Use of electronic health records to investigate the role of acute inflammation and infection in vascular disease

Caroline Minassian

July 2014

Department of Non-communicable Disease Epidemiology
Faculty of Epidemiology and Population Health
London School of Hygiene & Tropical Medicine,
University of London

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

This work was funded by the Wellcome Trust

Declaration

I, Caroline Minassian, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Caroline Minassian

July 2014

Abstract

Previous studies have demonstrated that acute systemic inflammation after surgery or infection is associated with a transient increase in the risk of vascular events. This suggests that vascular risk is not stable but fluctuates within short periods in response to inflammatory stimuli. While an association between respiratory tract infection and vascular events is well-documented, the effects of other acute infections and inflammatory stimuli are less certain.

The principal aim of this project was to investigate further the role of acute inflammation and infection in vascular disease, employing the unique opportunities offered by electronic health record databases. Two large observational studies were undertaken. First, the self-controlled case series method and Medicaid claims data from the United States were used to examine the short-term effects of invasive dental treatment, a novel acute inflammatory model, on the risk of vascular events. Second, a matched case-control study using primary care data from the United Kingdom General Practice Research Database investigated the role of acute maternal infection in the development of pre-eclampsia – a vascular disorder of pregnancy.

The case series analysis of 1152 adults with a vascular event demonstrated a transiently increased vascular event rate in the four weeks after invasive dental treatment relative to unexposed time periods. The analysis of 1533 pre-eclampsia cases and 14236 controls who had completed a pregnancy without pre-eclampsia revealed an increased risk of pre-eclampsia associated with antibiotic prescriptions and urinary infection, but not respiratory infection, during pregnancy.

The findings suggest that exposures sufficient to produce an acute inflammatory response may play an important role in the occurrence of vascular outcomes. Future research on the effects of other acute inflammatory triggers and the mediating mechanisms involved should help establish a clearer role for acute inflammation and infection in vascular disease and inform preventative strategies during periods of increased vascular risk.

Acknowledgements

First and foremost I would like to thank my supervisors, Professor Liam Smeeth and Dr Sara Thomas, for their invaluable guidance and encouragement throughout the duration of this project and for patiently reading draft versions of each Chapter.

I am also especially grateful for the advice and guidance I received from my other advisory committee members, Tim Clayton and Professor Aroon Hingorani.

Furthermore I would like to acknowledge Dr Francesco D'Aiuto, Professor Oona Campbell and Dr David Williams, with whom I had the pleasure of working on the pre-eclampsia and dental studies, for their expertise and interest and for providing many stimulating discussions.

Finally, I would like to express my deepest gratitude to my family, most of all my parents and sister Angela, and to Claudio, whose continuing love, support, patience and good humour enabled me to see this project through to completion.

Table of contents

List of Tables

List of Figures

Abbreviations

ART	Assisted reproductive technology
BMI	Body mass index
BNF	British National Formulary
CDT	Current Dental Terminology
CHD	Coronary heart disease
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
EDC	Estimated date of conception
EDD	Estimated date of delivery
EHR	Electronic health record
GPRD	General Practice Research Database
GP	General practitioner
HELLP	Haemolysis, Elevated Liver Enzymes, Low Platelets
HR	Hazard ratio
ICD-9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification
IgM	Immunoglobulin M
IMD	Index of Multiple Deprivation
IQR	Interquartile range
IR	Incidence ratio
LMP	Last menstrual period
MeSH	Medical Subject Headings
MI	Myocardial infarction
OR	Odds ratio
OXMIS	Oxford Medical Information System
RR	Relative risk
RTI	Respiratory tract infection
SCCS	Self-controlled case series
SES	Socioeconomic status
UK	United Kingdom
US	United States

UTI	Urinary tract infection
UTS	Up-to-standard

Chapter 1 Introduction

Vascular disease, notably coronary heart disease (CHD) and stroke, is a leading cause of chronic disease morbidity and mortality worldwide.[1,2] An estimated 15.6 million individuals died of cardiovascular disease (CVD) in 2010, the majority due to CHD (7.0 million) or stroke (5.9 million), representing a quarter of all global deaths.[3] The burden of CVD continues to rise and is projected to generate approximately 23.3 million deaths worldwide in 2030.[4]

The main pathological process underlying most vascular disease is atherosclerosis, the accumulation of lipid-containing material known as plaque or atheroma within the inner lining of blood vessels. It is a gradual process which can lead to the narrowing of the arteries if plaque deposits build up over time. When an artery becomes blocked due to plaque activation or rupture of unstable plaques resulting in thrombus formation or distal embolisation, this may culminate in an acute thrombotic event such as ischaemic stroke or myocardial infarction (MI).

While the mechanisms involved in the transition between stable and unstable atherosclerotic plaques are not completely understood, there is growing evidence that inflammation plays a key role.[5] Inflammatory and immune cells constitute an important component of plaques.[6] Furthermore, inflammation has been implicated in all phases of the atherosclerotic process: in early atherogenesis, in the progression of plaques, and finally in plaque activation and rupture resulting in arterial occlusion.[7] One of the earliest characteristics of atherosclerosis is the impairment in vascular endothelial function, which can derive from inflammation, and which has been shown to predict long-term atherosclerotic disease progression and vascular events.[8] Numerous prospective studies have shown that low-grade chronic systemic inflammation, as indicated by elevated levels of inflammatory biomarkers such as C-reactive protein (CRP), is associated with an increased risk of future CHD and ischaemic stroke, which persists after adjustment for conventional cardiovascular risk factors.[9,10] Among the potential sources of chronic inflammation which have been investigated are chronic infections such as periodontal disease, which has been shown to be associated with raised levels of CRP[11,12] and an increased risk of cardiovascular events in the long term[13,14]. While studies of specific pathogens,

including Chlamydia pneumoniae, herpes simplex virus and cytomegalovirus, have yielded mixed findings, their cumulative effect or overall "pathogen burden" has been shown to predict CRP levels and CHD risk.[15]

A possible aetiological role for acute inflammation in vascular disease has also received attention in recent years. There is accumulating evidence suggesting that episodes of acute bacterial or viral infection may trigger a short-term increase in the risk of vascular events. An increased risk of acute MI associated with recent acute respiratory tract infection (RTI) has been well-documented.[16] Evidence from observational studies also supports the notion that acute infection is an important trigger for ischaemic stroke, particularly in the week preceding stroke.[17] Four large studies using electronic primary care data have shown that acute RTI is associated with a transiently increased risk of vascular events.[18–21] The effect was strongest in the few days after infection (up to ten days), with relative risks (RR) ranging from 2.10 (95% confidence interval (CI) 1.38-3.21)[20] to 4.95 (95% CI 4.43-5.53)[19] for MI, and 1.92 (95% CI 1.24-2.97)[20] to 3.19 (95% CI 2.81-3.62)[19] for stroke, and gradually resolved over time. Three of these studies also investigated the effect of urinary tract infection (UTI): while one found no association with UTI,[18] two found a similar, transient increase in vascular event risk after UTI,[19,20] although in one the effect was confined to stroke[20]. These findings suggest that the risk of vascular events fluctuates transiently in response to acute inflammatory stimuli.

Various pathophysiological mechanisms have been proposed to explain the short-term increased risk of vascular events following acute infection observed in these earlier studies, including changes in plaque composition and stability, increased local coagulability due to disturbances in immunohaematological mechanisms, increased concentrations of CRP and proinflammatory cytokines, and endothelial dysfunction.[17] In support of the last, vaccination of healthy individuals has been shown to elicit a mild systemic inflammatory response which leads to a transient impairment in function of the arterial endothelium.[22] Such acute changes in the inflammatory state associated with acute infection may also give rise to a short-term alteration of endothelial function. Endothelial dysfunction may thus represent a common pathway through which acute infection/inflammation, among several other risk factors, may contribute to vascular risk.[19]

Another area of vascular disease in which inflammation has also been implicated is pre-eclampsia: a vascular disorder of unknown origin unique to pregnancy. Pre-eclampsia shares many features with atherosclerotic vascular disease. Notably, several well-established cardiovascular risk factors are associated with an increased risk of pre-eclampsia including a history of hypertension, obesity, diabetes and chronic renal disease.[23] Accordantly, women who develop pre-eclampsia are at an increased risk of CVD in later life.[24–26] A recent meta-analysis of studies assessing vascular risk after pre-eclampsia reported an approximately two-fold increased risk of CHD and stroke among women who had previously developed pre-eclampsia compared to parous women who had not.[27] A frequent finding in the uterine spiral arteries of women who develop pre-eclampsia is acute atherosis (lesions involving the accumulation of lipid-filled cells, resembling the early stages of atherosclerosis), which is thought to contribute to reduced placental perfusion.[28] Endothelial dysfunction is evident among women prior to the development of pre-eclampsia.[29] There are thus clear similarities between pre-eclampsia and other vascular diseases when investigating the role of inflammation.

1.1 Rationale for research

While an association between acute RTI and vascular events has been consistently demonstrated, the effects of other acute infections and inflammatory stimuli are less certain. Thus, a clear role for acute inflammation and infection in vascular disease has not yet been established. If acute inflammation does alter the occurrence of vascular events then similar effects should be seen for other inflammatory stimuli, and in other vascular disorders. Gaining a clearer understanding of how acute inflammation may be involved in vascular disease will give further insight into factors that influence the timing of vascular events, which in turn may help inform strategies for prevention or treatment at times of increased inflammation.

1.2 Aim and research questions

The overall aim of this project was to build on the previous work described above by investigating further the role of acute inflammation and infection in the occurrence of

vascular outcomes. To this end, two large observational studies were undertaken, each using electronic health records (EHRs) to address a specific research question.

1.2.1 Invasive dental treatment: a potential trigger for vascular events

The first study carried out for this thesis investigated a novel, acute inflammatory stimulus, invasive dental treatment, as a potential trigger for vascular events. While previous studies have examined whether invasive treatment of periodontal disease may be effective in reducing CVD risk by diminishing the infectious burden,[30] the aim of this study was to assess whether such treatment, or other invasive dental procedures sufficient to result in bacteraemia and induce an acute inflammatory response, may heighten the risk of vascular events in the short term.

The Medicaid claims database of the United States (US), in which both dental procedures and health outcomes are recorded, provided a unique opportunity to address this question. Using an innovative case-only design, the relative incidence of vascular events (ischaemic stroke and MI) in pre-defined periods after invasive dental treatment compared to all other observed time periods was estimated.

1.2.2 Acute maternal infections and pre-eclampsia

The second study undertaken as part of this thesis investigated the role of acute maternal infection in the pathogenesis of pre-eclampsia. The aim was to establish whether acute infections during pregnancy, sufficient to be likely to produce systemic effects, are associated with an increased risk of pre-eclampsia.

Using primary care data from the United Kingdom (UK) General Practice Research Database (GRPD) and a matched case-control study design, the effects of two specific acute infections on pre-eclampsia risk were assessed, namely UTI and RTI, in addition to maternal antibiotic drug prescriptions: a likely proxy for acute infection.

1.3 Outline of the thesis

The thesis comprises nine chapters. The background, methods and findings relating to the first study of invasive dental treatment and the risk of vascular events are presented first (Chapters 2 to 4); those relating to the second study of acute maternal

infection and pre-eclampsia are subsequently presented in Chapters 5 to 8. Both studies have been published. The papers "*Invasive dental treatment and risk for vascular events: a self-controlled case series*"[31] and "*Acute maternal infection and risk of pre-eclampsia: a population-based case-control study*"[32], are included as Appendices (Appendix A and Appendix B), together with additional supplementary material.

Chapter 2 presents a review of the published literature on the risk of vascular events associated with invasive dental treatment.

Chapter 3 describes the methods used in the study of invasive dental treatment and vascular events, including the study objectives, an overview of the Medicaid database, followed by a description of the case series method, a discussion of its strengths and advantages for the research question, and its application to the study.

The dental study findings are described in Chapter 4.

Chapter 5 presents a literature review of published articles on the association between pre-eclampsia and acute maternal infections.

Chapter 6 provides a detailed account of the methods used in the matched case-control study of pre-eclampsia and acute infection, including the study objectives, an overview of the GPRD, the matching procedure, the statistical methods used, and the analysis strategy.

Chapter 7 describes the algorithm developed as part of this project to identify and estimate the timing of pregnancies in the GPRD, for application in the pre-eclampsia study.

The results of the pre-eclampsia study are reported in Chapter 8.

In Chapter 9, the main findings of the dental and pre-eclampsia studies are summarised and brought into context of what was previously known on the topics, the suitability of the data sources to address the research questions are considered, and the implications of the findings for future research and clinical practice are discussed.

1.4 Funding

This project was funded by the Wellcome Trust.

Chapter 2 A literature review of invasive dental treatment and vascular events

This chapter reports on the literature review of studies assessing the association between invasive dental treatment and the risk of vascular events. The initial phase of the review was carried out prior to the planned analyses of the dental study (described in Chapter 3) and was subsequently updated for completeness. Following a brief overview of what is known of the link between dental infections, invasive dental treatment and CVD, the review methodology is described. Studies identified in the initial review phase, prior to completion of the dental study, are summarised first followed by additional studies identified in the updated search. The chapter concludes with a summary of the main findings of the review and a rationale for the dental study.

2.1 Background

Exposure to low-grade dental infections, particularly periodontal disease (a common chronic infection of the oral cavity caused by bacteria) has long been implicated in the aetiology of CVD. Several epidemiological studies have shown periodontal disease to be associated with raised levels of CRP and other inflammatory biomarkers,[11,12,33,34] and with endothelial dysfunction[35–37].

The first study reporting a link between dental infections and acute MI, published in 1989,[38] precipitated a number of subsequent studies examining the association between periodontal disease and cardiovascular events[39–41]. Meta-analyses of these studies have consistently shown an increased risk of cardiovascular events associated with periodontitis in the long term.[13,14,42,43] More recent population-based findings from the Scottish Health Survey have shown poor oral hygiene - a major cause of periodontal disease - to be associated with raised markers of inflammation (CRP and fibrinogen) and an increased risk of hospital admissions for both fatal and non-fatal cardiovascular events.[44]

2.1.1 Invasive dental treatment: an acute inflammatory stimulus

While treating periodontal disease may effect a positive influence on longer-term CVD risk by reducing the infectious burden,[30,45,46] studies have shown that intensive periodontal therapy can lead to a transient impairment of flow-mediated dilatation of the brachial artery (a measure of endothelial function), and raised markers of inflammation and endothelial activation in the week after treatment, followed by a longer-term improvement in these measures relative to baseline.[47–49] The more invasive the dental treatment, the more marked were these effects.[49] This work suggests that invasive dental treatment, particularly periodontal therapy, provides a useful model of acute inflammation. Furthermore, if the probability of a vascular event occurring is associated with changes in the underlying inflammatory state and endothelial function, then dental procedures sufficient to produce an acute inflammatory response may transiently increase the risk of vascular events, despite providing longer-term vascular benefits by reducing the infectious and inflammatory burden.

2.2 Methods

2.2.1 Aim of review

The aim of the literature review was to summarise published evidence on the effect of invasive dental treatment on the risk of vascular events, namely ischaemic stroke and MI.

2.2.2 Search strategy

The search was conducted using the Medline database which is indexed using the Medical Subject Headings (MeSH) system. A list of relevant MeSH keywords and free-text words for dental treatment and vascular events (ischaemic stroke and MI), was compiled. These were combined in an algorithm which was applied in Medline to identify all potentially relevant studies from inception to August 2010. The search was subsequently updated to February 2014, to identify any additional studies published after completion of the dental study (undertaken as part of this thesis, see Chapter 3).

For each study identified, the abstract was assessed with reference to the inclusion criteria described below. Reference lists of all studies fulfilling the inclusion criteria were scanned to identify any further studies or search terms that might have been missed in the initial search: subsequent searches were then conducted using any additional terms. For each included study, information on participant selection, exposure and outcome ascertainment, the main findings (including effect sizes and CIs when reported), and the extent of adjustment for confounding was recorded.

Full details of the search algorithm used are given in Appendix C-Table C.1.

2.2.3 Inclusion and exclusion criteria

Studies presenting original data, published in English, and which included fatal or non-fatal MI and/or stroke as an outcome and invasive dental treatment as an exposure were included. Invasive dental treatment was defined as any course of treatment or single procedure sufficient to result in bacteraemia, including periodontal therapy or dental extractions. Case reports, case-only studies with no comparison group (or with no comparison time period) and review articles without original data were excluded.

2.3 Results

The search generated 259 citations of which 249 were deemed not relevant (they did not report on the association between invasive dental treatment as defined above and vascular events). The remaining 10 publications were identified as potentially relevant. Of these, seven were excluded for the following reasons:

Four were case reports[50–52] or case series with no comparison group,[53] and a fifth lacked data relating invasive dental treatment and cardiovascular events[54].

The sixth publication reported on a multicentre pilot intervention study designed to assess the effects of periodontal therapy versus community dental care on the prevention of secondary cardiac events, and which found no difference in the incidence of adverse events between the treatment and control groups over a six month period.[55] However, the authors only presented findings for adverse cardiovascular and dental events combined. Furthermore, the nature of community

dental care was unclear as 48% of subjects randomized to community care received some preventive or periodontal treatment, which further complicates interpretation of the findings. Thus no conclusions could be drawn from this study on the effects of periodontal treatment on the risk of subsequent cardiovascular events.

Finally, a recent case-control study which examined the relationship between history of non-fatal MI and history of tooth extractions due to infection among adult males reported a positive association (OR 1.64, 95% CI 1.24-2.16).[56] However, this study was excluded due to uncertainty regarding the temporal sequence of events: both the disease history of MI and extractions were self-reported and their timing was not ascertained in the study, a limitation acknowledged by the authors. Furthermore, the reference group included, among those with no extractions, individuals with extractions due to trauma or other causes (not infection), and thus was not truly unexposed to invasive dental treatment.

2.3.1 Included studies

Three cohort studies fulfilled the inclusion criteria: two from Taiwan,[57,58] one from the US,[59] all three conducted within administrative healthcare claims databases. However, these studies were identified in the updated search (post-August 2010), after the dental study (described in Chapter 3) was completed. While the findings of these studies did not inform the dental study analyses, they are nevertheless relevant to this thesis, and are thus summarised below.

The most recent, a population-based Taiwanese study,[58] assessed the association between periodontal treatment and the incidence of ischaemic stroke among 719,436 beneficiaries of Taiwan's National Health Insurance program who were followed up over a ten year period (2000 to 2010). Comparing both treated and untreated individuals with periodontal disease to individuals without periodontal disease, the authors found a significantly lower rate of stroke in the intensive treatment periodontal disease group (Hazard ratio (HR) 0.95, 95% CI 0.91-0.99), and a significantly higher stroke rate among the untreated periodontal disease group (individuals with neither dental prophylaxis nor intensive treatment) (HR 1.15, 95% CI 1.07–1.24), after adjusting for age, sex, hypertension, diabetes and atrial fibrillation. Individuals with periodontal disease who received dental prophylaxis

only had the lowest stroke rate. The authors concluded that periodontal disease is an important risk factor for ischaemic stroke, and the treatment of periodontal disease reduces the incidence of stroke. However, the study had important limitations, for example, the potential for confounding by other unmeasured risk factors for stroke, notably smoking, body mass index (BMI), socioeconomic status (SES), alcohol consumption, diet, and other comorbidities such as inflammatory diseases. Smoking in particular is an important risk factor for both CVD and periodontal disease, and may influence the outcome of periodontal treatment. In addition, the apparent protective effect of treatment may in part be attributed to unmeasured differences in health-seeking behaviour between treated and untreated individuals. Finally, the study did not assess the timing of any increased risk following dental treatment; rather the focus was on the long-term effect of periodontal treatment on stroke risk up to ten years after treatment.

The earlier Taiwanese study,[57] conducted in the same database, examined the effect of tooth scaling (a component of periodontal therapy) on the risk of ischaemic stroke and MI among beneficiaries aged ≥50 years (10,887 exposed to tooth scaling at baseline and propensity score matched with 10,989 with no tooth scaling) over a seven year period (2000-2007). Tooth scaling was found to be associated with a reduced risk of developing MI (HR 0.69, 95% CI 0.57-0.85), and stroke (HR 0.85, 95% CI 0.78-0.93) after adjustment for age, gender, history of hypertension, hyperlipidaemia, dysrhythmia, diabetes and chronic kidney disease. However, this study was also prone to residual confounding due to a lack of adjustment for some important CVD risk factors, such as smoking, BMI, and other socioeconomic and lifestyle factors. Furthermore, the study also assessed long-term rather than acute effects of dental treatment on vascular risk.

The third study of 2035 beneficiaries of Medicare (the US health insurance program for individuals aged ≥65 years), who had all experienced an ischaemic vascular event, examined whether dental treatment in the one, two, three and six month periods immediately after this first event increased the risk of having a subsequent event.[59] During an average follow-up of four years, the researchers observed no increased risk of a second vascular event associated with dental treatment of any type, or with invasive procedures considered separately, performed in any of these

periods (up to six months) after the initial event. However, the study did not assess the timing of the second vascular event relative to the dental treatment. Furthermore, only high-risk individuals who had already suffered a vascular event were included, which limits the generalisability of the findings.

2.4 Comment

The initial phase of the literature search preceding the planned dental study analyses described in Chapter 3 yielded no studies that fulfilled the inclusion and exclusion criteria outlined above (section 2.2.3). In the updated search following completion of the dental study, three studies were identified which met the inclusion criteria. Thus, the rationale for the dental study is presented in light of what was already known on the association between invasive dental treatment and vascular risk, followed by a brief summary of what subsequent studies have shown.

2.4.1 Rationale for the dental study

While recent work has shown that invasive dental treatment such as periodontal therapy gives rise to an acute inflammatory response and transient impairment of endothelial function, it is not known whether such treatment confers an inflammation-induced fluctuation in vascular risk. Studies examining any such acute effect of invasive dental treatment are lacking. Such an effect, if observed, would have important implications for preventative measures at the time of invasive dental treatment, particularly among individuals at high risk of vascular events. Hence the dental study (described in Chapter 3) sought to test the hypothesis of an inflammation-induced, acute rise in vascular risk following exposure to invasive dental treatment.

2.4.2 Summary of evidence since the dental study

The three studies fulfilling the inclusion criteria, published after the dental study, each used a cohort design and were prone to residual confounding due to between-person differences in unmeasured, shared risk factors for both periodontal disease and vascular events such as SES or smoking. Such factors could play a role in explaining, at least in part, the protective effect of dental treatment on cardiovascular risk seen in the Taiwanese studies,[57,58] or the null effect observed in the US study[59].

Additionally, the focus of these studies was on the longer-term effect of dental treatment on vascular event risk over a period of several years. None of the studies assessed whether dental treatment might trigger a short-lived fluctuation in vascular event risk in the period immediately after treatment. Thus no conclusions could be drawn from these studies regarding any such acute effect of the dental treatment.

Chapter 3 Methods - Invasive dental treatment and vascular events

This chapter describes the methods of a self-controlled case series (SCCS) study using Medicaid administrative claims data to assess the risk of ischaemic stroke and MI following invasive dental treatment. All Medicaid enrolees exposed to invasive dental treatment and with a primary hospital discharge diagnosis of ischaemic stroke or MI occurring between January 2002 and December 2006 were selected. Hence the study only included exposed cases. Incidence ratios (IRs) and 95% CIs for vascular events occurring in periods immediately after invasive dental treatment versus all other observed time periods were derived from within-person comparisons using conditional Poisson regression.

3.1 Study hypothesis and objectives

The hypothesis was that invasive dental procedures sufficient to invoke an acute systemic inflammatory response may lead to a transient increased risk of vascular events (ischaemic stroke and MI).

The primary objective was to:

- Compare the risk of vascular events in periods following exposure to invasive dental treatment with the risk in periods not exposed to invasive dental treatment, and to quantify any increased risk.

Secondary objectives were to:

- Estimate separately the effect of invasive dental treatment on the risk of ischaemic stroke and on the risk of MI, in periods following the treatment.
- Investigate to what extent intra-person risk factors for vascular events that change with time may contribute to the effect of invasive dental treatment on the risk of vascular events (if such an effect is observed).

3.2 Description of the data source

3.2.1 The Medicaid Database

There are no longitudinal data linking dental procedures with health outcomes in the UK. Therefore the study used Medicaid claims data from the US, in which both dental and medical details are recorded. Medicaid is the US health care program established in 1965 to provide medical coverage for low-income individuals and families without private health insurance. Coverage includes physician and hospital bills, drug treatment costs, and long-term care. An estimated 50 million individuals received Medicaid assistance in 2009, corresponding to approximately one in six US citizens.[60]

Eligibility for enrolment in Medicaid is income-related, the main criterion being limited income and financial resources, and is evaluated monthly. The Medicaid database used for this study comprised pooled, anonymised claims data from nine geographically dispersed, anonymised states. It included details of all health care provided to beneficiaries, including inpatient and outpatient services, outpatient prescription drugs and details of enrolment, in addition to basic demographic information. The data have high levels of completeness and validity and undergo frequent quality checks to ensure selected fields are valid (including diagnosis codes, procedure codes, and dates of service), and that data across fields are reasonable against norms (e.g. diagnosis against gender or age).[61]

3.2.2 Data structure and key elements

Data were provided in separate SAS files. A unique person-level identifier allowed linkage of information across files. Most files contained multiple records per individual; the meaning of a single record varied across files. Files of interest for the study are described in Table 3.1 below.

Table 3.1 Medicaid files used for the dental study.

Data file	Each record represents:
Inpatient admissions	One hospital admission including the dates of admission and discharge, the primary diagnosis (the main reason for admission, usually the discharge diagnosis), the primary procedure performed during an admission, and up to 15 additional secondary diagnoses and procedures.
Inpatient Services	One service claim associated with an inpatient admission including the date of service, one procedure and up to two diagnoses. Multiple services make up one hospital admission.
Outpatient services	One service claim rendered in an outpatient facility, doctor's office, or hospital outpatient facility, including the date of service, one procedure and up to two diagnoses. Multiple services make up one outpatient visit.
Outpatient pharmaceutical claims	One prescription drug claim from mail-order programs or retail pharmacy including the drug code, therapeutic class, and the date the prescription was filled.
Enrolment	An individual's annual enrolment (January to December) with monthly flags indicating enrolment status, and demographic information including year of birth, gender and ethnicity.

3.2.2.1 Coding systems

In Medicaid, clinical diagnoses are coded using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), a classification system based on the World Health Organization's Ninth Revision, International Classification of Diseases (ICD-9).

Procedures are recorded using the Healthcare Common Procedure Coding System which includes two levels of codes. Level 1 comprises the American Medical Association's Current Procedural Terminology and includes medical, surgical and diagnostic procedures. Level 2 consists primarily of non-physician services and includes the American Dental Association's Current Dental Terminology (CDT),[62] a coding system for the billing of dental procedures and supplies.

Prescription drug claims are recorded using the National Drug Code coding system; each code belongs to a therapeutic class based on the American Hospital Formulary Service Classification Compilation.

3.3 The Self-controlled Case Series Method

This study used a case-only approach, the SCCS method,[63] to examine the risk of vascular events following exposure to invasive dental treatment.

3.3.1 Origin and description of the method

The SCCS method uses within-person comparisons to investigate the association between time-varying exposures and outcome events in a population of individuals all of whom experienced the outcome of interest. It is derived from a Poisson cohort model by conditioning on the number of events and exposure history experienced by an individual over a pre-defined observation period: the time during which if an event arose the individual would be sampled. While the method was originally developed to investigate associations between vaccination and acute adverse events,[64,65] it has subsequently been applied in other settings, for example to investigate the risk of MI,[21] stroke,[19] and deep vein thrombosis and pulmonary embolism[66] following acute infection, and it has been extensively used in pharmacoepidemiology[67–71].

The SCCS method provides an alternative to the more established cohort method for estimating the relative incidence of an event: that is the ratio of the rate of events in a defined period following exposure to the rate of events in the absence of exposure (the baseline period). A pictorial representation of the method is shown in Figure 3.1. Only cases are sampled: there is no comparison group of individuals. Comparisons are within-person. To take this into account, the likelihood is conditional on an outcome event having occurred during the observation period, and is thus based on the probability density that an individual's event occurred when it did in relation to exposure, given that their event occurred during the observation period.

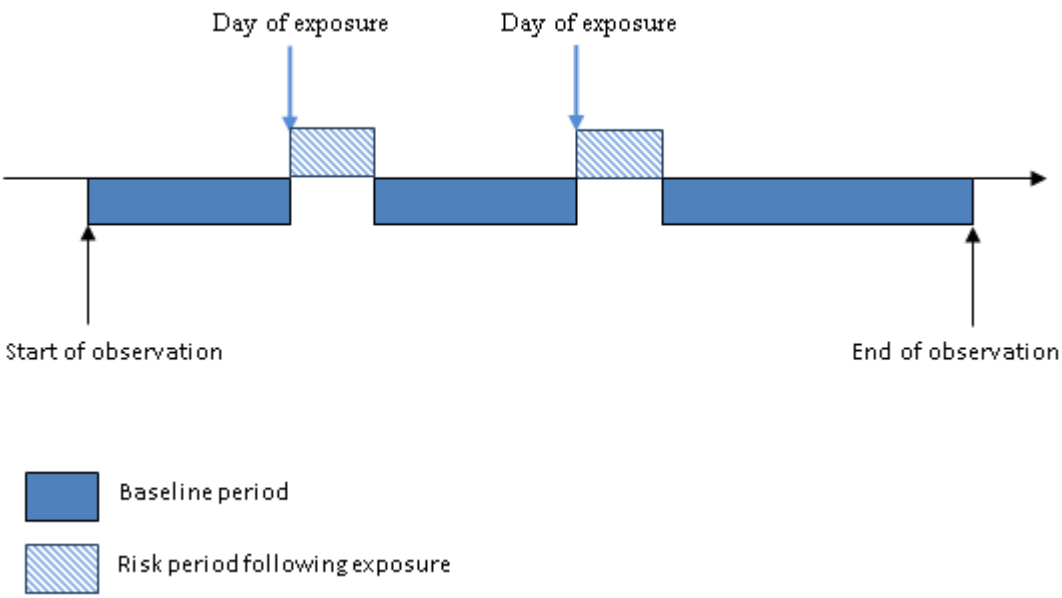


Figure 3.1 Pictorial representation of the self-controlled case series method.

The Figure represents a single participant experiencing two exposures during their observation period. The outcome event(s) could occur at any time during the observation period. The baseline period refers to all time an individual is observed and not "at risk" following an exposure.

3.3.2 Application of the method to this study

Individuals who have undergone invasive dental treatment may differ from those who have not in ways which can be difficult to measure and control for. Some of these differences may be associated with the future risk of vascular events which makes a conventional cohort design a less reliable approach for assessing this association. Therefore, the SCCS method was used to make within-person comparisons in individuals who experienced a vascular event (the outcome of interest). Because no comparisons are made between individuals, between-person confounding is not an issue when using this method.

Conditional Poisson regression was used to estimate the relative incidence of vascular events occurring during pre-defined risk periods after exposure to invasive dental treatment relative to all other observed time periods, in the same individuals. The null hypothesis was that the rate of vascular events remains constant from day to day and is not affected by exposure to invasive dental treatment. Further details of the analytical approach using this method for this study are provided in section 3.8.

3.3.3 Advantages

The SCCS design provided major advantages for the study question. The main advantage is that inference is within individuals; hence both recorded and unrecorded characteristics which may vary between individuals but are stable within individuals over the observation period, such as genetic factors, gender, SES and underlying health status, are implicitly controlled for. This minimised the potential for confounding in this study. The method only uses exposed cases (in this study, individuals who experienced both a vascular event and invasive dental treatment), which reduced the possibility of under-ascertainment of exposure to invasive dental treatment. In addition, it allowed the time-varying effect of age on the baseline incidence of vascular events to be controlled for. The method also often has high statistical efficiency relative to the cohort method from which it is derived.[63]

3.3.4 Limitations and assumptions

The SCCS method only produces estimates of relative incidence, not absolute incidence. Hence this study reports only IRs. The method also requires some variability in the timing of, or age at, the event: it would fail if all events occurred at the same age (an unlikely scenario, and not an issue in this study). In addition, the validity of the method rests on some important assumptions:[72] first, that the occurrence of an event does not affect an individual's subsequent exposure; second, that the occurrence of an event does not alter the duration of the observation period; and third, that events are independent within an individual. Details of how this study addressed these assumptions are discussed further in section 3.8.4.

3.4 Participants

Study participants were derived from a population of 9, 901, 464 Medicaid beneficiaries for whom data were available in the Medicaid database from 1st January 2002 to 31st December 2006. This comprised approximately 28 million person-years of observation.

All incident cases of ischaemic stroke or MI occurring during the study period were identified from primary discharge diagnoses on hospital admission records, coded using the ICD-9-CM classification system.

3.4.1 Eligibility criteria

Candidates were individuals who had a first hospital admission record for ischaemic stroke or MI at least 24 weeks after their enrolment in Medicaid began. This ensured a minimum of 24 weeks observation prior to the outcome, which reduced the possibility that an individual's first vascular event record was a repeated record for an earlier event.

3.4.1.1 Exclusions

The following three exclusion criteria were applied:

1. Age < 20 years at time of first stroke or MI.

Individuals were excluded if they were younger than 20 years of age at the time of their first hospital admission record for stroke or MI, because the aetiology of their stroke or MI could have differed from older individuals.

2. First vascular event occurred outside the maximum continuous enrolment period.

Eligibility for Medicaid is ascertained on a monthly basis, therefore gaps were sometimes found in an individual's enrolment. Medical events or procedures occurring during such gaps are unlikely to be recorded in the database. Thus, if an individual underwent dental procedures during a gap in enrolment, this could lead to misclassification of exposure to invasive dental treatment. To avoid this source of bias, each individual's maximum period of continuous enrolment was identified and their observation was restricted to this period. This ensured the study only included person-time during which individuals would have had health care services reimbursed by Medicaid if they had occurred. Individuals whose first recorded stroke or MI occurred outside this period were subsequently excluded from the relevant analyses.

3. No record of invasive dental treatment during the observation period.

All candidates not excluded for reasons 1 or 2 above were eligible for inclusion in the study. However, in a case series analysis, individuals who were not exposed during their observation period do not contribute to the estimate of association

between the exposure and outcome. The primary analysis was therefore restricted to eligible individuals who had both a vascular event and invasive dental treatment during their maximum continuous enrolment period in Medicaid.

3.4.2 Statistical power

A crude estimate of the number of potentially eligible individuals with a vascular event was derived from preliminary searches of beneficiaries' data. More than 30,000 individuals were identified with an ICD-9-CM diagnostic code for ischaemic stroke or MI (defined below in sections 3.5.1 and 3.5.2) during the study period. The binomial method[73] was used to estimate the power of the study to detect an IR for vascular events of 1.6 or greater in the first four weeks after invasive dental treatment compared to baseline.

Assuming at least 5% of potentially eligible individuals had undergone invasive dental treatment over a mean observation period of four years, the study had more than 90% power (at 5% significance) to detect an IR of at least 1.7 in the first four weeks after invasive dental treatment (compared to baseline); and more than 80% power to detect an IR of at least 1.6 in the first four weeks after treatment.

3.5 Outcome measures

The primary outcome of this study was the IR for vascular events (ischaemic stroke and MI) in the first four weeks after exposure to invasive dental treatment compared to baseline (unexposed time periods).

The accuracy of hospital discharge diagnostic codes for stroke and MI classifications in administrative claims databases has been examined and validated, with studies estimating positive predictive value of 90-96% for stroke[74,75] and 89-97% for MI[76–80].

3.5.1 Ischaemic stroke

Based on the criteria used by Tirschwell and Longstreth,[74] ischaemic stroke was defined as any one of the following ICD-9-CM codes as the primary discharge diagnosis on an inpatient admission record, with an admission date during the study period (1st January 2002 to 31st December 2006):

433.x1 Occlusion and stenosis of precerebral arteries (where "x" can vary to specify a specific arterial distribution, and the fifth digit 1 indicates "with cerebral infarction").

434.x1 Occlusion of cerebral arteries (where "x" can vary to specify the type of occlusion (thrombus, embolus or unspecified), and the fifth digit 1 indicates "with cerebral infarction").

436 Acute, but ill-defined, cerebrovascular disease.

The stroke was excluded if any one of the following ICD-9-CM codes for traumatic brain injury was recorded as a secondary diagnosis (in any of the 15 secondary diagnosis fields) for the same hospitalization: 800-804 Fracture of skull; 850-854 Intracranial injury, excluding those with skull fracture.

3.5.2 Myocardial infarction

Acute MI was defined according to the criteria used by Kiyota and co-workers,[80] as any one of the following ICD-9-CM codes as the primary discharge diagnosis on an inpatient admission record, with an admission date during the study period and a hospital length of stay lasting at least three days (unless the patient died, in which case less than three days was allowed) and no more than 180 days:

410.x1 Acute MI (where "x" can vary to specify the site of the MI, and the fifth digit 1 designates the initial episode of care for a newly diagnosed MI).

3.5.3 Timing of events

For both ischaemic stroke and MI, the date of admission to hospital was used to estimate the timing of the event.

3.5.4 Multiple events

The majority of individuals experienced a single vascular event during their observation period. However, a few individuals had multiple primary discharge diagnoses for stroke or MI on hospital admission records (see Chapter 4, Table 4.2). In this study, analyses were restricted to the first primary discharge diagnosis for a vascular event. This approach was taken in order to uphold an important assumption

of the case series method, that outcome events are independent within individuals, as discussed in section 3.8.4. Restricting to first events also avoided the difficulty in establishing whether these multiple primary discharge diagnoses represented multiple events or were in fact repeat hospitalisations for the same event.

3.6 Exposures

3.6.1 Invasive dental procedures

The exposure of interest in the study was invasive dental treatment. As mentioned earlier in section 3.2.2.1, dental procedures are recorded in Medicaid using the CDT coding system[62]. A complete list of CDT codes for invasive dental procedures was compiled in collaboration with a consultant in periodontology. The codes and corresponding terms are listed in Appendix D -Table D.1.

Data on claims for invasive dental procedures were extracted from inpatient and outpatient service records. All CDT codes for these procedure claims were further reviewed by the consultant periodontist and separated into two groups:

Invasive-inflammatory: dental procedures which may feasibly result in bacteraemia and induce an acute, local and systemic inflammatory response (Table 3.2).

Invasive-not inflammatory: dental procedures which were less severe and unlikely to induce an inflammatory response (Table 3.3).

Only invasive-inflammatory procedures were included in the study. These included periodontal therapy (surgical and non-surgical treatment involving tooth scaling, root planing and probing), and other invasive dental surgical procedures such as dental implant placement and tooth extractions, also known to be associated with bacteraemia[81,82] and with raised markers of inflammation[83].

Thus, exposure to invasive dental treatment was defined as any one of the CDT codes listed in Table 3.2 on inpatient or outpatient service records, with a procedure date during the observation period.

Table 3.2 Invasive-inflammatory dental procedures in Medicaid.

CDT [a] code	Description of procedure
D3410	Apicoectomy/periradicular surgery-anterior
D3421	Apicoectomy/periradicular surgery-bicuspid (first root)
D4210	Gingivectomy or gingivoplasty - four or more contiguous teeth or bounded teeth spaces per quadrant
D4211	Gingivectomy or gingivoplasty - one to three contiguous teeth or bounded teeth spaces per quadrant
D4341	Periodontal scaling and root planing - four or more teeth per quadrant
D4342	Periodontal scaling and root planing - one to three teeth, per quadrant
D6010	Surgical placement of implant body: endosteal implant
D7210	Surgical removal of erupted tooth requiring elevation of mucoperiosteal flap and removal of bone and/or section of tooth
D7230	Removal of impacted tooth-partially bony
D7240	Removal of impacted tooth-completely bony
D7241	Removal of impacted tooth-completely bony, with unusual surgical complications
D7250	Surgical removal of residual tooth roots (cutting procedure)
D7260	Oral antral fistula closure
D7261	Primary closure of a sinus perforation
D7280	Surgical access of an unerupted tooth
D7290	Surgical repositioning of teeth
D7310	Alveoloplasty in conjunction with extractions - four or more teeth or tooth spaces, per quadrant
D7311	Alveoloplasty in conjunction with extractions - one to three teeth or tooth spaces, per quadrant
D7320	Alveoloplasty not in conjunction with extractions - four or more teeth or tooth spaces, per quadrant
D7321	Alveoloplasty not in conjunction with extractions - one to three teeth or tooth spaces, per quadrant
D7340	Vestibuloplasty-ridge extension (second epithelialization)
D7410	Excision of benign lesion up to 1.25 cm
D7411	Excision of benign lesion greater than 1.25 cm
D7450	Removal of benign odontogenic cyst or tumor-lesion diameter up to 1.25 cm
D7460	Removal of benign nonodontogenic cyst or tumor-lesion diameter up to 1.25 cm
D7461	Removal of benign nonodontogenic cyst or tumor-lesion diameter greater than 1.25 cm
D7471	Removal of lateral exostosis (maxilla or mandible)
D7473	Removal of torus mandibularis
D7485	Surgical reduction of osseous tuberosity
D7510	Incision and drainage of abscess-intraoral soft tissue
D7520	Incision and drainage of abscess-extraoral soft tissue
D7540	Removal of reaction-producing foreign bodies-musculoskeletal system
D7550	Partial ostectomy/sequestrectomy for removal of non-vital bone
D7730	Mandible-open reduction
D7912	Complicated suture-greater than 5 cm
D7960	Frenulectomy (frenectomy or frenotomy)-separate procedure
D7970	Excision of hyperplastic tissue-per arch
D7972	Surgical reduction of fibrous tuberosity
D7999	Unspecified oral surgery procedure, by report

[a] Current Dental Terminology

Table 3.3 Invasive-not inflammatory dental procedures in Medicaid.

CDT[a] code	Description of procedure
D7111	Extraction, coronal remnants - deciduous tooth
D7140	Extraction, erupted tooth or exposed root (elevation and/or forceps removal)
D7220	Removal of impacted tooth-soft tissue
D7286	Biopsy of oral tissue - soft
D7971	Excision of pericoronal gingiva

[a] Current Dental Terminology

3.6.1.1 Defining treatment episodes

Some individuals had multiple records of invasive dental procedures. Within individuals, procedure records at least one week apart were considered to be repeat procedures; procedure records within one week of a previous record were excluded, as these were assumed to correspond to the same treatment episode. The methods used in the analysis to deal with repeat procedures are described in section 3.8.1.1.

3.6.2 Time-varying covariates

For descriptive purposes and analyses exploring the effects of time-varying covariates (see section 3.8.3), additional information was extracted on diseases and drug exposures which may have been temporally associated with both the occurrence of vascular events and invasive dental treatment and hence may have introduced within-person confounding.

3.6.2.1 Comorbidities

Study participants with a diagnosis of diabetes, hypertension, CHD or rheumatoid arthritis on inpatient admission or outpatient claim records prior to their invasive dental treatment were identified. These conditions were defined by the following ICD-9-CM diagnostic codes, recorded as the primary discharge diagnosis or any of the 15 secondary diagnoses for a hospital admission, or in either of the two diagnoses fields for an outpatient claim:

Diabetes: 250 (Diabetes mellitus);

Hypertension: 401-405 (Hypertensive disease);

CHD: 410-414 (Ischaemic heart disease) and 429.2 (Cardiovascular disease, unspecified);

Rheumatoid arthritis: 714 (Rheumatoid arthritis and other inflammatory polyarthropathies).

3.6.2.2 Drug exposures

Prescriptions within the antiplatelet, salicylate and non-steroidal anti-inflammatory drug (NSAID) therapeutic classes dispensed at any time before invasive dental treatment or up to one month after the treatment (see section 3.8.3) were identified from outpatient pharmaceutical claims records.

3.7 Data extraction and preparation

Data were extracted on demographic characteristics (year of birth, gender and ethnicity); enrolment details (start and end dates of continuous periods of enrolment in Medicaid); hospital admissions for vascular events (primary discharge diagnoses for ischaemic stroke and MI as defined in sections 3.5.1 and 3.5.2); claims for invasive dental procedures (CDT codes listed in Table 3.2); and potential time-varying covariates (diabetes, hypertension, CHD, and use of antiplatelets, salicylates and NSAIDs, as defined in section 3.6.2 above).

Key variables were checked for missing values and outliers (none were found). Dates corresponding to each individual's enrolment, inpatient admissions, inpatient and outpatient procedures, and drug prescriptions were all valid and within the study period. All date fields were complete.

Duplicate records for hospital admissions (when an individual had more than one admission record on the same day) were identified. Just 0.3% of all hospital admission records were duplicates and on all such duplicate records the individual's date of admission and discharge were the same. When an individual had a duplicate admission record for a vascular event, the record with the latest discharge date was selected. Thus, an individual's length of hospital stay and status at discharge (e.g. "died") were determined from the admission record with the latest discharge date. Information on age was limited to year of birth. Therefore, estimates of age were derived assuming each individual's date of birth to be 1st July of their birth year, allowing a maximum error margin of six months for age.

3.8 Statistical analysis

Analyses were performed to assess the short-term effect of invasive dental treatment on the risk of vascular events overall and separately by type of event (ischaemic stroke or MI).

3.8.1 Primary analysis

Individuals who experienced their first vascular event and at least one invasive dental procedure during their observation period were included in the primary analysis of vascular events. The observation period for each individual was the time during which if a vascular event occurred the individual would be sampled i.e. their continuous enrolment period in Medicaid between January 2002 and December 2006. Thus each individual was followed up from the start of their continuous enrolment period until they died or their continuous enrolment period ended (whichever occurred first), regardless of when they experienced their vascular event.

The risk period (during which invasive dental treatment might trigger an acute systemic inflammatory response) started on the day after an invasive dental procedure, extending up to 24 weeks, and was subdivided into the following periods: weeks 1-4, 5-8, 9-12, 13-16 and weeks 17-24, as the risk during the last eight weeks was assumed to be constant. All other observation time was taken as the baseline (unexposed) period. The choice of 24 weeks was based on previous work which suggested any increased risk would return to baseline by 24 weeks,[19,66] thus allowing the resolution of any increased risk to be fully described. The decision to start the risk period one day after a dental procedure was based on current evidence that the host response and vascular function are affected at their maximum 24 hours after invasive dental treatment.[47–49,84] Figure 3.2 illustrates the application of the method to this study and the time intervals used.

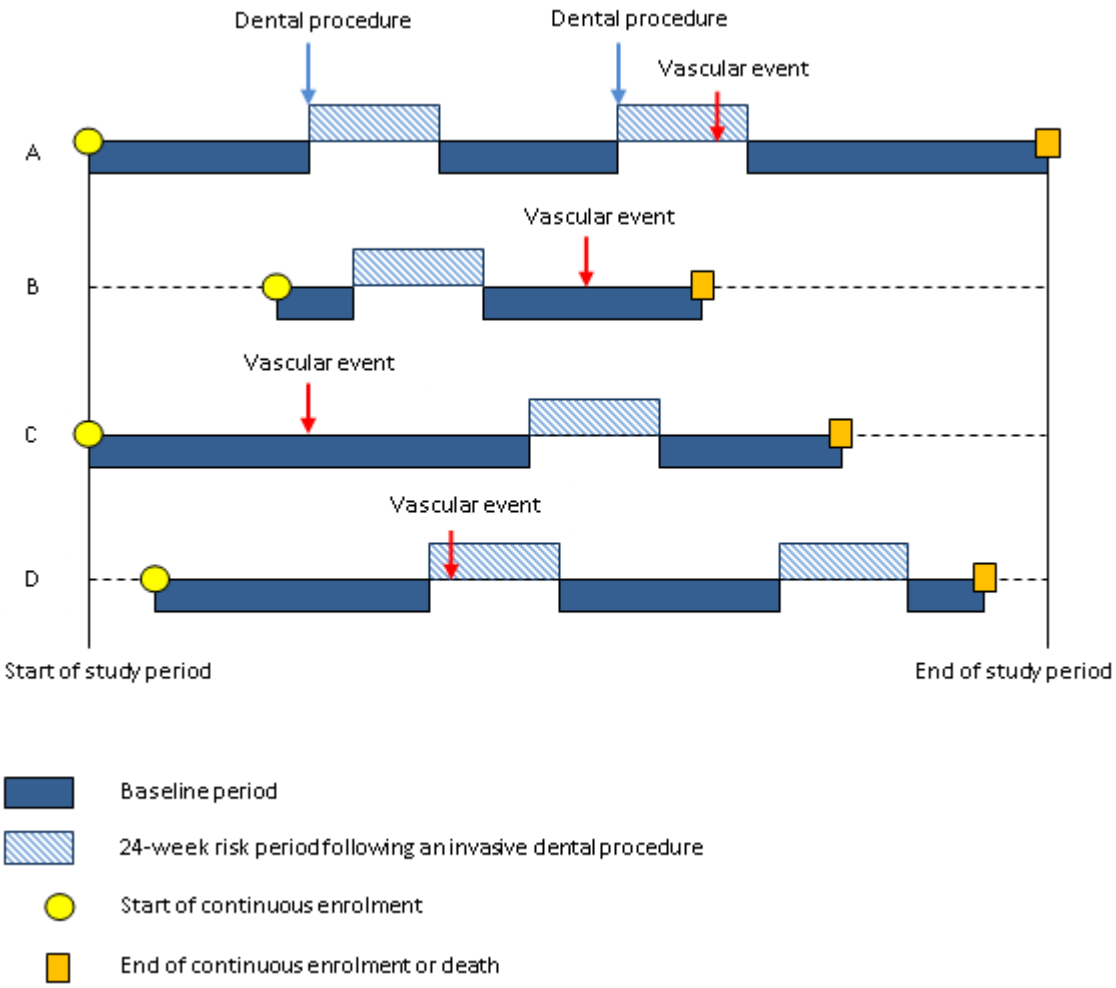


Figure 3.2 Pictorial representation of the timing of vascular events and dental procedures.

The Figure illustrates four possible scenarios for the timing of vascular events and invasive dental procedures, each representing a single participant: A was followed up for the duration of the study period, had two 24-week risk periods each following an invasive dental procedure and a vascular event during the second risk period; B was followed up for part of the study period, had one dental procedure followed by a vascular event at baseline; C was followed up from the start of the study period, had a vascular event at baseline prior to a dental procedure and died before the end of the study period; D was followed up for most of the study period, had two dental procedures and a vascular event during the first risk period. All participants included in a particular analysis had at least one dental procedure and at least one vascular event. Each risk period began the day after a procedure and lasted 24 weeks (not drawn to scale relative to length of baseline periods), divided into the following intervals: 1-4, 5-8, 9-12, 13-16 and 17-24 weeks.

Conditional Poisson regression was used to estimate IRs and 95% CIs for vascular events occurring within each stratum of the risk period compared to baseline. Age effects were adjusted for using 5-year age groups (20-24, 25-29, 30-34....≥85 years). Each individual's observation period was split into successive time intervals determined by changes in age group and exposure status, thus allowing individuals to contribute to different age groups over time. The crude age effect (IR for vascular events for a five-year increase in age) was estimated in a model including age group alone. Data were analysed using Stata software, version 10 (StataCorp., College Station, Texas).

3.8.1.1 Multiple invasive dental procedures

Some individuals had more than one invasive dental procedure during their observation period. When dealing with repeat procedures, each procedure was followed by a 24-week risk period. The same level of risk was assumed following each procedure, thus not allowing for a dose-effect. In the case of overlapping risk periods, when two or more procedures occurred within 24 weeks of each other, a simple convention was adopted which allowed later procedures to take precedence over earlier ones.[63] Thus, when an individual had two or more dental procedures and a later procedure occurred at some time during the risk period of an earlier procedure, a new 24-week risk period began from this time point. This meant that although the later procedure took precedence, it did not replace the earlier procedure; the earlier procedure was not ignored.

Figure 3.3 illustrates this convention with two possible scenarios. First, if an individual had a dental procedure followed by a vascular event two weeks later, and then a second dental procedure 20 weeks after the first, the vascular event would not have been classified as occurring during baseline; it would have been classified as occurring in the risk period corresponding to the first procedure, as shown in Scenario A. However, a vascular event occurring two weeks after the second dental procedure would have been classified as occurring during the risk period of this second procedure rather than during the risk period of the first, as shown in Scenario B. This convention was chosen as it reflects the actual exposure experience: in both scenarios the vascular event occurred two weeks after a dental procedure.

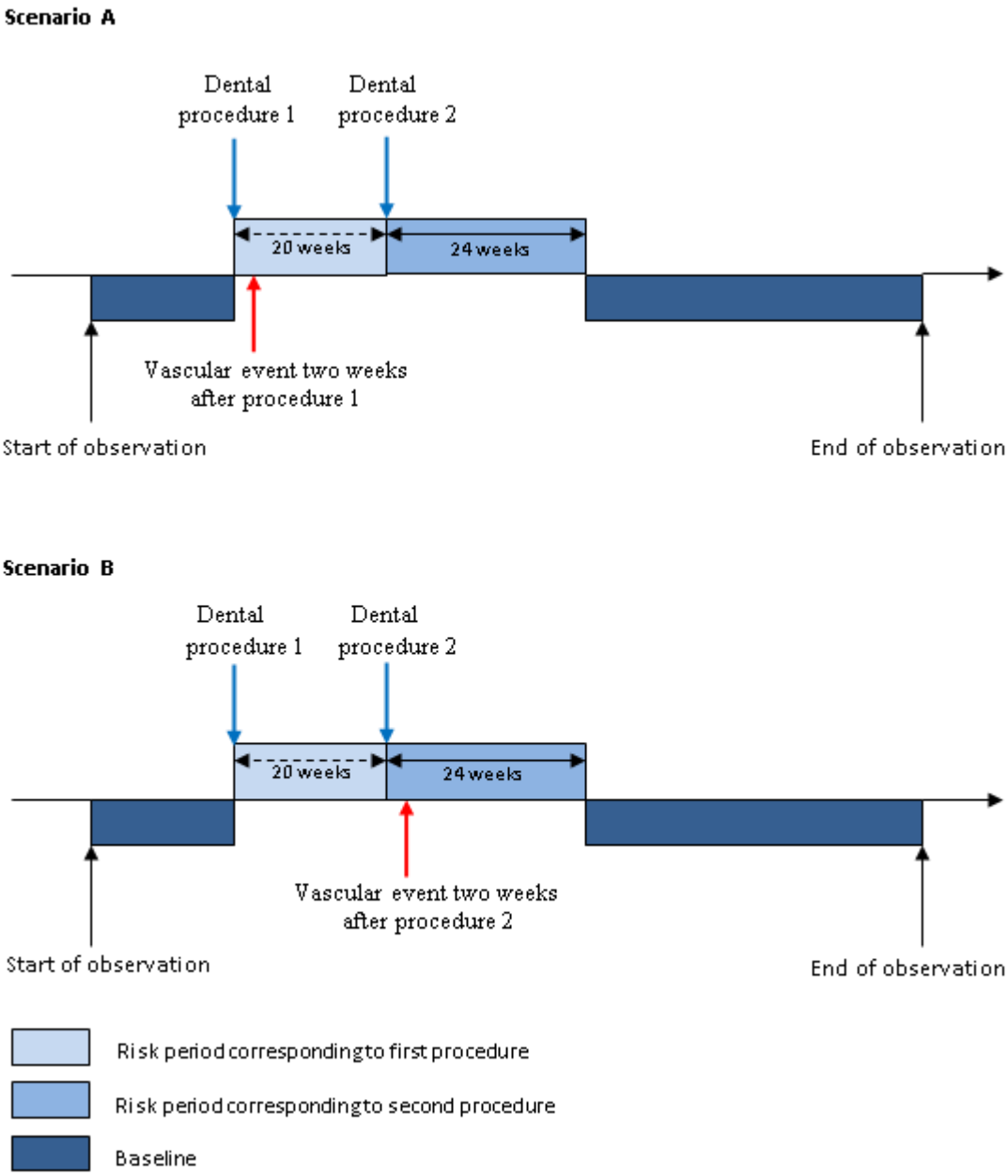


Figure 3.3 Pictorial representation of overlapping risk periods.

To eliminate the possibility that this convention (allowing later procedures to take precedence over earlier ones if risk periods overlap) might have contributed to an observed effect in earlier time frames, the primary analysis was repeated excluding individuals whose repeat procedures had overlapping risk periods.

3.8.2 Analyses by event type

The same methods as for the primary analysis (section 3.8.1) were used to assess the effect of invasive dental treatment on ischaemic stroke and MI separately.

Individuals whose first ischaemic stroke and at least one invasive dental procedure occurred during the observation period were included in the analysis of ischaemic stroke; individuals whose first MI and at least one invasive dental procedure occurred during the observation period were included in the analysis of MI. Thus, it was possible for an individual to be included in one of these analyses but not in the primary analysis of vascular events, if their first stroke (or MI) in the observation period was preceded by an earlier first MI (or stroke) before the start of the observation period.

3.8.3 Addressing time-varying confounding

As described in section 3.3.3, a major advantage of the SCCS method is that no comparisons are made between individuals; hence fixed covariates, measured and unmeasured, are implicitly controlled for in the analysis. However, there is still scope for confounding if intra-person risk factors for the study outcome (first vascular event) that change over time are also associated with the timing of exposure (invasive dental treatment).

Age

As the baseline risk of vascular events (the risk in the absence of exposure to invasive dental treatment) varies with age, each individual's follow-up was split into successive time intervals determined by changes in age (using 5-year groupings) and exposure status, as described previously in section 3.8.1. The time-varying effect of age was controlled for by including the age group factor as a covariate in each model. In addition, the primary analysis was repeated allowing for finer adjustment for age using 2-year groupings.

Withholding of antiplatelet or salicylate medication

It was recognised that the possible withholding of potentially protective antiplatelet or salicylate drugs prior to an invasive dental procedure among high-risk individuals on such treatment regimens may have introduced confounding. Among these patients, a brief period of stopping drug treatment was unlikely to be accurately recorded in their records. Therefore, a sensitivity analysis was done restricted to patients who had no recorded use of antiplatelets or salicylates prior to invasive

dental treatment. The rationale was that among such patients the issue of stopping medication at the time of dental treatment was unlikely to arise.

Use of NSAIDs after dental treatment

Individuals may have received NSAIDs after invasive dental procedures for pain control, and studies have suggested that the use of some NSAIDs may increase the risk of vascular events.[85] Thus the use of NSAIDs may also have confounded any association observed with invasive dental treatment. To address this possibility, a sensitivity analysis was conducted excluding individuals with a NSAID prescription around the time of their invasive dental treatment (from the four weeks before treatment up to four weeks after treatment), or those with a recorded diagnosis of rheumatoid arthritis at any time prior to invasive dental treatment (who were likely to be taking NSAIDs).

Pre-existing conditions

Confounding may also have arisen if diseases known to be associated with an increased risk of vascular events, specifically diabetes, hypertension or CHD, developed or worsened in the period leading up to invasive dental treatment. This was dealt with in sensitivity analyses excluding patients newly diagnosed with these conditions (defined in section 3.6.2.1) in the 12 months prior to invasive dental treatment. Since those who remained were either disease-free, or developed the disease after invasive dental treatment or more than a year before treatment, the scope for confounding was minimised.

3.8.4 Addressing the assumptions underlying the SCCS method

As stated previously in section 3.3.4, three key assumptions underlie the validity of the case series method. These assumptions and the methods used in this study to address them are discussed below.

Assumption 1: The occurrence of an outcome event should not affect the probability of subsequent exposure.

This is perhaps the most restrictive assumption underlying the SCCS method.[72] The assumption may not hold true if the event of interest increases the mortality rate (as

is the case for ischaemic stroke or MI). To address the issue of fatal vascular events, a sensitivity analysis was conducted excluding individuals who died during the hospital stay for their vascular event or whose enrolment ended within a month after their event, possibly indicating death.

Assumption 2: The occurrence of an outcome event should not censor or alter the duration of the observation period.

In a case series study, each individual's observation period is usually determined using pre-defined calendar time boundaries and/or age limits, and must be independent of the timing of the event. This assumption may also be violated when the outcome of interest is likely to increase the short-term death rate. Thus, the sensitivity analyses described above (relating to Assumption 1) also dealt with this assumption.

Assumption 3: Outcome events are independent within an individual.

The case series method requires that the occurrence of an outcome event should not affect the rate at which subsequent events may occur. If this assumption fails, a reasonable strategy is to restrict the analysis to first events, provided that these are not common.[63,86,87] In this study, analyses were confined to each case's first vascular event during the observation period (i.e. the first occurring during baseline or during a risk period). This was done because the recurrence times of each vascular outcome cannot be assumed to be independent within individuals: the occurrence of a first stroke or MI is known to increase the risk of further strokes or MIs. Primary discharge diagnoses for vascular events subsequent to the first event in an individual's observation period were not included in the analyses, yet each individual was followed up for the duration of their continuous enrolment period in Medicaid. Thus their pre-defined observation period was preserved.

3.8.5 Additional sensitivity analyses

Including unexposed cases

In a case series analysis, individuals not exposed at any time during follow-up do not contribute to the estimates of association between the exposure and outcome. However, including these unexposed individuals can help to control for confounding

by age, as they contribute information on the age-specific incidence of the outcome. A sensitivity analysis including unexposed cases (individuals who had a vascular event but no invasive dental treatment during their observation period) was performed in order to check that the effect estimates did not vary.

Restricting to dental extractions

Given that the vast majority of dental procedures included in the analyses were dental extractions (see Chapter 4, Table 4.4), a sensitivity analysis was conducted including only these homogenous exposures.

Restricting to "healthy" claimants

Finally, to assess whether a similar effect was observed among individuals with no history of diabetes, hypertension or CHD (as defined in section 3.6.2.1) prior to their invasive dental treatment, the primary analysis was repeated among this "healthy" subgroup.

3.9 Ethics approval

Ethics approval for this study was granted by the London School of Hygiene and Tropical Medicine Ethics Committee (application number 5284).

Chapter 4 Results - Invasive dental treatment and vascular events

This chapter reports on the SCCS analysis of invasive dental treatment and vascular events. The first two sections describe the identification of eligible cases and summarise their baseline characteristics. The remaining sections present the results of the case series analyses: the primary analysis, comparing the rate of vascular events in the six months after exposure to invasive dental treatment with the rate during unexposed periods; secondary analyses by event type (ischaemic stroke and MI), and a range of sensitivity analyses as outlined in Chapter 3. The chapter concludes with a discussion of the main findings, strengths and potential limitations of the study.

4.1 Identifying eligible individuals

The identification of eligible cases with a vascular event and the subset who had also been exposed to invasive dental treatment (and were thus included in the case series analyses) is described below and illustrated in participant flow diagrams: Figure 4.1 (primary analysis) and Appendix E-Figures E.1 and E.2 (secondary analyses).

4.1.1 Individuals with a first recorded vascular event

Study participants came from a base population of 9,901,464 individuals enrolled in Medicaid for all or part of the study period (from 1st January 2002 to 31st December 2006). 32060 individuals were identified with a hospital admission for ischaemic stroke (n=17741) and/or MI (n=14783) during the study period, of whom 11691 were excluded from the primary analysis on the basis of their first vascular event record, for one of the following reasons (outlined previously in Chapter 3, section 3.4.1.1) as shown in Figure 4.1:

- less than 24 weeks of observation prior to the first vascular event record (n=10822 excluded);
- age less than 20 years at the time of the first vascular event record (n=104 excluded);
- first vascular event record occurred outside the continuous enrolment period (n=765 excluded).

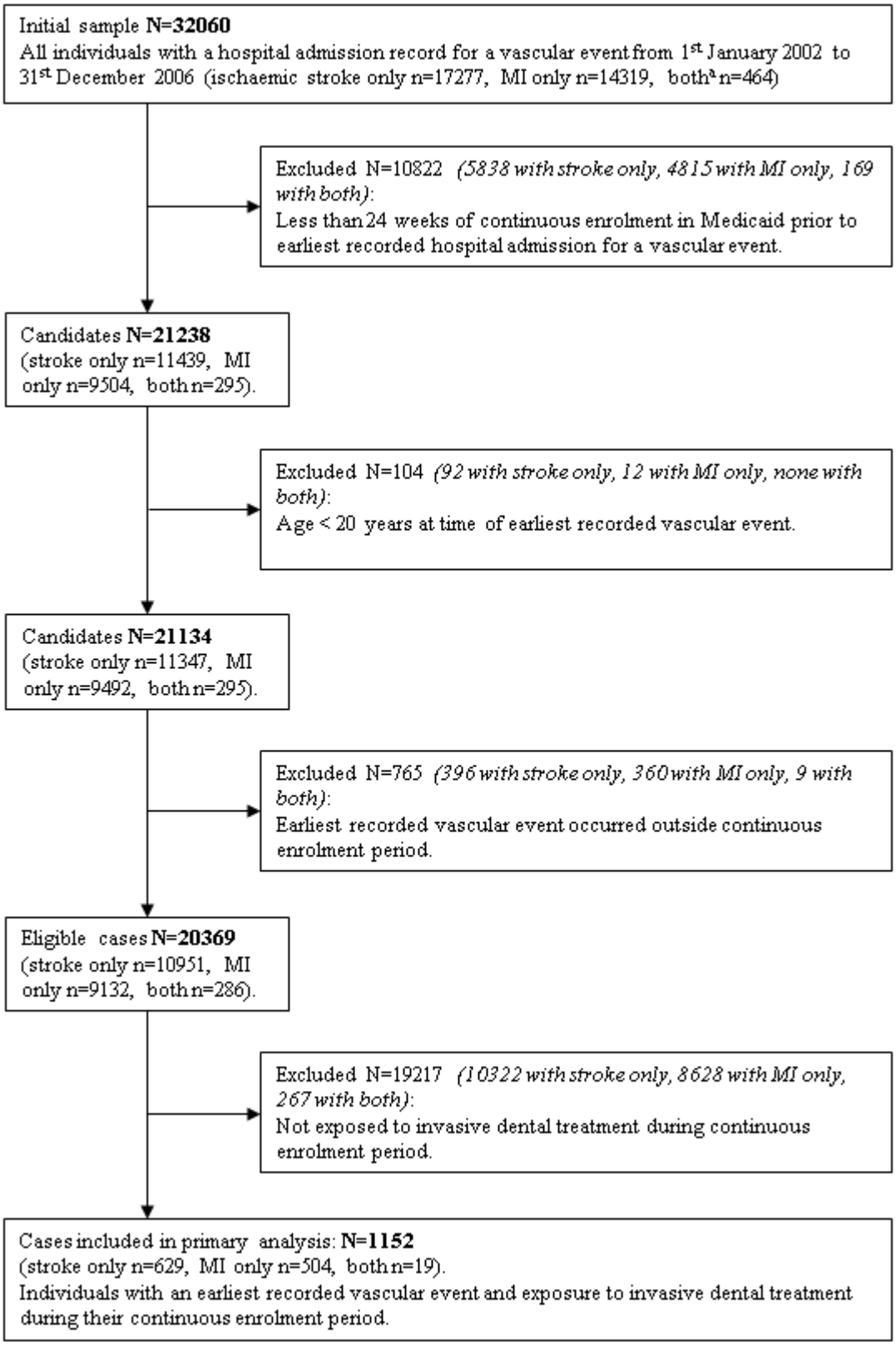


Figure 4.1 Participant flow diagram – primary analysis of vascular events.

MI=myocardial infarction
[a] Individuals who had both an ischaemic stroke and an MI during the study period.

These same exclusion criteria were applied to cases with a first ischaemic stroke (Appendix E-Figure E.1) and to cases with a first MI (Appendix E-Figure E.2), prior to the secondary analyses which assessed the effect of invasive dental treatment on ischaemic stroke and MI separately. Individuals were excluded from these analyses on the basis of the first event record of that type. Thus, 6457 cases with a first ischaemic stroke and 5299 with a first MI were excluded from the secondary analyses.

After applying these exclusions, 20369 cases with a first vascular event, 11284 with a first ischaemic stroke and 9484 with a first MI remained who were eligible for inclusion in the study.

4.1.2 Exposed cases

As described in Chapter 3, section 3.4.1.1, in a case series analysis only exposed cases contribute to estimates of the exposure effects: thus only cases who had been exposed to invasive dental treatment at least once during the observation period were included in the primary analysis of vascular events overall (n=1152), and in the analyses by event type: ischaemic stroke (n=650) and MI (n=525).

4.2 Descriptive data

4.2.1 Eligible cases

Of the 20369 eligible individuals with a first vascular event, the median age at the time of diagnosis was 67.3 years (interquartile range (IQR) 56.5-79.6), 34.3 percent were male, the median observation period was 3.8 years (IQR 2.1-5.0) and 7.7% died during their hospital stay. The predominance of female cases was in accordance with the demographic of the underlying population of Medicaid beneficiaries, of whom 58.1% (n=5,749,877) were female.

Eligible individuals with a first ischaemic stroke (n=11284) were slightly older at the time of diagnosis (median age 68.8 years, IQR 57.5-80.4) than eligible individuals with a first MI (n=9484) (median age 65.2 years, IQR 55.3-78.4), and fewer died during their hospital stay (5.6%) than those with MI (10.3%). The majority of both ischaemic stroke and MI cases were female, although a slightly larger proportion of

MIs were among males (37.7%) versus 31.4% of strokes. The observation periods of both groups were similar: median 3.8 years (IQR 2.1-5.0) for ischaemic stroke cases and 3.7 years (IQR 2.0-4.8) for MI cases.

4.2.2 Cases included in the case series analysis

1152 (5.7%) eligible individuals with a first vascular event had at least one invasive dental procedure during the observation period, and were thus included in the primary analysis of vascular events. 629 had an ischaemic stroke only, 504 had an MI only and 19 had both a stroke and an MI. 55.5% of these first vascular events were ischaemic strokes (n=639).

650 (5.8%) eligible individuals with a first ischaemic stroke and 525 (5.5%) with a first MI had at least one invasive dental procedure during the observation period, and were thus included in the secondary analyses by event type. These secondary analyses included four additional individuals (two in each analysis) who had experienced both an ischaemic stroke and an MI, but who were excluded from the primary analysis because their earlier vascular event met one or more of the exclusion criteria.

Table 4.1 summarises the characteristics of individuals included in the primary and secondary analyses.

Of the 1152 individuals included in the primary analysis, the median age at the time of diagnosis of the first vascular event was 57.7 years (IQR 47.6-69.9), 39.8% were male, the median observation period was 4.8 years (IQR 3.7-5.0) and 4.1% died during their hospital stay (2.6% of those first admitted for ischaemic stroke; 5.7% of those first admitted for MI). The majority of cases were of white (48.4%) or black (40.2%) ethnicity, 1.5% were Hispanic and the remaining 9.9% were classified as "other". A large majority of cases had pre-existing hypertension (70.2%) prior to their invasive dental treatment. Pre-existing diabetes (41.2 %) and CHD (40.8%) were also common.

Table 4.1 Characteristics of study participants.

Characteristic n (%)	**Vascular event patients** (N=1152)[a]	**Ischaemic stroke patients** (N=650)[b]	**Myocardial infarction patients** (N=525)[b]
Gender			
Male	458 (39.8)	233 (35.9)	236 (45.0)
Female	694 (60.2)	417 (64.2)	289 (55.1)
Ethnicity			
White	558 (48.4)	282 (43.4)	282 (53.7)
Black	463 (40.2)	303 (46.6)	171 (32.6)
Hispanic	17 (1.5)	9 (1.4)	8 (1.5)
Other	114 (9.9)	56 (8.6)	64 (12.2)
Age at first event (years)			
20-29	24 (2.1)	21 (3.2)	3 (0.6)
30-39	74 (6.4)	41 (6.3)	33 (6.3)
40-49	258 (22.4)	117 (18.0)	147 (28.0)
50-59	282 (24.5)	156 (24.0)	138 (26.3)
60-69	228 (19.8)	139 (21.4)	93 (17.7)
70-79	167 (14.5)	100 (15.4)	67 (12.8)
80-89	111 (9.6)	72 (11.1)	40 (7.6)
≥90	8 (0.7)	4 (0.6)	4 (0.8)
Median (IQR)	*57.7 (47.6-69.9)*	*59.2 (48.8-71.1)*	*55.4 (47.0-67.1)*
Diabetes diagnosed at any time prior to IDT	474 (41.2)	269 (41.4)	214 (40.8)
Hypertension diagnosed at any time prior to IDT	809 (70.2)	463 (71.2)	366 (69.7)
Coronary heart disease diagnosed at any time prior to IDT	470 (40.8)	211 (32.5)	278 (53.0)
Continuous enrolment in Medicaid (years) *Median (IQR)*	*4.8 (3.7-5.0)*	*4.8 (3.7-5.0)*	*4.7 (3.6-5.0)*

Abbreviations: IQR = interquartile range; IDT = invasive dental treatment

[a] Individuals included in the primary analysis

[b]23 patients experienced both an ischaemic stroke and a myocardial infarction during their observation period: 19 were included in each analysis (vascular events overall and by event type) and four (two from each analysis by event type) were excluded from the primary analysis of vascular events because their earlier event met the exclusion criteria.

Ischaemic stroke cases (n=650) were slightly older (median age at diagnosis 59.2 years, IQR 48.8-71.1) than MI cases (n=525) (median age 55.4 years, IQR 47.0-67.1), and were less likely to die during their hospital stay than MI cases (2.6% versus 5.7%). A larger proportion of MI cases were male (45.0% versus 35.9% of stroke cases), and of white ethnicity (53.7% versus 43.4% of stroke cases). The observation periods of both groups were similar (median 4.8 years (IQR 3.7-5.0) for ischaemic stroke and 4.7 years (IQR 3.6-5.0) for MI). The prevalence of pre-existing hypertension was similar and high among stroke cases (71.2%) and MI cases

(69.7%) and approximately 41% of both had pre-existing diabetes. Pre-existing CHD was more prevalent among MI cases (53.0%) than among stroke cases (32.5%).

89.1% of cases in the primary analysis had a single vascular event during the observation period. The remaining 10.9% had up to five primary discharge diagnoses for a vascular event; however, as described in Chapter 3, section 3.5.4, all analyses were restricted to first events. For descriptive purposes, the number of subsequent primary discharge diagnoses for vascular events excluded from the primary analysis, and from the analyses by event type, are shown in Table 4.2 below.

Table 4.2 Cases with a subsequent diagnosis for a vascular event and number of subsequent events excluded from the analyses.

Outcome	Number of cases included in analysis	Cases with a subsequent event[a] n (%)	Number of subsequent events excluded (range)
Vascular event	1152	126 (10.9)	158 (2-5)
Ischaemic stroke	650	73 (11.2)	93 (2-5)
Myocardial Infarction	525	38 (7.2)	46 (2-3)

[a] Primary discharge diagnosis of outcome (ischaemic stroke or MI) for a subsequent hospital admission

Table 4.3 summarises the exposure experience of cases over the observation period. 1574 episodes of invasive dental treatment were included in the primary analysis of vascular events. 74.7% of cases included in the primary analysis had just a single episode of invasive dental treatment over the observation period (n=861), 24.4% had between two and four episodes of treatment (n=281), and the remaining 0.9% had five or more treatment episodes (n=10). 204 cases (17.7%) had two or more episodes with overlapping risk periods. The median number of days between successive treatment episodes was 56.5 days (IQR 21-245).

Table 4.3 Episodes of invasive dental treatment among study participants

Episodes of invasive dental treatment	Vascular event patients N=1152[a] n (%)	Ischaemic stroke patients N=650 n (%)	Myocardial infarction patients N=525 n (%)
1 episode	861 (74.7)	493 (75.8)	385 (73.3)
2-4 episodes	281 (24.4)	150 (23.1)	137 (26.1)
≥5 episodes	10 (0.87)	7 (1.08)	3 (0.57)
Total episodes (range)	*1574 (1-11)*	*893 (1-11)*	*714 (1-5)*

[a] Individuals included in the primary analysis

The frequency distribution of dental procedures is shown in Table 4.4.

Table 4.4 Distribution of invasive dental procedures in study participants' records.

CDT[a] procedure code and description	n (%) individuals with at least one procedure record:		
	Vascular event patients (N=1152)[b]	Ischemic stroke patients (N=650)	Myocardial infarction patients (N=525)
D7210: surgical removal of erupted tooth requiring elevation of mucoperiosteal flap and removal of bone and/or section of tooth	847 (73.5)	499 (76.8)	364 (69.3)
D7250: surgical removal of residual tooth roots (cutting procedure)	151 (13.1)	90 (13.8)	67 (12.8)
D7310: alveoloplasty in conjunction with extractions - four or more teeth or tooth spaces, per quadrant	104 (9.0)	49 (7.5)	55 (10.5)
D7510: incision and drainage of abscess-intraoral soft tissue	25 (2.2)	15 (2.3)	11 (2.1)
D4341: periodontal scaling and root planing - four or more teeth per quadrant	23 (2.0)	7 (1.1)	16 (3.0)
D7320: alveoloplasty not in conjunction with extractions – four or more teeth or tooth spaces, per quadrant	16 (1.4)	9 (1.4)	8 (1.5)
D7240: removal of impacted tooth-completely bony	15 (1.3)	10 (1.5)	5 (1.0)
D7230: removal of impacted tooth-partially bony	13 (1.1)	8 (1.2)	6 (1.1)
D4211: gingivectomy or gingivoplasty - one to three contiguous teeth or bounded teeth spaces per quadrant	6 (0.5)	3 (0.5)	5 (1.0)
D7471: removal of lateral exostosis (maxilla or mandible)	5 (0.4)	3 (0.5)	2 (0.4)
D4210: gingivectomy or gingivoplasty - four or more contiguous teeth or bounded teeth spaces per quadrant	4 (0.3)	1 (0.2)	3 (0.6)
D7241: removal of impacted tooth-completely bony, with unusual surgical complications	4 (0.3)	3 (0.5)	1 (0.2)
D7999: unspecified oral surgery procedure, by report	4 (0.3)	0	4 (0.8)
D7540: removal of reaction-producing foreign bodies-musculoskeletal system	3 (0.3)	3 (0.5)	0
D3410: apicoectomy/periradicular surgery-anterior	2 (0.2)	1 (0.2)	1 (0.2)
D7960: frenulectomy (frenectomy or frenotomy)-separate procedure	2 (0.2)	0	2 (0.4)
D7970: excision of hyperplastic tissue-per arch	2 (0.2)	0	2 (0.4)
D3421: apicoectomy/periradicular surgery-bicuspid (first root)	1 (0.1)	1 (0.2)	0
D4342: periodontal scaling and root planing - one to three teeth, per quadrant	1 (0.1)	1 (0.2)	0
D7290: surgical repositioning of teeth	1 (0.1)	0	1 (0.2)
D7321: alveoloplasty not in conjunction with extractions – one to three teeth or tooth spaces, per quadrant	1 (0.1)	0	1 (0.2)
D7410: excision of benign lesion up to 1.25 cm	1 (0.1)	0	1 (0.2)
D7460: removal of benign nonodontogenic cyst or tumor-lesion diameter up to 1.25 cm	1 (0.1)	0	1 (0.2)
D7461: removal of benign nonodontogenic cyst or tumor-lesion diameter greater than 1.25 cm	1 (0.1)	0	1 (0.2)
D7520: incision and drainage of abscess-extraoral soft tissue	1 (0.1)	1 (0.2)	0
D7550: partial ostectomy/sequestrectomy for removal of non-vital bone	1 (0.1)	1 (0.2)	0

[a] Current Dental Terminology; [b] Individuals included in the primary analysis

The three most commonly occurring procedures (CDT codes D7210, D7250 and D7310) involved surgical removal of one or more tooth and/or bone, constituting 89.4% of all procedures included in the primary analysis. More than 90% of cases in the primary analysis (n=1059) had at least one of these extractions.

4.3 Primary analysis of vascular events

The primary analysis assessed the rate of vascular events in the 24 weeks after invasive dental treatment compared to all other observed time periods (baseline). Age-adjusted IRs and the number of vascular events occurring in each pre-defined, post-exposure risk period (weeks 1-4, 5-8, 9-12, 13-16 and weeks 17-24) and in all risk periods combined compared to baseline are shown in Table 4.5.

After adjusting for age in 5-year groups, the rate of vascular events (n=1152) was significantly raised in the first four weeks after invasive dental treatment compared to baseline (age-adjusted IR 1.50, 95% CI 1.09-2.06), and appeared to gradually resolve over the subsequent 20 weeks. Combining all risk periods yielded a less marked increased rate of vascular events over the entire 24 weeks after invasive dental treatment compared to baseline, which did not reach statistical significance (age-adjusted IR 1.15, 95% CI 0.98-1.36). No vascular events occurred on the same day as an invasive dental procedure. The earliest vascular events after invasive dental treatment occurred one day after the procedure (one ischaemic stroke and one MI); eight events occurred within the first week and 40 within the first four weeks after a procedure.

Considering the crude age effect alone, the rate of vascular events was found to increase steadily with age (IR for a 5-year increase in age 1.60, 95% CI 1.36-1.87). In order to maximise the control for confounding by age, the primary analysis was repeated including unexposed cases: individuals who had a vascular event but no invasive dental treatment during their observation period, and hence contributed only to the age effects. The inclusion of these additional cases (n=19217) did not alter the effect estimates of invasive dental treatment on vascular events (age-adjusted IR in weeks 1-4 post-treatment 1.50, 95% CI 1.09-2.06) (Appendix F-Table F.1). In addition, the primary analysis was repeated adjusting for age in 2-year (rather than 5-year) age groups. This finer level of stratification by age made no material difference

to the findings (age-adjusted IR in weeks 1-4 post treatment 1.49, 95% CI 1.09-2.06) (Appendix F-Table F.2).

Table 4.5 Results of the primary analysis: age-adjusted incidence ratios of a first vascular event in risk periods after invasive dental treatment.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event[b] (N=1152)		
Baseline period[c]	975	1
Post-procedure risk period		
Weeks 1-4	40	1.50 (1.09-2.06)
Weeks 5-8	29	1.11 (0.77-1.61)
Weeks 9-12	30	1.16 (0.81-1.68)
Weeks 13-16	25	0.96 (0.64-1.43)
Weeks 17-24	53	1.08 (0.82-1.43)
All risk periods (weeks 1-24)	*177*	*1.15 (0.98-1.36)*

[a] IR denotes age-adjusted incidence ratio.
[b] Vascular events are 639 ischemic strokes (55.5%) and 513 MIs (44.5%).
[c] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

4.4 Analyses by event type

Examining ischaemic stroke and MI separately yielded similar findings to the primary analysis, although these were not statistically significant.

The rate of MI (n=525) was higher in the first four weeks after invasive dental treatment compared to baseline (age-adjusted IR 1.56, 95% CI 0.98-2.47), and appeared to resolve thereafter. For ischaemic stroke (n=650), a slightly elevated risk was observed during the first four weeks after invasive dental treatment (age-adjusted IR 1.39, 95% CI 0.89-2.15), although the increase was less marked and the pattern of resolution over the subsequent 20 weeks was less clear. No ischaemic strokes or MIs occurred on the same day as an invasive dental procedure.

Table 4.6 shows the age-adjusted incidence rate ratios for ischaemic stroke and for MI in each pre-defined risk period following invasive dental treatment compared to baseline.

Table 4.6 Results of secondary analyses stratified by event type: age-adjusted incidence ratios of a first ischaemic stroke or myocardial infarction in risk periods after invasive dental treatment.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Ischaemic stroke (N=650)		
Baseline period[b]	553	1
Post-procedure risk period		
Weeks 1-4	21	1.39 (0.89-2.15)
Weeks 5-8	14	0.94 (0.55-1.60)
Weeks 9-12	18	1.21 (0.76-1.95)
Weeks 13-16	11	0.73 (0.40-1.32)
Weeks 17-24	33	1.18 (0.83-1.69)
All risk periods (weeks 1-24)	*97*	*1.10 (0.88-1.38)*
Myocardial infarction (N=525)		
Baseline period[b]	443	1
Post-procedure risk period		
Weeks 1-4	19	1.56 (0.98-2.47)
Weeks 5-8	16	1.35 (0.82-2.23)
Weeks 9-12	13	1.12 (0.64-1.95)
Weeks 13-16	14	1.20 (0.70-2.05)
Weeks 17-24	20	0.90 (0.57-1.42)
All risk periods (weeks 1-24)	*82*	*1.18 (0.93-1.50)*

[a] IR denotes age-adjusted incidence ratio.

[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

4.5 Sensitivity analyses

In addition to the analyses incorporating a more refined adjustment for age, a series of additional sensitivity analyses were conducted as outlined in Chapter 3, sections 3.8.3-3.8.5. The results of each of these analyses with respect to the primary outcome (age-adjusted IRs for vascular events in the first four weeks after exposure to invasive dental treatment compared to baseline) are described below and summarised in Table 4.7 (see Appendix F-Tables F.3-F.11 for detailed results of each of these analyses: age-adjusted IRs and the number of vascular events in each post-exposure risk period over 24 weeks).

4.5.1 Addressing time-varying confounding

In addition to the time-varying effect of age, which was taken into account in all analyses a priori, other possible sources of confounding were addressed in analyses excluding individuals who may have been at increased risk of vascular events at the time of their invasive dental treatment, as follows:

- individuals with antiplatelet or salicylate drug prescriptions (n=486) at any time prior to invasive dental treatment. Among this group, the possible withholding of medication prior to dental treatment because of concern for bleeding complications might have been an issue;
- individuals likely to be taking NSAIDs (n=687) around the time of invasive dental treatment (those with an NSAID prescription within four weeks either side of the dental treatment or with a rheumatoid arthritis diagnosis at any time prior to dental treatment);
- individuals newly diagnosed with diabetes (n=224), hypertension (n=398) or CHD (n=239) in the year before invasive dental treatment.

Each of these analyses yielded similar or stronger effects compared with the primary analysis, with age-adjusted IRs in weeks 1-4 following invasive dental treatment ranging from 1.46, 95% CI 1.02-2.10 when restricting to cases with no evidence of diabetes, to 2.23, 95% CI 1.56-3.18 when restricting to cases not taking antiplatelet or salicylate drugs (Table 4.7; Appendix F-Tables F.3-F.7).

4.5.2 Addressing fatal events

As discussed in Chapter 3, section 3.8.4, the validity of the case series method rests on the assumption that the occurrence of an outcome event does not affect the probability of exposure. To address the issue of fatal vascular events violating this assumption (individuals who die are no longer at risk of exposure to invasive dental treatment) the primary analysis was repeated excluding cases (n=83) who died during the hospital stay for their vascular event or whose enrolment in Medicaid ended within one month after their event (and hence may have died as a result of the event). This restriction made no material difference to the findings: age-adjusted IR in weeks 1-4 following invasive dental treatment 1.62, 95% CI 1.17-2.24 (Table 4.7; Appendix F-Table F.8).

4.5.3 Additional exclusions

Further sensitivity analyses were conducted as outlined in Chapter 3, excluding individuals with:

- two or more dental procedures with overlapping risk periods (n=204), to assess whether the decision to allow the later procedure to take precedence over the earlier one (when the risk periods after each procedure overlapped) might have contributed to the more marked effect observed in earlier (post-procedure) time frames;
- one or more invasive dental procedures that were not extractions (n=135), to assess whether a similar effect was observed when restricting the analysis to the more common, homogenous exposures (extractions);
- a history of diabetes, hypertension or CHD prior to invasive dental treatment (n=924), to assess whether a similar effect was observed amongst the remaining "healthy" subgroup.

These analyses made no material difference to the findings, and if anything yielded marginally stronger effects in weeks 1-4 following invasive dental treatment (Table 4.7; Appendix F-Tables F.9-F.11).

Table 4.7 Results of sensitivity analyses.

Analysis of vascular event rate in weeks 1-4 post-procedure compared to baseline[a]	Cases included in analysis (n)	IR[b] (95% CI)
Primary analysis:		
Vascular events[c]	*1152*	*1.50 (1.09-2.06)*
Sensitivity analyses excluding cases with:		
Antiplatelet or salicylate drug prescription record at any time prior to IDT (n=486 excluded)	666	2.23 (1.56-3.18)
NSAID prescription four weeks prior to four weeks post IDT or rheumatoid arthritis diagnosis at any time prior to IDT (n=687 excluded)	465	1.84 (1.17-2.89)
Earliest record of diabetes within 12 months prior to IDT (n=224 excluded)	928	1.46 (1.02-2.10)
Earliest record of hypertension within 12 months prior to IDT (n=398 excluded)	754	1.64 (1.12-2.40)
Earliest record of CHD within 12 months prior to IDT (n=239 excluded)	913	1.70 (1.21-2.40)
Enrolment ending or death within a month after the vascular event (n=83 excluded)	1069	1.62 (1.17-2.24)
Overlapping risk periods (n=204 excluded)	948	1.65 (1.17-2.33)
Procedures that were not extractions (n=135 excluded)	1017	1.58 (1.13-2.21)
Diagnosis of diabetes, hypertension or CHD at any time prior to IDT (n=924 excluded)	228	1.76 (0.92-3.36)

Abbreviations: IDT=invasive dental treatment; CHD=coronary heart disease

[a] Baseline period is all observation time except for 24 week risk period following an invasive dental procedure.

[b] IR denotes age-adjusted incidence ratio in weeks 1-4 following an invasive dental procedure.

[c] Vascular events are 639 ischaemic strokes (55.5%) and 513 MIs (44.5%).

4.6 Discussion

4.6.1 Summary of main findings

The study findings suggest that among adults aged 20 years or more, invasive dental treatment may be associated with an increased rate of vascular events in the first four weeks after treatment relative to all unexposed time periods (age-adjusted IR 1.50, 95% CI 1.09-2.06). The increased rate appeared to be transient, returning to baseline over the subsequent 20 weeks. Separate analyses of ischaemic stroke and MI suggested similar, transient increased rates of both of these outcomes in the four weeks after invasive dental treatment (age-adjusted IR 1.39, 95% CI 0.89-2.15 for ischaemic stroke and 1.56, 0.98-2.47 for MI), although the findings were not statistically significant.

4.6.2 Study strengths

In studies investigating the risk of vascular events following inflammatory exposures such as invasive dental treatment, the potential for confounding is great because individuals who undergo treatment may differ from those who do not in ways which are difficult to control for. Poor dental health and CVD share a number of aetiological factors, including socioeconomic and life-style factors such as cigarette smoking and diet. Furthermore, individuals who opt for dental treatment may be more likely to take other precautionary measures regarding general aspects of their health, including their cardiovascular health. The major strength of this study is the use of a case series analysis, which overcame the potential problem of confounding associated with the influence of such factors which may vary among individuals to which other observational study designs are prone. This was achieved by all comparisons being within-person; thus fixed covariates were implicitly controlled for. Confounding would only have occurred if intra-person risk factors for vascular events that change with time were also associated with the timing of invasive dental treatment. In addition, to produce the effect observed, any such factors would need to have a strong acute effect on vascular event risk and their time-dependent effect would need to be operating in a large proportion of included cases. Thus, while a small effect of such time varying factors cannot be excluded, the overall impact on the study results is likely to be minimal.

As expected, a clear age effect was observed demonstrating an increased risk of vascular events with increasing age; thus, all analyses were age-adjusted in 5-year age bands, and subsequently the primary analysis was repeated allowing for finer age-adjustment (2-year bands) which yielded similar results. The findings are therefore unlikely to be explained by the time-varying effect of age or age-related factors.

The influence of additional time-varying covariates identified as potential confounders were explored in sensitivity analyses, as described in section 4.5.1. In each of these analyses, a short-term increase in the rate of vascular events after invasive dental treatment persisted, indicating that these factors are unlikely to provide alternative explanations for the effect observed in this study. For example, possible confounding by the cessation of antiplatelet or salicylate medication before invasive dental treatment, which may feasibly trigger a subsequent vascular event in the short term, was addressed using a pragmatic approach of restricting the analysis to cases who had no recorded use of antiplatelet or salicylate drugs prior to their dental treatment. Because the issue of discontinuing medication at the time of dental treatment was unlikely to arise among these individuals, any observed increase in vascular event risk after dental treatment was unlikely to be attributable to a temporary cessation of antiplatelets or salicylates. Furthermore, because of the risk that aspirin cessation might precipitate a vascular event[88] and continued aspirin use during dental surgical procedures has been shown not to lead to excessive post-operative bleeding,[89] the continuation of antiplatelet therapy throughout such procedures is recommended practice in the US and elsewhere[90–92]. Thus, the withholding of such medication is unlikely to have been commonplace among the study population.

In addition, it was recognised that the development or worsening of diabetes, hypertension or CHD might increase both the risk of periodontal disease (and hence the likelihood of associated invasive dental procedures) and of vascular events. To allow for this, sensitivity analyses were performed restricted to cases with no evidence for each of these three conditions developing in the year leading up to invasive dental treatment. Since those remaining either had no pre-existing diabetes,

hypertension or CHD or they developed disease after their invasive dental treatment or more than a year before treatment, the scope for confounding was reduced.

The sensitivity analysis which excluded known or suspected fatal cases (described in section 4.5.2) demonstrated that the study findings were robust with regards to assumptions underlying the case series method.

Based on previous research showing that the host response and vascular function are affected at their maximum 24 hours after invasive dental treatment,[47–49,84] the decision was taken to start the risk period the day after the dental procedure; hence the day of the procedure contributed to the baseline (unexposed) period. This avoided the problem of vascular events on the same day as a procedure, that were a consequence of other factors unrelated to the dental treatment, being included in the risk estimates. Any bias arising from this convention would have led to an underestimate of effect. However, no vascular events occurred on the same day as a dental procedure, hence this particular source of bias was not a concern in this study.

When an individual had multiple invasive dental procedures with overlapping risk periods, all procedures were included, but the latest procedure was allowed to take precedence (see Figure 3.3, Chapter 3). The rationale for this choice was that the most proximate procedure to the vascular event was deemed the most relevant when examining the acute effect of invasive dental treatment on vascular event risk. The sensitivity analysis which excluded cases with overlapping risk periods yielded virtually identical results to the primary analysis, suggesting that this convention did not introduce any notable bias.

4.6.3 Potential limitations of the study

As described in Chapter 3, section 3.8.4, an important assumption of the case series method is that outcome events are independent. Thus all analyses were restricted to individuals' first vascular events occurring during the observation period. Although excluding subsequent primary discharge diagnoses for stroke and MI may have potentially underestimated the absolute risk of vascular events, this is unlikely to have had any material effect on the relative risk (the primary outcome of this study). A similar approach was taken in an earlier SCCS study exploring the risk of stroke and MI after acute infection and vaccination.[19]

It was recognized that the ascertainment of use of antiplatelet agents, salicylates or NSAIDs may have been incomplete as some patients are likely to have received these medicines both through prescription and over-the-counter. The Medicaid database does not capture over-the-counter medications, thus the possibility of residual confounding by differential use of these agents around the time of invasive dental treatment cannot be excluded.

Possible confounding by the proximity of invasive dental treatment to odontogenic infection or other acute conditions necessitating dental treatment must also be considered. The invasive dental procedures included in the main analysis did not necessarily follow an acute infection; however, data were not available on the reason for each dental procedure. While in the case of procedures carried out to treat an acute condition it was not possible to disentangle the effects of the condition from those of the treatment, it is unlikely that the entire association between invasive dental treatment and vascular events is due to such acute conditions. Even if the effect observed is in part attributed to the onset of acute infections coinciding with the timing of dental treatment, this would actually support an inflammatory mechanism for the association between invasive dental treatment and vascular events.

While the possibility of case-ascertainment bias cannot be excluded, whereby individuals may have been more likely to be diagnosed with a vascular event in the first month after a dental procedure than in earlier or later time periods, it is likely to be minimal given that both ischaemic stroke and MI are hard outcomes which manifest with clear clinical presentations. There was also scope for misclassification of exposure status. Not all Medicaid beneficiaries were necessarily eligible for dental care. While it was possible to determine who had made claims for dental care, there were no data indicating dental coverage. Individuals who did not qualify for dental coverage may have undergone invasive dental treatment (either self-funded or covered by another insurer), and this would not have been captured in the database. These individuals would thus have been misclassified as unexposed. However, this is unlikely to be a major problem in this study, as only individuals with at least one invasive dental procedure record (and thus with dental coverage) contributed to the

analyses, and the chance of their dental coverage changing over the course of their enrolment was likely to be small.

The study was based on claims data, and a potential weakness may relate to the skewed nature of the population eligible for Medicaid. Eligibility is income-related. Eligible groups include low-income adults and their children, and individuals with certain disabilities. It is possible, for example, that patients with diseases which put them at greater risk of thrombotic events may be more likely to enter the Medicaid program in order to pay for needed care including dental care. While this may raise the question of generalisability of the study findings to other populations, it is unlikely to have biased the effect estimates as these were derived from within-person comparisons, with each individual serving as his or her control. Furthermore, the sensitivity analysis which restricted to "healthy" claimants (individuals with no previous record of diabetes, hypertension or CHD) showed a similar effect to that obtained in the primary analysis, though with less precision due to the fewer cases in this subset.

The relatively small study population is another limitation. In a case series design, only individuals who are exposed at least once during follow-up contribute to the analyses. Given that invasive dental procedure claims were fairly uncommon among the study population, a relatively small proportion of the initial sample contributed to the analyses. The substantial reduction in sample size limited the power of the study to examine the effects of invasive dental treatment on ischaemic stroke and MI separately.

4.6.4 Conclusion

This study has shown that invasive dental procedures may be associated with a transient increase in the risk of ischaemic stroke and MI in adults in the four weeks after the dental treatment. The exact mechanisms underlying the observed association are uncertain. While the findings do not preclude the possibility that non-inflammatory mechanisms may be involved, for example, possible discontinuation of antiplatelet medication before invasive dental treatment, or increased stress due to pain arising from the dental treatment, they nevertheless support the hypothesis that invasive dental procedures sufficient to produce acute inflammation may play an

important role in the occurrence of vascular events. A discussion of the study findings in context of what was previously known on the relationship between invasive dental treatment and vascular risk is presented in the concluding chapter of this thesis (Chapter 9, section 9.1).

Chapter 5 A literature review of pre-eclampsia and acute maternal infection

This chapter reports on the literature review of studies assessing the association between acute maternal infections and pre-eclampsia. Following a brief overview of the clinical features of pre-eclampsia and the evidence implicating inflammation in its pathogenesis, the review methodology is described. The results of the review are presented in two parts: first, evidence on the association between UTI (the most commonly studied acute infection) and pre-eclampsia is summarised, followed by evidence on the effect of other acute infections. A summary of the main findings and rationale for the pre-eclampsia study (described in Chapter 6) concludes the chapter.

5.1 Background

Pre-eclampsia is a pregnancy-specific, multi-system vascular syndrome typically defined by the gestational onset of hypertension and proteinuria after 20 weeks' gestation. It is a major cause of maternal and perinatal morbidity and mortality, its incidence ranging from 2 to 8% in nulliparous women.[93] Globally, pre-eclampsia accounts for 10 to 15% of maternal deaths.[94]

The presentation and progression of pre-eclampsia are highly variable. It may develop from a mild to a severe disease state, the latter characterised by sustained, severe hypertension (blood pressure ≥110 mm Hg diastolic or ≥160 mm Hg systolic), nephrotic-range proteinuria (≥5g in 24 hours) or other evidence of end-organ damage, or it may be severe at the time of diagnosis.[94] In some women, pre-eclampsia progresses to a convulsive phase, eclampsia, a rare but serious complication affecting 1 to 2% of severe cases,[93] characterised by tonic-clonic seizures in a pregnant or recently delivered woman which may lead to coma. Other women may develop HELLP syndrome, a combined liver and blood clotting disorder characterised by "H", haemolysis (rupture of red blood cells), "EL", elevated liver enzymes in the blood (indicating liver dysfunction), and "LP", a low platelet count. HELLP complicates 10 to 20% of severe cases of pre-eclampsia[93] and may develop antepartum or postpartum.[95]

Despite advances in knowledge, our understanding of what causes pre-eclampsia remains incomplete, and we still have a limited ability to predict or prevent the disorder.[94] Pre-eclampsia is a complex disease, and numerous theories have attempted to explain its pathogenesis. These include abnormal placentation in early pregnancy, maternal immune mechanisms, genetic factors, an imbalance of pro- and anti-angiogenic proteins, and an excessive systemic inflammatory response.[23,96] While poor early placentation is especially implicated in early-onset pre-eclampsia, maternal cardiovascular and metabolic factors associated with endothelial dysfunction, such as obesity, might play a greater role in the origins of late-onset disease.[93,97] The aetiology of pre-eclampsia is thus generally considered to be multifactorial, involving a range of both maternal and placental contributions.[98] However, the common target of such factors is the maternal vascular endothelium, as evidenced by the characteristic widespread endothelial dysfunction observed among women preceding the onset of clinical disease.[29,99]

It has been proposed that the vascular endothelial dysfunction of pre-eclampsia is part of a generalised intravascular inflammatory response.[100] Indeed, a key feature of pre-eclampsia is the greater systemic inflammatory response of women who develop the syndrome compared to women who have normal pregnancies.[96] This suggests that inflammation may play a central role in the pathogenesis, although the exact cause of the inflammation is not completely understood. While reduced placental perfusion as a result of inadequate placentation may be an important inflammatory stimulus for many women who develop pre-eclampsia, it is not necessarily a strong component of all pre-eclamptic pregnancies.[101] It is likely that several factors, both placental and maternal in origin, contribute to the amplified maternal inflammatory response seen in pre-eclampsia and the development of the syndrome.[96]

Based on this notion, a growing body of research implicates infection, a common cause of inflammation and of endothelial dysfunction, in the aetiology of pre-eclampsia.[102] An increased risk of pre-eclampsia associated with maternal periodontal disease, a source of chronic infection and inflammation, has been well-documented.[103–105] Studies based on serological markers of other chronic infections have also yielded positive findings,[106–111] although temporal associations in these studies are uncertain.

Attention has also been directed towards acute infection possibly contributing to the development of the maternal syndrome. It is the potential role of acute infection in pre-eclampsia that this part of the thesis (Chapters 5-8) investigates.

5.2 Methods

5.2.1 Aim of review

The aim of the literature review was to summarise published evidence on the effect of acute maternal infections on the risk of pre-eclampsia.

5.2.2 Search strategy

A systematic review and meta-analysis of observational studies which examined the relationship between maternal infections and pre-eclampsia was published in 2008.[102] This was updated by searching the Medline database for additional studies published since the original review was conducted (from 2007 to February 2014). Following a similar search strategy to the original review, an algorithm was developed (see Appendix C-Table C.2) and applied in Medline using MeSH keywords and free-text words for infection and pre-eclampsia. Hence, the initial search encompassed all types of infection (both chronic and acute), consistent with the original review. However, given the specific interest and relevance of the effect of *acute* maternal infections to this project, these form the focus of this review.

The same procedures for identifying potentially relevant studies and extracting information were followed as for the literature review of invasive dental treatment and vascular events, described in Chapter 2, section 2.2.2.

5.2.3 Inclusion and exclusion criteria

Studies presenting original data, published in English, and which included pre-eclampsia, eclampsia, HELLP syndrome, or gestational hypertension with proteinuria as an outcome and also investigated acute maternal infection as an exposure, were included. Gestational hypertension was defined as blood pressure of at least 90 mm Hg (diastolic) or at least 140 mm Hg (systolic) after 20 weeks' gestation. Proteinuria was defined as the urinary excretion of ≥300 mg protein in 24 hours, or ≥300mg/L (≥ 1+ reading on dipstick) in a random urine sample. Acute

maternal infection could be defined clinically, or via the presence of a specific infectious agent indicating acute infection, or immunoglobulin M (IgM) antibodies to that specific infectious agent. Studies examining only the association between acute maternal infection and hypertensive states without proteinuria were excluded, as were case reports, case-only studies with no comparison group (or with no comparison time period), and review articles that lacked original data.

5.3 Results

The literature search generated 653 citations of which nine fulfilled the inclusion criteria. This gave rise to a total of 29 included studies from both the original 2008 review[102] and the present search combined.

The majority of studies (11 cohort,[112–122] six case-control,[123–128] three cross-sectional[129–131]) presented data on the relationship between pre-eclampsia and UTI, one of the most commonly occurring acute maternal infections.[132] The characteristics and main findings of these studies (17 from the original review;[112–126,129,130] three from the present search[127,128,131]) are summarised in Table 5.1 and discussed in more detail below (section 5.3.1).

The remaining studies presented data on other acute infections during pregnancy: six studies (one cohort[133] and five case-control[106,110,134–136]) examined the relationship between pre-eclampsia and the presence of IgM antibodies to Chlamydia pneumoniae infection, a recognised respiratory tract pathogen (although one case-control study was subsequently excluded as no antibodies were detected in either group)[136]; two population-based cross-sectional studies examined the association between pre-eclampsia and hospital diagnosed pneumonia,[137,138] and one case-control study assessed the association with IgM antibody seroprevalence to cytomegalovirus[139]. The key features and findings of these studies (three from the original review;[106,133,134] five from the present search[110,135,137–139]) are summarised in Table 5.2 and discussed below (section 5.3.2).

Table 5.1 Characteristics of studies examining the association between pre-eclampsia and maternal urinary tract infection and fulfilling the inclusion criteria.

First author, year published	Location	Study design	Sample size or cases/controls	Participant selection (study period)	Outcome or case definition	Type and timing of exposure; method of ascertainment	Adjustment or matching	Main findings (Odds Ratio, 95% CI)
Kashanian, 2011[128] [a]	Tehran, Iran	Case-control	318 cases with PE, 318 normotensive controls	Consecutive selection of controls (1 per case) from same hospital as case (2005-2006)	PE[b], obstetrician-diagnosed	UTI in pregnancy; ascertainment method not reported	Maternal and gestational age, history of pre-eclampsia, BMI, pre-existing hypertension or diabetes, parity, blood Rh, education level, anaemia, method of contraception	OR 2.2, 0.3-15.1
Shamsi, 2010[127] [a]	Karachi and Rawalpindi, Pakistan	Case-control	131 cases with PE, 262 controls without PE	Consecutive selection of controls (2 per case) from same hospital as case (2006-2007)	PE[b], obstetrician-diagnosed	UTI in pregnancy; maternal self-report in postpartum interview	Matched on hospital, day of delivery and parity. Adjusted for maternal age, SES, family history of diabetes mellitus, maternal weight, Rh factor	No association in adjusted analysis (OR not reported); Prevalence of infection in cases versus controls: 31.0% vs. 18.5% (p=0.006)
Mazor-Dray, 2009[131] [a]	Negev, Israel	Population-based cross-sectional	4,742 women with infection, 199,093 women without infection	All hospital deliveries (1988-2007)	PE, diagnosis recorded in perinatal database	UTI in pregnancy or at delivery; record of positive urine culture[c] with symptoms of dysuria, urgency and frequency in perinatal database	Maternal age and parity	OR 1.3, 1.1-1.4
Banhidy, 2007[130]	Hungary	Population-based cross-sectional	38,151	Mothers of newborns without congenital abnormalities selected from National birth Registry (1980-1996)	PE, eclampsia or pregnancy-induced hypertension with oedema and albuminuria, in antenatal logbook	UTI in pregnancy; positive urine culture, acute cystitis, cystopyelitis or pyelonephritis in antenatal logbook or maternal self-report in postpartum questionnaire	None	OR 1.3, 1.1-1.5
Villar, 2006[122]	Rosario, Argentina; Havana, Cuba; Jeddah, Saudi Arabia; Khon Kaen, Thailand	Cohort	32,147	Women enrolled in 53 randomly selected antenatal clinics and 5 hospitals (1996-1998)	PE[b], obstetrician-diagnosed	UTI in pregnancy; antenatal record, further ascertainment not reported	Maternal age, parity, reproductive tract infection or surgery, history of spontaneous abortion	OR 1.4, 1.1-1.7

First author, year published	Location	Study design	Sample size or cases/controls	Participant selection (study period)	Outcome or case definition	Type and timing of exposure; method of ascertainment	Adjustment or matching	Main findings (Odds Ratio, 95% CI)
Lee, 2000[121]	Taiwan	Retrospective cohort	29,735	All hospital deliveries (1990-1998)	PE[b], diagnosis recorded in hospital obstetric database	UTI in pregnancy; record of asymptomatic bacteriuria, cystitis or pyelonephritis and/or positive urine culture in obstetric database, or maternal self-report in first antenatal visit interview	Maternal age, parity, pre-pregnancy BMI, education, marital status, working during pregnancy, multiple gestation, infant gender, history of pre-eclampsia, diabetes mellitus, conception method, obstetric history, uterine fibroids	OR 4.8, 1.5-15.8
Mittendorf, 1996[126]	Boston, USA	Nested case-control	386 cases with PE, 2355 controls without PE	Random selection of controls from same hospital as case (1977-1980)	PE[b], obstetrician-diagnosed	UTI in pregnancy; ascertainment method not reported	Maternal age, parity, pre-pregnancy BMI, education, ethnicity, smoking, infant gender, prenatal care, history of abortion, working during pregnancy, marital status	OR (all women) 1.6, 1.1-2.5; OR (primiparas only) 5.3, 2.9-9.7
Abi-Said, 1995[125]	Houston, USA	Case-control	66 cases with eclampsia, 264 non pre-eclamptic controls	Consecutive selection of controls (4 per case) from same hospital as case (1977-1992)	Eclampsia, diagnosis recorded in hospital medical records	UTI in pregnancy; diagnosis in hospital records, further ascertainment not reported	Matched on hospital and month of delivery. Adjusted for maternal age, parity, ethnicity, prenatal care, obesity, history of diabetes	OR (eclampsia) 4.2, 1.3-14.1
Hsu, 1995[120]	Baltimore, USA	Retrospective cohort	13,852	All hospital deliveries (1983-1987)	Mild and severe PE, diagnosis recorded in hospital perinatal database	UTI in antepartum, intrapartum and postpartum periods; record of positive urine culture[c] with or without signs or symptoms of cystitis or pyelonephritis in perinatal database	Delivery by caesarean section, use of oxytocin, premature rupture of membranes, gestational age at delivery	OR for severe PE 2.6, 2.0-3.4; no association with mild PE (OR not reported)
Schieve, 1994[119]	Chicago, USA	Retrospective cohort	25,746	Perinatal registry cohort of mother/infant pairs (1988-1989)	PE included new onset hypertension without proteinuria, diagnosis in perinatal database	UTI in antepartum period; record of positive urine culture or clinical presentation (physician diagnosis of UTI or pyelonephritis) in perinatal database	Maternal age, ethnicity, outcome of previous pregnancy, hospital of delivery, genital tract infection	OR 1.4, 1.2-1.7

First author, year published	Location	Study design	Sample size or cases/controls	Participant selection (study period)	Outcome or case definition	Type and timing of exposure; method of ascertainment	Adjustment or matching	Main findings (Odds Ratio, 95% CI)
Qureshi, 1994[118]	Karachi, Pakistan	Cohort	1597	All women presenting for antenatal care at University medical centre (1988-1990)	PE, no further details	Bacteriuria at first antenatal visit; positive urine culture	None	No association (OR not reported)
Gilbert, 1986[117]	Melbourne, Australia	Cohort	340	Women attending antenatal care clinics	PE, no further details	Bacteriuria at first antenatal visit; positive urine culture	None	Bacteriuria associated with a 3-fold increased risk of PE ($p<0.05$)
Hill, 1986[124]	Augusta, USA	Case-control	100 primigravid cases with PE, 100 primigravid and 100 multigravid controls without PE	Not reported	PE defined as gestational hypertension with oedema and proteinuria	Asymptomatic bacteriuria at delivery; positive urine culture[c]	Matched on maternal age	Prevalence of infection in cases versus controls 19% vs. 3% (primigravidas) or vs. 6% (multigravidas) ($p<0.005$) (OR not reported)
Savige, 1983[123]	Melbourne, Australia	Case-control	51 cases with PE, 72 controls without PE	Not reported	PE defined as gestational hypertension with oedema, with or without proteinuria	Bacteriuria at delivery (cases), at <26 weeks' gestation (controls); positive urine culture	None	Bacteriuria associated with an increased risk of PE (OR not reported)
Brumfitt, 1975[129]	London, UK	Cross-sectional	426 women with infection, 477 women without infection	Unknown	PE, no further details	Bacteriuria at first antenatal visit; further ascertainment not reported	None	No association
Little, 1966[116]	London, UK	Cohort	5000	Women attending hospital antenatal clinics (1962-1965)	Pre-eclamptic toxaemia, not necessarily in presence of hypertension, proteinuria and oedema, obstetrician-diagnosed	Bacteriuria at first antenatal visit; positive urine culture[c] in 2 consecutive samples within 10 days apart	None	No association (Risk of PE in infected versus uninfected: 7.5% vs. 6.9%)

First author, year published	Location	Study design	Sample size or cases/controls	Participant selection (study period)	Outcome or case definition	Type and timing of exposure; method of ascertainment	Adjustment or matching	Main findings (Odds Ratio, 95% CI)
Stuart, 1965[115]	Jamaica	Cohort	88 women with infection, 729 women without infection all followed to term	Consecutive pregnant women attending antenatal clinics of the University College Hospital (1961-1963)	PE included new onset hypertension without proteinuria, obstetrician-diagnosed	Bacteriuria at first antenatal visit; positive urine culture[c] in ≥2 consecutive samples	None	Risk of PE in infected versus uninfected: 18.2% vs. 4.5% (p<0.001)
Kincaid-Smith, 1965[114]	Melbourne, Australia	Cohort	240 women with infection, 500 women without infection	Women recruited at antenatal clinic: infected women were compared with a random sample of uninfected women, all with completed pregnancies	Pre-eclamptic toxaemia defined as 2 or more of proteinuria, hypertension (≥140/90 mm Hg) or oedema, obstetrician-diagnosed	Bacteriuria at first antenatal visit before 26 weeks' gestation; positive urine culture[c] in single midstream urine specimen	None	Risk of PE in infected versus uninfected: 10.8% vs. 6.0% (p<0.05)
Low, 1964[113]	Toronto, Canada	Cohort	771	Unknown	PE, no further details	Asymptomatic bacteriuria at first antenatal visit; further ascertainment not reported	None	No association
Bryant, 1964[112]	Dallas, USA	Cohort	32 women with infection, 44 women without infection all followed to term	Women attending antenatal clinic	PE, no further details	Asymptomatic bacteriuria at first antenatal visit; further ascertainment not reported	Matched on maternal age, SES, parity, ethnicity	No association

OR denotes odds ratio adjusted for all specified variables; UTI=urinary tract infection; PE=pre-eclampsia; SES=socioeconomic status; BMI=body mass index

[a] studies identified in new search (since 2007)

[b] PE defined as de novo hypertension (blood pressure of at least 90 mm Hg diastolic or at least 140 mm Hg systolic) with proteinuria (≥300 mg in 24 hours, or ≥300mg/L (≥ 1+ on dipstick) in a urine specimen) after 20 weeks' gestation

[c] defined as >100,000 bacteria per ml of urine

5.3.1 Studies of urinary tract infection

Twenty studies conducted over a 47 year period (1964-2011) in a variety of geographical settings evaluated the relationship between UTI in pregnancy and pre-eclampsia (see Table 5.1). Of these, 13 studies (seven cohort,[114,115,117,119–122] four case-control,[123–126] two cross-sectional[130,131]) reported an association between UTI and an increased risk of pre-eclampsia, with odds ratio (OR) point estimates (when reported) ranging between 1.3 and 4.8 after adjustment for at least maternal age; whereas seven studies (four cohort,[112,113,116,118] two case-control,[127,128] one cross-sectional[129]) found no association. In the original review,[102] Conde-Agudelo et al reported a significant association between pre-eclampsia and maternal UTI (summary OR 1.57, 95% CI 1.45-1.70). However, there was considerable between-study heterogeneity (as confirmed by an I^2 of 79%) among the 17 studies included in their fixed-effects meta-analysis. Findings from the three most recent studies, identified in the present search, were conflicting: one, a large population-based cross-sectional study conducted in Israel reported an increased risk of pre-eclampsia among women with UTI in pregnancy after controlling for maternal age and parity (adjusted OR 1.3, 1.1-1.4),[131] whereas the two case-control studies, one conducted in Iran,[128] the other in Pakistan,[127] found no evidence for an association between UTI and pre-eclampsia after adjusting for maternal age, parity, maternal BMI or weight, Rhesus factor, and other potential confounders.

5.3.1.1 Possible reasons for the variability in study findings

There could be a number of reasons for the heterogeneity observed between studies. Many methodological aspects varied between studies, including the design, timing, geographical location, the definitions of pre-eclampsia and of UTI, and the extent of adjustment for potential confounders (eight studies reported adjusting for at least maternal age and parity,[112,121,122,125–128,131] whereas nine reported no adjustment for confounding[113–118,123,129,130]). In their meta-analysis, Conde-Agudelo et al explored possible sources of heterogeneity observed among the included studies, which could not be explained by differences in sample size (<100 pre-eclampsia cases versus ≥100 cases), year of publication (before 1990 versus after 1990) or definition of UTI used ("asymptomatic or symptomatic bacteriuria" versus "urinary tract infection") as the summary ORs obtained for these subgroups were similar to the overall summary

OR. However, smaller summary ORs (i.e. closer to the null) were obtained for studies that controlled for at least maternal age and some indicator of SES compared to studies that did not control for these (summary OR 1.40, 95% CI 1.27-1.55 versus 2.05, 1.77-2.39) and for studies rated as high quality (on the basis of several criteria including the method of participant selection, assessment of exposure and outcome, and control for confounding) compared to those rated as lower quality (summary OR 1.46, 95% CI 1.33-1.60 versus 1.99, 1.69-2.33). Study location also appeared to explain some of the heterogeneity, with European studies yielding the smallest summary OR (1.24, 1.08-1.41), and Australian studies the largest, by almost twofold (2.33, 1.52-3.56).

Differences in the case definition of pre-eclampsia used between studies might also explain some of the variability in the findings. Pre-eclampsia was most commonly defined as new onset hypertension in association with proteinuria after 20 weeks' gestation. However, some studies were less precise about the diagnosis of pre-eclampsia, so it is possible that some cases of pregnancy-induced hypertension, for example, were misclassified as pre-eclampsia. Indeed, three studies used a combined outcome of pre-eclampsia and pregnancy-induced hypertension;[115,119,123] all three reported a positive association with UTI. Two earlier studies of "pre-eclamptic toxaemia", diagnosed in the presence of two or more of proteinuria, hypertension or oedema,[114] or not otherwise specified,[116] reported conflicting findings. One study assessed the association with UTI according to the severity of pre-eclampsia and observed a positive association with severe pre-eclampsia (OR 2.6, 2.0-3.4), but no association with mild pre-eclampsia.[120] The single study of eclampsia reported a four-fold increased risk of eclampsia associated with UTI.[125]

Differences in the method and timing of exposure ascertainment might also explain some of the heterogeneity between studies. Although infection status was most often ascertained by direct measurement (confirmed by positive urine culture), or taken from medical records, three studies,[121,127,130] two of which reported a positive association with pre-eclampsia,[121,130] relied on maternal self-report of UTI in postpartum interview: thus the findings may be prone to recall bias if, for example, women with pre-eclampsia were more likely to report having a UTI during pregnancy than women who did not develop pre-eclampsia. A number of studies

only assessed the presence of UTI at one time point during pregnancy[112–115,117,118,123,124,129] and may have been particularly prone to exposure misclassification, for example, if some women classified as "unexposed" acquired an earlier or later UTI which was undetected. Additionally, the temporal association between UTI and pre-eclampsia was uncertain in some studies.[119,120,122–124,127,128,131] It was not always clear whether an episode of UTI preceded the clinical onset of pre-eclampsia, which renders the findings difficult to interpret.

The apparent relationship between UTI and pre-eclampsia observed in some studies might also be explained, at least in part, by confounding. None of the studies reported adjusting for renal disease, which might act as a confounder of the association observed. In one, pyelographic abnormalities were detected in more than 30% of women with bacteriuria in pregnancy.[114] These underlying morbidities or impairment of renal function could provide an alternative explanation for the increased risk of pre-eclampsia seen among women with UTI. Another potential explanation for the observed association may be increased ascertainment of UTI among women with pre-eclampsia. Patients whose pregnancies are categorised as high-risk may be seen by clinicians more often than those considered lower risk, hence potentially leading to more UTI diagnoses in women who develop pre-eclampsia.

5.3.2 Studies of other acute maternal infections

Eight studies conducted over a ten year period (2003-2012) in a variety of locations, evaluated the relationship between other acute (non-urinary tract) infections in pregnancy and pre-eclampsia (see Table 5.2). The five studies (one cohort,[133] four case-control[106,110,134,135]) which assessed the presence of IgM antibodies to Chlamydia pneumoniae during the second half of pregnancy or at delivery all reported no association between pre-eclampsia and Chlamydia pneumoniae IgM seroprevalence. Three of these adjusted for at least maternal age and gestational age.[106,110,135] However, the sample sizes of all five studies were small, with the number of cases ranging between 37 and 69, which limited the power of these studies to reliably assess the association. Furthermore, while the cohort study assessed maternal antibody status twice during pregnancy (approximately mid-way and at term)[133] all four case-control studies assessed antibody status just once towards the

end of pregnancy or at delivery[106,110,134,135]. Thus it was not possible to establish the temporal sequence of events (the onset of infection in relation to the onset of pre-eclampsia) in these studies.

Both cross-sectional studies of hospital-diagnosed pneumonia during pregnancy reported a significant positive association with pre-eclampsia after adjusting for maternal age, among other potential confounders. The most recent, conducted in Taiwan, observed a three-fold increased risk of pre-eclampsia/eclampsia,[138] while the earlier study from Israel observed a 2.6-fold increased risk of severe pre-eclampsia, though no association with mild pre-eclampsia[137]. However, the Israeli study provided no details on how pre-eclampsia (mild versus severe) was diagnosed. Thus, while the absence of an association with mild pre-eclampsia may reflect a true difference in the mechanisms involved in different subtypes, it may also be due to misclassification of pre-eclampsia in the mild cases. In addition, the timing of pneumonia during pregnancy was not reported in this study, which further complicates interpretation of the findings. However, the majority of women in the Taiwanese study who acquired pneumonia (93.6%) were hospitalised during the first trimester, prior to the onset of pre-eclampsia, thus the findings are compatible with a causal association.

The single study which assessed seroprevalence of maternal IgM antibodies to cytomegalovirus, a large population-based case-control study in Norway, found no evidence for an association with pre-eclampsia after adjusting for maternal age, smoking and parity (OR 1.07, 95% CI 0.48-2.36).[139] However, maternal antibody status was determined only once, approximately mid-way through pregnancy, thus women classified as seronegative at this time may have been previously infected during pregnancy, or may have acquired an infection later in pregnancy. Accordingly, the relationship between acute cytomegalovirus infection and pre-eclampsia has not been determined.

Table 5.2 Characteristics of studies examining the association between pre-eclampsia and other (non-UTI) acute maternal infections and fulfilling the inclusion criteria.

First author, year published	Location	Study design	Sample size or cases/controls	Participant selection (study period)	Outcome or case definition	Type and timing of exposure; method of ascertainment	Adjustment or matching	Main findings (Odds ratio, 95% CI)
Chlamydia pneumoniae								
Ustun, 2010[110] [a]	Malatya, Turkey	Case-control	40 cases with PE, 40 normotensive controls	Consecutive selection of controls (1 per case) from same hospital as case over 12 month period (year unspecified)	PE[b], obstetrician-diagnosed	CP infection in third trimester; IgM antibody status in serum sample	Matched on maternal and gestational age, BMI	No association between PE and seroprevalence of IgM antibodies to CP (30.0% in cases versus 27.5% in controls, p>0.05)
Xie, 2010[135] [a]	Vancouver, Canada	Case-control	50 cases with PE, 57 controls with normal pregnancies at term	Consecutive selection of 1-2 controls per case	PE[b], obstetrician-diagnosed	CP infection at >20 weeks' gestation; IgM antibody status in serum sample	Matched on maternal and gestational age, parity	No association between PE and seroprevalence of IgM antibodies to CP (2.0% in cases versus 0% in controls, p>0.05)
Aral, 2006[134]	Kahramanmaras, Turkey	Case-control	69 cases with PE, 47 controls with normal obstetric history	Not reported	PE, no further details	CP infection at delivery; IgM antibody status in serum sample	None	No association between PE and seroprevalence of IgM antibodies to CP (30.4% in cases versus 23.4% in controls, p>0.05)
Goulis, 2005[133]	London, UK	Cohort	32 multiparas and 37 primiparas	Pregnant women recruited in hospital for separate study of pre-eclampsia prevention (placebo arm only)	PE[b], obstetrician-diagnosed	CP infection at 16-22 and 28-40 weeks' gestation; IgM antibody status in serum sample	None	No association between PE and seroprevalence of IgM antibodies to CP. No difference in median IgM antibody level in pre-eclamptic versus normal pregnancies either mid-gestation or at term
Heine, 2003[106]	Pittsburgh, USA	Case-control	37 cases with PE, 37 controls with normal term pregnancies	Serum samples randomly selected from nulliparous pregnant women with and without pre-eclampsia at term	PE[b], obstetrician-diagnosed	CP infection at admission for labour/delivery; IgM antibody status in serum sample	Maternal and gestational age, smoking, ethnicity	No association between PE and seroprevalence of IgM antibodies to CP

First author, year published	Location	Study design	Sample size or cases/controls	Participant selection (study period)	Outcome or case definition	Type and timing of exposure; method of ascertainment	Adjustment or matching	Main findings (Odds ratio, 95% CI)
Pneumonia								
Chen, 2012[138 a]	Taipei, Taiwan	Population-based cross-sectional	1462 women with pneumonia, 7310 women without pneumonia	All women with pneumonia in pregnancy and random sample of uninfected women (5 per woman with pneumonia) selected from all live singleton births (in 2005)	PE or eclampsia, diagnosis recorded in national birth registry	Hospitalised with pneumonia during pregnancy; diagnosis (ICD-9-CM codes 480–483.8, 485–486 or 487.0) recorded in national health insurance dataset	Matched on maternal age; adjusted for education level, marital status, geographic region, gestational diabetes, gestational hypertension, CHD, anaemia, hyperlipidaemia, obesity, alcohol abuse, infant sex, parity, and paternal age	OR (PE/eclampsia) 3.05, 2.01-4.63
Romanyuk, 2011[137 a]	Be'er-Sheva, Israel	Population-based cross-sectional	181,765	All hospital deliveries (1988-2008)	Mild or severe PE, no further details	Hospitalised with pneumonia during pregnancy, physician-diagnosis confirmed by chest radiograph	Adjusted for maternal age, fertility treatments, placental abruption, intra-uterine growth restriction and previous caesarean delivery	OR for severe PE 2.6, 1.2-5.7; no association with mild PE (OR not reported)
Cytomegalovirus								
Strand, 2012[139 a]	Norway	Population-based, nested case-control	1470 cases with PE, 991 non pre-eclamptic controls	Cases and controls randomly selected from pregnancy cohort (1999-2006)	PE[b] or eclampsia, diagnosis recorded in national birth registry	CMV infection at 17-18 weeks' gestation; IgM antibody status in serum sample	Adjusted for maternal age, parity and smoking in pregnancy	No association between CMV IgM seropositivity and PE/eclampsia, OR 1.07, 0.48-2.36

OR denotes odds ratio adjusted for all specified variables; PE=pre-eclampsia; CP=Chlamydia pneumoniae; BMI=body mass index; CHD=coronary heart disease; ICD-9-CM = International Classification of Diseases, Ninth Revision, Clinical Modification; CMV=cytomegalovirus

[a] studies identified in new search (since 2007)

[b] PE defined as de novo hypertension (blood pressure of at least 90 mm Hg diastolic or at least 140 mm Hg systolic) with proteinuria (≥300 mg in 24 hours, or ≥300mg/L (≥ 1+ on dipstick) in a urine specimen) after 20 weeks' gestation.

5.4 Summary of review

This literature review of pre-eclampsia and acute infection has yielded some evidence for an increased pre-eclampsia risk associated with maternal UTI, although with conflicting findings from other studies. In addition, both studies of maternal pneumonia reported a significant positive association with pre-eclampsia, although in one the effect was confined to severe cases. However, there was no evidence for an association between pre-eclampsia and acute Chlamydia pneumoniae infection. The only study of maternal cytomegalovirus IgM antibody status found no association with pre-eclampsia, and data on other acute infections are lacking.

5.4.1 Rationale for the pre-eclampsia study

In some of these earlier studies, temporality of the association between infection and the development of pre-eclampsia was uncertain, which brings into question the causal nature of the association. In others, infection status was ascertained at a single time point, thus rendering these studies prone to misclassification of exposure. Studies of UTI may have been especially prone to bias by increased ascertainment of UTI in pregnancy among women at risk of pre-eclampsia, or to residual confounding by risk factors such as renal disease. Furthermore, factors such as gestational age at the time of infection or the number of infection episodes throughout pregnancy were not investigated in these studies. Thus a clear role for acute infection in the aetiology of pre-eclampsia has not yet been established.

The pre-eclampsia study described in the next Chapter sought to address these issues by examining, in addition to UTI, the effects of acute RTI, maternal antibiotic prescriptions (a proxy for acute infection) and their timing and frequency in pregnancy, on the risk of developing pre-eclampsia, with optimal adjustment for confounding. Studying two different infectious processes at different sites, in addition to reducing the problem of bias associated with any one particular infection, allowed assessment of whether any effect of acute infection on the risk of pre-eclampsia may be generic, and not specific to one type of infection.

Chapter 6 Methods - Acute maternal infection and pre-eclampsia

This chapter describes the methods of a matched case-control study of acute maternal infection and pre-eclampsia using data from the GPRD. Primiparous women aged at least 13 years and registered with a participating practice between January 1987 and October 2007 were eligible for inclusion. All cases of pre-eclampsia and a random sample of pregnant women without pre-eclampsia (controls) were selected. Cases were individually matched with up to ten controls on practice, calendar year of delivery and gestational age. ORs and 95% CIs for pre-eclampsia were estimated comparing women exposed and unexposed to acute infection using multivariable conditional logistic regression.

6.1 Study hypothesis and objectives

The hypothesis was that acute infections in pregnancy may lead to an increased risk of pre-eclampsia.

The primary objective was to:

- Measure the effects of i) acute UTI, ii) acute systemic RTI, and iii) antibiotic prescriptions in pregnancy (a proxy for acute infection) on the risk of pre-eclampsia.

Secondary objectives were to:

- Describe the occurrence of acute episodes of infection in each of the three pregnancy trimesters and assess the effect of the timing of infection on pre-eclampsia risk.
- Measure the effect of increasing numbers of episodes of acute infection on the risk of pre-eclampsia to establish whether there may be a dose-response relationship.

6.2 Description of the data source

6.2.1 The General Practice Research Database

The study used data from the Full Feature (pre-GOLD) version of the GPRD. The GPRD, founded in January 1987, is one of the world's largest and best established research databases of population-based electronic primary care data. At the time of this study the database held anonymised longitudinal patient records for over ten million patients registered to over 600 UK general practices. More than 98% of the UK population are registered with a general practitioner (GP) and practices contributing to the database are representative of practices throughout the UK in terms of their size, geographical distribution and the age and sex distribution of patients.[140]

Data are prospectively recorded at the patient level by each contributing practice and include medical diagnoses, prescribed medicines, hospital referrals, test results, lifestyle and demographic information, and additional clinical details on a variety of situations relating to patient care, including antenatal and postnatal care. Data recording guidelines to practices stipulate the recording of all significant clinical events in a patient's medical history, including a summary of events which occurred prior to a patient's registration with the practice, or prior to the practice contributing to the GPRD.[141]

In addition to being a rich data source, the GPRD has high data validity. A recent systematic review of validation studies of GPRD diagnoses estimated a median positive predictive value of 89% for recorded diagnoses.[142] Data are subject to ongoing evaluation, verification and validation procedures to ensure they are research-quality.[143] At the patient level, individual patients with non-contiguous or poorly recorded data are identified as unacceptable for use in research and excluded. At practice level, each practice contributing to the GPRD is assigned an "up-to-Standard" (UTS) date indicating when data recording by the practice adhered to specific quality measures based on an assessment of the completeness, continuity and plausibility of the data.[144] Thus, data recorded by a practice from the UTS date are deemed high quality and fit for use in research.

6.2.2 Data structure and key elements

Data on the defined study population were provided in multiple sets of text format files which could be linked using a unique patient identifier. Practice-level data were also provided, which could be linked to these patient-level data using a unique practice identifier.

The patient file contained basic demographic information including gender, year of birth, details of registration with the practice, and, for approximately half the patients, an Index of Multiple Deprivation (IMD) score based on the postcode of the patient. The practice file included the UTS date and last data collection date for each contributing practice, and an IMD score based on the practice postcode.

Clinical files comprised dated records of medical events, including clinical signs, symptoms and diagnoses, and feedback on diagnostic, therapeutic and surgical procedures. Test files included details of requests and results of diagnostic tests, and referral files documented patient referrals to secondary care and other services. Therapy files contained details of each drug prescription issued by the GP and the date of issue. The type and date of each patient consultation with their GP were provided in consultation files.

Additional information relating to current and past pregnancies, including the number of births or miscarriages, expected delivery date, and the weeks' gestation related to an antenatal booking, were recorded in the maternity file.

For the majority of patients, additional data were available on blood pressure readings, smoking, and/or anthropometric measures.

6.2.2.1 Coding systems

Clinical entries in the Full Feature GPRD were coded using the Oxford Medical Information System (OXMIS) and Read coding system which allowed linkage of codes to the corresponding medical terms provided in a look-up file. Prescription drugs were coded using the Multilex Product coding system which provides detailed information on the drug, dose and route of administration. The corresponding drug names, drug substances and British National Formulary (BNF) codes and chapter

headings could be referenced by linking the Multilex product code to the products look-up file.

GPRD Medical and Product dictionary browsers (version 0.3.7, Copyright © 2004) were also provided; these were used to build sets of medical and product codes to define specific events, diseases and drug treatments (see section 6.7.2).

6.3 Study design

A matched case-control study design was used to examine the association between acute maternal infections and pre-eclampsia. Cases (patients with pre-eclampsia) were individually matched with controls (patients who had completed a pregnancy without pre-eclampsia) on GP practice, calendar time of delivery, and gestational age defined as the duration of pregnancy starting from the first day of the woman's last menstrual period (LMP) before delivery. Further comment on the choice of these matching variables, the rationale for using a matched design and details of the matching procedure are provided in section 6.5

6.4 Participants

Participants were derived from a source population of all female patients registered with practices meeting GPRD quality standards during the study period: from 1st January 1987 to 31st October 2007.

Each participant's period of UTS observation (and hence, UTS data) was identified as follows: the UTS start date was defined as the later of the practice UTS date and the patient's registration date with the practice. Thus a patient who registered in 1987 at a practice which became UTS at the start of January 1990 had UTS data from 1st January 1990. The UTS end date was defined as the earlier of the date the patient transferred out of the practice, their date of death, or the practice's last data collection date (up to 31st October 2007).

6.4.1 Outcome

The outcome of the study was pre-eclampsia, defined as a clinical diagnosis of pre-eclampsia, eclampsia or the severe pre-eclampsia variant HELLP syndrome (haemolysis, elevated liver enzymes, low platelets).

For the identification of potential cases when requesting data from the GPRD, a set of Read/OXMIS codes was compiled (see Table 6.1) following the strategy outlined in section 6.7.2.1, below.

Because pre-eclampsia develops in the later stages of pregnancy, a patient must have completed a pregnancy to have had the opportunity to become a case. Therefore, only patients who had a *recorded completed pregnancy* in their primary care record were potentially eligible for inclusion in the study. Hence, for the identification of potential controls, an "end-of-pregnancy" code set was compiled of more than two thousand Read/OXMIS codes indicating that a patient had delivered, e.g. "birth details", or was in the final stages of pregnancy, e.g. "antenatal 37 week examination". Codes indicating early pregnancy loss due to miscarriage or termination (which could occur at any time prior to 24 weeks' gestation and most often in the first trimester) were not included since such pregnancies were likely to end before a woman had reached the required gestational age to be at risk of developing clinical signs of pre-eclampsia. Thus, for the purpose of this study, a completed pregnancy was defined as one ending (or soon to end) in a live birth or stillbirth.

Data were obtained from the GPRD on the following potential cases and controls, drawn from the source population on the basis of these pre-defined medical code sets:

Potential cases (n=3362): all patients with a medical code for pre-eclampsia, eclampsia or HELLP syndrome in UTS data;

Potential controls (n=93909): a random sample of patients with i) an end-of-pregnancy code in UTS data, and ii) no medical codes for pre-eclampsia, eclampsia, HELLP syndrome, or history of pre-eclampsia.

Table 6.1 Pre-eclampsia medical codes.

Medical code	Medical term
READ	
L124300	Mild or unspecified pre-eclampsia - not delivered
L126600	Eclampsia in labour
L126z00	Eclampsia NOS
L127.00	Pre-eclampsia or eclampsia with pre-existing hypertension
L127400	Pre-eclampsia or eclampsia with hypertension + p/n complication
L125200	Severe pre-eclampsia - delivered with postnatal complication
L124600	Pre-eclampsia, unspecified
L125100	Severe pre-eclampsia – delivered
L124.00	Mild or unspecified pre-eclampsia
L124.12	Toxaemia NOS
L126.00	Eclampsia
L126300	Eclampsia - not delivered
L127100	Pre-eclampsia or eclampsia with hypertension - delivered
L129.00	Moderate pre-eclampsia
L124000	Mild or unspecified pre-eclampsia unspecified
L125z00	Severe pre-eclampsia NOS
L126500	Eclampsia in pregnancy
L124z00	Mild or unspecified pre-eclampsia NOS
L126000	Eclampsia unspecified
L125.00	Severe pre-eclampsia
L126400	Eclampsia with postnatal complication
L127000	Pre-eclampsia or eclampsia with hypertension unspecified
L125000	Severe pre-eclampsia unspecified
L124100	Mild or unspecified pre-eclampsia – delivered
L124200	Mild or unspecified pre-eclampsia - delivered with p/n complication
L125400	Severe pre-eclampsia with postnatal complication
L126100	Eclampsia – delivered
L127300	Pre-eclampsia or eclampsia with hypertension - not delivered
L124.11	Mild pre-eclampsia
L124400	Mild or unspecified pre-eclampsia with p/n complication
L124500	Mild pre-eclampsia
L125300	Severe pre-eclampsia - not delivered
L12A.00	HELLP - Syndrome haemolysis, elevated liver enzyme low platelets
L12B.00	Proteinuric hypertension of pregnancy
L126200	Eclampsia - delivered with postnatal complication
L127200	Pre-eclampsia or eclampsia with hypertension - delivered+p/n complication
L127z00	Pre-eclampsia or eclampsia + pre-existing hypertension NOS
Lyu1.00	[X] Oedema,proteinuria+hypertension in pregnancy,childbirth,puerperium
Q000.11	Fetus affected by maternal toxaemia
OXMIS	
7623	Toxaemia pregnancy affecting foetus/newborn
6371PP	Eclampsia post-partum
7960TM	Toxaemia
6370A	Toxaemia pre-eclamptic
6370	Pregnancy pre-eclampsia
6371	Pregnancy eclampsia
6379	Toxaemia pregnancy

NOS=not otherwise specified; p/n=postnatal; the prefix [X] is used for codes introduced with the migration to ICD10 in April 1995.

6.4.2 Eligibility criteria

Pre-eclampsia is more common in primiparous than in multiparous pregnancies[145,146] and therefore a large proportion of potential cases (included on the basis of developing pre-eclampsia) would have been in their first completed pregnancy. Parity may also be associated with the risk of acquiring acute infections in pregnancy, and was thus considered a potential confounder. Once data on potential cases and controls had been obtained, the study population was restricted further to patients on the basis of their *first recorded completed pregnancy* (to reduce the scope for confounding by parity). The methods used to identify participants' first recorded completed pregnancies and to estimate the timing (start and end dates) of each pregnancy are reported in Chapter 7.

Patients aged 13 years or older at the end of their first recorded completed pregnancy within the UTS period were potentially eligible for inclusion in the study.

6.4.2.1 Case definition

Cases were defined as patients with a first-ever clinical diagnosis of pre-eclampsia (a Read/OXMIS code for pre-eclampsia, eclampsia or HELLP syndrome listed in Table 6.1) in their first recorded completed pregnancy.

While the onset of pre-eclampsia typically occurs during the third trimester of pregnancy, clinical signs of the disease can manifest from as early as 20 weeks' gestation up to 4 weeks postpartum[147], as illustrated in Figure 6.1.

Thus, to determine whether a patient's first-ever pre-eclampsia record corresponded to their first recorded completed pregnancy (and not to a previous unrecorded or subsequent pregnancy), a time period was pre-specified within which the pre-eclampsia record had to occur relative to the estimated date of delivery (EDD) of the first completed pregnancy (see Chapter 7 for details on how the EDD was derived). As illustrated in Figure 6.2, this time period started 28 weeks before the EDD and extended up to 15 weeks after the EDD. The choice of 28 weeks pre-EDD allowed for i) a first completed pregnancy of up to 42 weeks' gestation (starting from the first day of the woman's LMP before delivery), ii) a delay of up to six weeks in recording the delivery, and iii) an earliest pre-eclampsia diagnosis corresponding to this first

completed pregnancy at 20 weeks' gestation. The choice of 15 weeks post-EDD allowed for i) an earliest subsequent pregnancy starting one week after delivery (of the first completed pregnancy), and ii) an earliest pre-eclampsia diagnosis corresponding to this *subsequent* pregnancy at 20 weeks' gestation. Thus, cases whose earliest pre-eclampsia record was more than 28 weeks prior to the EDD or more than 15 weeks after the EDD were not included in the study, as it was assumed that their pre-eclampsia episode corresponded to an earlier (unrecorded) completed pregnancy or to a subsequent pregnancy.

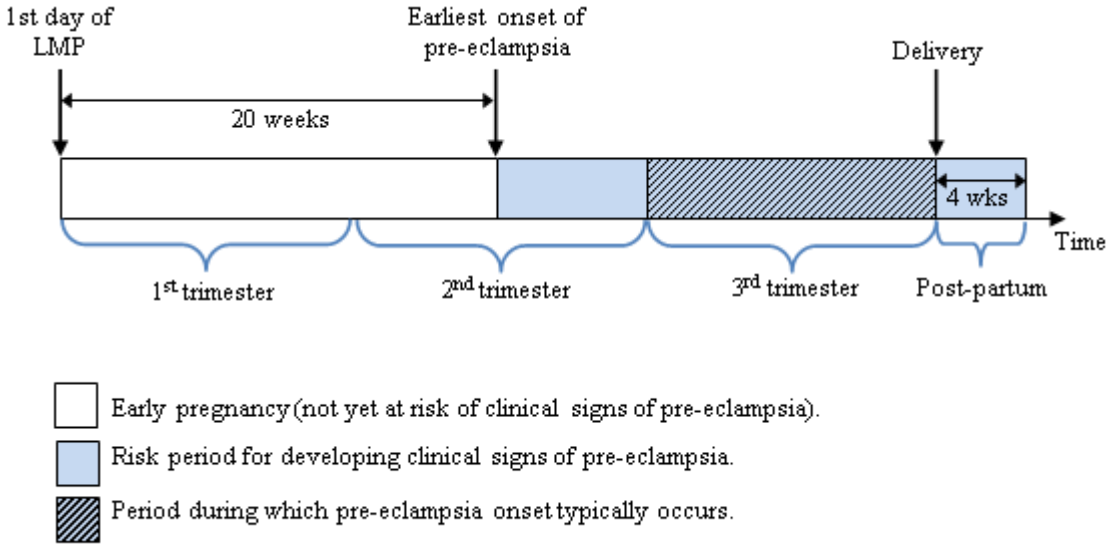


Figure 6.1 Pictorial representation of the timing of pre-eclampsia.

LMP=Last menstrual period

Pre-eclampsia is usually diagnosed in the presence of gestational hypertension associated with proteinuria. Thus for cases, additional data were extracted on hypertension diagnoses, blood pressure readings, the prescription of blood pressure lowering medication, and the presence of proteinuria. However, a pre-eclampsia diagnosis may be established in the absence of proteinuria by the presence of new onset hypertension in association with any of the following severe features: thrombocytopenia, impaired liver function, renal insufficiency, pulmonary oedema, or visual or cerebral disturbances.[148] Furthermore, given that a substantial proportion of women were likely to have received shared antenatal care, data on hypertension or proteinuria during pregnancy may have been incomplete (the absence of these conditions documented in patient records did not preclude the presence of pre-

eclampsia). For these reasons, data on hypertension and proteinuria did not form part of the case definition of pre-eclampsia and were used for descriptive purposes only.

Gestational hypertension was defined as one or more of the following during pregnancy:

- Read or OXMIS code for hypertension;
- Prescription for antihypertensive medication defined as any product under BNF chapter 2.21 (thiazides and related diuretics), 2.4 (beta-adrenoceptor blocking drugs), 2.5 (hypertension and heart failure) or 2.6.2 (calcium-channel blockers), and of 2.2.4 (potassium-sparing diuretics with other diuretics) any product which included "thiazide" as a drug substance;
- Blood pressure reading of at least 140 mm Hg (systolic) or at least 90 mm Hg (diastolic) on at least two occasions.

A record of either of the following during pregnancy was taken as evidence for proteinuria:

- Read or OXMIS code for proteinuria;
- Positive test result indicating more than a “trace” of protein in the urine.

6.4.2.2 Control definition

Controls were defined as patients with a first recorded completed pregnancy and with no clinical diagnosis of pre-eclampsia or history of pre-eclampsia recorded anywhere in their medical data. This ensured controls had no pre-eclampsia in their first recorded completed pregnancy, and no history of pre-eclampsia possibly relating to an earlier (unrecorded) pregnancy.

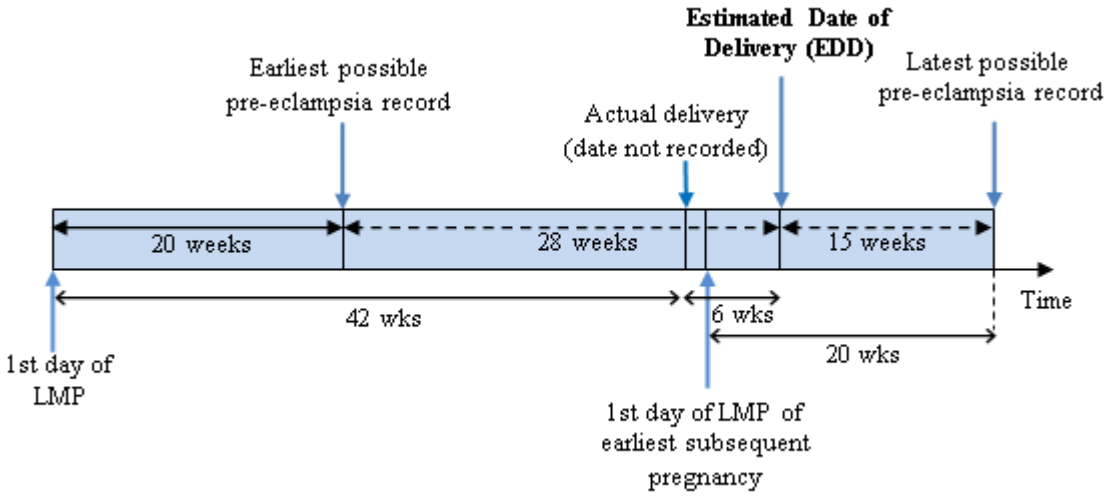


Figure 6.2 Linking the pre-eclampsia episode to the first completed pregnancy episode.

LMP=last menstrual period

6.4.2.3 Exclusions

The following four exclusion criteria were applied to cases and controls:

1. Uncertain timing of delivery

Patients with either of the following were excluded:

i. Delivery record on an invalid date (1st January 2500), which may represent a default date for a past delivery (see section 6.7.1);
ii. Earliest delivery record on the same date as a new-patient or well-patient health visit, which may represent a past delivery retrospectively recorded with the wrong date.

2. Evidence of an earlier (unrecorded) completed pregnancy

Patients were excluded if their records indicated that they had completed an earlier pregnancy prior to their first recorded completed pregnancy. This was to help ensure that cases and controls were primiparous at the time of selection into the study, thus further reducing the possibility of confounding by parity. Evidence of an earlier completed pregnancy was defined as any of the following records at least six weeks before the EDD of the first recorded completed pregnancy, thus allowing for a delay of up to six weeks in recording the latter:

i. Postnatal record (indicating that a patient had given birth without specifying the timing postpartum), e.g. a Read code for "Postpartum depression";
ii. "History of" delivery record, e.g. a Read code for "History of postpartum haemorrhage";
iii. Parity status of one or more births recorded in the Maternity file.

Records (of i, ii or iii) in the six weeks immediately preceding the EDD were not used as evidence of a previous pregnancy as they were considered more likely to correspond to the first recorded completed pregnancy whose outcome may have been recorded late.

3. New onset hypertension in pregnancy persisted after delivery

To strengthen the validity of the case definition of pre-eclampsia, patients with no evidence of pre-existing hypertension (no Read/OXMIS codes for hypertension or antihypertensive drug prescription records before pregnancy) but whose hypertension did not resolve following delivery (an antihypertensive drug prescription record six to 12 months after delivery) were excluded. This criterion helped to distinguish between true cases of pre-eclampsia and patients with essential or secondary hypertension which became clinically apparent during pregnancy.

4. Part of the gestational period was outside the UTS observation period

To ensure that the recording of diagnoses and events throughout the entire gestational period was research quality, patients whose pregnancies began prior to the UTS start date were excluded. Details of how each patient's estimated pregnancy start date was derived are provided in Chapter 7.

In addition, two **case-only** exclusion criteria were applied:

1. Uncertain timing of pre-eclampsia

Cases with either of the following were excluded:

i. Pre-eclampsia record on an invalid date (1st January 2500), which may represent a default date for a past pre-eclampsia episode;

ii. Earliest pre-eclampsia record on the same date as a new-patient or well-patient health visit, which may represent a past episode of pre-eclampsia retrospectively recorded with the wrong date.

2. Evidence of an earlier (unrecorded) pre-eclampsia episode

Cases whose records indicated an earlier pre-eclampsia episode (and hence an earlier unrecorded completed pregnancy) prior to the first recorded pre-eclampsia episode, e.g. a Read code for "History of pre-eclampsia", were excluded. This criterion helped to ensure that cases were primiparous at the point of selection.

6.4.3 Statistical power

Power calculations were derived using methods outlined by Dupont (1988) for matched case-control studies.[149] The statistical power of a matched case-control study can be increased by selecting more than one control per case.[150] Although any additional gain in power is generally considered to be minimal if the case-control ratio exceeds 1:4, increasing this ratio may be desirable if the prevalence of exposure among controls is expected to be less than 0.15,[151] or if analyses are to be stratified by other factors (potential confounders other than the matching variables).[150] Both of these circumstances applied in this study. In addition, the number of controls identified as eligible for matching greatly exceeded the number of cases (by more than twenty-fold): thus, increasing the control-per-case ratio beyond four posed no additional cost or effort in data collection. For these reasons, the decision was taken to select up to ten controls per case.

The power of the study to detect an OR for exposure to an acute maternal infection (UTI, RTI or antibiotic prescription during the gestational period) of at least 1.3 was estimated for a range of exposure prevalences among controls. Based on the number of pre-eclampsia cases identified as eligible for matching (n=1535), and allowing a case-control ratio of 1:10, the study had more than 90% power (at 5% significance) to detect the following:

- OR of 1.5 or more if the prevalence of an acute maternal infection among controls (p_0) was 5%;
- OR of 1.4 or more if p_0 was 10%;

- OR of 1.3 or more if p_0 was 15%.

6.5 Case-control matching

Matching in a case-control study refers to the procedure whereby one or more controls are selected for each case on the basis of similarity with respect to certain characteristics other than the exposure(s) being investigated. The rationale for matching is to make adjustment for confounding in the analysis more efficient.[150] Without matching, such adjustment may result in multiple strata with sparse data. Balancing the distribution of matching variables across strata results in gains in precision and hence more stable effect estimates (ORs) with smaller standard errors and thus narrower CIs. However, matching will only increase efficiency if the matching variables are associated with both the disease and exposure and are not on the causal pathway linking the two. Thus, only potential confounders of the pre-eclampsia and infection association were considered as candidate variables for matching in this study.

6.5.1 Matching procedure

With input from a statistician, an algorithm was developed to carry out a one-to-many individual matching of cases to controls. Three matching criteria were chosen on the basis that each was thought to be associated with both the risk of being diagnosed with pre-eclampsia and the risk of being diagnosed with (or treated for) acute infection, and were thus potential confounders: i) GP practice; ii) calendar time of delivery (allowing an absolute difference of up to 12 months between the case-control estimated delivery dates); and iii) gestational age. Matching on practice allowed for variability in recording and prescribing habits between practices, while at the same time providing some adjustment for unmeasured socioeconomic factors. Matching on delivery date within one year ensured that case and control pregnancies within matched sets were contemporaneous. Matching on gestational age ensured that the exposure period for cases and their matched controls was of a similar duration, hence allowing for a similar opportunity for infection.

The following steps were taken to perform the matching:

i. All potential case-control matches were identified on the basis of GP practice;
ii. Matches were excluded when case and control delivery dates were more than an absolute difference of 12 months apart;
iii. Matches were excluded when the control had a shorter pregnancy than the case. When a control had a longer pregnancy than the case, this was curtailed to match the case's gestational age at pre-eclampsia diagnosis, as outlined in section 6.6 below;
iv. Of the remaining matches, up to ten controls per case were selected at random without replacement.

The process generated matched sets, each comprising a single case and up to ten controls. Since cases and their matched controls were similar on these matching variables, their difference with respect to the outcome (pre-eclampsia) had to be attributable to other factors.

6.6 Exposures

In order to ascertain exposure to acute infections during pregnancy, it was first necessary to estimate the timing of each pregnancy. Full details of the methods used to date pregnancies in this study are provided in Chapter 7.

The exposure period for each participant began on the estimated pregnancy start date (see Chapter 7, section 7.2.2 for details of how this date was derived) and ended at the index date. The index date was defined as follows: for cases, it was the earlier of the date of the pre-eclampsia diagnosis and the EDD; for controls it was the date they reached the same gestational age as their matched case at the case's index date. Figure 6.3 illustrates the exposure period for a hypothetical case and matched control.

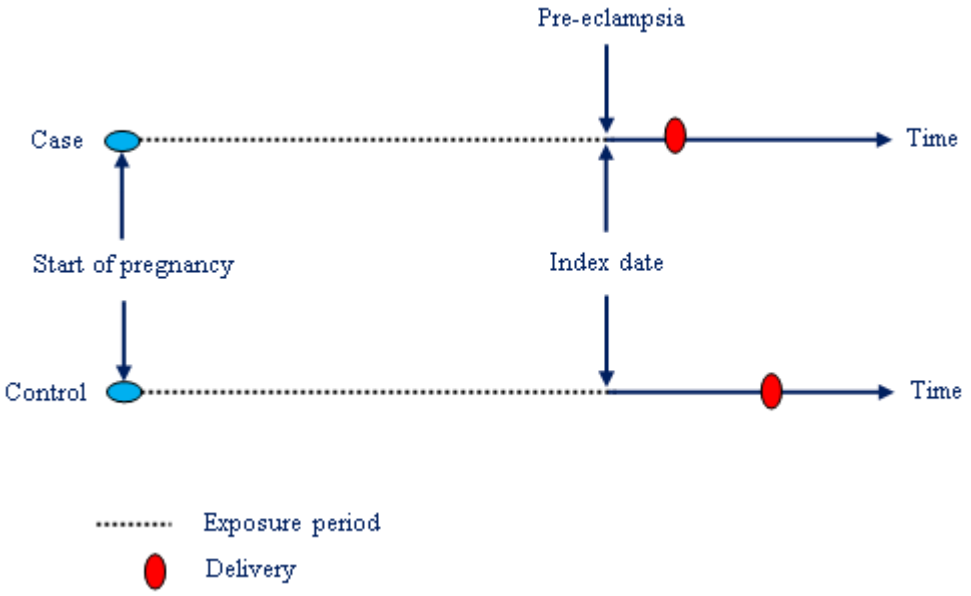


Figure 6.3 The exposure period for a hypothetical case and matched control: from the start of pregnancy to the index date.

Data were extracted on three exposures of interest over the exposure period:

- acute UTI (manifest as asymptomatic bacteriuria, cystitis, or pyelonephritis);
- acute RTI (excluding non-specific or minor upper respiratory infections and symptoms such as sore throat);
- antibiotic drug prescriptions.

The Read/OXMIS codes and terms used to define acute UTI and RTI are listed in Appendix G-Tables G.1 and G.2. A preliminary list of antibiotic drug codes was compiled based on pre-defined headings within chapter 5.1 "Antibacterial drugs" of the BNF (see Table 6.2, below). Additional searches were conducted at the drug substance level to identify any relevant products not found in the preliminary search for which the BNF codes were missing. More than two thousand product codes for antibiotic drugs were identified and included in the final code set.

Table 6.2 British National Formulary (BNF) codes and headings for antibiotics.

BNF code	BNF chapter heading
5.1.1	Penicillins
5.1.1.1	Benzylpenicillin & phenoxymethylpenicillin
5.1.1.2	Penicillinase-resistant penicillins
5.1.1.3	Broad-spectrum penicillins
5.1.1.4	Antipseudomonal penicillins
5.1.1.5	Mecillinams
5.1.2	Cephalosporins and other beta-lactams
5.1.3	Tetracyclines
5.1.4	Aminoglycosides
5.1.5	Macrolides
5.1.6	Clindamycin
5.1.7	Some other antibacterials
5.1.8	Sulphonamides & trimethoprim
5.1.11	Metronidazole and tinidazole
5.1.12	Quinolones
5.1.13	Urinary-tract infections

6.6.1 Quantifying and categorising exposure

When a patient had more than one record of UTI, RTI or antibiotic prescription, a minimum of 29 days between successive records of the same type was required for these to be considered distinct episodes of infection, rather than repeat consultations for the same infection.

Binary variables were created for each exposure of interest, indicating "exposed" versus "not exposed" at any time during the exposure period and during each of the three trimesters.

To investigate the effect of the timing of infection over the exposure period, categorical variables were created for each exposure, each with four levels representing the most proximate exposure to the index date:

i. no exposure (the reference category);
ii. trimester one only;
iii. trimester two (+/- trimester one);
iv. trimester three (+/- trimester one or two).

Thus, a patient diagnosed with an acute UTI in the first trimester and who experienced a second episode in the third trimester was assigned to exposure level iv.

To measure the effect of increasing episodes of infection, categorical variables were created for each exposure, each with three levels:

i. no episodes (the reference category);
ii. one episode;
iii. more than one episode.

6.6.2 Potential confounders

In addition to *maternal age* and the matching variables *practice*, *calendar year of delivery* and *gestational age at index date*, detailed code sets and algorithms were developed for extracting information on established or potential confounders of each pre-eclampsia-infection association of interest:

- Read/OXMIS codes for pre-existing comorbidities: renal disease, diabetes, hypertension, and asthma;
- Multilex product codes for insulin and for anti-diabetic, anti-hypertensive and anti-asthmatic drugs (used as evidence of pre-existing diabetes, hypertension and asthma in the absence of medical codes for these conditions);
- Read/OXMIS codes for multifetal gestation and previous early pregnancy loss;
- Lifestyle factors: pre-pregnancy BMI and maternal smoking (a known protective factor).

To address the possibility that infections may be more likely to be recorded among women who consult with their GP more frequently, data were extracted on the number of consultations and duration of follow-up each woman had prior to pregnancy. This allowed comparison of cases' and controls' pre-pregnancy consultation behaviour.

6.6.2.1 Estimating pre-pregnancy BMI

Data on pre-pregnancy BMI were derived from height and weight records or taken directly from BMI records (where available). Once all records of BMI, weight and height had been extracted, data were checked for outliers. Records which fell within the following pre-specified ranges of plausible values were retained:

- weight: 25.4-222 kilograms (kg);
- height: 1.3-2.3 meters (m);
- BMI (both recorded and derived measures): 15-50 kg/m^2.

When a patient had multiple records of height or weight on same day, the difference between the minimum and maximum values were generated; if the difference was ≤5 cm (height) or ≤2 kg (weight) the average of these values were taken, if the difference exceeded 5 cm or 2 kg the records were dropped.

Given that height is likely to be relatively stable beyond adolescence, the record closest to conception (either pre- or post-conception) was selected. Because an individual's weight can fluctuate over time, the most proximate record either pre-conception or up to eight weeks post-conception was selected as the best estimate of pre-pregnancy weight or BMI. Weight or BMI records more than eight weeks after conception may reflect gestational weight gain and were thus not used to estimate pre-pregnancy BMI.

While some patients had no information on BMI, others had both recorded and derived estimates. In such cases, the estimate closest to conception was selected. A categorical variable for pre-pregnancy BMI was thus generated according to standard categories for adults, with an additional category for "unknown":

i. Underweight (<18.5 kg/m^2)
ii. Normal (18.5-24.9 kg/m^2)
iii. Overweight (25.0-29.9 kg/m^2)
iv. Obese (≥30 kg/m^2)
v. Unknown

6.6.2.2 Estimating maternal smoking status

Data on maternal smoking were limited (>80% of cases and controls had no smoking status recorded during pregnancy), thus the decision was taken to supplement this with information on smoking status prior to conception. Because an individual's smoking status can change over time, the closest record prior to conception was selected as the best estimate of a patient's smoking status at the time of conception. When a patient had multiple records during pregnancy, possibly indicating more than

one status (current smoker, ex-smoker or non-smoker), "current" took precedence over "ex", and both of these superseded "non". Hence, two smoking variables were generated indicting smoking status before and during pregnancy, from which patients were assigned to one of the following categories:

1. Non-smoker (status "non" during pregnancy)
2. Ex-smoker (status "ex" during pregnancy)
3. Current smoker (status "current" during pregnancy)
4. Unknown-non (status "unknown" during pregnancy and "non" pre-pregnancy)
5. Unknown-ex (status "unknown" during pregnancy and "ex" pre-pregnancy)
6. Unknown-current (status "unknown" during pregnancy and "current" pre-pregnancy)
7. Unknown (status "unknown" both during and pre-pregnancy)

To assess whether information on smoking status pre-pregnancy could be used as a proxy for smoking status during pregnancy (when the latter was unknown), ORs for pre-eclampsia were calculated and compared for each smoking category relative to non-smokers (category 1). The rationale was that if, for example, women who smoked before pregnancy (according to their most proximate record prior to conception) continued to smoke during pregnancy, the ORs for categories 3 and 6 (relative to 1) would be similar. The similar effect estimates derived for categories 3 and 6, for categories 2 and 5, and the close to null value for category 4 (as shown in Appendix H-Table H.1) indicated that this was a reasonable assumption. Thus, these seven smoking categories were combined to generate a single variable that best captured maternal smoking status:

i. Non-smoker (status is "non" during pregnancy or status is "unknown" during pregnancy and "non" pre-pregnancy)
ii. Ex-smoker (status is "ex" during pregnancy or status is "unknown" during pregnancy and "ex" pre-pregnancy)
iii. Current smoker (status is "current" during pregnancy or status is "unknown" during pregnancy and "current" pre-pregnancy)
iv. Unknown (status is "unknown" both during and pre-pregnancy)

6.7 Data management

Despite the quality checks applied to GPRD data before they are made available for research, some additional checks, data cleaning and coding were necessary, as outlined below.

6.7.1 Extracting and cleaning data

Data were extracted on demographic characteristics (year of birth, practice, SES); medical codes used to identify cases and controls, exposures and potential confounders; prescription drug codes; and patient registration details. Key data fields were checked for missing values and outliers. Missing or incomplete dates were recorded in the GPRD as 1st January 2500. Records with this default date were thus assumed to represent past events or diagnoses for which the precise timing was uncertain.

Information on the day and month of birth were not provided. Therefore, each patient's date of birth was estimated as 1st July of their year of birth, thus allowing a maximum error margin of six months for age.

6.7.2 Creating code sets

In order to identify individuals with records of a particular disease, medical event or prescribed drugs to treat a condition, it was necessary to compile sets of codes defining each disease, event and drug treatment of interest. The procedure was iterative, involving the initial identification of all potentially relevant codes through searches of the GPRD dictionaries. The codes were then independently reviewed by two clinicians and final versions of each code set were agreed.

6.7.2.1 Medical code sets

The GPRD Medical dictionary browser was used to build sets of medical codes. The dictionary includes the GPRD assigned medical code for the type of event, the Read or OXMIS code for the event and a description of the medical term. Potential Read and OXMIS terms were identified using a two-stage search strategy. First, a list was compiled of key terms describing the event or disease. Using the wild card (*), these terms (or term stems) were used to search the GPRD dictionary. For example,

searching for *pre-eclam* identified terms including “Pre-eclampsia”, “Severe pre-eclampsia with postnatal complication” and “Pre-eclampsia or eclampsia with hypertension”. Read codes group and define diseases and other clinical events within a hierarchical structure, with top level codes for broad disease categories branching into more specific codes. Therefore, stage two involved identifying relevant top level Read codes and including the lower level codes.

The final sets of Read and OXMIS codes defining the study outcome (pre-eclampsia) and two exposures of interest (acute UTI and RTI) are listed in Table 6.1 and Appendix G-Tables G.1-G.2, respectively.

6.7.2.2 Product code sets

The GPRD Product dictionary browser was used to build sets of product codes. The dictionary includes the Multilex product code for the prescription, the product name, the composite drug substances and (where available) the corresponding BNF code and chapter heading. All prescription records had a Multilex code which could be referenced in the product dictionary. However, some Multilex codes had no corresponding BNF code if, for example, the drug was no longer licensed. Thus, to ensure all relevant products were included in a code set, a two-stage search strategy was implemented. First, all relevant BNF chapter headings were identified (Table 6.2) and the dictionary was searched for all products under these headings. Second, a list of all drug substances within the products identified in stage one was reviewed and additional searches were conducted at the drug substance level to identify any relevant products missed in the first stage.

6.8 Statistical analysis

Data analyses were conducted using Stata, release 12 (StataCorp., College Station, Texas).

6.8.1 Descriptive analyses

Characteristics of study participants including demographic and lifestyle factors, pre-existing morbidities, pregnancy characteristics, and the timing and number of acute infection episodes over the exposure period were summarised for cases and controls

separately. Proportions were calculated for categorical variables. For continuous variables, means (with standard deviations) or, where data were skewed, medians (with IQRs) were calculated.

For cases, the severity of pre-eclampsia was determined according to the Read/OXMIS codes for pre-eclampsia which made up a single episode, defined as all successively recorded codes within 28 days apart (as for infection episodes, section 6.6.1). When an episode comprised multiple codes indicating varying degrees of severity (ranging from non-specific to mild/moderate or severe pre-eclampsia, through to eclampsia or HELLP syndrome), the decision was taken to classify the case according to the most severe indication given the potential for the syndrome to develop. For example, where an episode contained a non-specific code and a code for severe pre-eclampsia, the case was classified as severe.

6.8.2 Primary analysis

To account for the individual matching carried out at the design stage, conditional logistic regression was used to estimate ORs and 95% CIs for pre-eclampsia comparing pregnant women exposed and not exposed to each exposure of interest: acute UTI, RTI or antibiotic prescriptions.

The primary analysis assessed the effect of each exposure of interest occurring at any time during the exposure period. First, univariable analyses were conducted to estimate crude effects for all explanatory variables, including the exposures of interest. Potential confounding factors (outlined in section 6.6.2) found to be independently associated with pre-eclampsia in univariable analyses (determined by a p-value ≤ 0.2) were subsequently assessed in more complex, multivariable models. Maternal age (controlled for in five-year age groups: <20, 20-24, 25-29, 30-34, 35-39, and ≥40) and pre-existing renal disease were included in all multivariable models a priori. The remaining potential confounders were each added to models in succession based on their strength of association with pre-eclampsia (in decreasing order), and were retained if they made an appreciable difference to the exposure of interest OR (determined by ≥10% change in either direction) and/or the likelihood ratio test indicated an improved model fit with their inclusion, or (in borderline cases) if their inclusion did not markedly compromise the precision of the exposure

effect. Variables excluded at earlier stages of the modelling process were later re-introduced to assess whether the presence of other factors altered their effect on the infection-pre-eclampsia association of interest.

6.8.3 Secondary analyses

Using the same methods as for the primary analysis and the categorical exposure variables described in section 6.6.1, the effects of infection occurring at different stages of pregnancy and of increasing episodes of infection over the exposure period were examined for each exposure of interest. To assess whether the timing of infection may be important, adjusted ORs and 95% CIs for pre-eclampsia were estimated comparing women exposed (according to the most proximate episode to the index date) versus women not exposed at any time during the exposure period. To assess whether there may be a dose-response effect of infection on pre-eclampsia risk, adjusted ORs and 95% CIs for pre-eclampsia were estimated for women with a single episode of infection, and for women with more than one episode, relative to women not exposed at any time during the exposure period.

6.8.4 Sensitivity analyses

Misclassification of UTI due to proteinuria

It was recognised that bias may have arisen due to possible misdiagnosis of UTI among patients with pre-eclampsia due to the identification of proteinuria. To address this possibility, a sensitivity analysis was done assessing the effects of early exposure to UTI or antibiotics in the first two trimesters only versus no exposure (to UTI or antibiotics) at any time during the exposure period, among a subset of the study population: cases who developed pre-eclampsia in the third trimester and their matched controls. The rationale was that these third trimester pre-eclampsia cases were unlikely to have had proteinuria detected during the first two trimesters, thus minimising the potential for such differential misclassification of exposure.

Incident versus past or prevalent infections

An inflated incidence rate of acute events, such as UTIs or RTIs, has been demonstrated in the period following registration with a practice, resolving to baseline over approximately six months.[152] In order to ensure that the infections

observed throughout the exposure period were incident and did not represent diagnoses for past infections retrospectively recorded within the first few months after a patient joined a practice, a sensitivity analysis was done excluding patients with less than six months UTS follow-up prior to their estimated pregnancy start date.

Pre-eclampsia diagnoses before year 2000

To address the possibility that pre-eclampsia diagnoses made in earlier years may have been less exact, analyses were restricted to pregnancies in the year 2000 onwards following publication of the first recommended consensus definition of pre-eclampsia.[153]

Timing and severity of pre-eclampsia

Pre-eclampsia is usually defined as "early-onset" when clinical manifestations occur before 34 weeks' gestation, and "late-onset" when occurring at or after 34 weeks' gestation.[154] To assess whether the effect of acute infection differed according to the timing of pre-eclampsia onset or the severity of the syndrome, separate analyses were performed for cases with early-onset pre-eclampsia (<34 weeks' gestation) and late-onset pre-eclampsia (≥34 weeks' gestation), and for cases with documented severe pre-eclampsia, eclampsia or HELLP syndrome.

Additional sensitivity analyses were performed, excluding the following:

- Patients aged less than 18 years as they may differ from older women with respect to their pregnancy outcomes and underlying risk profile;
- Patients with pre-existing hypertension, to reduce the possibility of misdiagnosis of pre-eclampsia;
- Controls with new onset hypertension during pregnancy which resolved in the 6-12 months after delivery (as determined by an absence of antihypertensive prescription records during this period), as these controls may have had pre-eclampsia even in the absence of a clinical diagnosis;
- Assisted reproductive technology (ART) pregnancies, defined as in vitro fertilization with or without intracytoplasmic sperm injection, and related

techniques including gamete intrafallopian transfer and embryo transfer, which may have a higher risk of developing pre-eclampsia.

6.9 Ethics approval

Ethics approval for the study was obtained from the Independent Scientific Advisory Committee (ISAC) (protocol 07_094) and the London School of Hygiene and Tropical Medicine Ethics Committee (application number 5283).

Chapter 7 Dating pregnancies in the GPRD

This chapter outlines the methods used to estimate the timing of pregnancies for participants included in the case-control study of pre-eclampsia (described previously in Chapter 6). It was crucial to establish when participants' pregnancies started and ended in this study, as this represented the exposure period of interest during which acute infections were identified.

Pregnancy is measured in trimesters from the first day of a woman's LMP (two weeks before conception) and normally lasting 37 to 42 weeks to delivery. While data pertaining to pregnancy are included in the GPRD, the timing of conception (or first day of the LMP) is rarely recorded. In addition, not all completed pregnancies have a timely, or indeed any, delivery record in the general practice data. The absence of such details on the precise timing of pregnancies in the GPRD presented a major challenge for the pre-eclampsia study, which relied on the ability to ascertain exposure to infections during the gestational period. Hence, development of a strategy to identify the start and end dates of pregnancy in GPRD patients was required.

7.1 Previous work to identify pregnancies in EHR databases

Previous approaches to identify pregnancies and classify gestational periods in the GPRD and in other EHR databases in the absence of LMP or conception data are outlined below.[155–158] While providing a useful basis on which to build a new strategy (described in section 7.2 below), each approach was limited in its ability to reliably estimate the timing of the start of pregnancy. Thus none were considered suitable for application in the pre-eclampsia study.

The earlier of two algorithms for identifying pregnancies in the GPRD, developed by Hardy et al,[155] used a "pregnancy-indicator" approach. This involved mapping early pregnancy markers (e.g. an antenatal visit, positive pregnancy test, or any pregnancy-related diagnosis or procedure) to pregnancy outcomes (e.g. birth, spontaneous abortion), allowing up to 280 days (40 weeks) between pregnancy markers and outcomes. The authors subsequently estimated the first pregnancy trimester as the 70 day period after the earliest pregnancy marker, allowing for the fact that the first

documentation of pregnancy (the earliest pregnancy marker) was likely to be several days/weeks after conception.[156]

A simpler approach to identify pregnancies in a Health Maintenance Organisation automated database was taken by Andrade et al,[157] whose "delivery date" algorithm involved counting back 270 days from the delivery date to estimate the timing of conception; hence the algorithm assumed all pregnancies were of equal duration (lasting 270 days from conception to delivery). The period between 181 and 270 days before delivery was considered a proxy for the first pregnancy trimester.

Despite their different approaches, both algorithms were similarly limited in their application: the pregnancy-indicator algorithm[156] excluded women whose first recorded antenatal visit was less than seven months before delivery (among whom were women who began antenatal care late and/or some who delivered prematurely), and the delivery date algorithm[157] excluded women with documented conditions associated with preterm birth. While the purpose of these exclusions was to reduce misclassification of first trimester exposures among women with shorter gestations or late entry into antenatal care, these exclusions also undermined the utility of the algorithms, particularly for studies such as the case-control study carried out for this thesis (described in Chapter 6), whose outcome of interest (pre-eclampsia) is associated with shorter gestations[97] and possibly also later antenatal care. A study comparing the performance of the two algorithms in identifying exposure to first trimester prescription medications found that Andrade et al's delivery date algorithm resulted in greater sensitivity (90.0%) and specificity (99.3%) for identifying medication use than Hardy et al's pregnancy-indicator algorithm (sensitivity: 56.4%, specificity 97.7%).[159] However, almost all of these pregnancies were among women who delivered at term; as expected, the sensitivity of the delivery date algorithm was markedly diminished for preterm deliveries (65.8%).

A more recent paper by Devine et al reported on the development of a computer-based algorithm for identifying pregnancies in the GPRD.[158] In contrast to the previous approaches described above which attempted to identify the start of pregnancy by counting back a fixed number of days from the pregnancy outcome, this method used a number of rules, allowing a variable number of days between the earliest pregnancy marker and the outcome, thus improving the identification of

pregnancies with outcomes other than full-term birth. The rationale was that pregnancies ending in stillbirth or live preterm delivery span a range of gestational ages (from as early as 24 weeks) and can appear to overlap with other pregnancies (and hence go undetected) when a fixed-day approach is used.

Despite the clear advantage of Devine et al's variable-day approach, it too was limited in its ability to estimate the precise timing of the start of pregnancy, as the earliest indication of pregnancy in a patient's records (e.g. "patient pregnant") is not a good proxy for the pregnancy start date as it would invariably be late. Notably, neither of the two GPRD-based algorithms[155,158] fully exploited information from individual pregnancy records that reported gestational age (e.g. "pregnancy prolonged – 41 weeks") or the time period postpartum ("Maternal postnatal 6 week exam"), to inform the timing of conception or delivery.

7.2 A new approach to dating pregnancies in the GPRD

Drawing from the previous approaches described above, and from discussions with researchers at the University of Nottingham and University College London who had undertaken pregnancy-related studies using EHRs, a new algorithm was developed for use in the pre-eclampsia study to estimate as accurately as possible the start and end dates of each participant's first recorded completed pregnancy. The algorithm is described in detail in sections 7.2.1 and 7.2.2 below. In brief, it involved extracting and using in a hierarchy all available general practice-recorded information on the timing of pregnancy from:

- antenatal records (medical codes indicating the weeks' gestation related to an antenatal booking);
- delivery records (medical codes indicating gestational age at delivery or the number of days or weeks postpartum);
- records indicating the expected date of delivery and/or the estimated date of conception (EDC) (when available).

Given that each woman must have completed a pregnancy in order to be considered eligible for the pre-eclampsia study, the process began with estimating the date of delivery (see section 7.2.1) corresponding to their first recorded completed

pregnancy. Subsequently, the pregnancy start date was determined (see section 7.2.2) based on gestational age and thus defined as the date of the first day of the woman's LMP before delivery. A common convention was used to estimate the timing of trimesters: first trimester (first day of the LMP to 13 weeks), second (weeks 14 to 26), and third (week 27 to delivery).

7.2.1 Estimating the date of delivery

As described in Chapter 6 (section 6.4.1), an "end-of-pregnancy" code set was compiled of Read/OXMIS codes which represented a completed pregnancy (i.e. codes specifying the birth outcome or mode of delivery, or indicating that the woman was close to term), using the strategy outlined in section 6.7.2.1. This "end-of-pregnancy" code set was used to estimate the date of delivery of case and control pregnancies, as follows.

First, each code was assigned to one of the following mutually exclusive categories:

1. Birth outcome, e.g. "Caesarean section";
2. Very late pregnancy: likely to be one to two days before delivery, e.g. "Premature rupture of membranes";
3. Very early postnatal: up to one week after delivery, e.g. "Repair of episiotomy";
4. Early postnatal: one to six weeks after delivery, e.g. "Postnatal – tenth day visit";
5. Late pregnancy: up to six weeks before delivery, e.g. "Cephalic version";
6. Other postnatal: any other postnatal code indicating a patient had given birth, but where the timing postpartum was uncertain e.g. "Postnatal depression".

Category **1-4** codes were combined to form a single set of delivery codes and extra information within these codes (when available) was used to derive the EDD, as outlined below. Category **5** codes were considered to be proxy delivery codes and hence were also used to determine the EDD. While category **6** codes indicated that a patient had given birth, they did not specify the time period postpartum, and hence could not be used to determine the EDD. However, any such codes recorded more than six weeks before the EDD were subsequently used as evidence of an earlier completed pregnancy, an exclusion criterion, as outlined in Chapter 6, section

6.4.2.3. Hence, only codes in categories 1-5 were used to estimate the date of delivery of participants' first completed pregnancies (see Appendix G-Table G.3).

Using these category 1-5 codes, the following steps were taken to derive the EDD, as illustrated in Figure 7.1:

Step 1. Identifying patients' earliest delivery records

Clinical, referral and maternity files of each potential case and control were searched for delivery codes (categories 1-4), and those recorded within ten years of the patient's year of birth (records which were likely to relate to the patient's own birth rather than her infant's birth) were excluded. Each patient's earliest delivery record (or records, if more than one on the same day) was selected.

The next step depended on whether the patient had at least one delivery record or no delivery records, as outlined below.

Step 2.i. Estimating the date of delivery for patients with a delivery code

For patients **with at least one delivery record** identified in Step 1, additional data were extracted on:

a. Late pregnancy (category 5) codes corresponding to an *earlier* pregnancy within the study period. This was to identify earlier completed pregnancies that had no corresponding delivery records. These codes were recorded more than 24 weeks prior to the patient's earliest delivery record, as illustrated in Figure 7.2. The choice of 24 weeks was based on the minimum gestation of a completed pregnancy (lasting 24 weeks from the first day of LMP), thus allowing a minimum of 24 weeks between two successive deliveries. When a patient had more than one late pregnancy code, codes recorded within six weeks of each other were assumed to correspond to the same pregnancy;
b. Information on the number of days or weeks postpartum within the earliest delivery record(s);
c. Late pregnancy (category 5) codes corresponding to the *same* pregnancy as the earliest delivery record (codes recorded less than 24 weeks prior to the earliest delivery record), also shown in Figure 7.2.

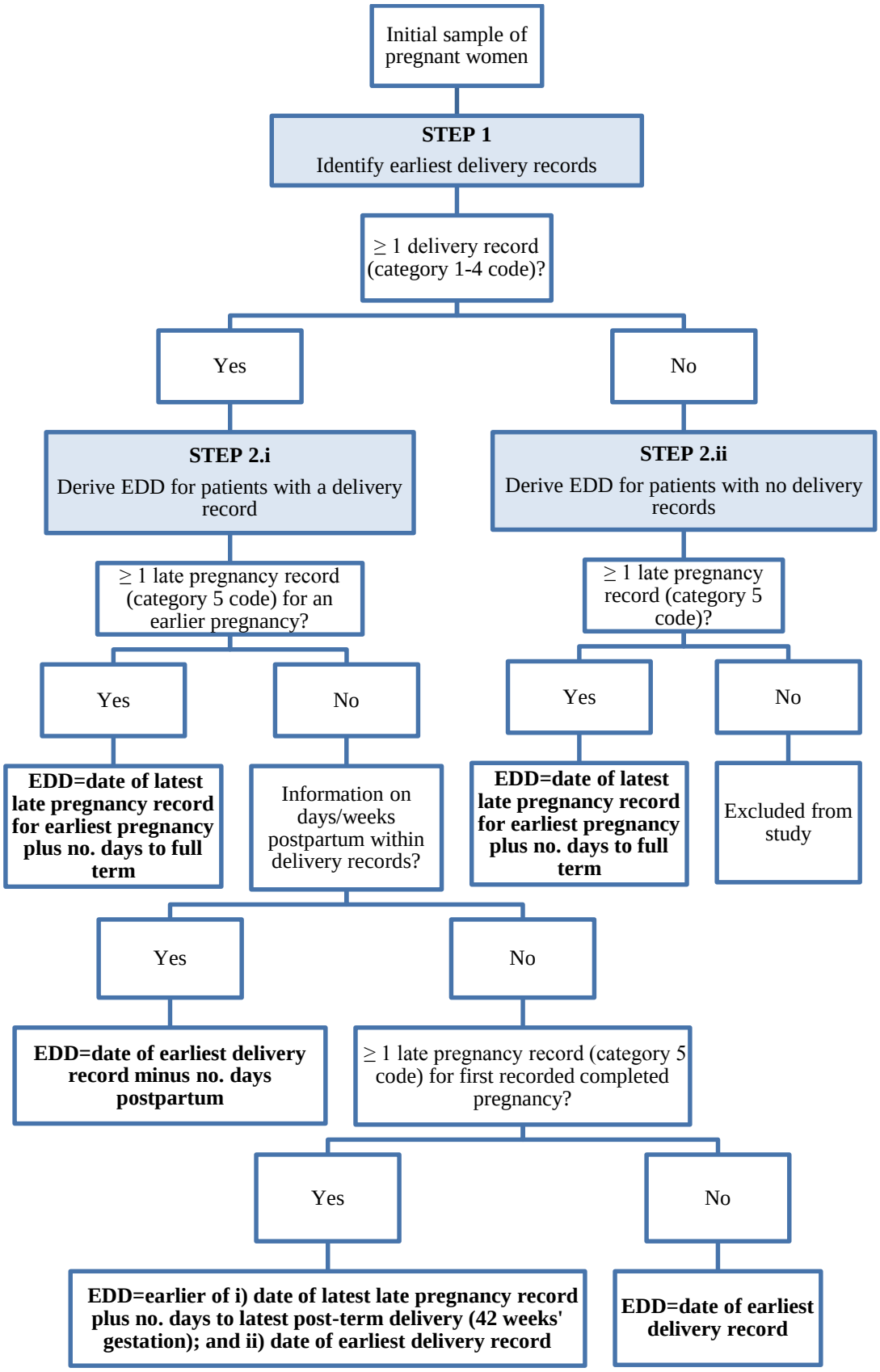


Figure 7.1 Deriving the estimated date of delivery (EDD) of participants' first recorded completed pregnancies.

Using all available information on **a, b,** and **c** and the following hierarchy of rules (summarised in Figure 7.1), a single best estimate of the date of delivery corresponding to a patient's first recorded completed pregnancy was derived:

- For patients with **a** (a late pregnancy record corresponding to an *earlier* pregnancy), the date of the *latest* **a** record corresponding to the *earliest* pregnancy was used to estimate the date of delivery, by adding on the appropriate number of weeks to full term (at 40 weeks' gestation), e.g. for a record of "Antenatal 36 week exam", four weeks were added to arrive at the EDD.
- For patients with **b** (information on days/weeks postpartum), and not **a**, the date of the earliest delivery record was adjusted by counting back the specified number of days, e.g. for a record of "Postnatal tenth day visit", ten days were subtracted to arrive at the EDD.
- For patients with **c** only (a late pregnancy record corresponding to the *same* pregnancy as the earliest delivery record), the date of the *latest* **c** record was identified and adjusted by adding on the appropriate number of weeks to post-term at 42 weeks (allowing for a latest possible delivery at 42 weeks), e.g. for a record of "Antenatal 41 week exam", one week was added. The earlier of this adjusted date and the date of the earliest delivery record was then selected as the EDD. Figure 7.3 illustrates this process using two hypothetical completed pregnancies, **A** (post-term) and **B** (preterm).
- For patients with no **a, b or c** records, the date of the earliest delivery record was selected as the EDD.

Step 2.ii. Estimating the date of delivery for patients without a delivery code

Clinical, referral and maternity files of patients **with no delivery records** identified in Step 1 were searched for late pregnancy records (category 5 codes). As for Step 2.i, successive records within six weeks of each other were assumed to correspond to the same pregnancy. The date of the *latest* late pregnancy record corresponding to the *earliest* pregnancy was selected. The date of delivery was then estimated by adding on the appropriate number of weeks to full term (e.g. for a record of "Antenatal 39 week exam", one week was added to arrive at the EDD).

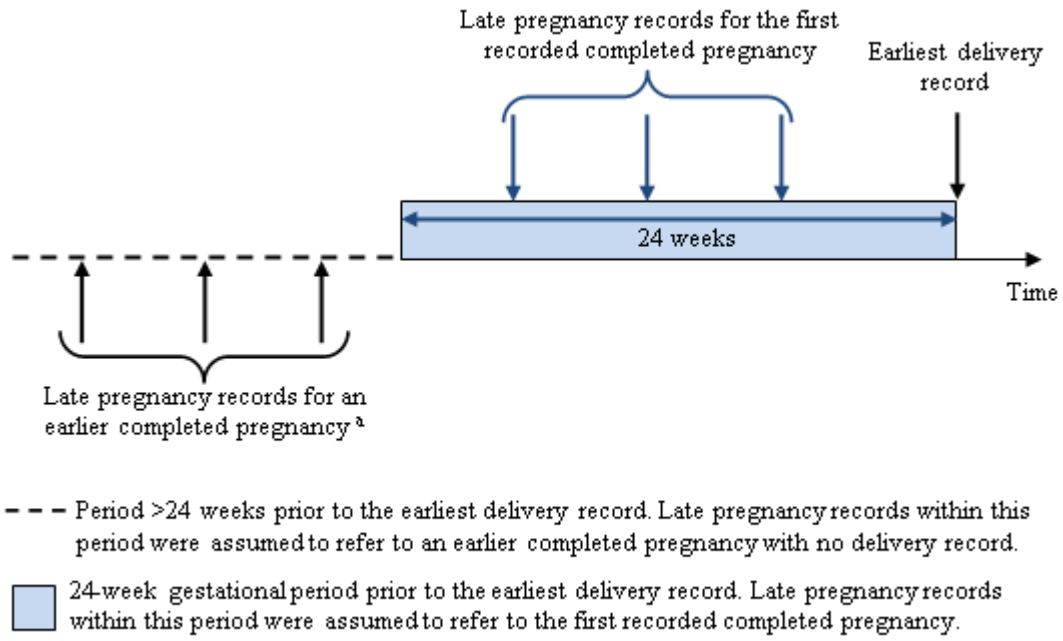


Figure 7.2 Mapping late pregnancy records onto pregnancy episodes.

[a] When an earlier completed pregnancy was identified on the basis of a late pregnancy record, that pregnancy was used in the study (see Section 7.2.1, Step 2.i).

After completing Steps 1-2.ii, all patients with a delivery record or a late pregnancy record were assigned an EDD for their first recorded completed pregnancy. Appendix I-Figures I.1 and I.2 illustrate the participant flow through this process for all potential cases and controls. Patients with no delivery or late pregnancy records, for whom it was not possible to estimate the date of delivery, were not eligible for the study (as described previously in Chapter 6, section 6.4.1).

The frequency distribution of codes used to estimate the date of delivery of study participants' first completed pregnancies is shown in Appendix G-Table G.3.

7.2.2 Estimating the pregnancy start date

Once each participant had been assigned an EDD corresponding to their first recorded completed pregnancy, the following steps were taken to estimate the pregnancy start date (the date of the first day of the woman's LMP before delivery), as illustrated in Figure 7.4.

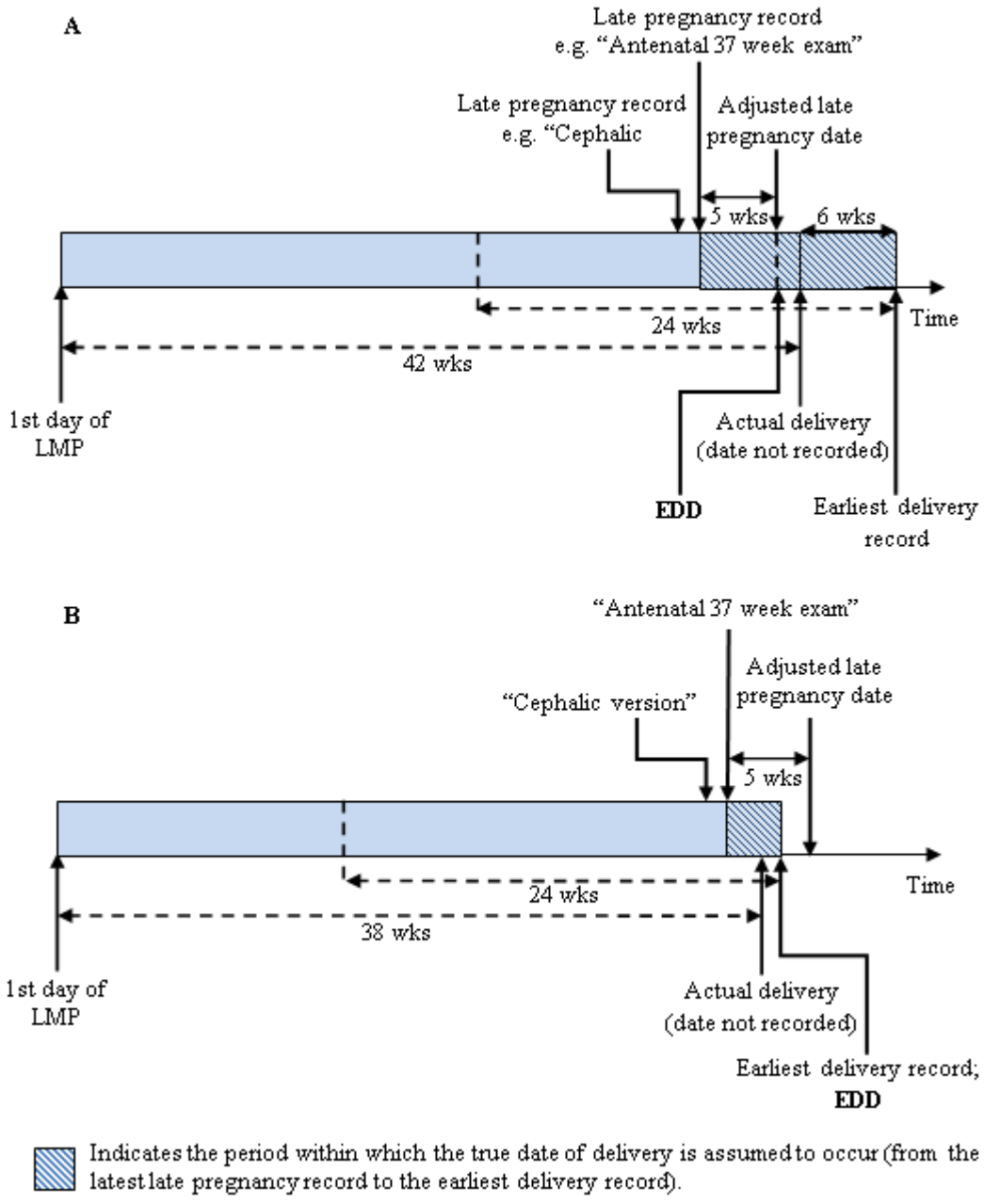


Figure 7.3 Deriving the estimated date of delivery (EDD) for patients with both a delivery record and a late pregnancy record corresponding to their first recorded completed pregnancy.

LMP=last menstrual period

The figure illustrates two hypothetical scenarios: **A,** a *post-term* pregnancy with delivery at 42 weeks; and **B,** a *preterm* pregnancy with delivery at 38 weeks. In both scenarios, the latest late pregnancy record is identified and an adjusted late pregnancy date is calculated by adding on the appropriate number of weeks to the latest possible delivery (at 42 weeks). However, in **A**, the adjusted date is *earlier* than the earliest delivery record, hence the former is selected as the EDD, whereas in **B**, the adjusted date is *later* than the earliest delivery record, hence the latter date is selected as the EDD.

Step 3. Extracting information on the timing of the start of pregnancy

Clinical, referral and maternity records of patients with an EDD were searched for the following:

d. Read/OXMIS antenatal codes with information on gestational age (e.g. "Antenatal ultrasound scan at 22 weeks"), or a recorded estimate of weeks' gestation related to an antenatal booking in the maternity file.
e. Read/OXMIS code for "Estimated date of conception" (EDC) or "Expected date of delivery", calculated manually by GPs or automatically by practice software as two weeks after the first day of the LMP (EDC) and 40 weeks after the first day of the LMP (expected date of delivery);
f. Read/OXMIS delivery codes providing information on gestational age at birth, e.g. "Baby premature 36 weeks".

Estimates of the pregnancy start date (i.e. the date of the first day of the LMP before delivery) were derived from each of these records, as described in Step 4, below. This resulted in multiple LMP estimates for some patients, sometimes relating to more than one pregnancy. LMP estimates corresponding to a patient's *first recorded completed pregnancy* were determined by allowing a minimum gestation of 24 weeks and a maximum gestation of 42 weeks between the LMP date and EDD. All remaining LMP estimates which did not correspond to a patient's first recorded completed pregnancy were excluded.

Step 4. Obtaining a best estimate of the pregnancy start date

Using all available information on **d, e,** and **f** (extracted in Step 3) and the following hierarchy of rules (summarised in Figure 7.4), a single best estimate of the pregnancy start date for each patient's first recorded completed pregnancy was derived:

- For patients with **d** (antenatal records indicating gestational age), the pregnancy start date was estimated by subtracting from the date of the antenatal record the specified number of weeks' gestation, e.g. subtracting 16 weeks from the date corresponding to a record of "Antenatal 16 weeks exam". If a patient had more than one "weeks' gestation" record corresponding to their first recorded completed pregnancy, the record specifying the *longest* weeks' gestation was

used to derive the pregnancy start date. The rationale was that estimates of gestational age from an antenatal examination or scan are likely to be more reliable than a patient's recall of their LMP, and the later the scan, the more reliable the estimate of weeks' gestation.

- For patients with **e** (EDC or "expected date of delivery") and not **d**, the pregnancy start date (based on gestational age) was estimated by subtracting 40 weeks from the expected date of delivery or two weeks from the EDC. If a patient had more than one EDC or "expected date of delivery" record corresponding to their first recorded completed pregnancy, the record yielding the *latest* LMP estimate was used to derive the pregnancy start date. The rationale was that the latest estimate was least likely to correspond to an earlier pregnancy (ending in miscarriage, for example).
- For patients with **f** only (information on gestational age at delivery), the pregnancy start date was estimated by subtracting from the date of the delivery record the specified number of weeks' gestation. When a delivery code indicated a range of gestational ages, for example, "Baby extremely premature 28-32 weeks" the mid-point (30 weeks) was used. If a patient had more than one record of gestational age at birth corresponding to their first recorded completed pregnancy, the *earliest* record was used to derive the pregnancy start date. The rationale was that the earliest record was most likely to represent the timing of delivery, with later records possibly reflecting some delay in recording.
- For patients with no **d, e or f** records, the pregnancy start date was estimated by subtracting 40 weeks from the EDD.

After completing Steps 3 and 4, each patient was assigned an estimated pregnancy start date corresponding to their first recorded completed pregnancy. Appendix I-Figures I.3 and I.4 illustrate the participant flow through this process for potential cases and controls with an EDD.

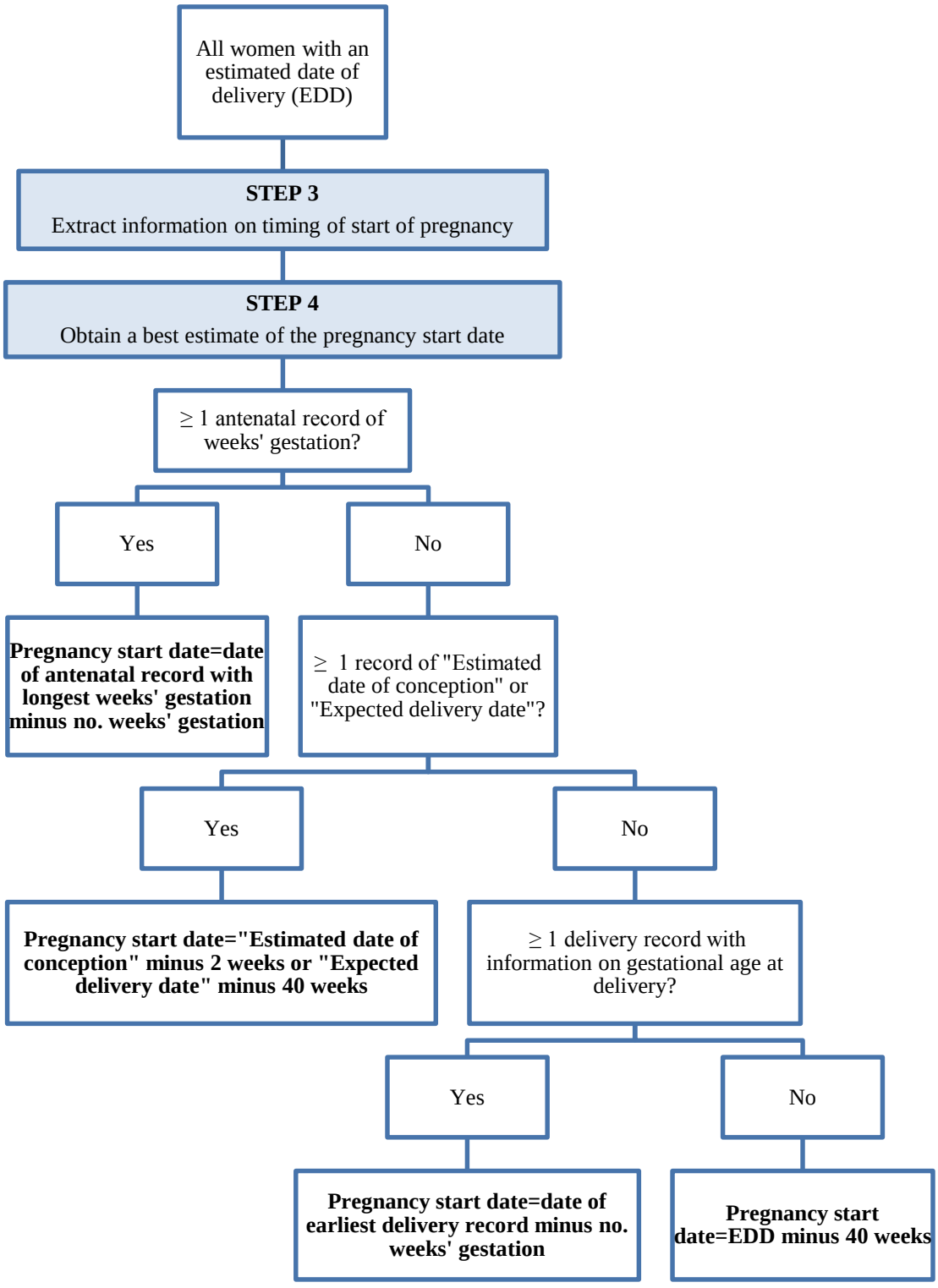


Figure 7.4 Estimating the pregnancy start date of participants' first completed pregnancies.

7.3 Conclusion

This Chapter has described the development of a new approach for dating pregnancies in the GPRD, and its application in the pre-eclampsia study described in Chapter 6. Descriptive data pertaining to the identified pregnancies are presented in the next Chapter among the wider findings of the analysis of acute maternal infection and pre-eclampsia.

Chapter 8 Results – Acute maternal infection and pre-eclampsia

This chapter reports on the findings of the matched case-control study described in Chapter 6, which assessed the effects of three exposures during pregnancy: antibiotic prescriptions (a proxy for acute infection), UTI and RTI, on the risk of pre-eclampsia. The first two sections describe the selection of cases and controls and summarise their demographic, clinical and pregnancy-related characteristics. The next three sections present the findings: the primary analysis, which assessed the effect of each exposure occurring at any time during pregnancy (prior to the index date); secondary analyses which explored the timing and number of episodes of each exposure; and a range of sensitivity analyses (outlined in Chapter 6). The chapter concludes with a summary of the main findings and discussion of the strengths and potential limitations of the study.

8.1 Identifying eligible cases and controls

Participants were drawn from a base population of all female patients registered with practices contributing UTS data to the GPRD during all or part of the study period from 1st January 1987 to 31st October 2007 inclusive.

Data were obtained on all women with a clinical diagnosis of pre-eclampsia during this period (n=3362 potential cases) and a large random sample of women who had a completed pregnancy recorded during this period and no diagnosis of pre-eclampsia ever recorded in their data (n=93909 potential controls).

After applying the eligibility, exclusion and matching criteria, as outlined in Chapter 6 (sections 6.4.2 and 6.5.1), 1533 cases (women who developed pre-eclampsia in their first recorded completed pregnancy) and 14236 controls (women with a first recorded completed pregnancy and no record of pre-eclampsia) were included in the primary analysis. The identification of these women is illustrated in participant flow diagrams Figure 8.1 and Figure 8.2. The most common reason for not being eligible for inclusion in the study was uncertainty about the timing of delivery.

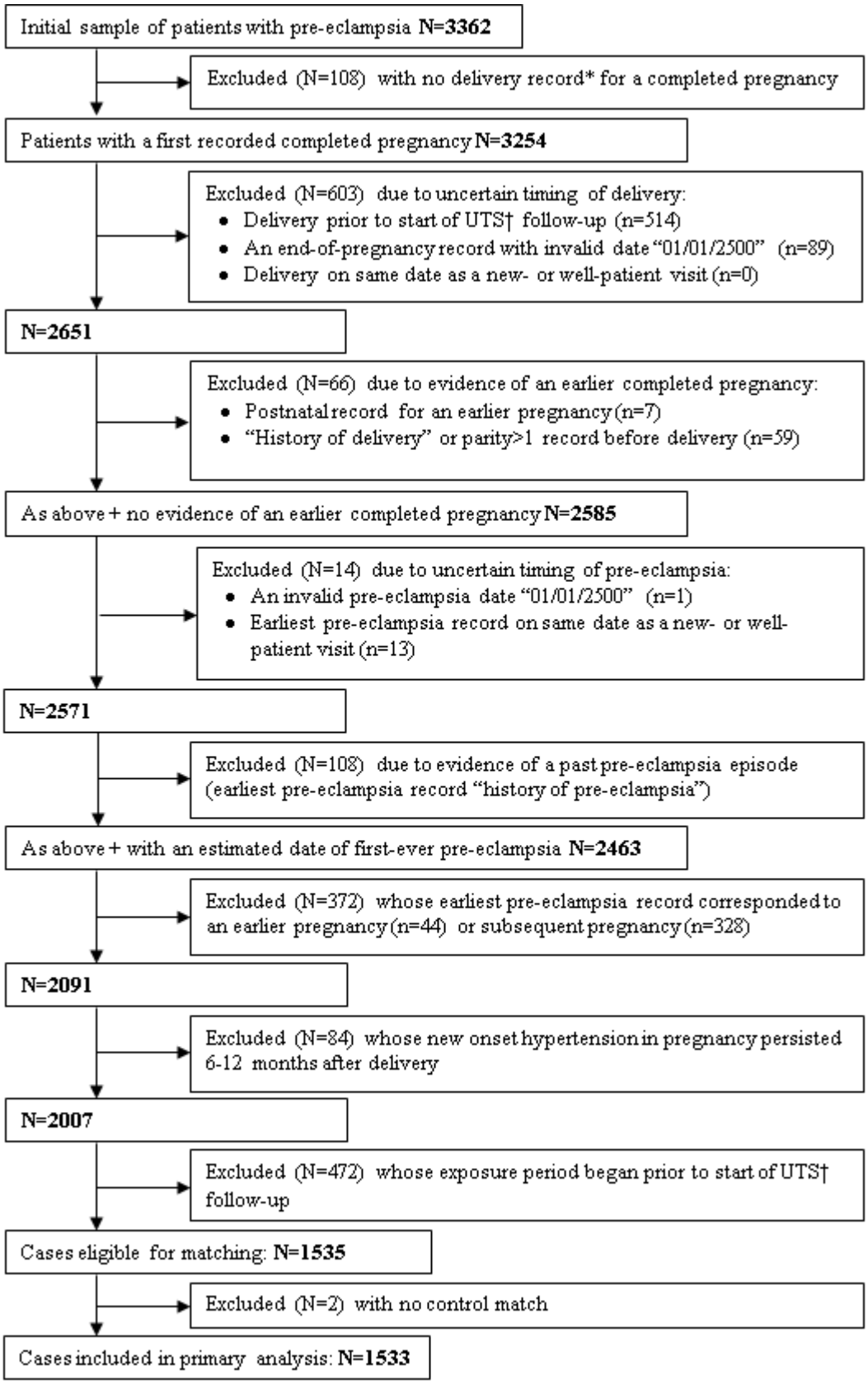


Figure 8.1 Participant flow – Cases.

*a record indicating the patient had delivered (e.g. birth details) or was soon to deliver (e.g. antenatal 37 week examination)
†UTS = up-to-standard (i.e. data meeting GPRD quality standards)

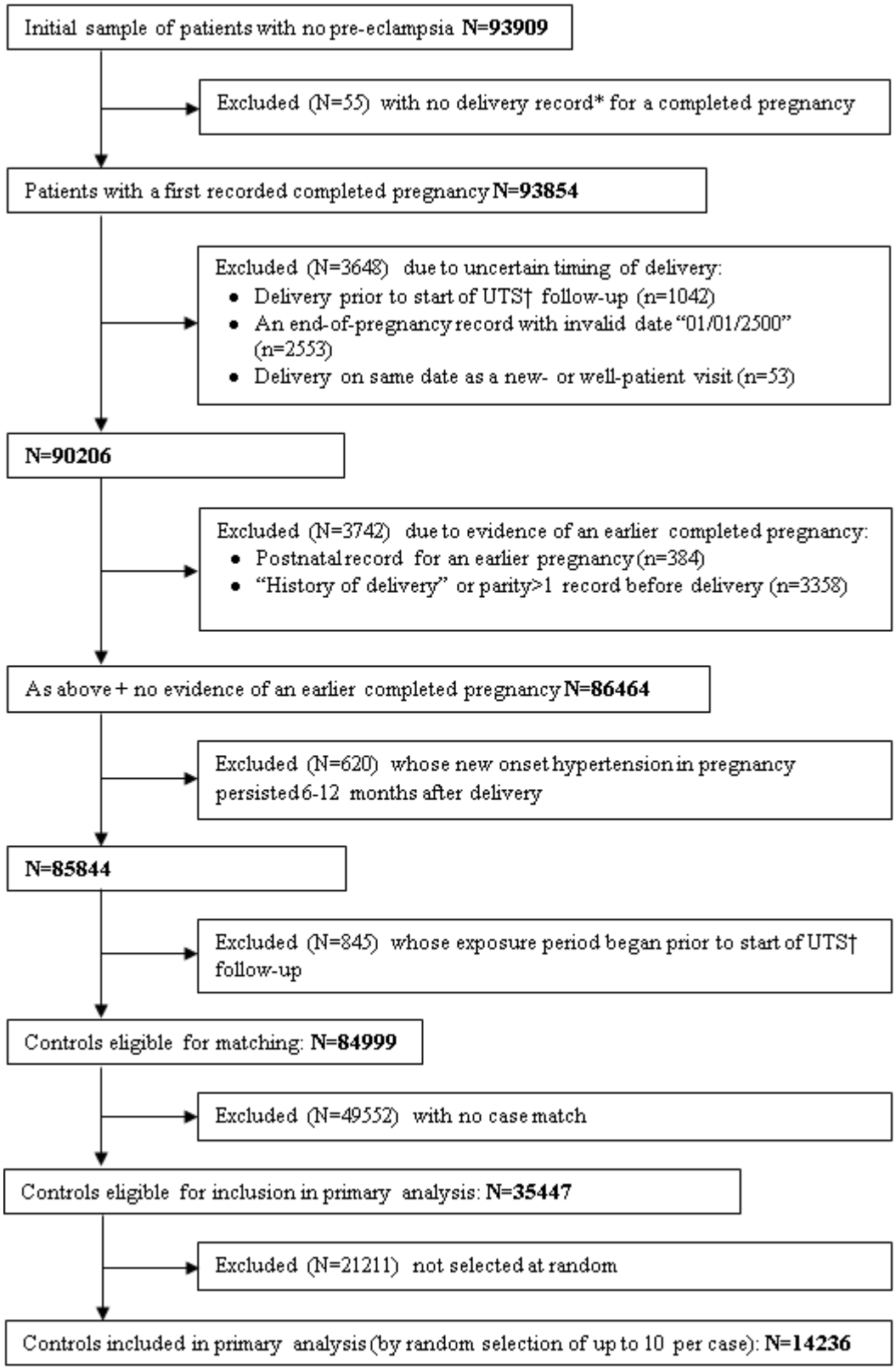


Figure 8.2 Participant flow – Controls.

*a record indicating the patient had delivered (e.g. birth details) or was soon to deliver (e.g. antenatal 37 week examination)
†UTS = up-to-standard (i.e. data meeting GPRD quality standards)

8.2 Descriptive data

8.2.1 Patient and pregnancy characteristics

Using the algorithm developed for dating pregnancies described in Chapter 7, estimates of gestational age at delivery were derived, ranging from 24.1 to 42.0 weeks, starting from the first day of a woman's LMP before delivery. The median gestational age at delivery was 40.0 weeks for both cases and controls (IQR 38.1-40.0 weeks for cases, 40.0-40.0 weeks for controls). More than half of cases and controls had no information in antenatal or delivery records indicating the likely timing of the LMP; for these women, the date of the first day of the LMP was assumed to occur 40 weeks prior to the EDD. However, among the 667 (43.5%) cases and 5278 (37.1%) controls for whom the estimated date of the first day of the LMP was derived from antenatal or delivery records, the median gestational age at delivery was 37.4 weeks (IQR 34.1-39.6) and 40.3 weeks (IQR 39.3-41.1), respectively.

Table 8.1 summarizes the risk profile, demographic and additional pregnancy characteristics of cases and controls. Cases and controls were of similar age at delivery (median 28.3 years for cases; 28.2 years for controls) and shared similar consultation behaviour before pregnancy (median 11 consultations over a median registration period of approximately 2.5 years). Cases were significantly more likely than controls to have a multifetal pregnancy (1.6% versus 0.9%, p=0.003), a history of hypertension (10.5% versus 6.2%, p<0.001) or diabetes (1.8% versus 1.2%, p=0.024). Pre-existing renal disease was uncommon, present in less than 0.3% of participants' records. Approximately one fifth of cases and controls had a record of early pregnancy loss prior to their first recorded completed pregnancy.

Among participants for whom data were available on pre-pregnancy BMI and/or smoking status, cases were significantly more likely to be overweight (OR 1.49, 95% CI 1.27-1.74) or obese (OR 2.16, 95% CI 1.79-2.60) before pregnancy (p trend<0.001), and less likely to smoke during pregnancy (OR 0.66, 95% CI 0.57-0.77), compared to controls.

Participants who were exposed to RTI in pregnancy were more likely to have pre-existing asthma (29.5%) than those not exposed to RTI (17.1%) (p<0.001), and were more likely to smoke (28.6%) than those not exposed (22.5%) (p<0.001).

Table 8.1 Characteristics of study participants.

Characteristic n (%)	Cases (N=1533)	Controls (N=14236)	OR[a] (95% CI)
Maternal age at delivery (years)			
<20	132 (8.6)	1470 (10.3)	1.00
20-24	340 (22.2)	2846 (20.0)	1.34 (1.08-1.66)
25-29	478 (31.2)	4492 (31.6)	1.21 (0.98-1.48)
30-34	406 (26.5)	3803 (26.7)	1.21 (0.98-1.49)
35-39	146 (9.5)	1348 (9.5)	1.23 (0.95-1.58)
40+	31 (2.0)	277 (2.0)	1.25 (0.82-1.91)
median, IQR	*28.3, 23.9-32.3*	*28.2, 23.9-32.1*	
Pre-pregnancy BMI (kg/m^2)			
<18.5 (underweight)	26 (1.7)	526 (3.7)	0.53 (0.36-0.80)
18.5-25 (normal)	620 (40.4)	6618 (46.5)	1.00
25-30 (overweight)	272 (17.7)	1959 (13.8)	1.49 (1.27-1.74)
30+ (obese)	192 (12.5)	946 (6.7)	2.16 (1.79-2.60)
unknown	423 (27.6)	4187 (29.4)	[b]
median, IQR	*24.1, 21.6-27.9*	*22.7, 20.7-25.6*	
Smoking status in pregnancy			
non-smoker	834 (54.4)	6680 (46.9)	1.00
ex-smoker	166 (10.8)	1476 (10.4)	0.90 (0.75-1.08)
current smoker	283 (18.5)	3311 (23.3)	0.66 (0.57-0.77)
unknown	250 (16.3)	2769 (19.5)	[b]
Practice level socioeconomic status[c]			
IMD score *[median, IQR]*	*16.2, 8.7-30.1*	*16.3, 8.4-30.2*	
Patient level socioeconomic status			
IMD score *[median, IQR]*	*14.3, 8.4-25.7*	*14.8, 8.3-26.4*	
unknown	744 (48.5)	6734 (47.3)	
Pre-existing hypertension	161 (10.5)	875 (6.2)	1.82 (1.52-2.18)
Pre-existing renal disease	4 (0.3)	25 (0.2)	1.55 (0.54-4.45)
Pre-existing diabetes	28 (1.8)	166 (1.2)	1.60 (1.06-2.41)
Pre-existing asthma	291 (19.0)	2509 (17.6)	1.10 (0.96-1.26)
Previous miscarriage or termination	298 (19.4)	2869 (20.2)	0.96 (0.84-1.09)
Multiple pregnancy	25 (1.6)	121 (0.9)	1.95 (1.26-3.02)
ART pregnancy	11 (0.7)	84 (0.6)	1.24 (0.65-2.35)
Consultations with GP pre-pregnancy *[median, IQR]*	*11, 4-27*	*11, 4-24*	
UTS follow-up pre-pregnancy (years) *[median, IQR]*	*2.4, 0.9-5.1*	*2.5, 1.1-5.3*	

Abbreviations: IQR=interquartile range; BMI=body mass index; IMD=Index of Multiple Deprivation score based on practice post-code (practice level socioeconomic status) or patient post-code (patient level socioeconomic status): the higher the score, the greater the deprivation; UTS=up-to-standard (i.e. data meeting GPRD quality standards); ART=assisted reproductive technology

[a]ORs adjusted for matched design

[b]individuals with missing data not included in OR estimates

[c]matching variable

The characteristics of matched study participants were similar to those who were eligible for matching; the latter, which included cases and controls with no match and additional controls not selected at random during the matching procedure, are described in Appendix J-Table J.1 for comparison.

8.2.2 Timing and severity of pre-eclampsia

Based on the estimated start and end dates of pregnancy (see Chapter 7 for details of how these were derived), the median gestational age of cases at pre-eclampsia diagnosis was 38.1 weeks (IQR 34.9-39.9 weeks). The majority of cases (79.5%) were late-onset, defined as an earliest pre-eclampsia diagnosis at 34 weeks' gestation or more; just 16 cases (1%) had a pre-eclampsia diagnosis before 20 weeks.

The majority of pre-eclampsia diagnoses were non-specific regarding severity (47.3%) or indicated mild to moderate disease (32.0%); the remaining 20.7% specified severe pre-eclampsia, eclampsia or HELLP syndrome (see Figure 8.3 below).

While more than half of cases (57.7%) had a record of hypertension in pregnancy, fewer (12.9%) had documented proteinuria, and just 166 cases (10.8%) had records of both conditions.

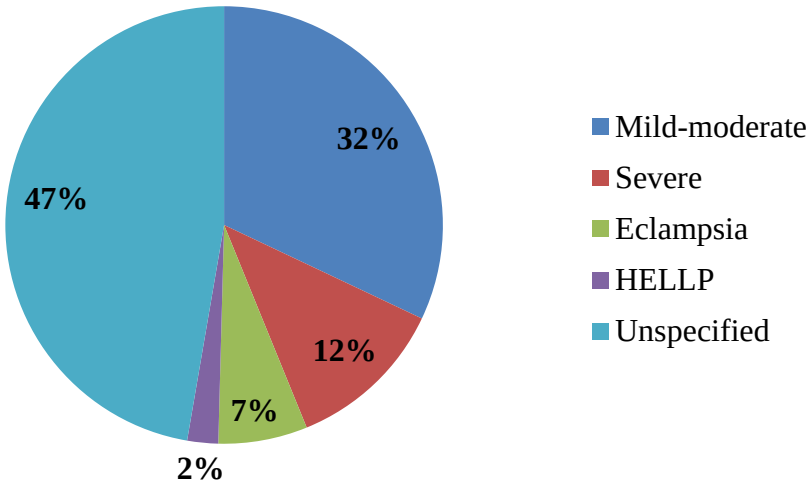


Figure 8.3 Classification of cases (N=1533) by severity or subtype.

8.2.3 Exposure to acute infections in pregnancy

During their first completed pregnancy, 528 (34.4%) cases and 4110 (28.9%) controls were prescribed an antibiotic drug, 182 (11.9%) cases and 1376 (9.7%) controls had one or more recorded UTI, and 77 (5.0%) cases and 781 (5.5%) controls had one or more recorded RTI. In each pregnancy trimester, cases were exposed more frequently than controls to antibiotic prescriptions and to UTI, whereas the frequency of RTI was similar among cases and controls (see Table 8.2).

Table 8.2 Frequency of maternal infections and antibiotic prescriptions.

Exposure during pregnancy[a] n (%)	Cases (N=1533)	Controls (N=14236)
Antibiotic prescription		
First trimester	221 (14.4)	1684 (11.8)
Second trimester	238 (15.5)	1952 (13.7)
Third trimester	203 (13.2)	1520 (10.7)
Any time in pregnancy	*528 (34.4)*	*4110 (28.9)*
Urinary tract infection		
First trimester	64 (4.2)	463 (3.3)
Second trimester	81 (5.3)	606 (4.3)
Third trimester	57 (3.7)	487 (3.4)
Any time in pregnancy	*182 (11.9)*	*1376 (9.7)*
Respiratory tract infection		
First trimester	31 (2.0)	293 (2.1)
Second trimester	29 (1.9)	307 (2.2)
Third trimester	24 (1.6)	218 (1.5)
Any time in pregnancy	*77 (5.0)*	*781 (5.5)*

Note some women had more than one exposure in the same (or in another) trimester.
[a]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the case's index date).

While the majority of exposed cases and controls experienced just a single episode of infection or antibiotic treatment during pregnancy, a few had more than one episode (range 1 to 6 episodes for antibiotics; 1 to 4 episodes for both UTI and RTI). The frequency distribution of the number of exposure episodes among cases and controls is shown in Table 8.3. Overall, 706 episodes of antibiotic treatment, 208 UTIs and 85 RTIs during pregnancy were recorded among cases during pregnancy; 5543 antibiotic treatment episodes, 1628 UTIs and 832 RTIs were recorded among controls.

Table 8.3 Frequency distribution of the number of episodes of maternal infection or antibiotic treatment.

Exposure during pregnancy[a] n (%)	Cases (N=1533)	Controls (N=14236)
Antibiotic prescription		
No episodes	1005 (65.6)	10126 (71.1)
1 episode	389 (25.4)	3026 (21.3)
2 episodes	109 (7.1)	804 (5.7)
≥3 episodes	130 (2.0)	280 (2.0)
Urinary tract infection		
No episodes	1351 (88.1)	12860 (90.3)
1 episode	158 (10.3)	1168 (8.2)
2 episodes	23 (1.5)	167 (1.2)
≥3 episodes	1 (0.1)	41 (0.3)
Respiratory tract infection		
No episodes	1456 (95.0)	13455 (94.5)
1 episode	70 (4.6)	734 (5.2)
2 episodes	6 (0.4)	44 (0.3)
≥3 episodes	1 (0.1)	3 (0.0)

Note some women had more than one exposure.

[a]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the case's index date).

The distribution of the timing of exposure episodes over the three trimesters by cases-control status is presented in Table 8.4; as shown, episodes were spread fairly evenly across the trimesters for both cases and controls.

Table 8.4 Frequency distribution of the timing of episodes of maternal infection and antibiotic treatment.

Exposure during pregnancy[a]	No. (%) exposure episodes			
	First trimester	Second trimester	Third trimester	Overall in pregnancy
Antibiotic prescription				
Cases (n exposed=528)	230 (32.6)	257 (36.4)	219 (31.0)	706 (100)
Controls (n exposed=4110)	1812 (32.7)	2102 (37.9)	1629 (29.4)	5543 (100)
Urinary tract infection				
Cases (n exposed=182)	64 (30.8)	85 (40.9)	59 (28.4)	208 (100)
Controls (n exposed=1376)	481 (29.6)	638 (39.2)	509 (31.3)	1628 (100)
Respiratory tract infection				
Cases (n exposed=77)	31 (36.5)	30 (35.3)	24 (28.2)	85 (100)
Controls (n exposed=781)	299 (35.9)	311 (37.4)	222 (26.7)	832 (100)

[a]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the at the case's index date)

Almost 90% of participants with a record of UTI or RTI in pregnancy also had an antibiotic prescription (89.3% of cases, 88.5% of controls), whereas less than half of participants with an antibiotic prescription during pregnancy had a record of UTI or RTI (42.8% of cases, 44.3% of controls).

8.3 Primary analysis

The primary analysis assessed the effects of antibiotic drug prescriptions, UTI and RTI, occurring at any time during pregnancy prior to the index date, on the risk of pre-eclampsia. Crude and adjusted ORs for the association between pre-eclampsia and each of these three primary exposures of interest are summarized in Table 8.5.

8.3.1 Univariable analyses

A crude positive association with pre-eclampsia was observed for both antibiotic prescriptions (crude OR 1.29, 95% CI 1.15-1.44) and UTI in pregnancy (crude OR 1.23, 95% CI 1.04-1.46). However, no such crude effect was observed for RTI (crude OR 0.91, 95% CI 0.71-1.15).

Table 8.5 The association between maternal infection and pre-eclampsia: crude and adjusted odds ratios for matched cases and controls.

Exposure in pregnancy[a]	Cases N=1533 n (%)	Controls N=14236 n (%)	Matched crude OR (95% CI)	Matched adjusted[b] OR (95% CI)
Antibiotic prescription	528 (34.4)	4110 (28.9)	1.29 (1.15-1.44)	1.28 (1.14-1.44)
Urinary tract infection	182 (11.9)	1376 (9.7)	1.23 (1.04-1.46)	1.22 (1.03-1.45)
Respiratory tract infection	77 (5.0)	781 (5.5)	0.91 (0.71-1.15)	0.91 (0.72-1.16)

[a]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the case's index date).
[b]ORs adjusted for maternal age; pre-gestational hypertension, diabetes and renal disease; and multifetal gestation. In addition, ORs for UTI and RTI are mutually adjusted for each other.

8.3.2 Multivariable analyses

Antibiotic prescriptions (adjusted OR 1.28, 95% CI 1.14-1.44) and UTI (adjusted OR 1.22, 95% 1.03-1.45) in pregnancy were associated with an increased risk of pre-eclampsia after controlling for maternal age; pre-gestational conditions - renal disease, diabetes and hypertension; multifetal gestation; and RTI in pregnancy (when assessing UTI only). There was no evidence for confounding by previous early pregnancy loss.

Consistent with the crude analysis, no association was observed between RTI and pre-eclampsia after adjustment for UTI in pregnancy; maternal age; pre-gestational renal disease, diabetes and hypertension; and multifetal gestation (adjusted OR 0.91,

95% CI 0.72-1.16). The inclusion of pre-existing asthma to the model did not alter the RTI effect estimate.

8.3.2.1 Further adjustment for pre-pregnancy BMI and smoking

As described previously in section 8.2.1, data on BMI (a well-established risk factor for pre-eclampsia) and smoking (a putative protective factor), were incomplete. Thus, the primary analysis was repeated among individuals for whom these data were available (1048 cases and 7216 matched controls), allowing for the additional adjustment. Table 8.6 presents the findings from both the crude and adjusted analyses. As shown, further adjustment for pre-pregnancy BMI and maternal smoking made no material difference to the effect estimates for UTI (adjusted OR 1.24, 95% CI 1.01-1.53), RTI (adjusted OR 0.90, 95% CI 0.66-1.23) or antibiotic prescriptions (adjusted OR 1.21, 95% CI 1.05-1.40).

Table 8.6 The association between maternal infection and pre-eclampsia: crude and adjusted odds ratios for matched cases (n=1048) and controls (n=7216) with data on BMI and smoking.

Exposure in pregnancy[a]	Matched crude OR (95% CI)	Matched adjusted[b] OR (95% CI)
Antibiotic prescription	1.23 (1.06-1.42)	1.21 (1.05-1.40)
Urinary tract infection	1.24 (1.01-1.52)	1.24 (1.01-1.53)
Respiratory tract infection	0.92 (0.68-1.25)	0.90 (0.66-1.23)

[a]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the case's index date).
[b]ORs adjusted for maternal age; pre-gestational hypertension, diabetes and renal disease; multifetal gestation, pre-pregnancy BMI and maternal smoking. In addition, ORs for UTI and RTI are mutually adjusted for each other.

8.4 Secondary analyses

Secondary analyses were performed to assess the effects of the timing of infection and the number of infection episodes throughout the gestational period.

Adjusted ORs and 95% CIs for pre-eclampsia associated with infection occurring at different stages of pregnancy are presented in Table 8.7. Antibiotic prescriptions in all three trimesters were associated with a significantly increased risk of pre-eclampsia. A small but significant trend of increased risk of pre-eclampsia was observed with increasing proximity of antibiotic prescription to delivery (p trend<0.001). No effect was observed for RTI at any time during pregnancy. There was no evidence to suggest a difference in the effect of UTI according to the timing

of UTI onset. Thus, the timing of infection did not appear to play an important role in the development of pre-eclampsia.

Table 8.7 The effect of infections at different stages of pregnancy on the risk of pre-eclampsia.

Latest onset of exposure during pregnancy n (%)	Cases (N=1533)	Controls (N=14236)	OR[a] (95% CI)
Antibiotic prescription			
Unexposed	1005	10126	1.00
First trimester	136	1039	1.31 (1.08-1.58)
Second trimester	189	1551	1.23 (1.04-1.45)
Third trimester	203	1520	1.33 (1.13-1.57)
Test for trend	*p<0.001*		*1.10 (1.05-1.16)[b]*
Urinary tract infection			
Unexposed	1351	12860	1.00
First trimester	49	363	1.24 (0.91-1.68)
Second trimester	76	526	1.36 (1.06-1.76)
Third trimester	57	487	1.07 (0.80-1.42)
Test for trend	*p=0.063*		*1.07 (1.00-1.16)[b]*
Respiratory tract infection			
Unexposed	1456	13455	1.00
First trimester	25	270	0.85 (0.56-1.30)
Second trimester	28	293	0.89 (0.60-1.32)
Third trimester	24	218	1.02 (0.66-1.56)
Test for trend	*p=0.644*		*0.97 (0.87-1.09)[b]*

[a]ORs adjusted for maternal age; pre-gestational hypertension, diabetes and renal disease; and multifetal gestation. In addition, ORs for UTI and RTI are mutually adjusted for each other.
[b]OR per unit increase in onset of exposure

Table 8.8 presents adjusted ORs and 95% CIs for pre-eclampsia associated with a single episode of infection, and more than one episode, during pregnancy. While a small but significant trend of increased pre-eclampsia risk with increasing episodes of antibiotic treatment (p trend<0.001) and UTI (p trend=0.044) was detected, the OR point estimates for increasing episodes of antibiotic treatment, UTI or RTI did not suggest a dose-response association with pre-eclampsia.

Table 8.8 The effect of increasing episodes of maternal infection on the risk of pre-eclampsia.

Dose of exposure during pregnancy n (%)	Cases (N=1533)	Controls (N=14236)	OR[a] (95% CI)
Antibiotic prescription			
No episodes	1005	10126	1.00
1 episode	389	3026	1.29 (1.13-1.46)
≥2 episodes	139	1084	1.28 (1.05-1.55)
Test for trend	*p<0.001*		*1.18 (1.08-1.28)[b]*
Urinary tract infection			
No episodes	1351	12860	1.00
1 episode	158	1168	1.25 (1.04-1.50)
≥2 episodes	24	208	1.08 (0.70-1.66)
Test for trend	*p=0.044*		*1.15 (1.00-1.33)[b]*
Respiratory tract infection			
No episodes	1456	13455	1.00
1 episode	70	734	0.89 (0.69-1.14)
≥2 episodes	7	47	1.29 (0.58-2.89)
Test for trend	*p=0.609*		*0.94 (0.76-1.18)[b]*

[a]ORs adjusted for maternal age; pre-gestational hypertension, diabetes and renal disease; and multifetal gestation. In addition, ORs for UTI and RTI are mutually adjusted for each other.
[b]OR per unit increase in dose of exposure

8.5 Sensitivity analyses

A number of sensitivity analyses were performed as outlined in Chapter 6, section 6.8.4. The results of these analyses are described below and summarised in Table 8.9 and Table 8.10.

To address the possibility that the detection of proteinuria among cases may have been misdiagnosed by the GP as a UTI (hence leading to differential misclassification of UTI), the primary analysis was repeated after confining the exposure window for UTI and antibiotic prescriptions to the first two trimesters only and excluding cases (n=41) with very early onset pre-eclampsia prior to the third trimester (and their matched controls). The findings were virtually identical to the primary analysis: adjusted OR for antibiotics 1.26, 95% CI 1.11-1.43 and for UTI 1.22, 1.01-1.49.

Consistent with the primary analysis, an increased risk was observed for both early-onset (n=315) and late-onset pre-eclampsia (n=1218) associated with maternal antibiotics prescriptions and UTI, with no evidence for a clear difference between these two subgroups (Table 8.9). Restricting the analysis to documented severe cases of pre-eclampsia, eclampsia or HELLP syndrome (n=317) yielded findings

consistent with the primary analysis: adjusted OR for antibiotics 1.29, 95% CI 1.00-1.67, UTI 1.66, 1.16-2.39 and RTI 1.24, 0.76-2.02.

Table 8.9 Adjusted odds ratios for early-onset (<34 weeks' gestation) and late-onset (≥34 weeks' gestation) pre-eclampsia.

	Matched adjusted[b] OR (95% CI)	
Exposure in pregnancy[a]	**Early-onset pre-eclampsia (n=315 cases)**	**Late-onset pre-eclampsia (n=1218 cases)**
Antibiotic prescription	1.54 (1.19-2.00)	1.22 (1.07-1.39)
Urinary tract infection	1.39 (0.94-2.06)	1.19 (0.99-1.44)
Respiratory tract infection	1.46 (0.88-2.42)	0.81 (0.61-1.07)

[a]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the case's index date).
[b]ORs adjusted for maternal age; pre-gestational hypertension, diabetes, renal disease; and multifetal gestation. In addition, ORs for UTI and RTI are mutually adjusted for each other.

Additional sensitivity analyses were conducted, as outlined in Chapter 6, excluding the following participants/pregnancies (excluded matched sets, n):

- cases and controls with less than six months UTS follow-up prior to the start of pregnancy (n=236);
- pregnancies which began prior to publication of the first recommended consensus definition of pre-eclampsia in 2000 (n=887);
- cases and controls aged <18 years at delivery (n=44);
- cases and controls with pre-existing hypertension (n=162);
- controls with new onset hypertension in pregnancy which resolved 6-12 months after delivery (n=0). The 292 controls who met this exclusion criterion did not result in a loss of matched sets as all cases still had at least one remaining matched control;
- ART pregnancies (n=11).

Each of these analyses yielded estimates similar to those obtained in the primary analysis (see Table 8.10).

Table 8.10 Results of sensitivity analyses by exclusion criteria.

		Matched adjusted[a] OR (95% CI) for pre-eclampsia		
	No. cases included	**Antibiotic prescription[b]**	**UTI[b]**	**RTI[b]**
Primary analysis	*1533*	*1.28 (1.14-1.44)*	*1.22 (1.03-1.45)*	*0.91 (0.72-1.16)*
Sensitivity analyses				
1. Excluding women with less than 6 months UTS data prior to conception	1297	1.27 (1.12-1.44)	1.25 (1.04-1.50)	0.88 (0.67-1.16)
2. Excluding pregnancies before year 2000	646	1.38 (1.16-1.66)	1.26 (0.97-1.65)	1.12 (0.76-1.64)
3. Excluding women aged <18 years	1489	1.28 (1.13-1.44)	1.20 (1.01-1.42)	0.90 (0.70-1.16)
4. Excluding women with pre-existing hypertension	1371	1.34 (1.18-1.51)	1.31 (1.09-1.57)	0.96 (0.75-1.23)
5. Excluding controls with new onset hypertension in pregnancy which resolved 6-12 months after delivery	1533	1.29 (1.15-1.44)	1.24 (1.04-1.46)	0.91 (0.72-1.17)
6. Excluding ART pregnancies	1522	1.28 (1.14-1.44)	1.21 (1.02-1.44)	0.91 (0.71-1.16)

Abbreviations: UTS=up-to-standard (i.e. data meeting GPRD quality standards). ART=assisted reproductive technology

[a]ORs adjusted for maternal age; pre-gestational hypertension (except for analysis 4), diabetes and renal disease; and multifetal gestation. In addition, ORs for UTI and RTI are mutually adjusted for each other.

[b]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the case's index date).

8.6 Discussion

8.6.1 Summary of main findings

The findings of this study suggest that women who acquire UTI during pregnancy, and women prescribed antibiotics during pregnancy (a likely proxy for acute infection) are at a higher risk of pre-eclampsia. The increased risk of pre-eclampsia developing in the third trimester following UTI or antibiotic prescriptions in the first two trimesters (before the likely detection of proteinuria among these third trimester cases) supports the notion that acute infections in pregnancy may play a role in the pathogenesis of pre-eclampsia. However, this study found no evidence for an increased risk of pre-eclampsia among women who acquire RTI during pregnancy.

The absence of an association with RTI is intriguing and warrants further investigation, although it does not preclude the possibility of a generic effect of acute infection on pre-eclampsia risk. The adjusted analyses suggest this finding is unlikely to be explained by the higher prevalence of maternal smoking (known to be associated with protection against pre-eclampsia) among women with RTI. While the finding may reflect the absence of a true RTI effect, it may also at least partly be due to incomplete ascertainment of RTI consultations. The definition of RTI used in this study excluded all non-specific RTI diagnoses (e.g. “Acute respiratory infection” or “Respiratory tract infection”) as it was unclear whether these were minor upper RTIs or more severe lower RTIs. While the exclusion of non-specific diagnoses increased the likelihood that the infections captured in the study were the more severe RTIs sufficient to produce systemic effects, the ascertainment of severe RTIs may have been incomplete, hence some individuals classified as unexposed to RTI may in fact have had a severe episode. It is likely that any such non-differential misclassification of RTI would lead to an underestimate of effect due to cases and controls being more homogeneous on exposure.

Neither the timing nor the number of episodes of UTI, RTI or antibiotic prescriptions during pregnancy appeared to play an important role in the development of pre-eclampsia.

8.6.2 Study strengths

A major strength of the study is the use of a population-based cohort of women from which all cases of pre-eclampsia in a first completed pregnancy and a random sample of primiparous controls without pre-eclampsia were selected. The nested case-control design avoided the common problem of selection bias inherent in many case-control studies, particularly those in which the base population giving rise to the cases is less clearly defined. Matching on practice allowed for variability in recording and prescribing habits between practices, and helped ensure that cases and controls were comparable on a range of socio-economic and environmental indicators. The additional criterion of allowing no more than 12 months between case and control delivery dates ensured pregnancies within matched sets were contemporaneous.

Another strength of this study is that data were available on a substantial number of well-known risk factors for pre-eclampsia, some of which, most notably renal disease and diabetes, were not accounted for in previous studies of UTI and pre-eclampsia[102,127,131]. The associations with UTI and antibiotic prescriptions persisted even after adjustment for maternal age; pre-existing renal disease, diabetes and hypertension; and multifetal gestation. The possibility of residual confounding cannot be excluded, if disease risk factors were not recorded for some women; for example, the low prevalence of pre-existing renal disease among cases (0.3%) and controls (0.2%) suggests ascertainment of renal disease may be limited to the more severe end of the disease spectrum. However, this is unlikely to have been a major concern since it is the more severe disease (stages 3-5) which predisposes to pre-eclampsia, rather than mild renal disease.[160] Missing information on maternal smoking and pre-pregnancy BMI limited the ability of this study to assess the effects of these risk factors in the complete study population. Nevertheless, additional adjustment for BMI and smoking made no material difference to the findings. The similar pre-pregnancy consultation behaviour of cases and controls suggests the findings are unlikely to be explained by possible increased ascertainment of infection among cases due to differential health-seeking behaviour.

The study population comprised women with a first documented completed pregnancy in their primary care record, defined as a medical code indicating a live birth, stillbirth, or that the woman was soon to deliver (e.g. “antenatal 37 week

examination"). Pregnancies resulting in early pregnancy loss (miscarriage or termination) were not included, as these were not considered to be completed pregnancies. A completed pregnancy record was an eligibility criterion in the study for two reasons: first, it ensured that all women had the opportunity to develop pre-eclampsia (typically occurring after 20 weeks' gestation), which enabled the selection of an appropriate control group; second, it allowed the timing of each woman's pregnancy to be estimated, this being essential for defining the exposure period for acute infection and for matching cases and controls on gestational age at the index date. Selecting women at a less well-defined stage of pregnancy, for example, on the basis of a miscarriage record, which could occur at any time prior to 24 weeks' gestation and most often in the first trimester, would have been problematic: most of these women were unlikely to have reached the required gestational age to be at risk of developing pre-eclampsia. The further criterion that the completed pregnancy must be the first in the primary care record, coupled with the exclusion of women with evidence of an earlier (unrecorded) completed pregnancy, helped ensure that the large majority of included pregnancies were primiparous, thus reducing the scope for confounding by parity. A further advantage of this approach is that it reduced the potential for confounding by change in paternity or by inter-pregnancy interval among multiparous women.[161]

While there is no universal agreement on the definition of pre-eclampsia,[98] a diagnosis has major consequences for a pregnant woman and is unlikely to be recorded speculatively. In 2000, the National High Blood Pressure Education Program Working Group developed diagnostic criteria for pre-eclampsia,[162] recommended in the American College of Obstetricians and Gynecologists practice guidelines for diagnosing pre-eclampsia[153]. To improve the validity of the case definition used in this study, women whose new onset hypertension in pregnancy did not resolve following delivery were excluded from the primary analysis, in keeping with this consensus definition. While the possibility of misclassification of pre-eclampsia cannot be ruled out, this criterion helped to distinguish cases of pre-eclampsia from women with essential or secondary hypertension which became clinically apparent during pregnancy. Furthermore, the sensitivity analysis which restricted to pregnancies (and hence pre-eclampsia diagnoses) in the year 2000 onwards made no material difference to the findings.

8.6.3 Potential limitations of the study

In the absence of systematically recorded information on the precise timing of pregnancy in the GPRD, information from antenatal, perinatal and postnatal records was used to estimate the date of delivery, the start of pregnancy, and the timing of trimesters for all primiparous pregnancies. Any imprecision in the pregnancy dates may have resulted in some misclassification of exposure; for example, if the estimated LMP date was late, some infections or antibiotic prescriptions occurring early in pregnancy might have been missed, whereas if the estimated LMP date was early, some pre-pregnancy infections/antibiotics may have been incorrectly assigned to the first trimester. Although the timing may have been inexact, the same methods were used for dating case and control pregnancies, so any such imprecision is likely to have been non-differential. The date of diagnosis of infection or antibiotic prescription was used rather than the date of onset of infection (the latter being unknown). However, the majority of patients, even with upper RTIs, attend their GP within three days of onset.[163] This small degree of imprecision in the timing of onset of infection is unlikely to have materially affected the results.

It was recognized that not all infections would lead to a GP consultation, so some episodes may not have been recorded. However, such infections are more likely to be minor or asymptomatic; those severe enough to induce systemic inflammation are more likely to result in a consultation and be detected. It is possible that the observed associations with maternal UTI and antibiotic prescriptions may partly be attributed to increased ascertainment of infections among women considered to be at high-risk for pre-eclampsia. However, the study only included primiparous women and hence may have been less prone to this particular source of bias since those at highest risk (i.e. women with a history of pre-eclampsia) were not included. Furthermore, the study also investigated the effect of acute RTI; the null effect observed for RTI suggests that such ascertainment bias is unlikely.

Possible misclassification of UTI among women with pre-eclampsia due to detection of protein in the urine was also considered to be a potential source of bias. This was addressed in a sensitivity analysis restricted to cases with pre-eclampsia in the third trimester (and their matched controls), and infections occurring in the first two trimesters (likely to precede the onset of proteinuria). The resulting effect estimates

for both UTI and antibiotic prescriptions were virtually identical to those obtained in the primary analysis, suggesting that any such bias was minimal and further strengthening the study findings.

More than half of women in the study with an antibiotic prescription in pregnancy had no urinary or respiratory indication, a finding which has previously been noted in primary care data.[164] While some antibiotics might have been prescribed prophylactically against recurrent infections, this is likely to have been a small minority: the majority would have been given for acute infections such as UTI, which is particularly common in pregnancy[132]. Nevertheless, it is possible (albeit unlikely) that the finding of an antibiotic effect may reflect an association with the drugs themselves rather than an association with acute infection, the main indication for their use.

Finally, the relatively few individuals with more than one episode of infection (or antibiotic prescriptions) during pregnancy limited the power of the study to reliably examine a dose-effect.

8.6.4 Conclusion

This study has shown that acute maternal UTI and antibiotic drug prescriptions in pregnancy (a likely proxy for infection), though not RTI in pregnancy, are associated with an increased risk of pre-eclampsia. While the underlying mechanism of this association could not be ascertained in this study, the findings support the notion that acute infections during pregnancy may contribute to the development of pre-eclampsia. A discussion of how the study findings compare with those from previous studies of pre-eclampsia and acute maternal infection is presented in the next chapter (Chapter 9, section 9.2).

Chapter 9 Discussion

This project has sought to establish a clearer role for acute inflammation and infection in vascular disease, by undertaking two large observational studies using EHRs. The first, a SCSS study reported in Chapters 3-4 (see also Appendix A) used Medicaid data to examine the risk of vascular events following invasive dental treatment.[31] The second, a matched case-control study (Chapters 6-8 and Appendix B) used GPRD data to investigate acute maternal infection as a possible trigger for pre-eclampsia.[32] This chapter summarises the key findings of these studies in the context of what previous studies of these associations have shown, considers possible mechanisms for the effects observed, and highlights some of the strengths and limitations of using EHRs to address these research questions. Finally, some areas for future research and implications for clinical practice are recommended.

9.1 Invasive dental treatment and vascular events

9.1.1 What was already known

A link between periodontal disease and CVD is well-established and treating periodontal disease is widely thought to bring long-term vascular benefits by reducing the burden of infection.[30] However, intervention studies of intensive periodontal therapy, including a randomized controlled trial comparing the intensive treatment with standard community-based care, have demonstrated that intensive periodontal therapy gives rise to an acute inflammatory response followed by transiently impaired flow-mediated dilatation and elevated markers of inflammation and endothelial activation in the week after therapy.[47–49] This suggests that invasive dental treatment may trigger a short-term increase in risk of vascular events, although prior to the dental study, no studies of any such acute effect arising from dental treatment had been reported.

9.1.2 What the dental study adds

The SCCS dental study (described in Chapters 3-4) has shown a 1.5-fold increased rate of vascular events associated with invasive dental treatment in the preceding four weeks, which gradually resolved over the subsequent 20 weeks. Similar effects

were observed for ischaemic stroke and MI separately, though with poorer precision (due to the fewer cases in these subsets) and not reaching statistical significance. The case-only approach used in this study made within-person comparisons, thus no comparison group was needed. This markedly reduced the scope for confounding to which a more conventional cohort design would have been susceptible, particularly when addressing a question of this nature as baseline characteristics related to vascular risk are likely to differ substantially between treated and untreated individuals. Furthermore, all analyses were age-adjusted using 5-year (and subsequently 2-year) age groups, hence the effects seen are very unlikely to be explained by increasing age.

The mechanisms through which invasive dental treatment may influence vascular event risk in the short term could not be reliably ascertained in this study (a limitation of the data source, see section 9.3.2.3 below). As discussed previously in Chapter 4, it is possible that non-inflammatory mechanisms might explain at least part of the effect observed; for example, acute stress, discontinuation of antiplatelet drugs or use of NSAIDs coinciding with the invasive dental treatment may all potentially trigger a vascular event. Nevertheless, the transient increased risk of vascular events observed in the first few weeks after the treatment is consistent with the short-lived inflammatory response observed after periodontal therapy, and confirms previous findings of a similar effect associated with other acute inflammatory exposures.[16,17,21] The dental study findings are thus compatible with an acute inflammatory response and associated short-term change in endothelial function after dental treatment mediating this increase in vascular risk.

Three recent cohort studies[57–59] (described in Chapter 2, section 2.3.1) have since been published which assessed the effect of invasive dental treatment on vascular risk up to several years after treatment. These studies reported a reduction in risk of vascular events associated with invasive dental treatment[57,58] or no treatment effect[59] over the longer-term; however, they did not investigate any *acute* effect of dental treatment on vascular risk.

9.2 Pre-eclampsia and acute maternal infection

9.2.1 What was already known

Previous research on acute infections during pregnancy and pre-eclampsia has yielded mixed findings. UTI, the most frequently studied acute maternal infection, has been shown to be associated with a 1.3- to 4.8-fold increased odds of pre-eclampsia in some studies,[114,115,117,119–126,130,131] and no association in others[112,113,116,118,127–129]. Two cohort studies of hospitalised pneumonia during pregnancy have reported a significant positive association with pre-eclampsia/eclampsia although no association with mild pre-eclampsia.[137,138] However, studies of acute Chlamydia pneumoniae infection and cytomegalovirus infection (as determined by the presence of IgM antibodies) found no association with pre-eclampsia.[106,110,133–135,139] The effects of other acute infections on the risk of pre-eclampsia are unknown.

9.2.2 What the pre-eclampsia study adds

The case-control study of pre-eclampsia (described in Chapters 6-8) has demonstrated a greater than 1.2-fold increased odds of pre-eclampsia associated with both maternal antibiotic prescriptions and UTI in pregnancy, independent of maternal age, multifetal gestation and pre-gestational conditions (hypertension, renal disease and diabetes). None of the earlier studies referred to above assessed the role of antibiotic prescriptions as a proxy for acute infection. Contrary to the two previous studies which found an increased risk of pre-eclampsia associated with maternal pneumonia, no association was observed between pre-eclampsia and acute RTI during pregnancy in this study. One explanation for this finding is that the definition of RTI used in the pre-eclampsia study encompassed a wider range of infections than pneumonia, some of which would have been less severe. The null effect of RTI is nevertheless consistent with previous studies showing no association with acute Chlamydia pneumoniae infection, a common cause of both upper and lower RTI[165].

As discussed previously in Chapter 8, the pre-eclampsia study has a number of advantages over the earlier studies, including:

- more complete adjustment for confounding (particularly renal disease and diabetes, which were not accounted for in most earlier studies of pre-eclampsia and UTI);
- the ability to establish clearly the temporal sequence between infection and pre-eclampsia onset (due to the data being prospectively recorded, see section 9.3.1.2, below);
- the assessment of more than one infection (RTI in addition to UTI); since both of these infections were susceptible to similar ascertainment bias (i.e. possible increased ascertainment of infections among cases), the finding of a positive association with UTI but no effect of RTI suggests that such ascertainment bias was unlikely.

Although it was not possible to establish the mechanism by which acute infections such as UTI may be associated with pre-eclampsia in this study (again, a limitation of the data source, discussed below in 9.3.2.3), various hypotheses have been proposed which implicate inflammation (a key feature of pre-eclampsia) as a mediating factor. For example, it has been suggested that acute infections may play a direct role in initiating pre-eclampsia by increasing the risk of acute uteroplacental atherosis[166] which may result in increased systemic inflammation and endothelial dysfunction preceding the clinical onset of disease. Acute infections may also contribute to the progression of pre-eclampsia by triggering the release of inflammatory cytokines into maternal circulation, hence amplifying the already increased level of inflammation in the pregnant women and altering vascular endothelial function.[167] Thus, there may be more than one mechanism through which acute infections, among other factors, may increase the risk of pre-eclampsia developing.

9.3 Strengths and limitations of electronic health data

EHRs are increasingly being used in epidemiological research, particularly in observational studies. Two of the most commonly used sources of EHRs for research are primary care and administrative claims databases, such as the GPRD and the Medicaid database used for this project. A clear advantage of using EHRs for research is that the data are pre-collected and readily available, and hence provide

unparalleled gains in efficiency over studies which involve more costly and time-consuming traditional methods of participant recruitment and data collection. However, the quality of EHR-based research crucially depends on the validity and completeness of the data. A discussion of the methodological strengths and limitations of the dental and pre-eclampsia studies has been reported in the results chapters of the thesis (Chapters 4 and 8). This section considers more specifically the strengths and potential limitations of the databases used to address the study questions.

9.3.1 Strengths

The Medicaid database and the GPRD have previously been described in Chapter 3 (section 3.2.1) and Chapter 6 (section 6.2.1), respectively. The databases share a number of key strengths as outlined below:

9.3.1.1 Large sample size

One of the main strengths of the databases for use in research is their size which allows for the study of rare outcomes and/or exposures with reduced concerns about loss of statistical power. The Medicaid database used for the dental study comprised data on more than nine million individuals. The ability to include such a large number of cases (more than 20,000) arising from this base population was of particular importance in the dental study given the relatively low frequency of exposure to invasive dental treatment. The pre-eclampsia study was able to include data on more than 1500 cases and 14000 controls, and was thus one of the largest studies to date assessing the role of acute infection in pre-eclampsia. Despite these large sample sizes, the dental study had limited power to examine stroke and MI separately, and the pre-eclampsia study was limited in its ability to reliably assess a dose-effect with increasing episodes of infection.

9.3.1.2 Prospectively collected data

A further common advantage of the GPRD and Medicaid databases is that data are collected routinely and prospectively. Medicaid captures all medical care provided to beneficiaries including dental care, which allowed the occurrence of invasive dental procedures to be linked with hospital diagnoses of vascular events and hence the

question of whether such procedures increase vascular risk could be addressed. The GRPD comprises a comprehensive record of patients' medical profiles, including details pertaining to pregnancy and its outcome, and thus presented a suitable data source for addressing the question of whether infections acquired during pregnancy pose an increased risk of pre-eclampsia. Because data are prospectively recorded, data on exposure to invasive dental procedures (Medicaid) and to infections and antibiotic prescriptions (GPRD) were not subject to recall bias (when the presence of disease influences the reporting of exposure) or observer bias (when knowledge of participants' disease status influences the ascertainment or recording of exposure).

9.3.1.3 Generalisability

A key strength of the GPRD is that it is broadly representative of the UK population.[143] The ability to select all cases of pre-eclampsia and a random sample of controls from the same well-defined population minimized the scope for selection bias in the pre-eclampsia study and ensured the results were generalisable. By contrast to the GPRD, the population served by Medicaid cannot be considered broadly representative of the overall US population given that eligibility is income-related and hence the majority of beneficiaries come from lower socioeconomic groups.[61] Nevertheless, the relative effect of invasive dental treatment on vascular event risk is unlikely to be different in low-income patients as compared with higher-income individuals.

9.3.1.4 Routine data quality checks

Both the GPRD and the Medicaid database are subject to ongoing internal quality assessments to ensure the research data are high-quality. In the GPRD, assessment is undertaken at the patient level (excluding patients with inconsistent or incomplete data in key areas including age, sex and registration details) and at practice level (ensuring, for example, that recording of patient referrals or prescriptions issued reach specified thresholds). The practice UTS date, based on the latter, indicates when the practice met quality standards and hence the point from which the data were deemed fit for research. In Medicaid, improper coding is flagged to recommend actions for improving data quality to the carrier or data processor.

9.3.2 Potential limitations

In addition to the strengths described above, some potential limitations of the databases need also be considered.

9.3.2.1 Validity of diagnoses

One of the most important concerns with using EHRs for epidemiological research is the validity of the data, particularly regarding clinical diagnoses. While EHRs provide a number of advantages for research as discussed above, it is important to consider the accuracy of clinical entries in the EHR for the study of a particular disease, given that the primary reason for data collection is for clinical use rather than for research. A large number of validation studies have assessed the validity of recording in the GPRD across a range of diagnoses and reassuringly, estimates of validity have consistently been high.[142,168] Similarly, the validity of hospital discharge diagnoses in Medicaid has been extensively examined with studies estimating positive predictive value of more than 90% for stroke and MI.[75,76]

9.3.2.2 Timing of events

For some events, the precise timing can be difficult to discern from the EHR. In Medicaid, the date of admission to hospital for a vascular event (as indicated by the primary discharge diagnosis) was used rather than the date of onset, the latter being unknown. However, for acute severe events such as ischaemic stroke and MI which often result in immediate hospitalisation, the admission date is likely to be a reasonable proxy. It is also possible that a few individuals may have experienced their event during their hospital stay (hence after the admission date) although this is unlikely as the primary discharge diagnosis (principal diagnosis) should reflect the main reason for the admission. Any small imprecision in the timing of the events is unlikely to have caused notable bias in the dental study.

As described in Chapter 7, a major challenge for the pre-eclampsia study was the need to develop an algorithm to estimate the likely start and end dates of participants' pregnancies in order to identify the relevant "risk" period for exposure to acute infection. The algorithm has not been validated, hence it was not possible to establish the degree of imprecision in the pregnancy dates. The main consequence of

any such imprecision for this study is possible misclassification of exposure early in pregnancy, although this is likely to be non-differential (as discussed in Chapter 8, section 8.6.3). Systematic recording of both the LMP and delivery dates in the GPRD would have reduced the scope for exposure misclassification and hence would be of great benefit to future studies of pregnancy in the GPRD.

9.3.2.3 Missing or incomplete data

Clinical outcomes

The Medicaid database comprises details of all health care provided to beneficiaries. Data are based on paid and adjudicated administrative claims for reimbursement of medical services and include information on diagnosed conditions and services performed. Hence the data should represent a virtually complete record of care received, though not of care needed (and not received) or undiagnosed conditions, neither of which were a particular concern for the dental study.

Although practices contributing to the GPRD are required to document all significant clinical events and diagnoses including referrals to specialists and hospital admissions, the recording of outcomes may be incomplete. However, any such under-ascertainment is likely to apply to the more minor medical events rather than significant events such as pre-eclampsia or infections severe enough to cause systemic effects (see Chapter 8, section 8.6.3). Hence this issue was not especially concerning in the pre-eclampsia study.

Lifestyle and anthropometrics

Both databases include some demographic data, such as year of birth, gender and (for Medicaid only) ethnicity. However, data on lifestyle and anthropometric factors (SES, smoking, alcohol use, body weight and height), which are often important confounding factors in many studies, are not readily available in Medicaid and may be incomplete or unavailable for some patients in the GPRD. The absence of such data was less of an issue for the dental study due to the case series design which implicitly controlled for between-person differences by making within-person comparisons. In the pre-eclampsia study, missing information on maternal smoking and pre-pregnancy BMI, two well-established risk factors for pre-eclampsia,

precluded the assessment of their effects in the complete study population. Nevertheless, their effects were assessed among the majority of women (who had available BMI and smoking data).

Prescription drugs

Prescriptions issued in primary care are computer-generated by the GP and so are automatically recorded in the GPRD. The therapy files therefore contain a complete record of prescriptions issued by the GP. However, prescriptions issued in secondary care are not accounted for in the GPRD. While this raises the question of possible incomplete ascertainment of antibiotic prescriptions in the pre-eclampsia study if some women were prescribed antibiotics in hospital antenatal clinics, it is likely that the majority of women would be referred to their GP for prescriptions due to cost. Furthermore, any such under-ascertainment of antibiotics is likely to have been more common among cases since women with high-risk pregnancies are more likely to be seen in hospital, hence resulting in an underestimation of the antibiotic effect.

In Medicaid, drug data relate to prescriptions which have been dispensed by the pharmacist rather than issued by the physician. Nevertheless, uncertainty remains as to whether the medicine was actually taken. Hence, it was not possible to ascertain patient compliance in either the GPRD or Medicaid. Any such under-ascertainment of drug use due to lack of compliance may have implications for studies examining drug effects. However, this was not a concern in the pre-eclampsia study given that antibiotic prescriptions (an exposure of interest) served as a proxy for acute infection independent of whether the drugs themselves were actually taken by the patient.

Finally, neither database capture non-prescription (over-the-counter) medicines. This was not an issue for the pre-eclampsia study as antibiotics are only available with prescription in the UK. Although drug effects were not assessed directly in the dental study, the possibility of residual confounding due to incomplete ascertainment of non-prescription aspirin or other NSAIDs was recognised (as discussed earlier in Chapter 4, section 4.6.3).

Data on underlying mechanisms

While the Medicaid database provided sufficient information to examine whether an association between invasive dental procedures and vascular events exists, it did not allow for an investigation of the implied mechanism. A potential pathogenic pathway underlying the effect observed may be the raised levels of CRP and other markers of systemic inflammation and endothelial dysfunction exhibited by patients who undergo invasive dental treatment. However such data on inflammatory biomarkers and endothelial function were lacking in Medicaid.

Similarly, the mechanisms by which UTI (and possibly other acute infections) might increase the risk of pre-eclampsia could not be reliably ascertained in the pre-eclampsia study. As discussed in Chapter 6, pre-eclampsia is a heterogeneous syndrome, and the mechanisms underlying the pathology may vary according to the specific clinical phenotype.[169] However, data on pre-eclampsia phenotypes were lacking in the GPRD (for example pre-eclampsia in association with a small-for-gestational-age infant or placental observations indicating atherosis) which limited the ability of the study to reliably assess whether acute infection may be implicated in different phenotypes. Nevertheless, development of an algorithm to date pregnancies in the pre-eclampsia study (described in Chapter 7) enabled the gestational age of cases at pre-eclampsia diagnosis to be estimated. Hence stratified analyses were undertaken among early-onset cases (thought to be placental-mediated and associated with placental insufficiency and fetal growth restriction) versus late-onset cases (linked to maternal factors such as BMI and thought to be associated with less severe outcomes)[170] to assess the role of acute infection in each of these subtypes. These analyses indicated no evidence for a difference in effect between the two groups (see Chapter 8, section 8.5).

9.4 Further areas for research

The studies presented in this thesis have shown that the risk of vascular outcomes may be increased in the short term following acute inflammatory exposures. However, further research is required to elucidate the underlying mechanisms of these associations.

The dental study assessed the effect of a broad range of invasive dental treatments, the majority of which were extractions. This work could be extended in further studies with larger sample sizes to examine the effects of different types of invasive dental treatment (for example periodontal therapy versus extractions with non-infectious causes such as trauma) on the short-term risk of vascular events. In addition, the effects of other medical procedures (outside the realm of dental treatment) that are likely to be associated with short-term inflammation could be investigated. Such studies may give further insight into specific situations and time periods of increased vascular risk and possibly identify particular groups of individuals who may benefit most from preventative interventions during short-term high-risk periods.

An important, related area for future research is the identification of interventions that may be effective in reducing vascular risk over the short term after inflammatory procedures. Among the possible candidate drugs for prophylactic use during transient periods of increased vascular risk are statins and antiplatelet agents, both widely used to reduce vascular risk over the longer term among high-risk individuals. However, to date there is limited evidence supporting the usefulness of such short-term prophylactic therapy. Studies assessing the effect of statin use during the perioperative period have yielded inconclusive findings.[171] A recent randomised placebo controlled trial of patients undergoing noncardiac surgery found no evidence for a protective effect of either aspirin or clonidine administered perioperatively.[172,173] Thus further studies are required to assess the benefit of administering such treatments during short-term periods of increased risk after invasive dental treatment and other inflammatory exposures.

The pre-eclampsia study carried out for this thesis investigated the effect of antibiotic prescriptions in pregnancy as a proxy for acute infection on pre-eclampsia risk. A different though related question which warrants addressing in future studies is whether treating acute maternal infections such as UTI with antibiotics may reduce the risk of pre-eclampsia developing. While such a study may feasibly be undertaken using EHRs, it may be prone to confounding by indication whereby differences in the underlying risk profile of pregnant women who do and do not receive antibiotic treatment for infection may be related to the risk of pre-eclampsia. Hence measures

to deal with this, for example using a propensity score approach, would need consideration. Furthermore, the uncertainty of compliance may complicate the interpretation of the findings of such a study. An additional issue is that the majority of symptomatic maternal UTIs are likely to be treated. A randomised controlled trial to evaluate the effect of screening for and treating bacteriuria among pregnant women would overcome these issues and provide the strongest evidence for an effect of screening and antibiotic administration on pre-eclampsia risk.

It has been suggested that particular clinical phenotypes of pre-eclampsia may differ in their underlying pathology.[169] Hence, an additional area which warrants investigating is whether acute infections such as UTI may be implicated in different pre-eclampsia phenotypes. The timing of onset and other clinical features pertaining to a particular pre-eclampsia phenotype could be established in a hospital setting. However, a very large multi-centre study would be required to accrue enough women with each phenotype in order to reliably assess the effect of infection on a specific phenotype. Future research in this area may further our understanding of the pathogeneses of this complex maternal syndrome and possibly identify groups of women at particularly high risk for whom preventative measures may be of particular benefit.

9.5 Implications for clinical practice

The recognition that individuals might have a transiently increased risk of vascular outcomes after acute inflammatory exposures such as invasive dental treatment and maternal UTI could have important implications for clinical practice.

The dental study findings presented in this thesis raise important questions relating to the management of patients undergoing invasive dental treatment or other inflammatory procedures, particularly of those individuals at high risk. For example, it is uncertain whether such procedures warrant routine pre-treatment cardiovascular risk evaluation, or whether patients should delay or even avoid such procedures at times of increased vascular risk. Confirmation of these findings in future studies would be useful for such decisions in clinical practice.

Furthermore, as discussed in section 9.4 above, the value of prophylactic interventions such as therapy with statins or antiplatelet medicines in transient periods of increased vascular risk is uncertain. Thus more research is needed before targeting specific therapies to individuals not already on such treatment regimens during short-term high-risk periods. However, the recommendation for patients to continue taking antiplatelet drugs or other cardioprotective medicines before and after invasive dental treatment when possible (among high-risk individuals already receiving these preventative medicines) seems reasonable on the basis of the dental study findings. The use of prophylactic antibiotics may also be prudent among high-risk individuals who undergo planned invasive dental treatment, in particular those with underlying periodontal disease or other dental infection (hence already at increased levels of inflammation).

Our incomplete understanding of the pathophysiology of pre-eclampsia has been a barrier to efforts to prevent and treat the syndrome. With the exception of a modest but significant protective effect of low-dose aspirin administered early in pregnancy in women at high risk of pre-eclampsia,[174] preventative interventions to date have been largely unsuccessful. If the association observed in the pre-eclampsia study between pre-eclampsia and acute UTI is in fact causal, this could have important implications for antenatal care programs, including the screening for and prompt treatment of asymptomatic bacteriuria (in addition to symptomatic UTI) in early pregnancy, which could have the potential to reduce the risk of pre-eclampsia. There may also be a role for prophylactic administration of low-dose antibiotics among women deemed at especially high risk of developing pre-eclampsia. However, such a strategy is less likely to be viable due to the potential threat of antibiotic drug resistance to public health. Crucially, the benefit of any such preventative interventions would require confirmation in a randomised trial setting and would need to outweigh any potential associated risks.

9.6 Overall conclusions

This thesis has reported on two large EHR-based observational studies which, using different study designs and data sources, have shown that vascular risk may be elevated in the short term following acute inflammatory exposures. The results of both studies are consistent with previous research on the effect of acute infection/inflammation on vascular outcomes, with the exception of the null effect observed of acute RTI on pre-eclampsia. The SCCS dental study has identified invasive dental treatment as a novel potential acute inflammatory trigger for vascular events. While the exact mechanisms for the associations observed in these studies remain uncertain, the findings lend support to the increasing body of evidence that acute inflammation may confer a transient increase in vascular risk.

Bibliography

1. Luepker R V. Cardiovascular disease: rise, fall, and future prospects. Annu Rev Public Health 2011;32:1–3.

2. Gersh BJ, Sliwa K, Mayosi BM, Yusuf S. Novel therapeutic concepts: the epidemic of cardiovascular disease in the developing world: global implications. Eur Heart J 2010;31:642–8.

3. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095–128.

4. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006;3:e442.

5. Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol 2009;54:2129–38.

6. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352:1685–95.

7. Packard RRS, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem 2008;54:24–38.

8. Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101:1899–906.

9. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350:1387–97.

10. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132–40.

11. Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E. Periodontal infections contribute to elevated systemic C-reactive protein level. J Periodontol 2001;72:1221–7.

12. Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S. Relationship between periodontal disease and C-reactive protein among adults in the Atherosclerosis Risk in Communities study. Arch Intern Med 2003;163:1172–9.

13. Janket S-J, Baird AE, Chuang S-K, Jones JA. Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:559–69.

14. Khader YS, Albashaireh ZSM, Alomari MA. Periodontal diseases and the risk of coronary heart and cerebrovascular diseases: a meta-analysis. J Periodontol 2004;75:1046–53.

15. Zhu J, Nieto FJ, Horne BD, Anderson JL, Muhlestein JB, Epstein SE. Prospective study of pathogen burden and risk of myocardial infarction or death. Circulation 2001;103:45–51.

16. Warren-Gash C, Smeeth L, Hayward AC. Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. Lancet Infect Dis 2009;9:601–10.

17. Emsley HC, Hopkins SJ. Acute ischaemic stroke and infection: recent and emerging concepts. Lancet Neurol 2008;7:341–53.

18. Meier CR, Jick SS, Derby LE, Vasilakis C, Jick H. Acute respiratory-tract infections and risk of first-time acute myocardial infarction. Lancet 1998;351:1467–71.

19. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. N Engl J Med 2004;351:2611–8.

20. Clayton TC, Thompson M, Meade TW. Recent respiratory infection and risk of cardiovascular disease: case-control study through a general practice database. Eur Heart J 2008;29:96–103.

21. Warren-Gash C, Hayward AC, Hemingway H, Denaxas S, Thomas SL, Timmis AD, et al. Influenza infection and risk of acute myocardial infarction in England and Wales: a CALIBER self-controlled case series study. J Infect Dis 2012;206:1652–9.

22. Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, et al. Acute systemic inflammation impairs endothelium-dependent dilatation in humans. Circulation 2000;102:994–9.

23. Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. Circulation 2011;123:2856–69.

24. Chesley LC. Recognition of the long-term sequelae of eclampsia. Am J Obstet Gynecol 2000;182:249–50.

25. Ray JG, Vermeulen MJ, Schull MJ, Redelmeier DA. Cardiovascular health after maternal placental syndromes (CHAMPS): population-based retrospective cohort study. Lancet 2005;366:1797–803.

26. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. Hypertension 2009;53:944–51.

27. Bellamy L, Casas J-P, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ 2007;335:974.

28. Staff AC, Johnsen GM, Dechend R, Redman CWG. Preeclampsia and uteroplacental acute atherosis: immune and inflammatory factors. J Reprod Immunol 2014;101-102:120–6.

29. Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. Lancet 2003;361:1511–7.

30. D'Aiuto F, Orlandi M, Gunsolley JC. Evidence that periodontal treatment improves biomarkers and CVD outcomes. J Clin Periodontol 2013;40 Suppl 1:S85–105.

31. Minassian C, D'Aiuto F, Hingorani AD, Smeeth L. Invasive dental treatment and risk for vascular events: a self-controlled case series. Ann Intern Med 2010;153:499–506.

32. Minassian C, Thomas SL, Williams DJ, Campbell O, Smeeth L. Acute maternal infection and risk of pre-eclampsia: a population-based case-control study. PLoS One 2013;8:e73047.

33. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van Der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol 2000;71:1528–34.

34. Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase Inflammatory Response to Periodontal Disease in the US Population. J Dent Res 2000;79:49–57.

35. Amar S, Gokce N, Morgan S, Loukideli M, Van Dyke TE, Vita JA. Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation. Arterioscler Thromb Vasc Biol 2003;23:1245–9.

36. Higashi Y, Goto C, Jitsuiki D, Umemura T, Nishioka K, Hidaka T, et al. Periodontal infection is associated with endothelial dysfunction in healthy subjects and hypertensive patients. Hypertension 2008;51:446–53.

37. Higashi Y, Goto C, Hidaka T, Soga J, Nakamura S, Fujii Y, et al. Oral infection-inflammatory pathway, periodontitis, is a risk factor for endothelial dysfunction in patients with coronary artery disease. Atherosclerosis 2009;206:604–10.

38. Mattila KJ, Nieminen MS, Valtonen V V, Rasi VP, Kesäniemi YA, Syrjälä SL, et al. Association between dental health and acute myocardial infarction. BMJ 1989;298:779–81.

39. Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for atherosclerosis, cardiovascular disease, and stroke. A systematic review. Ann Periodontol 2003;8:38–53.

40. Dietrich T, Jimenez M, Krall Kaye EA, Vokonas PS, Garcia RI. Age-dependent associations between chronic periodontitis/edentulism and risk of coronary heart disease. Circulation 2008;117:1668–74.

41. Persson GR, Persson RE. Cardiovascular disease and periodontitis: an update on the associations and risk. J Clin Periodontol 2008;35:362–79.

42. Bahekar AA, Singh S, Saha S, Molnar J, Arora R. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. Am Heart J 2007;154:830–7.

43. Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis. J Gen Intern Med 2008;23:2079–86.

44. De Oliveira C, Watt R, Hamer M. Toothbrushing, inflammation, and risk of cardiovascular disease: results from Scottish Health Survey. BMJ 2010;340:c2451.

45. D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J 2006;151:977–84.

46. Tonetti MS. Periodontitis and risk for atherosclerosis: an update on intervention trials. J Clin Periodontol 2009;36 Suppl 1:15–9.

47. D'Aiuto F, Parkar M, Tonetti MS. Periodontal therapy: a novel acute inflammatory model. Inflamm Res 2005;54:412–4.

48. D'Aiuto F, Parkar M, Tonetti MS. Acute effects of periodontal therapy on bio-markers of vascular health. J Clin Periodontol 2007;34:124–9.

49. Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, et al. Treatment of periodontitis and endothelial function. N Engl J Med 2007;356:911–20.

50. Chapman PJ, Penkeyman HW. Successful defibrillation of a dental patient in cardiac arrest. Aust Dent J 2002;47:176–7.

51. Shobha N, Bhatia R, Barber PA. Dental procedures and stroke: a case of vertebral artery dissection. J Can Dent Assoc 2010;76:a82.

52. Lopez R, Flavell S, Thomas C. A not very NICE case of endocarditis. BMJ Case Rep 2013;2013.

53. Niwa H, Sato Y, Matsuura H. Safety of dental treatment in patients with previously diagnosed acute myocardial infarction or unstable angina pectoris. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;89:35–41.

54. Scannapieco FA, Dasanayake AP, Chhun N. Does periodontal therapy reduce the risk for systemic diseases? Dent Clin North Am 2010;54:163–81.

55. Beck JD, Couper DJ, Falkner KL, Graham SP, Grossi SG, Gunsolley JC, et al. The Periodontitis and Vascular Events (PAVE) pilot study: adverse events. J Periodontol 2008;79:90–6.

56. Håheim LL, Olsen I, Rønningen KS. Association between tooth extraction due to infection and myocardial infarction. Community Dent Oral Epidemiol 2011;39:393–7.

57. Chen Z-Y, Chiang C-H, Huang C-C, Chung C-M, Chan W-L, Huang P-H, et al. The association of tooth scaling and decreased cardiovascular disease: a nationwide population-based study. Am J Med 2012;125:568–75.

58. Lee Y-L, Hu H-Y, Huang N, Hwang D-K, Chou P, Chu D. Dental prophylaxis and periodontal treatment are protective factors to ischemic stroke. Stroke 2013;44:1026–30.

59. Skaar D, O'Connor H, Lunos S, Luepker R, Michalowicz BS. Dental procedures and risk of experiencing a second vascular event in a Medicare population. J Am Dent Assoc 2012;143:1190–8.

60. Centers for Medicare and Medicaid Services. 2010 Actuarial report: on the financial outlook for Medicaid. Washington, DC: United States Department of Health and Human Services.; 2010.

61. Hennessy S, Carson J, Ray W, Strom BL. Medicaid databases. In: Strom B, editor. Pharmacoepidemiology. Chichester, UK: John Wiley & Sons, Ltd; 2007.

62. Current Dental Terminology 2007-2008. Chicago: American Dental Association; 2007.

63. Whitaker HJ, Farrington CP, Spiessens B, Musonda P. Tutorial in biostatistics: the self-controlled case series method. Stat Med 2006;25:1768–97.

64. Farrington CP. Relative incidence estimation from case series for vaccine safety evaluation. Biometrics 1995;51:228–35.

65. Farrington CP, Pugh S, Colville A, Flower A, Nash J, Morgan-Capner P, et al. A new method for active surveillance of adverse events from diphtheria/tetanus/pertussis and measles/mumps/rubella vaccines. Lancet 1995;345:567–9.

66. Smeeth L, Cook C, Thomas S, Hall AJ, Hubbard R, Vallance P. Risk of deep vein thrombosis and pulmonary embolism after acute infection in a community setting. Lancet 2006;367:1075–9.

67. Hocine M, Guillemot D, Tubert-Bitter P, Moreau T. Testing independence between two Poisson-generated multinomial variables in case-series and cohort studies. Stat Med 2005;24:4035–44.

68. Hubbard R, Farrington P, Smith C, Smeeth L, Tattersfield A. Exposure to tricyclic and selective serotonin reuptake inhibitor antidepressants and the risk of hip fracture. Am J Epidemiol 2003;158:77–84.

69. Douglas IJ, Evans SJ, Pocock S, Smeeth L. The risk of fractures associated with thiazolidinediones: a self-controlled case-series study. PLoS Med 2009;6:e1000154.

70. Gibson JE, Hubbard RB, Smith CJP, Tata LJ, Britton JR, Fogarty AW. Use of self-controlled analytical techniques to assess the association between use of prescription medications and the risk of motor vehicle crashes. Am J Epidemiol 2009;169:761–8.

71. Douglas IJ, Langham J, Bhaskaran K, Brauer R, Smeeth L. Orlistat and the risk of acute liver injury: self controlled case series study in UK Clinical Practice Research Datalink. BMJ 2013;346:f1936.

72. Whitaker HJ, Hocine MN, Farrington CP. The methodology of self-controlled case series studies. Stat Methods Med Res 2009;18:7–26.

73. Musonda P, Farrington CP, Whitaker HJ. Sample sizes for self-controlled case series studies. Stat Med 2006;25:2618–31.

74. Tirschwell DL, Longstreth WT. Validating Administrative Data in Stroke Research. Stroke 2002;33:2465–70.

75. Roumie CL, Mitchel E, Gideon PS, Varas-Lorenzo C, Castellsague J, Griffin MR. Validation of ICD-9 codes with a high positive predictive value for incident strokes resulting in hospitalization using Medicaid health data. Pharmacoepidemiol Drug Saf 2008;17:20–6.

76. Choma NN, Griffin MR, Huang RL, Mitchel EF, Kaltenbach LA, Gideon P, et al. An algorithm to identify incident myocardial infarction using Medicaid data. Pharmacoepidemiol Drug Saf 2009;18:1064–71.

77. Austin PC, Daly PA, Tu J V. A multicenter study of the coding accuracy of hospital discharge administrative data for patients admitted to cardiac care units in Ontario. Am Heart J 2002;144:290–6.

78. Levy AR, Tamblyn RM, Fitchett D, McLeod PJ, Hanley JA. Coding accuracy of hospital discharge data for elderly survivors of myocardial infarction. Can J Cardiol 1999;15:1277–82.

79. Petersen LA, Wright S, Normand SL, Daley J. Positive predictive value of the diagnosis of acute myocardial infarction in an administrative database. J Gen Intern Med 1999;14:555–8.

80. Kiyota Y, Schneeweiss S, Glynn RJ, Cannuscio CC, Avorn J, Solomon DH. Accuracy of Medicare claims-based diagnosis of acute myocardial infarction: estimating positive predictive value on the basis of review of hospital records. Am Heart J 2004;148:99–104.

81. Olsen I. Update on bacteraemia related to dental procedures. Transfus Apher Sci 2008;39:173–8.

82. Lockhart PB, Brennan MT, Sasser HC, Fox PC, Paster BJ, Bahrani-Mougeot FK. Bacteremia associated with toothbrushing and dental extraction. Circulation 2008;117:3118–25.

83. El-Sharrawy EA, El-Hakim IE, Sameeh E. Attenuation of C-reactive protein increases after exodontia by tramadol and ibuprofen. Anesth Prog 2006;53:78–82.

84. Graziani F, Cei S, Tonetti M, Paolantonio M, Serio R, Sammartino G, et al. Systemic inflammation following non-surgical and surgical periodontal therapy. J Clin Periodontol 2010;37:848–54.

85. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. BMJ 2006;332:1302–8.

86. Farrington CP, Whitaker HJ. Semiparametric analysis of case series data. J R Stat Soc Ser C Appl Stat 2006;55:553–94.

87. Farrington CP, Hocine MN. Within-individual dependence in self-controlled case series models for recurrent events. J R Stat Soc Ser C Appl Stat 2010;59:457–75.

88. Burger W, Chemnitius J-M, Kneissl GD, Rücker G. Low-dose aspirin for secondary cardiovascular prevention - cardiovascular risks after its perioperative withdrawal versus bleeding risks with its continuation - review and meta-analysis. J Intern Med 2005;257:399–414.

89. Brennan MT, Valerin M a., Noll JL, Napenas JJ, Kent ML, Fox PC, et al. Aspirin use and post-operative bleeding from dental extractions. J Dent Res 2008;87:740–4.

90. Brennan MT, Wynn RL, Miller CS. Aspirin and bleeding in dentistry: an update and recommendations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:316–23.

91. Pototski M, Amenábar JM. Dental management of patients receiving anticoagulation or antiplatelet treatment. J Oral Sci 2007;49:253–8.

92. Grines CL, Bonow RO, Casey DE, Gardner TJ, Lockhart PB, Moliterno DJ, et al. Prevention of premature discontinuation of dual antiplatelet therapy in patients with coronary artery stents. Circulation 2007;115:813–8.

93. Steegers EAP, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010;376:631–44.

94. Turner JA. Diagnosis and management of pre-eclampsia: an update. Int J Womens Health 2010;2:327–37.

95. Sibai BM. Diagnosis, controversies, and management of the syndrome of hemolysis, elevated liver enzymes, and low platelet count. Obstet Gynecol 2004;103:981–91.

96. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science 2005;308:1592–4.

97. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365:785–99.

98. Trogstad L, Magnus P, Stoltenberg C. Pre-eclampsia: Risk factors and causal models. Best Pract Res Clin Obstet Gynaecol 2011;25:329–42.

99. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. Circulation 2010;122:478–87.

100. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999;180:499–506.

101. Borzychowski AM, Sargent IL, Redman CWG. Inflammation and pre-eclampsia. Semin fetal neonatal Med 2006;11:309–16.

102. Conde-Agudelo A, Villar J, Lindheimer M. Maternal infection and risk of preeclampsia: systematic review and metaanalysis. Am J Obstet Gynecol 2008;198:7–22.

103. Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S. Periodontal disease and adverse pregnancy outcomes: a systematic review. BJOG 2006;113:135–43.

104. Cota LOM, Guimarães AN, Costa JE, Lorentz TCM, Costa FO. Association between maternal periodontitis and an increased risk of preeclampsia. J Periodontol 2006;77:2063–9.

105. Ruma M, Boggess K, Moss K, Jared H, Murtha A, Beck J, et al. Maternal periodontal disease, systemic inflammation, and risk for preeclampsia. Am J Obstet Gynecol 2008;198:389.e1–5.

106. Heine RP, Ness RB, Roberts JM. Seroprevalence of antibodies to Chlamydia pneumoniae in women with preeclampsia. Obstet Gynecol 2003;101:221–6.

107. Von Dadelszen P, Magee LA, Krajden M, Alasaly K, Popovska V, Devarakonda RM, et al. Levels of antibodies against cytomegalovirus and Chlamydophila pneumoniae are increased in early onset pre-eclampsia. BJOG 2003;110:725–30.

108. Pugliese A, Beltramo T, Todros T, Cardaropoli S, Ponzetto A. Interleukin-18 and gestosis: correlation with Helicobacter pylori seropositivity. Cell Biochem Funct 2008;26:817–9.

109. Aksoy H, Ozkan A, Aktas F, Borekci B. Helicobacter pylori seropositivity and its relationship with serum malondialdehyde and lipid profile in preeclampsia. J Clin Lab Anal 2009;23:219–22.

110. UstUn Y, Engin-UstUn Y, Ozkaplan E, Otlu B, Sait TekerekoGlu M. Association of Helicobacter pylori infection with systemic inflammation in preeclampsia. J Matern neonatal Med 2010;23:311–4.

111. Xie F, Hu Y, Magee LA, Money DM, Patrick DM, Krajden M, et al. An association between cytomegalovirus infection and pre-eclampsia: a case-control study and data synthesis. Acta Obstet Gynecol Scand 2010;89:1162–7.

112. Bryant RE, Windom RE, Vineyard JP, Sanford JP. Asymptomatic bacteriuria in pregnancy and its association with prematurity. J Lab Clin Med 1964;63:224–31.

113. Low JA, Johnston EE, McBride RL, Tuffnell PG. The significance of asymptomatic bacteriuria in the normal obstetric patient. Am J Obstet Gynecol 1964;90:897–906.

114. Kincaid-Smith P, Bullen M. Bacteriuria in pregnancy. Lancet 1965;1:395–9.

115. Stuart KL, Cummins GTM, Chin WA. Bacteriuria, prematurity, and the hypertensive disorders of pregnancy. BMJ 1965;1:554–6.

116. Little P. The incidence of urinary infection in 5000 pregnant women. Lancet 1966;2:925–8.

117. Gilbert GL, Garland SM, Fairley KF, McDowall DM. Bacteriuria due to ureaplasmas and other fastidious organisms during pregnancy: prevalence and significance. Pediatr Infect Dis J 1986;5:S239–243.

118. Qureshi R, Khan K, Darr O, Khattak N, Farooqui B, Rizvi J. Bacteriuria and pregnancy outcome: a prospective hospital-based study in Pakistani women. J Pak Med Assoc 1994;44:12–3.

119. Schieve LA, Handler A, Hershow R, Persky V, Davis F. Urinary tract infection during pregnancy: its association with maternal morbidity and perinatal outcome. Am J Public Health 1994;84:405–10.

120. Hsu CD, Witter FR. Urogenital infection in preeclampsia. Int J Gynecol Obstet 1995;49:271–5.

121. Lee CJ, Hsieh TT, Chiu TH, Chen KC, Lo LM, Hung TH. Risk factors for pre-eclampsia in an Asian population. Int J Gynecol Obstet 2000;70:327–33.

122. Villar J, Carroli G, Wojdyla D, Abalos E, Giordano D, Ba'aqeel H, et al. Preeclampsia, gestational hypertension and intrauterine growth restriction, related or independent conditions? Am J Obstet Gynecol 2006;194:921–31.

123. Savige JA, Gilbert GL, Fairley KF, McDowall DR. Bacteriuria Due to Ureaplasma urealyticum and Gardnerella vaginalis in Women with Preeclampsia. J Infect Dis 1983;148:605.

124. Hill JA, Devoe LD, Bryans CI. Frequency of asymptomatic bacteriuria in preeclampsia. Obstet Gynecololgy 1986;67:529–32.

125. Abi-Said D, Annegers JF, Combs-Cantrell D, Frankowski RF, Willmore LJ. Case-control study of the risk factors for eclampsia. Am J Epidemiol 1995;142:437–41.

126. Mittendorf R, Lain KY, Williams MA, Walker CK. Preeclampsia. A nested, case-control study of risk factors and their interactions. J Reprod Med 1996;41:491–6.

127. Shamsi U, Hatcher J, Shamsi A, Zuberi N, Qadri Z, Saleem S. A multicentre matched case control study of risk factors for preeclampsia in healthy women in Pakistan. BMC Womens Health 2010;10:14.

128. Kashanian M, Baradaran HR, Bahasadri S, Alimohammadi R. Risk factors for pre-eclampsia: a study in Tehran, Iran. Arch Iran Med 2011;14:412–5.

129. Brumfitt W. The effects of bacteriuria in pregnancy on maternal and fetal health. Kidney Int 1975;4(Suppl):S113–S119.

130. Bánhidy F, Ács N, Puhó EH, Czeizel AE. Pregnancy complications and birth outcomes of pregnant women with urinary tract infections and related drug treatments. Scand J Infect Dis 2007;39:390–7.

131. Mazor-Dray E, Levy A, Schlaeffer F, Sheiner E. Maternal urinary tract infection: is it independently associated with adverse pregnancy outcome? J Matern neonatal Med 2009;22:124–8.

132. Schnarr J, Smaill F. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. Eur J Clin Invest 2008;38 Suppl 2:50–7.

133. Goulis DG, Chappell L, Gibbs RGJ, Williams D, Dave JR, Taylor P, et al. Association of raised titres of antibodies to Chlamydia pneumoniae with a history of pre-eclampsia. BJOG 2005;112:299–305.

134. Aral M, Guven MA, Kocturk SA. Chlamydia pneumoniae seropositivity in women with pre-eclampsia. Int J Gynaecol Obstet 2006;92:77–8.

135. Xie F, Hu Y, Magee LA, Money DM, Patrick DM, Brunham RM, et al. Chlamydia pneumoniae infection in preeclampsia. Hypertens Pregnancy 2010;29:468–77.

136. Chrisoulidou A, Goulis DG, Iliadou PK, Dave JR, Bili H, Simms C, et al. Acute and chronic Chlamydia pneumoniae infection in pregnancy complicated with preeclampsia. Hypertens Pregnancy 2011;30:164–8.

137. Romanyuk V, Raichel L, Sergienko R, Sheiner E. Pneumonia during pregnancy: radiological characteristics, predisposing factors and pregnancy outcomes. J Matern Fetal Neonatal Med 2011;24:113–7.

138. Chen Y-H, Keller J, Wang I-T, Lin C-C, Lin H-C. Pneumonia and pregnancy outcomes: a nationwide population-based study. Am J Obstet Gynecol 2012;207:288.e1–288.e7.

139. Strand KM, Odland ML, Iversen AC, Nordbo SA, Vik T, Austgulen R. Cytomegalovirus antibody status at 17-18 weeks of gestation and pre-eclampsia: a case-control study of pregnant women in Norway. BJOG 2012;119:1316–23.

140. Lawrenson R, Williams T, Farmer R. Clinical information for research; the use of general practice databases. J Public Health Med 1999;21:299–304.

141. Medicines and Healthcare products Regulatory Agency. GPRD recording guidelines for vision users. London: Crown Publishing; 2004.

142. Herrett E, Thomas SL, Schoonen WM, Smeeth L, Hall AJ. Validation and validity of diagnoses in the General Practice Research Database: a systematic review. Br J Clin Pharmacol 2010;69:4–14.

143. Walley T, Mantgani A. The UK General Practice Research Database. Lancet 1997;350:1097–9.

144. Williams T, van Staa T, Puri S, Eaton S. Recent advances in the utility and use of the General Practice Research Database as an example of a UK Primary Care Data resource. Ther Adv Drug Saf 2012;3:89–99.

145. Luo Z-C, An N, Xu H-R, Larante A, Audibert F, Fraser WD. The effects and mechanisms of primiparity on the risk of pre-eclampsia: a systematic review. Paediatr Perinat Epidemiol 2007;21 Suppl 1:36–45.

146. Hernández-Díaz S, Toh S, Cnattingius S. Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. BMJ 2009;338:b2255.

147. Sibai BM, Stella CL. Diagnosis and management of atypical preeclampsia-eclampsia. Am J Obstet Gynecol 2009;200:481.e1–481.e7.

148. Homer CSE, Brown MA, Mangos G, Davis GK. Non-proteinuric pre-eclampsia: a novel risk indicator in women with gestational hypertension. J Hypertens 2008;26:295–302.

149. Dupont WD. Power calculations for matched case-control studies. Biometrics 1988;44:1157–68.

150. Rothman KJ, Greenland S, Lash TL. Design strategies to improve study accuracy. In: Rothman KJ, Greenland S, Lash TL, editors. Modern Epidemiology. Philadelphia: Lippincott Williams & Wilkins; 2008.

151. Hennessy S, Bilker WB, Berlin JA, Strom BL. Factors influencing the optimal control-to-case ratio in matched case-control studies. Am J Epidemiol 1999;149:195–7.

152. Lewis JD, Bilker WB, Weinstein RB, Strom BL. The relationship between time since registration and measured incidence rates in the General Practice Research Database. Pharmacoepidemiol Drug Saf 2005;14:443–51.

153. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet 2002;77:67–75.

154. Raymond D, Peterson E. A critical review of early-onset and late-onset preeclampsia. Obstet Gynecol Surv 2011;66:497–506.

155. Hardy JR, Holford TR, Hall GC, Bracken MB. Strategies for identifying pregnancies in the automated medical records of the General Practice Research Database. Pharmacoepidemiol Drug Saf 2004;13:749–59.

156. Hardy JR, Leaderer BP, Holford TR, Hall GC, Bracken MB. Safety of medications prescribed before and during early pregnancy in a cohort of 81 975 mothers from the UK General Practice Research Database. Pharmacoepidemiol Drug Saf 2006;15:555–64.

157. Andrade SE, Raebel MA, Morse AN, Davis RL, Chan KA, Finkelstein JA, et al. Use of prescription medications with a potential for fetal harm among pregnant women. Pharmacoepidemiol Drug Saf 2006;15:546–54.

158. Devine S, West S, Andrews E, Tennis P, Hammad TA, Eaton S, et al. The identification of pregnancies within the general practice research database. Pharmacoepidemiol Drug Saf 2010;19:45–50.

159. Toh S, Mitchell AA, Werler MM, Hernández-Díaz S. Sensitivity and specificity of computerized algorithms to classify gestational periods in the absence of information on date of conception. Am J Epidemiol 2008;167:633–40.

160. Munkhaugen J, Vikse BE. New aspects of pre-eclampsia: lessons for the nephrologist. Nephrol Dial Transplant 2009;24:2964–7.

161. Trogstad LI, Eskild A, Magnus P, Samuelsen SO, Nesheim BI. Changing paternity and time since last pregnancy; the impact on pre-eclampsia risk. A study of 547 238 women with and without previous pre-eclampsia. Int J Epidemiol 2001;30:1317–22.

162. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183:S1–S22.

163. Little P, Williamson I, Warner G, Gould C, Gantley M, Kinmonth AL. Open randomised trial of prescribing strategies in managing sore throat. BMJ 1997;314:722–7.

164. Petersen I, Gilbert R, Evans S, Ridolfi A, Nazareth I. Oral antibiotic prescribing during pregnancy in primary care: UK population-based study. J Antimicrob Chemother 2010;65:2238–46.

165. Miyashita N, Fukano H, Yoshida K, Niki Y, Matsushima T. Chlamydia pneumoniae infection in adult patients with persistent cough. J Med Microbiol 2003;52:265–9.

166. Von Dadelszen P, Magee LA. Could an infectious trigger explain the differential maternal response to the shared placental pathology of preeclampsia and normotensive intrauterine growth restriction? Acta Obstet Gynecol Scand 2002;81:642–8.

167. Herrera JA, Chaudhuri G, López-Jaramillo P. Is infection a major risk factor for preeclampsia? Med Hypotheses 2001;57:393–7.

168. Khan NF, Harrison SE, Rose PW. Validity of diagnostic coding within the General Practice Research Database: a systematic review. Br J Gen Pract 2010;60:e128–36.

169. Vatten LJ, Skjaerven R. Is pre-eclampsia more than one disease? BJOG 2004;111:298–302.

170. Valensise H, Vasapollo B, Gagliardi G, Novelli GP. Early and late preeclampsia: two different maternal hemodynamic states in the latent phase of the disease. Hypertension 2008;52:873–80.

171. Kapoor AS, Kanji H, Buckingham J, Devereaux PJ, McAlister FA. Strength of evidence for perioperative use of statins to reduce cardiovascular risk: systematic review of controlled studies. BMJ 2006;333:1149.

172. Devereaux PJ, Mrkobrada M, Sessler DI, Leslie K, Alonso-Coello P, Kurz A, et al. Aspirin in patients undergoing noncardiac surgery. N Engl J Med 2014;370:1494–503.

173. Devereaux PJ, Sessler DI, Leslie K, Kurz A, Mrkobrada M, Alonso-Coello P, et al. Clonidine in patients undergoing noncardiac surgery. N Engl J Med 2014;370:1504–13.

174. Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S, et al. Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. Obstet Gynecol 2010;116:402–14.

Appendix A Published paper "Invasive dental treatment and risk for vascular events: a self-controlled case series" and reproduction permission

ACP
American College of Physicians
Internal Medicine | Doctors for Adults

WAA1418472

April 1, 2014

Caroline Minassian
London School of Hygiene & Tropical Medicine
Keppel St
London
WC1E 7HT

Dear Ms. Minassian:

Thank you for your request for electronic format of the following from *Annals of Internal Medicine:*

Minassian, C., D'Aiuto, F., Hingorani, A. D., & Smeeth, L. (2010). Invasive dental treatment and risk for vascular events: a self-controlled case series. Annals of Internal Medicine, 153(8), 499–506. doi:10.1059/0003-4819-153-8

Permission is granted to print the preceding material with the understanding that you will give appropriate credit to *Annals of Internal Medicine* as the original source of the material. Any translated version must carry a disclaimer stating that the American College of Physicians is not responsible for the accuracy of the translation. This permission grants non-exclusive, worldwide rights for this edition in electronic format for not for profit only. ACP does not grant permission to reproduce entire articles or chapters on the Internet unless explicit permission is given. This letter represents the agreement between ACP and Caroline Minassianfor request WAA1418472 and supersedes all prior terms from the requestor. The Annals of Internal Medicine wants to encourage users to go to the original article on the website for scientific integrity, in the event there are retractions and corrections.

Thank you for your interest in Annals of Internal Medicine. If you have any further questions or would like to discuss the matter further, please contact me at 856-489-4446 or fax 856-489-4449.

Sincerely,

Gina Brown
Permissions Coordinator

Annals of Internal Medicine | ORIGINAL RESEARCH

Invasive Dental Treatment and Risk for Vascular Events

A Self-Controlled Case Series

Caroline Minassian, MSc; Francesco D'Aiuto, PhD; Aroon D. Hingorani, PhD; and Liam Smeeth, PhD

Background: Treatment of periodontal disease may reduce cardiovascular risk in the longer term, but studies have suggested a link among dental procedures, acute inflammation, and endothelial dysfunction. However, whether such acute inflammatory effects translate into a short-lived increased risk for vascular events is not known.

Objective: To investigate whether invasive dental treatment transiently increases the risk for vascular events.

Design: Self-controlled case series.

Setting: Data came from the U.S. Medicaid claims database.

Patients: All persons exposed to invasive dental treatment with a primary hospital discharge diagnosis of ischemic stroke ($n = 650$) or myocardial infarction ($n = 525$) from 2002 to 2006.

Measurements: The incidence of ischemic stroke and myocardial infarction in periods immediately after invasive dental treatment was compared with the incidence in all other observed time periods. Incidence ratios and 95% CIs were calculated.

Results: The rate of vascular events significantly increased in the first 4 weeks after invasive dental treatment (incidence ratio, 1.50 [95% CI, 1.09 to 2.06]) and gradually returned to the baseline rate within 6 months. The positive association remained after exclusion of persons with diabetes, hypertension, or coronary artery disease or persons with prescriptions for antiplatelet or salicylate drugs before treatment.

Limitations: Power to examine the effects of invasive dental treatment on stroke and myocardial infarction separately was limited because of the low frequency of invasive dental procedures. Lack of information about use of over-the-counter drugs limited the ability to assess confounding by possible withholding of antiplatelet or salicylate drugs before invasive dental treatment or by the use of nonsteroidal anti-inflammatory drugs after treatment.

Conclusion: Invasive dental treatment may be associated with a transient increase in the risk for vascular events. However, the absolute risks are minimal, and the long-term benefits on vascular health will probably outweigh the short-lived adverse effects.

Primary Funding Source: Wellcome Trust.

Ann Intern Med. 2010;153:499-506. www.annals.org
For author affiliations, see end of text.

There is considerable interest in the role of inflammatory mechanisms in the occurrence of cardiovascular events. Local inflammation—the process by which the body responds to injury or infection—plays an important role in the pathogenesis of the atherosclerotic lesion (1). Moreover, long-term, low-grade chronic systemic inflammation has been linked to adverse cardiovascular outcomes (2). Acute inflammation after surgery (3), or bacterial infection (4), has also been associated with a short-term increase in the risk for vascular events, with endothelial dysfunction representing a possible common pathway through which several risk factors, including inflammation, may influence the atherogenic process (5, 6).

Epidemiologic data implicate exposure to low-grade dental infection—particularly periodontitis (a common chronic infection of the oral cavity caused by bacteria)—in the cause of cardiovascular disease. Such infections have been found to be associated with elevated levels of C-reactive protein and other inflammatory biomarkers (7), endothelial dysfunction (8), atherosclerosis, and an increased risk for stroke and myocardial infarction (9). Recent studies have shown that intensive periodontal treatment leads to transiently impaired, flow-mediated dilatation (a measure of endothelial function) and increased markers of inflammation and endothelial activation in the week after treatment followed by a longer-term improvement relative to baseline (10, 11). The more invasive the dental treatment (12), the more marked the changes.

Ischemic stroke and myocardial infarction share a common pathophysiologic process: arterial thrombosis occurring in a background of atherosclerosis. We have previously established that infections cause a transient increased risk for both myocardial infarction and stroke (6). If the likelihood of a vascular event is associated with variations in the underlying inflammatory state and endothelial function, then invasive dental treatment sufficient to produce an inflammatory response may transiently increase the risk for vascular events—namely myocardial infarction and stroke—despite providing longer term vascular benefits due to reducing the infectious burden. To test this hypothesis of a transient increased risk, we examined the incidence

See also:

Print
Editors' Notes 500
Editorial comment. 542
Summary for Patients. I-45

Web-Only
Appendix
Appendix Tables
Appendix Figures
CME quiz
Conversion of graphics into slides

Context

Chronic inflammatory states, such as periodontal disease, are increasingly believed to play a role in the cause of cardiovascular disease.

Contribution

Using data from a large administrative database, researchers found that adults who underwent discrete invasive dental procedures have an increased risk for myocardial infarction or stroke in the 4 weeks immediately after the procedure, but not at later times.

Implication

Acute dental inflammation may transiently increase cardiovascular disease risk.

—The Editors

of ischemic stroke and myocardial infarction after invasive dental treatment by using Medicaid claims data from the United States and the self-controlled case series method.

METHODS

Medicaid Database

Medicaid is a federally funded, state-administered health care program for persons without private insurance coverage. It is used by approximately 13% of U.S. citizens and provides inpatient, outpatient, drug treatment, and long-term care. The Medicaid claims database contains pooled data from 9 geographically dispersed states, including medical and dental records and details of interventional treatment, prescription drug claims, and enrollment. The data have high levels of completeness and validity (13). Eligibility is income-related and evaluated monthly. All information obtained from the database is anonymous.

Case Series Method

We examined the risk for vascular events after exposure to invasive dental procedures. Persons who have had invasive dental treatment may differ from those who have not in ways that can be difficult to measure and control for. Some of these differences may also be associated with the future risk for vascular events, which makes a conventional cohort design a less reliable source of information on this association. Therefore, we used the self-controlled case series method (14), which relies on within-person comparisons in a sample of persons, all of whom had an outcome of interest. The main advantage of this method is that inference is within a person; hence, fixed confounders (those that do not vary with time during the observation period) are implicitly controlled for. We derived incidence ratios of events occurring during predefined risk periods after an exposure, relative to all other observed time periods for each person. Our null hypothesis was that rates of vascular events remain constant from day to day and are not affected by exposure to invasive dental treatment. The **Figure** illustrates this method and the time intervals used.

A key assumption underlying the case series method is that events are independent within a person. This does not hold for vascular events because the occurrence of an ischemic stroke or myocardial infarction increases the probability of subsequent events. In the case in which this assumption fails, a reasonable strategy is to restrict the analysis to first events, provided that these are not common (14, 15). We therefore chose not to look at recurrent events and instead used the first event in the study period to assess the effect of invasive dental treatment on vascular events. Our approach is reasonable because both ischemic stroke and myocardial infarction are relatively uncommon conditions.

Participants

Participants were derived from a population of 9 901 464 persons for whom data were available in the Medicaid database from January 2002 to December 2006. This comprised approximately 28 million person-years of observation. All incident cases of ischemic stroke or myocardial infarction occurring during this period were identified from primary discharge diagnoses that were coded by using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), classification system.

Candidates were those who had a first hospitalization record for ischemic stroke or myocardial infarction at least 24 weeks after their enrollment period began. This ensured a minimum of 24 weeks of observation before each outcome, thus allowing participants' exposure status to be ascertained throughout this period. Restricting to the first event record avoided the problem of repeated coding of a single event within the database. Because participants who were eligible had at least 24 weeks of continuous observation before their first event record, we could be confident that their first event record was not a repeated record for an earlier event. We excluded persons if they were younger than 20 years at the time of their first hospitalization record for stroke or myocardial infarction because the cause of the event could have differed from that of older persons. Because eligibility for Medicaid health care is ascertained on a monthly basis, gaps were often found in a person's enrollment. Events or procedures occurring during such gaps are unlikely to be recorded in the database. This could lead to misclassification of exposure status. To avoid this, we identified each person's maximum period of continuous enrollment and restricted the person's follow-up to this period. Persons whose stroke or myocardial infarction occurred outside this period were subsequently excluded from the relevant analyses.

All candidates not excluded for these reasons were eligible for the study. However, in a case series analysis, persons not exposed during their observation period do not contribute to the estimates of association between exposure and outcome. The primary analysis was therefore restricted

to eligible persons who had both an event and invasive dental treatment during their continuous enrollment period.

Exposure

Data were extracted on claims for invasive dental procedures. Dental procedures are recorded in Medicaid by using the Current Dental Terminology coding system (16). We defined invasive dental procedures as those that may feasibly result in bacteremia and induce an inflammatory response. These included periodontal therapy and other invasive dental surgery, such as simple or complicated tooth extractions, also known to be associated with bacteremia (17, 18) and raised markers of inflammation (19). Some persons had several records of procedures, sometimes within a few days of one another. We defined procedures recorded at least 1 week apart as repeated procedures, and we excluded, for each person, all procedure records occurring within 1 week of a previous record, assuming these to be repeated records of the same treatment program. When addressing repeated procedures, we assumed the risk to be the same after each procedure, thus not allowing for a dose effect. **Appendix Table 1** (available at www.annals.org) describes all of the invasive dental procedures found in the study participants' records.

Outcome Measures

The accuracy of hospital discharge diagnostic codes for stroke and myocardial infarction classifications in administrative claims databases has been examined and validated (20, 21). On the basis of the criteria by Tirschwell and Longstreth (20), we defined ischemic stroke as any one of the following ICD-9-CM primary discharge diagnostic codes on inpatient admission records: 433.x1 (in which "x" can vary to specify a specific arterial distribution), 434 (excluding 434.x0), and 436. If any traumatic brain injury (ICD-9-CM codes 800 to 804 or 850 to 854) was recorded for the same hospitalization, the stroke was excluded. We defined myocardial infarction, according to criteria used by Kiyota and coworkers (21), as an ICD-9-CM primary discharge diagnostic code of 410.x1 and a hospital length of stay lasting from 3 to 180 days. If the patient died during hospitalization, the length of stay could be less than 3 days.

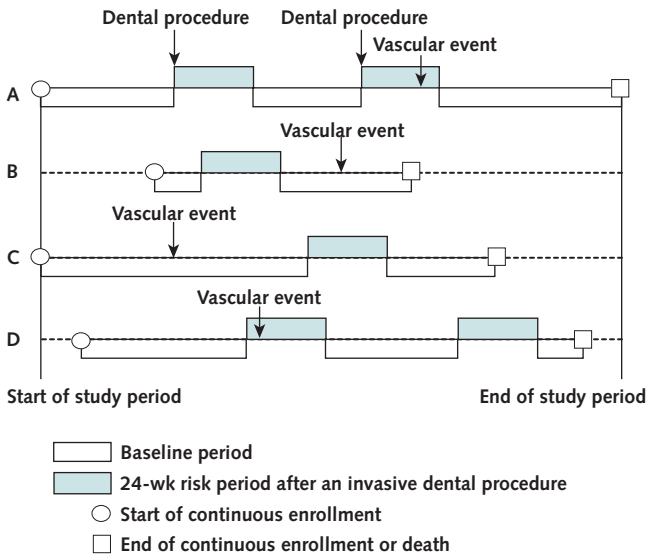
For descriptive purposes and sensitivity analyses, we identified persons with a diagnosis of diabetes, hypertension, coronary artery disease, or rheumatoid arthritis on inpatient admission or outpatient claim records before their invasive dental treatment. We defined each condition according to the following ICD-9-CM diagnostic codes: diabetes (code 250), hypertension (codes 401 to 405), coronary artery disease (codes 410 to 414 and 429.2), and rheumatoid arthritis (code 714).

Statistical Analysis

The exposed period started 1 day after an invasive dental procedure and extended up to 24 weeks later. It was subdivided into 1 to 4, 5 to 8, 9 to 12, 13 to 16, and 17 to 24 weeks because we assumed the risk to be similar during the last 8 weeks. All other observation time was considered the baseline (unexposed) period. Persons who were exposed to at least 1 invasive dental procedure were included in the primary analysis. For persons who had more than 1 procedure during the observation period, each procedure was followed by a 24-week exposed period. Our decision to start the exposed period 1 day after a procedure is based on current evidence that the host response and vascular function are affected at their maximum 24 hours after invasive dental treatment (10–12). We used a 24-week exposed period on the basis of previous work, which suggested any increased risk would have returned to baseline by 24 weeks (6, 22), and thus we would be able to fully describe the resolution of any increased risk. In the case of overlapping risk periods, we adopted a simple convention: later procedures take precedence over earlier ones (14).

Analyses were done for vascular events overall and separately by event type (ischemic stroke or myocardial infarction). We estimated incidence ratios and 95% CIs for events occurring within each stratum of the exposed period compared with baseline by using conditional Poisson re-

Figure. **Pictorial representation of the case series method.**



Four possible scenarios for the timing of vascular events and invasive dental procedures (each representing a single participant) are shown. **A.** Participant is followed for the duration of the study period, has two 24-week risk periods (each after an invasive dental procedure), and has a vascular event during the second risk period. **B.** Participant is followed for part of the study period and has 1 dental procedure followed by a vascular event at baseline. **C.** Participant is followed from the start of the study period, has a vascular event at baseline before a dental procedure, and dies before the end of the study period. **D.** Participant is followed for most of the study period, has 2 dental procedures, and has a vascular event during the first risk period. All participants included in a particular analysis had at least 1 exposure and at least 1 vascular event. Each risk period began the day after a procedure, lasted 24 weeks (not drawn to scale relative to length of baseline periods), and was divided into the following intervals: 1 to 4, 5 to 8, 9 to 12, 13 to 16, and 17 to 24 weeks.

gression. We adjusted for age in 5-year age groups (for example, 20 to 24 years, 25 to 29 years, and 30 to 34 years). Each person's observation was split into successive intervals determined by changes in age group and exposure status, thus allowing persons to contribute to different age groups over time. In a case series analysis, persons not exposed at any time during follow-up do not contribute to the estimates of the association between exposure and outcome. However, including these unexposed persons can help control for confounding by age because they contribute information on the age-specific incidence of the outcome of interest. We did a sensitivity analysis including unexposed cases to check that the estimates did not vary.

The validity of the case series method rests on the assumption that the probability of exposure is not affected by the occurrence of an outcome event. This may not hold true if the event of interest increases the mortality rate (as is the case for ischemic stroke or myocardial infarction); therefore, we conducted a sensitivity analysis excluding persons who died during their hospital stay for the vascular event or whose enrollment ended within 1 month of their event (possibly indicating death). Although fixed covariates are implicitly controlled for in a case series analysis, we recognized that there may be potential for confounding by possible withholding of antiplatelet or salicylate medications before invasive dental treatment among high-risk persons receiving such drug regimens. We therefore did a sensitivity analysis restricted to patients who had no recorded use of antiplatelet or salicylate agents before invasive dental treatment. The rationale for this is that among such patients, cessation of drug therapy at the time of dental treatment is unlikely to occur. Thus, any observed increased risk for a vascular event after the dental therapy is unlikely to be attributable to cessation of antiplatelet or salicylate therapy. To address the possibility that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) after invasive dental procedures for pain control may confound the association observed, we did an additional sensitivity analysis excluding persons with a recorded diagnosis of rheumatoid arthritis at any time before invasive dental treatment (who were probably taking NSAIDs) or with an NSAID prescription around the time of their dental treatment (4 weeks before to 4 weeks after treatment). Similarly, the potential for confounding by the development of diabetes, hypertension, or coronary artery disease in the period leading up to invasive dental treatment was addressed in sensitivity analyses excluding patients with these conditions newly diagnosed within the year before dental treatment.

To eliminate the possibility that our convention (allowing later procedures to take precedence over earlier ones if the risk periods overlapped) might contribute to an observed effect in earlier time frames, we did additional sensitivity analyses by excluding persons with overlapping risk periods and persons with repeated procedures. Finally, given that most dental procedures included in our analyses were extractions, we repeated our analyses, restricting to only these homogeneous exposures. Data were analyzed by using Stata software, version 10 (StataCorp, College Station, Texas). The **Appendix** (available at www.annals.org) provides further details of our analysis.

Role of the Funding Source

A Wellcome Trust Senior Fellowship grant and a senior fellowship from the British Heart Foundation funded this study. The funding sources had no role in the design, conduct, and reporting of the study or in the decision to submit this manuscript for publication.

RESULTS

A total of 32 060 persons were identified from the Medicaid database with a hospitalization for ischemic stroke (n = 17 741) or myocardial infarction (n = 14 783); 11 691 were excluded for 1 of the following reasons: Less than 24 weeks of observation had passed before their first event record (n = 10 822); they were younger than 20 years at the time of their first event (n = 104); or the first event occurred outside the continuous enrollment period (n = 765). Among the remaining 20 369 eligible persons, the median age at the time of diagnosis was 67.3 years (interquartile range [IQR], 56.5 to 79.6 years), 34.3% were men, the mean observation period was 3.4 years, and 7.7% died during their hospital stay. Among eligible persons with ischemic stroke (n = 11 284), the median age at the time of diagnosis was 68.8 years (IQR, 57.5 to 80.4 years), 31.4% were men, the mean observation period was 3.4 years, and 5.6% died during their hospital stay. Among eligible persons with myocardial infarction (n = 9484), the median age at the time of diagnosis was 65.2 years (IQR, 55.3 to 78.4 years), 37.7% were men, the mean observation period was 3.3 years, and 10.3% died during their hospital stay.

Only cases that had been exposed to an invasive dental procedure at least once during follow-up were included in the primary analysis of vascular events, overall and by event type. **Table 1** provides demographic details of these persons. The identification of the 1152 persons included in the primary analysis of vascular events is illustrated in **Appendix Figure 1** (available at www.annals.org). The mean duration of total observation for patients with vascular events was 4.2 years (4.2 years for patients with ischemic stroke and 4.1 years for patients with myocardial infarction). During the observation period, 1152 (5.7%) eligible persons with a vascular event (629 with ischemic stroke only, 504 with myocardial infarction only, and 19 with both) had 1 or more invasive dental procedures; 861 (74.7%) of whom had a single exposure period, 281 (24.4%) had 2 to 4 exposure periods, and 10 (0.9%) had 5 or more exposure periods. Of these 1152 exposed persons, 4.1% died during their hospital stay (2.6% of those first hospitalized for ischemic stroke and 5.7% of those first hospitalized for myocardial infarction). The median number of days between adjacent procedures was 56.5 days

(IQR, 21 to 245 days). A total of 89% of all invasive dental procedures included in the primary analysis were extractions, and more than 95% of persons had at least 1 extraction (**Appendix Table 1**). **Table 2** shows the number of exposed persons who had an ischemic stroke or myocardial infarction and the age-adjusted incidence ratios after invasive dental treatment.

The rate of vascular events ($n = 1152$) significantly increased in the first 4 weeks after invasive dental treatment compared with the baseline (unexposed) period (incidence ratio, 1.50 [95% CI, 1.09 to 2.06]) and decreased thereafter. No events occurred on the same day as an invasive dental procedure. Examining stroke and myocardial infarction separately yielded similar findings, although these were not statistically significant. The rate of myocardial infarction ($n = 525$) was higher in the first 4 weeks after an invasive dental treatment compared with baseline (incidence ratio, 1.56 [CI, 0.98 to 2.47]) and seemed to decrease over 24 weeks. For ischemic stroke ($n = 650$), a slightly elevated risk was seen during the first 4 weeks after an invasive dental treatment (incidence ratio, 1.39 [CI, 0.89 to 2.15]), although this was less marked and the pattern of resolution was less clear. Repeating the analyses to include unexposed cases did not materially alter the estimates of the effect of invasive dental procedures on ischemic stroke or myocardial infarction.

We conducted further sensitivity analyses: restricting to persons whose enrollment continued for at least 1 month after their vascular event and hence did not die immediately or shortly after stroke or myocardial infarction; excluding persons with overlapping risk periods; excluding persons with repeated procedures; excluding persons probably taking NSAIDs around the time of dental treatment (those with a rheumatoid arthritis diagnosis at any time before treatment or an NSAID prescription 4 weeks before or after treatment); restricting our exposure to extractions; and restricting to persons who were healthy, as defined by an absence of diabetes, hypertension, or coronary artery disease diagnoses at any time before invasive dental treatment. To assess whether any observed effect could be attributable to persons stopping antiplatelet or salicylate therapy before their dental treatment, we did an analysis restricted to persons with no antiplatelet or salicylate drug prescriptions at any time before their dental treatment. Among this group, stopping therapy was unlikely to be an issue. Finally, to assess whether the development of diabetes, hypertension, or coronary artery disease might confound the observed association between invasive dental treatment and vascular events, we excluded persons with these conditions newly diagnosed within 1 year before their dental treatment. These analyses made no material difference to our findings, and if anything, they yielded a marginally stronger effect 1 to 4 weeks after dental treatment. **Table 3** summarizes the results of the sensitivity analyses.

***Table 1.* Characteristics of Study Participants**

Characteristic	Patients With Vascular Events ($n = 1152$)*	Patients With Ischemic Stroke ($n = 650$)†	Patients With Myocardial Infarction ($n = 525$)†
Men, *n (%)*	458 (39.8)	233 (35.9)	236 (45.0)
Women, *n (%)*	694 (60.2)	417 (64.2)	289 (55.1)
Ethnicity, *n (%)*			
White	558 (48.4)	282 (43.4)	282 (53.7)
Black	463 (40.2)	303 (46.6)	171 (32.6)
Hispanic	17 (1.5)	9 (1.4)	8 (1.5)
Other	114 (9.9)	56 (8.6)	64 (12.2)
Age at first event, *n (%)*			
20–29 y	24 (2.1)	21 (3.2)	3 (0.6)
30–39 y	74 (6.4)	41 (6.3)	33 (6.3)
40–49 y	258 (22.4)	117 (18.0)	147 (28.0)
50–59 y	282 (24.5)	156 (24.0)	138 (26.3)
60–69 y	228 (19.8)	139 (21.4)	93 (17.7)
70–79 y	167 (14.5)	100 (15.4)	67 (12.8)
80–89 y	111 (9.6)	72 (11.1)	40 (7.6)
≥90 y	8 (0.7)	4 (0.6)	4 (0.8)
Diabetes diagnosis at any time before IDT, *n (%)*	474 (41.2)	269 (41.4)	214 (40.8)
Hypertension diagnosis at any time before IDT, *n (%)*	809 (70.2)	463 (71.2)	366 (69.7)
Coronary artery disease diagnosis at any time before IDT, *n (%)*	470 (40.8)	211 (32.5)	278 (53.0)

IDT = invasive dental treatment.
* Persons included in the primary analysis of vascular events.
† Twenty-three patients had both an ischemic stroke and a myocardial infarction during their observation period: 19 were included in each analysis (vascular events overall and by event type), and 4 (2 from each analysis, by event type) were excluded from the primary analysis of vascular events because their earlier event met the exclusion criteria.

DISCUSSION

Our study has shown that invasive dental procedures may be associated with a transient increase in the risk for stroke and myocardial infarction in the first 4 weeks after treatment. These findings provide further evidence to support the link between acute inflammation and the risk for vascular events.

In studies investigating the risk for vascular events after inflammatory exposures, the potential for confounding is great because persons who have invasive dental treatment may differ from those who do not in ways that are difficult to control for. The major strength of our study is the use of a case series analysis in which within-person comparisons are done, thereby overcoming the problem of potential confounding associated with the influence of risk factors, which may vary among persons. Confounding would occur only if intraperson risk factors for vascular events that change with time are also associated with the timing of invasive dental treatment. In addition, to produce the effect observed, any such factors would need to have a large

Table 2. **Age-Adjusted Incidence Ratios of a First Vascular Event in Risk Periods After Exposure to Invasive Dental Treatment**

Outcome and Risk Period	Cases, *n*	Age-Adjusted Incidence Ratio (95% CI)
Vascular event (*n* = 1152)*		
Risk period after procedure		
1–4 wk	40	1.50 (1.09–2.06)
5–8 wk	29	1.11 (0.77–1.61)
9–12 wk	30	1.16 (0.81–1.68)
13–16 wk	25	0.96 (0.64–1.43)
17–24 wk	53	1.08 (0.82–1.43)
Baseline period†	975	1.00
Ischemic stroke (*n* = 650)		
Risk period after procedure		
1–4 wk	21	1.39 (0.89–2.15)
5–8 wk	14	0.94 (0.55–1.60)
9–12 wk	18	1.21 (0.76–1.95)
13–16 wk	11	0.73 (0.40–1.32)
17–24 wk	33	1.18 (0.83–1.69)
Baseline period†	553	1.00
Myocardial infarction (*n* = 525)		
Risk period after procedure		
1–4 wk	19	1.56 (0.98–2.47)
5–8 wk	16	1.35 (0.82–2.23)
9–12 wk	13	1.12 (0.64–1.95)
13–16 wk	14	1.20 (0.70–2.05)
17–24 wk	20	0.90 (0.57–1.42)
Baseline period†	443	1.00

* Vascular events are 639 ischemic strokes (55.5%) and 513 myocardial infarctions (44.5%).
† Baseline period is all observation time except for the 24-wk period after an invasive dental procedure.

acute effect and their time-dependent effect would need to operate in a large proportion of included participants. Possible confounding by the development of diabetes, hypertension, or coronary artery disease; the cessation of antiplatelet or salicylate medications before invasive dental treatment; or the use of NSAIDs after treatment for pain control were addressed in sensitivity analyses that excluded persons with these newly diagnosed conditions, those with recorded use of antiplatelet or salicylate drugs before dental treatment, and those probably taking NSAIDs around the time of dental treatment. These exclusions made no material difference to our findings. Nevertheless, we recognize that our ascertainment of use of antiplatelet agents, salicylates, or NSAIDs may be incomplete because some patients probably received these agents both through prescription and over the counter. Because the database does not capture over-the-counter use, we cannot exclude the possibility of residual confounding by differential use of these agents around the time of invasive dental treatment. Further sensitivity analyses demonstrated that our results were robust with regard to assumptions underlying the within-person case series.

In our study, the exposed period starts 1 day after an invasive dental procedure; hence, the day of a dental procedure contributes to the baseline period. This avoids the problem of events occurring on the same day as a procedure, which are a consequence of some other factors unrelated to the dental treatment included in our risk estimates. Any bias occurring from this convention would lead to an underestimate of effect. However, this is of no concern in our study because no vascular events occurred on the same day as a procedure.

A further strength of the study is that the Medicaid database has high levels of completeness and validity (13). It contains records of all medical care provided to eligible persons, therefore eliminating the problems of recall or interviewer bias in both exposure and outcome. Neverthe-

Table 3. **Results of Sensitivity Analyses**

Analysis of Vascular Event Risk*	Cases Included in Analysis, *n*	Age-Adjusted Incidence Ratio (95% CI)†
Primary analysis		
Vascular events‡	1152	1.50 (1.09–2.06)
Sensitivity analyses, by exclusion criteria		
Overlapping risk periods (204 excluded)	948	1.65 (1.17–2.33)
Several invasive dental procedures (291 excluded)	861	1.53 (1.04–2.25)
Procedures that were not extractions (135 excluded)	1017	1.58 (1.13–2.21)
Enrollment ending or death within 1 mo after vascular event (83 excluded)	1069	1.62 (1.17–2.24)
Antiplatelet or salicylate drug prescription record at any time before IDT (486 excluded)	666	2.23 (1.56–3.18)
NSAID prescription 4 wk before to 4 wk after IDT or rheumatoid arthritis diagnosis at any time before IDT (687 excluded)	465	1.84 (1.17–2.89)
Earliest record of diabetes within 12 mo before IDT (224 excluded)	928	1.46 (1.02–2.10)
Earliest record of hypertension within 12 mo before IDT (398 excluded)	754	1.64 (1.12–2.40)
Earliest record of coronary artery disease within 12 mo before IDT (239 excluded)	913	1.70 (1.21–2.40)
Diagnosis of diabetes, hypertension, or coronary artery disease at any time before IDT (924 excluded)	228	1.76 (0.92–3.36)

IDT = invasive dental treatment; NSAID = nonsteroidal anti-inflammatory drug.
* 1–4 wk after procedure compared with baseline. Baseline period is all observation time except for the 24-wk risk period after an invasive dental procedure.
† 1–4 wk after an invasive dental procedure.
‡ Vascular events are 639 ischemic strokes (55.5%) and 513 myocardial infarctions (44.5%).

less, we cannot exclude the possibility of case ascertainment bias, whereby patients with events may be more likely to be designated as having an outcome in the first month after a dental procedure than later. There is also some scope for misclassification of exposure status. We cannot determine who had dental coverage—only who made dental claims. If some persons did not qualify for dental coverage yet had undergone an invasive dental treatment (either self-funded or covered by another insurer), this would not be captured in the database. These persons would be misclassified as unexposed, which could lead to an underestimate of effect. This is unlikely to be a major problem in our study because only those persons with an invasive dental procedure record (and thus with dental coverage) contributed to the analyses, and the chance of their dental coverage changing during enrollment is probably small.

Our study was based on claims data, and a potential weakness may relate to the skewed nature of the population eligible for Medicaid. Eligibility is income-related, which raises the question of generalizability of these findings to other populations. Eligible groups include low-income adults and their children and persons with certain disabilities. Patients with diseases that put them at greater risk for thrombotic events may be more likely to enter the Medicaid program to pay for needed care, including dental care. However, this is more of a problem in descriptive studies and less of a concern in an analytical study such as ours, particularly because each person serves as his or her own control. The relatively small study population is another limitation. In a case series design, only persons exposed at least once during follow-up contribute to the analyses. Given that invasive dental procedure claims were fairly uncommon, a relatively small proportion of our initial study sample contributed to the analyses. This resulted in a loss of power, which unfortunately limited our ability to examine the effects of invasive dental treatment on stroke and myocardial infarction separately. Nevertheless, the stringent criteria used to define our exposure and outcomes and the suitability of our statistical approach together make the case for the validity of our findings.

Increasing evidence implicates low-grade dental infections, such as periodontitis, in the cause of systemic diseases. Several epidemiologic studies have shown that periodontal disease is associated with elevated markers of inflammation (23–25) and increased cardiovascular disease risk in the long term (9, 26, 27). Treatment of periodontitis may yield a positive influence on longer term cardiovascular disease risk by reducing the infectious burden (28). Recent studies have found that intensive periodontal therapy produces an acute systemic inflammatory response 1 week in duration and a transient impairment of endothelial function followed by a subsequent improvement relative to baseline (10–12). Our findings of a small but statistically significant association between invasive dental treatment and vascular event risk over the short term are consistent with these earlier studies. Although we cannot exclude the possibility that other mechanisms may be involved, such as elevated stress due to pain arising from invasive dental treatment, possible discontinuation of antiplatelet or salicylate therapy before treatment, or the use of NSAIDs after treatment, our findings lend support to the hypothesis that inflammation may play an important role in the occurrence of vascular events.

Although the mechanisms are uncertain, we conclude that invasive dental treatment may be associated with a transient increase in the risk for stroke and myocardial infarction in adults. The short-lived adverse effects are nevertheless likely to be outweighed by long-term benefits of invasive dental treatment to vascular health.

From London School of Hygiene and Tropical Medicine, University College London Eastman Dental Institute, and University College London, London, United Kingdom.

Acknowledgment: The authors thank GlaxoSmithKline for supplying the Medicaid data and for their advice on using the database. The company had no role in the study design, analysis, interpretation of the findings, or preparation of this manuscript.

Grant Support: In part by a Wellcome Trust Senior Fellowship grant (Dr. Smeeth) and a senior fellowship from the British Heart Foundation (Dr. Hingorani). Dr. D'Aiuto holds a Clinical Senior Lectureship Award supported by the United Kingdom Clinical Research Collaboration. Drs. Hingorani and D'Aiuto work at University College London Hospital–University College London, who received a proportion of funding from the Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme.

Potential Conflicts of Interest: Dr. Hingorani: *Grants received/pending:* British Heart Foundation and Medical Research Council Research Award on Biomarkers, with Pfizer as a co-funder. *Employment:* Editorial board member of the *Drug and Therapeutics Bulletin*, a BMJ Group publication. *Other:* Received honoraria for speaking at educational meetings and teaching a course on cardiovascular risk. Some of this money was donated to medical charities. Dr. Smeeth: *Grants received/pending:* Wellcome Trust Senior clinical fellowship. *Other:* GlaxoSmithKline provided access to the data but had no role in the study design, analysis, or interpretation of the manuscript. Disclosures can also be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M10-0574.

Reproducible Research Statement: *Study protocol and statistical code*: Available from Ms. Minassian (e-mail, caroline.minassian@lshtm.ac.uk). *Data set*: Not available.

Requests for Single Reprints: Liam Smeeth, PhD, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom; e-mail, liam.smeeth@lshtm.ac.uk.

Current author addresses and author contributions are available at www.annals.org.

References

1. **Hansson GK.** Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352:1685-95. [PMID: 15843671]
2. **Libby P, Ridker PM, Maseri A.** Inflammation and atherosclerosis. Circula-

tion. 2002;105:1135-43. [PMID: 11877368]
3. **Mamode N, Cobbe S, Pollock JG.** Infarcts after surgery [Editorial]. BMJ. 1995;310:1215-6. [PMID: 7767182]
4. **Meier CR, Jick SS, Derby LE, Vasilakis C, Jick H.** Acute respiratory-tract infections and risk of first-time acute myocardial infarction. Lancet. 1998;351:1467-71. [PMID: 9605802]
5. **Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, et al.** Acute systemic inflammation impairs endothelium-dependent dilatation in humans. Circulation. 2000;102:994-9. [PMID: 10961963]
6. **Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P.** Risk of myocardial infarction and stroke after acute infection or vaccination. N Engl J Med. 2004;351:2611-8. [PMID: 15602021]
7. **Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E.** Periodontal infections contribute to elevated systemic C-reactive protein level. J Periodontol. 2001;72:1221-7. [PMID: 11577954]
8. **Higashi Y, Goto C, Hidaka T, Soga J, Nakamura S, Fujii Y, et al.** Oral infection-inflammatory pathway, periodontitis, is a risk factor for endothelial dysfunction in patients with coronary artery disease. Atherosclerosis. 2009;206:604-10. [PMID: 19410250]
9. **Scannapieco FA, Bush RB, Paju S.** Associations between periodontal disease and risk for atherosclerosis, cardiovascular disease, and stroke. A systematic review. Ann Periodontol. 2003;8:38-53. [PMID: 14971247]
10. **D'Aiuto F, Parkar M, Tonetti MS.** Periodontal therapy: a novel acute inflammatory model. Inflamm Res. 2005;54:412-4. [PMID: 16283108]
11. **D'Aiuto F, Parkar M, Tonetti MS.** Acute effects of periodontal therapy on bio-markers of vascular health. J Clin Periodontol. 2007;34:124-9. [PMID: 17214734]
12. **Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, et al.** Treatment of periodontitis and endothelial function. N Engl J Med. 2007;356:911-20. [PMID: 17329698]
13. **Strom BL.** Medicaid databases. In: Hennessy S, Carson JL, Ray WA, Strom BL, eds. Pharmacoepidemiology. 4th ed. Hoboken, NJ: J Wiley; 2005.
14. **Whitaker HJ, Farrington CP, Spiessens B, Musonda P.** Tutorial in biostatistics: the self-controlled case series method. Stat Med. 2006;25:1768-97. [PMID: 16220518]
15. **Farrington CP, Hocine MN.** Within-individual dependence in self-controlled case series models for recurrent events. J R Stat Soc Ser C Appl Stat 2010;59:457-75.
16. **American Dental Association.** Current Dental Terminology 2007-2008. Chicago: American Dental Association; 2007.
17. **Olsen I.** Update on bacteraemia related to dental procedures. Transfus Apher Sci. 2008;39:173-8. [PMID: 18753008]
18. **Lockhart PB, Brennan MT, Sasser HC, Fox PC, Paster BJ, Bahrani-Mougeot FK.** Bacteremia associated with toothbrushing and dental extraction. Circulation. 2008;117:3118-25. [PMID: 18541739]
19. **El-Sharrawy EA, El-Hakim IE, Sameeh E.** Attenuation of C-reactive protein increases after exodontia by tramadol and ibuprofen. Anesth Prog. 2006;53:78-82. [PMID: 17175820]
20. **Tirschwell DL, Longstreth WT Jr.** Validating administrative data in stroke research. Stroke. 2002;33:2465-70. [PMID: 12364739]
21. **Kiyota Y, Schneeweiss S, Glynn RJ, Cannuscio CC, Avorn J, Solomon DH.** Accuracy of Medicare claims-based diagnosis of acute myocardial infarction: estimating positive predictive value on the basis of review of hospital records. Am Heart J. 2004;148:99-104. [PMID: 15215798]
22. **Smeeth L, Cook C, Thomas S, Hall AJ, Hubbard R, Vallance P.** Risk of deep vein thrombosis and pulmonary embolism after acute infection in a community setting. Lancet. 2006;367:1075-9. [PMID: 16581406]
23. **Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U.** Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol. 2000;71:1528-34. [PMID: 11063384]
24. **Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS.** Acute-phase inflammatory response to periodontal disease in the US population. J Dent Res. 2000;79:49-57. [PMID: 10690660]
25. **Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S.** Relationship between periodontal disease and C-reactive protein among adults in the Atherosclerosis Risk in Communities study. Arch Intern Med. 2003;163:1172-9. [PMID: 12767953]
26. **Dietrich T, Jimenez M, Krall Kaye EA, Vokonas PS, Garcia RI.** Age-dependent associations between chronic periodontitis/edentulism and risk of coronary heart disease. Circulation. 2008;117:1668-74. [PMID: 18362228]
27. **Persson GR, Persson RE.** Cardiovascular disease and periodontitis: an update on the associations and risk. J Clin Periodontol. 2008;35:362-79. [PMID: 18724863]
28. **D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS.** Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J. 2006;151:977-84. [PMID: 16644317]

Current Author Addresses: Ms. Minassian and Dr. Smeeth: Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom.
Dr. D'Aiuto: Periodontology Unit, University College London Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD, United Kingdom.
Dr. Hingorani: Genetic Epidemiology Group, Department of Epidemiology and Public Health, University College London, 1-19 Torrington Place, London WC1E 6BT, United Kingdom.

Author Contributions: Conception and design: L. Smeeth, F. D'Aiuto.
Analysis and interpretation of the data: C. Minassian, L. Smeeth, A.D. Hingorani, F. D'Aiuto.
Drafting of the article: C. Minassian, L. Smeeth, A.D. Hingorani, F. D'Aiuto.
Critical revision of the article for important intellectual content: C. Minassian, L. Smeeth, A.D. Hingorani, F. D'Aiuto.
Final approval of the article: C. Minassian, L. Smeeth, A.D. Hingorani, F. D'Aiuto.
Statistical expertise: C. Minassian, L. Smeeth.
Obtaining of funding: L. Smeeth.
Administrative, technical, or logistic support: F. D'Aiuto.

29. **Farrington CP.** Relative incidence estimation from case series for vaccine safety evaluation. Biometrics. 1995;51:228-35. [PMID: 7766778]
30. **Farrington P, Pugh S, Colville A, Flower A, Nash J, Morgan-Capner P, et al.** A new method for active surveillance of adverse events from diphtheria/tetanus/pertussis and measles/mumps/rubella vaccines. Lancet. 1995;345:567-9. [PMID: 7619183]
31. **Hocine M, Guillemot D, Tubert-Bitter P, Moreau T.** Testing independence between two Poisson-generated multinomial variables in case-series and cohort studies. Stat Med. 2005;24:4035-44. [PMID: 16320271]
32. **Hubbard R, Farrington P, Smith C, Smeeth L, Tattersfield A.** Exposure to tricyclic and selective serotonin reuptake inhibitor antidepressants and the risk of hip fracture. Am J Epidemiol. 2003;158:77-84. [PMID: 12835289]
33. **Douglas IJ, Evans SJ, Pocock S, Smeeth L.** The risk of fractures associated with thiazolidinediones: a self-controlled case-series study. PLoS Med. 2009;6:e1000154. [PMID: 19787025]
34. **Gibson JE, Hubbard RB, Smith CJ, Tata LJ, Britton JR, Fogarty AW.** Use of self-controlled analytical techniques to assess the association between use of prescription medications and the risk of motor vehicle crashes. Am J Epidemiol. 2009;169:761-8. [PMID: 19181876]
35. **Whitaker HJ, Hocine MN, Farrington CP.** The methodology of self-controlled case series studies. Stat Methods Med Res. 2009;18:7-26. [PMID: 18562396]
36. **Farrington CP, Whitaker HJ.** Semiparametric analysis of case series data. J R Stat Soc Ser C Appl Stat 2006;55:1-28.

Appendix

The following provides a brief overview of the self-controlled case series (SCCS) method and further details of its application to our study on invasive dental treatment and risk for vascular events. For readers interested in using the SCCS method, the Web site run by the statistician who developed the method (http://statistics.open.ac.uk/sccs/) provides a tutorial (14) and files to download to implement the method in several statistical software packages: Stata (StataCorp); SAS (SAS Institute, Cary, North Carolina); R (R Foundation for Statistical Computing, Vienna, Austria); GLIM (Royal Statistical Society, London, United Kingdom); and GenStat (VSN International, Hemel Hempstead, United Kingdom).

Background and Description of SCCS Method

The SCCS method uses within-person comparisons in a population of persons who had the outcome of interest to investigate the association between time-varying exposures and outcome events. It is derived from a Poisson cohort model by conditioning on the number of events and exposure history that a person has during a predefined observation period: the time during which, if an event occurred, the person would be sampled. Although the method was originally developed to investigate associations between vaccination and acute adverse events (29, 30), it has subsequently been applied in other settings (for example, to investigate the risk for myocardial infarction and stroke [6] and deep venous thrombosis and pulmonary embolism [22] after acute infection) and has been extensively used in pharmacoepidemiology (31–34).

The SCCS method provides an alternative to the more established cohort method for estimating the relative incidence of an event (that is, the ratio of the rate of events in a defined period after exposure to the rate of events in the absence of exposure). **Appendix Figure 2** illustrates this method. Only cases are sampled—there is no comparison control group of persons. Comparisons are within person. To take this into account, the likelihood is conditional on an outcome event having occurred during the observation period. Thus, the likelihood is based on the probability density that a person's event occurred when it did in relation to exposure, given that the event occurred during the observation period.

Advantages

The main advantage of the SCCS method is that inference is within persons; hence, fixed or stable characteristics, such as genetic factors, sex, socioeconomic status, and underlying health status (individual characteristics that do not vary over the observation period) are implicitly controlled for. The method uses only case patients, which reduces the cost and effort involved in data collection and provides consistent estimates of the relative incidence of events. In addition, it allows age or temporal variation in baseline incidence to be controlled for. It also often has high statistical efficiency relative to the cohort method from which it is derived.

Limitations and Assumptions

The SCCS method produces only estimates of relative incidence and not absolute incidence. Hence, our study reports only incidence ratios. The method also requires some variability in the time or age at event: It would fail if all events occurred at the same age (an unlikely scenario and not an issue in our study). In addition, the validity of the method rests on some important assumptions (35). First, the occurrence of an event does not affect a person's subsequent exposure; second, the occurrence of an event does not alter the duration of the observation period; and third, events are independent within a person. In the context of our study, these assumptions are discussed in the section, Addressing the Assumptions Underlying the SCCS Method.

Application of the SCCS Method to Our Study

To examine the risk for vascular events after exposure to invasive dental procedures, we used the SCCS method because persons who have had invasive dental treatment may differ from those who have not in ways that can be difficult to measure and control for. Some of these differences may also be associated with the future risk for vascular events, which makes a conventional cohort design a less reliable approach for examining this association.

Persons who had a vascular event and at least 1 invasive dental procedure during their observation period were included in the primary analysis. The observation period for each person was the time during which, if a vascular event occurred, the person would be sampled (that is, the continuous enrollment period in Medicaid from January 2002 to December 2006). Thus, each person was followed from the start of his or her continuous enrollment period until he or she died or the continuous enrollment period ended (whichever occurred first), regardless of when the vascular event occurred. We took into account repeated invasive dental procedures during the observation period, assuming the same level of risk after each procedure. By using conditional Poisson regression, we derived incidence ratios of vascular events occurring during predefined risk periods extending up to 24 weeks after an invasive dental procedure, relative to all other observed time periods. Our null hypothesis was that rates of vascular events remain constant from day to day and are not affected by exposure to invasive dental treatment.

Although fixed covariates are implicitly controlled for in a case series analysis, there is still scope for confounding if intraperson risk factors for vascular events that change with time are also associated with the timing of invasive dental treatment. As the baseline risk for vascular events varies with age (that is, the risk in the absence of exposure to invasive dental treatment), we split each person's follow-up into successive intervals determined by changes in age (by using 5-year groupings) and exposure status. The time-varying effect of age was thus controlled for by including the age group factor as a covariate in each model.

We recognized that there may be potential for confounding by the development of diabetes, hypertension, or coronary artery disease; possible withholding of antiplatelet or salicylate medication before invasive dental treatment; or the use of NSAIDs after dental treatment for pain control. Therefore, we conducted sensitivity analyses excluding persons with these conditions newly diagnosed during the year before invasive dental treatment, those with recorded use of antiplatelet or salicylate drugs before dental treatment (who thus had the opportunity to withhold from their medication), or those with a recorded diagnosis of rheumatoid arthritis before dental treatment (who were probably taking NSAIDs) or with an NSAID prescription around the time of their dental treatment. These exclusions made no material difference to our findings or conclusions.

Addressing the Assumptions Underlying the SCCS Method

Assumption 1: The Occurrence of an Event Should Not Affect the Probability of Subsequent Exposure. This is perhaps the most restrictive assumption underlying the SCCS method (36). Other than the vascular outcome itself being fatal (thus curtailing the probability of exposure), we can think of no major factors likely to alter exposure to invasive dental treatment after a vascular event. To address the issue of fatal vascular events, we conducted a sensitivity analysis excluding persons who died during their hospital stay for their vascular event or whose enrollment ended within a month of their event, possibly indicating death. Excluding all such possible deaths did not materially alter our findings or conclusions. We found a marginally stronger effect in 1 to 4 weeks after invasive dental treatment.

Assumption 2: The Occurrence of the Event Should Not Censor or Alter the Duration of the Observation Period. In a case series study, each person's observation period is usually determined by using predefined calendar time boundaries, age limits, or both and must be independent of the timing of the event. This assumption may also be violated when the event of interest is likely to increase the short-term death rate. Thus, the sensitivity analyses described previously also addressed this assumption.

Assumption 3: Events Are Independent Within a Person. The case series method requires that the occurrence of an event should not affect the rate at which subsequent events may occur. If this assumption fails, a reasonable strategy is to restrict the analysis to first events, provided that these are not common (14, 15, 36). We restricted our analyses to the first event during the observation period (that is, the first occurring during baseline or a risk period). We did this because the recurrence times of events under study (ischemic stroke and myocardial infarction) cannot be assumed to be independent within persons. Occurrence of a first stroke or myocardial infarction is known to increase the risk for further strokes or myocardial infarctions. All events subsequent to the first in a person's observation period were not included in the analysis, yet each person was followed for the duration of his or her continuous enrollment period. Thus, his or her predefined observation period was preserved. Although excluding subsequent events could underestimate the absolute risk for events, this is unlikely to have any material effect on the relative risk (the outcome of our study). A similar approach was taken in a study exploring the risk for myocardial infarction and stroke after acute infection and vaccination (6). **Appendix Table 2** shows the number of subsequent events excluded from each of our analyses.

Overlapping Risk Periods

Some persons had several dental procedures during their observation period. When 2 or more procedures occur within 24 weeks of each other, the risk periods for these procedures overlap. A simple convention to address overlapping risk periods is that later exposures take precedence over earlier ones (14). We used this convention in our study; thus, when a person had 2 or more dental procedures and a later procedure occurred at some point during the risk period of an earlier procedure, a new 24-week risk period started from that point. This means that the later procedure takes precedence, although it does not replace the earlier procedure. The earlier procedure is not ignored. **Appendix Figure 3** illustrates our convention with 2 possible scenarios. First, if

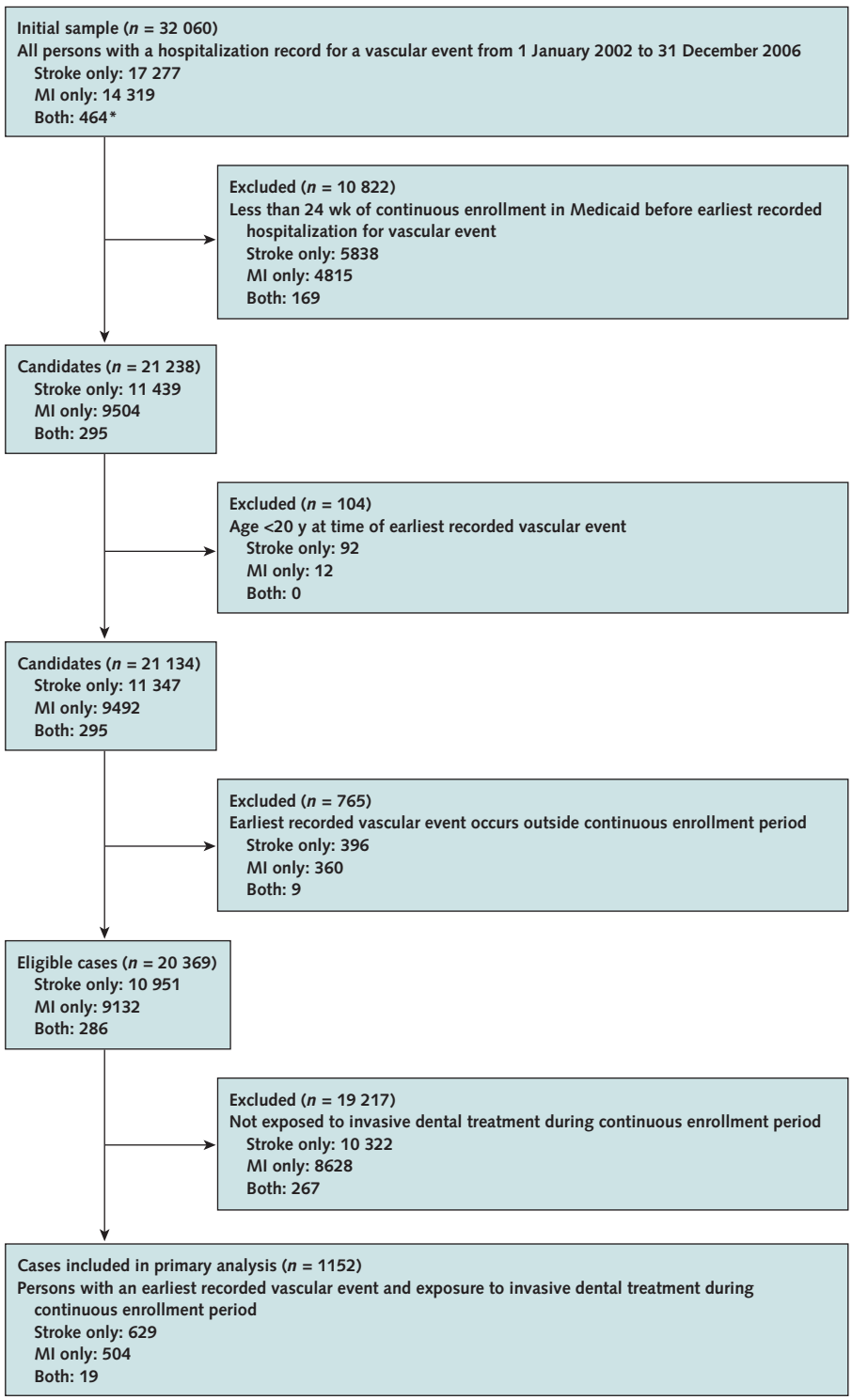
a person had a dental procedure followed by a vascular event 2 weeks later and then a second dental procedure 20 weeks after the first, the vascular event would not be classified as occurring during baseline; it would be classified as occurring in the risk period corresponding to the first procedure (**Appendix Figure 3,** *scenario A*). However, a vascular event occurring 2 weeks after the second dental procedure would be classified as occurring during the risk period of this second procedure rather than during the risk period of the first (**Appendix Figure 3,** *scenario B*). This convention reflects the actual exposure experience: In both scenarios, the event occurred 2 weeks after exposure.

Appendix Table 1. **Distribution of Invasive Dental Procedures Found in Study Participants' Records**

Dental Procedure Code*	Description	Persons With ≥1 Procedure Record, *n* (%)		
		Patients With Vascular Events (*n* = 1152)†	Patients With Ischemic Stroke (*n* = 650)	Patients With Myocardial Infarction (*n* = 525)
D7210	Surgical removal of erupted tooth requiring elevation of mucoperiosteal flap and removal of bone, section of tooth, or both	847 (73.5)	499 (76.8)	364 (69.3)
D7250	Surgical removal of residual tooth roots (cutting procedure)	151 (13.1)	90 (13.8)	67 (12.8)
D7310	Alveoloplasty in conjunction with extractions (≥4 teeth or tooth spaces per quadrant)	104 (9.0)	49 (7.5)	55 (10.5)
D7510	Incision and drainage of abscess (intraoral soft tissue)	25 (2.2)	15 (2.3)	11 (2.1)
D4341	Periodontal scaling and root planning (≥4 teeth per quadrant)	23 (2.0)	7 (1.1)	16 (3.0)
D7320	Alveoloplasty not in conjunction with extractions (≥4 tooth spaces per quadrant)	16 (1.4)	9 (1.4)	8 (1.5)
D7240	Removal of impacted tooth (completely bony)	15 (1.3)	10 (1.5)	5 (1.0)
D7230	Removal of impacted tooth (partially bony)	13 (1.1)	8 (1.2)	6 (1.1)
D4211	Gingivectomy or gingivoplasty (1–3 contiguous teeth or bounded teeth spaces per quadrant)	6 (0.5)	3 (0.5)	5 (1.0)
D7471	Removal of lateral exostosis (maxilla or mandible)	5 (0.4)	3 (0.5)	2 (0.4)
D4210	Gingivectomy or gingivoplasty (≥4 contiguous teeth or bounded teeth spaces per quadrant)	4 (0.3)	1 (0.2)	3 (0.6)
D7241	Removal of impacted tooth (completely bony, with unusual surgical complications)	4 (0.3)	3 (0.5)	1 (0.2)
D7999	Unspecified oral surgery procedure, by report	4 (0.3)	0 (0)	4 (0.8)
D7540	Removal of reaction-producing foreign bodies (musculoskeletal system)	3 (0.3)	3 (0.5)	0 (0)
D3410	Apicoectomy or periradicular surgery (anterior)	2 (0.2)	1 (0.2)	1 (0.2)
D7960	Frenulectomy (frenectomy or frenotomy) as a separate procedure	2 (0.2)	0 (0)	2 (0.4)
D7970	Excision of hyperplastic tissue (per arch)	2 (0.2)	0 (0)	2 (0.4)
D3421	Apicoectomy or periradicular surgery (bicuspid [first root])	1 (0.1)	1 (0.2)	0 (0)
D4342	Periodontal scaling and root planning (1–3 teeth per quadrant)	1 (0.1)	1 (0.2)	0 (0)
D7290	Surgical repositioning of teeth	1 (0.1)	0 (0)	1 (0.2)
D7321	Alveoloplasty not in conjunction with extractions (1–3 teeth or tooth spaces per quadrant)	1 (0.1)	0 (0)	1 (0.2)
D7410	Excision of benign lesion ≤1.25 cm	1 (0.1)	0 (0)	1 (0.2)
D7460	Removal of benign nonodontogenic cyst or tumor (lesion diameter ≤1.25 cm)	1 (0.1)	0 (0)	1 (0.2)
D7461	Removal of benign nonodontogenic cyst or tumor (lesion diameter >1.25 cm)	1 (0.1)	0 (0)	1 (0.2)
D7520	Incision and drainage of abscess (extraoral soft tissue)	1 (0.1)	1 (0.2)	0 (0)
D7550	Partial ostectomy or sequestrectomy for removal of nonvital bone	1 (0.1)	1 (0.2)	0 (0)

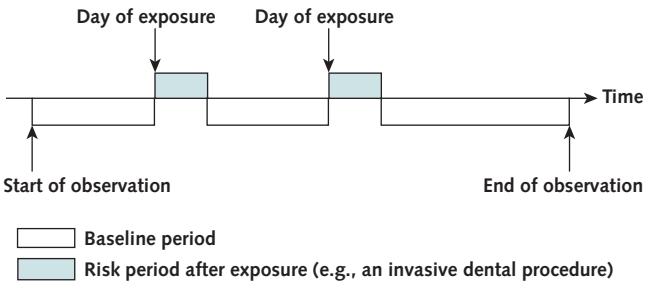
* From Current Dental Terminology (16).
† Persons included in the primary analysis of vascular events.

Appendix Figure 1. **Study flow diagram.**



MI = myocardial infarction.
* Individuals who had both an ischemic stroke and an MI during the study period.

Appendix Figure 2. **Pictorial representation of the self-controlled case series method.**



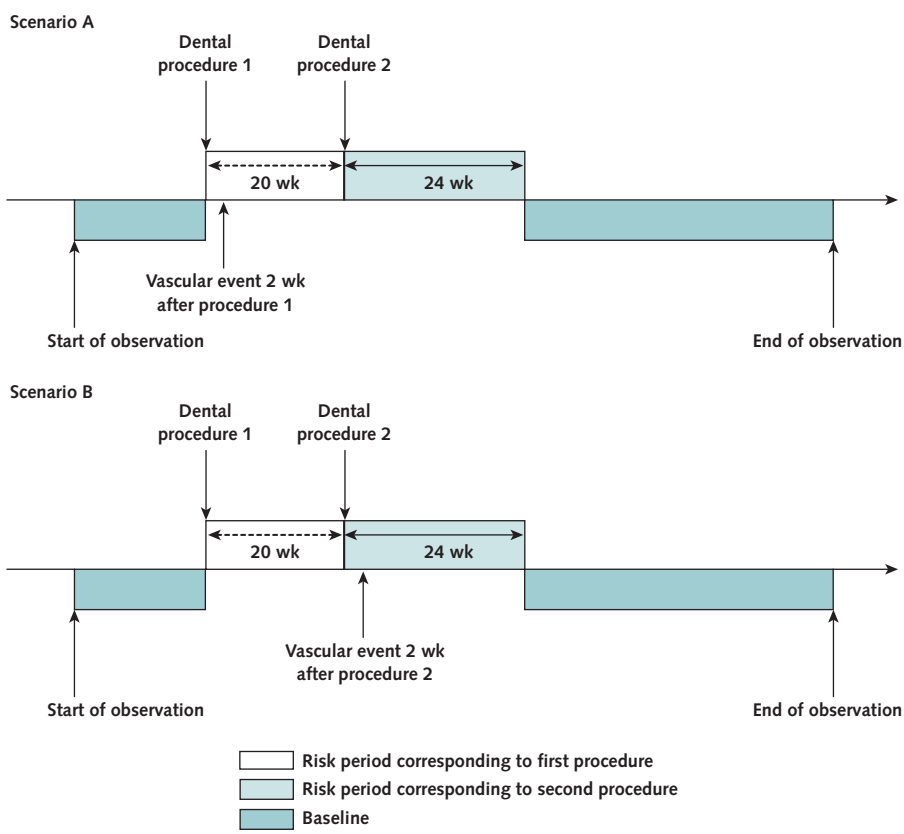
A single participant who had 2 exposures during the observation period is shown. The outcome event could occur at any time during the observation period.

Appendix Table 2. **Cases With a Subsequent Event and Number of Subsequent Events Excluded From Analysis**

Outcome	Cases Included, *n*	Cases With a Subsequent Event, *n* (%)	Subsequent Events Excluded (Range), *n*
Vascular event*	1152	126 (10.9)	158 (2–5)
Ischemic stroke	650	73 (11.2)	93 (2–5)
Myocardial infarction	525	38 (7.2)	46 (2–3)

* Ischemic stroke or myocardial infarction.

Appendix Figure 3. **Pictorial representation of overlapping risk periods.**



Appendix B Published paper "Acute maternal infection and risk of pre-eclampsia: a population-based case-control study"

OPEN ACCESS Freely available online

PLOS ONE

Acute Maternal Infection and Risk of Pre-Eclampsia: A Population-Based Case-Control Study

Caroline Minassian[1]*, Sara L. Thomas[1], David J. Williams[2], Oona Campbell[1], Liam Smeeth[1]

1 Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom, **2** Institute for Women's Health, University College London Hospital, London, United Kingdom

Abstract

Background: Infection in pregnancy may be involved in the aetiology of pre-eclampsia. However, a clear association between acute maternal infection and pre-eclampsia has not been established. We assessed whether acute urinary tract infection, respiratory tract infection, and antibiotic drug prescriptions in pregnancy (a likely proxy for maternal infection) are associated with an increased risk of pre-eclampsia.

Methods and Findings: We used a matched nested case-control design and data from the UK General Practice Research Database to examine the association between maternal infection and pre-eclampsia. Primiparous women aged at least 13 years and registered with a participating practice between January 1987 and October 2007 were eligible for inclusion. We selected all cases of pre-eclampsia and a random sample of primiparous women without pre-eclampsia (controls). Cases (n = 1533) were individually matched with up to ten controls (n = 14236) on practice and year of delivery. We calculated odds ratios and 95% confidence intervals for pre-eclampsia comparing women exposed and unexposed to infection using multivariable conditional logistic regression. After adjusting for maternal age, pre-gestational hypertension, diabetes, renal disease and multifetal gestation, the odds of pre-eclampsia were increased in women prescribed antibiotic drugs (adjusted odds ratio 1.28;1.14–1.44) and in women with urinary tract infection (adjusted odds ratio 1.22;1.03–1.45). We found no association with maternal respiratory tract infection (adjusted odds ratio 0.91;0.72–1.16). Further adjustment for maternal smoking and pre-pregnancy body mass index made no difference to our findings.

Conclusions: Women who acquire a urinary infection during pregnancy, but not those who have a respiratory infection, are at an increased risk of pre-eclampsia. Maternal antibiotic prescriptions are also associated with an increased risk. Further research is required to elucidate the underlying mechanism of this association and to determine whether, among women who acquire infections in pregnancy, prompt treatment or prophylaxis against infection might reduce the risk of pre-eclampsia.

Citation: Minassian C, Thomas SL, Williams DJ, Campbell O, Smeeth L (2013) Acute Maternal Infection and Risk of Pre-Eclampsia: A Population-Based Case-Control Study. PLoS ONE 8(9): e73047. doi:10.1371/journal.pone.0073047

Editor: Andrew Dewan, Yale School of Public Health, United States of America

Received April 4, 2013; **Accepted** July 16, 2013; **Published** September 3, 2013

Funding: This study was funded by a Wellcome Trust Senior Research Fellowship in Clinical Science awarded to Professor LS (grant number 098504/Z/12/Z). Dr DJW receives part of his funding from UCL/UCLH Biomedical Research Centre. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: caroline.minassian@lshtm.ac.uk

Introduction

Pre-eclampsia is a multi-system vascular syndrome of pregnancy defined by the gestational onset of hypertension and proteinuria, typically occurring after 20 weeks' gestation. It is a major cause of maternal and perinatal morbidity and mortality worldwide, its incidence ranging between 2% and 8% in nulliparous women. [1] Despite advances in knowledge, we still have a limited ability to predict or prevent pre-eclampsia. While its aetiology is generally considered to be multifactorial, involving both maternal and placental contributions, [2] there is increasing evidence that inflammation plays a central pathogenic role. [3] Impaired vascular endothelial function, which can derive from inflammation, is evident among women prior to developing pre-eclampsia. [4] Poor placental perfusion as a result of inadequate placentation is a key inflammatory stimulus for many women with pre-eclampsia. However, any factor that provokes the maternal systemic inflammatory response, such as infection, may contribute to the overall inflammatory burden and the development of pre-eclampsia.

A growing body of evidence suggests that infection, a common cause of inflammation and of endothelial dysfunction, may be involved in the aetiology of pre-eclampsia. [5] An increased risk of pre-eclampsia associated with maternal periodontal disease has been well-documented.[6–8] Studies based on serological markers of chronic infections have also yielded positive findings,[9–14] although temporal associations in these studies are uncertain.

Acute maternal infections such as urinary tract infection (UTI) may also play a role in pre-eclampsia, possibly by amplifying the maternal systemic inflammatory response. A meta-analysis of observational studies examining the relationship between maternal infections and pre-eclampsia [5] reported a summary odds ratio for pre-eclampsia of 1.57 (95% CI 1.45–1.70) in women with UTI in pregnancy. However, there was marked heterogeneity between

studies and results were inconsistent. Findings from two more recent studies are conflicting: one, a large population-based cohort study [15] reported an increased risk of pre-eclampsia among women with maternal UTI, while a case-control study found no association. [16] Factors such as the timing of infection in relation to pre-eclampsia were not investigated in these studies, and the findings may have been confounded by renal disease, or biased by increased ascertainment of UTI in pregnancy, particularly among women at risk of pre-eclampsia. Data on the effects of other acute maternal infections are lacking. Thus a clear role for acute infection in the aetiology of pre-eclampsia has not been established.

The large sample size afforded by the UK General Practice Research Database (GPRD) provided a unique opportunity to address these issues. We assessed the gestational onset of UTI, as well as respiratory tract infection (RTI), and maternal antibiotic prescriptions (a likely proxy for infection). Examining the role of antibiotic prescriptions and infections in different organ systems would, if positive, suggest that the effect of acute infection on the risk of pre-eclampsia is generic and not specific to one type of infection.

Methods

The General Practice Research Database

The GPRD is an electronic UK population-based primary care database. Established in January 1987, it holds anonymised longitudinal patient records, routinely recorded as part of patients' normal care, for over 10 million patients registered to over 600 general practices. More than 98% of the UK population are registered with a general practitioner (GP) and practices contributing to the database are representative of practices throughout the UK. [17] In addition to being a rich data source, the GPRD has high data validity. [18] Each participating practice is assigned an "up-to-standard" date indicating when data recording complied with specific quality measures (based on an assessment of the completeness, continuity and plausibility of data). Data are subject to ongoing evaluation, verification and validation procedures to ensure they are research-quality [19].

Ethics Statement

The electronic health records used for this study comprised data from the Full Feature GPRD obtained under licence from the UK Medicines and Healthcare Products Regulatory Agency. GPRD data are used extensively for public health research, and the GPRD has stringent procedures for maintaining confidentiality of personal data. All data provided to researchers are anonymised to ensure that individual patients cannot be identified. In addition, patients have the right to opt out from the use of their anonymised data. The use of these data for this study was approved by the Independent Scientific Advisory Committee of the GPRD (protocol 07_094) and by the London School of Hygiene and Tropical Medicine Ethics Committee (application number 5283).

Study Design and Participants

We used a matched nested case-control study design to examine the association between acute maternal infections and pre-eclampsia. Participants were derived from a source population of all female patients registered with a practice contributing to the GPRD between 1st January 1987 and 31st October 2007 inclusive and who had a pregnancy during this period.

Eligibility criteria. Because pre-eclampsia usually develops in the later stages of pregnancy, a woman must have completed her pregnancy to have the opportunity to become a case. Therefore, only women with a documented completed pregnancy, defined as an end-of-pregnancy record indicating the woman had delivered a live birth or stillbirth (e.g. "birth details"), or was soon to deliver (e.g. "antenatal 37 week examination"), were potential candidates. Pregnancies resulting in miscarriage or termination were not included since they were likely to end before a woman had reached the required gestational age to be at risk of being diagnosed with pre-eclampsia. As pre-eclampsia is much more common in nulliparous women, [20] we restricted the study population to women with a first documented completed pregnancy during the study period.

Women aged at least 13 years at delivery and whose data throughout the gestational period (from conception to delivery) were within up-to-standard follow-up were eligible for inclusion. The follow-up criterion helped ensure that diagnoses and events pertaining to the pregnancy were captured. Clinical entries in the data were coded using the Oxford Medical Information System (OXMIS) and Read coding system. We selected as potential cases all those with a clinical diagnosis of pre-eclampsia, defined as a Read/OXMIS code for pre-eclampsia, eclampsia or the severe pre-eclampsia variant HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), in their first documented completed pregnancy. We selected as potential controls a random sample of eligible women with no diagnosis of pre-eclampsia anywhere in their medical data. This ensured controls had no pre-eclampsia in their first documented completed pregnancy, and no history of pre-eclampsia possibly relating to an earlier (unrecorded) pregnancy.

Exclusions. To help ensure cases and controls were primiparous, we excluded women with evidence for an earlier completed pregnancy (e.g. a record of "previous caesarean section" before their earliest delivery record). To distinguish cases of pre-eclampsia from women with essential or secondary hypertension which became clinically apparent during pregnancy, we excluded women with no evidence of high blood pressure until pregnancy but whose hypertension did not resolve six to 12 months post-delivery. The identification of cases and controls for inclusion in the study is illustrated in **Figure 1**.

Matching. Cases were matched with controls on GP practice to allow for variability in recording and prescribing habits between practices, and on year of delivery (an absolute difference of up to 12 months between cases' and controls' estimated delivery dates) to ensure they were contemporaneous. All possible case-control matches were identified and up to ten controls per case were selected at random without replacement. Although any additional gain in power is minimal if the case-control ratio exceeds 1:4, the number of eligible controls exceeded the number of cases by more than twenty-fold: thus, increasing the control-per-case ratio beyond four posed no additional cost or effort in data collection.

Dating Pregnancies

In the absence of systematically recorded information on the exact timing of pregnancy, we used information from antenatal records indicating gestational age, delivery records indicating the number of days or weeks postnatal, and recorded estimates of the expected date of delivery and first day of a woman's last menstrual period (LMP) to obtain our best estimates of the start and end of each woman's completed pregnancy. We estimated the timing of trimesters adopting a common convention: first trimester (first day of LMP to 13 weeks), second (weeks 14 to 26), and third (week 27 to delivery).

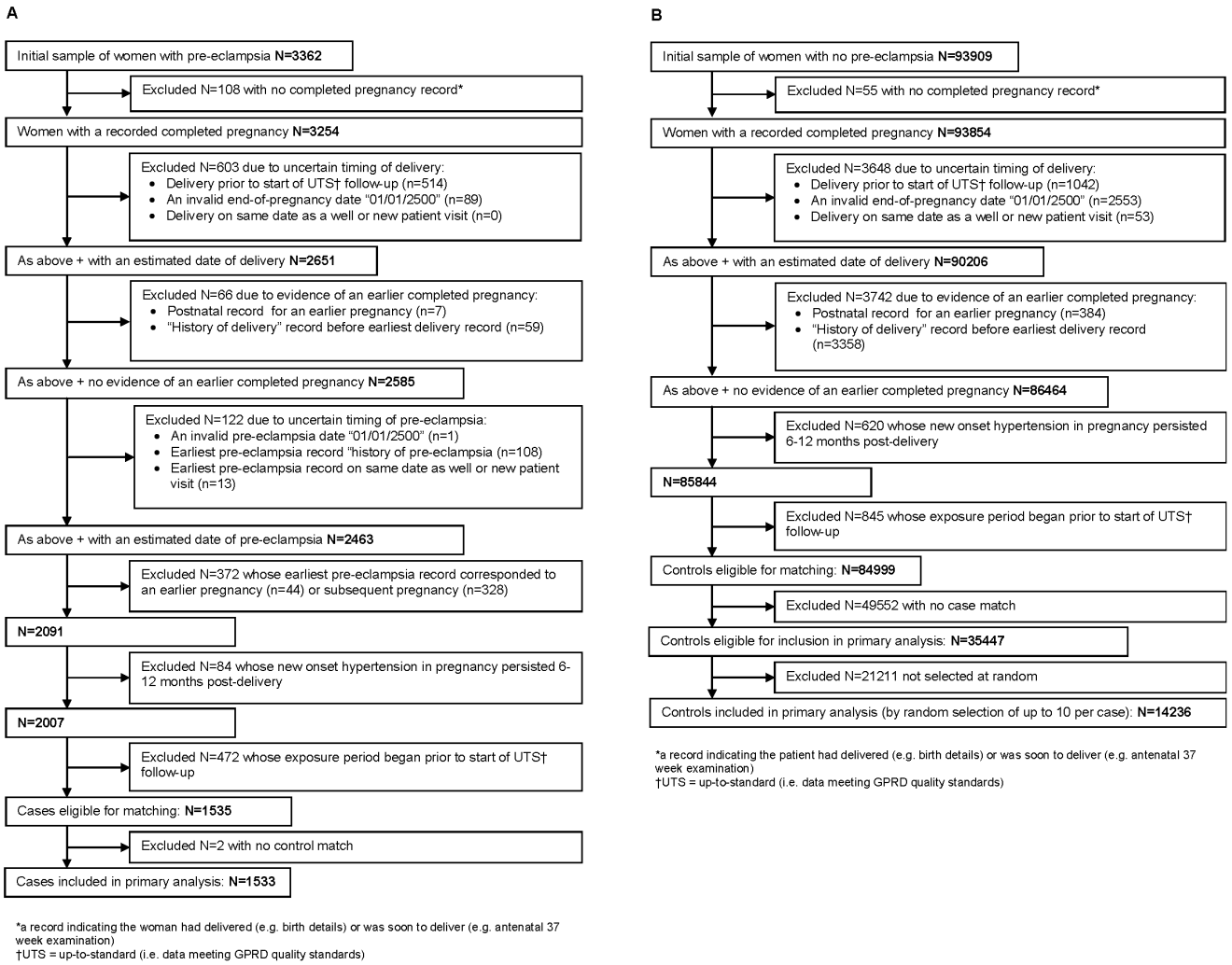


Figure 1. Identification of study participants included in the primary analysis: A) cases (n = 1533); B) controls (n = 14236).
doi:10.1371/journal.pone.0073047.g001

Exposures

The exposure period for each participant began on the first day of LMP and ended at the index date, defined as the date of pre-eclampsia diagnosis (for cases). For controls, the index date was the date they reached the same gestational age as their matched case at pre-eclampsia diagnosis. This was to ensure the duration of the exposure period for cases and their matched controls was comparable.

We extracted data on Read/OXMIS codes for acute UTIs (manifest as asymptomatic bacteriuria, cystitis, or pyelenephritis) and RTIs (excluding non-specific or minor upper RTIs and symptoms such as sore throat), and on antibiotic drug prescriptions over the exposure period. When a woman had more than one record of infection or antibiotic prescription, a minimum of 29 days between records of the same type was required for these to be considered distinct episodes of infection (rather than repeat records for the same infection).

Potential confounders. Data on the following potential risk factors were extracted: maternal age; pre-gestational renal disease, diabetes, hypertension and asthma; multifetal gestation; pre-pregnancy body mass index (BMI); maternal smoking (a known protective factor); previous early pregnancy loss; and assisted reproductive technology (ART), defined as in vitro fertilization and related techniques (including gamete intrafallopian transfer and embryo transfer). To address the possibility that some infections may be more likely to be recorded among women who consult with their GP more frequently, we measured the number of consultations and duration of follow-up each woman had prior to pregnancy. This allowed comparison of cases' and controls' pre-pregnancy consultation behaviour.

Statistical Analysis

We used multivariable conditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals for pre-eclampsia comparing pregnant women exposed and not exposed to each type of infection or to antibiotic prescriptions. The primary analysis assessed the effect of each exposure at any time during the exposure period. Subsequent analyses explored the effects of increasing episodes of infection over the exposure period. Potential confounding factors associated with pre-eclampsia in crude analyses were assessed in more complex models and retained if they made an appreciable difference to the infection (or antibiotics) OR. Maternal age (controlled for in five-year age groups) and pre-existing renal disease were included in all models a priori. Likelihood ratio tests were used to assess statistical significance.

To reduce the possibility of misdiagnosis of UTI among women with pre-eclampsia due to identification of proteinuria, we repeated the analyses for UTI and antibiotics among cases who developed pre-eclampsia in the third trimester and their matched controls, and assessed the effect of exposure to UTI or antibiotics in the first and second trimesters only versus no exposure at any time in pregnancy. These third trimester pre-eclampsia cases were unlikely to have proteinuria detected during the first two trimesters, thus minimising the potential for such misclassification.

In sensitivity analyses, we excluded the following: women aged less than 18 years as their pregnancy outcomes and underlying risk profile may differ from older women; women with pre-existing hypertension, to rule out the possibility of misdiagnosis of pre-eclampsia; controls with new onset hypertension during pregnancy which resolved shortly after delivery, as they may have had pre-eclampsia even in the absence of a clinical diagnosis; and ART pregnancies which may have a higher risk of developing pre-eclampsia. To reduce the possibility that events we identified throughout the exposure period (e.g. infections) may have referred to past diagnoses that were recorded retrospectively within the first few months after a patient joined a practice, we extended the up-to-standard follow-up criterion to include only women with at least six months up-to-standard follow-up prior to conception. To address the possibility that pre-eclampsia diagnoses made in earlier years were less exact, we restricted our analyses to pregnancies in year 2000 onwards following publication of the first recommended consensus definition of pre-eclampsia. [21] Finally, to assess whether the effect of infection differed according to the timing of onset of pre-eclampsia, or the severity, we conducted separate analyses for cases with early-onset (<34 weeks' gestation) versus late-onset (≥34 weeks' gestation) pre-eclampsia, and for cases with documented severe pre-eclampsia, eclampsia or HELLP syndrome.

Data were analysed using Stata, release 12 (StataCorp., College Station, Texas).

Results

Data were obtained on all women with a clinical diagnosis of pre-eclampsia during the study period (3362 potential cases) and a large random sample of women who had a pregnancy during this period and no recorded diagnosis of pre-eclampsia (93909 potential controls). After applying the eligibility, exclusion and matching criteria, 1533 pre-eclampsia cases and 14236 controls were included in the primary analysis (see **Figure 1**). The commonest reason for not being eligible was uncertainty about the timing of pregnancy. **Table 1** summarizes the demographic and risk profile of study participants. The median gestational age of cases at pre-eclampsia diagnosis was 38.1 weeks (interquartile range (IQR) 34.9 to 39.9 weeks), and most cases (79.5%) were late-onset (≥34 weeks' gestation). The majority of pre-eclampsia diagnoses were non-specific regarding severity (47.3%) or mild (32.0%); the remaining 20.7% were severe pre-eclampsia, eclampsia or HELLP syndrome. Cases and controls were of similar age at delivery (median 28.3 years for cases; 28.2 years for controls) and shared similar pre-pregnancy consultation behaviour (median 11 consultations over 2.5 years). A higher proportion of cases were overweight or obese (30.3%) compared to controls (20.4%), and cases were less likely to smoke (18.5%) than controls (23.3%).

During their first completed pregnancy, 528 (34.4%) cases and 4110 (28.9%) controls were prescribed an antibiotic drug, 182 (11.9%) cases and 1376 (9.7%) controls had one or more UTI, and 77 (5.0%) cases and 781 (5.5%) controls had one or more RTI. The timing of each exposure by pregnancy trimester is shown in **Table 2**. Less than half of women with an antibiotic prescription in pregnancy had a record of UTI or RTI (42.8% of cases; 44.3% of controls).

Crude and adjusted ORs for the association between maternal infection and pre-eclampsia are summarized in **Table 3**. Antibiotic prescriptions (adjusted OR 1.28; 1.14–1.44) and UTI (adjusted OR 1.22; 1.03–1.45) in pregnancy were associated with an increased risk of pre-eclampsia after controlling for maternal age; pre-gestational renal disease, diabetes and hypertension; and multifetal gestation. We found no evidence for confounding by prior early pregnancy loss. Findings were virtually identical when we addressed the potential for differential misclassification of UTI due to detection of proteinuria in cases by repeating analyses after confining the exposure window for UTI and antibiotics to the first two trimesters and excluding cases (n = 41) with very early onset pre-eclampsia prior to the third trimester: adjusted OR for antibiotics 1.26 (1.11–1.43) and UTI 1.22 (1.01–1.49). Further adjustment for pre-pregnancy BMI and maternal smoking among individuals with BMI and smoking data (1048 cases and 7216 controls) made no material difference to our findings (**Table S1**).

Women exposed to RTI in pregnancy were more likely to have pre-existing asthma (29.5%) than women not exposed to RTI (17.1%), and were more likely to smoke (28.6%) than those not exposed (22.5%). We found no association between RTI and pre-eclampsia in either the crude or adjusted analyses. The inclusion of pre-existing asthma to our model did not alter the RTI OR.

The frequency distribution of the number of episodes (none, one, more than one) of infection or antibiotic treatment is shown in **Table S2**. No evidence of a dose-response association was demonstrated (data not shown). Consistent with the primary analysis, we observed an increased risk of both early- and late-onset pre-eclampsia associated with maternal antibiotics prescriptions and UTI, with no evidence for a clear difference between these two sub-groups (**Table S3**). We conducted additional sensitivity analyses as outlined in the methods. Each of these analyses yielded estimates similar to those obtained in our primary analysis (**Table S4**).

Discussion

Our study has shown that women who acquire UTI during pregnancy, and women prescribed antibiotics during pregnancy (a likely proxy for acute infection) are at an increased risk of pre-eclampsia. The increased risk of pre-eclampsia developing in the third trimester following UTI or antibiotic prescriptions in the first two trimesters suggests that acute maternal infection may play a role in the pathogenesis of pre-eclampsia. However, we found no evidence for an increased risk of pre-eclampsia among women who acquire RTI during pregnancy.

A major strength of our study is the use of a population-based cohort of women from which we selected all cases of pre-eclampsia in a first completed pregnancy and a random sample of primiparous controls without pre-eclampsia. Our nested case-control design avoids the common problem of selection bias inherent in many case-control studies, particularly those in which the base population giving rise to the cases is less well-defined. Matching on GP practice allowed for variability in recording and prescribing habits between practices, and helped ensure that cases and controls were comparable on a range of socio-economic and environmental indicators. The additional criterion of allowing no more than 12 months between case and control delivery dates ensured pregnancies within matched sets were contemporaneous.

Table 1. Characteristics of study participants.

Characteristic n (%)	Cases (N = 1533)	Controls (N = 14236)
Maternal age at delivery (yrs)		
<20	132 (8.6)	1470 (10.3)
20–24	340 (22.2)	2846 (20.0)
25–29	478 (31.2)	4492 (31.6)
30–34	406 (26.5)	3803 (26.7)
35–39	146 (9.5)	1348 (9.5)
40–44	29 (1.9)	239 (1.7)
≥45	2 (0.1)	38 (0.3)
median, IQR	*28.3, 23.9–32.3*	*28.2, 23.9–32.1*
Pre-pregnancy BMI (kg/m^2)		
<18.5 (underweight)	26 (1.7)	526 (3.7)
18.5–25 (normal)	620 (40.4)	6618 (46.5)
25–30 (overweight)	272 (17.7)	1959 (13.8)
30+ (obese)	192 (12.5)	946 (6.7)
unknown	423 (27.6)	4187 (29.4)
median, IQR	*24.1, 21.6–27.9*	*22.7, 20.7–25.6*
Smoking status in pregnancy		
non-smoker	834 (54.4)	6680 (46.9)
ex-smoker	166 (10.8)	1476 (10.4)
current smoker	283 (18.5)	3311 (23.3)
unknown	250 (16.3)	2769 (19.5)
Practice level socioeconomic status[a]		
IMD score *[median, IQR]*	*16.2, 8.7–30.1*	*16.3, 8.4–30.2*
Patient level socioeconomic status		
IMD score *[median, IQR]*	*14.3, 8.4–25.7*	*14.8, 8.3–26.4*
unknown	744 (48.5)	6734 (47.3)
Pre-existing hypertension	161 (10.5)	875 (6.2)
Pre-existing renal disease	4 (0.3)	25 (0.2)
Pre-existing diabetes	28 (1.8)	166 (1.2)
Pre-existing asthma	291 (19.0)	2509 (17.6)
Previous miscarriage or termination	298 (19.4)	2869 (20.2)
Multiple pregnancy	25 (1.6)	121 (0.9)
ART pregnancy	11 (0.7)	84 (0.6)
Consultations with GP pre-pregnancy *[median, IQR]*	*11, 4–27*	*11, 4–24*
UTS follow-up pre-pregnancy (yrs) *[median, IQR]*	*2.4, 0.9–5.1*	*2.5, 1.1–5.3*

Abbreviations: IMD = Index of Multiple Deprivation score based on practice post-code (practice level socioeconomic status) or patient post-code (patient level socioeconomic status). The higher the score the greater the deprivation. UTS = up-to-standard (i.e. data meeting GPRD quality standards). ART = assisted reproductive technology.

[a]matching variable.

doi:10.1371/journal.pone.0073047.t001

Another strength of this study is that we were able to include data on a substantial number of well-known risk factors for pre-eclampsia, some of which, most notably renal disease and diabetes, were not accounted for in previous studies of UTI and pre-eclampsia. [5,15,16] The associations with UTI and antibiotics persisted even after adjustment for maternal age; pre-existing renal disease, diabetes and hypertension; and multifetal gestation. We cannot exclude the possibility of residual confounding if disease risk factors were not recorded for some women; for example, the low prevalence of pre-existing renal disease among cases (0.3%) and controls (0.2%) suggests ascertainment of renal disease may be limited to the more severe end of the disease spectrum. However, this is unlikely to be a major concern since it is the more severe disease (stages 3–5) which predisposes to pre-eclampsia, rather than mild renal disease. [22] Missing information on maternal smoking and pre-pregnancy BMI limited our ability to assess their effects in the entire study population. Nevertheless, additional adjustment for BMI and smoking made no material difference to our findings. The similar pre-pregnancy consultation behaviour of cases and controls suggests our findings are unlikely to be explained by possible increased ascertainment of infection among cases due to differential health-seeking behaviour.

We were able to restrict the study population to women in their first documented completed pregnancy in their primary care

Table 2. Frequency of maternal infection or antibiotic treatment in pregnancy and by pregnancy trimester.

Exposure in pregnancy[a] n (%)	Cases (N = 1533)	Controls (N = 14236)
Antibiotic treatment		
Any time in pregnancy	528 (34.4)	4110 (28.9)
First trimester	221 (14.4)	1684 (11.8)
Second trimester	238 (15.5)	1952 (13.7)
Third trimester	203 (13.2)	1520 (10.7)
Urinary tract infection		
Any time in pregnancy	182 (11.9)	1376 (9.7)
First trimester	64 (4.2)	463 (3.3)
Second trimester	81 (5.3)	606 (4.3)
Third trimester	57 (3.7)	487 (3.4)
Respiratory tract infection		
Any time in pregnancy	77 (5.0)	781 (5.5)
First trimester	31 (2.0)	293 (2.1)
Second trimester	29 (1.9)	307 (2.2)
Third trimester	24 (1.6)	218 (1.5)

Note some women had more than one exposure in the same (or in another) trimester.

[a]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the case's index date).

doi:10.1371/journal.pone.0073047.t002

record, and excluded women with evidence of an earlier (unrecorded) completed pregnancy, for example, a record indicating parity>0 prior to the earliest delivery record. While not guaranteeing that this was their first ever completed pregnancy, it was likely to be the first for a large majority. In addition, because the risk of infections is unlikely to have a strong relationship with parity, the scope for confounding by parity is limited. A further advantage of our approach is that it reduced the potential for confounding by change in paternity or by inter-pregnancy interval among multiparas [23].

While there is no universal agreement on the definition of pre-eclampsia, [2] a diagnosis has major consequences for a pregnant woman and is unlikely to be recorded speculatively. In 2000, the National High Blood Pressure Education Program Working Group developed diagnostic criteria for pre-eclampsia, [21] recommended in the American College of Obstetricians and Gynecologists practice guidelines for diagnosing pre-eclampsia. [24] To improve the validity of our case definition we excluded from the primary analysis women whose new onset hypertension in pregnancy did not resolve following delivery, in line with this consensus definition. While we cannot rule out the possibility of misclassification of pre-eclampsia, this criterion helped distinguish cases of pre-eclampsia from women with essential or secondary hypertension that became clinically apparent during pregnancy. Furthermore, restricting our analyses to pregnancies (and hence pre-eclampsia diagnoses) in year 2000 onwards made no material difference to our findings.

We used information from antenatal, perinatal and postnatal records to estimate the date of conception, delivery, and trimesters for all primiparous pregnancies. Although the timing may be inexact, the same method was used for dating case and control pregnancies, so any imprecision is likely to be non-differential. The main consequence for this study is that some infections early in pregnancy may have been missed if they were misclassified as occurring prior to conception. We used the date of diagnosis (or antibiotic prescription) rather than the date of onset of infection. However, the majority of patients, even with upper RTIs, attend their general practitioner within three days of onset. [25] This small degree of imprecision is unlikely to materially affect our results.

We recognize that not all infections lead to a GP consultation, so some may not have been recorded. However, such infections are more likely to be minor or asymptomatic; those severe enough to cause systemic inflammation are more likely to result in a consultation and be detected. We cannot rule out the possibility that the observed associations with maternal UTI and antibiotic prescriptions may in part be attributed to increased ascertainment of infections among women with problematic pregnancies. However, unlike previous studies we also investigated the effect of acute RTI; the null effect we observed for RTI suggests that ascertainment bias is unlikely.

Possible misclassification of UTI among women with pre-eclampsia due to detection of proteinuria was addressed by restricting the exposure period for infection to the first two trimesters, prior to the onset of pre-eclampsia in the third trimester. The resulting effect estimates for both UTI and antibiotics were virtually identical to those obtained in the primary analysis.

In our study, more than half of women with an antibiotic prescription in pregnancy had no urinary or respiratory indication, a finding which has previously been noted in primary care data. [26] While some antibiotics might have been prescribed prophylactically against recurrent infections, this is likely to be a small minority: the majority will be given for acute infections such as UTIs which are particularly common in pregnancy. [27] Nevertheless, we cannot exclude the possibility that our finding of an antibiotic effect may reflect an association with the drugs

Table 3. The association between maternal infection and pre-eclampsia: crude and adjusted odds ratios for matched cases (n = 1533) and controls (n = 14236).

Exposure in pregnancy[a]	Matched crude OR (95% CI)	Matched adjusted[b] OR (95% CI)
Antibiotic treatment	1.29 (1.15–1.44)	1.28 (1.14–1.44)
Urinary tract infection	1.23 (1.04–1.46)	1.22 (1.03–1.45)
Respiratory tract infection	0.91 (0.71–1.15)	0.91 (0.72–1.16)

[a]any time from 1st day of last menstrual period (LMP) to index date (for cases this is the date of pre-eclampsia, for controls this is the date they reached the same gestational age as their matched case at the case's index date).

[b]ORs adjusted for maternal age; pre-gestational hypertension, diabetes and renal disease; and multifetal gestation. In addition, ORs for UTI and RTI are mutually adjusted for.

doi:10.1371/journal.pone.0073047.t003

themselves rather than an association with acute infection (the main indication for their use).

Various hypotheses have been proposed to explain the mechanism by which maternal infection may be associated with pre-eclampsia. A key feature of pre-eclampsia is the greater systemic inflammatory response of women who develop the syndrome compared to women who have normal pregnancies, [28] which suggests that inflammation may play an important role in the pathogenesis. Acute infections such as UTI are an important source of inflammation. Thus, the underlying mechanism of infection may be indirect, by enhancing the maternal systemic inflammatory response. It may also include direct effects of infectious agents increasing the risk of acute uteroplacental atherosis, [29] resulting in increased systemic inflammation and vascular endothelial dysfunction preceding the clinical onset of pre-eclampsia. Although the exact mechanism of the association is uncertain, our finding of an increased risk of pre-eclampsia associated with both acute UTI and maternal antibiotic prescriptions lends support to the hypothesis that maternal infection may play a pathogenic role. The relatively few individuals with more than one episode of infection limited our ability to reliably examine a dose-effect.

The absence of an association with RTI in our study is intriguing and warrants further investigation, although it does not preclude the possibility of a generic effect of infection on pre-eclampsia risk. Our adjusted analyses suggest this finding is unlikely to be explained by the higher prevalence of maternal smoking (known to protect against pre-eclampsia) among women with RTI. However, it may in part be due to incomplete ascertainment of RTI consultations. We excluded from our definition of RTI any non-specific RTI diagnoses (e.g. a record of "Acute respiratory infection" or "Respiratory tract infection") as it was unclear whether these were minor upper RTIs or more severe lower RTIs. We expect any such non-differential misclassification would lead to an underestimate of effect. The increased risk of pre-eclampsia we observed among pregnant women with UTI and with antibiotic prescriptions (a proxy for any acute maternal infection, including but not restricted to UTI or RTI) is consistent with a generic effect, suggesting as it does that the effect may not be specific to one type of infection.

We conclude that acute maternal UTI and antibiotic drug prescriptions in pregnancy (a likely proxy for infection) are associated with an increased risk of pre-eclampsia. Further research is required to elucidate the underlying mechanism of this association and to determine whether, among women who acquire infections in pregnancy, prompt treatment or prophylaxis against infection might reduce the risk of pre-eclampsia.

Supporting Information

Table S1 The association between maternal infection and pre-eclampsia: crude and adjusted odds ratios for matched cases and controls with data on pre-pregnancy BMI and smoking status in pregnancy (n = 1048 cases; n = 7216 controls).
(DOCX)

Table S2 Episodes of exposure to maternal infection or antibiotic treatment in pregnancy.
(DOCX)

Table S3 Adjusted odds ratios for early-onset (<34 weeks' gestation) and late-onset (≥34 weeks' gestation) pre-eclampsia.
(DOCX)

Table S4 Results of sensitivity analyses.
(DOCX)

Acknowledgments

We thank Tim Clayton (London School of Hygiene and Tropical Medicine) for his advice on developing a matching algorithm.

Author Contributions

Conceived and designed the experiments: LS. Analyzed the data: CM. Wrote the paper: CM. Acquisition of data: LS CM. Interpretation of data: CM LS SLT DJW OC. Critical revision of article for important intellectual content: CM LS SLT DJW OC. Final approval of the version to be published: CM LS SLT DJW OC.

References

1. Steegers EAP, von Dadelszen P, Duvekot JJ, Pijnenborg R (2010) Pre-eclampsia. Lancet 376: 631–644. doi:10.1016/S0140-6736(10)60279-6.
2. Trogstad L, Magnus P, Stoltenberg C (2011) Pre-eclampsia: Risk factors and causal models. Best practice & research Clinical obstetrics & gynaecology. doi:10.1016/j.bpobgyn.2011.01.007.
3. Borzychowski AM, Sargent IL, Redman CWG (2006) Inflammation and pre-eclampsia. Seminars in fetal and neonatal medicine 11: 309–316. doi:10.1016/j.siny.2006.04.001.
4. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ (2010) Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. Circulation 122: 478–487. doi:10.1161/CIRCULATIONAHA.109.895458.
5. Conde-Agudelo A, Villar J, Lindheimer M (2008) Maternal infection and risk of preeclampsia: systematic review and metaanalysis. American journal of obstetrics and gynecology 198: 7–22. doi:10.1016/j.ajog.2007.07.040.
6. Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S (2006) Periodontal disease and adverse pregnancy outcomes: a systematic review. BJOG?: an international journal of obstetrics and gynaecology 113: 135–143. doi:10.1111/j.1471-0528.2005.00827.x.
7. Ruma M, Boggess K, Moss K, Jared H, Murtha A, et al. (2008) Maternal periodontal disease, systemic inflammation, and risk for preeclampsia. American journal of obstetrics and gynecology 198: 389.e1–5. doi:10.1016/j.ajog.2007.12.002.
8. Cota LOM, Guimarães AN, Costa JE, Lorentz TCM, Costa FO (2006) Association between maternal periodontitis and an increased risk of preeclampsia. Journal of periodontology 77: 2063–2069. doi:10.1902/jop.2006.060061.
9. Von Dadelszen P, Magee LA, Krajden M, Alasaly K, Popovska V, et al. (2003) Levels of antibodies against cytomegalovirus and Chlamydophila pneumoniae are increased in early onset pre-eclampsia. BJOG?: an international journal of obstetrics and gynaecology 110: 725–730.
10. Heine RP, Ness RB, Roberts JM (2003) Seroprevalence of antibodies to Chlamydia pneumoniae in women with preeclampsia. Obstetrics and Gynecology 101: 221–226.
11. UstUn Y, Engin-UstUn Y, Ozkaplan E, Otlu B, Sait TekerekoGlu M (2010) Association of Helicobacter pylori infection with systemic inflammation in preeclampsia. The journal of maternal-fetal and neonatal medicine 23: 311–314. doi:10.3109/14767050903121456.
12. Aksoy H, Ozkan A, Aktas F, Borekci B (2009) Helicobacter pylori seropositivity and its relationship with serum malondialdehyde and lipid profile in preeclampsia. Journal of clinical laboratory analysis 23: 219–222. doi:10.1002/jcla.20330.
13. Pugliese A, Beltramo T, Todros T, Cardaropoli S, Ponzetto A (2008) Interleukin-18 and gestosis?: correlation with Helicobacter pylori seropositivity. Cell Biochemistry and Function 26: 817–819. doi:10.1002/cbf.
14. Xie F, Hu Y, Magee LA, Money DM, Patrick DM, et al. (2010) An association between cytomegalovirus infection and pre-eclampsia: a case-control study and data synthesis. Acta obstetricia et gynecologica Scandinavica 89: 1162–1167. doi:10.3109/00016349.2010.499449.
15. Mazor-Dray E, Levy A, Schlaeffer F, Sheiner E (2009) Maternal urinary tract infection: is it independently associated with adverse pregnancy outcome? The journal of maternal-fetal and neonatal medicine 22: 124–128. doi:10.1080/14767050802488246.
16. Shamsi U, Hatcher J, Shamsi A, Zuberi N, Qadri Z, et al. (2010) A multicentre matched case control study of risk factors for preeclampsia in healthy women in Pakistan. BMC women's health 10: 14. doi:10.1186/1472-6874-10-14.

17. Lawrenson R, Williams T, Farmer R (1999) Clinical information for research; the use of general practice databases. Journal of public health medicine 21: 299–304.
18. Herrett E, Thomas SL, Schoonen WM, Smeeth L, Hall AJ (2010) Validation and validity of diagnoses in the General Practice Research Database: a systematic review. British journal of clinical pharmacology 69: 4–14. doi:10.1111/j.1365-2125.2009.03537.x.
19. Walley T, Mantgani A (1997) The UK General Practice Research Database. Lancet 350: 1097–1099.
20. Hernández-Díaz S, Toh S, Cnattingius S (2009) Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. BMJ (Clinical research ed) 338: b2255.
21. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (2000) American Journal of Obstetrics and Gynecology 183: S1–S22. doi:10.1067/mob.2000.107928.
22. Munkhaugen J, Vikse BE (2009) New aspects of pre-eclampsia: lessons for the nephrologist. Nephrology, dialysis, transplantation?: official publication of the European Dialysis and Transplant Association - European Renal Association 24: 2964–2967. doi:10.1093/ndt/gfp341.
23. Trogstad LI, Eskild A, Magnus P, Samuelsen SO, Nesheim BI (2001) Changing paternity and time since last pregnancy; the impact on pre-eclampsia risk. A study of 547 238 women with and without previous pre-eclampsia. International journal of epidemiology 30: 1317–1322.
24. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. (2002) International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics 77: 67–75.
25. Little P, Williamson I, Warner G, Gould C, Gantley M, et al. (1997) Open randomised trial of prescribing strategies in managing sore throat. BMJ 314: 722–727.
26. Petersen I, Gilbert R, Evans S, Ridolfi A, Nazareth I (2010) Oral antibiotic prescribing during pregnancy in primary care: UK population-based study. The Journal of antimicrobial chemotherapy 65: 2238–2246. doi:10.1093/jac/dkq307.
27. Schnarr J, Smaill F (2008) Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. European journal of clinical investigation 38 Suppl 2: 50–57. doi:10.1111/j.1365-2362.2008.02009.x.
28. Redman CW, Sargent IL (2005) Latest advances in understanding preeclampsia. Science (New York, NY) 308: 1592–1594.
29. Von Dadelszen P, Magee LA (2002) Could an infectious trigger explain the differential maternal response to the shared placental pathology of preeclampsia and normotensive intrauterine growth restriction? Acta obstetricia et gynecologica Scandinavica 81: 642–648.

Appendix C Medline search strategies for the dental and pre-eclampsia study literature reviews

All MeSH keywords were searched as exploded terms (thus including all sub-terms in the hierarchy). All subheadings were included. For free-text searches, mp=title, abstract and subject headings. Truncation of a search term is represented by an asterisk (*); the wildcard (?) within a term allows a character to be present or absent; the adjacency operator (ADJn) retrieves records containing terms within "n" words of each other, in any order.

Table C.1 Medline search algorithm for studies of invasive dental treatment and vascular events.

	Search History MEDLINE
1	exp dentistry, operative/ or exp endodontics/ or exp oral surgical procedures/ or exp periodontics/ or exp surgery, oral/
2	((dental or periodont* or oral surgical) adj1 (treatment* or procedure* or therap* or surgery or extraction* or operation*)).mp.
3	exp Myocardial Infarction/
4	(myocardial infarct* or MI or AMI or cardiac infarct* or acute infarct* or heart infarct* or coronary infarct* or STEMI or coronary event* or cardiovascular event* or vascular event* or coronary attack* or heart attack* or q wave infarct* or myocardial thrombosis or coronary thrombosis).mp.
5	brain ischemia/ or exp stroke/
6	(ischemic stroke or brain attack or acute isch?emic cerebrovascular syndrome or cerebral infarct* or brain isch?emia or anterior circulation infarct* or TACI or PACI or lacunar infarct* or LACI or posterior circulation infarct* or POCI or cerebral isch?emia or cerebrovascular accident* or CVA or cerebrovascular event*).mp.
7	1 or 2
8	3 or 4 or 5 or 6
9	**7 and 8**
10	limit 9 to (english language and (classical article or clinical conference or clinical trial or comparative study or controlled clinical trial or government publications or journal article or meta analysis or randomized controlled trial))

Table C.2 Medline search algorithm for studies of maternal infection and pre-eclampsia.

	Search History MEDLINE
1	exp Inflammation/
2	exp Infection/
3	(inflammation or infection).mp.
4	exp Hypertension, Pregnancy-Induced/
5	(pre?eclampsia or eclampsia or pre?eclamptic pregnancy or HELLP or gestosis or EPH?gestosis or (pregnancy adj2 tox?emia) or maternal tox?emia or pregnancy?induced hypertension or hypertensive disorders of pregnancy or gestational hypertension or pregnancy?associated hypertension or pregnancy hypertension or proteinuric hypertension).mp.
6	1 or 2 or 3
7	4 or 5
8	**6 and 7**
9	limit 8 to (english language and yr="2007 -Current" and (classical article or clinical conference or clinical trial or comparative study or controlled clinical trial or government publications or journal article or meta analysis or randomized controlled trial))

Appendix D Invasive dental procedure codes

Table D.1 Current Dental Terminology codes for invasive dental procedures.

CDT[a] code	Description of procedure
D3410	Apicoectomy/periradicular surgery-anterior
D3421	Apicoectomy/periradicular surgery-bicuspid (first root)
D3425	Apicoectomy/periradicular surgery-molar (first root).
D3426	Apicoectomy/periradicular surgery (each additional root)
D3450	Root amputation-per root
D3920	Hemisection (including any root removal), not including root canal therapy
D4210	Gingivectomy or gingivoplasty - four or more contiguous teeth or bounded teeth spaces per quadrant
D4211	Gingivectomy or gingivoplasty - one to three contiguous teeth or bounded teeth spaces per quadrant
D4230	Anatomical crown exposure - four or more contiguous teeth per quadrant
D4231	Anatomical crown exposure - one to three teeth per quadrant
D4240	Gingival flap procedure, including root planing - four or more contiguous teeth or bounded teeth spaces per quadrant
D4241	Gingival flap procedure, including root planing - one to three contiguous teeth or bounded teeth spaces per quadrant
D4245	Apically positioned flap
D4249	Clinical crown lengthening-hard tissue
D4260	Osseous surgery (including flap entry and closure) - four or more contiguous teeth or bounded teeth spaces per quadrant
D4261	Osseous surgery (including flap entry and closure) - one to three contiguous teeth or bounded teeth spaces per quadrant
D4263	Bone replacement graft - first site in quadrant
D4264	Bone replacement graft - each additional site in quadrant
D4266	Guided tissue regeneration - resorbable barrier, per site
D4267	Guided tissue regeneration - nonresorbable barrier, per site, (includes membrane removal)
D4268	Surgical revision procedure, per tooth
D4270	Pedicle soft tissue graft procedure
D4271	Free soft tissue graft procedure (including donor site surgery)
D4273	Subepithelial connective tissue graft procedures, per tooth
D4274	Distal or proximal wedge procedure (when not performed in conjunction with surgical procedures in the same anatomical area)
D4275	Soft tissue allograft
D4276	Combined connective tissue and double pedicle graft, per tooth
D4341	Periodontal scaling and root planing - four or more teeth per quadrant
D4342	Periodontal scaling and root planing - one to three teeth, per quadrant
D6010	Surgical placement of implant body: endosteal implant
D6012	Surgical placement of interim implant body for transitional prosthesis: endosteal implant
D6040	Surgical placement: eposteal implant
D6050	Surgical placement: transosteal implant
D7111	Extraction, coronal remnants - deciduous tooth
D7140	Extraction, erupted tooth or exposed root (elevation and/or forceps removal)

CDT[a] code	Description of procedure
D7210	Surgical removal of erupted tooth requiring elevation of mucoperiosteal flap and removal of bone and/or section of tooth
D7220	Removal of impacted tooth-soft tissue
D7230	Removal of impacted tooth-partially bony
D7240	Removal of impacted tooth-completely bony
D7241	Removal of impacted tooth-completely bony, with unusual surgical complications
D7250	Surgical removal of residual tooth roots (cutting procedure)
D7260	Oral antral fistula closure
D7261	Primary closure of a sinus perforation
D7270	Tooth reimplantation and/or stabilization of accidentally evulsed or displaced tooth
D7272	Tooth transplantation (includes reimplantation from one site to another and splinting and/or stabilization)
D7280	Surgical access of an unerupted tooth
D7281	Surgical exposure of impacted or unerupted tooth to aid eruption
D7285	Biopsy of oral tissue - hard (bone, tooth)
D7286	Biopsy of oral tissue - soft
D7290	Surgical repositioning of teeth
D7292	Surgical placement: temporary anchorage device [screw retained plate] requiring surgical flap
D7293	Surgical placement: temporary anchorage device requiring surgical flap
D7294	Surgical placement: temporary anchorage device without surgical flap
D7310	Alveoloplasty in conjunction with extractions - four or more teeth or tooth spaces, per quadrant
D7311	Alveoloplasty in conjunction with extractions - one to three teeth or tooth spaces, per quadrant
D7320	Alveoloplasty not in conjunction with extractions - four or more teeth or tooth spaces, per quadrant
D7321	Alveoloplasty not in conjunction with extractions - one to three teeth or tooth spaces, per quadrant
D7340	Vestibuloplasty-ridge extension (second epithelialization)
D7350	Vestibuloplasty-ridge extension (including soft tissue grafts, muscle re-attachments, revision of soft tissue attachment, and management of hypertrophied and hyperplastic tissue)
D7410	Excision of benign lesion up to 1.25 cm
D7411	Excision of benign lesion greater than 1.25 cm
D7412	Excision of benign lesion, complicated
D7413	Excision of malignant lesion up to 1.25 cm
D7414	Excision of malignant lesion greater than 1.25 cm
D7415	Excision of malignant lesion, complicated
D7440	Excision of malignant tumor-lesion diameter up to 1.25 cm
D7441	Excision of malignant tumor-lesion diameter greater than 1.25 cm
D7450	Removal of benign odontogenic cyst or tumor-lesion diameter up t0 1.25 cm
D7451	Removal of benign odontogenic cyst or tumor-lesion diameter greater than 1.25 cm
D7460	Removal of benign nonodontogenic cyst or tumor-lesion diameter up to 1.25 cm
D7461	Removal of benign nonodontogenic cyst or tumor-lesion diameter greater than 1.25 cm
D7471	Removal of lateral exostosis (maxilla or mandible)
D7472	Removal of torus palatinus

CDT[a] code	Description of procedure
D7473	Removal of torus mandibularis
D7485	Surgical reduction of osseous tuberosity
D7490	Radical resection of maxilla or mandible
D7510	Incision and drainage of abscess-intraoral soft tissue
D7511	Incision and drainage of abscess - intraoral soft tissue - complicated (includes drainage of multiple fascial spaces)
D7520	Incision and drainage of abscess-extraoral soft tissue
D7521	Incision and drainage of abscess - extraoral soft tissue - complicated (includes drainage of multiple fascial spaces)
D7530	Removal of foreign body from mucosa, skin, or subcutaneous alveolar tissue
D7540	Removal of reaction-producing foreign bodies-musculoskeletal system
D7550	Partial ostectomy/sequestrectomy for removal of non-vital bone
D7560	Maxillary sinusotomy for removal of tooth fragment or foreign body
D7610	Maxilla-open reduction (teeth immobilized if present)
D7630	Mandible-open reduction (teeth immobilized if present)
D7650	Malar and/or zygomatic arch-open reduction
D7671	Alveolus - open reduction, may include stabilization of teeth
D7680	Facial bones-complicated reduction with fixation and multiple surgical approaches
D7710	Maxilla-open reduction
D7730	Mandible-open reduction
D7750	Malar and/or zygomatic arch-open reduction
D7770	Alveolus - open reduction stabilization of teeth
D7771	Alveolus, closed reduction stabilization of teeth
D7780	Facial bones-complicated reduction with fixation and multiple surgical approaches
D7810	Open reduction of dislocation
D7840	Condylectomy
D7850	Surgical discectomy; with/without implant
D7854	Synovectomy
D7856	Myotomy
D7858	Joint reconstruction
D7860	Arthrotomy
D7865	Arthroplasty
D7873	Arthroscopy-surgical: lavage and lysis of adhesions
D7874	Arthroscopy-surgical: disc repositioning and stabilization
D7875	Arthroscopy-surgical: synovectomy
D7876	Arthroscopy-surgical: discectomy
D7877	Arthroscopy-surgical: debridement
D7911	Complicated suture-up to 5 cm
D7912	Complicated suture-greater than 5 cm
D7920	Skin graft (identify defect covered, location, and type of graft)
D7940	Osteoplasty-for orthognathic deformities
D7941	Osteotomy - mandibular rami
D7943	Osteotomy - mandibular rami with bone graft; includes obtaining the graft
D7944	Osteotomy-segmented or subapical

CDT[a] code	Description of procedure
D7945	Osteotomy-body of mandible
D7946	Le Fort i (maxilla-total)
D7947	Le Fort i (maxilla-segmented)
D7948	Le Fort ii or Le Fort iii (osteoplasty of facial bones for midface hypoplasia or retrusion)-without bone graft
D7949	Le Fort ii or Le Fort iii-with bone graft
D7950	Osseous, osteoperiosteal, or cartilage graft of the mandible or maxilla - autogenous or nonautogenous, by report
D7951	Sinus augmentation with bone or bone substitutes
D7953	Bone replacement graft for ridge preservation - per site
D7955	Repair of maxillofacial soft and/or hard tissue defect
D7960	Frenulectomy (frenectomy or frenotomy)-separate procedure
D7963	Frenuloplasty
D7970	Excision of hyperplastic tissue-per arch
D7971	Excision of pericoronal gingiva
D7972	Surgical reduction of fibrous tuberosity
D7980	Sialolithotomy
D7981	Excision of salivary gland, by report
D7982	Sialodochoplasty
D7983	Closure of salivary fistula
D7991	Coronoidectomy
D7996	Implant-mandible for augmentation purposes (excluding alveolar ridge), by report
D7999	Unspecified oral surgery procedure, by report

[a] Current Dental Terminology

Appendix E Participant flow for secondary analyses of ischaemic stroke and myocardial infarction

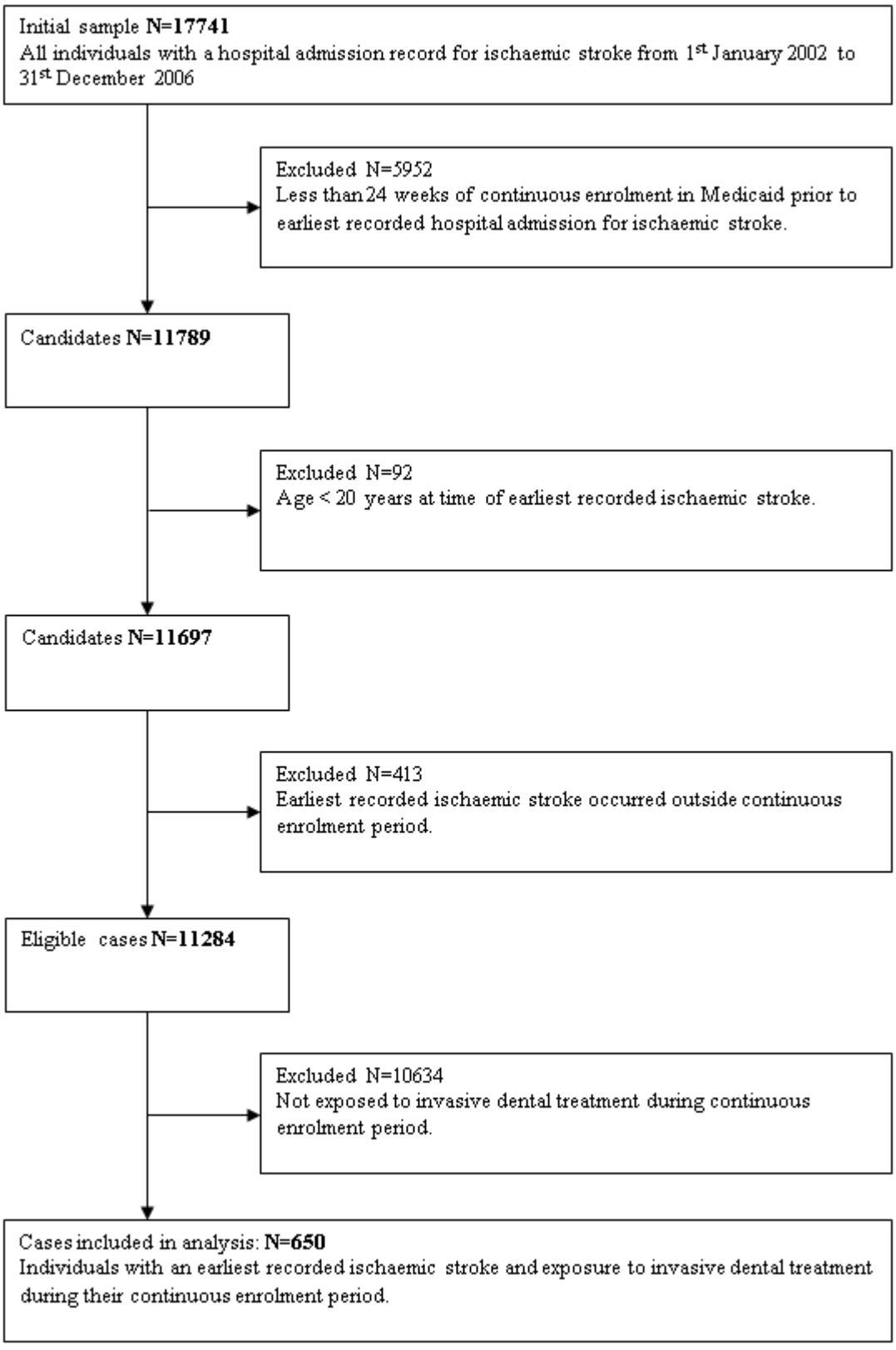


Figure E.1 Participant flow - Ischaemic stroke.

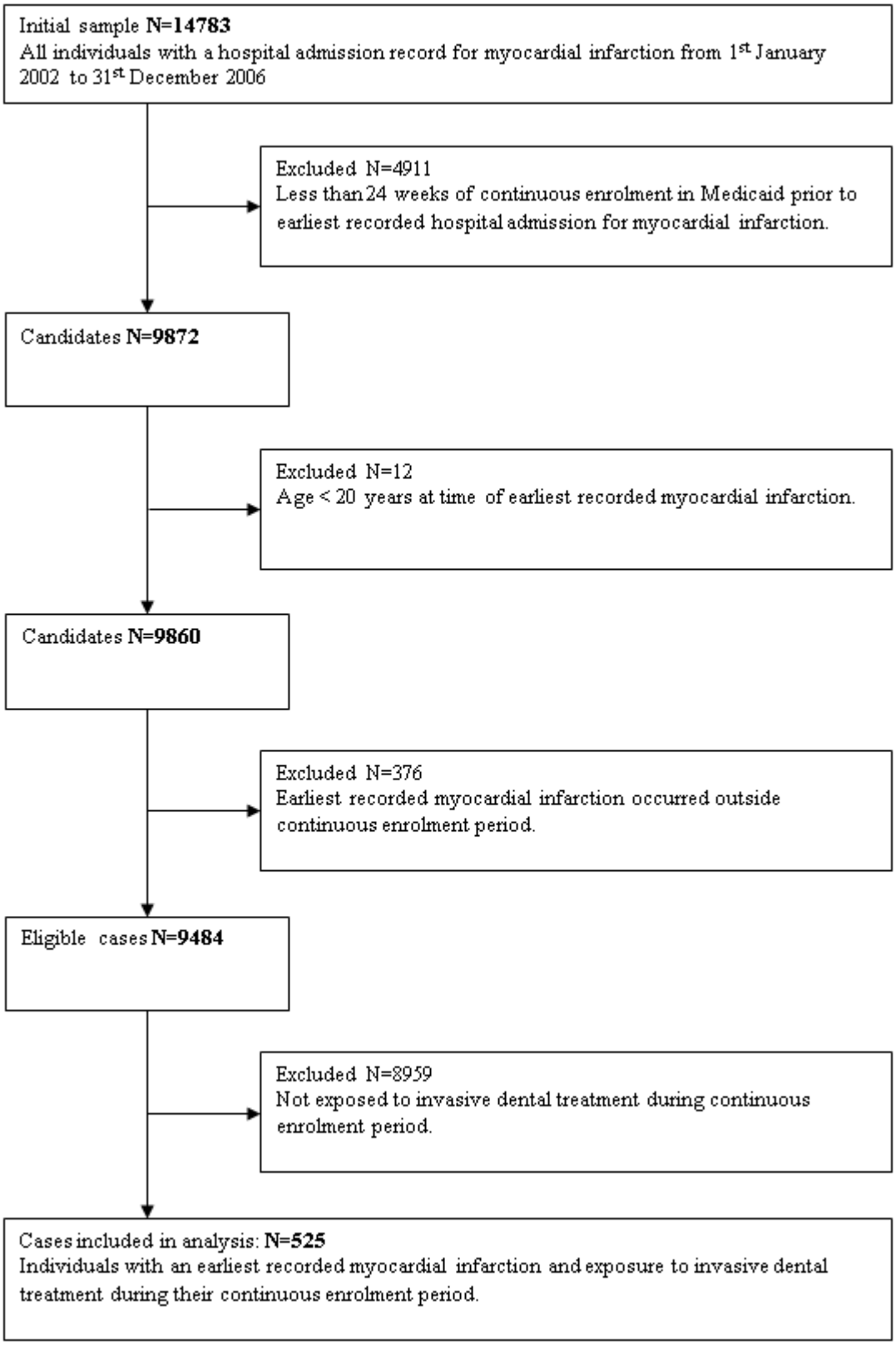


Figure E.2 Participant flow - Myocardial infarction.

Appendix F Results of sensitivity analyses of the effect of invasive dental treatment on vascular event risk

Table F.1 Results including unexposed cases.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=13894[b])		
Baseline period[c]	13717	1
Post-procedure risk period		
Weeks 1-4	40	1.50 (1.09-2.06)
Weeks 5-8	29	1.11 (0.77-1.61)
Weeks 9-12	30	1.16 (0.80-1.67)
Weeks 13-16	25	0.95 (0.64-1.42)
Weeks 17-24	53	1.09 (0.83-1.44)

[a] IR denotes age-adjusted incidence ratio.
[b] note that 6475 unexposed cases were dropped from the analysis as they only contributed to a single age-band (and hence did not contribute to the age effects).
[c] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.2 Results with finer adjustment for age in 2-year age groups.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=1152)		
Baseline period[b]	975	1
Post-procedure risk period		
Weeks 1-4	40	1.49 (1.09-2.06)
Weeks 5-8	29	1.11 (0.76-1.60)
Weeks 9-12	30	1.16 (0.80-1.67)
Weeks 13-16	25	0.96 (0.64-1.42)
Weeks 17-24	53	1.07 (0.81-1.42)

[a] IR denotes age-adjusted incidence ratio.
[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.3 Results excluding individuals with an antiplatelet or salicylate drug prescription record prior to invasive dental treatment.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=666)		
Baseline period[b]	543	1
Post-procedure risk period		
Weeks 1-4	33	2.23 (1.56-3.18)
Weeks 5-8	24	1.63 (1.08-2.46)
Weeks 9-12	21	1.42 (0.91-2.20)
Weeks 13-16	15	1.00 (0.60-1.68)
Weeks 17-24	30	1.04 (0.72-1.51)

[a] IR denotes age-adjusted incidence ratio.
[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.4 Results excluding individuals with a rheumatoid arthritis diagnosis prior to invasive dental treatment or an NSAID prescription in the period four weeks prior to four weeks post invasive dental treatment.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=465)		
Baseline period[b]	386	1
Post-procedure risk period		
Weeks 1-4	20	1.84 (1.17-2.89)
Weeks 5-8	15	1.40 (0.83-2.35)
Weeks 9-12	12	1.13 (0.63-2.01)
Weeks 13-16	8	0.74 (0.37-1.50)
Weeks 17-24	24	1.17 (0.77-1.77)

[a] IR denotes age-adjusted incidence ratio.
[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.5 Results excluding individuals with an earliest diagnosis of diabetes within 12 months prior to invasive dental treatment.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=928)		
Baseline period[b]	781	1
Post-procedure risk period		
Weeks 1-4	31	1.46 (1.02-2.10)
Weeks 5-8	25	1.21 (0.81-1.80)
Weeks 9-12	24	1.17 (0.78-1.76)
Weeks 13-16	20	0.97 (0.62-1.52)
Weeks 17-24	47	1.21 (0.90-1.63)

[a] IR denotes age-adjusted incidence ratio.
[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.6 Results excluding individuals with an earliest diagnosis of hypertension within 12 months prior to invasive dental treatment.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=754)		
Baseline period[b]	641	1
Post-procedure risk period		
Weeks 1-4	28	1.64 (1.12-2.40)
Weeks 5-8	17	1.02 (0.63-1.65)
Weeks 9-12	20	1.20 (0.76-1.87)
Weeks 13-16	17	1.01 (0.62-1.64)
Weeks 17-24	31	0.97 (0.67-1.40)

[a] IR denotes age-adjusted incidence ratio.
[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.7 Results excluding individuals with an earliest diagnosis of coronary heart disease within 12 months prior to invasive dental treatment.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=913)		
Baseline period[b]	762	1
Post-procedure risk period		
Weeks 1-4	35	1.70 (1.21-2.40)
Weeks 5-8	24	1.19 (0.79-1.79)
Weeks 9-12	25	1.25 (0.84-1.87)
Weeks 13-16	21	1.04 (0.67-1.61)
Weeks 17-24	46	1.20 (0.89-1.62)

[a] IR denotes age-adjusted incidence ratio.
[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.8 Results excluding individuals who died during their hospital stay following admission for a vascular event or whose enrolment ended within a month after their event.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=1069)		
Baseline period[b]	904	1
Post-procedure risk period		
Weeks 1-4	39	1.62 (1.17-2.24)
Weeks 5-8	27	1.15 (0.78-1.69)
Weeks 9-12	26	1.12 (0.76-1.66)
Weeks 13-16	23	0.98 (0.65-1.49)
Weeks 17-24	50	1.14 (0.86-1.52)

[a] IR denotes age-adjusted incidence ratio.
[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.9 Results excluding individuals with multiple invasive dental procedures with overlapping risk periods.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=948)		
Baseline period[b]	810	1
Post-procedure risk period		
Weeks 1-4	34	1.65 (1.17-2.33)
Weeks 5-8	22	1.08 (0.71-1.66)
Weeks 9-12	22	1.11 (0.72-1.70)
Weeks 13-16	20	1.03 (0.66-1.60)
Weeks 17-24	40	1.06 (0.77-1.47)

[a] IR denotes age-adjusted incidence ratio.
[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.10 Results for individuals exposed only to dental extractions (the three most common procedures*).

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=1017)		
Baseline period[b]	863	1
Post-procedure risk period		
Weeks 1-4	36	1.58 (1.13-2.21)
Weeks 5-8	25	1.12 (0.75-1.67)
Weeks 9-12	26	1.18 (0.79-1.74)
Weeks 13-16	21	0.94 (0.61-1.46)
Weeks 17-24	46	1.09 (0.81-1.47)

*D7210: surgical removal of erupted tooth requiring elevation of mucoperiosteal flap and removal of bone and/or section of tooth; D7250: surgical removal of residual tooth roots (cutting procedure); D7310: alveoloplasty in conjunction with extractions - four or more teeth or tooth spaces, per quadrant

[a] IR denotes age-adjusted incidence ratio.

[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Table F.11 Results excluding individuals with a diagnosis of diabetes, coronary heart disease or hypertension at any time prior to invasive dental treatment.

Outcome and risk period	No. of cases	IR[a] (95% CI)
Vascular event (N=228)		
Baseline period[b]	192	1
Post-procedure risk period		
Weeks 1-4	10	1.76 (0.92-3.36)
Weeks 5-8	6	1.05 (0.46-2.38)
Weeks 9-12	5	0.84 (0.34-2.06)
Weeks 13-16	4	0.66 (0.24-1.78)
Weeks 17-24	11	0.93 (0.50-1.73)

[a] IR denotes age-adjusted incidence ratio.

[b] Baseline period is all observation time except for the 24 week period following an invasive dental procedure.

Appendix G Read and OXMIS codes defining exposure to UTI and RTI and frequency distribution of delivery codes

Table G.1 Urinary tract infection medical codes.

Medical code	Medical term
READ	
1AG..00	Recurrent urinary tract infections
1J4..00	Suspected UTI
46B3.00	Urine bacteria test: positive
46H..11	Bacteria in urine O/E
46H4.00	Urine microscopy: bacteria present
A981100	Acute gonococcal cystitis
A981111	Bladder gonorrhoea - acute
K100600	Calculous pyelonephritis
K101.00	Acute pyelonephritis
K101000	Acute pyelonephritis without medullary necrosis
K101100	Acute pyelonephritis with medullary necrosis
K101200	Acute pyelitis
K101300	Acute pyonephrosis
K101z00	Acute pyelonephritis NOS
K102.00	Renal and perinephric abscess
K102000	Renal abscess
K102100	Perinephric abscess
K102200	Renal carbuncle
K102z00	Renal and perinephric abscess NOS
K10y.00	Pyelonephritis and pyonephrosis unspecified
K10y000	Pyelonephritis unspecified
K10y100	Pyelitis unspecified
K10y200	Pyonephrosis unspecified
K10y400	Pyelitis in diseases EC
K10yz00	Unspecified pyelonephritis NOS
K15..00	Cystitis
K150.00	Acute cystitis
K152000	Subacute cystitis
K155.00	Recurrent cystitis
K15z.00	Cystitis NOS
K190.00	Urinary tract infection, site not specified
K190000	Bacteriuria, site not specified
K190011	Asymptomatic bacteriuria
K190200	Post operative urinary tract infection
K190300	Recurrent urinary tract infection
K190311	Recurrent UTI
K190z00	Urinary tract infection, site not specified NOS
K213.00	Prostatocystitis
Kyu5100	[X]Other cystitis
L09y400	Urinary tract infection following abortive pregnancy
L165.00	Asymptomatic bacteriuria in pregnancy
L165000	Asymptomatic bacteriuria in pregnancy unspecified
L165100	Asymptomatic bacteriuria in pregnancy - delivered

Medical code	Medical term
L165200	Asymptomatic bacteriuria in pregnancy - delivered with p/n complication
L165300	Asymptomatic bacteriuria in pregnancy - not delivered
L165400	Asymptomatic bacteriuria in pregnancy with p/n complication
L165z00	Asymptomatic bacteriuria in pregnancy NOS
L166.00	Genitourinary tract infections in pregnancy
L166.11	Cystitis of pregnancy
L166000	Genitourinary tract infection in pregnancy unspecified
L166100	Genitourinary tract infection in pregnancy - delivered
L166200	Genitourinary tract infection in pregnancy - delivered +p/n complication
L166300	Genitourinary tract infection in pregnancy - not delivered
L166400	Genitourinary tract infection in pregnancy with p/n complication
L166500	Infections of kidney in pregnancy
L166600	Urinary tract infection following delivery
L166800	Urinary tract infection complicating pregnancy
L166z00	Genitourinary tract infection in pregnancy NOS
L166z11	UTI - urinary tract infection in pregnancy
L177.00	Infections of bladder in pregnancy
Lyu2300	[X]Infections of other parts of urinary tract in pregnancy
Lyu2400	[X]Other+unspecified genitourinary tract infection in pregnancy
Lyu6100	[X]Other genitourinary tract infections following delivery
OXMIS	
5901	Pyelitis
5901A	Pyelitis acute
5901NA	Pyelonephritis acute
5901PC	Pyelocystitis
5901PN	Pyelonephritis
592 PN	Pyelonephritis calculous
595	Cystitis
595 A	Cystitis acute
595 AH	Cystitis acute haemorrhagic
595 BR	Recurrent cystitis
599 A	UTI (urinary tract infection)
599 AA	Urinary tract infection acute
599 AR	Abscess urinary
599 D	Urinary tract infection recurrent
599 GI	Genito-urinary infection
6350CG	Pyelocystitis pregnancy
6350CP	Pyelocystitis puerperium
6350G	Pyelitis pregnancy
6350P	Pyelitis puerperium
6359A	Pregnancy cystitis
6359B	Puerperal cystitis
6359G	Urinary infection pregnancy
6359P	Urinary infection puerperium
7891	Bacilluria
7891A	Bacteriuria asymptomatic
7891AA	Bacteriuria
7891BP	Pyuria bacterial

O/E=on examination; NOS=not otherwise specified; EC=elsewhere classified; p/n=postnatal;
The prefix [X] is used for codes introduced with the migration to ICD10 in April 1995.

Table G.2 Respiratory tract infection medical codes.

Medical code	Medical term
READ	
16L..00	Influenza-like symptoms
2DB5.00	O/E - tonsils - quinsy present
2DB5.11	O/E - quinsy present
2DB6.00	O/E - follicular tonsillitis
2DB7.00	O/E - exudate on tonsils
65VA.00	Notification of whooping cough
7531100	Drainage of peritonsillar abscess
7531111	Drainage of quinsy
A022200	Salmonella pneumonia
A320.00	Faucial diphtheria
A321.00	Nasopharyngeal diphtheria
A322.00	Anterior nasal diphtheria
A323.00	Laryngeal diphtheria
A32z.00	Diphtheria NOS
A33..00	Whooping cough
A330.00	Bordetella pertussis
A331.00	Bordetella parapertussis
A33y.00	Whooping cough - other specified organism
A33yz00	Other whooping cough NOS
A33z.00	Whooping cough NOS
A34..00	Streptococcal sore throat and scarlatina
A340300	Streptococcal tonsillitis
A380300	Septicaemia due to streptococcus pneumoniae
A383000	Fusobacterial necrotising tonsillitis
A3BXA00	Mycoplasma pneumoniae [PPLO] cause/dis classifd/oth chaptr
A3By400	Pleuropneumonia-like organism (PPLO) infection
A521.00	Varicella pneumonitis
A54x400	Herpes simplex pneumonia
A551.00	Postmeasles pneumonia
A730.00	Ornithosis with pneumonia
A785000	Cytomegaloviral pneumonitis
A789300	HIV disease resulting in pneumocystis carinii pneumonia
A79A.00	Respiratory syncytial virus infection
AB24.11	Pneumonia - candidal
AB40500	Histoplasma capsulatum with pneumonia
AB41500	Histoplasma duboisii with pneumonia
AB4z500	Histoplasmosis with pneumonia
AD04.00	Toxoplasma pneumonitis
AD63.00	Pneumocystosis
Ayu3800	[X]Diphtheria, unspecified
Ayu3900	[X]whooping cough due to other bordetella species
Ayu3A00	[X]whooping cough, unspecified
AyuK900	[X]mycoplasma pneumoniae [PPLO]cause/dis classifd/oth chaptr
F030800	Encephalitis due to influenza-specific virus not identified
F030A00	Encephalitis due to influenza-virus identified
G520300	Acute myocarditis - influenzal
H03..00	Acute tonsillitis
H03..11	Throat infection - tonsillitis
H03..12	Tonsillitis
H031.00	Acute follicular tonsillitis
H034.00	Acute gangrenous tonsillitis
H035.00	Acute bacterial tonsillitis
H036.00	Acute viral tonsillitis
H037.00	Recurrent acute tonsillitis

Medical code	Medical term
H03z.00	Acute tonsillitis NOS
H04..00	Acute laryngitis and tracheitis
H040.00	Acute laryngitis
H040400	Acute haemophilus influenzae laryngitis
H040600	Acute suppurative laryngitis
H040w00	Acute viral laryngitis unspecified
H041.00	Acute tracheitis
H041000	Acute tracheitis without obstruction
H041100	Acute tracheitis with obstruction
H041z00	Acute tracheitis NOS
H042.00	Acute laryngotracheitis
H042.11	Laryngotracheitis
H042000	Acute laryngotracheitis without obstruction
H042100	Acute laryngotracheitis with obstruction
H042z00	Acute laryngotracheitis NOS
H043.00	Acute epiglottitis (non strep)
H043.11	Viral epiglottitis
H043000	Acute epiglottitis without obstruction
H043100	Acute epiglottitis with obstruction
H043200	Acute obstructive laryngitis
H043211	Croup
H043z00	Acute epiglottitis NOS
H044.00	Croup
H04z.00	Acute laryngitis and tracheitis NOS
H052.00	Pharyngotracheitis
H053.00	Tracheopharyngitis
H055.00	Pharyngolaryngitis
H06..00	Acute bronchitis and bronchiolitis
H060.00	Acute bronchitis
H060.11	Acute wheezy bronchitis
H060100	Acute membranous bronchitis
H060200	Acute pseudomembranous bronchitis
H060300	Acute purulent bronchitis
H060400	Acute croupous bronchitis
H060500	Acute tracheobronchitis
H060600	Acute pneumococcal bronchitis
H060700	Acute streptococcal bronchitis
H060800	Acute haemophilus influenzae bronchitis
H060900	Acute neisseria catarrhalis bronchitis
H060A00	Acute bronchitis due to mycoplasma pneumoniae
H060B00	Acute bronchitis due to coxsackievirus
H060C00	Acute bronchitis due to parainfluenza virus
H060D00	Acute bronchitis due to respiratory syncytial virus
H060E00	Acute bronchitis due to rhinovirus
H060F00	Acute bronchitis due to echovirus
H060w00	Acute viral bronchitis unspecified
H060x00	Acute bacterial bronchitis unspecified
H060z00	Acute bronchitis NOS
H061.00	Acute bronchiolitis
H061000	Acute capillary bronchiolitis
H061200	Acute bronchiolitis with bronchospasm
H061300	Acute exudative bronchiolitis
H061500	Acute bronchiolitis due to respiratory syncytial virus
H061600	Acute bronchiolitis due to other specified organisms
H061z00	Acute bronchiolitis NOS
H062.00	Acute lower respiratory tract infection
H06z.00	Acute bronchitis or bronchiolitis NOS

Medical code	Medical term
H06z000	Chest infection NOS
H06z011	Chest infection
H06z100	Lower resp tract infection
H06z112	Acute lower respiratory tract infection
H06z200	Recurrent chest infection
H07..00	Chest cold
H0y..00	Other specified acute respiratory infections
H14y500	Caseous tonsillitis
H15..00	Peritonsillar abscess - quinsy
H15..11	Quinsy
H1y2100	Pharynx or nasopharynx cellulitis
H1y2200	Parapharyngeal abscess
H1y2600	Pharynx or nasopharynx abscess
H1yz000	Abscess of trachea
H2...00	Pneumonia and influenza
H20..00	Viral pneumonia
H20..11	Chest infection - viral pneumonia
H200.00	Pneumonia due to adenovirus
H201.00	Pneumonia due to respiratory syncytial virus
H202.00	Pneumonia due to parainfluenza virus
H20y.00	Viral pneumonia NEC
H20z.00	Viral pneumonia NOS
H21..00	Lobar (pneumococcal) pneumonia
H21..11	Chest infection - pneumococcal pneumonia
H22..00	Other bacterial pneumonia
H22..11	Chest infection - other bacterial pneumonia
H220.00	Pneumonia due to klebsiella pneumoniae
H221.00	Pneumonia due to pseudomonas
H222.00	Pneumonia due to haemophilus influenzae
H222.11	Pneumonia due to haemophilus influenzae
H223.00	Pneumonia due to streptococcus
H223000	Pneumonia due to streptococcus, group B
H224.00	Pneumonia due to staphylococcus
H22y.00	Pneumonia due to other specified bacteria
H22y000	Pneumonia due to escherichia coli
H22y011	E.coli pneumonia
H22y100	Pneumonia due to proteus
H22y200	Pneumonia - legionella
H22yX00	Pneumonia due to other aerobic gram-negative bacteria
H22yz00	Pneumonia due to bacteria NOS
H22z.00	Bacterial pneumonia NOS
H23..00	Pneumonia due to other specified organisms
H23..11	Chest infection - pneumonia organism OS
H230.00	Pneumonia due to Eaton's agent
H231.00	Pneumonia due to mycoplasma pneumoniae
H232.00	Pneumonia due to pleuropneumonia like organisms
H233.00	Chlamydial pneumonia
H23z.00	Pneumonia due to specified organism NOS
H24..00	Pneumonia with infectious diseases EC
H24..11	Chest infection with infectious disease EC
H240.00	Pneumonia with measles
H241.00	Pneumonia with cytomegalic inclusion disease
H242.00	Pneumonia with ornithosis
H243.00	Pneumonia with whooping cough
H243.11	Pneumonia with pertussis
H244.00	Pneumonia with tularaemia
H245.00	Pneumonia with anthrax

Medical code	Medical term
H246.00	Pneumonia with aspergillosis
H247.00	Pneumonia with other systemic mycoses
H247000	Pneumonia with candidiasis
H247100	Pneumonia with coccidioidomycosis
H247200	Pneumonia with histoplasmosis
H247z00	Pneumonia with systemic mycosis NOS
H24y.00	Pneumonia with other infectious diseases EC
H24y000	Pneumonia with actinomycosis
H24y100	Pneumonia with nocardiasis
H24y200	Pneumonia with pneumocystis carinii
H24y300	Pneumonia with Q-fever
H24y400	Pneumonia with salmonellosis
H24y500	Pneumonia with toxoplasmosis
H24y600	Pneumonia with typhoid fever
H24y700	Pneumonia with varicella
H24yz00	Pneumonia with other infectious diseases EC NOS
H24z.00	Pneumonia with infectious diseases EC NOS
H25..00	Bronchopneumonia due to unspecified organism
H25..11	Chest infection - unspecified bronchopneumonia
H26..00	Pneumonia due to unspecified organism
H26..11	Chest infection - pnemonia due to unspecified organism
H260.00	Lobar pneumonia due to unspecified organism
H260000	Lung consolidation
H261.00	Basal pneumonia due to unspecified organism
H262.00	Postoperative pneumonia
H27..00	Influenza
H270.00	Influenza with pneumonia
H270.11	Chest infection - influenza with pneumonia
H270000	Influenza with bronchopneumonia
H270100	Influenza with pneumonia, influenza virus identified
H270z00	Influenza with pneumonia NOS
H271.00	Influenza with other respiratory manifestation
H271000	Influenza with laryngitis
H271100	Influenza with pharyngitis
H271z00	Influenza with respiratory manifestations NOS
H27y.00	Influenza with other manifestations
H27y000	Influenza with encephalopathy
H27y100	Influenza with gastrointestinal tract involvement
H27yz00	Influenza with other manifestations NOS
H27z.00	Influenza NOS
H27z.11	Flu like illness
H27z.12	Influenza like illness
H28..00	Atypical pneumonia
H29..00	Avian influenza
H2y..00	Other specified pneumonia or influenza
H2z..00	Pneumonia or influenza NOS
H30..11	Chest infection - unspecified bronchitis
H300.00	Tracheobronchitis NOS
H301.00	Laryngotracheobronchitis
H3y0.00	Chronic obstruct pulmonary disease with acute lower respiratory infection
H501400	Purulent pleurisy
H510900	Pneumococcal pleurisy
H510A00	Staphylococcal pleurisy
H510B00	Streptococcal pleurisy
H511.00	Bacterial pleurisy with effusion
H511000	Pneumococcal pleurisy with effusion
H511100	Staphylococcal pleurisy with effusion

Medical code	Medical term
H511200	Streptococcal pleurisy with effusion
H511z00	Bacterial pleurisy with effusion NOS
H530200	Gangrenous pneumonia
H530300	Abscess of lung with pneumonia
H564.00	Bronchiolitis obliterans organising pneumonia
Hyu0400	[X]Flu+oth respiratory manifestations,'flu virus identified
Hyu0500	[X]influenza+other manifestations,influenza virus identified
Hyu0600	[X]influenza+other respiratory manifestations,virus not identified
Hyu0700	[X]influenza+other manifestations, virus not identified
Hyu0800	[X]other viral pneumonia
Hyu0900	[X]pneumonia due to other aerobic gram-negative bacteria
Hyu0A00	[X]other bacterial pneumonia
Hyu0B00	[X]pneumonia due to other specified infectious organisms
Hyu0C00	[X]pneumonia in bacterial diseases classified elsewhere
Hyu0D00	[X]pneumonia in viral diseases classified elsewhere
Hyu0E00	[X]pneumonia in mycoses classified elsewhere
Hyu0F00	[X]pneumonia in parasitic diseases classified elsewhere
Hyu0G00	[X]pneumonia in other diseases classified elsewhere
Hyu0H00	[X]other pneumonia, organism unspecified
Hyu1.00	[X]other acute lower respiratory infections
Hyu1000	[X]acute bronchitis due to other specified organisms
Hyu1100	[X]Acute bronchiolitis due to other specified organisms
Hyu2800	[X]Other abscess of pharynx
SP13200	Post operative chest infection
OXMIS	
331	Parapertussis
339	Whooping cough
0339P	Pertussis
0340L	Streptococcal laryngitis
0340PN	Septic pharyngitis
0340T	Tonsillitis streptococcal
136 C	Pneumocystosis
136 LG	Legionnaires' disease
460 C	Influenza-like illness
463	Tonsillitis acute
463 A	Tonsillitis
463 B	Follicular tonsillitis
463 BC	Tonsillitis bacterial
464 A	Laryngitis acute
464 B	Laryngitis
464 BV	Laryngitis viral
464 C	Tracheitis acute
464 D	Tracheitis
464 E	Croup
464 LA	Laryngotracheitis acute
464 LT	Laryngotracheitis
464 P	Tracheitis purulent
464 TP	Tracheal suppuration
465 LP	Pharyngolaryngitis
465 TP	Tracheopharyngitis
466 A	Bronchiolitis
466 B	Bronchiolitis acute
466 C	Bronchitis acute
466 CR	Croup bronchial
466 D	Bronchitis purulent
466 V	Viral bronchitis
470	Influenza

Medical code	Medical term
470 F	Flu
470 P	Parainfluenza virus infection
471	Pneumonia influenzal
472 A	Influenzal bronchitis
472 B	Tracheitis influenzal
472 H	Influenza haemorrhagic
472 L	Laryngitis with influenza
480	Virus pneumonia
480 A	Syncytial virus respiratory infection
481 A	Pneumonia pneumococcal
481 B	Lobar pneumonia
481 BA	Pneumonia basal
481 BC	Consolidation lung
4820K	Pneumonia klebsiella
4823	Pneumonia staphylococcal
483 AP	Pneumonia primary atypical
483 AT	Pneumonia atypical
483 E	Pneumonia eaton's agent
483 M	Pneumonia mycoplasal
485	Bronchopneumonia
486	Pneumonia
486 CA	Pneumonia cold agglutinin positive
490 CT	Catarrhal bronchitis
490 LT	Laryngotracheobronchitis
490 T	Tracheobronchitis
491 AC	Bronchitis acute on chronic
500 B	Tonsillitis recurrent
500 TD	Tonsils discharging
501 CP	Cellulitis peritonsillar
501 CT	Cellulitis tonsil
501 LA	Abscess lingual tonsil
501 N	Quinsy
501 NA	Abscess intratonsillar
501 PA	Abscess tonsillopharyngeal
501 PB	Abscess peritonsillar
501 PC	Peritonsillar abscess
501 PD	Peritonsillitis
501 PT	Abscess post-tonsillar
501 TA	Abscess tonsil
508 GC	Abscess postpharyngeal
508 GD	Abscess retropharyngeal
508 GM	Abscess postnasal
508 GN	Abscess nasopharyngeal
508 GP	Abscess postlaryngeal
508 GR	Abscess retrolaryngeal
508 K	Abscess upper respiratory
508 KA	Abscess throat
508 KE	Abscess epiglottis
508 KF	Abscess fauces
508 KL	Abscess larynx
508 KP	Abscess pharyngeal
508 L	Epiglottitis
508 LP	Cellulitis pharynx
5192LN	Lung infection
5199DP	Respiratory tract infection postoperative
5199E	Infection chest
5199RN	Recurrent chest infection

Medical code	Medical term
K2301	Drainage abscess tonsillar

O/E=on examination; OS=otherwise specified; NOS=not otherwise specified; EC=elsewhere classified; NEC=not elsewhere classified; The prefix [X] is used for codes introduced with the migration to ICD10 in April 1995.

Table G.3 Frequency distribution of end-of-pregnancy Read/OXMIS codes used to estimate the timing of delivery for study participants' first recorded completed pregnancies (1533 cases and 14236 controls).

Medical code	Medical term	Case records (N=2815)		Control records (N=26068)	
		n	%	n	%
63...00	Birth details	738	26.2	7312	28.0
7F19.00	Normal delivery	322	11.4	5177	19.9
635..00	Maturity of baby	186	6.6	1778	6.8
L20..11	Spontaneous vaginal delivery	58	2.1	1087	4.2
650 AM	Normal delivery (mother)	53	1.9	851	3.3
L34..00	Trauma to perineum and vulva during delivery	62	2.2	673	2.6
632..00	Length of labour	54	1.9	594	2.3
L398.00	Caesarean delivery	126	4.5	488	1.9
L398400	Delivery by emergency caesarean section	116	4.1	399	1.5
Ly0..00	Spontaneous vertex delivery	26	0.9	405	1.6
L398200	Caesarean section - pregnancy at term	81	2.9	266	1.0
7F13.00	Other caesarean delivery	65	2.3	267	1.0
7F17.11	Ventouse delivery	17	0.6	262	1.0
L396.11	Ventouse delivery	17	0.6	261	1.0
L395.00	Forceps delivery	26	0.9	249	1.0
650 AP	SVD (spontaneous vertex delivery)	15	0.5	237	0.9
7F12.00	Elective caesarian delivery	21	0.7	227	0.9
K755 AB	Forceps delivery	21	0.7	195	0.7
63E2.00	Normal birth	18	0.6	186	0.7
L3495P	Postnatal visit	23	0.8	178	0.7
7F16.00	Forceps cephalic delivery	21	0.7	176	0.7
7F13111	Lower uterine segment caesarean section (LSCS) NEC	42	1.5	150	0.6
7F13300	Emergency caesarean section	42	1.5	147	0.6
K755 M	Forceps delivery (mother)	21	0.7	166	0.6
K769 M	Delivery caesarian section (mother)	43	1.5	137	0.5
7F17.00	Vacuum delivery	14	0.5	163	0.6
7F16z00	Forceps cephalic delivery NOS	15	0.5	149	0.6
K766	Caesarian section lower segment	39	1.4	124	0.5
K7581M	Ventouse extraction delivery (mother)	17	0.6	136	0.5
T318	Child born	13	0.5	124	0.5
7F17z00	Vacuum delivery NOS	10	0.4	103	0.4
62R..00	Postnatal visits	21	0.7	88	0.3
633..00	Outcome of delivery	11	0.4	91	0.3
635..11	Full term baby	6	0.2	95	0.4
7F12111	Elective lower uterine segment caesarean section (LSCS)	15	0.5	82	0.3
633a.00	Birth of child	9	0.3	82	0.3
63D2.00	Placenta normal O/E	9	0.3	81	0.3
650 AA	Labour	7	0.2	80	0.3
L20..00	Normal delivery in a completely normal case	3	0.1	79	0.3
63Z..11	Apgar normal	5	0.2	77	0.3
ZV27.00	[V]Outcome of delivery	9	0.3	71	0.3
63Z..00	Birth details NOS	10	0.4	63	0.2
L36..00	Postpartum haemorrhage (PPH)	16	0.6	56	0.2
Z257.14	FTND - Full term normal delivery	4	0.1	65	0.2
K750 AB	Induction labour	21	0.7	47	0.2
Y61 N	Postnatal examination normal	13	0.5	50	0.2
650 A	Pregnancy normal delivery	3	0.1	60	0.2
K7581	Ventous assisted delivery	4	0.1	59	0.2
62S..11	Postnatal exam. - maternal	16	0.6	43	0.2

Medical code	Medical term	Case records (N=2815)		Control records (N=26068)	
		n	%	n	%
650 BP	Premature labour	1	0.0	57	0.2
7F13100	Lower uterine segment caesarean delivery NEC	12	0.4	44	0.2
L341.00	Second degree perineal tear during delivery	2	0.1	51	0.2
7F12z00	Elective caesarean delivery NOS	4	0.1	48	0.2
14Y6.00	Born by emergency caesarean section	4	0.1	48	0.2
L398300	Delivery by elective caesarean section	9	0.3	41	0.2
L3z..00	Complications of labour and delivery NOS	4	0.1	44	0.2
62S..00	Maternal P/N 6 week exam.	9	0.3	38	0.1
6313.00	Consultant unit birth	3	0.1	43	0.2
L222.12	Breech delivery	5	0.2	38	0.1
6331.00	Single live birth	4	0.1	36	0.1
7F17.12	Ventouse extraction	2	0.1	36	0.1
ZV27.11	[V]Live birth	3	0.1	34	0.1
7799B	Stillbirth	8	0.3	28	0.1
Z251.00	Mother delivered	2	0.1	34	0.1
64B2.11	Baby normal at birth	4	0.1	31	0.1
Q032.00	Fetus or neonate affected by forceps delivery	2	0.1	32	0.1
7789CF	Caesarian section (baby)	5	0.2	28	0.1
7789TE	Ventouse birth extraction (baby)	3	0.1	30	0.1
633..11	Livebirth	2	0.1	30	0.1
63E1.00	Spontaneous onset of labour	6	0.2	26	0.1
7F16400	Low forceps cephalic delivery	0	0.0	32	0.1
634..13	Male baby	5	0.2	25	0.1
7789A1	Baby normal at birth	3	0.1	26	0.1
L210100	Twin pregnancy - delivered	5	0.2	22	0.1
7789NA	Normal apgar rating	2	0.1	25	0.1
K769 EC	Elective caesarian section	2	0.1	24	0.1
Q034.00	Fetus or neonate affected by caesarean section	6	0.2	20	0.1
7789CE	Caesarian section birth (baby)	5	0.2	20	0.1
Z257.15	ND - Normal delivery	0	0.0	24	0.1
7789NB	Normal birth (baby)	0	0.0	24	0.1
6311.00	Home birth	0	0.0	23	0.1
0389B	Umbilical sepsis	0	0.0	23	0.1
K760	Episiotomy	1	0.0	22	0.1
K7581B	Ventouse extraction delivery (baby)	1	0.0	20	0.1
6341.00	Baby male	0	0.0	21	0.1
7F21000	Manual removal of placenta from delivered uterus	3	0.1	17	0.1
7F1B000	Episiotomy to facilitate delivery	1	0.0	18	0.1
634..12	Female baby	1	0.0	18	0.1
657 B	Twin pregnancy delivery	2	0.1	16	0.1
Z257.11	Normal delivery	3	0.1	15	0.1
Q4z..15	Stillbirth NEC	6	0.2	11	0.0
6342.00	Baby female	2	0.1	15	0.1
Y61 M	Postnatal examination minor problem	5	0.2	11	0.0
7F12100	Elective lower uterine segment caesarean delivery	4	0.1	11	0.0
L14..11	Premature labour	3	0.1	12	0.0
Q48D.00	[X] stillbirth	5	0.2	10	0.0
637..00	Birth head circumference	2	0.1	13	0.0
650 N	Delivery no details	0	0.0	15	0.1
Z257.00	Delivery normal	0	0.0	15	0.1
64B2.00	Child birth exam. - normal	3	0.1	12	0.0
Q033.00	Fetus or neonate affected by vacuum extraction delivery	0	0.0	14	0.1

Medical code	Medical term	Case records (N=2815)		Control records (N=26068)	
		n	%	n	%
L340.00	First degree perineal tear during delivery	2	0.1	12	0.0
14Y5.00	Born by ventouse delivery	0	0.0	14	0.1
L395.12	Neville - Barnes forceps delivery	0	0.0	13	0.0
L360.11	Retained placenta NOS	1	0.0	12	0.0
14Y2.00	Born by elective caesarean section	1	0.0	12	0.0
62R1.00	P/N - first day visit	1	0.0	11	0.0
Q41y111	Perinatal transient vaginal bleeding	1	0.0	11	0.0
661 J	Premature delivery (mother)	8	0.3	4	0.0
L142.11	Premature delivery	10	0.4	2	0.0
7775DH	Hospital confinement (baby)	1	0.0	10	0.0
62SZ.00	Maternal P/N 6 week exam. NOS	2	0.1	9	0.0
14Y0.00	Born by caesarean section	1	0.0	10	0.0
650 CA	Normal labour	1	0.0	10	0.0
7F11z00	Other induction of labour NOS	2	0.1	9	0.0
658	Perineal laceration at delivery	0	0.0	11	0.0
6351.00	Baby premature 36-38 weeks	3	0.1	7	0.0
L397.00	Breech extraction	0	0.0	10	0.0
652 R	Retained placenta	0	0.0	10	0.0
650 C	Normal birth (confinement)	1	0.0	9	0.0
L396.00	Vacuum extractor delivery	0	0.0	10	0.0
635..13	Premature baby	5	0.2	5	0.0
L395200	Low forceps delivery	1	0.0	9	0.0
Z257.12	Spontaneous vaginal delivery	2	0.1	7	0.0
657 D	Labour difficult	0	0.0	9	0.0
Z257100	Spontaneous vertex delivery	0	0.0	9	0.0
653	PPH (postpartum haemorrhage)	2	0.1	7	0.0
L142.00	Early onset of delivery	1	0.0	8	0.0
635..12	Postmature baby	0	0.0	9	0.0
T801	Labour induction nonsurgical	4	0.1	5	0.0
633..14	Twin birth	3	0.1	6	0.0
6349E	Labour premature	0	0.0	8	0.0
656 B	Delivery breech	0	0.0	8	0.0
L28y.13	Amniotic fluid leaking	0	0.0	8	0.0
14Y3.00	Born by normal vaginal delivery	0	0.0	8	0.0
L342.00	Third degree perineal tear during delivery	1	0.0	7	0.0
K755 AA	Forceps extraction midcavity	0	0.0	8	0.0
Z254500	Delivered by caesarean section - pregnancy at term	2	0.1	6	0.0
7789CG	Delivery caesarian section (baby)	1	0.0	7	0.0
7F14.00	Breech extraction delivery	1	0.0	6	0.0
63E..00	Labour details	1	0.0	6	0.0
L1A..00	Sublux of symphysis pubis in pregnancy childbirth and puerperium	0	0.0	7	0.0
Z241100	Onset of labour induced	2	0.1	5	0.0
K7561	Forceps extraction low with episiotomy	1	0.0	6	0.0
7F16900	Kielland forceps cephalic delivery with rotation	1	0.0	6	0.0
K7562	Forceps extraction low	0	0.0	6	0.0
7F18.00	Cephalic vaginal delivery abnormal presentation head - no instrument	0	0.0	6	0.0
7789ND	Neonatal death	3	0.1	3	0.0
Z257.13	SVD - Spontaneous vaginal delivery	0	0.0	6	0.0
7F10z12	ARM (Artificial rupture of the membranes)	0	0.0	6	0.0
7789FC	Forceps birth (baby)	2	0.1	4	0.0
650 DB	Delivery gp unit (mother)	0	0.0	6	0.0
7F19100	Water birth delivery	0	0.0	6	0.0

Medical code	Medical term	Case records (N=2815)		Control records (N=26068)	
		n	%	n	%
6333.00	Twins - both live born	2	0.1	4	0.0
7789AH	Normal baby delivered normally	2	0.1	4	0.0
L112100	Placental abruption - delivered	1	0.0	5	0.0
656 BM	Delivery breech (mother)	0	0.0	5	0.0
7F16300	Mid forceps cephalic delivery NEC	2	0.1	3	0.0
Q213.11	Fetal distress in labour - liveborn	1	0.0	4	0.0
7F11.00	Other induction of labour	1	0.0	4	0.0
7F15100	Assisted breech delivery	0	0.0	5	0.0
14Y1.00	Born by forceps delivery	0	0.0	5	0.0
63F..00	Birth details not known	1	0.0	4	0.0
7F13z00	Other caesarean delivery NOS	3	0.1	2	0.0
K7551KD	Keillands delivery (mother)	0	0.0	5	0.0
657 BB	Twins non identical delivered	0	0.0	5	0.0
Z254200	Delivered by low forceps delivery	2	0.1	3	0.0
6349LA	Leaking amniotic fluid	0	0.0	5	0.0
Z254900	Vaginal delivery	1	0.0	4	0.0
6371PP	Eclampsia post partum	5	0.2	0	0.0
ZV27.12	[V]stillbirth	1	0.0	4	0.0
636..00	Birthweight of baby	1	0.0	4	0.0
Q4z..12	Neonatal death	1	0.0	4	0.0
7F19z00	Normal delivery NOS	0	0.0	4	0.0
L340000	First degree perineal tear during delivery, unspecified	0	0.0	4	0.0
Q421.11	ABO isoimmunisation of the newborn	0	0.0	4	0.0
L356.14	Symphysis pubis separation	3	0.1	1	0.0
650 DA	Delivery domicillary (mother)	0	0.0	4	0.0
L360.00	Third-stage postpartum haemorrhage	1	0.0	3	0.0
656 TR	Transverse lie delivery	0	0.0	4	0.0
K7601	Episiotomy repair	0	0.0	4	0.0
7789NC	Birth no details	0	0.0	4	0.0
L341000	Second degree perineal tear during delivery, unspecified	0	0.0	4	0.0
L291.00	Failed medical or unspecified induction	2	0.1	2	0.0
639..00	Apgar at 1 minute	1	0.0	3	0.0
657 T	Delay 2nd stage (labour)	0	0.0	4	0.0
6335.00	Twins - both still born	0	0.0	4	0.0
9N05.00	Seen in postnatal clinic	0	0.0	4	0.0
62O5.00	Spontaneous membrane rupture	0	0.0	3	0.0
633Z.00	Outcome of delivery NOS	0	0.0	3	0.0
650 AN	Pregnancy uncomplicated delivery	0	0.0	3	0.0
L452400	Obstetric nonpurulent mastitis with postnatal complication	0	0.0	3	0.0
L200.00	Normal delivery but ante- or post- natal conditions present	0	0.0	3	0.0
L222.11	Assisted breech delivery	0	0.0	3	0.0
Q212.00	Liveborn with prelabour fetal distress	0	0.0	3	0.0
7F1B300	Manual dilatation of cervix	0	0.0	3	0.0
7789BB	Breech birth (baby)	1	0.0	2	0.0
7F10.00	Surgical induction of labour	3	0.1	0	0.0
62S7.00	Postnatal examination normal	0	0.0	3	0.0
Z254300	Delivered by mid-cavity forceps delivery	0	0.0	3	0.0
L213200	Multiple delivery, all by caesarean section	0	0.0	3	0.0
L304100	Persistent occipitopost/occipitoant position - delivered	0	0.0	3	0.0
7F14100	Forceps to aftercoming head (breech)	0	0.0	3	0.0

Medical code	Medical term	Case records (N=2815)		Control records (N=26068)	
		n	%	n	%
661 D	Postmature at delivery (mother)	0	0.0	3	0.0
63A..00	Apgar at 5 minutes	1	0.0	2	0.0
7F1..00	Induction and delivery operations	1	0.0	2	0.0
L126100	Eclampsia - delivered	3	0.1	0	0.0
6G...00	Postnatal care	2	0.1	1	0.0
L3X..00	Intrapartum haemorrhage, unspecified	1	0.0	1	0.0
ZV27000	[V]Single live birth	0	0.0	2	0.0
L182400	Anaemia in the puerperium - baby previously delivered	0	0.0	2	0.0
62NE.00	A/N 39 week examination	2	0.1	0	0.0
777 B	Premature baby	2	0.1	0	0.0
653 A	Postpartum haemorrhage immediate	0	0.0	2	0.0
L452.00	Obstetric nonpurulent mastitis	0	0.0	2	0.0
8CH..00	Post partum care	0	0.0	2	0.0
7F16100	High forceps cephalic delivery NEC	0	0.0	2	0.0
L342z00	Third degree perineal tear during delivery NOS	0	0.0	2	0.0
62R6.00	P/N - sixth day visit	0	0.0	2	0.0
7F21.00	Manual removal retained products conception delivered uterus	0	0.0	2	0.0
62RA.00	P/N - tenth day visit	0	0.0	2	0.0
Z29..00	Postnatal examination observations	1	0.0	1	0.0
650 DC	Delivery in hospital (mother)	0	0.0	2	0.0
634..11	Delivery - sex of baby	0	0.0	2	0.0
L398z00	Caesarean delivery NOS	0	0.0	2	0.0
L356.13	Pubic symphysis separation	0	0.0	2	0.0
6779C	Postpartum haemorrhage delayed	0	0.0	2	0.0
657 SS	Delivery delay in second stage	0	0.0	2	0.0
L37z.00	Retained placenta or membranes with no haemorrhage NOS	0	0.0	2	0.0
L396z00	Vacuum extractor delivery NOS	1	0.0	1	0.0
Z246311	Onset of labour pains	0	0.0	2	0.0
ZV27.13	[V]Birth - type	2	0.1	0	0.0
657 BA	Twins identical delivered	0	0.0	2	0.0
L341z00	Second degree perineal tear during delivery NOS	1	0.0	1	0.0
L305.00	Shoulder dystocia	0	0.0	2	0.0
L312100	Other uterine inertia - delivered	0	0.0	2	0.0
L340400	Fourchette tear during delivery	0	0.0	2	0.0
651 AD	Delivery after antepartum haemorrhage	1	0.0	1	0.0
L330.00	Prolapse of cord	0	0.0	2	0.0
62Q..00	Postnatal care provider	0	0.0	2	0.0
62Q2.00	P/N care from G.P.	0	0.0	2	0.0
6353.00	Baby extremely prem.28-32 week	2	0.1	0	0.0
ZV24.11	[V]Postnatal care and examination	0	0.0	2	0.0
L340500	Vulval tear during delivery	0	0.0	2	0.0
Q404.11	Umbilical stump infection of the newborn	0	0.0	2	0.0
L395.11	Keilland's forceps delivery	1	0.0	1	0.0
L15..00	Prolonged or post-term pregnancy	2	0.1	0	0.0
Q30..00	Respiratory distress syndrome	2	0.1	0	0.0
62R3.00	P/N - third day visit	0	0.0	2	0.0
L442.12	Episiotomy breakdown	0	0.0	2	0.0
L400.00	Puerperal endometritis	1	0.0	1	0.0
62RZ.00	Postnatal visit NOS	1	0.0	1	0.0
650 EP	Delivery epidural	0	0.0	2	0.0
6349PL	Premature labour undelivered	0	0.0	2	0.0

Medical code	Medical term	Case records (N=2815)		Control records (N=26068)	
		n	%	n	%
Z254700	Deliveries by vacuum extractor	0	0.0	2	0.0
6352.00	Baby v. premature 32-36 weeks	1	0.0	1	0.0
Z254A00	Abnormal delivery	0	0.0	2	0.0
6779E	Postnatal haemorrhage	0	0.0	1	0.0
Ly1..00	Spontaneous breech delivery	0	0.0	1	0.0
L343.00	Fourth degree perineal tear during delivery	0	0.0	1	0.0
L340600	Vaginal tear during delivery	0	0.0	1	0.0
L282z00	Prolonged spontaneous/unspecified rupture of membranes NOS	0	0.0	1	0.0
656 A	Malpresentation at delivery	0	0.0	1	0.0
L309.00	Failed ventouse extraction unspecified	0	0.0	1	0.0
6355.00	Baby post-mature	0	0.0	1	0.0
L142100	Early onset of delivery - delivered	0	0.0	1	0.0
Q31y500	Neonatal snuffles	0	0.0	1	0.0
L340100	First degree perineal tear during delivery - delivered	0	0.0	1	0.0
Q031600	Fetus/neonate affected by disproportion during labour/delivery	0	0.0	1	0.0
L344000	Unspecified perineal laceration during delivery, unspecified	0	0.0	1	0.0
ZV29100	[V]Newborn receiving special care	0	0.0	1	0.0
7F13y00	Other specified other caesarean delivery	0	0.0	1	0.0
6779G	Retained placenta fragments puerperium	0	0.0	1	0.0
Q206100	Birth plexus injury - Erb-Duchenne	0	0.0	1	0.0
L322.00	Prolonged second stage	1	0.0	0	0.0
62R7.00	P/N - seventh day visit	0	0.0	1	0.0
L250100	Fetus with central nervous system malformation - delivered	0	0.0	1	0.0
6359.00	Baby premature 38 weeks	1	0.0	0	0.0
6363.00	Baby BW = 10%-24% (2850-3149g)	0	0.0	1	0.0
K762	Delivery assisted breech	0	0.0	1	0.0
Z254E00	Multiple birth	0	0.0	1	0.0
L341200	Second degree perineal tear during delivery with p/n problem	0	0.0	1	0.0
F4F5400	Neonatal nasolacrimal duct obstruction	0	0.0	1	0.0
678 BL	Mastitis lactating	0	0.0	1	0.0
636..11	Birthweight	0	0.0	1	0.0
7F1A000	Caesarian hysterectomy	0	0.0	1	0.0
7F15y00	Other specified other breech delivery	0	0.0	1	0.0
Q214.11	Fetal distress, unspecified when, liveborn	0	0.0	1	0.0
L37..00	Retained placenta or membranes with no haemorrhage	0	0.0	1	0.0
Q030.11	Fetus affected by breech delivery	0	0.0	1	0.0
6332.00	Single stillbirth	0	0.0	1	0.0
M261700	Acne neonatorum	0	0.0	1	0.0
L39y.00	Other complications of labour and delivery	0	0.0	1	0.0
L4...00	Complications of the puerperium	0	0.0	1	0.0
Q404100	Omphalitis	0	0.0	1	0.0
Q036.00	Fetus or neonate affected by precipitate delivery	0	0.0	1	0.0
L181500	Postpartum thyroiditis	0	0.0	1	0.0
L391.00	Obstetric shock	0	0.0	1	0.0
Z263F00	Spontaneous forewater rupture of membranes	0	0.0	1	0.0
7F19000	Manually assisted vaginal delivery	0	0.0	1	0.0
Q....00	Perinatal conditions	0	0.0	1	0.0
7F25.13	Monitoring during labour	0	0.0	1	0.0

Medical code	Medical term	Case records (N=2815)		Control records (N=26068)	
		n	%	n	%
L344.00	Unspecified perineal laceration during delivery	0	0.0	1	0.0
7F11100	Induction of labour using prostaglandins	1	0.0	0	0.0
7F10z00	Surgical induction of labour NOS	1	0.0	0	0.0
L3...00	Complications occurring during labour and delivery	0	0.0	1	0.0
L341100	Second degree perineal tear during delivery - delivered	0	0.0	1	0.0
L354.12	High vaginal tear - obstetric	0	0.0	1	0.0
ZV27200	[V]Twins, both live born	0	0.0	1	0.0
L125400	Severe pre-eclampsia with postnatal complication	1	0.0	0	0.0
Q48D100	[X]Macerated stillbirth	0	0.0	1	0.0
L244400	Other uterine/pelvic floor abnormality - baby delivered previously	0	0.0	1	0.0
ZV29200	[V]Newborn receiving intensive care	0	0.0	1	0.0
7F16000	High forceps cephalic delivery with rotation	0	0.0	1	0.0
Z241.00	Labour established	0	0.0	1	0.0
Q11z.00	Born premature NOS	1	0.0	0	0.0
Q48y600	Early neonatal death	0	0.0	1	0.0
7710C	Cord compressed (baby)	0	0.0	1	0.0
7F23.11	Immediate repair of obstetric tear	0	0.0	1	0.0
Q215.00	Severe birth asphyxia - apgar score less than 4 at 1 minute	0	0.0	1	0.0
L125100	Severe pre-eclampsia - delivered	1	0.0	0	0.0
Z246211	Start of labour	0	0.0	1	0.0
63B..00	Apgar at 10 minutes	0	0.0	1	0.0
6354.00	Baby full term maturity	0	0.0	1	0.0
L126600	Eclampsia in labour	1	0.0	0	0.0
657 E	Dystocia	0	0.0	1	0.0
K7551	Forceps extraction midcavity with episiotomy	0	0.0	1	0.0
Q214.00	Liveborn with fetal distress, unspecified	0	0.0	1	0.0
L281.00	Premature rupture of membranes	1	0.0	0	0.0
L213100	Multiple delivery, all by forceps and vacuum extractor	0	0.0	1	0.0
L345.12	Vulval and perineal haematoma during delivery	0	0.0	1	0.0
650 G	Delivery premature in hospital/maternity	1	0.0	0	0.0
L394600	Haematoma of obstetric wound	0	0.0	1	0.0
L394500	Infection of obstetric surgical wound	0	0.0	1	0.0
657 C	Labour difficult atony uterus	0	0.0	1	0.0
L321.00	Prolonged labour unspecified	0	0.0	1	0.0
Lyu6A00	[X]Infection of caesarian section wound following delivery	0	0.0	1	0.0
6312.00	GP unit birth	0	0.0	1	0.0
777 D	Dysmaturity newborn	0	0.0	1	0.0
L452100	Obstetric nonpurulent mastitis - delivered	0	0.0	1	0.0
L150100	Post-term pregnancy - delivered	1	0.0	0	0.0
L281z00	Premature rupture of membranes NOS	0	0.0	1	0.0
63D6.00	Placenta incomplete	0	0.0	1	0.0
L225.00	Face presentation	0	0.0	1	0.0
L291.11	Failed medical induction of labour	1	0.0	0	0.0
L398100	Caesarean delivery - delivered	0	0.0	1	0.0
K777 AC	Suture obstetric laceration	0	0.0	1	0.0
62T..00	Misc. Postnatal data	0	0.0	1	0.0
L395500	Mid-cavity forceps with rotation	0	0.0	1	0.0
Z239100	Uterine contractions present	0	0.0	1	0.0

Medical code	Medical term	Case records (N=2815)		Control records (N=26068)	
		n	%	n	%
L126400	Eclampsia with postnatal complication	1	0.0	0	0.0
L371.00	Retained portion of placenta or membranes - no haemorrhage	0	0.0	1	0.0
7781	Postmature (baby)	0	0.0	1	0.0
L396100	Vacuum extractor delivery - delivered	0	0.0	1	0.0
L395z00	Forceps delivery NOS	0	0.0	1	0.0
L127100	Pre-eclampsia or eclampsia with hypertension - delivered	1	0.0	0	0.0
L30..00	Obstructed labour	0	0.0	1	0.0
L124200	Mild or unspecified pre-eclampsia - delivered with p/n comp	1	0.0	0	0.0
L34zz00	Vulval/perineal trauma during delivery NOS	0	0.0	1	0.0
K7541	Forceps extraction high with episiotomy	0	0.0	1	0.0
L28y300	Ragged membranes	1	0.0	0	0.0
63E3.00	Normal labour	0	0.0	1	0.0
L461.00	Cracked nipple in pregnancy, the puerperium or lactation	0	0.0	1	0.0
L340300	Labial tear during delivery	0	0.0	1	0.0
Q48D000	[X]Fresh stillbirth	0	0.0	1	0.0
ZV27100	[V]Single stillbirth	0	0.0	1	0.0
7F23.00	Immediate repair of obstetric laceration	0	0.0	1	0.0

NEC=not elsewhere classified; NOS=not otherwise specified; O/E=on examination; the prefix [V] is used for codes corresponding to the ICD10 chapter that records reasons other than illness for contact with the Health Service (e.g. childbirth); P/N=postnatal; the prefix [X] is used for codes introduced with the migration to ICD10 in April 1995; A/N=antenatal; BW=birth weight

Appendix H Odds ratios for pre-eclampsia associated with smoking (seven category variable)

Table H.1 Odds ratios for the association between pre-eclampsia and maternal smoking (seven categories using information on smoking status during and pre-pregnancy).

Maternal smoking status	Cases N=1533 n (%)	Controls N =14236 n (%)	Pre-eclampsia odds ratio (95% CI)
In pregnancy			
1. non-smoker	162 (10.6)	1184 (8.3)	1.00
2. ex-smoker	66 (4.3)	590 (4.1)	0.82 (0.60-1.12)
3. current smoker	65 (4.2)	720 (5.1)	0.65 (0.48-0.88)
In pregnancy, pre-pregnancy			
4. unknown, non-smoker	672 (43.8)	5496 (38.6)	0.87 (0.72-1.06)
5. unknown, ex-smoker	100 (6.5)	886 (6.2)	0.80 (0.61-1.05)
6. unknown, current smoker	218 (14.2)	2591 (18.2)	0.58 (0.46-0.72)
7. unknown, unknown	250 (16.3)	2769 (19.5)	0.60 (0.48-0.76)

Appendix I Participant flow through the dating pregnancy algorithm

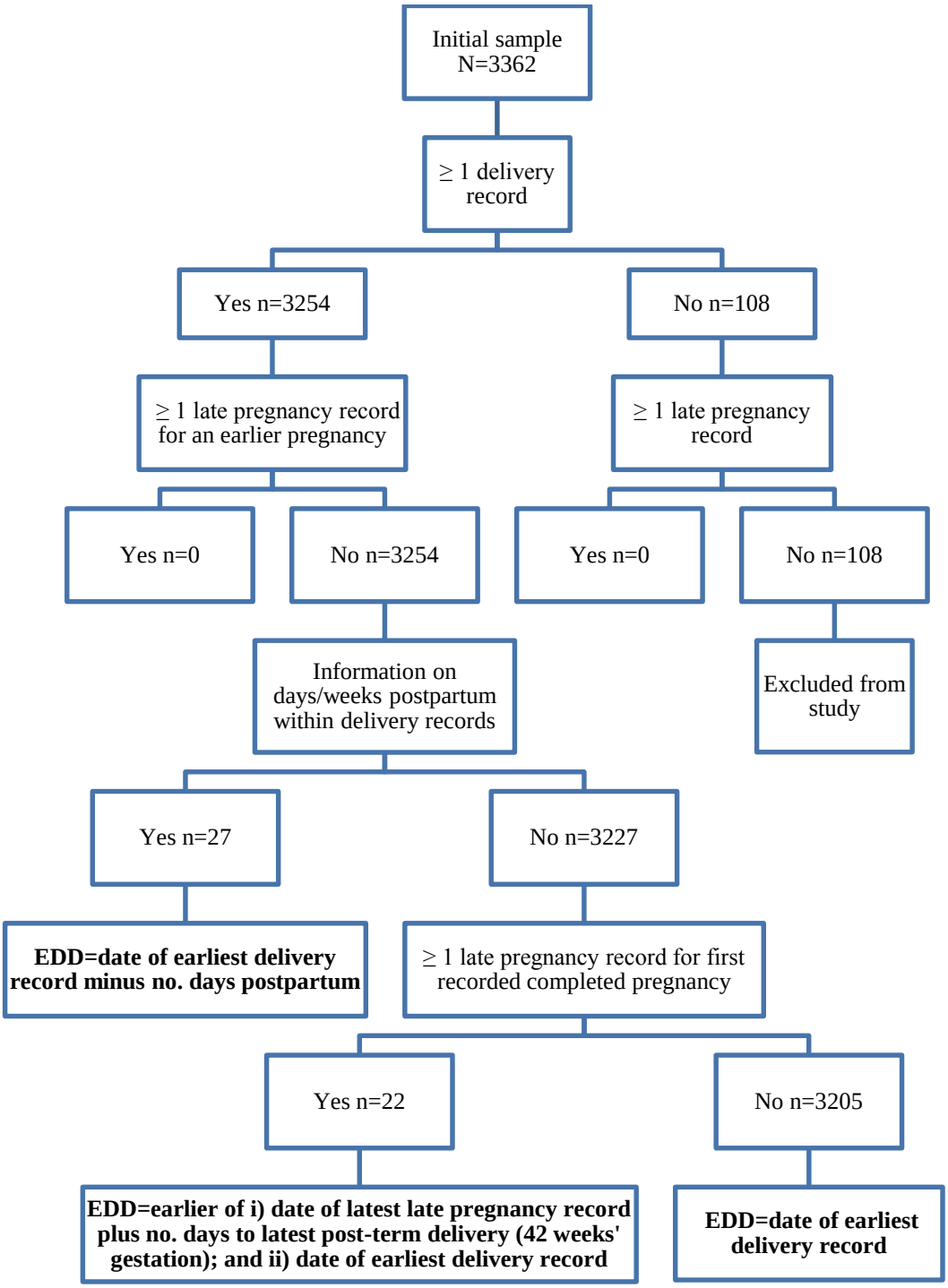


Figure I.1 Deriving the estimated date of delivery (EDD) of potential cases' first recorded completed pregnancies (n=3362).

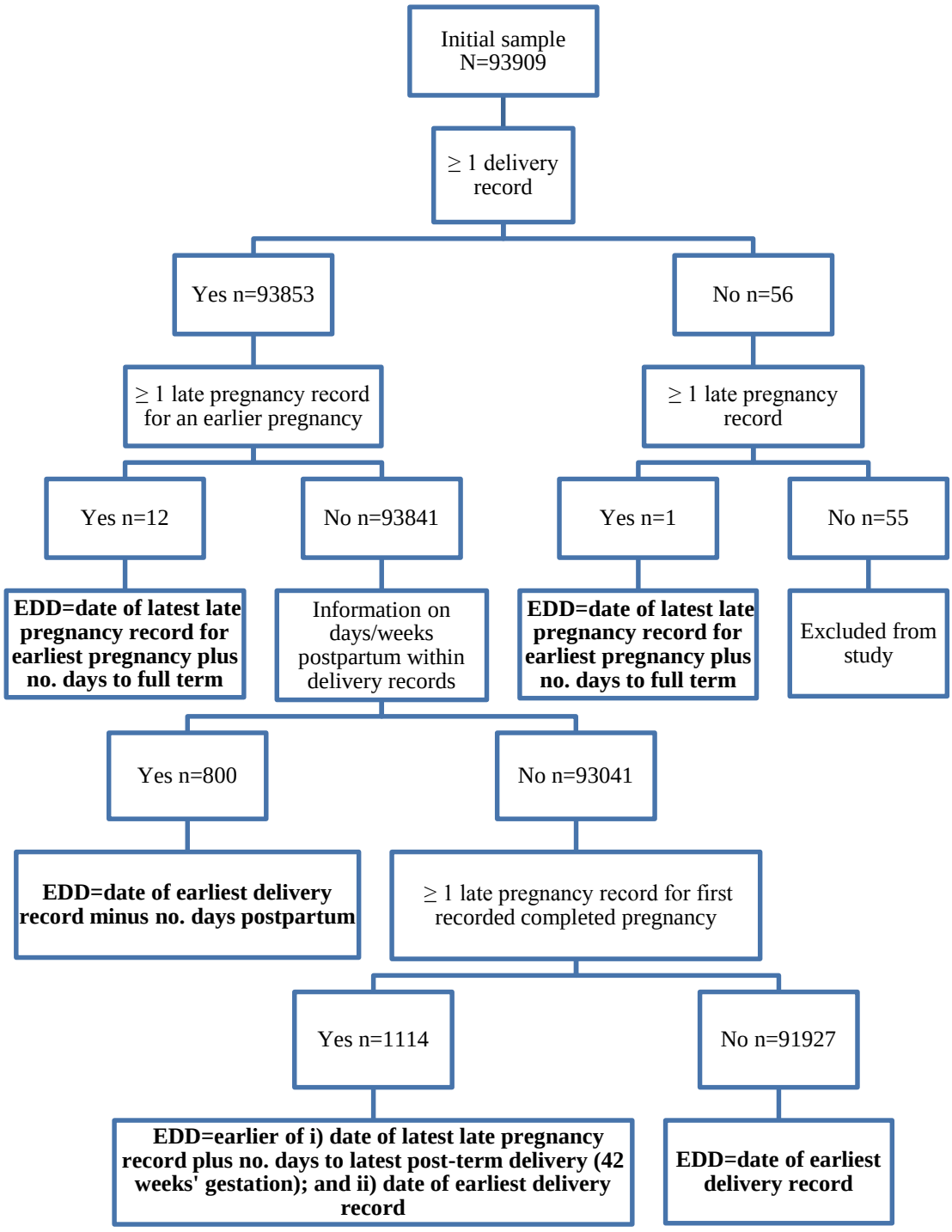


Figure I.2 Deriving the estimated date of delivery (EDD) of potential controls' first recorded completed pregnancies (n=93909).

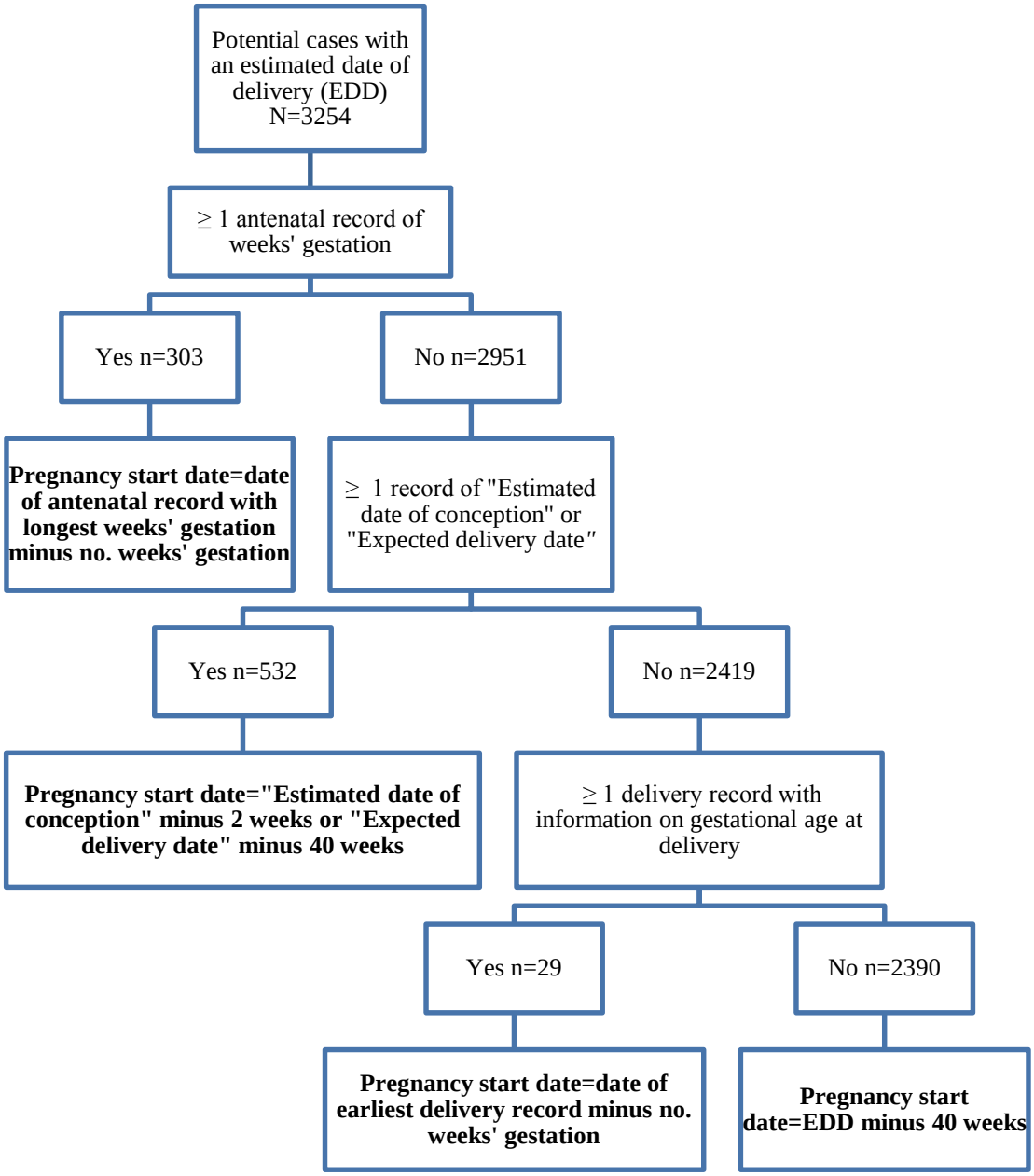


Figure I.3 Estimating the pregnancy start date for potential cases with an estimated date of delivery (EDD) (n=3254).

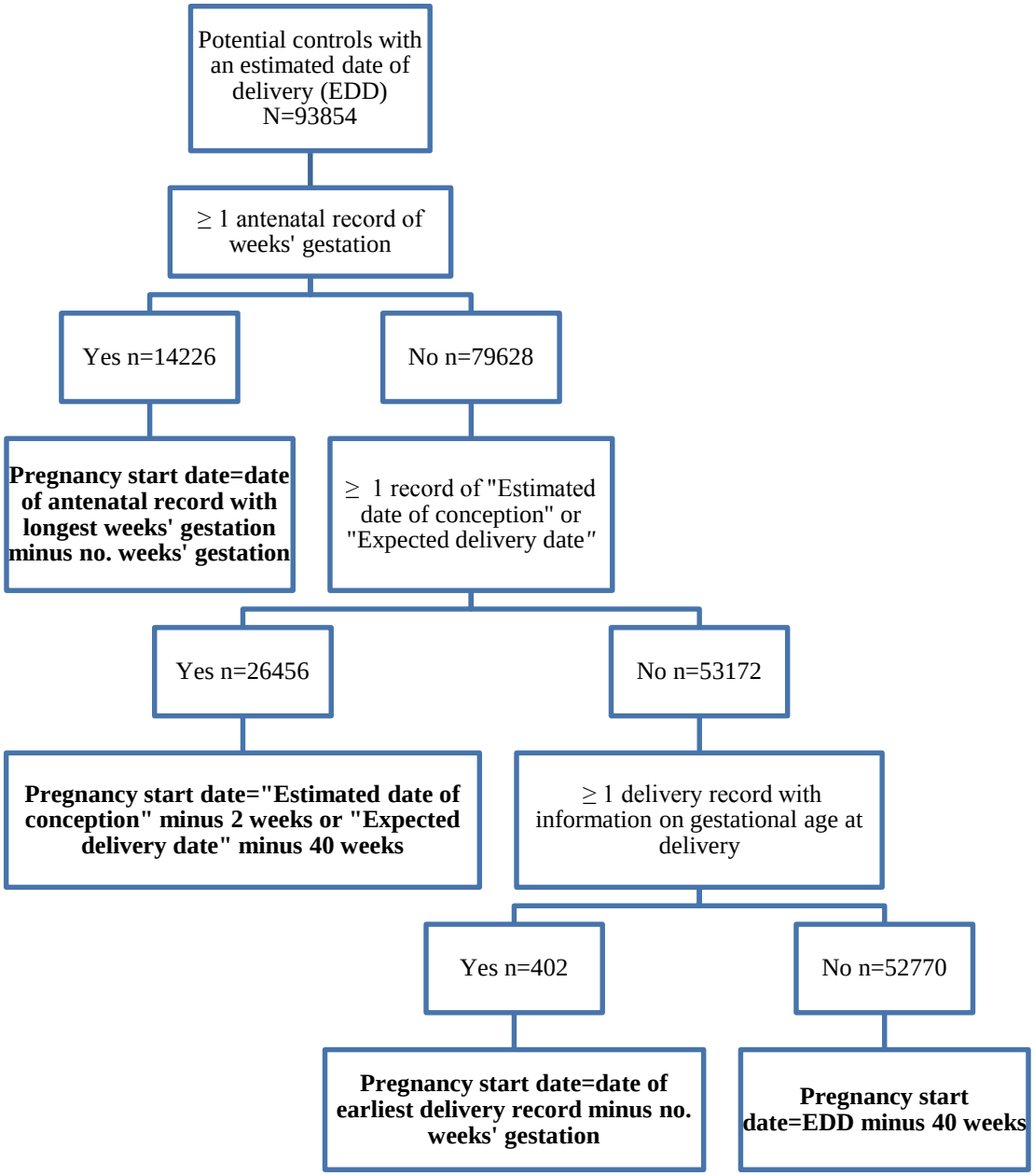


Figure I.4 Estimating the pregnancy start date for potential controls with an estimated date of delivery (EDD) (n=93854).

Appendix J Characteristics of cases and controls eligible for matching in the pre-eclampsia study

Table J.1 Characteristics of potential study participants eligible for matching.

Characteristic n (%)	Potential cases (N=1535)	Potential controls (N=84999)
Maternal age at delivery (years)		
<20	133 (8.7)	9621 (11.3)
20-24	340 (22.2)	17093 (20.1)
25-29	478 (31.1)	25139 (29.6)
30-34	407 (26.5)	22414 (26.4)
35-39	146 (9.5)	8912 (10.5)
40-44	31 (2.0)	1820 (2.1)
median, IQR	*28.3, 23.9-32.3*	*28.3, 23.5-32.3*
Pre-pregnancy BMI (kg/m^2)		
<18.5 (underweight)	26 (1.7)	3377 (4.0)
18.5-25 (normal)	620 (40.4)	40000 (47.1)
25-30 (overweight)	272 (17.7)	12300 (14.5)
30+ (obese)	192 (12.5)	5921 (7.0)
missing	425 (27.7)	23401 (27.5)
median, IQR	*24.1, 21.6-27.9*	*22.8, 20.7-25.7*
Smoking status in pregnancy		
non-smoker	835 (54.4)	40869 (48.1)
ex-smoker	166 (10.8)	9331 (11.0)
current smoker	284 (18.5)	20014 (23.6)
unknown	250 (16.3)	14785 (17.4)
Practice level socioeconomic status		
IMD score *[median, IQR]*	*16.2, 8.7-30.1*	*18.4, 9.9-33.9*
Patient level socioeconomic status		
IMD score *[median, IQR]*	*14.3, 8.4-25.7*	*15.5, 8.8-28.3*
missing	745 (48.5)	38585 (45.4)
Pre-existing hypertension	161 (10.5)	5509 (6.5)
Pre-existing renal disease	4 (0.3)	159 (0.2)
Pre-existing diabetes	28 (1.8)	1099 (1.3)
Pre-existing asthma	291 (19.0)	16169 (19.0)
Previous miscarriage or termination	298 (19.4)	17231 (20.3)
Multiple pregnancy	25 (1.6)	722 (0.9)
ART pregnancy	11 (0.7)	496 (0.6)
Consultations with GP pre-pregnancy *[median, IQR]*	*11, 4-27*	*12, 5-27*
UTS follow-up pre-pregnancy (years) *[median, IQR]*	*2.4, 0.9-5.1*	*2.9, 1.2-6.0*

Abbreviations: IQR=interquartile range; BMI=body mass index; IMD=Index of Multiple Deprivation score based on practice post-code (practice level socioeconomic status) or patient post-code (patient level socioeconomic status): the higher the score, the greater the deprivation; UTS=up-to-standard (i.e. data meeting GPRD quality standards); ART=assisted reproductive technology