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# Self-controlled designs to control confounding

Pouwels, Koen

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# Self-controlled designs to control confounding

Koen Pouwels

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# Self-controlled designs to control confounding

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CHAPTER 1

# General introduction

Ideally, randomized controlled trials (RCTs) are used to evaluate both causal intended and unintended effects of interventions. If the intervention is allocated randomly, the trial has a large number of participants and there is no substantial loss to follow-up, groups of patients with and without the intervention will on average have similar risks of outcomes, except for the potential benefits and risks caused by the intervention itself. Unfortunately, it is often unethical or not feasible to perform a RCT [1]. For example, it would be unethical to design a RCT deliberately exposing patients to a potentially harmful exposure such as diethylstilboestrol (DES).
Moreover, RCTs are often too expensive or not feasible when studying the effect of exposures on rare outcomes or exposure-outcome relationships with long induction periods [1]. When it is unethical or not feasible to perform a RCT, non-randomized observational studies are essential to guide health care decision making [2]. Often, in absence of RCTs, the best available evidence for decision-making will come from observational studies.

However, in daily life, exposures are generally not allocated by a random process. By the art of medicine, drugs are prescribed to patients in need of treatment, persons who regularly exercise have a healthier life style in general than persons who rarely exercise, subjects that do not use alcohol may do this because of an underlying disease, etc. As a consequence, observational studies that study the effects of exposures are prone to confounding bias [3].

Although several empirical examples have shown that observational studies tend to find similar effect estimates as RCTs [4-7], such non-randomized designs have been criticized because of notorious examples in which observational studies found contrasting results with RCTs. For example, findings from large RCTs refuted observational studies that suggested a protective effect of hormone replacement therapy against coronary heart disease [8]. While several observational studies suggested a protective effect of beta-carotene consumption against lung cancer among smokers, large RCTs showed no beneficial effect [9]. Another textbook example is the case of vitamin E supplementation and the risk of cardiovascular events, where observational studies did find a protective effect whereas RCTs did not [10]. Differences in effect estimates between observational and randomized studies are often attributed to unmeasured or inadequately measured confounders. For example, consumption of vitamin E supplements may correlate with a healthy life style. Consequently, patients with a relatively healthy life style are compared with patients with a less healthy life style; hence effect estimates will be biased by difficult to measure differences in average prognosis between comparison groups. It is therefore essential to adequately measure, adjust for, and report about all relevant confounders. Unfortunately, information on important potential confounders is often lacking from routine health care databases. Even when the effect estimates are adjusted for measured

potential confounders as in the examples mentioned above, biased effect estimates may be obtained, especially when difficult to measure patient characteristics or confounding domains are expected to bias the association of interest.

Given the vulnerability of observational studies to confounding, complete and transparent reporting about confounding is necessary to enable readers to assess the validity of study findings. Nevertheless, poor quality of reporting of confounding has previously been observed [11]. Acknowledging the widespread problem of inadequate reporting, the 'STrenghtening the Reporting of OBservational studies in Epidemiology' (STROBE) guideline was developed and published in 2007 [12]. This guideline includes several items related to the reporting of confounding and is endorsed by a growing number of biomedical journals. In this thesis we aim to assess whether the reporting of confounding improved in articles published after the publication of the STROBE guideline compares with articles published before that guideline.

One of the more novel developments with regard to the control of confounding in observational studies is the application of a self-controlled or case-only design. Examples are self-controlled case-series [13, 14], case-crossover design [15] and sequence symmetry analysis [16] which have been developed to overcome the problem of confounding by difficult to measure patient characteristics. The underlying idea of these designs is that patients can serve as their own controls, which reduces confounding by factors that are stable over time. This may include characteristics that are often not available to researchers such as chronic use of nonprescription drugs, health behaviors, tendency to seek professional care, occupation, etcetera. Although self-controlled designs are increasingly being used in recent years [17], empirical comparisons of such designs with each other, with more traditional observational designs and with randomized controlled trials are scarce. In this thesis we aim to apply and empirically compare various available self-controlled designs to quantify and control for confounding with other designs and to apply novel techniques.

As study objects both acute and chronic drug therapies with unintended and intended outcomes will be researched in this thesis. First, the association between angiotensin-converting enzyme inhibitors and urinary tract infections will be evaluated, because we have access to data from both randomized trial and large routine health care databases. Another advantage of assessing this association to research the self-controlled designs is that angiotensin-converting enzyme inhibitors are prescribed to patients with risk-factors for urinary tract infections such as diabetes and renal impairment [18]. Consequently, there is a high potential for confounding by indication for this association in the absence of accurately measured information about potential renal problems.

Second, we aim to study the effect of concomitant use of selective serotonin reuptake inhibitors (SSRIs) and nonsteroidal anti-inflammatory drugs (NSAIDs) on the occurrence of peptic ulcer. Previously, a conventional cohort study compared concomitant use of SSRIs with NSAIDs with concomitant use of tri-cyclic antidepressants (TCAs) and NSAIDs without adjusting for potential differences between these two groups of patients [19]. It has been shown that heavy alcohol use is a very strong confounder when evaluating the association between SSRI use and gastrointestinal bleeding [20]. Since TCAs can potentiate the effects of alcohol due to their antihistaminic effects, while SSRIs have minimal effects on alcohol pharmacokinetics and pharmacodynamics [21, 22], heavy alcohol use is likely less common among TCA users than SSRI users due to channeling by the physician. Consequently, this association has strong potential for confounding and is an interesting study object.

A third empirical study will be focused on the association between antibiotic use during pregnancy and development of asthma among preschool children. There is an ongoing debate about whether the increased risk observed in several conventional observational studies is due to unmeasured confounding [23-25]. Two recent studies suggested that the increased risk was due to confounding [23, 24]. However, both studies were vulnerable to different biases. In the current thesis, different novel methods to quantify and minimize confounding will be applied in order to evaluate whether the increased risk is indeed largely due to confounding bias.

Although individual studies comparing different designs and single-study comparisons can identify and highlight different strengths and weaknesses of case-only designs, systematic comparisons are needed to obtain more insights into the merits of case-only designs and possible limitations. Therefore, the concordance between case-only and cohort or case-control studies in published empirical studies will also be evaluated in a systematic way. A secondary aim is to identify predictors of discrepancies between case-only designs and traditional designs, with specific focus on potentially important underlying assumptions of the self-controlled case-series and case-crossover design.

## Thesis objectives

The studies presented in this thesis will focus on two main objectives: (1) to evaluate the reporting of confounding in observational cohort and case-control studies before and after the publication of an important reporting guideline for observational studies (STROBE) [26], and (2) to evaluate how self-controlled, or case-only, designs can be used to quantify and adjust for confounding. Various self-controlled designs will be

object of study: prescription sequence symmetry analysis [16], case-crossover [15], case-sibling [27], time-trend-control-sibling and self-controlled case-series [14].

## Thesis outline

In **chapter 2** the reporting of confounding in observational cohort and case-control studies on interventions for which a beneficial effect was hypothesized before and after the publication of an important reporting guideline for observational studies (STROBE) is presented.
In **chapter 3** the effect of pravastatin on recurrent urinary tract infections is evaluated in a *post hoc* analysis of a randomized controlled trial. In addition, the effect of fosinopril, an angiotensin-converting enzyme inhibitor, on acute urinary tract infections is assessed.
In **chapter 4** the association of angiotensin-converting enzyme inhibitors with the risk of acute urinary tract infections is assessed using a prescription sequence symmetry analysis. In **chapter 5**, the association between angiotensin-converting enzyme inhibitors and urinary tract infections is further evaluated using a case-crossover design. An empirical comparison with the prescription sequence symmetry analysis and the post-hoc analysis of the randomized controlled trial (chapter 3) is used to discuss some important differences between the sequence symmetry design and the case-crossover design. In **chapter 6**, the association of combined use of selective serotonin reuptake inhibitors and nonsteroidal anti-inflammatory drugs with the risk of starting peptic ulcer treatment is evaluated using a prescription sequence symmetry design. A comparison is made with a previously published cohort study that used the same database but did not adjust for potential confounders.
In **chapter 7** the association between antibiotic use during pregnancy and the development of asthma in preschool children is analysed using different confounding-minimizing designs, including a case-control and case-sibling design. In addition, we will develop a method that can address time-trend bias in case-sibling designs in this chapter. In **chapter 8** the concordance between case-only and case-control or cohort studies in empirical studies is evaluated in a systematic way. In addition, predictors of discrepancies between both types of designs are identified.
**Chapter 9** provides a general discussion of our findings and future perspectives.

## References


1. Wilcken B. Rare diseases and the assessment of intervention: what sorts of clinical trials can we use? J Inherit Metab *Dis* 2001;24**:**291-8.
2. Concato J. Is it time for medicine-based evidence? JAMA 2012;307:1641-3.
3. Lash TL, Fox MP, Fink AK. Applying quantitative bias analysis to epidemiologic data. Springer Science & Business Media; 2011.
4. Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the hierarchy of research designs. N Engl J Med 2000;342:1887-92.
5. Benson K, Hartz AJ. A comparison of observational studies and randomized, controlled trials. N Engl J Med 2000;342:1878-86.
6. Golder S, Loke YK, Bland M. Meta-analyses of adverse effects data derived from randomised controlled trials as compared to observational studies: methodological overview. PLoS Med 2011;8:e1001026.
7. Ioannidis JP, Haidich AB, Pappa M, et al. Comparison of evidence of treatment effects in randomized and nonrandomized studies. JAMA 2001;286:821-30.
8. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. Am J Epidemiol 2003;158:915-20.
9. Stram DO, Huberman M, Wu AH. Is residual confounding a reasonable explanation for the apparent protective effects of beta-carotene found in epidemiologic studies of lung cancer in smokers? Am J Epidemiol 2002;155:622-8.
10. Eidelman RS, Hollar D, Hebert PR, Lamas GA, Hennekens CH. Randomized trials of vitamin E in the treatment and prevention of cardiovascular disease. Arch Intern Med 2004;164:1552-6.
11. Groenwold RH, Van Deursen AM, Hoes AW, Hak E. Poor quality of reporting confounding bias in observational intervention studies: a systematic review. Ann Epidemiol 2008;18:746-51.
12. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, et al. Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ 2007;335:806-8.
13. Farrington CP, Nash J, Miller E. Case series analysis of adverse reactions to vaccines: a comparative evaluation. Am J Epidemiol 1996;143:1165-73.
14. Farrington CP. Relative incidence estimation from case series for vaccine safety evaluation. Biometrics 1995;51:228-35.
15. Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, Muller JE. Triggering of acute myocardial infarction by heavy physical exertion. Protection against triggering by regular exertion. Determinants of Myocardial Infarction Onset Study Investigators. N Engl J Med 1993;329:1677-83.
16. Hallas J. Evidence of depression provoked by cardiovascular medication: a prescription sequence symmetry analysis. Epidemiology 1996;7:478-84.
17. Consiglio GP, Burden AM, Maclure M, McCarthy L, Cadarette SM. Case-crossover study design in pharmacoepidemiology: systematic review and recommendations. Pharmacoepidemiol Drug Saf 2013;22:1146-53.
18. Rutten GEHM, de Grauw WJC, Nijpels G, et al. NHG-standaard diabetes mellitus type 2 (tweede herziening). Huisarts Wet 2006;49:137-52.
19. de Jong JC, van den Berg PB, Tobi H, de Jong-van den Berg LT. Combined use of SSRIs and NSAIDs increases the risk of gastrointestinal adverse effects. Br J Clin Pharmacol 2003;55:591-5.
20. Opatrny L, Delaney JA, Suissa S. Gastro-intestinal haemorrhage risks of selective serotonin receptor antagonist therapy: a new look. Br J Clin Pharmacol 2008;66:76-81.

21. Harvey BH. The neurobiology and pharmacology of depression. A comparative overview of serotonin selective antidepressants. S Afr Med J 1997;87:540,50,552.
22. Fraser AG. Pharmacokinetic interactions between alcohol and other drugs. Clin Pharmacokinet 1997;33:79-90.
23. Murk W, Risnes KR, Bracken MB. Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. Pediatrics 2011;127:1125-38.
24. Ortqvist AK, Lundholm C, Kieler H, et al. Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis. BMJ 2014;349:g6979.
25. Stokholm J, Sevelsted A, Bonnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. Lancet Respir Med 2014;2:631-7.
26. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med 2007;4:e296.
27. Donovan SJ, Susser E. Commentary: Advent of sibling designs. Int J Epidemiol 2011;40:345-9.

CHAPTER 2

# Quality of reporting of confounding before and after the STROBE statement

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## Abstract

### Background

Poor quality of reporting of confounding has been observed in observational studies prior the STrenghtening the Reporting of Observational studies in Epidemiology (STROBE) statement, a reporting guideline for observational studies. We assessed whether the reporting of confounding improved after the STROBE statement.

### Methods

We searched MEDLINE for all articles about observational cohort and case–control studies on interventions with a hypothesized beneficial effect in five general medical and five epidemiologic journals published between January 2010 and December 2012. We abstracted data for the baseline period before the publication of the STROBE statement (January 2004–April 2007) from a prior study. Six relevant items related to confounding were scored for each article. A comparison of the median number of items reported in both periods was made.

### Results

In total, 174 articles published before and 220 articles published after the STROBE statement were included. The median number reported items was similar before and after the publication of the STROBE statement [median, 4; interquartile range [IQR], 3–5 vs. median, 4; IQR, 3.75–5]. However, the distribution of the number of reported items shifted somewhat to the right ($P = 0.01$).

### Conclusions

Although the quality of reporting about confounding improved in certain aspects, the overall quality remains suboptimal. Research is needed into the development and evaluation of strategies to improve the quality of reporting and adherence to reporting guidelines.

## Introduction

There is a growing interest into widespread problems affecting the validity and reliability of published health care research [1-4]. Inadequate reporting is a widespread problem and has been frequently observed in publications of animal and other preclinical studies, observational studies, diagnostic studies, clinical prediction research, surveys and qualitative studies, and randomized trials [3]. Several studies indicate that it is often impossible to replicate studies, partly due to poor reporting [5-7]. Complete and transparent reporting is necessary to enable readers to assess the reliability and validity of study findings. Although poor reporting may have some correlation with the risk of bias [8], the reporting quality of a study does not necessarily reflect the methodological quality of the study [9-10]. Hence, without adequate reporting, it is difficult or impossible to assess the strengths and weaknesses of the study and to replicate the study. Furthermore, inadequate reporting wastes the time and resources invested in the conduct of research [3].

Guidelines on the reporting of research can improve the quality of reporting, especially if those guidelines are supported and adopted by journals [11-13]. Several guidelines have been developed to improve the quality of reporting of studies, including CONsolidated Standards of Reporting Trials (CONSORT), STrenghtening the Reporting of Observational studies in Epidemiology (STROBE), Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), Standards for Reporting of Diagnostic Accuracy (STARD), Animal Research: Reporting of In Vivo Experiments, Standards for Quality Improvement Reporting Excellence, and Consolidated Health Economic Evaluation Reporting Standards [14]. A comprehensive list of reporting guidelines can be found elsewhere (http://www.equator-network.org/http://www.equator-network.org/) [14]. The STROBE statement was developed to improve the reporting of observational studies and published in 2007 [15]. The adoption of this guideline differs per journal, although it seems intuitive to assume that more active endorsement would result in better reporting quality. Some journals actively endorse the use of the STROBE guideline and require the submission of the STROBE checklist (http://www.strobe-statement.org/fileadmin/Strobe/uploads/checklists/STROBE_checklist_v4_combined.pdf), such as The BMJ and recently PLOS Medicine [17], whereas other journals only endorse the use of the STROBE statement in their Instructions for Authors (e.g., Lancet) or do not mention the STROBE statement at all (e.g., New England Journal of Medicine).

It is well known that observational studies are prone to confounding because interventions are often prescribed to patients based on the perceived risk of the outcome instead of randomly assigned as in randomized controlled trials [18-19]. Moreover, especially for

preventive interventions, patients who initiate and adhere to the intervention of interest may be more health conscious, have a more healthy lifestyle, and may also adhere better to other preventive interventions [20-21].

Despite the vulnerability of observational studies to confounding, poor quality of reporting of confounding has previously been observed [22]. Included articles were published before the STROBE statement, and it was suggested that this statement, which was intended to improve the reporting of observational studies, could have a considerable impact on the reporting of confounding [22].

To enable an adequate assessment of the likelihood that a study is affected by unmeasured or residual confounding, several items should be reported and discussed. This is acknowledged by the designers of the STROBE statement, who included several items related to the reporting of confounding in the STROBE checklist: item 7 requires that all potential confounders are clearly defined; item 12 requires that all statistical methods, including those used to control for confounding are described; item 14a requires that characteristics of study participants and information on exposures and potential confounders are given; item 16 requires the reporting of unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision together with a clear description of the confounders that were adjusted for and why they were included. In addition, the following items which are more general statements related to bias may improve the reporting of confounding: item 9 requires that any efforts to address potential sources of bias are described in the method section, and item 19 requires that the limitation of the study is discusses, taking into account sources of potential bias or imprecision, thereby discussing both the directions as magnitude of any potential bias.

Because multiple items related to confounding are included in the STROBE statement, we were interested whether the reporting of confounding improved over time.

Our primary objective was to assess whether the reporting of confounding improved in articles after the publication of the STROBE statement compared with articles published before that statement. In secondary analyses, we evaluated whether reporting was better for journals that published the STROBE statement, endorsed the STROBE statement in their Instructions for Authors, and/or required the completion of the STROBE checklist when submitting an observational study. We hypothesized that the reporting of confounding would have been improved after publication of the STROBE guideline, especially in journals that endorsed the STROBE statement.

## Methods

As we intended to make a comparison with articles published before and after the STROBE statement, similar methods were used as previously described [22]. We searched the MEDLINE database to find observational studies that were published from January 2010 through December 2012 in the same five epidemiologic journals and five general medical journals. The five epidemiologic journal included International Journal of Epidemiology, Epidemiology, Journal of Clinical Epidemiology, American Journal of Epidemiology, and Journal of Epidemiology and Community Health. The five general medical journals included New England Journal of Medicine, The Lancet, Journal of the American Medical Association, The BMJ, and Annals of Internal Medicine. Those journals were selected in the previous study based on their high-impact factor [22]. Of these, five journals published the STROBE statement (The Lancet, The BMJ, Annals of Internal Medicine, Epidemiology, and Journal of Clinical Epidemiology). Of the journals that published the STROBE statement, The Lancet, The BMJ, and Annals of Internal Medicine also refer to the STROBE statement in their Instructions for Authors, whereas none of the other journals did endorse the STROBE statement in their instructions to authors. The BMJ was the only included journal that required the completion of the STROBE checklist when submitting an observational study.

### Data selection

The search strategy aimed at identifying observational cohort and case–control studies that evaluated a hypothesized beneficial (preventive) effect of an intervention on a clinical outcome. Hence, studies on adverse effects were excluded. We did not include randomized controlled trials, meta-analyses, letters, comments, editorials, studies in which the primary outcome (as indicated by the authors or the outcome mentioned in the abstract) was intermediate (e.g., cholesterol levels instead of cardiovascular disease), nonintervention studies (e.g., effect of weather on myocardial infarction incidence), before–after studies, or non-English studies.

The search strategy is listed in Appendix 1. In studies with multiple outcomes, we assessed the reporting related to the primary outcome. We excluded studies for which the allocation of the exposure of interest was likely determined by a random process as mentioned in Section 2 or anywhere else in the article as confounding will likely not play a role in such studies. For example, we excluded a study in which dispensation of proprietary vs. generic formulations of antiretroviral therapy was not driven by patient characteristics, but by the availability of drugs, with an effort to maintain a given patient on the same formulation from month to month. This resulted in a natural experiment that was close to a randomized trial, with a small likelihood of confounding.

We exported retrieved citations to Refworks (ProQuest, Ann Arbor, Michigan). Title and abstract screening were performed including all possibly relevant evaluations for further review. The full text of all remaining studies was retrieved and reviewed for eligibility.

### Data extraction

Details on a number of basic study characteristics and items related to reporting of confounding were independently extracted by two researchers (K.B.P. and N.N.W.). Disagreements were resolved by consensus. Basic study characteristics were journal type (general medical or epidemiologic), study design (cohort or case–control), publication year, whether the journal published the STROBE statement, type of intervention, and type of outcome. To facilitate a comparison with the previous assessment of reporting of confounding prior the publication of the STROBE statement [22], the same information on the design and analytical details concerning confounding were extracted (Table 2.1) [22]. It was assessed whether the following items were reported: characteristics of key confounders as well as reasons why potential confounders were selected for analysis and included in the final model; methods to control for confounding (e.g., stratification, multivariate regression, propensity score matching etc.); and both the crude as well as the adjusted effect estimate, in case only an adjusted effect estimate was reported, it was considered sufficient if the crude effect estimate could be calculated using data from the article. Furthermore, it was evaluated whether qualitative statements on the likelihood and direction of the potential impact of unmeasured confounders were reported. Finally, we assessed whether a quantitative sensitivity analysis to estimate the potential impact of unmeasured confounders on the effect estimate was included in the published article.

The original data from the previous study [22] were obtained to enable a comparison of a period before the STROBE statement was published (January 2004–April 2007; previous study) with the period after the STROBE statement (January 2010–December 2012; present study).

### Comparisons and data analysis

In primary analysis, a comparison was made between the quality of reporting of confounding before vs. after the publication of the STROBE statement.

Three secondary analyses were performed. First, articles from journals that published the STROBE statement—used as an indicator that the journal acknowledges the importance of adequate reporting—(The Lancet, BMJ, Annals of Internal Medicine, Epidemiology, and Journal of Clinical Epidemiology) were compared with articles from journals that did

not publish the STROBE statement (American Journal of Epidemiology, International Journal of Epidemiology, New England Journal of Medicine, Journal of the American Medical Association, and Journal of Epidemiology and Community Health).

Second, we compared journals that published the STROBE statement and included an endorsement of the STROBE guidelines in their author instructions (The Lancet, The BMJ, and Annals of Internal Medicine) with journals that did neither (American Journal of Epidemiology, International journal of Epidemiology, New England Journal of Medicine, Journal of the American Medical Association, and Journal of Epidemiology and Community Health).

Third, The BMJ, the only included journal that required the completion of the STROBE checklist when submitting an observational study, was compared with the two journals that only endorsed the STROBE statement in their author instructions (The Lancet and Annals of Internal Medicine). This was done to evaluate whether such more active endorsement of the STROBE statement would result in better reporting of confounding.

Our primary outcome consisted of the same eight-item score that was created and used previously, excluding items 1 and 8 from Table 2.1 that are not addressed by the STROBE statement [22]. Hence, a six-item score was created with equal weights given to each item. For the comparison of the overall quality of reporting, a comparison was made between the median number of reported items (maximum of 6) before and after the STROBE statement using the Mann–Whitney *U*-test. In addition, relative risks 95% confidence interval (CI) were calculated to represent changes in the individual items. Statistical analyses were performed using the R statistical software package version 3.0.2.

#### Sensitivity analysis

Of the eight items related to confounding that we considered important, two items are not included in the STROBE statement. The reason why potential confounders were selected for analyses and the application of a quantitative bias analysis are both not mentioned in that guideline. Therefore, in the primary analysis, we used a six-item score, excluding the two items that are not addressed by the STROBE statement. However, because both items are important for evaluating the likelihood and potential impact of unmeasured confounding [23-28], we performed a sensitivity analysis in which we used the same eight-item score that was created and used previously [8], including these two items.

## Results

The MEDLINE search identified 2,651 publications (Fig. 2.1). After screening the titles and abstracts of all retrieved publications, we reviewed 408 full-text articles and subsequently included 220 articles in the final analysis (appendix 2).

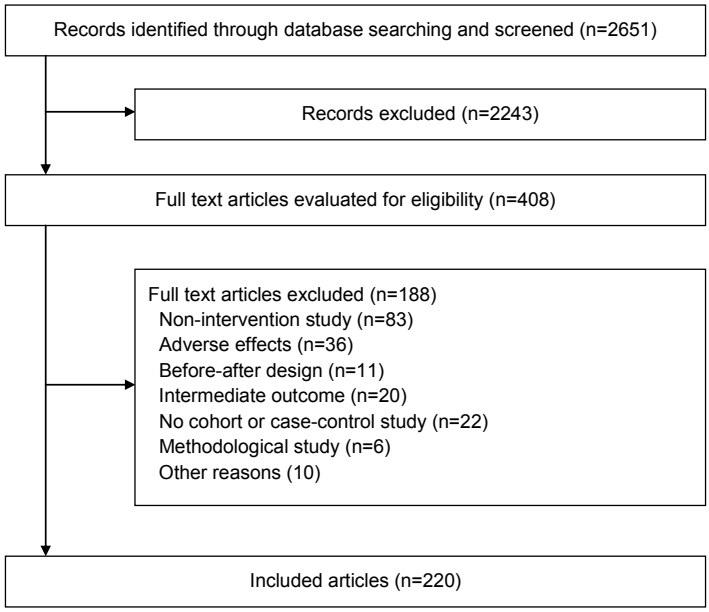


**Figure 2.1** Flow diagram for study selection.

Of those studies, 125 (56.8%) were published in general medical journals and 95 (43.2%) in epidemiologic journals. Of the included articles, 66 were published in 2010, 75 in 2011, and 79 in 2012. There were more cohort studies (181, 82.3%) than case–control studies (39, 17.7%) included. Among general medical journals, only 11.2% of studies were case–control studies. Most studies were published in journals that did not publish the STROBE statement (151, 68.6%). In 72 articles (32.7%), the effects of diets were studied; in 52 (23.6%), the effects of drugs; in 24 (10.9%), the effects of surgical procedures; in 27 (12.3%), the effects of medical strategies (e.g., the association between mechanical ventilation and survival among patients with acute lung injury); 20 (9.1%) articles described the effects of behavioral interventions (e.g., the effect of physical activity on mortality); 12 (5.5%) reported the effects of vaccination; 7 (3.2%) the effects of screening or preventive measures, and 6 (2.7%) about other interventions such as hospital recognition of nursing excellence.

Table 2.1 shows the frequencies of items in the reporting of confounding in observational intervention studies. Low frequencies were observed for the reporting of reasons why potential confounders were selected for analysis, the reporting of reasons to include confounders in the final model, reporting of comments on the direction of the potential effect of unmeasured confounding, and the use of sensitivity analysis to quantify this potential effect (Table 2.1). When interpreting these results, it should be noted that the reasons why potential confounders were selected for analysis and the use of sensitivity analysis to quantify the potential effect of unmeasured confounding are not included in the STROBE statement. Nevertheless, compared with the period before the STROBE statement, the reporting of reasons why potential confounders were selected for analysis improved. Other items that were more frequently reported were comments on the likelihood of unmeasured confounding, and qualitative statements about the direction unmeasured confounder(s) would likely bias the results (Table 2.1). However, reports included less frequently the reasons to include confounders in the final model. The other items did not change significantly.

The median number of items reported was similar before and after the publication of the STROBE statement [before: median, 4; interquartile range [IQR], 3–5; after: median, 4; IQR, 3.75–5]. However, the distribution of the number of items reported shifted somewhat to the right with less articles with a low number of items and more articles with a high number of items (Fig. 2, $P = 0.01$). When in sensitivity analysis, items 1 and 8 from Table 2.1 were included in the summary score, this shift became slightly stronger (median, 4; IQR, 3–5 vs. median, 4; IQR, 4–5; $P = 0.0007$).

When journals that published the STROBE statement in 2007—used as an indicator that the journal acknowledges the importance of adequate reporting—were compared with journals that did not, median number of items reported were not statistically significant higher for journals that published the STROBE statement (median, 4; IQR, 4–5 vs. median, 4; IQR, 3–5; $P = 0.26$). Similar results were obtained when comparing journals that published the STROBE statement and included an endorsement of the STROBE guidelines in their author instructions with journals that did neither (median, 4; IQR, 4–5 vs. median, 4; IQR, 3–5;$P = 0.33$). Articles from the BMJ, the only included journal that required the submission of a completed STROBE checklist, did not have a better reporting of confounding than journals that endorsed the STROBE statement in their author instructions but did not require the completion of the checklist (median, 4; IQR, 4–5 vs. median, 4; IQR, 3.5–5; $P = 0.72$).

**Table 2.1.** Frequencies of important items in the reporting of confounding in observational studies.

| Item | Studies with adequate reporting (2004-2007, n=174) | Studies with adequate reporting (2010-2012, n=220) | RR (95% CI) |
|---|---|---|---|
| Reporting of reasons why potential confounders are selected for analysis | 18 (10.3%) | 55 (25.0%) | 2.42 (1.49-3.96) |
| Reporting of reasons to include confounders in the final model [a,b] | 88 (50.6%) | 88 (40.0%) | 0.79 (0.64-0.98) |
| Reporting of characteristics of key confounders [c] | 139 (79.9%) | 175 (79.5%) | 1.00 (0.90-1.11) |
| Reporting of any method used to control for confounding [d] | 171 (98.3%) | 218 (99.1%) | 1.01 (0.98-1.04) |
| Reporting of both crude and adjusted effect estimate [b] | 136 (78.2%) | 160 (72.7%) | 0.93 (0.83-1.04) |
| Comment on likelihood unmeasured confounding [e] | 102 (58.6%) | 186 (84.5%) | 1.44 (1.27-1.67) |
| Qualitative statement direction unmeasured confounding [e] | 27 (15.5%) | 71 (32.2%) | 2.08 (1.41-3.10) |
| Quantitative bias analysis for unmeasured confounding | 4 (2.3%) | 8 (3.6%) | 1.58 (0.52-4.88) |

[a] STROBE item 7: Clearly define all outcomes exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.
[b] STROBE item 16(a): Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included.
[c] STROBE item 14(a): Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders; give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
[d] STROBE item 12(a): Describe all statistical methods, including those used to control for confounding.
[e] STROBE item 19: Discuss limitation of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.

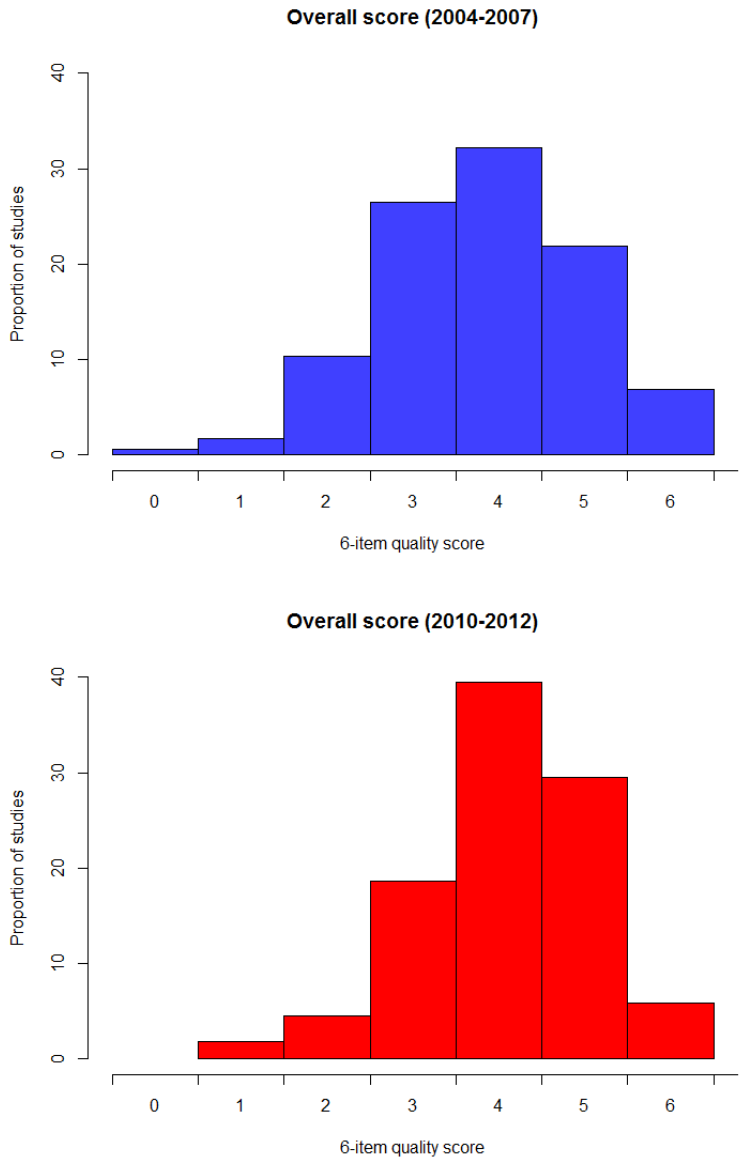


**Figure 2.2.** Histograms of the overall scores for the quality of reporting of confounding. Blue bars represent the proportion of studies with a score of 0-6 on the quality of reporting of confounding for studies published between January 2004 and April 2007. Red bars represent the proportion of studies with a score of 0-6 on the quality of reporting of confounding for studies published between January 2010 and December 2012.

## Discussion

Although the quality of reporting of confounding in articles on observational interventions improved in certain aspects because the introduction of the STROBE statement, this study shows that the overall quality of reporting remains suboptimal.

Quantitative bias analyses were still very rare (reported in 3.6% of the articles), whereas such analyses can be very informative and potentially avoid unnecessary harm to patients and waste of time and resources invested in new research [22]. Despite the increasing number of articles in the literature emphasizing the importance of quantitative bias analysis[25,28-34], such analyses are still rarely applied as observed previously [22]. Although we acknowledge that there are situations where quantitative bias analysis may not be very useful, such as analyses with very wide conventional confidence intervals, we do think that the STROBE statement should ask authors to report on quantitative bias analysis or—if not conducted—report why not.

It is difficult to imagine that the current practice of systematically ignoring sources of uncertainty other than random error is the way forward. Especially with the increasing interest in and use of big data [35,36], very narrow confidence intervals can be expected. Without a quantitative bias analysis, researchers and decision makers risk largely underestimating the true uncertainty in these circumstances. In the future, reporting of quantitative bias analysis and the other items from our Table 2.1 enables a better assessment of the risk and impact of confounding using, for example, the currently developed "Cochrane risk of bias assessment tool for nonrandomized studies of interventions" [37].

The reasons why potential confounders were selected for analysis and included in the final analysis were also frequently missing from the included articles. Reporting of both items is important, as causal inference from observational data depends not only on the data, but also on the design of the study and subject-specific knowledge [26-28]. Without a structured way to obtain subject matter knowledge, it will be difficult to define a causal structure, a prerequisite to adequately select a variable as a potential confounder [26-28]. Hernan et al. previously showed the importance of communicating which strategy is used to select the confounders included in the final model [27]. Ideally, causal diagrams are used to summarize and communicate the causal structure assumed by the researchers. Of the articles in the 2010–2012 cohort, 0.9% included a causal diagram. Such diagrams may also enable the researcher to determine the direction of the bias caused by unmeasured confounding [38], another item that was frequently not reported.

Reporting of both crude and adjusted effect estimates remained similar. If both effect estimates are reported, readers can judge by how much, and in what direction, potential confounders changed the effect estimate [39]. Together with a distribution of the confounders among exposed and unexposed or cases and controls, this information can be used to understand the data behind the reported associations. Unfortunately, almost 20% of the included studies did not report the characteristics of all key confounders. Only the reasons why potential confounders are selected for analysis (item 1), comments on likelihood of unmeasured confounding (item 6), and qualitative statements about the direction of unmeasured confounding (item 7) were more frequently reported over time.

This is the first study that evaluated whether the reporting of confounding improved after the publication of the STROBE statement. Moreover, this is the first study that evaluated whether the reporting of confounding is better in journals with a more active endorsement of the STROBE guideline.

This study has some potential limitations. Although most evaluated items are included in the STROBE checklist, the application of a quantitative bias analysis and an item about the reason why potential confounders are selected for analysis are not mentioned in that guideline. Therefore, one may expect that these items would not increase substantially over time as a result of the STROBE statement. However, after including both items, the difference before and after the STROBE statement became larger instead of smaller, indicating that including these items would not result in an underestimation of the impact of the STROBE statement.

We focused on studies published in a selection of high-impact general medical and epidemiologic journals. Such high-impact general journals may have a better reporting quality than lower impact and specialist journals [40], resulting in an overestimate of the quality of reporting of confounding in all published studies on observational medical interventions.

The observational nature of the before–after comparison may have masked effects of the STROBE guideline, due to underlying trends. Because we were mainly interested in the question whether the reporting improved since the previous study that was performed prior the STROBE statement [22], we did not directly evaluate the impact of the STROBE statement [15], the launch of the EQUATOR Network and its activities [14], the previous study showing poor reporting of confounding in observational research [22], or other articles that showed the importance of adequate reporting or the lack of adequate reporting in different journals [41-45] using for example a time-series analysis. For such an analysis, as done by Bastuji-Gain et al. [46], the potential lag time

between implementation and effect would be ideally known, including other events and interventions happening in between plus the exact dates. Moreover, there was no trend seen in the previous study in the median number of items reported over time using data from 2004 to 2007 [22] nor in the years 2010 to 2012 (2010: 4; IQR, 4–5; 2011: 4; IQR, 3–5; 2012: 4; IQR, 4–5).

Our data are in agreement with previous studies that showed that especially the reporting of selection of confounders for analysis needs improvement [22, 47-49]. Moreover, in agreement with other studies [22], key issues related to unobserved confounding are not addressed and/or underreported. Although there is evidence that reporting guidelines such as the CONSORT and STARD statement improve the completeness of reporting [50-52], the effect of the STROBE statement on the quality of reporting is less clear [44,46,52]. Despite we did find that the reporting of confounding improved slightly over time and was better after than before the STROBE statement was published, journals that published the STROBE statement or were more actively endorsing the STROBE statement did not have a statistically significant better reporting of confounding. This finding is in agreement with a recent systematic review that did not find a clear relationship between journals' endorsement of reporting guidelines (BMJ economic checklist, CONSORT for harms, PRISMA, QUOROM, STARD, STRICTA, and STROBE) and the completeness of reporting [52].

In conclusion, reporting of confounding in articles on observational interventions remained suboptimal. Although we acknowledge that improving the quality of reporting of confounding does not solve the whole problem of published research that cannot be replicated and for which it is unclear how reliable and valid the study finding are, there is still room and need for improvement. How such improvements should be accomplished remains a difficult issue. Publishing the STROBE statement or endorsing it in the instructions for authors does not seem to be enough. The recently implemented strategy of PLOS Medicine is an interesting solution [17]. Requiring authors to submit a checklist with sufficient text excerpted from the manuscript to explain how they accomplished all applicable items [17] may results in better adherence to the guideline. In addition, adequate reporting and knowledge about the existence of the different reporting guidelines listed on the EQUATOR network Web site should preferably become part of the core training of current and future scientists, to make them more aware of the importance of adequate reporting already at the beginning of their study. Furthermore, we would like to encourage research into the development and evaluation of strategies to improve the quality of reporting, thereby reducing the waste.

## References


1. Ioannidis JP. Why most published research findings are false. *PLoS Med* 2005;2:e124.
2. Ioannidis JP, Greenland S, Hlatky MA, Khoury MJ, Macleod MR, Moher D, et al. Increasing value and reducing waste in research design, conduct, and analysis. *Lancet* 2014;383:166-75.
3. Glasziou P, Altman DG, Bossuyt P, Boutron I, Clarke M, Julious S, et al. Reducing waste from incomplete or unusable reports of biomedical research. *Lancet* 2014;383:267-76.
4. Lang TA, Secic M. How to report statistics in medicine: annotated guidelines for authors, editors, and reviewers. 2nd ed. Philadelphia: ACP Press 2006.
5. Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, et al. A call for transparent reporting to optimize the predictive value of preclinical research. *Nature* 2012;490:187-91.
6. Prinz F, Schlange T, Asadullah K. Believe it or not: how much can we rely on published data on potential drug targets? *Nat Rev Drug Discov* 2011;10:712-c1.
7. Begley CG, Ellis LM. Drug development: Raise standards for preclinical cancer research. *Nature* 2012;483:531-3.
8. Schulz KF, Chalmers I, Hayes RJ, Altman DG. Emperical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. JAMA 1995;273:408-12.
9. Huwiler-Muntener K, Juni P, Junker C, Egger M. Quality of reporting of randomized trials as a measure of methodological quality. JAMA 2002;287:2801-4.
10. Soares HP, Daniels S, Kumar A, Clarke M, Scott C, Swann S, et al. Bad reporting does not mean bad methods for randomised trials: observational study of randomised controlled trials performed by the Radiation Therapy Oncology Group. BMJ 2004;382:22-4.
11. Turner L, Shamseer L, Altman DG, Schulz KF, Moher D. Does use of the CONSORT Statement impact the completeness of reporting of randomised controlled trials published in medical journals? A Cochrane review. *Syst Rev* 2012;1:60.
12. Moher D, Jones A, Lepage L, CONSORT Group (Consolidated Standards for Reporting of Trials). Use of the CONSORT statement and quality of reports of randomized trials: a comparative before-and-after evaluation. *JAMA* 2001;285:1992-5.
13. Hopewell S, Dutton S, Yu LM, Chan AW, Altman DG. The quality of reports of randomised trials in 2000 and 2006: comparative study of articles indexed in PubMed. *BMJ* 2010;340:c723.
14. The EQUATOR Network | Enhancing the QUAlity and Transparancy Of health Research. Available at: http://www.equator-network.org/. Accessed 5/2, 2014.
15. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 2007;335:806-8.
16. The British Medical Journal. Resource for authors. Available: http://www.bmj.com/about-bmj/resources-authors/article-types/research. Accessed 11 November 2014.
17. The PLOS Medicine Editors. Observational studies: Getting clear about transparancy. *PLoS Med* 2014;11:e1001711.
18. Hak E, Verheij TJ, Grobbee DE, Nichol KL, Hoes AW. Confounding by indication in non-experimental evaluation of vaccine effectiveness: the example of prevention of influenza complications. *J Epidemiol Community Health* 2002;56:951-5.
19. Pouwels KB, Hak E. Re: "a prospective study of statin drug use and lower urinary tract symptoms in older men". *Am J Epidemiol* 2014;179:927.

20. Simpson SH, Eurich DT, Majumdar SR, Padwal RS, Tsuyuki RT, Varney J, et al. A meta-analysis of the association between adherence to drug therapy and mortality. *BMJ* 2006;333:15.
21. Dormuth CR, Patrick AR, Shrank WH, Wright JM, Glynn RJ, Sutherland J, et al. Statin adherence and risk of accidents: a cautionary tale. *Circulation* 2009;119:2051-7.
22. Groenwold RH, Van Deursen AM, Hoes AW, Hak E. Poor quality of reporting confounding bias in observational intervention studies: a systematic review. *Ann Epidemiol* 2008;18:746-51.
23. Lash TL, Fox MP, Fink AK. Applying quantitative bias analysis to epidemiological data. New York: Springer; 2009.
24. Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, Greenland S. Good practices for quantitative bias analysis. Int J Epidemiol 2014;43:1969-85.
25. Greenland S. Multiple-bias modelling for analysis of observational data. *Journal of the Royal Statistical Society: Series A (Statistics in Society)* 2005;168:267-306.
26. Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol* 2002;155:176-84.
27. Robins JM. Data, design, and background knowledge in etiologic inference. *Epidemiology* 2001;12:313-320.
28. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology* 1999;10:37-48.
29. Groenwold RH, Nelson DB, Nichol KL, Hoes AW, Hak E. Sensitivity analyses to estimate the potential impact of unmeasured confounding in causal research. *Int J Epidemiol* 2010;39:107-17.
30. McCandless LC. Statin use and fracture risk: can we quantify the healthy-user effect? *Epidemiology* 2013;24:743-52.
31. Phillips CV. Quantifying and reporting uncertainty from systematic errors. *Epidemiology* 2003;14:459-66.
32. Lash TL, Silliman RA. A sensitivity analysis to separate bias due to confounding from bias due to predicting misclassification by a variable that does both. *Epidemiology* 2000;11:544-9.
33. Steenland K, Greenland S. Monte Carlo sensitivity analysis and Bayesian analysis of smoking as an unmeasured confounder in a study of silica and lung cancer. *Am J Epidemiol* 2004;160:384-92.
34. Greenland S. Interval estimation by simulation as an alternative to and extension of confidence intervals. *Int J Epidemiol* 2004;33:1389-97.
35. Lynch C. Big data: How do your data grow? *Nature* 2008;455:28-9.
36. Toh S, Platt R. Is size the next big thing in epidemiology? *Epidemiology* 2013;24:349-51.
37. Sterne JAC, Higgins JPT, Reeves BC, on behalf of the development group, for ACROBAT-NRSI. A Cochrance Risk Of Bias Assessment Tool: for Non-Randomized Studies of Interventions (ACROBAT-NRSI). Version 1.0.0. Available at http://www.riskofbias.info Accessed February 23, 2015.
38. VanderWeele TJ, Hernan MA, Robins JM. Causal directed acyclic graphs and the direction of unmeasured confounding bias. *Epidemiology* 2008;19:720-728.
39. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS Med* 2007;4:e297.
40. Mills E, Wu P, Gagnier J, Heels-Ansdell D, Montori VM. An analysis of general medical and specialist journals that endorse CONSORT found that reporting was not enforced consistently. *J Clin Epidemiol* 2005;58:662-7.
41. Chalmers I, Glasziou P. Avoidable waste in the production and reporting of research evidence. *Lancet* 2009;374:86-9.

42. Peng RD, Dominici F, Zeger SL. Reproducible epidemiologic research. *Am J Epidemiol* 2006;163:783-9.

43. Galera Llorca J, Lahoz Grillo R, Roig Loscertales F. The reporting of observational studies: analysis using the STROBE statement. *Rev Esp Salud Publica* 2011;85:583-91.

44. Cobo E, Cortes J, Ribera JM, Cardellach F, Selva-O'Callaghan A, Kostov B, et al. Effect of using reporting guidelines during peer review on quality of final manuscripts submitted to a biomedical journal: masked randomised trial. *BMJ* 2011;343:d6783.

45. Hirst A, Altman DG. Are peer reviewers encouraged to use reporting guidelines? A survey of 116 health research journals. *PLoS One* 2012;7:e35621.

46. Bastuji-Garin S, Sbidian E, Gaudy-Marqueste C, Ferrat E, Roujeau JC, Richard MA, et al. Impact of STROBE statement publication on quality of observational study reporting: interrupted time series versus before-after analysis. *PLoS One* 2013;8(8):e64733.

47. Pocock SJ, Collier TJ, Dandreo KJ, de Stavola BL, Goldman MB, Kalish LA, et al. Issues in the reporting of epidemiological studies: a survey of recent practice. *BMJ* 2004;329:883.

48. Mullner M, Matthews H, Altman DG. Reporting on statistical methods to adjust for confounding: a cross-sectional survey. *Ann Intern Med* 2002;136:122-6.

49. Delaney M, Meyer E, Cserti-Gazdewich C, Haspel RL, Lin Y, Morris A, et al. A systematic assessment of the quality of reporting for platelet transfusion studies. *Transfusion* 2010;50:2135-44.

50. Turner L, Shamseer L, Altman DG, Weeks L, Peters J, Kober T, et al. Consolidated standards of reporting trials (CONSORT) and the completeness of reporting of randomised controlled trials (RCTs) published in medical journals. *Cochrane Database Syst Rev* 2012;11:MR000030.

51. Smidt N, Rutjes AW, van der Windt DA, Ostelo RW, Bossuyt PM, Reitsma JB, et al. The quality of diagnostic accuracy studies since the STARD statement: has it improved? *Neurology* 2006;67:792-7.

52. Stevens A, Shamseer L, Weinstein E, Yazdi F, Turner L, Thielman J, et al. Relation of completeness of reporting of health research to journals' endorsement of reporting guidelines: systematic review. *BMJ* 2014;348:g3804.

# APPENDICES

## APPENDIX 1

## Search strategy

The search strategy consisted of the following search terms:

#1 "N Engl J Med"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#2 "Lancet"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#3 "JAMA"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#4 "Ann Intern Med"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#5 "BMJ"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#6 "Am J Epidemiol"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#7 "Int J Epidemiol"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#8 "Epidemiology"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#9 "J Clin Epidemiol"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

#10 "J Epidemiol Community Health"[jour] and (cohort[All Fields] or longitudinal[All Fields] or ("longitudinal studies"[MeSH Terms] or ("longitudinal"[All Fields] and "studies"[All Fields]) or "longitudinal studies"[All Fields] or "prospective"[All Fields]) or follow-up[All Fields] or cross-sectional[All Fields] or ("retrospective studies"[MeSH Terms] or ("retrospective"[All Fields] and "studies"[All Fields]) or "retrospective studies"[All Fields] or "retrospective"[All Fields]) or case-control[All Fields])

All searches (#1 - #10) were limited to the period 1-1-2010 to 31-12-2012 and were performed using the following restrictions:

> not (letter[pt] or review[pt] or editorial[pt] or meta-analysis[pt] or comment[pt] or randomized controlled trial[pt] or practice guideline[pt] or Patient Education Handout[pt] or Published Erratum[pt] or Case Reports[pt] or Interview[pt] or Historical Article[pt] or Clinical Conference[pt] or Retracted Publication[pt] or Retraction of Publication[pt] or News[pt])

APPENDIX 2

# Characteristics of included studies

**Table 2.A1.** Characteristics of included studies

| Author, year | Journal | Study type | Intervention | Outcome |
|---|---|---|---|---|
| Jia, 2012 [1] | Lancet | Cohort | Antiretroviral therapy | HIV infection of HIV-negative partner |
| Kokkinos, 2012 [2] | Lancet | Cohort | Statins | Mortality |
| Misegades, 2012 [3] | JAMA | Case-control | DTaP vaccination | Childhood pertussis |
| Lund, 2012 [4] | JAMA | Cohort | Renin-angiotensin system antagonists | Mortality |
| Wu, 2012 [5] | JAMA | Cohort | Nucleoside analogues | Hepatitis B virus-related hepatocellular carcinoma recurrence |
| Nielsen, 2012 [6] | N Engl J Med | Cohort | Statins | Cancer-related mortality |
| Roumie, 2012 [7] | Ann Intern Med | Cohort | Sulfonylurea vs metformin monotherapy | Cardiovascular events |
| Jakola, 2012 [8] | JAMA | Cohort | Strategy favoring early surgical resection vs strategy favoring watchful waiting | Survival |
| Liao, 2012 [9] | N Engl J Med | Cohort | Aspirin | Mortality |
| Paik, 2012 [10] | BMJ | Cohort | Calcium intake | Primary hyperparathyroidism |
| Tornqvist, 2012 [11] | BMJ | Cohort | Intended intraoperative cholangiography | Mortality |
| Bangalore, 2012 [12] | JAMA | Cohort | Beta-blockers | Composite of cardiovascular death, nonfatal MI, or nonfatal stroke |
| Neovius, 2012 [13] | JAMA | Cohort | Bariatric surgery | Annual hospital days and nonprimary care outpatient visits |

| | | | | |
|---|---|---|---|---|
| Adams, 2012 [14] | JAMA | Cohort | Gastric bypass | Weight loss, Diabetes mellitus, hypertension, dyslipidemia, and health-related quality of life |
| Stoffels, 2012 [15] | JAMA | Cohort | Single-photon emission computed tomography/ computed tomography (SPECT/CT)-aided Sentinel lymph node excision (SLNE) | Metastatic node detection, and disease-free survival |
| Andersson, 2012 [16] | JAMA | Cohort | Clopidogrel | All-cause mortality, cardiovascular mortality, and a composite of recurrent myocardial infarction and all-cause mortality |
| Rizzuto, 2012 [17] | BMJ | Cohort | Healthy lifestyle behaviours | Median age at death |
| Carlsson, 2012 [18] | N Engl J Med | Cohort | Bariatric surgery | Type 2 diabetes |
| Braeckman, 2012 [19] | BMJ | Case-control | Rotavirus vaccination | Rotavirus gastroenteritis hospital admissions |
| Fraser, 2012 [20] | N Engl J Med | Cohort | Ventricular assist device | Survival |
| Tseng, 2012 [21] | JAMA | Cohort | Cataract surgery | Hip fractures |
| Williams, 2012 [22] | JAMA | Cohort | Endoscopic vs open vein-graft harvesting | Mortality |
| Limketkai, 2012 [23] | JAMA | Cohort | Antiviral therapy | Composite of end-stage liver disease, hepatocellular carcinoma, or death |
| Menne, 2012 [24] | BMJ | Case-control | Treatment strategies for enterohaemorrhagic Escherichia coli O104:H4 induced haemolytic uraemic syndrome | Dialysis, seizures, mechanical ventilation, and death |

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|---|---|---|---|---|
| Shirani, 2012 [25] | JAMA | Cohort | Interferon beta | Progression of disability (Time to sustained score of 6 on the Expanded Disability Status Scale) |
| Di Giuseppe, 2012 [26] | BMJ | Cohort | Moderate alcohol consumption | Rheumatoid arthritis |
| O'Reilly, 2012 [27] | Lancet | Case-control | Oral poliovirus vaccination | Poliomyelitis |
| Zhang, 2012 [28] | JAMA | Cohort | Herpes zoster vaccination | Herpes zoster infection |
| McMinn, 2012 [29] | BMJ | Cohort | Cemented vs uncemented total hip replacement | Mortality, and revision |
| Bonser, 2012 [30] | Lancet | Cohort | Lungs from donors with a positive smoking history vs waiting list | Survival |
| Shiomi, 2012 [31] | BMJ | Cohort | Door to balloon time | Composite of death and congestive heart failure |
| Winner, 2012 [32] | N Engl J Med | Cohort | Long-acting reversible contraception | Unintended pregnancies |
| Wallace, 2012 [33] | N Engl J Med | Cohort | Nighttime intensivist staffing | Mortality |
| Freedman, 2012 [34] | N Engl J Med | Cohort | Coffee drinking | Mortality |
| Stock, 2012 [35] | BMJ | Cohort | Elective induction of labour | Perinatal mortality, mode of delivery, postpartum haemorrhage, obstetric anal sphincter injury, and admission to a neonatal or special care baby unit |
| Smith, 2012 [36] | JAMA | Cohort | Brachytherapy vs whole-breast irradiation | Mastectomy and death |
| Athan, 2012 [37] | JAMA | Cohort | Cardiac device removal | In-hospital and 1-year mortality |

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| Lake, 2012 [38] | JAMA | Cohort | Hospital recognition of nursing excellence | 7-day, 28-day, and hospital stay mortality, nosocomial infection, and severe (grade 3 or 4) intraventricular haemorrhage |
| Tan, 2012 [39] | JAMA | Cohort | Partial vs radical nephrectomy | Overall and kidney cancer-specific survival |
| Jackson, 2012 [40] | JAMA | Cohort | Open vs endovascular repair of intact abdominal aortic aneurysm | Mortality |
| Galvagno, 2012 [41] | JAMA | Cohort | Helicopter vs ground emergency medical services | Survival to hospital discharge and discharge disposition |
| Zhu, 2012 [42] | JAMA | Cohort | Bevacizumab | Survival |
| Svanstrom, 2012 [43] | JAMA | Cohort | Losartan vs candesartan | Mortality |
| Needham, 2012 [44] | BMJ | Cohort | Lung protective mechanical ventilation | Mortality |
| Wahbi, 2012 [45] | JAMA | Cohort | Electrophysiological study with prophylactic permanent pacing | Survival |
| Bretler, 2012 [46] | BMJ | Cohort | Discontinuation of hormone replacement therapy after myocardial infarction | Reinfarction, cardiovascular mortality, and all cause mortality 30 to 360 days after discharge |
| Weintraub, 2012 [47] | N Engl J Med | Cohort | Percutaneous coronary intervention vs coronary-artery bypass grafting | Mortality |

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| Hagihara, 2012 [48] | JAMA | Cohort | Prehospital epinephrine | Return of spontaneous circulation before hospital arrival, survival at 1 month after cardiac arrest, survival with good or moderate cerebral performance, and survival with no, mild, or moderate neurological disability |
| Yang, 2012 [49] | JAMA | Cohort | Cardiovascular health metrics (not smoking; being physically active; having normal blood pressure, blood glucose and total cholesterol levels, and weight; and eating a healthy diet) | All-cause, cardiovascular disease, and ischemic heart disease mortality |
| Andrae, 2012 [50] | BMJ | Cohort | Cervical cancer screening | Cure proportions and five year relative survival ratios |
| Emborgh, 2011 [51] | BMJ | Cohort | Adjuvanted monovalent vaccine against pandemic influenza A/H1N1 | Laboratory confirmed H1N1 infection and influenza related hospital admission with laboratory confirmed H1N1 infection |
| Campos-Rodriquez, 2012 [52] | Ann Intern Med | Cohort | Continuous positive airway pressure | Cardiovascular death |
| Choi, 2012 [53] | BMJ | Case-control | Calcium channel blockers and losartan | Gout |
| Sjostrom, 2012 [54] | JAMA | Cohort | Bariatric surgery | Cardiovascular mortality and a composite of myocardial infarction and stroke |

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| Prieto-Alhambra, 2011 [55] | BMJ | Cohort | Bisphosphonates | Revision arthroplasties occurring after surgery |
| Carlo, 2011 [56] | JAMA | Cohort | Antenatal corticosteroids | Mortality and neurodevelopmental impairment at 18 to 22 months' corrected age |
| Agarwal, 2012 [57] | Lancet | Cohort | Surveillance policy vs chemotherapy | Rates of human chorionic gonadotropin normalisation, relapse, and death |
| Birthplace in England Collaborative Group, 2011 [58] | BMJ | Cohort | Planned place of birth | Composite of perinatal mortality and intrapartum related neonatal morbidities |
| Kiefer, 2011 [59] | JAMA | Cohort | Valvular surgery | In-hospital and 1-year mortality |
| Mathurin, 2011 [60] | N Engl J Med | Cohort | Early liver transplantation | Survival |
| De Tisi, 2011 [61] | Lancet | Cohort | Epilepsy surgery | Seizure and patterns of seizure outcome |
| Roth, 2011 [62] | JAMA | Cohort | Prenatal folic acid supplements | Severe language delay in offspring |
| Noah, 2011 [63] | JAMA | Cohort | Referral to extracorporeal membrane oxygenation center | In-hospital mortality |
| Morse, 2011 [64] | JAMA | Cohort | Hospital compliance with asthma care quality measures | Postdischarge emergency department utilization and asthma-related readmission rates at 7, 30, and 90 days |
| Parsons, 2011 [65] | JAMA | Cohort | Lymph node evaluation | Node positivity and mortality |

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| Reis, 2011 [66] | Ann Intern Med | Cohort | Lifestyle factors (dietary intake, body weight and height, physical activity, smoking, and alcohol consumption) | Diabetes |
| Wen, 2011 [67] | Lancet | Cohort | Physical activity | Mortality |
| Mozaffarian, 2011 [68] | Ann Intern Med | Cohort | Long-chain omega-3 fatty acids | Congestive heart failure |
| Montgomery, 2011 [69] | N Engl J Med | Cohort | Desensitization with plasmapheresis and administration of low-dose intravenous immune globulin prior renal transplantation | Mortality |
| Crowe, 2011 [70] | BMJ | Cohort | Vegetarian diet and dietary fibre intake | Diverticular disease |
| Wisnivesky, 2011 [71] | BMJ | Cohort | Postoperative platinum based chemotherapy | Mortality |
| Chiuve, 2011 [72] | JAMA | Cohort | Adherence to a low-risk, healthy lifestyle (not smoking, body mass index of less than 25, exercise duration of 30 minutes/day or longer and top 40% of the alternate Mediterranean diet score) | Sudden cardiac death |
| Wijeysundera, 2011 [73] | BMJ | Cohort | Resting echocardiography within 6 months before surgery | Postoperative survival (30 days and 1 year) and length of hospital stay |
| Wang, 2011 [74] | JAMA | Cohort | Door-in to door-out time | Door-to-balloon time and in-hospital mortality |
| Solomon, 2011 [75] | JAMA | Cohort | Disease-modifying antirheumatic drugs | Diabetes mellitus |
| Maciejewski, 2011 [76] | JAMA | Cohort | Bariatric surgery | Mortality |

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| Warensjo, 2011 [77] | BMJ | Cohort | Dietary intake of calcium | Fractures of any type and hip fractures |
| Short, 2011 [78] | BMJ | Cohort | Beta blockers | Mortality, emergency oral corticosteroid use, and respiratory related hospital admission |
| Stolarz-Skrzypek, 2011 [79] | JAMA | Cohort | Sodium | Mortality and changes in blood pressure |
| Gershon, 2011 [80] | Ann Intern Med | Cohort | Inhaled long-acting beta-agonist vs anticholinergics | Mortality |
| HIV-CAUSAL Collaberation, 2011 [81] | Ann Intern Med | Cohort | Optimal CD4 cell count to initiate combined antiretroviral therapy (cART) | Mortality and composite of AIDS-defining illness or death |
| Douglas, 2011 [82] | BMJ | Cohort | Statins | Mortality within six months of diagnosis of pneumonia |
| Skowronski, 2011 [83] | BMJ | Case-control | AS03 adjuvanted pandemic H1N1 vaccination | Medically attended, laboratory confirmed pandemic H1N1 illness |
| Driver, 2011 [84] | BMJ | Case-control | Non-steroidal anti-inflammatory drugs | Parkinson's disease |
| Tseng, 2011 [85] | JAMA | Cohort | Herpes zoster vaccination | Herpes zoster disease |
| Brenner, 2011 [86] | Ann Intern Med | Case-control | Colonoscopy | Colorectal cancer |
| Graff-Iversen, 2012 [87] | J Epidemiol Community Health | Cohort | Low-frequent use of alcohol | Mortality |
| Hildebrand, 2012 [88] | Am J Epidemiol | Cohort | Caffeinated coffee, decaffeinated coffee, and tea intake | Oral/pharyngeal cancer mortality |
| He, 2012 [89] | Am J Epidemiol | Cohort | Type of fish consumed and fish preparation methods | Pancreatic cancer |

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| Misirli, 2012 [90] | Am J Epidemiol | Cohort | Traditional Mediterranean diet and major food groups | Cerebrovascular disease and mortality from cerebrovascular disease |
| Cozier, 2012 [91] | Am J Epidemiol | Cohort | Oral contraceptive use and female hormone use | Sarcoidosis |
| Mannino, 2012 [92] | Am J Epidemiol | Cohort | MF59 adjuvanted trivalent inactivated vaccine vs nonadjuvanted trivalent inactivated vaccine | Hospitalization for influenza or pneumonia |
| Xue, 2012 [93] | Am J Epidemiol | Cohort | Change in physical activity levels | Mortality |
| Zhang, 2012 [94] | Int J Epidemiol | Case-control | Isoflavonoids | Coronary heart disease |
| Paranjothy, 2012 [95] | Int J Epidemiol | Case-control | Diet in first trimester of pregnancy | Fetal gastroschisis |
| Vanhala, 2012 [96] | Am J Epidemiol | Cohort | Omega-3 and omega-6 polyunsaturated fatty acids | Metabolic syndrome |
| Epstein, 2012 [97] | Am J Epidemiol | Cohort | Dietary fatty acid intake | Prostate cancer mortality |
| Yang, 2012 [98] | Int J Epidemiol | Cohort | Regular alcohol drinking | Overall and cause-specific mortality |
| Bradshaw, 2012 [99] | Am J Epidemiol | Case-control | Dietary pattern | Head and neck squamous cell carcinoma |
| Henny, 2012 [100] | J Epidemiol Community Health | Cohort | Regular participation in Periodic Health Examination | All-cause, cancer, and cardiovascular disease mortality |
| Lee, 2012 [101] | Am J Epidemiol | Cohort | Physical activity | Type 2 diabetes |
| Li, 2012 [102] | Epidemiology | Cohort | Maternal use of folic acid without other vitamins | Nonsyndromic cleft lip with or without cleft palate and cleft palate alone |

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| Mansournia, 2012 [103] | Epidemiology | Cohort | Physical activity | Functional performance and self-reported knee pain |
| Pasternak, 2012 [104] | Am J Epidemiol | Cohort | Dihydropyridine calcium channel blockers | Parkinson's disease |
| Zhou, 2012 [105] | Am J Epidemiol | Cohort | Total fluid intake | Bladder cancer |
| Gerber, 2012 [106] | Am J Epidemiol | Cohort | Smoking reduction | Mortality |
| Kesse-Guyot, 2012 [107] | Am J Epidemiol | Cohort | n-3 polyunsaturated fatty acid intake | Depressive symptoms |
| Au Yeung, 2012 [108] | Am J Epidemiol | Cohort | Moderate alcohol use | Cognitive function |
| Cole, 2012 [109] | Am J Epidemiol | Cohort | Antiretroviral therapy | Composite of AIDS or death |
| Gulsvik, 2012 [110] | Int J Epidemiol | Cohort | Physical activity | Mortality from all causes, ischaemic heart disease and stroke |
| Lo-Ciganic, 2012 [111] | Epidemiology | Case-control | Aspirin, nonaspirin nonsteroidal anti-inflammatory drugs, and acetaminophin | Ovarian cancer |
| Yamaji, 2012 [112] | Am J Epidemiol | Case-control | 25-hydroxyvitamin D | Colorectal adenoma |
| Varraso, 2012 [113] | Am J Epidemiol | Cohort | Dietary patterns, food intakes, and nutrient intakes | Venous thromboembolism |
| Morales, 2011 [114] | Epidemology | Cohort | Maternal 25-hydroxyvitamin D status | Lower respiratory infections, wheezing, and asthma |
| Gong, 2011 [115] | Am J Epidemiol | Cohort | Surgical resection | Survival |
| Ahern, 2011 [116] | Am J Epidemiol | Cohort | Vitamin K antagonists | Site-specific cancers |
| Lucas, 2011 [117] | Am J Epidemiol | Cohort | Physical activity | Clinical depression |
| Sjolander, 2011 [118] | Am J Epidemiol | Cohort | Timing of dialysis initiation | Survival |
| Yonkers, 2011 [119] | Epidemiology | Cohort | Antidepressants use | Major depressive episode during pregnancy |
| Kerr, 2011 [120] | Am J Epidemiol | Cohort | Alcohol consumption | Mortality |

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| Fung, 2011 [121] | Am J Epidemiol | Cohort | Dietary Approaches to Strop Hypertension diets, overall, animal-based, and vegetable-based low-carbohydrate-diets, and major plant food groups | Postmenopausal breast cancer |
| Brantsaeter, 2011 [122] | Am J Epidemiol | Cohort | Milk-based probiotic product consumption during pregnancy | Preeclampsia |
| Kasperzyk, 2011 [123] | Am J Epidemiol | Case-control | Nutrient intake and multivitamin use | Hodgkin lymphoma |
| Zhang, 2011 [124] | Am J Epidemiol | Cohort | Aspirin use | Colon cancer |
| Ahrens, 2011 [125] | Epidemiology | Case-control | Maternal intake of folic acid from supplement and diet | Spina bifida |
| Anderson, 2011 [126] | Am J Epidemiol | Case-control | Ultraviolet sunlight, time spent outdoors, and sun protection practices | Breast cancer |
| Nilsen, 2011 [127] | Am J Epidemiol | Cohort | Physical exercise | Chronic pain in the low back and neck/ shoulders |
| Schmidt, 2011 [128] | Epidemiology | Case-control | Maternal vitamin intake before and during pregnancy | Autism |
| Engel, 2011 [129] | Am J Epidemiol | Cohort | Menopausal hormone therapy | Major osteoporotic fractures |
| Larsson, 2011 [130] | Am J Epidemiol | Cohort | Dietary potassium, calcium, and magnesium intake | Stroke |
| Shrubsole, 2011 [131] | Am J Epidemiol | Cohort | Dietary methionine, folate, vitamin B(6), vitamin B(12), niacin, and riboflavin intake | Breast cancer |
| Nichols, 2011 [132] | Am J Epidemiol | Case-control | Bilateral oophorectomy with hysterectomy | Invasive breast cancer |

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| Murcia, 2011 [133] | Am J Epidemiol | Cohort | Maternal iodine intake from diet and supplements | Infant neurodevelopment |
| Park, 2011 [134] | Am J Epidemiol | Cohort | Multivitamin use | Mortality and cancer |
| Wang, 2011 [135] | Am J Epidemiol | Cohort | Vitamin E intake | Amyotrophic lateral sclerosis |
| Hjellvik, 2011 [136] | Epidemiology | Cohort | Boiled coffee intake | Type 2 diabetes |
| Mackey, 2011 [137] | Am J Epidemiol | Cohort | Physical activity | Hip fracture |
| Northstone, 2011 [138] | J Epidemiol Community Health | Cohort | Dietary patterns | IQ |
| Ford, 2011 [139] | Int J Epidemiol | Cohort | 25-hydroxyvitamin D | Mortality |
| Weinstein, 2011 [140] | Am J Epidemiol | Case-control | 25-hydroxyvitamin D | Colon and rectal cancer |
| Cox, 2011 [141] | Am J Epidemiol | Case-control | School milk consumption | Colorectal cancer |
| Prentice, 2011 [142] | Epidemiology | Cohort | Energy and protein consumption | Cardiovascular disease |
| Pierce, 2011 [143] | Am J Epidemiol | Cohort | Dietary patterns | Skin lesion |
| Inoue, 2011 [144] | J Epidemiol Community Health | Cohort | Alcohol intake | All-cause and major causes of mortality |
| Baer, 2011 [145] | Am J Epidemiol | Cohort | Lifestyle and dietary factors | All-cause and cause-specific mortality |
| Sato, 2011 [146] | J Epidemiol Community Health | Cohort | Alcohol consumption patterns | Type 2 diabetes |
| Press, 2011 [147] | Am J Epidemiol | Case-control | Ovariectomy, hysterectomy, and tubal sterilization | Breast cancer |
| Musselman, 2011 [148] | Am J Epidemiol | Case-control | Maternal dieary intake patterns | Pediatric germ cell tumors |
| Erichsen, 2011 [149] | Am J Epidemiol | Case-control | Statins | Gallstone disease |
| Landrum, 2011 [150] | Am J Epidemiol | Cohort | Timing of hepatitis B virus immunization | Hepatitis B virus infection |

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| Beydoun, 2011 [151] | J Epidemiol Community Health | Cohort | Statins | Dementia and mild cognitive imparment |
| Leung, 2011 [152] | J Epidemiol Community Health | Case-control | Moderate coffee consumption | Hepatocellular carcinoma |
| Heesch, 2011 [153] | J Epidemiol Community Health | Cohort | Leisure-time physical activity and walking | Mental health (Goldberg Anxiety and Depression Scale) |
| Mineharu, 2011 [154] | J Epidemiol Community Health | Cohort | Coffee and green, black and oolong teas and caffeine intake | Cardiovascular disease mortality |
| Mozaffarian, 2010 [155] | Ann Intern Med | Cohort | Trans-palmitoleate | Type 2 diabetes |
| Ruidavets, 2010 [156] | BMJ | Cohort | Alcohol intake patterns | Angina pectoris and composite of myocardial infarction or coronary death |
| Chan, 2010 [157] | JAMA | Cohort | Automated external defibrillator | Survival to hospital discharge |
| Kirkegaard, 2010 [158] | BMJ | Cohort | Adherence to lifestyle recommendations (based on physical activity, waist circumference, smoking, alcohol intake and diet) | Colorectal cancer |
| Neily, 2010 [159] | JAMA | Cohort | Medical team training program for operating room personnel | Mortality |
| Bobrow, 2010 [160] | JAMA | Cohort | Chest compression-only bystander cardiopulmonary resuscitation vs conventional cardiopulmonary resuscitation | Survival |

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| Yu, 2010 [161] | BMJ | Cohort | Oseltamivir | Radiographically confirmed pneumonia, duration of fever, and viral RNA shedding |
| Fung, 2010 [162] | Ann Intern Med | Cohort | Animal-based and vegetable-based low-carbohydrate diet | Mortality |
| Domchek, 2010 [163] | JAMA | Cohort | Mastectomy and salpingo-oophorectomy | Breast and ovarian cancer, cancer-specific mortality, and overall mortality |
| Summers, 2010 [164] | Lancet | Cohort | Controlled cardiac death vs. brain death kidney transplantation | Graf survival and long-term renal function |
| Ramnarayan, 2010 [165] | Lancet | Cohort | Specialist retrieval teams | Mortality in paediatric intensive care unit and length of stay in paediatric intensive care unit |
| Ritchie, 2010 [166] | BMJ | Cohort | Fruit and vegetable consumption | Diagnosis of mild cognitive impairment or dementia |
| Cooper-DeHoff [167] | JAMA | Cohort | Tight blood pressure control | Composite of death, nonfatal myocardial infarction, or nonfatal stroke |
| Kimber, 2010 [168] | BMJ | Cohort | Opiate substitution treatment | Injection free period and mortality |
| Graham, 2010 [169] | JAMA | Cohort | Rosiglitazone vs pioglitazone | Acute myocardial infarction, stroke, heart failure, death, and composite of acute myocardial infarction, stroke, heart failure, or death |

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| O'Donnell [170] | Lancet | Case-control | Diet risk score and regular physical activity | All stroke, ischaemic stroke, and intracerebral haemorrhagic stroke |
| Johansson, 2010 [171] | JAMA | Case-control | Vitamin B(2), B(6), B(9), and B(12), methionine, and homocysteine | Lung cancer |
| Lindenauer, 2010 [172] | JAMA | Cohort | Low doses of steroids administered orally vs higher doses administered intravenously | Composite of treatment failure, defined as the initiation of mechanical ventilation after the second hospital day, inpatient mortality, or readmission for acute exacerbation of COPD within 30 days of discharge; length of stay and hospital costs |
| De Palma, 2010 [173] | BMJ | Case-control | Rotavirus vaccination | Laboratory confirmed rotavirus diarrhoea requiring hospital admission |
| Marso, 2010 [174] | JAMA | Cohort | Vascular closure devices vs bivalirudin | Periprocedural bleeding |
| Lambert, 2010 [175] | JAMA | Cohort | Timeliness of reperfusion | Death at 30 days and at 1 year and composite of death or hospital readmission for acute myocardial infarction or congestive heart failure at 1 year |

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| Rothberg, 2010 [176] | JAMA | Cohort | Antibiotic therapy | Composite of treatment failure, defined as the initiation of mechanical ventilation after the second hospital day, inpatient mortality, or readmission for acute exacerbation of COPD within 30 days of discharge; length of stay and hospital costs |
| Hippisley-Cox [177] | BMJ | Cohort | Statins | Cardiovascular disease, moderate or serious myopathic events, moderate or serious liver dysfunction, acute renal failure, venous thromboembolism, Parkinson's disease, dementia, rheumatoid arthritis, cataract, osteoporotic fracture, gastric cancer, oesophageal cancer, colon cancer, lung cancer, melanoma, renal cancer, breast cancer, or prostate cancer |
| Dheda, 2010 [178] | Lancet | Cohort | Moxifloxacin treatment, number of drugs used in a regimen | Mortality |
| Hernandez, 2010 [179] | JAMA | Cohort | Outpatient follow-up within 7 days after discharge from a heart failure hospitalization | Readmission within 30 days |

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| Tseng, 2010 [180] | JAMA | Cohort | Pneumococcal vaccination | Acute myocardial infarction and stroke |
| Walker, 2010 [181] | Lancet | Cohort | Co-trimoxazole prophylaxis after combination antiretroviral therapy initiation | Mortality |
| Kitamura, 2010 [182] | N Engl J Med | Cohort | Public-access automated external defibrillators | 1-month rate of survival with minimal neurological impairment |
| Ray, 2010 [183] | Ann Intern Med | Cohort | Concurrent use of clopidogrel and proton-pump inhibitors | Hospitalizations for gastroduodenal bleeding and serious cardiovascular disease |
| Hannaford, 2010 [184] | BMJ | Cohort | Oral contraception use | All-cause and cause-specific mortality |
| Kitamura, 2010 [185] | Lancet | Cohort | Bystander cardiopulmonary resuscitation | Favourable neurological outcome 1 month after an out-of-hospital cardiac arrest |
| Brookhart, 2010 [186] | JAMA | Cohort | Use of erythropoiesis-stimulating agents and intravenous iron | One-year mortality |
| Todo, 2010 [187] | Lancet | Cohort | Complete, systematic pelvic lymphadenectomy vs combined pelvic and para-aortic lymphadenectomy | Survival |
| Shuhaiber, 2010 [188] | BMJ | Cohort | Preoperative placement of a left ventricular assist device | Survival |
| Wijesundera, 2010 [189] | BMJ | Cohort | Non-invasive cardiac stress testing performed within six months before surgery | Postoperative one year survival and length of stay in hospital |

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| Boger-Megiddo, 2010 [190] | BMJ | Case-control | Diuretics plus beta blockers, diuretics plus calcium channel blockers, and diuretics plus angiotensin converting enzyme inhibitors or angiotensin receptor blockers | Myocardial infarction or stroke |
| Jenab, 2010 [191] | BMJ | Case-control | Dietary intake of vitamin D and calcium | Colorectal cancer |
| Schaer, 2010 [192] | Ann Intern Med | Case-control | Antihypertensive drugs | Atrial fibrillation |
| Li, 2010 [193] | BMJ | Cohort | Angiotensin receptor blockers vs other cardiovascular drugs | Alzheimer's disease, dementia, admission to a nursing home, and mortality |
| Duell, 2010 [194] | Am J Epidemiol | Cohort | Hormone use | Gastric adenocarcinoma |
| Chang, 2010 [195] | Am J Epidemiol | Cohort | Alcohol consumption | B-cell non-Hodgkin lymphoma and multiple myeloma |
| Boggs, 2010 [196] | Am J Epidemiol | Cohort | Fruit and vegetable intake | Breast cancer |
| Tarrant, 2010 [197] | Epidemiology | Cohort | Breast-feeding | Public hospital admissions for respiratory infections, gastrointestinal infections, and all infectious diseases |
| Lobo, 2010 [198] | Am J Epidemiol | Cohort | Low-to-moderate alcohol consumption | Severe cognitive decline |
| Salinas, 2010 [199] | Am J Epidemiol | Case-control | Aspirin and other nonsteroidal anti-inflammatory drugs | Prostate cancer |
| Abnet, 2010 [200] | Am J Epidemiol | Case-control | 25-hydroxyvitamin D | Upper gastrointestinal cancers of the stomach and oesophagus |

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| Zeleniuch-Jacquotte, 2010 [201] | Am J Epidemiol | Case-control | 25-hydroxyvitamin D | Endometrial cancer |
| Gallicchio, 2010 [202] | Am J Epidemiol | Case-control | 25-hydroxyvitamin D | Kidney cancer |
| Zheng, 2010 [203] | Am J Epidemiol | Case-control | 25-hydroxyvitamin D | Epithelial ovarian cancer |
| Stolzenberg-Solomon, 2010 [204] | Am J Epidemiol | Case-control | 25-hydroxyvitamin D | Pancreatic cancer |
| Purdue, 2010 [205] | Am J Epidemiol | Case-control | 25-hydroxyvitamin D | Non-hodgkin lymphoma |
| Costenbader, 2010 [206] | Am J Epidemiol | Cohort | Dietary antioxidant intake | Rheumatoid arthritis and systemic lupus erythematosus |
| Kim, 2010 [207] | Am J Epidemiol | Case-control | Long-chain omega-3 polyunsaturated fatty acid intake | Distal large bowel cancer |
| Villegas, 2010 [208] | Int J Epidemiol | Cohort | Dietary patterns | Type 2 diabetes |
| Kaluza, 2010 [209] | Am J Epidemiol | Cohort | Dietary calcium and magnesium intake | All-cause, cardiovascular disease, and cancer mortality |
| Delellis Henderson, 2010 [210] | Am J Epidemiol | Cohort | Hormone therapy use | Invasive colon cancer |
| Ma, 2010 [211] | Am J Epidemiol | Cohort | Diet and lifestyle | Acute myeloid leukaemia |
| Kesavan, 2010 [212] | Am J Epidemiol | Cohort | Magnesium and iron intake | Pancreatic cancer |
| Wise, 2010 [213] | Am J Epidemiol | Cohort | Dairy intake | Uterine leiomyoma |
| Cole, 2010 [214] | Am J Epidemiol | Cohort | Highly active antiretroviral therapy | Composite of AIDS or death |
| Vitonis, 2010 [215] | Epidemiology | Cohort | Physical activity | Endometriosis |
| Marron, 2010 [216] | Int J Epidemiol | Case-control | Cessation of alcohol drinking or tobacco smoking | Head and neck cancer |

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| Robinson, 2010 [217] | J Epidemiol Community Health | Cohort | Maternal smoking cessation during pregnancy | Child behavioural problems |
| Oien, 2010 [218] | J Epidemiol Community Health | Cohort | Cod liver oil and fish consumption during pregnancy and if the first year of life | Parental reported asthma and eczema at 2 years of age |
| Heroux, 2010 [219] | Int J Epidemiol | Cohort | Dietary patterns | Mortality |
| Epplein, 2010 [220] | Am J Epidemiol | Cohort | Fruit and vegetable consumption | Distal gastric cancer |

1. Jia Z, Ruan Y, Li Q, Xie P, Li P, Wang X, et al. Antiretroviral therapy to prevent HIV transmission in serodiscordant couples in China (2003-11): a national observational cohort study. *Lancet* 2012; Nov 30.

2. Kokkinos PF, Faselis C, Myers J, Panagiotakos D, Doumas M. Interactive effects of fitness and statin treatment on mortality risk in veterans with dyslipidaemia: a cohort study. *Lancet* 2013; Feb 2;381(9864):394-9.

3. Misegades LK, Winter K, Harriman K, Talarico J, Messonnier NE, Clark TA, et al. Association of childhood pertussis with receipt of 5 doses of pertussis vaccine by time since last vaccine dose, California, 2010. *JAMA* 2012; Nov 28;308(20):2126-32.

4. Lund LH, Benson L, Dahlstrom U, Edner M. Association between use of renin-angiotensin system antagonists and mortality in patients with heart failure and preserved ejection fraction. *JAMA* 2012; Nov 28;308(20):2108-17.

5. Wu CY, Chen YJ, Ho HJ, Hsu YC, Kuo KN, Wu MS, et al. Association between nucleoside analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. *JAMA* 2012; Nov 14;308(18):1906-14.

6. Nielsen SF, Nordestgaard BG, Bojesen SE. Statin use and reduced cancer-related mortality. *N Engl J Med* 2012; Nov 8;367(19):1792-802.

7. Roumie CL, Hung AM, Greevy RA, Grijalva CG, Liu X, Murff HJ, et al. Comparative effectiveness of sulfonylurea and metformin monotherapy on cardiovascular events in type 2 diabetes mellitus: a cohort study. *Ann Intern Med* 2012; Nov 6;157(9):601-10.

8. Jakola AS, Myrmel KS, Kloster R, Torp SH, Lindal S, Unsgard G, et al. Comparison of a strategy favoring early surgical resection vs a strategy favoring watchful waiting in low-grade gliomas. *JAMA* 2012; Nov 14;308(18):1881-8.

9. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med* 2012; Oct 25;367(17):1596-606.

10. Paik JM, Curhan GC, Taylor EN. Calcium intake and risk of primary hyperparathyroidism in women: prospective cohort study. *BMJ* 2012; Oct 17;345:e6390.

11. Tornqvist B, Stromberg C, Persson G, Nilsson M. Effect of intended intraoperative cholangiography and early detection of bile duct injury on survival after cholecystectomy: population based cohort study. *BMJ* 2012; Oct 11;345:e6457.

12. Bangalore S, Steg G, Deedwania P, Crowley K, Eagle KA, Goto S, et al. beta-Blocker use and clinical outcomes in stable outpatients with and without coronary artery disease. *JAMA* 2012; Oct 3;308(13):1340-9.

13. Neovius M, Narbro K, Keating C, Peltonen M, Sjoholm K, Agren G, et al. Health care use during 20 years following bariatric surgery. *JAMA* 2012; Sep 19;308(11):1132-41.

14. Adams TD, Davidson LE, Litwin SE, Kolotkin RL, LaMonte MJ, Pendleton RC, et al. Health benefits of gastric bypass surgery after 6 years. *JAMA* 2012; Sep 19;308(11):1122-31.

15. Stoffels I, Boy C, Poppel T, Kuhn J, Klotgen K, Dissemond J, et al. Association between sentinel lymph node excision with or without preoperative SPECT/CT and metastatic node detection and disease-free survival in melanoma. *JAMA* 2012; Sep 12;308(10):1007-14.

16. Andersson C, Lyngbaek S, Nguyen CD, Nielsen M, Gislason GH, Kober L, et al. Association of clopidogrel treatment with risk of mortality and cardiovascular events following myocardial infarction in patients with and without diabetes. *JAMA* 2012; Sep 5;308(9):882-9.

17. Rizzuto D, Orsini N, Qiu C, Wang HX, Fratiglioni L. Lifestyle, social factors, and survival after age 75: population based study. *BMJ* 2012; Aug 29;345:e5568.

18. Carlsson LM, Peltonen M, Ahlin S, Anveden A, Bouchard C, Carlsson B, et al. Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects. *N Engl J Med* 2012; Aug 23;367(8):695-704.

19. Braeckman T, Van Herck K, Meyer N, Pircon JY, Soriano-Gabarro M, Heylen E, et al. Effectiveness of rotavirus vaccination in prevention of hospital admissions for rotavirus gastroenteritis among young children in Belgium: case-control study. *BMJ* 2012; Aug 8;345:e4752.

20. Fraser CD,Jr, Jaquiss RD, Rosenthal DN, Humpl T, Canter CE, Blackstone EH, et al. Prospective trial of a pediatric ventricular assist device. *N Engl J Med* 2012; Aug 9;367(6):532-41.

21. Tseng VL, Yu F, Lum F, Coleman AL. Risk of fractures following cataract surgery in Medicare beneficiaries. *JAMA* 2012; Aug 1;308(5):493-501.

22. Williams JB, Peterson ED, Brennan JM, Sedrakyan A, Tavris D, Alexander JH, et al. Association between endoscopic vs open vein-graft harvesting and mortality, wound complications, and cardiovascular events in patients undergoing CABG surgery. *JAMA* 2012; Aug 1;308(5):475-84.

23. Limketkai BN, Mehta SH, Sutcliffe CG, Higgins YM, Torbenson MS, Brinkley SC, et al. Relationship of liver disease stage and antiviral therapy with liver-related events and death in adults coinfected with HIV/HCV. *JAMA* 2012; Jul 25;308(4):370-8.

24. Menne J, Nitschke M, Stingele R, Abu-Tair M, Beneke J, Bramstedt J, et al. Validation of treatment strategies for enterohaemorrhagic Escherichia coli O104:H4 induced haemolytic uraemic syndrome: case-control study. *BMJ* 2012; Jul 19;345:e4565.

25. Shirani A, Zhao Y, Karim ME, Evans C, Kingwell E, van der Kop ML, et al. Association between use of interferon beta and progression of disability in patients with relapsing-remitting multiple sclerosis. *JAMA* 2012; Jul 18;308(3):247-56.

26. Di Giuseppe D, Alfredsson L, Bottai M, Askling J, Wolk A. Long term alcohol intake and risk of rheumatoid arthritis in women: a population based cohort study. *BMJ* 2012; Jul 10;345:e4230.

27. O'Reilly KM, Durry E, ul Islam O, Quddus A, Abid N, Mir TP, et al. The effect of mass immunisation campaigns and new oral poliovirus vaccines on the incidence of poliomyelitis in Pakistan and Afghanistan, 2001-11: a retrospective analysis. *Lancet* 2012; Aug 4;380(9840):491-8.

28. Zhang J, Xie F, Delzell E, Chen L, Winthrop KL, Lewis JD, et al. Association between vaccination for herpes zoster and risk of herpes zoster infection among older patients with selected immune-mediated diseases. *JAMA* 2012; Jul 4;308(1):43-9.

29. McMinn DJ, Snell KI, Daniel J, Treacy RB, Pynsent PB, Riley RD. Mortality and implant revision rates of hip arthroplasty in patients with osteoarthritis: registry based cohort study. *BMJ* 2012; Jun 14;344:e3319.

30. Bonser RS, Taylor R, Collett D, Thomas HL, Dark JH, Neuberger J, et al. Effect of donor smoking on survival after lung transplantation: a cohort study of a prospective registry. *Lancet* 2012; Aug 25;380(9843):747-55.

31. Shiomi H, Nakagawa Y, Morimoto T, Furukawa Y, Nakano A, Shirai S, et al. Association of onset to balloon and door to balloon time with long term clinical outcome in patients with ST elevation acute myocardial infarction having primary percutaneous coronary intervention: observational study. *BMJ* 2012; May 23;344:e3257.

32. Winner B, Peipert JF, Zhao Q, Buckel C, Madden T, Allsworth JE, et al. Effectiveness of long-acting reversible contraception. *N Engl J Med* 2012; May 24;366(21):1998-2007.

33. Wallace DJ, Angus DC, Barnato AE, Kramer AA, Kahn JM. Nighttime intensivist staffing and mortality among critically ill patients. *N Engl J Med* 2012; May 31;366(22):2093-101.

34. Freedman ND, Park Y, Abnet CC, Hollenbeck AR, Sinha R. Association of coffee drinking with total and cause-specific mortality. *N Engl J Med* 2012; May 17;366(20):1891-904.

35. Stock SJ, Ferguson E, Duffy A, Ford I, Chalmers J, Norman JE. Outcomes of elective induction of labour compared with expectant management: population based study. *BMJ* 2012; May 10;344:e2838.

36. Smith GL, Xu Y, Buchholz TA, Giordano SH, Jiang J, Shih YC, et al. Association between treatment with brachytherapy vs whole-breast irradiation and subsequent mastectomy, complications, and survival among older women with invasive breast cancer. *JAMA* 2012; May 2;307(17):1827-37.

37. Athan E, Chu VH, Tattevin P, Selton-Suty C, Jones P, Naber C, et al. Clinical characteristics and outcome of infective endocarditis involving implantable cardiac devices. *JAMA* 2012; Apr 25;307(16):1727-35.

38. Lake ET, Staiger D, Horbar J, Cheung R, Kenny MJ, Patrick T, et al. Association between hospital recognition for nursing excellence and outcomes of very low-birth-weight infants. *JAMA* 2012; Apr 25;307(16):1709-16.

39. Tan HJ, Norton EC, Ye Z, Hafez KS, Gore JL, Miller DC. Long-term survival following partial vs radical nephrectomy among older patients with early-stage kidney cancer. *JAMA* 2012; Apr 18;307(15):1629-35.

40. Jackson RS, Chang DC, Freischlag JA. Comparison of long-term survival after open vs endovascular repair of intact abdominal aortic aneurysm among Medicare beneficiaries. *JAMA* 2012; Apr 18;307(15):1621-8.

41. Galvagno SM,Jr, Haut ER, Zafar SN, Millin MG, Efron DT, Koenig GJ,Jr, et al. Association between helicopter vs ground emergency medical services and survival for adults with major trauma. *JAMA* 2012; Apr 18;307(15):1602-10.

42. Zhu J, Sharma DB, Gray SW, Chen AB, Weeks JC, Schrag D. Carboplatin and paclitaxel with vs without bevacizumab in older patients with advanced non-small cell lung cancer. *JAMA* 2012; Apr 18;307(15):1593-601.

43. Svanstrom H, Pasternak B, Hviid A. Association of treatment with losartan vs candesartan and mortality among patients with heart failure. *JAMA* 2012; Apr 11;307(14):1506-12.

44. Needham DM, Colantuoni E, Mendez-Tellez PA, Dinglas VD, Sevransky JE, Dennison Himmelfarb CR, et al. Lung protective mechanical ventilation and two year survival in patients with acute lung injury: prospective cohort study. *BMJ* 2012; Apr 5;344:e2124.

45. Wahbi K, Meune C, Porcher R, Becane HM, Lazarus A, Laforet P, et al. Electrophysiological study with prophylactic pacing and survival in adults with myotonic dystrophy and conduction system disease. *JAMA* 2012; Mar 28;307(12):1292-301.

46. Bretler DM, Hansen PR, Sorensen R, Lindhardsen J, Ahlehoff O, Andersson C, et al. Discontinuation of hormone replacement therapy after myocardial infarction and short term risk of adverse cardiovascular events: nationwide cohort study. *BMJ* 2012; Mar 27;344:e1802.

47. Weintraub WS, Grau-Sepulveda MV, Weiss JM, O'Brien SM, Peterson ED, Kolm P, et al. Comparative effectiveness of revascularization strategies. *N Engl J Med* 2012; Apr 19;366(16):1467-76.

48. Hagihara A, Hasegawa M, Abe T, Nagata T, Wakata Y, Miyazaki S. Prehospital epinephrine use and survival among patients with out-of-hospital cardiac arrest. *JAMA* 2012; Mar 21;307(11):1161-8.

49. Yang Q, Cogswell ME, Flanders WD, Hong Y, Zhang Z, Loustalot F, et al. Trends in cardiovascular health metrics and associations with all-cause and CVD mortality among US adults. *JAMA* 2012; Mar 28;307(12):1273-83.

50. Andrae B, Andersson TM, Lambert PC, Kemetli L, Silfverdal L, Strander B, et al. Screening and cervical cancer cure: population based cohort study. *BMJ* 2012; Mar 1;344:e900.

51. Emborg HD, Krause TG, Hviid A, Simonsen J, Molbak K. Effectiveness of vaccine against pandemic influenza A/H1N1 among people with underlying chronic diseases: cohort study, Denmark, 2009-10. *BMJ* 2011; Jan 25;344:d7901.

52. Campos-Rodriguez F, Martinez-Garcia MA, de la Cruz-Moron I, Almeida-Gonzalez C, Catalan-Serra P, Montserrat JM. Cardiovascular mortality in women with obstructive sleep apnea with or without continuous positive airway pressure treatment: a cohort study. *Ann Intern Med* 2012; Jan 17;156(2):115-22.

53. Choi HK, Soriano LC, Zhang Y, Rodriguez LA. Antihypertensive drugs and risk of incident gout among patients with hypertension: population based case-control study. *BMJ* 2012; Jan 12;344:d8190.

54. Sjostrom L, Peltonen M, Jacobson P, Sjostrom CD, Karason K, Wedel H, et al. Bariatric surgery and long-term cardiovascular events. *JAMA* 2012; Jan 4;307(1):56-65.

55. Prieto-Alhambra D, Javaid MK, Judge A, Murray D, Carr A, Cooper C, et al. Association between bisphosphonate use and implant survival after primary total arthroplasty of the knee or hip: population based retrospective cohort study. *BMJ* 2011; Dec 6;343:d7222.

56. Carlo WA, McDonald SA, Fanaroff AA, Vohr BR, Stoll BJ, Ehrenkranz RA, et al. Association of antenatal corticosteroids with mortality and neurodevelopmental outcomes among infants born at 22 to 25 weeks' gestation. *JAMA* 2011; Dec 7;306(21):2348-58.

57. Agarwal R, Teoh S, Short D, Harvey R, Savage PM, Seckl MJ. Chemotherapy and human chorionic gonadotropin concentrations 6 months after uterine evacuation of molar pregnancy: a retrospective cohort study. *Lancet* 2012; Jan 14;379(9811):130-5.

58. Birthplace in England Collaborative Group, Brocklehurst P, Hardy P, Hollowell J, Linsell L, Macfarlane A, et al. Perinatal and maternal outcomes by planned place of birth for healthy women with low risk pregnancies: the Birthplace in England national prospective cohort study. *BMJ* 2011; Nov 23;343:d7400.

59. Kiefer T, Park L, Tribouilloy C, Cortes C, Casillo R, Chu V, et al. Association between valvular surgery and mortality among patients with infective endocarditis complicated by heart failure. *JAMA* 2011; Nov 23;306(20):2239-47.

60. Mathurin P, Moreno C, Samuel D, Dumortier J, Salleron J, Durand F, et al. Early liver transplantation for severe alcoholic hepatitis. *N Engl J Med* 2011; Nov 10;365(19):1790-800.

61. de Tisi J, Bell GS, Peacock JL, McEvoy AW, Harkness WF, Sander JW, et al. The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: a cohort study. *Lancet* 2011; Oct 15;378(9800):1388-95.

62. Roth C, Magnus P, Schjolberg S, Stoltenberg C, Suren P, McKeague IW, et al. Folic acid supplements in pregnancy and severe language delay in children. *JAMA* 2011; Oct 12;306(14):1566-73.

63. Noah MA, Peek GJ, Finney SJ, Griffiths MJ, Harrison DA, Grieve R, et al. Referral to an extracorporeal membrane oxygenation center and mortality among patients with severe 2009 influenza A(H1N1). *JAMA* 2011; Oct 19;306(15):1659-68.

64. Morse RB, Hall M, Fieldston ES, McGwire G, Anspacher M, Sills MR, et al. Hospital-level compliance with asthma care quality measures at children's hospitals and subsequent asthma-related outcomes. *JAMA* 2011; Oct 5;306(13):1454-60.

65. Parsons HM, Tuttle TM, Kuntz KM, Begun JW, McGovern PM, Virnig BA. Association between lymph node evaluation for colon cancer and node positivity over the past 20 years. *JAMA* 2011; Sep 14;306(10):1089-97.

66. Reis JP, Loria CM, Sorlie PD, Park Y, Hollenbeck A, Schatzkin A. Lifestyle factors and risk for new-onset diabetes: a population-based cohort study. *Ann Intern Med* 2011; Sep 6;155(5):292-9.

67. Wen CP, Wai JP, Tsai MK, Yang YC, Cheng TY, Lee MC, et al. Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. *Lancet* 2011; Oct 1;378(9798):1244-53.

68. Mozaffarian D, Lemaitre RN, King IB, Song X, Spiegelman D, Sacks FM, et al. Circulating long-chain omega-3 fatty acids and incidence of congestive heart failure in older adults: the cardiovascular health study: a cohort study. *Ann Intern Med* 2011; Aug 2;155(3):160-70.

69. Montgomery RA, Lonze BE, King KE, Kraus ES, Kucirka LM, Locke JE, et al. Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* 2011; Jul 28;365(4):318-26.

70. Crowe FL, Appleby PN, Allen NE, Key TJ. Diet and risk of diverticular disease in Oxford cohort of European Prospective Investigation into Cancer and Nutrition (EPIC): prospective study of British vegetarians and non-vegetarians. *BMJ* 2011; Jul 19;343:d4131.

71. Wisnivesky JP, Smith CB, Packer S, Strauss GM, Lurslurchachai L, Federman A, et al. Survival and risk of adverse events in older patients receiving postoperative adjuvant chemotherapy for resected stages II-IIIA lung cancer: observational cohort study. *BMJ* 2011; Jul 14;343:d4013.

72. Chiuve SE, Fung TT, Rexrode KM, Spiegelman D, Manson JE, Stampfer MJ, et al. Adherence to a low-risk, healthy lifestyle and risk of sudden cardiac death among women. *JAMA* 2011; Jul 6;306(1):62-9.

73. Wijeysundera DN, Beattie WS, Karkouti K, Neuman MD, Austin PC, Laupacis A. Association of echocardiography before major elective non-cardiac surgery with postoperative survival and length of hospital stay: population based cohort study. *BMJ* 2011; Jun 30;342:d3695.

74. Wang TY, Nallamothu BK, Krumholz HM, Li S, Roe MT, Jollis JG, et al. Association of door-in to door-out time with reperfusion delays and outcomes among patients transferred for primary percutaneous coronary intervention. *JAMA* 2011; Jun 22;305(24):2540-7.

75. Solomon DH, Massarotti E, Garg R, Liu J, Canning C, Schneeweiss S. Association between disease-modifying antirheumatic drugs and diabetes risk in patients with rheumatoid arthritis and psoriasis. *JAMA* 2011; Jun 22;305(24):2525-31.

76. Maciejewski ML, Livingston EH, Smith VA, Kavee AL, Kahwati LC, Henderson WG, et al. Survival among high-risk patients after bariatric surgery. *JAMA* 2011; Jun 15;305(23):2419-26.

77. Warensjo E, Byberg L, Melhus H, Gedeborg R, Mallmin H, Wolk A, et al. Dietary calcium intake and risk of fracture and osteoporosis: prospective longitudinal cohort study. *BMJ* 2011; May 24;342:d1473.

78. Short PM, Lipworth SI, Elder DH, Schembri S, Lipworth BJ. Effect of beta blockers in treatment of chronic obstructive pulmonary disease: a retrospective cohort study. *BMJ* 2011; May 10;342:d2549.

79. Stolarz-Skrzypek K, Kuznetsova T, Thijs L, Tikhonoff V, Seidlerova J, Richart T, et al. Fatal and nonfatal outcomes, incidence of hypertension, and blood pressure changes in relation to urinary sodium excretion. *JAMA* 2011; May 4;305(17):1777-85.

80. Gershon A, Croxford R, To T, Stanbrook MB, Upshur R, Sanchez-Romeu P, et al. Comparison of inhaled long-acting beta-agonist and anticholinergic effectiveness in older patients with chronic obstructive pulmonary disease: a cohort study. *Ann Intern Med* 2011; May 3;154(9):583-92.

81. HIV-CAUSAL Collaboration, Cain LE, Logan R, Robins JM, Sterne JA, Sabin C, et al. When to initiate combined antiretroviral therapy to reduce mortality and AIDS-defining illness in HIV-infected persons in developed countries: an observational study. *Ann Intern Med* 2011; Apr 19;154(8):509-15.

82. Douglas I, Evans S, Smeeth L. Effect of statin treatment on short term mortality after pneumonia episode: cohort study. *BMJ* 2011; Apr 6;342:d1642.

83. Skowronski DM, Janjua NZ, De Serres G, Hottes TS, Dickinson JA, Crowcroft N, et al. Effectiveness of AS03 adjuvanted pandemic H1N1 vaccine: case-control evaluation based on sentinel surveillance system in Canada, autumn 2009. *BMJ* 2011; Feb 3;342:c7297.

84. Driver JA, Logroscino G, Lu L, Gaziano JM, Kurth T. Use of non-steroidal anti-inflammatory drugs and risk of Parkinson's disease: nested case-control study. *BMJ* 2011; Jan 20;342:d198.

85. Tseng HF, Smith N, Harpaz R, Bialek SR, Sy LS, Jacobsen SJ. Herpes zoster vaccine in older adults and the risk of subsequent herpes zoster disease. *JAMA* 2011; Jan 12;305(2):160-6.

86. Brenner H, Chang-Claude J, Seiler CM, Rickert A, Hoffmeister M. Protection from colorectal cancer after colonoscopy: a population-based, case-control study. *Ann Intern Med* 2011; Jan 4;154(1):22-30.

87. Graff-Iversen S, Jansen MD, Hoff DA, Hoiseth G, Knudsen GP, Magnus P, et al. Divergent associations of drinking frequency and binge consumption of alcohol with mortality within the same cohort. *J Epidemiol Community Health* 2013; Apr;67(4):350-7.

88. Hildebrand JS, Patel AV, McCullough ML, Gaudet MM, Chen AY, Hayes RB, et al. Coffee, tea, and fatal oral/pharyngeal cancer in a large prospective US cohort. *Am J Epidemiol* 2013; Jan 1;177(1):50-8.

89. He K, Xun P, Brasky TM, Gammon MD, Stevens J, White E. Types of fish consumed and fish preparation methods in relation to pancreatic cancer incidence: the VITAL Cohort Study. *Am J Epidemiol* 2013; Jan 15;177(2):152-60.

90. Misirli G, Benetou V, Lagiou P, Bamia C, Trichopoulos D, Trichopoulou A. Relation of the traditional Mediterranean diet to cerebrovascular disease in a Mediterranean population. *Am J Epidemiol* 2012; Dec 15;176(12):1185-92.

91. Cozier YC, Berman JS, Palmer JR, Boggs DA, Wise LA, Rosenberg L. Reproductive and hormonal factors in relation to incidence of sarcoidosis in US Black women: The Black Women's Health Study. *Am J Epidemiol* 2012; Oct 1;176(7):635-41.

92. Mannino S, Villa M, Apolone G, Weiss NS, Groth N, Aquino I, et al. Effectiveness of adjuvanted influenza vaccination in elderly subjects in northern Italy. *Am J Epidemiol* 2012; Sep 15;176(6):527-33.

93. Xue QL, Bandeen-Roche K, Mielenz TJ, Seplaki CL, Szanton SL, Thorpe RJ, et al. Patterns of 12-year change in physical activity levels in community-dwelling older women: can modest levels of physical activity help older women live longer?. *Am J Epidemiol* 2012; Sep 15;176(6):534-43.

94. Zhang X, Gao YT, Yang G, Li H, Cai Q, Xiang YB, et al. Urinary isoflavonoids and risk of coronary heart disease. *Int J Epidemiol* 2012; Oct;41(5):1367-75.

95. Paranjothy S, Broughton H, Evans A, Huddart S, Drayton M, Jefferson R, et al. The role of maternal nutrition in the aetiology of gastroschisis: an incident case-control study. *Int J Epidemiol* 2012; Aug;41(4):1141-52.

96. Vanhala M, Saltevo J, Soininen P, Kautiainen H, Kangas AJ, Ala-Korpela M, et al. Serum omega-6 polyunsaturated fatty acids and the metabolic syndrome: a longitudinal population-based cohort study. *Am J Epidemiol* 2012; Aug 1;176(3):253-60.

97. Epstein MM, Kasperzyk JL, Mucci LA, Giovannucci E, Price A, Wolk A, et al. Dietary fatty acid intake and prostate cancer survival in Orebro County, Sweden. *Am J Epidemiol* 2012; Aug 1;176(3):240-52.

98. Yang L, Zhou M, Sherliker P, Cai Y, Peto R, Wang L, et al. Alcohol drinking and overall and cause-specific mortality in China: nationally representative prospective study of 220,000 men with 15 years of follow-up. *Int J Epidemiol* 2012; Aug;41(4):1101-13.

99. Bradshaw PT, Siega-Riz AM, Campbell M, Weissler MC, Funkhouser WK, Olshan AF. Associations between dietary patterns and head and neck cancer: the Carolina head and neck cancer epidemiology study. *Am J Epidemiol* 2012; Jun 15;175(12):1225-33.

100. Henny J, Paulus A, Helfenstein M, Godefroy T, Gueguen R. Relationship between the achievement of successive periodic health examinations and the risk of dying. Appraisal of a prevention scheme. *J Epidemiol Community Health* 2012; Dec;66(12):1092-6.

101. Lee DC, Park I, Jun TW, Nam BH, Cho SI, Blair SN, et al. Physical activity and body mass index and their associations with the development of type 2 diabetes in korean men. *Am J Epidemiol* 2012; Jul 1;176(1):43-51.

102. Li S, Chao A, Li Z, Moore CA, Liu Y, Zhu J, et al. Folic acid use and nonsyndromic orofacial clefts in China: a prospective cohort study. *Epidemiology* 2012; May;23(3):423-32.

103. Mansournia MA, Danaei G, Forouzanfar MH, Mahmoodi M, Jamali M, Mansournia N, et al. Effect of physical activity on functional performance and knee pain in patients with osteoarthritis : analysis with marginal structural models. *Epidemiology* 2012; Jul;23(4):631-40.

104. Pasternak B, Svanstrom H, Nielsen NM, Fugger L, Melbye M, Hviid A. Use of calcium channel blockers and Parkinson's disease. *Am J Epidemiol* 2012; Apr 1;175(7):627-35.

105. Zhou J, Smith S, Giovannucci E, Michaud DS. Reexamination of total fluid intake and bladder cancer in the Health Professionals Follow-up Study Cohort. *Am J Epidemiol* 2012; Apr 1;175(7):696-705.

106. Gerber Y, Myers V, Goldbourt U. Smoking reduction at midlife and lifetime mortality risk in men: a prospective cohort study. *Am J Epidemiol* 2012; May 15;175(10):1006-12.

107. Kesse-Guyot E, Touvier M, Andreeva VA, Jeandel C, Ferry M, Hercberg S, et al. Cross-sectional but not longitudinal association between n-3 fatty acid intake and depressive symptoms: results from the SU.VI.MAX 2 study. *Am J Epidemiol* 2012; May 15;175(10):979-87.

108. Au Yeung SL, Jiang CQ, Cheng KK, Liu B, Zhang WS, Lam TH, et al. Evaluation of moderate alcohol use and cognitive function among men using a Mendelian randomization design in the Guangzhou biobank cohort study. *Am J Epidemiol* 2012; May 15;175(10):1021-8.

109. Cole SR, Hudgens MG, Tien PC, Anastos K, Kingsley L, Chmiel JS, et al. Marginal structural models for case-cohort study designs to estimate the association of antiretroviral therapy initiation with incident AIDS or death. *Am J Epidemiol* 2012; Mar 1;175(5):381-90.

110. Gulsvik AK, Thelle DS, Samuelsen SO, Myrstad M, Mowe M, Wyller TB. Ageing, physical activity and mortality--a 42-year follow-up study. *Int J Epidemiol* 2012; Apr;41(2):521-30.

111. Lo-Ciganic WH, Zgibor JC, Bunker CH, Moysich KB, Edwards RP, Ness RB. Aspirin, nonaspirin nonsteroidal anti-inflammatory drugs, or acetaminophen and risk of ovarian cancer. *Epidemiology* 2012; Mar;23(2):311-9.

112. Yamaji T, Iwasaki M, Sasazuki S, Sakamoto H, Yoshida T, Tsugane S. Association between plasma 25-hydroxyvitamin D and colorectal adenoma according to dietary calcium intake and vitamin D receptor polymorphism. *Am J Epidemiol* 2012; Feb 1;175(3):236-44.

113. Varraso R, Kabrhel C, Goldhaber SZ, Rimm EB, Camargo CA,Jr. Prospective study of diet and venous thromboembolism in US women and men. *Am J Epidemiol* 2012; Jan 15;175(2):114-26.

114. Morales E, Romieu I, Guerra S, Ballester F, Rebagliato M, Vioque J, et al. Maternal vitamin D status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. *Epidemiology* 2012; Jan;23(1):64-71.

115. Gong Z, Holly EA, Bracci PM. Survival in population-based pancreatic cancer patients: San Francisco Bay area, 1995-1999. *Am J Epidemiol* 2011; Dec 15;174(12):1373-81.

116. Ahern TP, Pedersen L, Svaerke C, Rothman KJ, Sorensen HT, Lash TL. The association between vitamin K antagonist therapy and site-specific cancer incidence estimated by using heart valve replacement as an instrumental variable. Am J Epidemiol 2011; Dec 15;174(12):1382-90.

117. Lucas M, Mekary R, Pan A, Mirzaei F, O'Reilly EJ, Willett WC, et al. Relation between clinical depression risk and physical activity and time spent watching television in older women: a 10-year prospective follow-up study. Am J Epidemiol 2011; Nov 1;174(9):1017-27.

118. Sjolander A, Nyren O, Bellocco R, Evans M. Comparing different strategies for timing of dialysis initiation through inverse probability weighting. Am J Epidemiol 2011; Nov 15;174(10):1204-10.

119. Yonkers KA, Gotman N, Smith MV, Forray A, Belanger K, Brunetto WL, et al. Does antidepressant use attenuate the risk of a major depressive episode in pregnancy?. Epidemiology 2011; Nov;22(6):848-54.

120. Kerr WC, Greenfield TK, Bond J, Ye Y, Rehm J. Racial and ethnic differences in all-cause mortality risk according to alcohol consumption patterns in the national alcohol surveys. Am J Epidemiol 2011; Oct 1;174(7):769-78.

121. Fung TT, Hu FB, Hankinson SE, Willett WC, Holmes MD. Low-carbohydrate diets, dietary approaches to stop hypertension-style diets, and the risk of postmenopausal breast cancer. Am J Epidemiol 2011; Sep 15;174(6):652-60.

122. Brantsaeter AL, Myhre R, Haugen M, Myking S, Sengpiel V, Magnus P, et al. Intake of probiotic food and risk of preeclampsia in primiparous women: the Norwegian Mother and Child Cohort Study. Am J Epidemiol 2011; Oct 1;174(7):807-15.

123. Kasperzyk JL, Chang ET, Birmann BM, Kraft P, Zheng T, Mueller NE. Nutrients and genetic variation involved in one-carbon metabolism and Hodgkin lymphoma risk: a population-based case-control study. Am J Epidemiol 2011; Oct 1;174(7):816-27.

124. Zhang X, Smith-Warner SA, Chan AT, Wu K, Spiegelman D, Fuchs CS, et al. Aspirin use, body mass index, physical activity, plasma C-peptide, and colon cancer risk in US health professionals. Am J Epidemiol 2011; Aug 15;174(4):459-67.

125. Ahrens K, Yazdy MM, Mitchell AA, Werler MM. Folic acid intake and spina bifida in the era of dietary folic acid fortification. Epidemiology 2011; Sep;22(5):731-7.

126. Anderson LN, Cotterchio M, Kirsh VA, Knight JA. Ultraviolet sunlight exposure during adolescence and adulthood and breast cancer risk: a population-based case-control study among Ontario women. Am J Epidemiol 2011; Aug 1;174(3):293-304.

127. Nilsen TI, Holtermann A, Mork PJ. Physical exercise, body mass index, and risk of chronic pain in the low back and neck/shoulders: longitudinal data from the Nord-Trondelag Health Study. Am J Epidemiol 2011; Aug 1;174(3):267-73.

128. Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, et al. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology 2011; Jul;22(4):476-85.

129. Engel P, Fabre A, Fournier A, Mesrine S, Boutron-Ruault MC, Clavel-Chapelon F. Risk of osteoporotic fractures after discontinuation of menopausal hormone therapy: results from the E3N cohort. Am J Epidemiol 2011; Jul 1;174(1):12-21.

130. Larsson SC, Virtamo J, Wolk A. Potassium, calcium, and magnesium intakes and risk of stroke in women. Am J Epidemiol 2011; Jul 1;174(1):35-43.

131. Shrubsole MJ, Shu XO, Li HL, Cai H, Yang G, Gao YT, et al. Dietary B vitamin and methionine intakes and breast cancer risk among Chinese women. Am J Epidemiol 2011; May 15;173(10):1171-82.

132. Nichols HB, Visvanathan K, Newcomb PA, Hampton JM, Egan KM, Titus-Ernstoff L, et al. Bilateral oophorectomy in relation to risk of postmenopausal breast cancer: confounding by nonmalignant indications for surgery?. Am J Epidemiol 2011; May 15;173(10):1111-20.

133. Murcia M, Rebagliato M, Iniguez C, Lopez-Espinosa MJ, Estarlich M, Plaza B, et al. Effect of iodine supplementation during pregnancy on infant neurodevelopment at 1 year of age. Am J Epidemiol 2011; Apr 1;173(7):804-12.

134. Park SY, Murphy SP, Wilkens LR, Henderson BE, Kolonel LN. Multivitamin use and the risk of mortality and cancer incidence: the multiethnic cohort study. Am J Epidemiol 2011; Apr 15;173(8):906-14.

135. Wang H, O'Reilly EJ, Weisskopf MG, Logroscino G, McCullough ML, Schatzkin A, et al. Vitamin E intake and risk of amyotrophic lateral sclerosis: a pooled analysis of data from 5 prospective cohort studies. Am J Epidemiol 2011; Mar 15;173(6):595-602.

136. Hjellvik V, Tverdal A, Strom H. Boiled coffee intake and subsequent risk for type 2 diabetes. Epidemiology 2011; May;22(3):418-21.

137. Mackey DC, Hubbard AE, Cawthon PM, Cauley JA, Cummings SR, Tager IB, et al. Usual physical activity and hip fracture in older men: an application of semiparametric methods to observational data. Am J Epidemiol 2011; Mar 1;173(5):578-86.

138. Northstone K, Joinson C, Emmett P, Ness A, Paus T. Are dietary patterns in childhood associated with IQ at 8 years of age? A population-based cohort study. J Epidemiol Community Health 2012; Jul;66(7):624-8.

139. Ford ES, Zhao G, Tsai J, Li C. Vitamin D and all-cause mortality among adults in USA: findings from the National Health and Nutrition Examination Survey Linked Mortality Study. Int J Epidemiol 2011; Aug;40(4):998-1005.

140. Weinstein SJ, Yu K, Horst RL, Ashby J, Virtamo J, Albanes D. Serum 25-hydroxyvitamin D and risks of colon and rectal cancer in Finnish men. Am J Epidemiol 2011; Mar 1;173(5):499-508.

141. Cox B, Sneyd MJ. School milk and risk of colorectal cancer: a national case-control study. Am J Epidemiol 2011; Feb 15;173(4):394-403.

142. Prentice RL, Huang Y, Kuller LH, Tinker LF, Horn LV, Stefanick ML, et al. Biomarker-calibrated energy and protein consumption and cardiovascular disease risk among postmenopausal women. Epidemiology 2011; Mar;22(2):170-9.

143. Pierce BL, Argos M, Chen Y, Melkonian S, Parvez F, Islam T, et al. Arsenic exposure, dietary patterns, and skin lesion risk in bangladesh: a prospective study. Am J Epidemiol 2011; Feb 1;173(3):345-54.

144. Inoue M, Nagata C, Tsuji I, Sugawara Y, Wakai K, Tamakoshi A, et al. Impact of alcohol intake on total mortality and mortality from major causes in Japan: a pooled analysis of six large-scale cohort studies. J Epidemiol Community Health 2012; May;66(5):448-56.

145. Baer HJ, Glynn RJ, Hu FB, Hankinson SE, Willett WC, Colditz GA, et al. Risk factors for mortality in the nurses' health study: a competing risks analysis. Am J Epidemiol 2011; Feb 1;173(3):319-29.

146. Sato KK, Hayashi T, Harita N, Koh H, Maeda I, Endo G, et al. Relationship between drinking patterns and the risk of type 2 diabetes: the Kansai Healthcare Study. J Epidemiol Community Health 2012; Jun;66(6):507-11.

147. Press DJ, Sullivan-Halley J, Ursin G, Deapen D, McDonald JA, Strom BL, et al. Breast cancer risk and ovariectomy, hysterectomy, and tubal sterilization in the women's contraceptive and reproductive experiences study. Am J Epidemiol 2011; Jan 1;173(1):38-47.

148. Musselman JR, Jurek AM, Johnson KJ, Linabery AM, Robison LL, Shu XO, et al. Maternal dietary patterns during early pregnancy and the odds of childhood germ cell tumors: A Children's Oncology Group study. Am J Epidemiol 2011; Feb 1;173(3):282-91.

149. Erichsen R, Froslev T, Lash TL, Pedersen L, Sorensen HT. Long-term statin use and the risk of gallstone disease: A population-based case-control study. Am J Epidemiol 2011; Jan 15;173(2):162-70.

150. Landrum ML, Hullsiek KH, Chun HM, Crum-Cianflone NF, Ganesan A, Weintrob AC, et al. The timing of hepatitis B virus (HBV) immunization relative to human immunodeficiency virus (HIV) diagnosis and the risk of HBV infection following HIV diagnosis. Am J Epidemiol 2011; Jan 1;173(1):84-93.

151. Beydoun MA, Beason-Held LL, Kitner-Triolo MH, Beydoun HA, Ferrucci L, Resnick SM, et al. Statins and serum cholesterol's associations with incident dementia and mild cognitive impairment. J Epidemiol Community Health 2011; Nov;65(11):949-57.

152. Leung WW, Ho SC, Chan HL, Wong V, Yeo W, Mok TS. Moderate coffee consumption reduces the risk of hepatocellular carcinoma in hepatitis B chronic carriers: a case-control study. J Epidemiol Community Health 2011; Jun;65(6):556-8.

153. Heesch KC, Burton NW, Brown WJ. Concurrent and prospective associations between physical activity, walking and mental health in older women. J Epidemiol Community Health 2011; Sep;65(9):807-13.

154. Mineharu Y, Koizumi A, Wada Y, Iso H, Watanabe Y, Date C, et al. Coffee, green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. J Epidemiol Community Health 2011; Mar;65(3):230-40.

155. Mozaffarian D, Cao H, King IB, Lemaitre RN, Song X, Siscovick DS, et al. Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study. Ann Intern Med 2010; Dec 21;153(12):790-9.

156. Ruidavets JB, Ducimetiere P, Evans A, Montaye M, Haas B, Bingham A, et al. Patterns of alcohol consumption and ischaemic heart disease in culturally divergent countries: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). BMJ 2010; Nov 23;341:c6077.

157. Chan PS, Krumholz HM, Spertus JA, Jones PG, Cram P, Berg RA, et al. Automated external defibrillators and survival after in-hospital cardiac arrest. JAMA 2010; Nov 17;304(19):2129-36.

158. Kirkegaard H, Johnsen NF, Christensen J, Frederiksen K, Overvad K, Tjonneland A. Association of adherence to lifestyle recommendations and risk of colorectal cancer: a prospective Danish cohort study. BMJ 2010; Oct 26;341:c5504.

159. Neily J, Mills PD, Young-Xu Y, Carney BT, West P, Berger DH, et al. Association between implementation of a medical team training program and surgical mortality. JAMA 2010; Oct 20;304(15):1693-700.

160. Bobrow BJ, Spaite DW, Berg RA, Stolz U, Sanders AB, Kern KB, et al. Chest compression-only CPR by lay rescuers and survival from out-of-hospital cardiac arrest. JAMA 2010; Oct 6;304(13):1447-54.

161. Yu H, Liao Q, Yuan Y, Zhou L, Xiang N, Huai Y, et al. Effectiveness of oseltamivir on disease progression and viral RNA shedding in patients with mild pandemic 2009 influenza A H1N1: opportunistic retrospective study of medical charts in China. BMJ 2010; Sep 28;341:c4779.

162. Fung TT, van Dam RM, Hankinson SE, Stampfer M, Willett WC, Hu FB. Low-carbohydrate diets and all-cause and cause-specific mortality: two cohort studies. Ann Intern Med 2010; Sep 7;153(5):289-98.

163. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA 2010; Sep 1;304(9):967-75.

164. Summers DM, Johnson RJ, Allen J, Fuggle SV, Collett D, Watson CJ, et al. Analysis of factors that affect outcome after transplantation of kidneys donated after cardiac death in the UK: a cohort study. Lancet 2010; Oct 16;376(9749):1303-11.

165. Ramnarayan P, Thiru K, Parslow RC, Harrison DA, Draper ES, Rowan KM. Effect of specialist retrieval teams on outcomes in children admitted to paediatric intensive care units in England and Wales: a retrospective cohort study. Lancet 2010; Aug 28;376(9742):698-704.

166. Ritchie K, Carriere I, Ritchie CW, Berr C, Artero S, Ancelin ML. Designing prevention programmes to reduce incidence of dementia: prospective cohort study of modifiable risk factors. BMJ 2010; Aug 5;341:c3885.

167. Cooper-DeHoff RM, Gong Y, Handberg EM, Bavry AA, Denardo SJ, Bakris GL, et al. Tight blood pressure control and cardiovascular outcomes among hypertensive patients with diabetes and coronary artery disease. JAMA 2010; Jul 7;304(1):61-8.

168. Kimber J, Copeland L, Hickman M, Macleod J, McKenzie J, De Angelis D, et al. Survival and cessation in injecting drug users: prospective observational study of outcomes and effect of opiate substitution treatment. BMJ 2010; Jul 1;341:c3172.

169. Graham DJ, Ouellet-Hellstrom R, MaCurdy TE, Ali F, Sholley C, Worrall C, et al. Risk of acute myocardial infarction, stroke, heart failure, and death in elderly Medicare patients treated with rosiglitazone or pioglitazone. JAMA 2010; Jul 28;304(4):411-8.

170. O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, et al. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. Lancet 2010; Jul 10;376(9735):112-23.

171. Johansson M, Relton C, Ueland PM, Vollset SE, Midttun O, Nygard O, et al. Serum B vitamin levels and risk of lung cancer. JAMA 2010; Jun 16;303(23):2377-85.

172. Lindenauer PK, Pekow PS, Lahti MC, Lee Y, Benjamin EM, Rothberg MB. Association of corticosteroid dose and route of administration with risk of treatment failure in acute exacerbation of chronic obstructive pulmonary disease. JAMA 2010; Jun 16;303(23):2359-67.

173. de Palma O, Cruz L, Ramos H, de Baires A, Villatoro N, Pastor D, et al. Effectiveness of rotavirus vaccination against childhood diarrhoea in El Salvador: case-control study. BMJ 2010; Jun 15;340:c2825.

174. Marso SP, Amin AP, House JA, Kennedy KF, Spertus JA, Rao SV, et al. Association between use of bleeding avoidance strategies and risk of periprocedural bleeding among patients undergoing percutaneous coronary intervention. JAMA 2010; Jun 2;303(21):2156-64.

175. Lambert L, Brown K, Segal E, Brophy J, Rodes-Cabau J, Bogaty P. Association between timeliness of reperfusion therapy and clinical outcomes in ST-elevation myocardial infarction. JAMA 2010; Jun 2;303(21):2148-55.

176. Rothberg MB, Pekow PS, Lahti M, Brody O, Skiest DJ, Lindenauer PK. Antibiotic therapy and treatment failure in patients hospitalized for acute exacerbations of chronic obstructive pulmonary disease. JAMA 2010; May 26;303(20):2035-42.

177. Hippisley-Cox J, Coupland C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. BMJ 2010; May 20;340:c2197.

178. Dheda K, Shean K, Zumla A, Badri M, Streicher EM, Page-Shipp L, et al. Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study. Lancet 2010; May 22;375(9728):1798-807.

179. Hernandez AF, Greiner MA, Fonarow GC, Hammill BG, Heidenreich PA, Yancy CW, et al. Relationship between early physician follow-up and 30-day readmission among Medicare beneficiaries hospitalized for heart failure. JAMA 2010; May 5;303(17):1716-22.

180. Tseng HF, Slezak JM, Quinn VP, Sy LS, Van den Eeden SK, Jacobsen SJ. Pneumococcal vaccination and risk of acute myocardial infarction and stroke in men. JAMA 2010; May 5;303(17):1699-706.

181. Walker AS, Ford D, Gilks CF, Munderi P, Ssali F, Reid A, et al. Daily co-trimoxazole prophylaxis in severely immunosuppressed HIV-infected adults in Africa started on combination antiretroviral therapy: an observational analysis of the DART cohort. Lancet 2010; Apr 10;375(9722):1278-86.

182. Kitamura T, Iwami T, Kawamura T, Nagao K, Tanaka H, Hiraide A, et al. Nationwide public-access defibrillation in Japan. N Engl J Med 2010; Mar 18;362(11):994-1004.

183. Ray WA, Murray KT, Griffin MR, Chung CP, Smalley WE, Hall K, et al. Outcomes with concurrent use of clopidogrel and proton-pump inhibitors: a cohort study. Ann Intern Med 2010; Mar 16;152(6):337-45.

184. Hannaford PC, Iversen L, Macfarlane TV, Elliott AM, Angus V, Lee AJ. Mortality among contraceptive pill users: cohort evidence from Royal College of General Practitioners' Oral Contraception Study. BMJ 2010; Mar 11;340:c927.

185. Kitamura T, Iwami T, Kawamura T, Nagao K, Tanaka H, Nadkarni VM, et al. Conventional and chest-compression-only cardiopulmonary resuscitation by bystanders for children who have out-of-hospital cardiac arrests: a prospective, nationwide, population-based cohort study. Lancet 2010; Apr 17;375(9723):1347-54.

186. Brookhart MA, Schneeweiss S, Avorn J, Bradbury BD, Liu J, Winkelmayer WC. Comparative mortality risk of anemia management practices in incident hemodialysis patients. JAMA 2010; Mar 3;303(9):857-64.

187. Todo Y, Kato H, Kaneuchi M, Watari H, Takeda M, Sakuragi N. Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. Lancet 2010; Apr 3;375(9721):1165-72.

188. Shuhaiber JH, Hur K, Gibbons R. The influence of preoperative use of ventricular assist devices on survival after heart transplantation: propensity score matched analysis. BMJ 2010; Feb 10;340:c392.

189. Wijeysundera DN, Beattie WS, Austin PC, Hux JE, Laupacis A. Non-invasive cardiac stress testing before elective major non-cardiac surgery: population based cohort study. BMJ 2010; Jan 28;340:b5526.

190. Boger-Megiddo I, Heckbert SR, Weiss NS, McKnight B, Furberg CD, Wiggins KL, et al. Myocardial infarction and stroke associated with diuretic based two drug antihypertensive regimens: population based case-control study. BMJ 2010; Jan 25;340:c103.

191. Jenab M, Bueno-de-Mesquita HB, Ferrari P, van Duijnhoven FJ, Norat T, Pischon T, et al. Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations:a nested case-control study. BMJ 2010; Jan 21;340:b5500.

192. Schaer BA, Schneider C, Jick SS, Conen D, Osswald S, Meier CR. Risk for incident atrial fibrillation in patients who receive antihypertensive drugs: a nested case-control study. Ann Intern Med 2010; Jan 19;152(2):78-84.

193. Li NC, Lee A, Whitmer RA, Kivipelto M, Lawler E, Kazis LE, et al. Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: prospective cohort analysis. BMJ 2010; Jan 12;340:b5465.

194. Duell EJ, Travier N, Lujan-Barroso L, Boutron-Ruault MC, Clavel-Chapelon F, Palli D, et al. Menstrual and reproductive factors, exogenous hormone use, and gastric cancer risk in a cohort of women from the European Prospective Investigation Into Cancer and Nutrition. Am J Epidemiol 2010; Dec 15;172(12):1384-93.

195. Chang ET, Clarke CA, Canchola AJ, Lu Y, Wang SS, Ursin G, et al. Alcohol consumption over time and risk of lymphoid malignancies in the California Teachers Study cohort. Am J Epidemiol 2010; Dec 15;172(12):1373-83.

196. Boggs DA, Palmer JR, Wise LA, Spiegelman D, Stampfer MJ, Adams-Campbell LL, et al. Fruit and vegetable intake in relation to risk of breast cancer in the Black Women's Health Study. Am J Epidemiol 2010; Dec 1;172(11):1268-79.

197. Tarrant M, Kwok MK, Lam TH, Leung GM, Schooling CM. Breast-feeding and childhood hospitalizations for infections. Epidemiology 2010; Nov;21(6):847-54.

198. Lobo E, Dufouil C, Marcos G, Quetglas B, Saz P, Guallar E, et al. Is there an association between low-to-moderate alcohol consumption and risk of cognitive decline?. Am J Epidemiol 2010; Sep 15;172(6):708-16.

199. Salinas CA, Kwon EM, FitzGerald LM, Feng Z, Nelson PS, Ostrander EA, et al. Use of aspirin and other nonsteroidal antiinflammatory medications in relation to prostate cancer risk. Am J Epidemiol 2010; Sep 1;172(5):578-90.

200. Abnet CC, Chen Y, Chow WH, Gao YT, Helzlsouer KJ, Le Marchand L, et al. Circulating 25-hydroxyvitamin D and risk of esophageal and gastric cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol 2010; Jul 1;172(1):94-106.

201. Zeleniuch-Jacquotte A, Gallicchio L, Hartmuller V, Helzlsouer KJ, McCullough ML, Setiawan VW, et al. Circulating 25-hydroxyvitamin D and risk of endometrial cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol 2010; Jul 1;172(1):36-46.

202. Gallicchio L, Moore LE, Stevens VL, Ahn J, Albanes D, Hartmuller V, et al. Circulating 25-hydroxyvitamin D and risk of kidney cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol 2010; Jul 1;172(1):47-57.

203. Zheng W, Danforth KN, Tworoger SS, Goodman MT, Arslan AA, Patel AV, et al. Circulating 25-hydroxyvitamin D and risk of epithelial ovarian cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol 2010; Jul 1;172(1):70-80.

204. Stolzenberg-Solomon RZ, Jacobs EJ, Arslan AA, Qi D, Patel AV, Helzlsouer KJ, et al. Circulating 25-hydroxyvitamin D and risk of pancreatic cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol 2010; Jul 1;172(1):81-93.

205. Purdue MP, Freedman DM, Gapstur SM, Helzlsouer KJ, Laden F, Lim U, et al. Circulating 25-hydroxyvitamin D and risk of non-hodgkin lymphoma: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol 2010; Jul 1;172(1):58-69.

206. Costenbader KH, Kang JH, Karlson EW. Antioxidant intake and risks of rheumatoid arthritis and systemic lupus erythematosus in women. Am J Epidemiol 2010; Jul 15;172(2):205-16.

207. Kim S, Sandler DP, Galanko J, Martin C, Sandler RS. Intake of polyunsaturated fatty acids and distal large bowel cancer risk in whites and African Americans. Am J Epidemiol 2010; May 1;171(9):969-79.

208. Villegas R, Yang G, Gao YT, Cai H, Li H, Zheng W, et al. Dietary patterns are associated with lower incidence of type 2 diabetes in middle-aged women: the Shanghai Women's Health Study. Int J Epidemiol 2010; Jun;39(3):889-99.

209. Kaluza J, Orsini N, Levitan EB, Brzozowska A, Roszkowski W, Wolk A. Dietary calcium and magnesium intake and mortality: a prospective study of men. Am J Epidemiol 2010; Apr 1;171(7):801-7.

210. Delellis Henderson K, Duan L, Sullivan-Halley J, Ma H, Clarke CA, Neuhausen SL, et al. Menopausal hormone therapy use and risk of invasive colon cancer: the California Teachers Study. Am J Epidemiol 2010; Feb 15;171(4):415-25.

211. Ma X, Park Y, Mayne ST, Wang R, Sinha R, Hollenbeck AR, et al. Diet, lifestyle, and acute myeloid leukemia in the NIH-AARP cohort. Am J Epidemiol 2010; Feb 1;171(3):312-22.

212. Kesavan Y, Giovannucci E, Fuchs CS, Michaud DS. A prospective study of magnesium and iron intake and pancreatic cancer in men. Am J Epidemiol 2010; Jan 15;171(2):233-41.

213. Wise LA, Radin RG, Palmer JR, Kumanyika SK, Rosenberg L. A prospective study of dairy intake and risk of uterine leiomyomata. Am J Epidemiol 2010; Jan 15;171(2):221-32.

214. Cole SR, Jacobson LP, Tien PC, Kingsley L, Chmiel JS, Anastos K. Using marginal structural measurement-error models to estimate the long-term effect of antiretroviral therapy on incident AIDS or death. Am J Epidemiol 2010; Jan 1;171(1):113-22.

215. Vitonis AF, Hankinson SE, Hornstein MD, Missmer SA. Adult physical activity and endometriosis risk. Epidemiology 2010; Jan;21(1):16-23.

216. Marron M, Boffetta P, Zhang ZF, Zaridze D, Wunsch-Filho V, Winn DM, et al. Cessation of alcohol drinking, tobacco smoking and the reversal of head and neck cancer risk. Int J Epidemiol 2010; Feb;39(1):182-96.

217. Robinson M, McLean NJ, Oddy WH, Mattes E, Bulsara M, Li J, et al. Smoking cessation in pregnancy and the risk of child behavioural problems: a longitudinal prospective cohort study. J Epidemiol Community Health 2010; Jul;64(7):622-9.

218. Oien T, Storro O, Johnsen R. Do early intake of fish and fish oil protect against eczema and doctor-diagnosed asthma at 2 years of age? A cohort study. J Epidemiol Community Health 2010; Feb;64(2):124-9.

219. Heroux M, Janssen I, Lam M, Lee DC, Hebert JR, Sui X, et al. Dietary patterns and the risk of mortality: impact of cardiorespiratory fitness. Int J Epidemiol 2010; Feb;39(1):197-209.

220. Epplein M, Shu XO, Xiang YB, Chow WH, Yang G, Li HL, et al. Fruit and vegetable consumption and risk of distal gastric cancer in the Shanghai Women's and Men's Health studies. Am J Epidemiol 2010; Aug 15;172(4):397-406.

CHAPTER 3

# Effect of pravastatin and fosinopril on recurrent urinary tract infections

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## Abstract

### Background

Recurrent urinary tract infections (UTIs) are a problem affecting both women and men. Animal experiments and *in vitro* studies indicate that statins might prevent recurrent UTIs. We assessed the effects of pravastatin on UTI antibiotic prescribing among adults.

### Methods

A *post hoc* analysis was conducted with data from PREVEND IT, a trial among participants randomized to receive pravastatin, fosinopril or placebo in a 2×2 factorial design over 4 years. Trial data were linked to the pharmacy prescription database IADB.nl. The primary outcome was the number of prescriptions with a nitrofurantoin derivate, a sulphonamide or trimethoprim as a proxy for UTI antibiotic prescribing. In primary analysis, generalized estimating equations were used to estimate the effect on the occurrence of UTI antibiotic prescriptions (including both first and subsequent prescriptions). Cox regression was used to determine the effect on first and second (recurrent) UTI antibiotic prescriptions.

### Results

Of the 864 trial participants, 655 were eligible for analysis. During an average follow-up of 3.8 years, 112 (17%) participants received at least one UTI antibiotic prescription. Intention-to-treat analyses showed that pravastatin was associated with a reduced occurence of UTI antibiotic prescriptions (relative risk, 0.43; 95% CI, 0.21–0.88) and occurrence of second UTI antibiotic prescriptions [hazard ratio (HR), 0.25; 95% CI, 0.08–0.77]. No significant effect on occurrence of first UTI antibiotic prescriptions was found (HR, 0.83; 95% CI, 0.57–1.20). Fosinopril was associated with an increased occurrence of first UTI antibiotic prescriptions (HR, 1.82; 95% CI, 1.16–2.88). Combination therapy with fosinopril and pravastatin did not significantly influence the occurrence of UTI antibiotic prescriptions.

### Conclusions

This study suggests that pravastatin can reduce the occurrence of recurrent UTIs. Larger studies among patients with recurrent UTIs are warranted.

## Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans [1]. Cystitis in particular is very common, with an annual incidence of 70 per 1000 women and 10 per 1000 men [1]. Recurrent UTIs are a considerable problem, affecting ~25% of women within 6 months of an acute UTI episode [2]. Recurrent UTIs are also a problem in men [3].

Recently, it has been shown that uropathogenic *Escherichia coli* are invasive to bladder and kidney epithelial cells [2]. Bacterial invasion facilitates the establishment of a quiescent intracellular reservoir (QIR) [2]. The bacteria that form the QIR can persist for months following initial infection, resist antibiotic treatment [4] and can serve as the source for recurrent UTIs [5].

Bacterial invasion into the bladder epithelium involves Rac1, a Rho GTPase [6-9]. Because statins can reduce the amount of Rac1 associated with the membrane [10-14], they might inhibit bacterial invasion. Pre-clinical studies indicate that statins indeed can reduce bacterial invasion [9, 15-17].

This might prevent the formation of a QIR. Therefore, we hypothesized that statins may reduce the occurrence of recurrent UTIs, as their source could be removed. Statin treatment may result in a decreased duration or severity of first UTIs, but their occurrence is most likely less affected, since removing the source of recurrent UTIs does not substantially influence the occurrence of first (non-recurrent) UTIs.

Two observational studies assessed the effect of statins on the risk of contracting UTIs. One found that statin therapy was associated with a 9% decreased UTI risk [18], whereas the other observed a 5% increased risk [19]. These studies were vulnerable to unmeasured confounding bias, because both studies were non-randomized and important risk factors for UTIs, such as kidney disorders and/or urinary tract abnormalities, were not measured and patients having these conditions were not excluded.

Hence, we investigated the effect of statins on the occurrence of (recurrent) UTIs using a randomized design. Data from the Prevention of REnal and Vascular ENdstage Disease Intervention Trial (PREVEND IT) [20] were linked to a large prescription database to estimate *post hoc* the effect of pravastatin on the occurrence of UTIs compared with placebo. We further assessed whether the effect was larger for subsequent UTIs than for first UTIs.

## Methods

PREVEND IT is a randomized, double-blind, placebo-controlled trial with a 2×2 factorial design, which aimed to determine whether treatment with pravastatin and/or fosinopril can prevent cardiovascular and renal disease in non-hypertensive, non-hypercholesterolaemic adults with persistent microalbuminuria. Participants were randomized to 40 mg of pravastatin or matching placebo and to 20 mg of fosinopril or matching placebo. Details of the PREVEND IT objectives, design and methods have been described previously [20, 21] and are summarized below.

The PREVEND IT study protocol was approved by the institutional review board of the University Medical Center Groningen and was conducted in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all participants before randomization. The key entry criteria for participation in PREVEND IT were persistent microalbuminuria (one urinary albumin concentration >10 mg/L in an early morning spot urine test and at least one of 15–300 mg/24 h in two 24 h urine samples), absence of antihypertensive and lipid-lowering medication, blood pressure <160/100 mmHg and total cholesterol <8.0 or <5.0 mmol/L in the case of previous myocardial infarction. From April 1998 to June 1999, 864 subjects were included in PREVEND IT and were randomized to the study medication for 4 years.

Most participants in PREVEND IT were inhabitants of the city of Groningen. The IADB.nl database (IADB), a community-based pharmacy database, contains detailed patient-specific drug prescription information on almost all inhabitants of the city of Groningen [22] and was linked to PREVEND IT data. The IADB contains, among other data, information on the date of prescription, number of days the drug was prescribed for and the number of defined daily doses based on the WHO definition [22]. Prescription drugs are classified according to the Anatomical Therapeutic Chemical (ATC) system and the IADB population is considered representative of the Dutch population in terms of drug use [23]. All PREVEND IT participants included in this study gave informed consent to link their data with pharmacy-dispensing data. For the present *post hoc* analyses, individuals were excluded if no pharmacy data during the 6 months prior to the start of the trial could be linked. Further, individuals who received a prescription of nitrofurantoin, a sulphonamide or trimethoprim (used as a proxy for UTI antibiotic prescribing, see outcome definitions) during this pre-trial period were excluded.

### Outcome definitions

The primary outcome was defined as the occurence of prescriptions with nitrofurantoin (ATC code J01XE) or sulphonamides or trimethoprim (ATC code J01E) during follow-

up. To exclude relapses due to insufficient or incorrect treatment, a new UTI antibiotic prescription was defined as a UTI antibiotic prescription occurring ≥30 days after a previous UTI antibiotic prescription. During the research period (1998–2003), in 96% of the cases that nitrofurantoin was prescribed in the Netherlands, it was prescribed for a UTI [24]. For sulphonamides or trimethoprim the corresponding specificity was 82% [24]. The sensitivity of our proxy was estimated at 75% [24,25].

**Statistical analyses**

Statistical analyses were performed according to the intention-to-treat (ITT) and per-protocol principles, with the use of two-sided tests and STATA 12 and SPSS 18 software.

For ITT analyses, follow-up time was defined as the period from the date of the start of randomization to the end of the trial (4 years) or right censoring (loss to follow-up) in the prescription database. The association between the treatment arm and occurence of UTI antibiotic prescriptions was determined using a multivariate negative binomial generalized estimating equation (GEE) with an autoregressive correlation structure and robust standard errors, and the results are presented as the relative risk (RR) with the corresponding 95% confidence interval (95% CI). We clustered on the patient level, because the risk of contracting a UTI increases after a first UTI [2, 26, 27] and UTI antibiotic prescriptions within one person are consequently correlated, especially when following shortly one after another.

If the mechanism behind a potential reduced risk of infections would indeed be related to reducing the formation of QIRs, one would expect that a protective effect would be larger for recurrent than for first UTIs. Therefore, secondary ITT time-to-event analyses were performed with Cox regression to estimate the effect on the first (time to first UTI antibiotic prescription) or subsequent UTI antibiotic prescriptions (time between first and second UTI antibiotic prescription), with the results presented as hazard ratios (HR) and 95% CIs. Since one cannot acquire a second UTI without experiencing a first UTI, this latter analysis was restricted to patients that experienced a first UTI.

Per-protocol analyses were performed using the same regression techniques as for the ITT analyses. Follow-up time was defined as the period from the start of study medication use to the moment the participant did not adhere to the study protocol. Possible reasons were non-adherence to the study medication, crossover between treatment groups, use of study medication outside the study protocol or right censoring in the prescription database.

We further explored in separate analyses the effect of fosinopril. Treatment with angiotensin-converting enzyme inhibitors (ACEIs) can result in a decrease of the urine output in healthy elderly persons [28]. We therefore secondarily hypothesized that fosinopril may increase the risk of UTIs and that effect modification may be present for recurrent infections, but not for first infections. Effect modification on an additive scale was assessed by incorporating an interaction term between fosinopril and pravastatin into the models. Because power calculations showed that our study likely lacked statistical power for identifying effect modification, especially for the analysis of time between first and second events, a *P* value of <0.2 was considered significant for analyses of interactions [29]. For Cox regression analyses, we used the delta method to calculate the CIs for the relative excess risk due to interaction (RERI) on an additive scale [30]. Given the biological mechanism that could explain an interaction between fosinopril and pravastatin for recurrent infections, pravastatin and combination therapy were analysed separately. We also calculated the effect of pravastatin, regardless of possible effect modification by fosinopril.

## Results

### ITT analyses

Of the 864 trial participants, 655 could be included in the analyses (Table 3.1). Reasons for exclusion were receiving a UTI antibiotic prescription in the 6 months prior to the study (placebo, *n*=6; pravastatin, *n*=3; fosinopril, *n*=4; and combination therapy, *n*=3) or having no pharmacy data available during the full 6 months prior to the study (placebo, *n*=41; pravastatin, *n*=56; fosinopril, *n*=51; and combination therapy, *n*=45). Excluded patients were more frequently male, were slightly younger and had a higher glomerular filtration rate than patients that met the inclusion criteria.

During an average follow-up of 3.8 years, 17 subjects (11%) allocated to pravastatin received at least one UTI antibiotic prescription. For placebo, fosinopril and combination therapy, these numbers were 34 (20%), 30 (19%) and 31 (18%), respectively. Of those subjects allocated to pravastatin that received a first UTI antibiotic prescription, four (24%) subjects experienced also a second UTI during follow-up. For subjects allocated to placebo, fosinopril and combination therapy, these figures were 16 (47%), 15 (50%) and 11 (35%), respectively. Of all men, 38 (19%) received a UTI antibiotic prescription, while 74 (60%) women received a UTI antibiotic prescription during follow-up. The use of other antibiotics used to treat UTIs and commonly prescribed drugs during follow-up was similar in all groups (Table 3.2).

**Table 3.1.** Baseline characteristics (n=655)

| Characteristics | Placebo n=169 | Pravastatin n=158 | Fosinopril n=160 | Pravastatin & Fosinopril n=168 | Excluded patients n=209 |
|---|---|---|---|---|---|
| Age (y) | 50.6 ± 11.6 | 51.5 ± 11.7 | 50.3 ± 11.5 | 51.5 ± 12.1 | 47.6 ± 11.7 |
| Male gender (%) | 59.2 | 65.8 | 59.4 | 64.9 | 73.2 |
| Sys blood pressure (mm Hg) | 131.8 ± 16.0 | 132.5 ± 17.4 | 132.0 ± 16.0 | 130.9 ± 16.6 | 130.7 ± 15.0 |
| Dia blood pressure (mm Hg) | 76.6 ± 9.7 | 76.9 ± 8.3 | 76.5 ± 9.0 | 76.3 ± 9.0 | 75.7 ± 9.1 |
| Total cholesterol (mmol/L) | 5.6 ± 1.0 | 5.6 ± 0.9 | 5.8 ± 1.0 | 5.8 ± 1.0 | 5.6 ± 1.0 |
| Serum creatinine (mmol/L) | 82.6 ± 13.5 | 83.4 ± 12.9 | 84.4 ± 14.1 | 86.6 ± 14.7 | 85.0 ± 13.3 |
| Urinary albumin excretion (mg/24 h)[a] | 24.6 (26.3) | 22.1 (22.4) | 25.5 (21.8) | 23.9 (26.8) | 22.6 (23.5) |
| eGFR, (mL/min/1.73 $m^2$) | 73.6 ± 28.9 | 70.3 ± 32.6 | 73.4 ± 28.1 | 70.8 ± 30.2 | 84.2 ± 13.2 |
| Body mass index (kg/$m^2$) | 27.5 ± 4.9 | 26.9 ± 4.7 | 27.0 ± 4.3 | 27.0 ± 4.1 | 26.1 ± 4.3 |
| Smoking (%) | 52.1 | 49.4 | 40.6 | 49.4 | 52.2 |
| Blood pressure lowering medication (%) | 2.4 | 1.3 | 0 | 3.0 | 1.9 |
| Glucose lowering medication (%) | 1.8 | 1.3 | 0 | 3.0 | 0 |
| Lipid lowering medication (%) | 1.2 | 0.6 | 1.3 | 1.2 | 1.0 |

Abbreviation: eGFR, estimated glomerular filtration rate
[a] median (IQR)

GEE analysis showed that pravastatin reduced the occurrence of UTI antibiotic prescriptions (RR, 0.43; 95% CI, 0.21–0.88) compared with placebo. Pravastatin further reduced the hazard of a first UTI antibiotic prescription (HR, 0.58; 95% CI, 0.32–1.03 and second UTI antibiotic prescription (HR, 0.25; 95% CI, 0.08–0.77; Table 3.3). Allocation to combination therapy was not associated with the occurence of UTI antibiotic prescriptions (RR, 1.04; 95% CI, 0.57–1.89) or the hazard of a first UTI antibiotic prescription (HR, 1.07; 95% CI, 0.66–1.75). A non-significant reduction in the occurrence of second UTI antibiotic prescriptions was observed in those patients (HR, 0.48; 95% CI, 0.22–1.05).

**Table 3.2.** Drug use during follow-up

| Drug | Placebo* | Pravastatin* | Fosinopril* | Pravastatin & Fosinopril* |
|---|---|---|---|---|
| Total other antibiotics | 84 (50%) | 88 (56%) | 79 (49%) | 94 (56%) |
| Other antibiotics used for UTI treatment (Amoxicillin, Amoxicillin-clavulanate, Quinolones) | 47 (28%) | 44 (28%) | 44 (28%) | 54 (32%) |
| NSAIDs | 86 (51%) | 88 (56%) | 85 (53%) | 98 (58%) |
| Drugs for acid related disorders | 33 (20%) | 33 (21%) | 39 (24%) | 38 (23%) |
| Antifungals for dermatological use | 32 (19%) | 33 (21%) | 36 (23%) | 40 (24%) |

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; UTI, urinary tract infection
* Data presented as number of persons receiving at least one prescription during follow-up

**Table 3.3.** Intention to treat analyses

| Characteristics | Total number of UTIs | Risk of an UTI (RR, 95% CI)[a] | Hazard of a first UTI (HR, 95% CI)[a] | Hazard of a second UTI (HR, 95% CI)[b] |
|---|---|---|---|---|
| Placebo | 66 | Ref. | Ref. | Ref. |
| Pravastatin | 25 | 0.43 (0.21-0.88) | 0.58 (0.32-1.03) | 0.25 (0.08-0.77) |
| Pravastatin + fosinopril | 62 | 1.04 (0.57-1.89) | 1.07 (0.66-1.75) | 0.48 (0.22-1.05) |
| *Pravastatin vs. no pravastatin* | | | | |
| No pravastatin | 130 | Ref. | Ref. | Ref. |
| Pravastatin | 87 | 0.71 (0.44-1.15) | 0.83 (0.57-1.20) | 0.48 (0.25-0.89) |

Abbreviations: UTI, urinary tract infection; RR, relative risk; CI, confidence interval; HR, hazard ratio; Ref., reference category
[a] adjusted for sex
[b] adjusted for urinary albumin excretion

At an α level of 0.20, there was a significant interaction on an additive scale between fosinopril and pravastatin, when evaluating the time between first and second UTI antibiotic prescription (RERI, –4.13; 80% CI, –8.07 to –0.19) and the total occurence of UTI antibiotic prescriptions ($P$=0.10). In contrast, there was no significant interaction between fosinopril and pravastatin for the time to first UTI antibiotic prescription (RERI, –0.59; 80% CI, –1.78–0.60).

#### Pravastatin versus no pravastatin and fosinopril versus no fosinopril

When pravastatin was compared with no pravastatin, a non-significant reduction in the risk of receiving UTI antibiotic prescriptions (RR, 0.71; 95% CI, 0.44–1.15) was found. Pravastatin treatment further resulted in a non-significant reduction in the

hazard of receiving a first UTI antibiotic prescription (HR, 0.83; 95% CI, 0.57–1.20), but a significant reduction in the hazard of a second UTI antibiotic prescription (HR, 0.48; 95% CI, 0.25–0.89; Table 3.3). When compared with no fosinopril, allocation to fosinopril resulted in a non-significant increase in the hazard of receiving a first UTI antibiotic prescription (HR, 1.28; 95% CI, 0.89–1.86).

#### Per-protocol analyses

During an average time at risk of 2.7 years, 12 subjects (8%) allocated to pravastatin received at least one UTI antibiotic prescription. These figures were 18 (11%), 24 (15%) and 24 (14%) for placebo, fosinopril and combination therapy, respectively.

Of the pravastatin-treated subjects that received a first UTI antibiotic prescription, two (17%) also received a second one. For placebo, fosinopril and combination therapy, these numbers were 7 (39%), 10 (42%) and 8 (33%), respectively. In total, 27 (13%) males and 51 (43%) females received a UTI antibiotic prescription.

Per-protocol analysis showed that pravastatin resulted in a non-significant reduction in the frequency of UTI antibiotic prescriptions (RR, 0.54; 95% CI, 0.23–1.28) and the occurrence of first UTI antibiotic prescriptions (HR, 0.83; 95% CI, 0.40–1.73) when compared with placebo (Table 3.4). The occurrence of second UTI antibiotic prescriptions was significantly reduced (HR, 0.19; 95% CI, 0.38–0.95) with pravastatin treatment.

Combination therapy resulted in a non-significant increase in the risk of receiving UTI antibiotic prescriptions (RR, 1.39; 95% CI, 0.64–3.02) and the hazard of a first UTI antibiotic prescription (HR, 1.79; 95% CI, 0.97–3.31). This combination therapy resulted in a non-significant reduction in the occurrence of second UTI antibiotic prescriptions (HR, 0.43; 95% CI, 0.15–1.23).

#### Pravastatin versus no pravastatin and fosinopril versus no fosinopril

When comparing pravastatin with no pravastatin, a non-significant reduction in the frequency of UTI antibiotic prescriptions in the pravastatin treatment group was found (RR, 0.79; 95% CI, 0.46–1.37) (Table 3.4). Pravastatin treatment had no effect on the time to first UTI antibiotic prescription (HR, 1.02; 95% CI, 0.65–1.59) and resulted in a non-significant reduction in the hazard of receiving a second UTI antibiotic prescription (HR, 0.53; 95% CI, 0.46–1.37). If fosinopril was compared with no fosinopril therapy, a significant increase in the hazard of receiving a first UTI antibiotic prescription was found (HR, 1.82; 95% CI, 1.16–2.88).

**Table 3.4.** Per-protocol analyses.

| Characteristics | Total number of UTIs | Risk of an UTI (RR, 95% CI)[a] | Hazard of a first UTI (HR, 95% CI)[a] | Hazard of a second UTI (HR, 95% CI)[b] |
|---|---|---|---|---|
| Placebo | 36 | Ref. | Ref. | Ref. |
| Pravastatin | 15 | 0.54 (0.23-1.28) | 0.83 (0.40-1.73) | 0.19 (0.38-0.95) |
| Pravastatin + fosinopril | 41 | 1.39 (0.64-3.02) | 1.79 (0.97-3.31) | 0.43 (0.15-1.23) |
| *Pravastatin vs. no pravastatin* | | | | |
| No pravastatin | 80 | Ref. | Ref. | Ref. |
| Pravastatin | 56 | 0.79 (0.46-1.37) | 1.02 (0.65-1.59) | 0.53 (0.46-1.37) |

Abbreviations: UTI, urinary tract infection; RR, relative risk; CI, confidence interval; HR, hazard ratio; Ref., reference category

[a] adjusted for sex

[b] adjusted for urinary albumin excretion

## Discussion

We found that allocation to pravastatin without coadministration of fosinopril was associated with a reduction in the occurence of UTI antibiotic prescriptions and the hazard of a second (recurrent) UTI antibiotic prescription, but had no influence on first UTI antibiotic prescriptions. The results of the per-protocol and ITT analyses were not substantially different from each other. The observed difference between second and first UTI antibiotic prescriptions is compatible with our hypothesis that statins exert a higher effect on recurrent UTIs than on first UTIs.

Per-protocol analysis further showed an increased hazard of receiving a first UTI antibiotic prescription in patients using fosinopril, indicating that fosinopril increases the risk of contracting UTIs.

Together with the finding that exfoliated intracellular bacterial communities and filamentous bacteria are present in the urine samples of women with acute cystitis [31], our results indicate that QIR formation also occurs in humans. The finding that pravastatin can reduce the occurrence of (recurrent) UTI antibiotic prescriptions is further supported by the correlation found between elevated cholesterol levels and an increased incidence of UTIs in children [32].

Previously, a population-based non-randomized study observed a small increased risk of contracting UTIs associated with statin use (HR, 1.05; 99% CI, 1.00–1.11) [19]. By exploring the effect on time to first events, most of the UTIs in that study were likely first UTIs, on which the effect of statin therapy in our study was limited. This limited effect on first UTIs might also explain why no significant effect is found on the

occurence of UTI antibiotic prescriptions in our per-protocol analysis. After all, more than half of the total number of UTI antibiotic prescriptions was a first UTI antibiotic prescription.

Another non-randomized study observed a smaller decrease in the number of UTIs associated with statin use (OR, 0.91; 95% CI, 0.85–0.98) [18] than we estimated using either the per-protocol (RR, 0.54; 95% CI, 0.23–1.28) or ITT analyses (RR, 0.43; 95% CI, 0.21–0.88). This difference in the magnitude of the effect estimate might be caused by unmeasured confounding due to the non-randomized character of that study and the lack of information on potentially important risk factors for UTIs [18].

An alternative explanation of our findings could be that UTIs might have been treated with other antibiotics more frequently in the pravastatin group. Therefore, we evaluated the use of amoxicillin, amoxicillin/clavulanate and quinolones as, together with our proxy, these antibiotics cover >98% of all UTIs that are treated with antibiotics. The similar use of these other antibiotics is not in accordance with this alternative explanation.

Another alternative hypothesis could be that by reducing membrane-associated Rac1, patients on pravastatin have a much higher bacterial load prior to active disease and are thus be more likely to have systemic disease instead of UTIs. However, the number of persons that received other antibiotics was similar between the placebo and pravastatin groups, indicating that patients allocated to pravastatin did not receive more alternative antibiotics. We did not assess whether patients on pravastatin were more frequently hospitalized for bacteraemia or sepsis, as such hospitalizations are likely very uncommon in our small and relatively healthy study population [33, 34].

The similar use of other commonly used drugs among the different treatment groups indicates that the pravastatin-treated patients were also not less likely to receive drug prescriptions in general.

An important strength of our study is the analysis of data from a randomized placebo-controlled trial, thereby limiting potential unmeasured confounding. Although we excluded some patients, primarily because of unsuccessful linkage of pharmacy data to study participants, measured potential confounders were equally distributed between treatment groups for the primary analysis, except for a small difference in gender. This indicates that unmeasured potential confounders were also likely equally distributed between the treatment groups.

Our study has potential limitations. First, we used specific antibiotic prescriptions obtained from a pharmacy description database as a proxy for UTIs. The sensitivity

and specificity are based on studies using data from the Second Dutch National Survey of General Practice (DNSGP-2) [24, 25]. The patient population of DNSGP-2 partly overlaps with that of IADB [35, 36]. Moreover, because Akkerman *et al.* [24] and Ong *et al.* [25] both estimated the sensitivity and specificity of our proxy using data from the same time period as PREVEND IT took place, both high test characteristics are likely also applicable to our study.

In the placebo group, 34 subjects received a first UTI antibiotic prescription, while based on age- and gender-specific figures from the general Dutch population (Statistics Netherlands), 22 first UTIs would be expected. However, persons with microalbuminuria are at increased risk of developing *de novo* renal function impairment [37]. Because an impaired renal function might increase the risk for UTIs [38-40], the study population might have had an increased risk of contracting a UTI compared with the general population.

Although misclassification of the outcome may have occurred, we expect this to be random, because we used the same objective outcome (i.e. antibiotic prescriptions) for the different treatment groups. Random misclassification of the outcome across treatment groups biases the effect towards a null finding (no effect). Therefore, using a proxy has likely not resulted in an overestimation of the effect of pravastatin.

Second, although we excluded patients ($n$=16) who had a UTI in the previous 6 months based on antibiotic prescriptions, it is still possible that patients with asymptomatic UTIs during these months were included. If these patients were unevenly distributed between treatment groups, this could have resulted in different baseline risks for the different treatment groups.

Third, although a decrease in the urine output has been shown in previous studies [28], no studies are available that showed that ACEIs also increase the occurrence of UTIs. Because bacterial clearance from the urinary tract is partially dependent on urine output [41], ACEIs might increase the frequency of UTIs.

Because patients might stop taking fosinopril after experiencing an adverse effect and the increased risk for UTIs is likely quickly reversible by non-adherence [42, 43], the estimated increased hazard of receiving a first UTI antibiotic prescription in patients on fosinopril treatment was more clearly present in per-protocol analysis than in ITT analysis.

This increased risk might be partly due to the concomitant use of non-steroidal anti-inflammatory drugs (NSAIDs) [28]. Of patients treated with fosinopril, 21% received

three or more NSAID prescriptions during the time at risk. Moreover, many people buy NSAIDs over the counter. The interaction between fosinopril and pravastatin, when evaluating the time between first and second events, further supports the hypothesis that fosinopril can increase the risk of contracting UTIs, due to reduced urine output. This secondary analysis should be interpreted with caution given the limited power to detect an interaction. However, given the possible biological mechanism behind a reduced risk, we do feel that the separated analyses for pravastatin and combination therapy best represent the possible true effect of pravastatin for outcomes including recurrent events.

Fourth, this study was not designed and powered for the current *post hoc* analysis. Therefore, statistical significance could not be reached for some of the secondary analyses. Larger studies with preferably hard endpoints instead of proxies are needed.

Fifth, reasons for censoring were not known and could therefore not be adjusted for using for example inverse probability of censoring weighting.Finally, our study sample was predominantly healthy and male, and we excluded patients with antibiotic prescriptions for UTIs in the previous 6 months, which could limit the generalizability of our results. It is not likely that such individuals who have no recurrent UTIs and no other indication for treatment with statins will be treated with a statin to prevent UTIs. Therefore, future research should preferably focus on individuals with recurrent UTIs and/or a high risk for recurrent UTIs.

In summary, this *post hoc* analysis suggests that pravastatin can reduce the risk of recurrent UTIs, possibly by inhibiting bacterial invasion. Larger studies with persons that have a high risk for recurrent UTIs are needed to evaluate whether our findings are likely causal.

## References


1. van Pinxteren B, van Vliet SM, Wiersma TJ, et al. Summary of the practice guideline 'urinary-tract infections' (second revision) from the Dutch college of general practitioners. Ned Tijdschr Geneeskd. 2006;150:718–22.
2. Hunstad DA, Justice SS. Intracellular lifestyles and immune evasion strategies of uropathogenic *Escherichia coli*. Annu Rev Microbiol. 2010;64:203–21.
3. Lipsky BA. Urinary tract infections in men: epidemiology, pathophysiology, diagnosis, and treatment. Ann Intern Med. 1989;110:138–50.
4. Mulvey MA, Schilling JD, Hultgren SJ. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. Infect Immun. 2001;69:4572–9.
5. Schilling JD, Mulvey MA, Vincent CD, et al. Bacterial invasion augments epithelial cytokine responses to *Escherichia coli* through a lipopolysaccharide-dependent mechanism. J Immunol. 2001;166:1148–55.
6. Duncan MJ, Li G, Shin JS, et al. Bacterial penetration of bladder epithelium through lipid rafts. J Biol Chem. 2004;279:18944–51.
7. Martinez JJ, Hultgren SJ. Requirement of rho-family GTPases in the invasion of type 1-piliated uropathogenic *Escherichia coli*. Cell Microbiol. 2002;4:19–28.
8. Song J, Bishop BL, Li G, et al. TLR4-initiated and cAMP-mediated abrogation of bacterial invasion of the bladder. Cell Host Microbe. 2007;1:287–98.
9. Burnham CA, Shokoples SE, Tyrrell GJ. Rac1, RhoA, and Cdc42 participate in HeLa cell invasion by group B *Streptococcus*. FEMS Microbiol Lett. 2007;272:8–14.
10. Cordle A, Koenigsknecht-Talboo J, Wilkinson B, et al. Mechanisms of statin-mediated inhibition of small G-protein function. J Biol Chem. 2005;280:34202–9.
11. Rashid M, Tawara S, Fukumoto Y, et al. Importance of Rac1 signaling pathway inhibition in the pleiotropic effects of HMG-CoA reductase inhibitors. Circ J. 2009;73:361–70.
12. Dechend R, Gieffers J, Dietz R, et al. Hydroxymethylglutaryl coenzyme A reductase inhibition reduces *Chlamydia pneumoniae*-induced cell interaction and activation. Circulation. 2003;108:261–5.
13. Antoniades C, Bakogiannis C, Tousoulis D, et al. Preoperative atorvastatin treatment in CABG patients rapidly improves vein graft redox state by inhibition of Rac1 and NADPH-oxidase activity. Circulation. 2010;122:S66–73.
14. Maack C, Kartes T, Kilter H, et al. Oxygen free radical release in human failing myocardium is associated with increased activity of rac1-GTPase and represents a target for statin treatment. Circulation. 2003;108:1567–74.
15. Zaas DW, Duncan M, Rae Wright J, et al. The role of lipid rafts in the pathogenesis of bacterial infections. Biochim Biophys Acta. 2005;1746:305–13.
16. Horn MP, Knecht SM, Rushing FL, et al. Simvastatin inhibits *Staphylococcus aureus* host cell invasion through modulation of isoprenoid intermediates. J Pharmacol Exp Ther. 2008;326:135–43.
17. Rosch JW, Boyd AR, Hinojosa E, et al. Statins protect against fulminant pneumococcal infection and cytolysin toxicity in a mouse model of sickle cell disease. J Clin Invest. 2010;120:627–35.
18. Fleming DM, Verlander NQ, Elliot AJ, et al. An assessment of the effect of statin use on the incidence of acute respiratory infections in England during winters 1998–1999 to 2005–2006. Epidemiol Infect. 2010;138:1281–8.
19. Smeeth L, Douglas I, Hall AJ, et al. Effect of statins on a wide range of health outcomes: a cohort study validated by comparison with randomized trials. Br J Clin Pharmacol. 2009;67:99–109.

20. Diercks GF, Janssen WM, van Boven AJ, et al. Rationale, design, and baseline characteristics of a trial of prevention of cardiovascular and renal disease with fosinopril and pravastatin in nonhypertensive, nonhypercholesterolemic subjects with microalbuminuria (the Prevention of REnal and Vascular ENdstage Disease Intervention Trial [PREVEND IT]) Am J Cardiol. 2000;86:635–8.
21. Asselbergs FW, Diercks GF, Hillege HL, et al. Effects of fosinopril and pravastatin on cardiovascular events in subjects with microalbuminuria. Circulation. 2004;110:2809–16.
22. *Pharmacy-dispensing Database of IADB.nl*. http://www.iadb.nl. (2 October 2012, date last accessed)
23. Tobi H, van den Berg P, de Jong-van den Berg L. The InterAction database: synergy of science and practice in pharmacy. Lect Notes Comput Sci. 2000;1933:93–108.
24. Akkerman AE, Kuyvenhoven MM, Verheij TJ, et al. Antibiotics in Dutch general practice: nationwide electronic GP database and national reimbursement rates. Pharmacoepidemiol Drug Saf. 2008;17:378–83.
25. Ong DS, Kuyvenhoven MM, van Dijk L, et al. Antibiotics for respiratory, ear and urinary tract disorders and consistency among GPs. J Antimicrob Chemother. 2008;62:587–92.
26. Russo TA, Stapleton A, Wenderoth S, et al. Chromosomal restriction fragment length polymorphism analysis of *Escherichia coli* strains causing recurrent urinary tract infections in young women. J Infect Dis. 1995;172:440–5.
27. Foxman B. Recurring urinary tract infection: incidence and risk factors. Am J Public Health. 1990;80:331–3.
28. Juhlin T, Bjorkman S, Hoglund P. Cyclooxygenase inhibition causes marked impairment of renal function in elderly subjects treated with diuretics and ACE-inhibitors. Eur J Heart Fail. 2005;7:1049–56.
29. Marshall SW. Power for tests of interaction: effect of raising the type I error rate. Epidemiol Perspect Innov. 2007;4:4.
30. Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. Int J Epidemiol 2012;41:514-520.
31. Rosen DA, Hooton TM, Stamm WE, et al. Detection of intracellular bacterial communities in human urinary tract infection. PLoS Med. 2007;4:e329.
32. Gulati S, Kher V, Arora P, et al. Urinary tract infection in nephrotic syndrome. Pediatr Infect Dis J. 1996;15:237–40.
33. Pedersen G, Schonheyder HC, Sorensen HT. Source of infection and other factors associated with case fatality in community-acquired bacteremia—a Danish population-based cohort study from 1992 to 1997. Clin Microbiol Infect. 2003;9:793–802.
34. Elhanan G, Sarhat M, Raz R. Empiric antibiotic treatment and the misuse of culture results and antibiotic sensitivities in patients with community-acquired bacteraemia due to urinary tract infection. J Infect. 1997;35:283–8.
35. *Second Dutch National Survey of General Practice*. www.nivel.nl/pdf/ns2_r0_h14.pdf. (2 October 2012, date last accessed)
36. Pechlivanoglou P, van der Veen WJ, Bos JH, et al. Analyzing generic and branded substitution patterns in the Netherlands using prescription data. BMC Health Serv Res. 2011;11:89.
37. Verhave JC, Gansevoort RT, Hillege HL, et al. An elevated urinary albumin excretion predicts de novo development of renal function impairment in the general population. Kidney Int Suppl. 2004;92:S18–21.
38. Funfstuck R, Ott U, Naber KG. The interaction of urinary tract infection and renal insufficiency. Int J Antimicrob Agents. 2006;28(Suppl 1):S72–7.
39. Gilbert DN. Urinary tract infections in patients with chronic renal insufficiency. Clin J Am Soc Nephrol. 2006;1:327–31.

40. Yamasaki T, Naganuma T, Iguchi T, et al. Association between chronic kidney disease and small residual urine volumes in patients with benign prostatic hyperplasia. Nephrology (Carlton) 2011;16:335–9.
41. Beetz R. Mild dehydration: a risk factor of urinary tract infection? Eur J Clin Nutr. 2003;57(Suppl 2):S52–8.
42. Navis G, Faber HJ, de Zeeuw D, et al. ACE inhibitors and the kidney. A risk–benefit assessment. Drug Saf. 1996;15:200–11.
43. Whelton A, Schulman G, Wallemark C, et al. Effects of celecoxib and naproxen on renal function in the elderly. Arch Intern Med. 2000;160:1465–70.

CHAPTER 4

# Angiotensin-converting enzyme inhibitor treatment and the development of urinary tract infections: a prescription sequence symmetry analysis

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## Abstract

### Background

Angiotensin-converting enzyme inhibitors (ACEi) can reduce urine output, especially when treatment is started. Since bacterial clearance from the urinary tract is dependent on urine output, it was hypothesized that ACEi may also increase the risk of urinary tract infections (UTIs). Our objective was to assess the risk of UTIs associated with ACEi therapy initiation in the general population.

### Methods

A prescription sequence symmetry analysis was performed with the IADB.nl pharmacy prescription database. We selected all patients from the IADB who were incident users of both ACEi and nitrofurantoin (a proxy for UTIs). A relatively short maximum time-span of 4 weeks between both prescriptions was used to limit time-varying confounding. The sequence ratio was calculated by dividing the number of individuals starting ACEi first and nitrofurantoin second by the number of individuals starting nitrofurantoin first and ACEi second. We adjusted for trends in prescribing and estimated 95% confidence intervals using exact confidence intervals for binomial distributions. To evaluate whether the effect is specific to ACEi and to assess whether the possible mechanism behind an increased risk of UTIs is related to the renin-angiotensin-aldosterone system, we also estimated the risk for β-adrenoceptor antagonists (β-blockers).

### Results

In total, 22,959 incident users of ACEi therapy were eligible for analyses. Of these, 161 patients started ACEi therapy within 4 weeks prior to or after nitrofurantoin therapy initiation. A total of 101 (63%) started ACEi therapy first followed by nitrofurantoin treatment, while 60 (37%) patients started nitrofurantoin treatment first, which corresponds to an adjusted sequence ratio (ASR) of 1.68 (95% CI 1.21-2.36). No association was found between β-blockers and UTI treatment (ASR 1.01, 95%CI 0.74-1.38).

### Conclusions

A significant excess of patients received UTI medication prescriptions following the first month after ACEi initiation. This prescription sequence asymmetry suggests that ACEi initiation increases the risk of developing UTIs.

## Introduction

Angiotensin-converting enzyme inhibitors (ACEi) are one of the most frequently prescribed classes of antihypertensive drugs. ACEi are prescribed for the management of various cardiovascular and renal diseases, including diabetic and non-diabetic nephropathy. Although ACEi therapy has renoprotective effects in chronic kidney disease [1, 2], treatment with ACEi can occasionally lead to renal failure [3-5]. A possible mechanism behind this adverse event is that ACEi have a relatively greater dilating effect on the efferent than the afferent arterioles by reducing angiotensin-II levels. This hemodynamic effect can result in a decreased glomerular filtration rate (GFR) and urine output [6, 7]. An adverse hemodynamic effect is particularly relevant in patients with a reduced circulating volume, since the GFR is then more dependent on angiotensin-II levels [6]. However, treatment with ACEi can also result in decreased urine output in healthy elderly patients without known risk factors for adverse renal effects with ACEi therapy [7]. Such an adverse effect usually develops immediately after ACEi treatment has been started, but it has also occasionally been observed later in the treatment course, after months or even years [6]. Although this adverse effect is almost always reversible [8, 9], it is not uncommon that ACEi therapy leads to a reduced GFR and urine output without such severe consequences as oliguria, anuria, or acute renal failure [6, 7]. We hypothesized that, since bacterial clearance from the urinary tract is dependent on urine output [10], ACEi may also be associated with an increased risk of the development of urinary tract infections (UTIs), especially early in the treatment course. In a recent post hoc analysis of a randomized controlled trial, we indeed observed an increased risk of developing UTIs after ACEi initiation in adults with micro-albuminuria [11]. We now present the results from a population-based prescription sequence symmetry analysis [12], to assess the risk of developing UTIs after ACEi therapy initiation.

## Methods

### Setting

This prescription sequence symmetry study was performed with the University of Groningen IADB.nl pharmacy prescription database, which contains prescription data from 1994 through 2011 from approximately 55 community pharmacies, and covers an estimated population of 500,000 patients [13]. Registration in the database is irrespective of healthcare insurance, age, and gender. Prescription rates among the database population have been found to be representative for The Netherlands as a whole, and the database is widely used in research [14]. Each prescription record contains information on the date of dispensing, the quantity dispensed, the dose regimen, the number of days the prescription is valid, the prescribing physician, and the Anatomical

Therapeutic Chemical (ATC) code. Each patient has a unique anonymous identifier; date of birth and gender are known. Due to a high patient pharmacy commitment in The Netherlands and sophisticated pharmacy software, the medication records for each patient are virtually complete, except for over-the-counter drugs and medication dispensed during hospitalization [15].

#### Study population and outcome definition

The study population comprised all patients from the IADB who were incident users of both ACEi (ATC code C09A or C09B) and antibiotic UTI therapy with nitrofurantoin

(J01XE). Incidence was defined as not having been prescribed the drug in question for at least 12 months, while being captured in the database for at least 12 months prior to the first prescription.

Because patient diagnoses were not available from the IADB, UTIs were defined on the basis of the first start of a nitrofurantoin course. In The Netherlands, guidelines regarding the primary care treatment of UTIs changed in 2005, due to increased bacterial resistance to trimethoprim [16]. Nitrofurantoin became the first-choice drug for uncomplicated UTIs. Indeed, the IADB recorded an increase in nitrofurantoin prescriptions and a decrease in trimethoprim after 2005 [13]. Nitrofurantoin is almost exclusively used to treat uncomplicated UTIs; trimethoprim is more frequently used for other indications such as respiratory tract infections [17]. We defined a UTI as a nitrofurantoin prescription and used data between January 2006 and December 2011 in order to obtain a proxy with both high specificity and high sensitivity.

In 2001, when nitrofurantoin was prescribed, 96 % of the cases were for a UTI [17]. At that time, nitrofurantoin was used in approximately 33 % of cystitis cases [18].

However, due to the guideline change in 2005, the specificity, and especially the sensitivity, of nitrofurantoin is higher in our study. The use of nitrofurantoin in our database was, on average, approximately two times higher during 2006–2011 than in 2001, while the age and gender distribution remained similar. Simultaneously, the use of sulphonamides and trimethoprim in our database halved between these periods. Since the age-specific annual incidence of UTIs was similar in both periods in The Netherlands (Statistics Netherlands [http://www.cbs.nl]), we estimated the sensitivity of our proxy at 66 %.

**Statistical analyses**

Incident use of ACEi or nitrofurantoin therapy was defined as absence of a prescription of the particular medical drug in the 12 months prior to the dispensing date. A prescription sequence symmetry analysis was performed to evaluate whether nitrofurantoin was prescribed more often following the start of ACEi therapy than the reverse in the period between first dispensing dates [12]. Such an analysis is a subtype of a case-crossover study, in which the sequence order is largely independent of time-invariant patient confounders as, similar to traditional case-crossover studies, patients serve as their own control group. However, this design is still vulnerable to confounding by indication, especially when using longer time intervals. When using short maximum time-spans between both prescriptions, confounding by indication due to diseases that slowly progress is largely eliminated, although confounding by sudden-onset diseases or sudden increases in disease severity might still be present. To reduce time-varying confounding, we examined a relatively short timespan of 4 weeks in the primary analysis. Moreover, a reduced urine output and GFR after ACEi initiation have been reported in relatively short-term studies ranging from 7 days to 8 weeks [7, 19].

The sequence ratio (SR) was calculated by dividing the number of individuals starting ACEi first and nitrofurantoin second by the number of individuals starting nitrofurantoin treatment first and ACEi second. If no association exists between the drugs, patients should have an equal probability of receiving the drugs in either order. If there is an association, such that ACEi increase the risk of a UTI, ACEi should be prescribed more often prior to the start of nitrofurantoin, and the SR would be above one.

Case-crossover studies and prescription sequence symmetry analyses are vulnerable to time trends. We therefore adjusted the crude SR for time trends in use of the study drugs. The adjusted sequence ratio (ASR) was obtained by dividing the crude SR by a null ratio, i.e. the crude SR obtained assuming no association between both drugs based on overall prescribing of both drugs in the total IADB population [12, 20]. For example, if nitrofurantoin were to be prescribed with increasing incidence while the prescribing of ACEi was stable over time, there would be a non-specific excess of nitrofurantoin prescribed last. Assuming no associations between both drugs, one would obtain a null ratio above one based on overall prescribing of both drugs in the total population. The ASR would then consequently be lower than the crude SR. The ASR is an estimate of the incidence ratio of nitrofurantoin prescribing in ACEi-exposed versus non-exposed person time [12, 20]. For the primary analysis, the ASR was calculated for ACEi and UTI treatment within 4 weeks. We estimated 95 % confidence intervals using exact confidence intervals for binomial distributions [12, 21]. Statistical analyses were performed using STATA 12 software.

**Secondary analysis**

It is possible that patients started ACEi treatment because they recently experienced a stroke. As the rates of infections are increased after a stroke [22], we conducted a secondary analysis to see whether excluding patients who recently experienced a stroke would change the ASR. Most patients who experience a stroke are subsequently treated with a platelet aggregation inhibitor or a vitamin K antagonist. Therefore, we identified patients with stroke as those patients starting treatment with one of these drugs within 1 month before or on the same day as ACEi treatment initiation. Additionally, we evaluated whether a possible association would be influenced by time-varying prescribing of diuretics. We also evaluated whether our findings would change if we excluded patients receiving second- or third-choice (during the study period of 2006–2011) UTI antibiotic treatment (trimethoprim and fosfomycin; ATC codes J01EE, J01EA, and J01XX01) in the year before their first nitrofurantoin prescription. As adults with diabetes are at increased risk of UTIs [23], subgroup analyses with patients with and without diabetes were performed. Patients with diabetes were defined as individuals having received at least one prescription with glucose-lowering drugs (ATC codes A10A or A10B) in the year prior to and including the date of ACEi therapy initiation. For ACEi therapy, a Chi square test was used to assess whether there was a difference in the ASR among patients with and without diabetes. To evaluate whether the development of diabetes occurring concurrently with ACEi therapy initiation might explain the association, we assessed whether the SR was lower in more chronic patients with at least three prescriptions of oral glucose-lowering drugs and/or insulin during the year before starting ACEi treatment.

To evaluate whether the effect is specific to ACEi and to assess whether the possible mechanism behind an increased risk of UTI is related to the renin–angiotensin aldosterone system, we estimated the ASR for β-adrenoceptor antagonists (β-blockers). These anti-hypertensive drugs are also known to affect the renin–angiotensin–aldosterone system. The same inclusion criteria were used as for the analysis with ACEi. We did not estimate the ASR for angiotensin-receptor blockers, as most patients who developed a UTI within 28 days of starting angiotensin-receptor blockers were first treated with ACEi and/or experienced more UTIs in the year prior to the treatment initiation. Calcium channel blockers were not used as a control because they might decrease the antibacterial function of uroepithelial cells [24].

Furthermore, we calculated the SRs for receiving UTI treatment within different time-spans since ACEi or b-blocker therapy initiation, ranging from 1 to 8 weeks. For this analysis, each week was treated separately to show the epidemic curve. To reduce random fluctuation, we used a 3-week moving average for calculation of the SRs.

In line with the literature, we hypothesized that the strongest effect, if present, should be within the smaller time spans allowing for some time to develop a UTI (2–4 weeks). Additionally, we plotted the number of individuals with nitrofurantoin prescriptions within 6 months of ACEi therapy initiation to visually explore whether an increase in nitrofurantoin prescriptions is seen after starting ACEi treatment.

## Results

In total, 27,101 incident users of ACEi therapy were identified between January 2006 and December 2011. After excluding all patients who were not present in the database 12 months prior to their nitrofurantoin prescription, who received a first nitrofurantoin prescription more than 1 year prior to ACEi therapy initiation, or who received a nitrofurantoin course prior to January 2006, a total of 22,959 patients were eligible for analysis.

The mean age of these patients at the start of ACEi therapy was 61.8 (SD 13.9), 42.7% were female, 16.5% had recorded use of glucose-lowering drugs and/or insulin, 39.6% recorded use of diuretics and 22.8% recorded use of nonsteroidal anti-inflammatory drugs. Of these incident ACEi users, 582 patients received their first nitrofurantoin prescription in the year prior to starting ACEi therapy and 681 patients started their first nitrofurantoin course in the year after initiating ACEi therapy. Of these, 161 patients started ACEi therapy within 4 weeks prior to or after nitrofurantoin therapy initiation. The mean age of these patients at ACEi initiation was 69.3 years (SD 13.4); 78.9% were female, 26.1% had recorded use of glucose-lowering drugs and/or insulin, 54.0% recorded use of diuretics and 24.2 % recorded use of nonsteroidal anti-inflammatory drugs.

Of all 161 study patients, 101 (63%) started ACEi therapy first followed by nitrofurantoin treatment, while 60 (37%) patients started nitrofurantoin treatment first with a corresponding statistically significant ASR of 1.68 (95% CI 1.21–2.36) (Table 4.1). For all analyses, adjustment for trends in prescribing did not substantially change the SR, as the null ratios ranged from 0.999 to 1.002. This indicates that the prescribing of ACEi and b-blockers did not increase or decrease more than nitrofurantoin prescribing over time within the short time-spans used.

Excluding patients receiving other antibiotic treatment indicated for UTIs before receiving their nitrofurantoin prescription did not substantially change the ASR (1.56,

**Table 4.1.** Symmetry analysis with selected antihypertensive drugs. Analyses are performed with all persons starting angiotensin-converting enzyme inhibitor or β-blocker therapy within four weeks of nitrofurantoin therapy initiation.

| Drug | Nitrofurantoin prescribed second/first (n) | Sequence Ratio (95% CI)[a] |
|---|---|---|
| ACEi | 101/60 | 1.68 (1.21-2.36) |
| β-blocker | 87/86 | 1.01 (0.74-1.38) |

[a] Adjusted for trends in prescribing.
ACEi = angiotensin-converting enzyme inhibitors.

95 % CI 1.11–2.20). Moreover, excluding patients who may have started with ACEi treatment because they recently experienced a stroke resulted in a slight increase of the ASR (1.86, 95% CI 1.28–2.75). Additionally, when patients starting treatment with diuretics within 1 month of ACEi treatment were excluded, the ASR (1.59, 95 % CI 1.05–2.44) was similar to our primary analysis. No association was found between b-blocker therapy initiation and UTI treatment (Table 4.1). Subgroup analysis for diabetes patients on anti-diabetic therapy (n = 42) showed a higher ASR ($p<0.05$) for diabetes patients than for non-diabetes patients (Table 4.2).

**Table 4.2.** Symmetry analysis with ACEi among patients with and without diabetes. Analyses are performed with all patients starting angiotensin-converting enzyme inhibitor therapy within four weeks of nitrofurantoin therapy initiation.

| Patients | Nitrofurantoin prescribed second/first (n) | Sequence Ratio (95% CI)[a] |
|---|---|---|
| Diabetes | 32/10 | 3.20 (1.53-7.29) |
| No diabetes | 69/50 | 1.38 (0.94-2.03) |

[a] Adjusted for trends in prescribing.
ACEi = angiotensin-converting enzyme inhibitors.

The ASR was not lower when restricting the analysis to more chronic patients with diabetes instead of including all patients with diabetes (ASR 4.0 vs. ASR 3.2). A histogram of the prescription asymmetry of first nitrofurantoin prescriptions within 6 months of ACEi therapy initiation is shown in Figure 4.1. Nitrofurantoin prescriptions increased sharply within 1 month after ACEi therapy started. From this figure, it appears that nitrofurantoin prescription numbers were not random prior to the initiation of ACEi treatment, but instead show a steady increase.

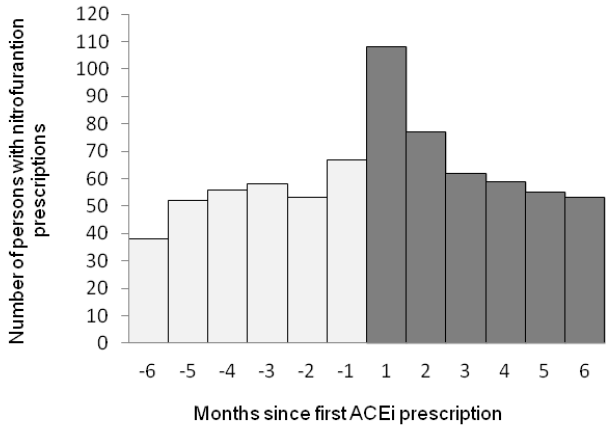


**Figure 4.1.** Prescription asymmetry of first nitrofurantoin prescriptions within 6 months of angiotensin-converting enzyme inhibitor therapy initiation (n=738). ACEi = angiotensin-converting enzyme inhibitors.

**However, when looking at the 4 weeks prior to ACEi treatment, such a pattern is not apparent (Figure 4.2). A steady increase in the number of nitrofurantoin prescriptions was also observed prior to the initiation of b-blockers (Figure 4.3).**

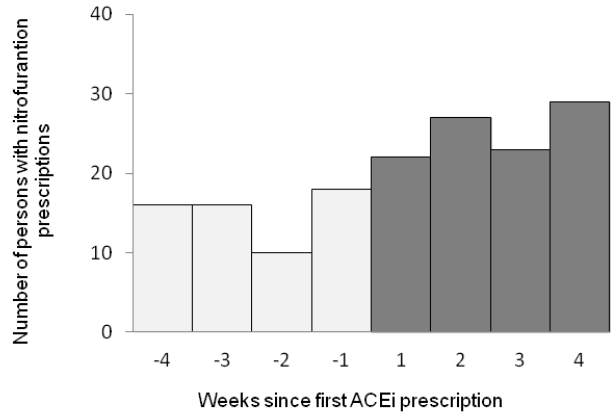


**Figure 4.2.** Prescription asymmetry of first nitrofurantoin prescriptions within 4 weeks of angiotensin-converting enzyme inhibitor therapy initiation. ACEi = angiotensin-converting enzyme inhibitors.

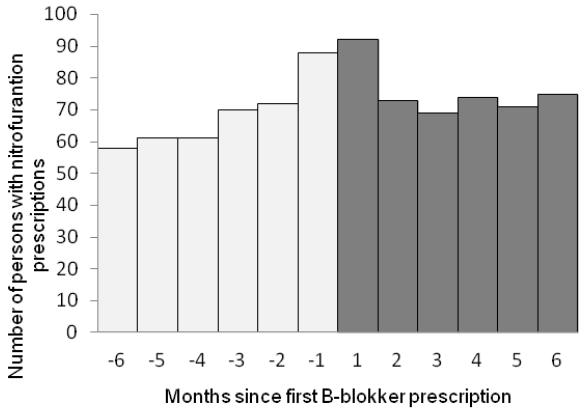


**Figure 4.3.** Prescription asymmetry of first nitrofurantoin prescriptions within 6 months of β-blocker therapy initiation (n=864).

Both findings indicate that confounding by disease severity would be present if time-spans longer than 4 weeks were used to evaluate the associations between ACEi or b-blockers and UTIs. However, this bias is likely largely eliminated by using a short time-span of 4 weeks, as the number of nitrofurantoin prescriptions does not clearly increase during the 4 weeks prior to ACEi initiation. In addition, we did not find an association between b-blockers and UTI treatment, despite a steady increase in the number of nitrofurantoin prescriptions during the months prior to initiation with this antihypertensive drug. Figure 4 shows that the risk of developing UTIs is especially high shortly after ACEi therapy initiation, but is elevated during all evaluated weeks (see also Table 4.3). In contrast, the SR for β-blockers fluctuates around one during the same period.

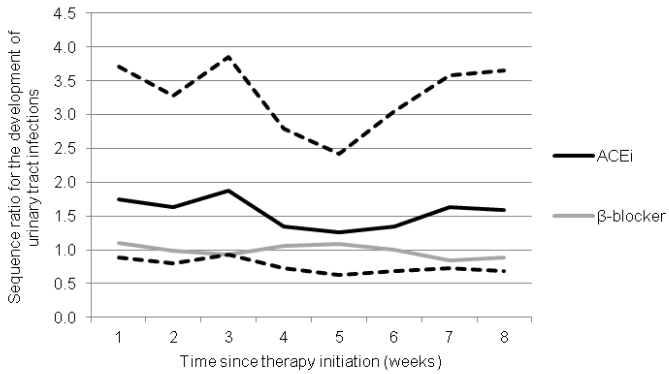


**Figure 4.4.** Sequence ratios for the development of urinary tract infections using different time-spans since angiotensin-converting enzyme inhibitors/β-blocker therapy initiation. The dashed lines

represent the lower and upper confidence intervals of the sequence ratio for angiotensin-converting enzyme inhibitors. ACEi = angiotensin-converting enzyme inhibitors.

**Table 4.3.** Symmetry analysis with ACEi and β-blockers using different time-spans since therapy initiation

| Time since therapy initiation (weeks)[a] | Nitrofurantoin prescribed second/first (n) | Sequence ratio (95% CI) |
|---|---|---|
| *ACEi* | | |
| 1 | 25/14 | 1.75 (0.9-3.7) |
| 2 | 24/15 | 1.64 (0.8-3.3) |
| 3 | 26/14 | 1.88 (0.9-3.8) |
| 4 | 24/18 | 1.34 (0.7-2.8) |
| 5 | 22/18 | 1.26 (0.6-2.4) |
| 6 | 20/15 | 1.34 (0.7-3.1) |
| 7 | 19/12 | 1.63 (0.7-3.6) |
| 8 | 17/11 | 1.59 (0.7-3.7) |
| *β-blockers* | | |
| 1 | 22/20 | 1.10 (0.6-2.3) |
| 2 | 22/22 | 0.99 (0.5-1.9) |
| 3 | 21/22 | 0.93 (0.5-1.8) |
| 4 | 22/20 | 1.07 (0.5-1.9) |
| 5 | 19/18 | 1.09 (0.5-2.1) |
| 6 | 16/16 | 1.00 (0.4-2.0) |
| 7 | 14/17 | 0.84 (0.4-1.8) |
| 8 | 15/17 | 0.88 (0.4-2.1) |

[a] Each week is treated separately to show the epidemic curve. To reduce random fluctuation, a 3-week moving average for calculation of the sequence ratios.
**ACEi** = angiotensin-converting enzyme inhibitors.

## Discussion

The results of this population-based prescription sequence symmetry analysis are compatible with the hypothesis that there is a statistically significant increased risk of starting UTI antibiotic therapy after the initiation of ACEi therapy, notably within the first month. The study confirms a post hoc analysis of a randomized controlled trial in which we observed an increased risk of developing UTIs in patients aged 28 to 75 years

on fosinopril treatment [11]. Given that the mechanism underlying this increased risk is likely reversible by either changing the dose or switching to another antihypertensive drug [6], it is not surprising that the increased risk for UTIs is mainly seen during the first month after initiation of ACEi treatment. Moreover, as with renal failure, reduced urine output resulting in UTIs will likely develop early in the course when ACEi treatment is started. Importantly, in the specific group of patients with chronic kidney disease, longer-term ACEi therapy may even reduce the risk of developing UTIs because of its renoprotective effects in these patients [8].

Secondary analysis showed that the association between ACEi therapy and UTI antibiotic prescriptions was stronger among patients with diabetes than patients without diabetes. This increased risk among patients with diabetes might be because diabetes is strongly associated with renal impairment [25, 26], which increases the risk of ACEi-induced renal failure [25, 26], and possibly the short-term risk of ACEi-associated reduced urine output. However, as our database contains no diagnoses and clinical information for individual patients, we could not establish whether these patients with diabetes indeed had more frequent renal impairment.

Alternatively, this increased risk might be due to confounding by the presence of diabetes itself, since patients who have diabetes are more likely to be prescribed ACEi and are more likely to have UTIs. However, the association is likely not confounded by the presence of diabetes, because an ASR of 4.0 was obtained when only patients with at least three prescriptions of glucose-lowering drugs and/or insulin during the year before starting ACEi treatment were included.

Inherent to observational studies, those who start ACEi treatment may have different risk factors than those who do not initiate this treatment [27], which may confound the association between ACEi therapy and UTI development. For example, as ACEi are considered to be more renoprotective than antihypertensive agents not affecting the renin–angiotensin pathway [28–30], patients who start ACEi therapy are more likely to have risk factors for renal impairment. Different design and analytical techniques exist to control for such confounding bias [31]. Though suited for a limited number of epidemiological questions, a powerful design technique is the case-crossover study, and the prescription sequence symmetry analysis is a variation on this design. By performing a prescription sequence symmetry analysis, we controlled for time invariant (unmeasured) confounding, since patients act as their own controls.

Furthermore, no trends in prescribing that vary within the short time-spans were present in this study.

However, the prescription sequence symmetry analysis is still vulnerable to confounding by disease severity or sudden-onset diseases. The increased risk of UTI after starting with ACEi treatment might be due to a change in disease severity unrelated to the effects of ACEi. Although Figure 4.1 and 4.3 both suggest that confounding by disease severity or disease progression might bias analyses using longer time intervals between both prescriptions, Figure 4.2 and the results of the prescription sequence symmetry analysis with b-blockers both suggest that confounding by disease severity is largely eliminated when using a short maximum time-span of 4 weeks.

An alternative explanation for our findings could be that some of the patients who developed a UTI after starting ACEi treatment started this therapy because they recently experienced a sudden-onset disease. It is possible that patients started ACEi treatment because they recently experienced a stroke, which is often a sudden-onset disease. Because rates of infections are increased after an acute stroke, more patients might get UTIs after than before ACEi therapy initiation [22]. Most patients who experience a stroke are subsequently treated with a platelet aggregation inhibitor or a vitamin K antagonist. When patients starting one of these drugs within 1 month before or on the same day as ACEi treatment initiation were excluded from our primary analysis, the ASR slightly increased (ASR 1.86, 95 % CI 1.28–2.75). This indicates that our results were not affected much by acute strokes among patients starting ACEi treatment. When patients starting treatment with diuretics within 1 month of ACEi treatment were also excluded, the ASR (1.59, 95 % CI 1.05–2.44) was similar to our primary analysis (ASR 1.68, 95 % CI 1.21–2.36), indicating that our results were not confounded by time-varying prescribing of diuretics.

We performed a secondary analysis with b-blockers to evaluate whether the mechanism behind an increased risk could be related to the renin–angiotensin–aldosterone system. The lack of an effect of b-blockers on the development of UTIs indicates that either the effect of b-blockers on renin and/or angiotensin-II is not strong enough to increase the risk of UTIs, or that another unknown mechanism might be involved. Additionally, this finding indicates that there is a real class effect of ACEi, and that potential confounding by hypertension or bias due to an increase in physician contacts after starting antihypertensive treatment were eliminated.

This study has potential limitations. First, we used nitrofurantoin prescriptions obtained from a pharmacy prescription database as a proxy for UTIs. While the specificity of this proxy is very high [17], the sensitivity is estimated to be somewhat lower, at 66 % [13, 18]. Although we may have consequently missed some UTIs, it is unlikely that UTIs are systematically treated differently just before than just after ACEi therapy initiation.

Moreover, excluding patients receiving other antibiotics that are used to treat UTIs did not substantially alter our findings. Second, because we used a prescription sequence symmetry analysis, reverse causality might have influenced the results [32]. Although the sharp increase in the number of patients receiving nitrofurantoin prescriptions following ACEi therapy initiation supports the hypothesis that ACEi treatment initiation increases the risk of developing UTIs, this phenomenon would also have been observed if the probability of receiving ACEi therapy reduces after getting a nitrofurantoin prescription. However, both drugs are not used for the same indication and, to our knowledge, there is no reason why physicians should avoid using ACEi in patients who have recently experienced a UTI. The fact that UTIs are not a well-documented side effect of ACEi therapy further reduces the probability that reverse causality biased the results. A prescription sequence symmetry analysis would be more prone to reverse causality bias with well documented side effects, as physicians may try to avoid prescribing a drug that further increases the risk of a recently experienced disease.

Finally, as with all prescription sequence symmetry analyses, only patients using both drugs within a certain period were included in the analyses. Therefore, generalizability of our results might be limited, as only a small fraction of all incident ACEi users was included in the analyses. Patients included in the primary analysis were slightly older, more frequently female, and had more frequently recorded use of diuretics and glucose-lowering drugs and/or insulin compared with all incident ACEi users from our database. However, this might not be surprising as all these factors are risk factors or indicators of diseases that increase the risk for UTIs [23, 33]. On the other hand, we previously found, in a post hoc analysis of a randomized controlled trial, an increased risk of developing UTIs after fosinopril therapy initiation in a patient population without diabetes [11]. This suggests that, although the magnitude of the risk may vary, different patient populations are at increased risk of developing UTIs after starting ACEi therapy.

In summary, we found a significant excess of patients receiving UTI medication prescriptions following the first month after ACEi initiation. This prescription sequence asymmetry suggests that ACEi therapy initiation, at least during the first month, increases the risk of developing UTIs.

## References


1. Ruggenenti P, Fassi A, Ilieva AP, et al. Preventing microalbuminuria in type 2 diabetes. N Engl J Med 2004 Nov; 351 (19): 1941-51.
2. Ruggenenti P, Perna A, Gherardi G, et al. Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet 1999 Jul; 354 (9176): 359-64.
3. Oster JR, Materson BJ. Renal and electrolyte complications of congestive heart failure and effects of therapy with angiotensin-converting enzyme inhibitors. Arch Intern Med 1992 Apr; 152 (4): 704-10.
4. Dietz R, Nagel F, Osterziel KJ. Angiotensin-converting enzyme inhibitors and renal function in heart failure. Am J Cardiol 1992 Oct; 70 (10): 119C-25C.
5. Suki WN. Renal hemodynamic consequences of angiotensin-converting enzyme inhibition in congestive heart failure. Arch Intern Med 1989 Mar; 149 (3): 669-73.
6. Schoolwerth AC, Sica DA, Ballermann BJ, Wilcox CS. Renal considerations in angiotensin converting enzyme inhibitor therapy: a statement for healthcare professionals from the Council on the Kidney in Cardiovascular Disease and the Council for High Blood Pressure Research of the American Heart Association. Circulation 2001 Oct; 104 (16): 1985-91.
7. Juhlin T, Bjorkman S, Hoglund P. Cyclooxygenase inhibition causes marked impairment of renal function in elderly subjects treated with diuretics and ACE-inhibitors. Eur J Heart Fail 2005 Oct; 7 (6): 1049-56.
8. Navis G, Faber HJ, de Zeeuw D, de Jong PE. ACE inhibitors and the kidney. A risk-benefit assessment. Drug Saf 1996 Sep; 15 (3): 200-11.
9. Whelton A, Schulman G, Wallemark C, et al. Effects of celecoxib and naproxen on renal function in the elderly. Arch Intern Med 2000 May; 160 (10): 1465-70.
10. Beetz R. Mild dehydration: a risk factor of urinary tract infection? Eur J Clin Nutr 2003 Dec; 57 Suppl. 2: S52-8.
11. Pouwels KB, Visser ST, Hak E. Effect of pravastatin and fosinopril on recurrent urinary tract infections. J Antimicrob Chemother. Epub 2012 Oct 30.
12. Hallas J. Evidence of depression provoked by cardiovascular medication: a prescription sequence symmetry analysis. Epidemiology 1996 Sep; 7 (5): 478-84.
13. Pharmacy-dispensing database of IADB.nl [online]. Available from URL: http://www.iadb.nl/ [Accessed 2012 Nov 15].
14. Monster TB, Janssen WM, de Jong PE, de Jong-van den Berg LT. Pharmacy data in epidemiological studies: an easy to obtain and reliable tool. Pharmacoepidemiol Drug Saf 2002 Jul-Aug; 11 (5): 379-84.
15. Leufkens HGM, Urguhart J. Automated pharmacy record linkage in the Netherlands. In: Strom BL, editors. Pharmacoepidemiology. Chichester: John Wiley & Sons, Ltd, 2005: 311-322.
16. van Pinxteren B, van Vliet SM, Wiersma TJ, Goudswaard AN. Summary of the practice guideline 'Urinary-tract infections' (second revision) from the Dutch College of General Practitioners [in Dutch]. Ned Tijdschr Geneeskd 2006 Apr; 150 (13): 718-22.
17. Akkerman AE, Kuyvenhoven MM, Verheij TJ, van Dijk L. Antibiotics in Dutch general practice: nationwide electronic GP database and national reimbursement rates. Pharmacoepidemiol Drug Saf 2008 Apr; 17 (4): 378-83.
18. Ong DS, Kuyvenhoven MM, van Dijk L, Verheij TJ. Antibiotics for respiratory, ear and urinary tract disorders and consistency among GPs. J Antimicrob Chemother 2008 Sep; 62 (3): 587-92.
19. van der Ent M, Remme WJ, de Leeuw PW, Bartels GL. Renal hemodynamic effects in patients with moderate to severe heart failure during chronic treatment with trandolapril. Cardiovasc Drugs Ther 1998 Sep; 12 (4): 395-403.

20. Tsiropoulos I, Andersen M, Hallas J. Adverse events with use of antiepileptic drugs: a prescription and event symmetry analysis. Pharmacoepidemiol Drug Saf 2009 Jun; 18 (6): 483-91.
21. Morris JA, Gardner MJ. Calculating confidence intervals for relative risks (odds ratios) and standardised ratios and rates. Br Med J (Clin Res Ed) 1988 May; 296 (6632): 1313-6.
22. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: a systematic review and meta-analysis. BMC Neurol 2011 Sep; 11: 110.
23. Muller LM, Gorter KJ, Hak E, et al. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 2005 Aug; 41 (3): 281-8.
24. Mannhardt W, Putzer M, Zepp F, Schulte-Wissermann H. Host defense within the urinary tract. II. Signal transducing events activate the uroepithelial defense. Pediatr Nephrol 1996 Oct; 10 (5): 573-7.
25. Ritz E, Rychlik I, Locatelli F, Halimi S. End-stage renal failure in type 2 diabetes: A medical catastrophe of worldwide dimensions. Am J Kidney Dis 1999 Nov; 34 (5): 795-808.
26. Meyers JL, Candrilli SD, Kovacs B. Type 2 diabetes mellitus and renal impairment in a large outpatient electronic medical records database: rates of diagnosis and antihyperglycemic medication dose adjustment. Postgrad Med 2011 May; 123 (3): 133-43.
27. Rossing P, Parving HH, de Zeeuw D. Renoprotection by blocking the RAAS in diabetic nephropathy--fact or fiction? Nephrol Dial Transplant 2006 Sep; 21 (9): 2354-7; discussion 2357-8.
28. De Grauw WJC, Kaasjager HAH, Bilo HJG, et al. Landelijke transmurale afspraak chronische nierschade. Huisarts Wet 2009; 52 (12): 586-97.
29. Farmacotherapeutisch kompas. Tractus circulatorius – antihypertensive [online]. Available from URL: http://www.fk.cvz.nl/default.asp?soort=inleidendetekst&naam=inl%20antihypertensiva/ [Accessed 2012 Nov 25].
30. Rutten GEHM, de Grauw WJC, Nijpels G, et al. NHG-standaard diabetes mellitus type 2 (tweede herziening). Huisarts Wet 2006; 49 (3): 137-52.
31. Schneeweiss S. Developments in post-marketing comparative effectiveness research. Clin Pharmacol Ther 2007 Aug; 82 (2): 143-56.
32. Maclure M, Fireman B, Nelson JC, et al. When should case-only designs be used for safety monitoring of medical products? Pharmacoepidemiol Drug Saf 2012 Jan; 21 Suppl. 1: 50-61.
33. Venmans LM, Gorter KJ, Rutten GE, Schellevis FG, Hoepelman AI, Hak E. A clinical prediction rule for urinary tract infections in patients with type 2 diabetes mellitus in primary care. Epidemiol Infect 2009 Feb; 137 (2): 166-72.

CHAPTER 5

# ACE inhibitors and the risk of urinary tract infections: Comparison of a case-crossover and prescription sequence symmetry design

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## Abstract

### Background

In a post-hoc analysis of a randomized controlled trial (RCT) and a prescription sequence symmetry analysis (PSSA) we observed that angiotensin-converting enzyme inhibitor (ACEi) use was associated with an increased risk of urinary tract infections (UTIs). We evaluated the same association using a case-crossover design.

### Methods

A case-crossover design was performed with the University Groningen prescription database (IADB.nl). The first date of incident use of nitrofurantoin, used as a proxy for UTIs, was defined as the index date. The risk period was defined as 30 days before the index date and the control period as 60-90 days before that date. A person was considered exposed to ACEi if there was at least three days' supply within the time-window. In secondary analysis the definitions were set similar to the previous PSSA. Conditional logistic regression with adjustments for potential time-varying confounders was applied to obtain adjusted odds ratios.

### Results

There were 51,249 patients that received a first nitrofurantoin prescription and met eligibility criteria. Of these, 276 patients were only exposed to ACEi during the risk window and 150 patients only during the control window (crude OR 1.84, 95%CI 1.51-2.25; adjusted OR 1.74, 95%CI 1.42-2.13). When using similar criteria as in the PSSA, the case-crossover estimates were slightly higher (adjusted OR 2.09, 95%CI 1.68-2.61).

### Conclusions

These findings suggest that ACEi use increases the risk of developing first UTIs. Despite the similarities between the case-crossover design and the PSSA, the PSSA led to slightly lower effect estimates than the case-crossover design and the RCT.

## Introduction

Randomized clinical trials (RCTs) are considered to provide evidence of the highest grade on treatment effects whereas observational studies are viewed less valid because they are prone to confounding [1]. However, often it is not feasible or unethical to perform a RCT and observational studies on treatment effects are required to guide health care policy. To prevent or limit confounding in observational research several design methods have been developed. One group of methods that can overcome the challenging task of identifying a control group with similar patient characteristics are case-only studies. Examples include the self-controlled case-series [2], case-crossover studies [3] and sequence symmetry analyses [4, 5]. The advantage of these methods is that patients can serve as their own controls, thereby eliminating confounding by (unmeasured) factors that are stable over time.

Recently, we applied a prescription sequence symmetry analysis to estimate the association between angiotensin-converting enzyme inhibitors (ACEi) use and urinary tract infections (UTIs) (sequence ratio = 1.56, 95% confidence interval (CI) 1.11-2.20)) [6]. That study was conducted to further support findings from a post-hoc analysis of a randomized clinical trial in which fosinopril, an ACEi, was associated with an increased risk of incident UTIs (hazard ratio = 1.82, 95% CI 1.16-2.88) [7]. We hypothesized that the underlying mechanism may be related to an ACEi-induced reduction of the urine output [6, 8, 9]. As bacterial clearance from the urinary tract is dependent on the urine output [10], ACEi initiation may result in an increased risk of first UTIs.

In the prescription sequence symmetry analysis we could not adjust for time-varying confounders and the design has been criticized because of a lower sensitivity to detect adverse drug events than conventional observational designs [11]. Since the prescription sequence symmetry analysis can be regarded as a variation on the case-crossover design [12], it would be informative to compare the results of these two designs evaluating the same association in the same database. However, such a comparison is currently lacking from the existing literature, although a few comparisons with other designs than the case-crossover have been made [11, 13, 14]. We aimed to re-evaluate the association between ACEi initiation and UTIs using the case-crossover design, while adjusting for several potential time-varying confounders. As a secondary objective we used this empirical comparison to discuss some important differences between the sequence symmetry design and the case-crossover design.

## Methods

### Data source, setting and study population

The design of the earlier reported prescription sequence symmetry analysis has been published elsewhere [6]. Briefly, the analyses were performed using data retrieved from the University Groningen IADB.nl database (IADB), a community-based pharmacy prescription database containing detailed prescription data from 1994 through 2011 from approximately 55 community pharmacy, covering an estimated population of 500,000 patients [15]. All patients that were incident users of both ACEi and nitrofurantoin (a proxy for UTIs) between January 2006 and December 2011 were selected in that study. First starters or incident users were defined as patients who did not have a prescription for the study drug over a time period of 12 months or more and presence in the database for at least 12 months prior to the first prescription. The prescription sequence symmetry analysis was performed to determine whether nitrofurantoin was more often initiated after than before ACEi initiation. To reduce the probability of time-varying confounding a relatively short time-span of 4 weeks was used. The sequence ratio was calculated by dividing the number of individuals starting ACEi first and nitrofurantoin second by the number of individuals starting both drugs in the reverse order.

For the present study we used the same database to conduct a case-crossover analysis. In the primary analysis, a case-crossover design was performed using data retrieved from the IADB covering the whole data-capturing period from 1994 to 2011.

A secondary analysis was performed, thereby keeping the definitions and in- and exclusion criteria as similar as possible to the original study [6]. As the guidelines for treating UTIs have changed over the years in the Netherlands, different criteria were used to exclude persons with previous UTIs in both sets (see below).

### Case-crossover design

The case-crossover design was first introduced in 1991 by Maclure [3]. With this design patients (cases) serve as their own control, thereby limiting selection bias and confounding by time-invariant characteristics. A within-person comparison is made by comparing the probability of exposure in the period just before the outcome event (risk period) with the probability of exposure in control period(s). The crude odds ratio can be calculated by dividing the number of individuals being exposed only in the risk period by the number of individuals being exposed only in the control period. However, when time-varying confounders are present, conditional logistic regression can be used to adjust for these confounders. We matched 1 control period per case, thereby avoiding

bias due to non-exchangeability of the distribution of exposures between multiple control intervals [16].

**Primary Analysis**

The date of incident use of nitrofurantoin was defined as the index date. Incident use was defined as absence of a prescription of nitrofurantoin in the 12 months prior to the dispensing date. We excluded persons using other antibiotics indicated for use against UTIs prior to their first nitrofurantoin prescription (amoxicillin, amoxicillin/clavulanate, quinolones, fosfomycin, methenamine, sulfonamides or trimethoprim). We defined the risk period as 30 days before the index date and the control period as 60-90 days before the index day. A binary exposure indicator was created for the risk and control period, such that a person was considered exposed to ACEi if there was at least 3 days' supply within the window, thereby assuming that the exposure started on the day of dispensing. Seven days were added to the days' supply dispensed for every dispensation to allow for modest non-adherence. In addition, binary exposures were created using the same criteria for prescription drugs that could act as potential time-varying confounders. Potential time-varying confounders were selected on prior knowledge and included the following prescription drugs: β-adrenoceptor antagonists (β-blockers), calcium channel blockers, angiotensin-receptor blockers, diuretics, lipid modifying agents, non-steroidal anti-inflammatory drugs and glucose lowering drugs. Statistical analyses were performed using SPSS version 20 (SPSS Inc, Chicago, IL).

**Secondary Analyses**

To be able to make a fair comparison with the published prescription sequence symmetry analysis [6], a secondary analysis was performed, thereby keeping the definitions and in- and exclusion criteria as similar as possible to the original study.[6] The same study period was used (January 2006 – December 2011) and the risk period was defined as the 28 days before the index date and the control period as 56-84 days before the index date. In addition, individuals were considered exposed to ACEi if they had at least 1 days' supply within the window, as done with the prescription sequence symmetry analysis [6]. Patients receiving the second- or third-choice (during the period of 2006-2011) UTI antibiotic treatment (trimethoprim and fosfomycin) in the year before their first nitrofurantoin prescription were excluded from the analysis. In sensitivity analysis we defined the risk period as 28 days before the index date and the control period as 29-56 days before the index date.

## Results

There were 116,974 patients that received a nitrofurantoin prescription between January 1994 and December 2011. After excluding all patients who were not present in the database 12 months prior to their nitrofurantoin prescription or who previously received antibiotics used to treat UTIs, a total of 51,249 cases remained. Of these, 3966 patients were exposed to ACEi in the year before their first nitrofurantoin prescription. The characteristics of these patients during both comparison time windows are shown in Table 5.1. Demographic characteristics were evaluated at the index date.

The case-crossover estimates of the association between ACEi use and UTI medication are shown in Table 5.2. The crude odds ratio was 1.84 (95% CI 1.51-2.25), which slightly decreased after adjustment for time-varying prescribing of diuretics (OR 1.74, 95% CI 1.42-2.13) or for all factors listed in Table 5.1 (OR 1.74 95% CI 1.41-2.15). Although the estimates were calculated using conditional logistic regression, the crude odds ratio could also be obtained by dividing the number of patients that are exposed in the risk window and unexposed in the control window by the number of patients that are unexposed in the risk window and exposed in the control window (276 / 150 = 1.84). When the analysis were restricted to individuals within the same age-category as the previous post-hoc analysis of the randomized controlled trial (28 to 75 years of age), the adjusted odds ratio increased to 1.90 (95% CI 1.44-2.50).

### Secondary analysis

When the same study period and similar criteria were used as done with the previous prescription sequence symmetry analysis [6], an adjusted odds ratio of 2.09 (95% CI 1.68-2.61) was obtained. Using a control window immediately before the risk window did not substantially affect this estimate (aOR 2.10, 95% CI 1.59-2.77).

Restricting this analysis to individuals aged 28-75 years of age, resulted in an adjusted odds ratio of 2.29 (95% CI 1.70-3.06).

**Table 5.1.** Characteristics of patients exposed to ACEi in the year prior their first nitrofurantoin prescription (N=3966).

| | |
|---|---|
| Median age (years); (IQR) | 72 (62-80) |
| Women; N (%) | 3197 (81) |

| | Risk window<br>N (%) | Control window<br>N (%) |
|---|---|---|
| *Exposure to* | | |
| ACEi | 3492 (88) | 3366 (85) |
| β-blockers | 1622 (41) | 1593 (40) |
| Calcium channel blockers | 786 (20) | 774 (20) |
| Angiotensin receptor blockers | 206 (5) | 192 (5) |
| Diuretics | 1755 (44) | 1692 (43) |
| Glucose-lowering medication | 979 (25) | 943 (24) |
| Lipid modifying medication | 1381 (35) | 1366 (34) |
| NSAIDs | 429 (11) | 392 (10) |

Abbreviations: ACEi, angiotensin-converting enzyme inhibitors; NSAIDs, non-steroidal anti-inflammatory drugs

**Table 5.2.** Case-crossover estimates for the association between ACEi and UTIs

| | | Risk window | |
|---|---|---|---|
| | | Exposed | Unexposed |
| Control window | Exposed | 3216 | 150 |
| | Unexposed | 276 | 324 |
| Crude OR (95% CI) | 1.84 (1.51 – 2.25) | | |
| Adjusted OR (95% CI)[a] | 1.74 (1.42 – 2.13) | | |
| Adjusted OR (95%CI)[b] | 1.74 (1.41 – 2.15) | | |

[a]adjusted for time-varying use of diuretics

[b]adjusted for time-varying use of diuretics, β-blockers, calcium channel blockers, angiotensin receptor blockers, glucose-lowering medication, lipid modifying medication and non-steroidal anti-inflammatory drugs.

## Discussion

This population-based case-crossover study showed that recent use of ACEi was associated with an increased risk of starting UTI antibiotic therapy. The results confirm findings from a previous post-hoc analysis of a randomized controlled trial (HR, 1.82; 95% CI, 1.16–2.88) [7] and a prescription sequence symmetry analysis [6].

Secondary analyses showed that, using the same database and in- and exclusion criteria as similar as possible, a case-crossover design resulted in higher risk estimates than a prescription sequence symmetry design (OR 2.09, 95% CI 1.68-2.61 vs. SR 1.56, 95% CI 1.11-2.20). There are several explanations for this observation. The first one is the use of different risk estimates. However, it can be shown that the sequence ratio equals a rate ratio if there is no loss to follow-up and the same time at risk is used [4, 17], which is similar to an odds ratio obtained using a case-crossover analysis with the same short risk-intervals [18].

Another possibility is that the case-crossover analysis adjusted for several potential time-varying confounders, while no adjustment was possible using the prescription sequence symmetry analysis. However, the unadjusted case-crossover estimate was also higher than the effect estimate obtained with the prescription sequence symmetry analysis.

Another explanation may be that the prescription sequence symmetry analysis provides conservative estimates compared to other methods, as found in an empirical comparison with a cohort and nested case-control design [11]. A theoretical comparison with the self-controlled case-series could partly explain why sequence symmetry designs could provide more conservative estimates than other designs. We did not add an empirical comparison with a self-controlled case-series design as urinary tract infections within individuals are not independent of each other, which makes self-controlled case series invalid (although recently a modification was proposed to overcome this problem) [19]. Suppose one would assess the effect of drug A initiation on the occurrence of event B, thereby assuming that the occurrence of event B does not modify the risk of a subsequent event B. A self-controlled case-series would use information about the incidence of event B before and after starting with drug A within patients. Thus, if a patient experiences both an event before and after starting with drug A, both events are used in the analysis. In contrast, the variant of the sequence symmetry analysis we previously applied only takes into account the event before starting with drug A and disregards the event after starting with drug A, thereby leading to a lower effect estimate compared to a self-controlled case-series analysis.

A prescription sequence symmetry analysis can also be performed in a way that takes into account information about events before and after drug initiation within patients [20]. Instead of selecting first time users of both drugs or first time users and first events, the occurrence of both new and recurrent events before and after initiation with the drug of interest is then used to calculate a rate ratio. This rate ratio can be calculated by dividing the number of patients experiencing events only after therapy initiation by the number of patients experiencing events only before therapy initiation. However, as with the self-controlled case-series, this way of analysing the data would in our case result in an overestimation of the risk, as patients that experience an UTI before ACEi initiation would have subsequently a higher risk to experience another UTI after ACEi initiation, while a first UTI after ACEi initiation does not modify the probability of experiencing an UTI before ACEi initiation. To avoid such overestimation, and because recurrent UTIs may be more likely complicated UTIs which are more likely to be treated with other antibiotics than nitrofurantoin, we decided in our previous study [6] to analyse the data in a similar way as Hallas proposed [21].

Alternatively, despite using the same database and similar in- and –exclusion criteria, the difference may be explained by the fact that both methods partly use different patients and patient-time in the analysis. The sequence ratio is partly based on patients with a first UTI prior to ACEi initiation, while the case-crossover design selects only patients using ACEi prior to their first UTI. In our case-crossover analysis we separated the risk and control window by 30 days to overcome potential carry-over effects, although sensitivity analysis suggested that carry-over effects did not play an important role. In our previous prescription sequence symmetry analysis carry-over effects did not play a role since the sequence ratio was purely based on the order of first-time prescriptions.

An important strength of this case-crossover study is that we controlled for time-invariant confounders and largely controlled for confounders that do change minimally or slowly over time, regardless of whether these confounders were measured or not. In addition, we controlled for a large set of measured potential time-varying confounders, thereby reducing the probability that the results are affected by time-varying confounding. Further, the data were obtained from a widely researched pharmacy dispensing database which is representative for the general Dutch population in terms of drug use [15].

By using the same database and similar in- and exclusion criteria we could make an empirical comparison between the case-crossover design and a previously published prescription sequence symmetry analysis [6]. Although a few comparisons between the sequence symmetry design and other more conventional designs do exist [11,13, 14], this is the first study making the intuitive comparison with a case-crossover study.

Another strength of this study is that we evaluated a relatively unknown adverse effect. If we would have made a comparison between the case-crossover design and a prescription sequence symmetry analysis while evaluating a well-known effect, different conclusions could have been reached. A prescription sequence symmetry analysis could overestimate the effect with well-known effects, as physicians may try to avoid prescribing a drug that further increases the risk of a recently experienced event. Therefore, sequence symmetry designs seem to be most useful to detect new adverse events [6, 11, 21] although it has also been used for the evaluation of well-known adverse events [13, 22-25].

This study also has some important limitations. The case-crossover design was originally intended to study brief exposures that have an immediate effect (e.g. the association between heavy physical exertion and acute myocardial infarction) [26]. Although ACEi are in general used more chronically, the fact that the increased risk is mainly expected shortly after treatment initiation and the presence of non-adherence make the application of a case-crossover design using an exposure window of 28-30 days possible. In case all patients exposed to ACEi were receiving a life-long therapy and were perfectly adherent, a case-crossover analysis would not be possible in contrast to a prescription sequence symmetry analysis.

When comparing the results of both non-experimental designs with the post-hoc analysis of the randomized controlled trial, one should take into account that the findings from the randomized controlled trial were obtained during an average follow-up of 2.7 years (HR, 1.82; 95% CI, 1.16–2.88) of patients with microalbuminuria [7]. When the follow-up of the post-hoc analysis was restricted to 1 year, the hazard ratio was slightly higher with HR 2.43 (95% CI 1.11-5.33), although confidence intervals widely overlap (KB Pouwels, unpublished data, 2013). The size of the effect may therefore not be directly comparable with the RCT findings, although the direction of the effect is the same with all designs. For a more thorough investigation of the performance in terms of bias and precision of the case-crossover design and the prescription sequence symmetry analysis simulation studies should be performed.

Furthermore, we used nitrofurantoin prescriptions as a proxy for urinary tract infections. Although nitrofurantoin is almost exclusively used to treat urinary tract infections, this proxy has a somewhat lower sensitivity [6]. Since this misclassification is likely random, this would result in an underestimation of the effect for both designs.

We did not have information about drug use during hospitalizations and over the counter drugs. The majority of urinary tract infections will be treated outside the hospital and both antibiotics and ACEi are not available over the counter in the

Netherlands. However, our results may be affected by immeasurable time bias during hospitalizations. Despite these limitations, we did find results that were similar to that found in a randomized setting [7].

In addition, the case-crossover and prescription sequence symmetry design produce different effect estimates (odds ratio vs sequence ratio) that may only be comparible under certain conditions. A sequence ratio and a risk ratio should approximate each other when the length of follow-up is the same in the cohort as in the prescription sequence symmetry analysis [27]. On its turn an odds ratio approximates a risk ratio if the outcome is rare. Since less than 1% of patients initiating ACEi treatment received a nitrofurantoin prescription within a time-window of 4 weeks, it seems reasonable to consider the outcome as rare in the prescription sequence symmetry analysis, Similarly, first nitrofurantoin prescriptions are expected to be rare whitin 4 weeks of (re-)initiating or stopping ACEi therapy, a situation in which non-collapsibillity of the odds ratio is of less concern.

Finally, our results may not be generalizable to all urinary tract infections. We only considered first urinary tract infections in our analyses. Although ACEi initiation is associated with an increased risk of first UTIs, ACEi treatment may even reduce the risk of recurrent UTIs. As ACEi can inhibit rac1 membrane expression [28, 29], they may reduce bacterial invasion [30-33] and subsequently prevent the establishment of a reservoir of recurrent UTIs [34]. If this effect is stronger than the ACEi-associated reduction in urine output [8, 9], ACEi may decrease the risk of recurrent urinary tract infections, despite increasing the risk of first UTIs. However, awareness of the association between ACEi therapy initiation and the risk of first UTIs may improve adherence, especially if future research does show that ACEi can reduce the risk of recurrent UTIs.

In conclusion, these findings further support the hypothesis that ACEi therapy initiation is associated with an increased risk of developing first UTIs, even after adjustment for all potential time-invariant and several time-varying confounders. Furthermore, despite the similarities between the case-crossover design and the prescription sequence symmetry design and the use of the same database, the prescription sequence symmetry design led to slightly lower effect estimates than the case-crossover design and the analysis in a randomized setting.

## References


1. Groenwold RH, Hak E, Hoes AW. Quantitative assessment of unobserved confounding is mandatory in nonrandomized intervention studies. *J Clin Epidemiol* 2009;62:22-28.
2. Farrington CP. Relative incidence estimation from case series for vaccine safety evaluation. *Biometrics* 1995;51:228-235.
3. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. *Am J Epidemiol* 1991;133:144-153.
4. Hallas J. Evidence of depression provoked by cardiovascular medication: a prescription sequence symmetry analysis. *Epidemiology* 1996;7:478-484.
5. Tsiropoulos I, Andersen M, Hallas J. Adverse events with use of antiepileptic drugs: a prescription and event symmetry analysis. *Pharmacoepidemiol Drug Saf* 2009;18:483-491.
6. Pouwels KB, Visser ST, Bos HJ, Hak E. Angiotensin-Converting Enzyme Inhibitor Treatment and the Development of Urinary Tract Infections: A Prescription Sequence Symmetry Analysis. *Drug Saf* 2013;36:1079-1086.
7. Pouwels KB, Visser ST, Hak E. Effect of pravastatin and fosinopril on recurrent urinary tract infections. *J Antimicrob Chemother* 2013;68:708-714.
8. Schoolwerth AC, Sica DA, Ballermann BJ, Wilcox CS, Council on the Kidney in Cardiovascular Disease and the Council for High Blood Pressure Research of the American Heart Association. Renal considerations in angiotensin converting enzyme inhibitor therapy: a statement for healthcare professionals from the Council on the Kidney in Cardiovascular Disease and the Council for High Blood Pressure Research of the American Heart Association. *Circulation* 2001;104:1985-1991.
9. Juhlin T, Bjorkman S, Hoglund P. Cyclooxygenase inhibition causes marked impairment of renal function in elderly subjects treated with diuretics and ACE-inhibitors. *Eur J Heart Fail* 2005;7:1049-1056.
10. Beetz R. Mild dehydration: a risk factor of urinary tract infection? *Eur J Clin Nutr* 2003;57 Suppl 2:S52-8.
11. Corrao G, Botteri E, Bagnardi V, et al. Generating signals of drug-adverse effects from prescription databases and application to the risk of arrhythmia associated with antibacterials. *Pharmacoepidemiol Drug Saf* 2005;14:31-40.
12. Maclure M, Mittleman MA. Should we use a case-crossover design? *Annu Rev Public Health* 2000;21:193-221.
13. Wahab IA, Pratt NL, Wiese MD, Kalisch LM, Roughead EE. The validity of sequence symmetry analysis (SSA) for adverse drug reaction signal detection. *Pharmacoepidemiol Drug Saf* 2013;22:496-502.
14. Pratt NL, Roughead LE, Peck R, Andrew G. Self-controlled case series and prescription event symmetry analysis: How different are they really? *Pharmacoepidemiol Drug Saf* 2010;19:S229.
15. Visser ST, Schuiling-Veninga CC, Bos JH, de Jong-van den Berg LT, Postma MJ. The population-based prescription database IADB.nl: its development, usefulness in outcomes research and challenges. *Expert Rev Pharmacoecon Outcomes Res* 2013;13:285-292.
16. Vines SK, Farrington CP. Within-subject exposure dependency in case-crossover studies. *Stat Med* 2001;20:3039-3049.
17. Garrison SR, Dormuth CR, Morrow RL, Carney GA, Khan KM. Nocturnal leg cramps and prescription use that precedes them: a sequence symmetry analysis. *Arch Intern Med* 2012;172:120-126.
18. Mittleman MA, Maclure M, Robins JM. Control sampling strategies for case-crossover studies: an assessment of relative efficiency. *Am J Epidemiol* 1995;142:91-98.

19. Simpson SE. A positive event dependence model for self-controlled case series with applications in postmarketing surveillance. *Biometrics* 2013;69:128-36.
20. Thacker EL, Schneeweiss S. Initiation of acetylcholinesterase inhibitors and complications of chronic airways disorders in elderly patients. *Drug Saf* 2006;29:1077-1085.
21. Hallas J. Evidence of depression provoked by cardiovascular medication: a prescription sequence symmetry analysis. *Epidemiology* 1996;7:478-484.
22. Vegter S, de Jong-van den Berg LT. Misdiagnosis and mistreatment of a common side-effect--angiotensin-converting enzyme inhibitor-induced cough. *Br J Clin Pharmacol* 2010;69:200-203.
23. van Boven JF, de Jong-van den Berg LT, Vegter S. Inhaled corticosteroids and the occurrence of oral candidiasis: a prescription sequence symmetry analysis. *Drug Saf* 2013;36:231-236.
24. Vegter S, de Boer P, van Dijk KW, Visser S, de Jong-van den Berg LT. The effects of antitussive treatment of ACE inhibitor-induced cough on therapy compliance: a prescription sequence symmetry analysis. *Drug Saf* 2013;36:435-439.
25. Pouwels KB, Kalkman GA, Schagen D, Visser ST, Hak E. Is combined use of SSRIs and NSAIDs associated with an increased risk of starting peptic ulcer treatment? (Letter) *Br J Clin Pharmacol* 2013;doi:10.1111/bcp.12300
26. Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, Muller JE. Triggering of acute myocardial infarction by heavy physical exertion. Protection against triggering by regular exertion. Determinants of Myocardial Infarction Onset Study Investigators. *N Engl J Med* 1993;329:1677-1683.
27. Garrison SR, Dormuth CR, Morrow RL, Camey GA, Khan KM. Nocturnal leg cramps and prescription use that precedes them: a sequence symmetry analysis. Arch Intern Med 2012;172(2):120-126.
28. Groban L, Lindsey S, Wang H, et al. Differential effects of late-life initiation of low-dose enalapril and losartan on diastolic function in senescent Fischer 344 x Brown Norway male rats. *Age (Dordr)* 2012;34:831-843.
29. Kamioka M, Ishibashi T, Sugimoto K, et al. Blockade of renin-angiotensin system attenuates advanced glycation end products-mediated signaling pathways. *J Atheroscler Thromb* 2010;17:590-600.
30. Duncan MJ, Li G, Shin JS, Carson JL, Abraham SN. Bacterial penetration of bladder epithelium through lipid rafts. *J Biol Chem* 2004;279:18944-18951.
31. Song J, Bishop BL, Li G, Duncan MJ, Abraham SN. TLR4-initiated and cAMP-mediated abrogation of bacterial invasion of the bladder. *Cell Host Microbe* 2007;1:287-298.
32. Burnham CA, Shokoples SE, Tyrrell GJ. Rac1, RhoA, and Cdc42 participate in HeLa cell invasion by group B *Streptococcus*. *FEMS Microbiol Lett* 2007;272:8-14.
33. Martinez JJ, Hultgren SJ. Requirement of Rho-family GTPases in the invasion of Type 1-piliated uropathogenic *Escherichia coli*. *Cell Microbiol* 2002;4:19-28.
34. Hunstad DA, Justice SS. Intracellular lifestyles and immune evasion strategies of uropathogenic *Escherichia coli*. *Annu Rev Microbiol* 2010;64:203-221.

CHAPTER 6

# Is combined use of SSRIs and NSAIDs associated with an increased risk of starting peptic ulcer treatment? Reanalysis of a cohort study using a prescription sequence symmetry design

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## Abstract

### Background

A Dutch cohort study reported that the combined use of SSRIs with NSAIDs synergistically increases the risk of initiating a treatment course with peptic ulcer drugs (used as a proxy for uncomplicated gastrointestinal adverse effects) compared to TCA users. However that study was prone to unmeasured confounding, as no adjustment for potential differences between SSRI and TCA users was performed. We evaluated the same association in the same database using a prescription sequence symmetry analysis.

### Methods

Drug dispensing data between 1994 and 2011 were retrieved from the IADB.nl database. A prescription sequence symmetry analysis was used to assess whether peptic ulcer drugs were prescribed more often following SSRI therapy initiation in combination with NSAIDs, than the other way around. The association between NSAIDs alone and peptic ulcer drugs was also evaluated, as a positive control.

### Results

In total, 50,350 incident SSRI users were identified. Of these patients, 277 were incident users of both SSRIs and peptic ulcer drugs within a four week time-span. Less patients received peptic ulcer drugs after SSRI therapy initiation than the other way around (126 vs. 151), corresponding to an adjusted sequence ratio (ASR) of 0.83 (95% confidence interval [CI] 0.65-1.06). The ASR of concurrent use of SSRIs and NSAIDs (1.48, 95% CI, 0.90-2.49) did not exceed the ASR of NSAIDs alone (2.50, 95% CI, 2.27-2.76).

### Conclusions

Our findings indicate that at least part of the previously reported association between combined use of SSRIs with NSAIDs and peptic ulcer initiation might be attributed to unmeasured or residual confounding.

## Introduction

Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressant drugs that are thought to increase the risk of gastrointestinal adverse events [1]. De Jong *et al.*, reported that the combined use of nonsteroidal anti-inflammatory drugs (NSAIDs) with SSRIs synergistically increased the risk of initiating a treatment course with peptic ulcer drugs as a proxy for gastrointestinal adverse effects (RR 12.4, 95% CI 3.2-48.0). In an attempt to limit confounding by indication, tricyclic antidepressant (TCA) users were used as a control group. As the observed association was not adjusted for potential differences between TCA and SSRI users [1], channelling bias or unmeasured confounding may have affected the estimation of the adverse effects.

We re-analysed the study of De Jong *et al.* [1] to evaluate whether the dramatic increased risk associated with combined use of SSRIs and NSAIDs could be (partly) explained by differences in patient characteristics between SSRI and TCA users.

A prescription sequence symmetry analysis was used to overcome the problem of finding an appropriate control group. The main advantage of this method is that the patient population serves as its own control group, thereby controlling for time-invariant (unmeasured) confounding.

## Methods

A prescription sequence symmetry analysis was applied to data from the same pharmacy prescription database (IADB.nl) that de Jong *et al.* [1] used. This database contains prescription data from 1994 till 2011 from approximately 54 Dutch community pharmacies and covers an estimated population of 500,000 patients [2]. The database has been shown to be representative for the Netherlands in terms of drug use [3]. The database contains, among other data, information on the date of prescription, number of days the drug was prescribed for and the number of prescribed daily doses based on the WHO definition [2]. The medication records for each patient are virtually complete, except for over the counter (OTC) drugs and medication dispensed during hospitalization [4].

### Study population and outcome definition

From the IADB.nl database incident users of SSRIs (Anatomical Therapeutic Chemical classification [ATC] code N06AB), TCAs (ATC code N06AA), NSAIDs (ATC code

M01A) and peptic ulcer drugs (ATC code A02B) were selected. Only people who were 18 years or older during the first incident prescriptions were included.

Incident users of a drug were defined as users who did not use the drug in a period of 6 months before the first prescription, while being captured in the database for at least that period of time. All patients being prescribed thrombocyte aggregation inhibitors (ATC code B01A) or systemic corticosteroids (ATC code H02) in a period of 6 months before the incident use of the above mentioned drugs (SSRIs, TCAs, NSAIDs and peptic ulcer drugs) were excluded. The outcome was defined as a first start of a peptic ulcer drug course ($H_2$-receptor blocking agents, proton pump inhibitors, prostaglandins [ATC code A02B]).

**Statistical analysis**

A prescription sequence symmetry analysis was performed to evaluate whether peptic ulcer drugs were prescribed more often following the drugs of interest than the reverse [5]. When assessing the association between initiating treatment A and B, the sequence ratio (SR) is calculated by dividing the number of individuals starting treatment A first and treatment B second by the number of individuals starting treatment B first and treatment A second. If there would be no association between the drugs, patients should have equal probabilities to receive the drugs in either order, yielding a SR of 1. However if drug A has a side-effect that is treated with drug B, more patients would be prescribed drug B after drug A than the reverse, yielding a SR above 1.

Since prescription sequence symmetry analyses are vulnerable to trends in prescribing, we adjusted the crude SR for time trends in use of the study drugs. The adjusted SR was obtained by dividing the crude SR by a null ratio, i.e. the SR obtained assuming no association between both drugs based on overall prescribing of both drugs in the total IADB population, taking into account the exclusion criteria [5, 6]. For concurrent use of NSAIDs and SSRIs or TCAs we used the null-ratios obtained for NSAIDs alone. The adjusted SR is an estimate of the incidence ratio of peptic ulcer prescribing in exposed versus non-exposed person time [5, 6].

For the primary analysis, only patients for which the time-span between both incident prescriptions was between 2 and 28 days were included to limit time-varying confounding. We estimated 95% confidence intervals (95% CIs) of SRs using exact confidence intervals for binomial distributions using STATA 12 software (StataCorp LP, College Station, TX, USA).

**Sensitivity analyses**

In sensitivity analyses we evaluated whether our results were sensitive to certain choices in the design. First, because knowledge about a possible side-effect might influence the prescribing of general practitioners, we assessed whether restricting our analyses to the period before July 2000, the cut-off date De Jong *et al.* used [1], would change our results. Second, we evaluated the effect of using a drug-free run-in period of two years instead of half a year. Third, we calculated the ASR for SSRI and peptic ulcer drugs within different time-spans to evaluate whether the possible association would be stable using different time-windows ranging from two to eight weeks. Fourth, we evaluated whether excluding individuals using ATC code 'M01AX' (Other anti-inflammatory and anti-rheumatic agents, non-steroids) influenced the results. Finally, we restricted our analyses to patients that started with SSRI therapy within 90 days before the first NSAID prescription to exclude patients that already used SSRIs for a long period, before their first NSAID prescription.

## Results

In total, 50,350 incident adult patients who initiated SSRI treatment were identified between July 1994 and December 2011. The median age of these patients at the start of SSRI therapy was 43 [interquartile range (IQR), 25], 64.0% was female and 25.8% had recorded use of NSAIDs in the year prior to SSRI therapy initiation. After excluding patients that used peptic ulcer drugs within 1 day of SSRI therapy initiation and those that used NSAIDs, systemic corticosteroids or thrombocyte aggregation inhibitors, 277 patients that received a peptic ulcer drug course within 4 weeks prior to or after SSRI treatment initiation were identified. The median age of these patients at the start of SSRI treatment was 42 (IQR 23) and 61.7% was female.

Of these 277 patients, 126 (45%) started SSRI therapy prior to peptic ulcer drug treatment, while 151 (55%) patients started peptic ulcer drug treatment first, which corresponds to an adjusted sequence ratio of 0.83 (95% CI, 0.65-1.06) (Table 6.1).

Concurrent use of SSRIs and NSAIDs was associated with a statistically non-significant increase in the risk of starting peptic ulcer drug treatment (aSR, 1.48, 95% CI 0.90-2.49), which did not exceed the risk estimated for NSAID treatment alone (aSR 2.50, 95% 2.27-2.76). Similar results were obtained for TCA treatment alone and concurrent use of TCAs and NSAIDs (Table 6.1).

**Table 6.1.** Symmetry analysis of selected drug therapy initiation within 4 weeks of peptic ulcer drug therapy initiation using a drug-free run-in period of half a year.

| Drug | Peptic ulcer drug prescribed first/second (n) | Adjusted sequence ratio (95% CI)[a] |
|---|---|---|
| SSRI | 151/126 | 0.83 (0.65-1.06) |
| NSAID | 571/1445 | 2.50 (2.27-2.76) |
| SSRI+NSAID | 28/42 | 1.48 (0.90-2.49) |
| TCA | 68/63 | 0.92 (0.65-1.32) |
| TCA+NSAID | 11/16 | 1.44 (0.63-3.43) |

SSRI, selective serotonin reuptake inhibitor; NSAID, non-steroidal anti-inflammatory drugs; TCA, tricyclic antidepressant; CI, confidence interval.
[a] adjusted for trends in prescribing

**Sensitivity analysis**

Restricting the analysis to data obtained before the end of the study period of De Jong *et al.* [1], did not substantially change the results. No association was found between SSRIs and peptic ulcer drug initiation (aSR 0.85, 95% CI 0.54-1.33), while an association between NSAIDs and peptic ulcer drugs was still present (ASR 1.91, 95% CI 1.61-2.29). In addition, the risk of concomitant use of NSAIDs and SSRIs was still not higher than the risk for NSAIDs alone.

The results were insensitive to prolonging the drug-free run-in period from half a year to two years. Using this prolonged run-in period, no association was found between SSRIs and peptic ulcer drugs (ASR 0.98, 95% CI 0.74-1.30). Additionally, the sequence ratio for concomitant use of NSAIDs and SSRIs (aSR 1.38, 95% CI 0.75-2.59) remained lower than for NSAIDs alone (aSR 2.96, 95% CI 2.61-3.36).

When using different maximum time-spans between both first prescriptions, ranging from 1 to 8 weeks, no association was present between SSRIs and peptic ulcer drugs. The sequence ratios were relatively stable within these different maximum time-spans between the prescriptions, ranging from an aSR of 0.87 (95% CI 0.70-1.09) to 1.00 (95% CI 0.73-1.37). The results for concomitant use of NSAIDs and SSRIs were also

relatively stable using these different time-spans, ranging from an aSR of 1.24 (95% CI 0.76-2.12) to 1.67 (95% CI 0.88-3.35).

Excluding individuals using ATC code 'M01AX' did not change the results, as none of the patients receiving a first peptic ulcer drug course within 28 days of starting with concomitant use of NSAIDs and SSRIs did use a drug that falls in this category.

When restricting our analyses to patients that started with SSRI therapy within 90 days before the first NSAID prescription, the aSR was not higher for concurrent use of SSRIs and NSAIDs than for NSAIDs alone using both a run-in period of half a year (2.23 vs 2.50) and a run-in period of 2 years (1.32 vs 2.96).

Only 3 patients initiated SSRI and NSAID therapy on the same day, without receiving prophylactic peptic ulcer drugs. Two patients received first this combination of drugs and then peptic ulcer drugs, while one patient received those drugs in the reverse order.

## Discussion

This prescription sequence symmetry analysis suggests that concurrent use of SSRIs and NSAIDs is not associated with an increased risk of starting peptic ulcer treatment compared to NSAIDs alone. Our results are in contrast with the study of De Jong *et al.* who found that combined use of NSAIDs with SSRIs synergistically increased the risk of initiating a treatment course with peptic ulcer drugs.

Given that we used the same database and similar inclusion and exclusion criteria as de Jong *et al.* [1], the main difference between their study and ours is that we used an alternative design that reduces time-invariant (unmeasured) confounding. In accordance with our results, Dall *et al.,* who applied a case-control design in which they controlled for various potential confounders including alcohol abuse, did not observe an increased risk of uncomplicated peptic ulcers associated with combined use of SSRIs and NSAIDs [7]. These data suggest that the synergistically increased risk of combined use of SSRIs and NSAIDs found by De Jong *et al.* [1], might be, at least partly, contributed to unmeasured confounding. The study of De Jong *et al.* was vulnerable to confounding bias, because they compared SSRI users with TCA users, without adjusting for any potential differences between these different types of patients [1].

For example, it has been shown that heavy alcohol use is a strong confounder when evaluating the association between SSRI use and GI bleeding [8]. This can be explained by the fact that alcohol abuse is a well-known risk factor for both GI adverse events and

depression. TCAs can potentiate the effects of alcohol due to their antihistaminic effects, while SSRIs have minimal antihistaminic properties [9] and consequently do have minimal effects on alcohol pharmacokinetics and pharmacodynamics [10]. Therefore, alcohol abuse might be less common among TCA users than among SSRI users, due to channelling by the physician. In our database, SSRI users were indeed approximately 2 times more likely to use drugs indicated for treatment of alcohol dependence (ATC code N07BB) than TCA users.

The main strength of our study is that we controlled for time-invariant (unmeasured) confounding, because the patient population serves as its own control. By using relatively short time-spans between SSRI and peptic ulcer drug prescriptions we further reduced (unmeasured) time-varying confounding.

However, the prescription sequence symmetry analysis is still vulnerable to confounding by disease severity or sudden-onset diseases. Despite figure 1 suggests that confounding by disease severity or other time-varying variables might result in spurious inflations of the risk of starting peptic ulcer treatment; we did not find an increased risk associated with SSRIs, indicating that our results are not largely affected by changes in disease severity.

Alternatively, it is possible that we could not detect an increased risk using our study design, because we could not capture peptic ulcer drugs courses prescribed during hospitalizations or measure over-the-counter NSAID and peptic ulcer drug use. However, De Jong *et al.* did previously find and increased risk in patients concurrently using SSRIs and NSAIDs, while coping with the same limitations [1]. Moreover, the well-known increased risk of gastrointestinal adverse effect after starting with NSAID treatment [11, 12] was conformed in our study. Therefore, the lack of an increased risk associated with SSRI use, whether or not used concurrently with NSAIDs, is likely not related to these potential limitations.

It is possible that using a drug-free run-in period of half a year was not long enough to guarantee incident use of the study-drugs. We used a run-in period of half a year as we tried to use the same restrictions as De Jong *et al.* did [1], to be able to make a fair comparison. However, sensitivity analysis showed that prolonging the drug-free run-in period to two years did not substantially change the SR estimates.

While De Jong *et al.* assessed the effect of concurrent use of NSAIDs and SSRIs in starters [1], we assessed the effect of co-administration of NSAIDs in more chronic SSRI users. Because patients that experience adverse events with SSRIs may self-select

themselves out of longer treatment [13], starters might have a different risk than chronic SSRI users. However, restricting our analyses to patients that started with SSRI therapy within 90 days before the first NSAID prescription did not substantially change the SR estimates.

As we restricted our analyses to relatively short time-spans to reduce the potential influence of time-varying confounding, we could not detect a risk that develops later in the course of treatment. However, De Jong *et al.* found an increased risk for concomitant use of SSRIs and NSAIDs during an average follow-up of 21 days [1]. In addition, Dall *et al.* found that the risk of uncomplicated peptic ulcers is strongest during the first 30 days of SSRI treatment [7], indicating that our time-span of four weeks should be long enough to capture potential drug-related increases in peptic ulcer drug prescribing.

In conclusion, our results suggest that the observations reported by de Jong *et al*. [1] might, at least partly, be attributed to unmeasured confounding. Although comparison with a drug that is used to treat the same indication does limit confounding, it does not eliminate all potential confounders. Therefore, when limited data on potential confounders are available, a self-controlled design can have added value, because it reduces time-invariant confounding [14].

## References


1. de Jong JC, van den Berg PB, Tobi H, de Jong-van den Berg LT. Combined use of SSRIs and NSAIDs increases the risk of gastrointestinal adverse effects. Br J Clin Pharmacol 2003;55:591-5.
2. Pharmacy-dispensing database of IADB.nl. Available at http://www.iadb.nl. (last accessed 16 november 2012).
3. Tobi H, van den Berg P, de Jong-van den Berg L. The InterAction Database: Synergy of Science and Practice in Pharmacy. Lect Notes Comput Sci 2000;1933:93-108.
4. Leufkens HGM, Urguhart J. Automated pharmacy record linkage in the Netherlands. In: Pharmacoepidemiology, Fourth Edition, ed Strom BL, Chichester, UK: John Wiley & Sons, Ltd; 2007.
5. Hallas J. Evidence of depression provoked by cardiovascular medication: a prescription sequence symmetry analysis. Epidemiology 1996; 7: 478-84.
6. Tsiropoulos I, Andersen M, Hallas J. Adverse events with use of antiepileptic drugs: a prescription and event symmetry analysis. Pharmacoepidemiol Drug Saf 2009;18:483-91.
7. Dall M, Schaffalitzky de Muckadell OB, Lassen AT, Hallas J. There is an association between selective serotonin reuptake inhibitor use and uncomplicated peptic ulcers: a population-based case-control study. Aliment Pharmacol Ther 2010;32:1383-91.
8. Opatrny L, Delaney JA, Suissa S. Gastro-intestinal haemorrhage risks of selective serotonin receptor antagonist therapy: a new look. Br J Clin Pharmacol 2008;66:76-81.
9. Harvey BH. The neurobiology and pharmacology of depression. A comparative overview of serotonin selective antidepressants. S Afr Med J 1997;87:540,50,552.
10. Fraser AG. Pharmacokinetic interactions between alcohol and other drugs. Clin Pharmacokinet 1997;33:79-90.
11. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 1999;340:1888-99.
12. Ofman JJ, MacLean CH, Straus WL, Morton SC, Berger ML, Roth EA, Shekelle P. A metaanalysis of severe upper gastrointestinal complications of nonsteroidal antiinflammatory drugs. J Rheumatol 2002;29:804-12.
13. Andrade C, Sandarsh S, Chethan KB, Nagesh KS. Serotonin reuptake inhibitor antidepressants and abnormal bleeding: a review for clinicians and a reconsideration of mechanisms. J Clin Psychiatry 2010;71:1565-75.
14. Maclure M, Fireman B, Nelson JC, Hua W, Shoaibi A, Paredes A, Madigan D. When should case-only designs be used for safety monitoring of medical

    products? Pharmacoepidemiol Drug Saf 2012;21:50-61.

CHAPTER 7

# Antibiotic use during pregnancy and the development of asthma in preschool children: the influence of confounding

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## Abstract

### Background

Recent reported associations between prenatal antibiotic use and childhood asthma may have been influenced by unmeasured confounders or time-trends in exposure prevalence. We aimed to assess the association between prenatal antibiotic use and asthma in preschool children with a case-sibling and case-control design.

### Methods

We conducted a case-sibling study in which 1,228 children with asthma were compared to 1,228 siblings without asthma, using data from the prescription database IADB.nl. In addition, a case-control study was conducted. Maternal exposure was defined as ≥1 day of supply of antibiotics during pregnancy. Asthma in preschool children was defined as ≥3 prescriptions for anti-asthma medication within a year before the fifth birthday. Conditional logistic regression was used to estimate crude and adjusted odds ratios (aOR). The case-sibling results were additionally adjusted for potential time-trends in antibiotic exposure.

### Results

Both the case-sibling and case-control analysis yielded similar increased risks of asthma in preschool children when antibiotics were used in the third trimester of pregnancy (aOR 1.37;95%CI 1.02-1.83 and aOR 1.40;95%CI 1.15-1.47). Time trend analyses showed that results were not influenced by a time trend in antibiotic exposure. Significant increased risks of asthma in preschool children after exposure to antibiotics in any trimester of pregnancy was observed in the case-control analysis only (aOR 1.46;95%CI 1.34-1.59).

### Conclusions

Exposure to antibiotics in the third trimester of pregnancy appeared to be associated with a small increased risk of asthma in preschool children. This association appeared not to be influenced by time-invariant confounders or time trends in antibiotic exposure.

## Introduction

The prevalence of atopic diseases, like asthma, has increased dramatically in developed countries [1]. Latest evidence suggests that exposure to environmental factors and drugs during early life or pregnancy may play a role in this sudden increase in atopic disease development [2, 3]. Currently, there is an on-going debate whether antibiotic use during pregnancy could increase the child's asthma risk [4-12].

According to the hygiene hypothesis, children who are less frequently exposed to micro-organisms at birth and in early life are prone to developing atopic diseases [13, 14]. Antibiotics taken during pregnancy can result in the alteration of vaginal bacterial flora during birth and may ultimately predispose the immune system to develop towards an atopic state [15, 16]. Therefore the association between antibiotics during pregnancy and asthma in the offspring would be expected stronger for exposure periods shortly before birth, i.e. stronger in the third than the first trimester. Several studies reported associations between antibiotic use during pregnancy and asthma development in children [4-12]. A recent study reported that the association between antibiotic use and childhood asthma was not restricted to the pregnancy period only, suggesting that antibiotic use is a marker of the mother's general propensity for infections [10]. However they did not account for the correlation between antibiotic use before, during and after pregnancy. In addition, a Swedish case-sibling study stated that previous found associations can be explained by time-invariant confounders shared within families [12]. However, potential time-trends in the underlying cohort could have influenced the results of the case-sibling study, especially since exposure periods were compared within the same person i.e. exposure periods were per definition always different periods in time [17, 18]. Firstborn children are at higher risk of developing asthma than their later born siblings and it is therefore likely that birth sequence is unevenly distributed between case and control siblings [19, 20]. If there was a time-trend in exposure frequencies in the underlying cohort, an uneven distribution of birth sequence could have introduced time-trend bias.

We aimed to assess the association between antibiotic use in pregnancy and the development of asthma in preschool children, by applying a case-sibling design. To evaluate the influence of time-invariant confounding, results of the case-sibling analysis were compared with results obtained using a matched case-control design. In our secondary analyses, we evaluated if found associations were restricted to the pregnancy period only while adjusting for correlations between pre- and postnatal antibiotic use. In addition, we evaluated the influence of potential time trends in exposure frequencies in the case-sibling analysis.

## Methods

### Setting

Data for this study were collected from the IADB.nl database, a prescription database from the University of Groningen in the Netherlands. The IADB.nl contains prescription records of community pharmacies and covers a population of approximately 500,000 patients, including a mother-infant subset of approximately 40,000 children and their mothers, living in the Northern part of the Netherlands. The database is representative of the Netherlands as a whole and is described in detail elsewhere [21, 22]. Over the counter medication and medication dispensed during hospitalization were not recorded in the database.

### Study designs

We applied both a case-sibling and a matched case-control design. In the case-sibling analysis, preschool children with asthma were compared with their own siblings without asthma, thereby minimizing (un)measured time-invariant confounding (i.e. covariates that have similar statistical distributions in both pregnancies, such as maternal genetic predisposition and other stable familial factors) [23, 24]. In addition, results of the case-sibling analysis were adjusted for potential time trends (see also Appendix 1). In the more conventional case-control study, cases were not restricted to children with eligible siblings resulting in higher statistical power. Because exposure status was compared between children with asthma and randomly selected children without asthma, this design is per definition more vulnerable to potential confounding.

Moreover, a maternal-paternal comparison was performed, using the case-sibling subjects. We analysed paternal exposure to systemic antibiotics during pregnancy in the subset of children where paternal data were present as a potential measure for infections in the household, while adjusting for potential confounders [25].

### Study population

The study population was restricted to all preschool children present in the database from birth until the age of 5 years. Children who were part of multiple births were excluded from the study population.

### Case definitions

Children were defined as cases if they had received at least 3 prescriptions for asthma medication (ATC-group R03) within a 12 month period before the fifth

birth day according to the primary care drug treatment guidelines [26]. In the case-sibling analysis a case was eligible for inclusion if there was at least one available control, i.e. one sibling without any prescriptions from the ATC-group R03 before his or her fifth birthday. Sibling controls were matched to cases with a 1:1 ratio, thereby preventing the introduction of bias due to exposure dependency between control siblings [27]. Therefore, if multiple sibling controls were available for a case, one eligible sibling control was randomly selected and matched to the case. In the case-control analyses, cases were eligible for inclusion, irrespective of their sibling status. Controls in the case-control analyses were defined as all children that had not received prescriptions for asthma medication before their fifth birthday. Controls were matched to cases on birth date (±3 years) with a 6:1 ratio.

**Exposure to antibiotics in and after pregnancy**

In the Netherlands antibiotics are prescription drugs and no over the counter sale is allowed. Maternal exposure was defined as at least a one day supply of systemic antibiotics (ATC-group J01) during pregnancy. Since the actual conception date was unknown, pregnancy was defined as the birth date of the child minus 273 days. Subtypes of antibiotics were stratified into beta-lactam penicillins (ATC-group J01C), Sulphonamides (ATC-group J01E), Macrolides (ATC-group J01F), Nitrofurantoin (ATC-group J01XE) and other antibiotics (remaining subgroups of ATC J01). Exposure was further stratified according to trimester of exposure, consisting of 13 weeks each (first 1-91 days; second: 92-189 days; third 190-273 days), thereby adjusting for exposure in the other trimesters. Since only the child's birth date is known, the theoretical conception data was determined as the data of birth minus 273 weeks (39 weeks). To evaluate whether associations were restricted to the pregnancy period only, we assessed the effect of maternal antibiotic use in the 13 weeks after delivery while adjusting for antibiotic use during pregnancy.

**Covariates**

Potential confounders were gender of the child and birth order [19, 20, 28]. We did not adjust for antibiotic use in the preschool children as early symptoms of undiagnosed asthma are often treated with antibiotics [7, 29]. Adjustment for antibiotic use in preschool children would consequently result in an underestimation of the true effect, if present, due to protopathic bias [7, 30, 31]. Maternal characteristics that are measured in the database and could potentially be confounders or correlated with confounders of the association between maternal antibiotic use and asthma in the offspring are: age at delivery [32], the use of asthma medication (ATC-group R03) [32], use of acid suppressive drugs (ATC-group A02B) [23, 24], use of anti-depressant drugs (ATC-group N06) [33], use of drugs indicated for allergic dermatitis (ATC-group D07), use of drugs

indicated for allergic rhinitis (ATC-group R01AD, R01AC), and use of insulin (ATC -group A10A) [32, 33] during pregnancy (defined as having at least a one day supply of the drug class). Though the genetic predisposition for the development of asthma is equal between cases and controls in the case-sibling analysis, the allergic status of the mother can be different [34]. Hence, we also performed a sub-analysis on a subgroup of children whose mothers who did not use asthma medication during either pregnancy.

### Statistical analyses

We used conditional logistic regression to obtain the odds ratios (OR) and their corresponding 95% confidence intervals (95%CIs) in both the case-sibling and matched case-control analyses. In multivariate analyses, OR's were adjusted for variables that were significantly associated ($p<0.05$) with both the outcome (asthma of the child) and the exposure (antibiotic use during pregnancy) in univariate analyses. Each remaining covariate was further assessed for possible confounding by adding it to the multivariate regression to evaluate whether it resulted in a more than 10% change in the effect estimate. The distributions of covariates were measured with paired tests. In addition, OR's for the development of asthma after exposure to antibiotics anytime during pregnancy and during different trimesters of pregnancy were adjusted for potential time-trends in the case-sibling analysis (see Appendix 7.1).

Since data on smoking, respiratory infections and asthma status during pregnancy are not available in the IADB.nl, both the case-sibling and the case-control design cannot adjust for these potential time-varying confounders. Therefore we used a simplified sensitivity analysis proposed by VanderWeele et al. [35] to assess the impact of unmeasured confounders on our main analysis of the case-sibling study. A detailed description of this method is available in appendix 7.2. All analyses were conducted using the IBM SPSS Statistics 20 version and R (version 3.0.2).

## Results

### Case-sibling analysis

In the case-sibling analysis 1,228 children with asthma were included as cases and 1,228 siblings without asthma were included as controls. Analysis of the distribution of covariates between cases and controls showed that case siblings were more often male and were more often born before than after their control siblings. Mothers were older at delivery and used more acid suppressive drugs during the pregnancy that resulted in a case (Table 7.1). Children who were exposed to antibiotics during pregnancy were more often born before their sibling. Mothers who used antibiotics during pregnancy

also received anti-asthma medication and acid suppressive drugs more often during that pregnancy (appendix 7.3).

**Table 7.1.** Distribution of covariates between cases and controls in case-sibling and case-control analysis

| | Case-sibling analysis | | | Case-control analysis | | |
|---|---|---|---|---|---|---|
| | n (%) | n (%) | | n (%) | n (%) | |
| | Cases (N=1228) | Controls (N=1228) | P-value | Cases (N=3,754) | Controls (N=22,523) | P-value |
| ***Child characteristics*** | | | | | | |
| Male gender | 815 (66.4) | 565 (46.0) | <0.001 | 2372 (63.2) | 10,808 (48.0) | <0.001 |
| Mean age at index date asthma (years) | 1,63 | | | 1.65 | | |
| Birth order: First born | 444 (36.2) | 784 (63.8) | <0.001 | NA | NA | |
| Use of ABs before index date | 939 (76.5) | 631 (51.4) | <0.001 | 2932 (78.1) | 9564 (42.5) | <0.001 |
| ***Mother characteristics*** | | | | | | |
| Mean age (years) | 29.8 | 29.0 | <0.001 | 30.3 | 30.6 | <0.001 |
| Use of medication for atopic diseases during pregnancy | 314 (25.6) | 320 (26.1) | 0.774 | 1069 (28.5) | 4410 (19.6) | <0.001 |
| Asthma medication | 73 (5.9) | 82 (6.7) | 0.321 | 301 (8.0) | 698 (3.1) | <0.001 |
| Drugs for atopic dermatitis | 123 (10.0) | 110 (9.0) | 0.362 | 405 (10.8) | 1936 (8.6) | <0.001 |
| Drugs for rhinitis | 171 (13.9) | 181 (14.7) | 0.535 | 595 (15.8) | 2342 (10.4) | <0.001 |
| Use of acid suppressive drugs during pregnancy (ATC A02B) | 51 (4.2) | 26 (2.1) | 0.001 | 196 (5.2) | 627 (2.8) | <0.001 |
| Use of insulin during pregnancy | 10 (0.8) | 8 (0.7) | 0.687 | 27 (0.7) | 157 (0.7) | 0.879 |
| Use of antidepressants during pregnancy | 30 (2.4) | 27 (2.2) | 0.749 | 101 (2.7) | 439 (1.9) | 0.003 |
| ***Father characteristics*** | | | | | | |
| Data present (N) | 903 | 835 | | 2544 | 13626 | |
| Use of medication for atopic diseases during pregnancy | 223 (24.7) | 189 (22.6) | 0.176 | 738 (29.0) | 2957 (21.7) | <0.001 |
| Asthma medication | 74 (8.2) | 49 (5.9) | 0.012 | 251 (9.9) | 595 (4.4) | <0.001 |
| Drugs for atopic dermatitis | 99 (11.0) | 97 (11.6) | 1.000 | 312 (12.3) | 1427 (10.5) | 0.025 |
| Drugs for rhinitis | 94 (10.4) | 80(9.6) | 0.494 | 349 (13.7) | 1438 (10.6) | <0.001 |

Of the cases 24,5% were exposed to antibiotics during pregnancy, while 22,3% were exposed in the control group. The use of antibiotics during pregnancy in general did not yield a significant increase in risk for the development of asthma in preschool children (aOR 1.06 95%CI 0.85-1.32). Stratification on trimester of exposure yielded a small but statistically significant increase in risk for the development of asthma in preschool children (aOR 1.37 95%CI 1.02-1.83) among those exposed in the third trimester, independent of exposure in the other trimesters. In addition, when we restricted case-status to children that continued anti-asthma treatment until at least the age of 3, the aOR after exposure

in third trimester increased to 1.51 (95%CI 1.07-2.14). In contrast the risk of asthma development among those with exposure in the first trimester was significantly decreased (aOR 0.70 95%CI 0.50-0.98). This decreased risk explains the difference between the effects in the third trimester compared to the null effect of exposure at any time during pregnancy. Subsequent analyses of first trimester exposure found that the decreased risk was present primarily in tetracycline exposure between cases (n=8; 0.7%) and controls (n=15; 1.2%)). When we assessed the effect of exposure in the 13 weeks after delivery the aOR was 1.03 (95%CI 0.80-1.32). There were no significant increases in risk for the development of childhood asthma after stratification on the subtypes penicillin's, sulfonamides, macrolides and nitrofurantoin (Table 7.2). When the analysis of the association between antibiotic use in third trimester and the development of asthma in the offspring was restricted to children from mothers who were not using any asthma medication during pregnancy, the aOR was attenuated to 1.22 (95%CI 0.90-1.67).

#### Influence of postnatal antibiotic use and potential time-trends

When we assessed the effect of maternal antibiotic use in the 13 weeks after delivery the aOR was 1.03 (95%CI 0.80-1.32). In addition, the case-sibling analysis appeared not to be influenced by a time-trend in exposure frequency, since the time-trends adjusted odds ratio was similar to the original case-sibling odds ratio (1.06 95%CI 0.81-1.36 vs 1.06 95%CI 0.85-1.32) (Table 7.2). Time-trends adjusted odds ratios for antibiotic use in the different trimesters of pregnancy were also similar to the odds ratios of the original case-sibling analysis (Table 7.2).

#### Maternal-paternal comparison and sensitivity analysis

The analysis of paternal exposure to antibiotics during the third trimester of pregnancy as proxy for infections in the household and other factors shared by both parents showed no significant increased risk of asthma (aOR 1.11 95%CI 0.70-1.74).

**Table 7.2.** Unadjusted and adjusted conditional odds ratios for the development of asthma in preschool children after exposure to antibiotic drugs during pregnancy in the case-sibling and case-time-control analysis

| | **Case-sibling analysis** | | | | **Case-time-control analysis** | |
|---|---|---|---|---|---|---|
| | Cases (N=1228) N (%) | Controls (N=1228) N (%) | Unadjusted conditional OR (95% CI) | Adjusted conditional OR (95% CI)* | Adjusted time-trend OR (95% CI)* | Adjusted case-time-control OR (95% CI)* |
| **Exposure to any antibiotic during pregnancy** | 301 (24.5) | 274 (22.3) | 1.16 (0.94-1.42) | 1.06 (0.85-1.32) | 1.00 (0.87-1.15) | 1.06 (0.81-1.36) |
| **Trimester of exposure** | | | | | | |
| Trimester 1 | 89 (7.2) | 100 (8.1) | 0.88 (0.65-1.19) | 0.70 (0.50-0.98) ‡ | 1.00 (0.81-1.25) ‡ | 0.70 (0.45-1.03) ‡ |
| Trimester 2 | 128 (10.4) | 112 (9.1) | 1.17 (0.89-1.54) | 1.25 (0.92-1.69) ‡ | 1.01 (0.82-1.24) ‡ | 1.24 (0.85-1.79) ‡ |
| Trimester 3 | 161 (13.1) | 118 (9.6) | 1.49 (1.14-1.95) | ***1.37 (1.02-1.83) ‡*** | 0.91 (0.75-1.09) ‡ | ***1.51 (1.05-2.13) ‡*** |
| **Subgroup of antibiotics**** | | | | | | |
| Beta lactam Penicillins | 242 (19.7) | 208 (16.9) | 1.23 (0.99-1.53) | 1.13 (0.90-1.43) | | |
| Sulfonamides & trimetoprim | 34 (2.8) | 33 (2.7) | 1.03 (0.63-1.70) | 1.06 (0.62-1.82) | | |
| Macrolides | 14 (1.1) | 14 (1.1) | 1.00 (0.45-2.23) | 0.96 (0.41-2.28) | | |
| Nitrofurantoin | 45 (3.7) | 42 (3.4) | 0.93 (0.59-1.45) | 0.93 (0.57-1.51) | | |
| Other | 15 (1.2) | 23 (1.9) | 0.64 (0.33-1.24) | 0.63 (0.31-1.30) | | |

** Adjusted for gender, birth order, maternal age at delivery and maternal use of acid suppressive drugs during pregnancy*
*‡ Odds ratios were additionally adjusted for exposure in other trimesters*
**** Women can be exposed to more than one subgroup of antibiotics*

Effect estimates were almost identical for maternal exposure in the third trimester when restricted to this subset or the main analysis (aOR 1.39 vs aOR 1.37).

Appendix 7.2 shows the effects of a hypothetical unmeasured confounder in the case-sibling analysis. A binary confounder, with a prevalence of 10% among unexposed, that could have biased the third-trimester results from 1.00 to 1.37 should have a strong association with the exposure and/or the outcome (Appendix 7.2), thereby exceeding the effects of any measured confounder. For this scenario, if the prevalence of the binary confounder is 2 times higher than the prevalence among unexposed women, the OR between this confounder and asthma in the offspring should be 6.9 to bias the effect estimate from 1.00 to 1.37. However, for this scenario a confounder with an OR of 1.21 could bias the results from statistically non-significant to statistically significant.

### Case-control analysis

In the case-control analysis we included 3,754 children with asthma and 22,523 children without asthma (controls). Univariate analysis identified gender, maternal age at delivery, maternal use of acid suppressive drugs, antidepressant drugs and drugs for atopic disease during pregnancy as potential confounders (appendix 7.3).

25,4% of cases and 17,8% of controls were exposed to antibiotics during pregnancy. The use of antibiotics during pregnancy in general yielded a small but significant increase in the risk of childhood asthma (aOR 1.45 95%CI 1.33-1.58). Risks were even higher when children were exposed to higher doses (DDDs) during pregnancy (aOR 1.90 95%CI 1.62-2.22). After adjusting for antibiotic exposure during other trimesters, the risk of childhood asthma was highest in the third trimester, although confidence intervals overlap (table 7.3). Stratification on the different subtypes of antibiotics yielded significant increases in risk for the development of childhood asthma only for the penicillin, sulfonamide and macrolide subtypes (table 7.3). Exposure to nitrofurantoin was not statistically significantly associated with the development of asthma in preschool children (aOR 1.10 95%CI 0.91-1.33).

## Discussion

Exposure to antibiotics during pregnancy was associated with a small increased risk of asthma in preschool children in our case-control analysis. In the case-sibling analysis, this association for exposure anytime during the whole pregnancy was considerably reduced, suggesting that confounding by time-invariant factors may have played an important role. However, when stratified by trimester both designs found an association between

exposure to antibiotics in the third trimester and an increased risk of asthma in preschool children. The similar estimates and confidence intervals of the case-sibling and case-control study for the second and third trimester, suggest that the influence of potential time-invariant confounding is minimal. Given these very similar effect estimates, the discrepancy between both designs after exposure anytime during pregnancy is almost solely caused by the significant decreased risk found in the first trimester of the case-sibling design. Secondary analyses showed that results of the case-sibling analysis were restricted to the pregnancy period only and were not influenced by a time-trend in antibiotic exposure.

**Table 7.3.** Unadjusted and adjusted conditional odds ratios for the development of asthma in preschool children after exposure to antibiotic drugs during pregnancy in the case-control analysis

| | **Case-control analysis** | | | |
|---|---|---|---|---|
| | Cases (N=3754) N (%) | Controls (N=22524) N (%) | Unadjusted conditional OR (95% CI) | Adjusted conditional OR (95% CI)* |
| **Exposure to any antibiotic during pregnancy** | 952 (25.4) | 4017 (17.8) | 1.57 (1.45-1.71) | 1.45 (1.33-1.58) |
| **Trimester of exposure** | | | | |
| Trimester 1 | 336 (9.0) | 1,401 (6.2) | 1.49 (1.31-1.68) | 1.23 (1.08-1.41) ‡ |
| Trimester 2 | 404 (10.8) | 1603 (7.1) | 1.57 (1.40-1.77) | 1.30 (1.15-1.47) ‡ |
| Trimester 3 | 477 (12.7) | 1880 (8.3) | 1.61 (1.44-1.79) | 1.40 (1.25-1.57) ‡ |
| **Subgroup of antibiotics**** | | | | |
| Beta lactam Penicillins | 773 (20.6) | 3159 (14.0) | 1.60 (1.46-1.74) | 1.48 (1.35-1.62) |
| Sulfonamides & trimetoprim | 105 (2.8) | 393 (1.7) | 1.63 (1.31-2.03) | 1.49 (1.19-1.87) |
| Macrolides | 50 (1.3) | 190 (0.8) | 1.60 (1.17-2.17) | 1.37 (1.00-1.87) |
| Nitrofurantoin | 140 (3.7) | 710 (3.2) | 1.19 (0.99-1.44) | 1.10 (0.91-1.33) |
| Other | 56 (1.5) | 250 (1.1) | 1.35 (1.01-1.81) | 1.20 (0.89-1.61) |

**Interpretation and comparison with other studies**

To be able to conclude whether time-invariant confounding largely explains the association between antibiotic exposure during pregnancy and asthma in (preschool) children, as suggested by the recent studies of Örtqvist *et al.*[12] and Stockholm *et al.* [10], the reason for the discrepant findings between trimesters should be explored.

When assessing exposure anytime during pregnancy, results of the case-sibling analysis are in agreement with these other two studies. However, while we did find a difference between trimesters, Örtqvist et al. did not. [12] This discrepancy may be explained by different analytical choices. Örtqvist et al included multiple control siblings per case, which may have introduced bias due to exposure dependencies between control siblings [27]. Moreover, they did not take into account that antibiotic exposure in one trimester likely correlates with such exposure in other trimesters. In addition, they adjusted for a different subset of potential time-varying confounders and may have had different antibiotic prescription patterns. Moreover, they did not evaluate whether there may be a time-trend in exposure or confounder distribution that may have affected their results. Time-trends may vary between countries because of differences in prescription patterns, guidelines or reimbursements. Therefore, previous reported results could have been influenced by time-bias while our results were not. For example, if in their case-sibling analysis cases were more frequently born before their control siblings, and there would be an increasing trend in the prescribing of antibiotics in pregnant women, results will be biased towards no or a protective effect. In our analysis, selected cases were more often born after their control sibling due to the manner children entered the database [21]. Alternatively, given the limited sample size for the stratified analyses in comparison with the other study our results for the different trimester may be chance findings.

An important limitation of the study of Stokholm *et al.* is that they did not take into account in their main analyses that antibiotic exposures before, during and after pregnancy are likely correlated [10]. In a sensitivity analysis they estimated the effect of exposure before and after pregnancy in women that were not exposed during pregnancy, but results were significantly lower for the period from birth to 40 weeks postpartum (1.42 vs 1.18). This suggests that this population was not comparable to the population in the main analysis or that the effect post-partum was partly explained by exposure during pregnancy.

Given the potential limitations of prior studies that tried to eliminate confounding by designs, the findings of the differences in trimester may be caused by a biological mechanism. The highest risk found in the third trimester is in agreement with the hypothesis that antibiotic drug use during pregnancy alters the vaginal bacterial flora at delivery. Different studies indicate that antibiotic use can have long-term altering effects on the vaginal bacterial flora [5, 15], explaining the slight increased asthma risks found after antibiotic drug use in the second trimester.

In contrast to the case-control analysis, our case-sibling analysis did find a decreased risk for use in the first trimester. Subsequent analyses found that the decreased risk in the case-sibling analysis was present primarily in tetracycline exposure between cases

(n=8; 0.7%) and controls (n=15; 1.2%)). Recent studies showed that tetracyclines may lower IgE-levels [36-39]. A reduction in cord blood IgE levels - a predictor of allergic disorders in children, especially among children with a family history of allergic diseases is likely more relevant in mothers with elevated IgE levels and atopic diseases [40]. This may explain the difference between both designs, because all mothers in the case-sibling gave birth to a child with asthma, and most mothers of controls in the case-control did not. Similarly, confounding by allergic status of the mother may explain why another case-control study did not find an association between prenatal exposure to tetracyclines and childhood asthma [11].

**Strengths and limitations**

A major strength of the present study is the replication of findings in the two study designs. Application of the case-sibling study enabled us to minimize the potential influence of time-invariant confounders that are potential strong risk factors for asthma development. Comparing the results of the case-sibling with the case-control study allowed us to gain insight into the influence of confounding. In addition, this is the first study that evaluated the presence of a time-trend bias in a case-sibling design and corrected for that trend. In contrast to two other recent attempts to evaluate the influence of confounding [10, 12], we took into account that exposure to antibiotics at different time-points are likely correlated within the same mother.

Another strength of our study is that data were obtained from the prescription database IADB.nl [21]. Validation of the identification of mother-infant pairs showed high accuracy, hence potential information bias was minimal. Since we made use of a prescription database, recall bias with respect to maternal medication use during pregnancy was not present.

This study also has potential limitations. First, though we minimized potential confounding influences by design, we cannot rule out the possibility of some unmeasured time-varying confounding in the case-sibling analysis, such as smoking, infections and allergic state of the mother [35, 40].

If smoking did confound our estimate, an increased risk would also be expected for exposure to antibiotics in the three months after delivery since smoking would increase the risk of infection regardless of pregnancy. It is unlikely that women smoke during the pregnancy and stop immediately after the child is born. However, no increased risk was observed after postnatal use of antibiotics, indicating that the results are not confounded by smoking. Moreover, almost identical results were obtained in the case-sibling study

of Örtqvist et al before and after adjustment for maternal smoking, indicating that this is not an important confounder for this association using a sibling-design.

Several studies evaluated the possibility of confounding by respiratory infections by investigating whether specific groups of antibiotics were associated with asthma [8, 11, 12]. Amoxicillin is in those studies often considered as an 'airway antibiotic', however, at least in the Dutch situation, this would be an inadequate categorization as amoxicillin was during the study period the first-choice treatment for urinary tract infections in pregnant women. In our study, in contrast to for example penicillin, exposure to nitrofurantoin showed no increase in the child's asthma risk. Given the limited effect of nitrofurantoin on the vaginal flora and *Lactobacillus* colonization [41], this further supports the hypothesis that a reduced microbial exposure during delivery can predispose the child to allergic diseases like asthma. However, since penicillins, such as amoxicillin are also indicated for respiratory infections, it may also indicate that respiratory infections do confound the association and urinary tract infections do not. Since data on types of infections were not available, we performed a maternal-paternal comparison with data from the case-sibling analysis. Paternal exposure to antibiotics was used as a proxy for infections in the household and other factors shared by both parents that may change between pregnancies. Paternal exposure did not significantly increase the risk of asthma in the child and the point estimate was also lower for paternal than for maternal exposure (aOR 1.11 vs 1.37). However, since pathogens will not always be transmitted to the mother a lower effect estimate would also be expected a priori for paternal exposure. Thus we cannot exclude indication bias due to respiratory infections.

The allergic state of the mother, or disease severity, could also change between pregnancies and be associated with the child's asthma risk. When our analysis was restricted to a subgroup of children whose mothers had not used asthma medication during either pregnancy the child's asthma risk was attenuated but antibiotic use during third trimester was still associated with a 22% increase in the child's asthma risk.

Since we could not rule out potential confounding by time-varying disease severity and (respiratory) infections, we performed sensitivity analyses to assess how strong such an unmeasured confounder should be to account for the observed risk in the third trimester. These analyses showed that potential unmeasured time-varying confounders should have a much stronger association with the outcome and exposure than measured confounders to fully explain our findings. However, given the relatively low power of our case-sibling study, a weaker unmeasured confounder could render our results statistically non-significant.

The IADB.nl contains records of dispensed prescriptions of participating pharmacies and not the actual use of medication. It is possible that some women did not take all of the medication which may have led to small amount of exposure misclassification and an overestimation of actual use. In addition, pregnancy was a standardized at 273 days preceding the date of birth, which also could have led to exposure misclassification. This could have led to over- as well as underestimation of actual use of antibiotic drugs during each trimester of pregnancy.

The database lacks information about indications, therefore case status was determined based on dispensed prescriptions for anti-asthma medications [26]. To ensure high specificity of case selection, cases were required to receive at least 3 prescriptions for anti-asthma medication within a 12 month period. Because of this specific inclusion of cases some children with mild symptoms or untreated asthma may have been missed. However, a recent study of our group in the same source population, showed that still 49% of preschool children with an asthma diagnosis could be identified (positive predictive value 0.77) when the same medication proxy as in this study was used (manuscript in preparation). Given these accuracy measures, this would likely not have materially influenced the results of our study.

Fifth, the database does not include information on the delivery method. Caesarean sections are associated with an increased risk of childhood asthma. Since rates of caesarean sections are low in the Netherlands (1.0-5.5%), this would not have a substantial influence [42]. In addition, the database lacks information on the dispensation of in-hospital and thereby intrapartum antibiotic use [16].

In conclusion, exposure to antibiotics in the third trimester of pregnancy appeared to be associated with a small increased risk of asthma in preschool children. This association did not appear to be influenced by time-invariant confounders or time trends in antibiotic exposure. More studies are now warranted to focus on whether there is indeed a difference for the different trimesters and on the potential mechanisms of the associations.

## References


1. Lai CK, Beasley R, Crane J, Foliaki S, Shah J, Weiland S. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC).Thorax.2009;64:476–483.
2. Henderson AJ, Warner JO. Fetal origins of asthma. Semin Fetal Neonatal Med. 2012;17:82-91.
3. Prescott SL, Clifton V. Asthma and pregnancy: emerging evidence of epigenetic interactions in utero. Curr Opin Allergy Clin Immunol. 2009;9:417– 426.
4. Benn CS, Thorsen P, Jensen JS et al. Maternal vaginal microflora during pregnancy and the risk of asthma hospitalization and use of antiasthma medication in early childhood. J Allergy Clin Immunol. 2002;110:72–77.
5. McKeever TM, Lewis SA, Smith C, Hubbard R. The importance of prenatal exposures on the development of allergic disease: a birth cohort study using the West Midlands General Practice Database. Am J Respir Crit Care Med. 2002;166:827– 832.
6. Rusconi F, Galassi C, Forastiere F et al. Maternal complications and procedures in pregnancy and at birth and wheezing phenotypes in children. Am J Respir Crit Care Med 2007;175:16-21.
7. Murk W, Risnes KR, Bracken MB. Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. Pediatrics. 2011;127:1125-38.
8. Stensballe LG, Simonsen J, Jensen SM, Bonnelykke K, Bisgaard H. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. J Pediatr. 2013;162:832-838.
9. Källén B, Finnström O, Nygren KG, Otterblad Olausson P. Maternal drug use during pregnancy and asthma risk among children. Pediatr Allergy Immunol 2013;24:28-32.
10. Stokholm J, Sevelsted A, Bonnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. Lancet Respir Med. 2014;2:631-7.
11. Metsälä J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. Clin Exp Allergy. 2015;45(1):137-45.
12. Örtqvist AK, Lundholm C, Kieler H, Ludvigsson JF, Fall T, Ye W, Almqvist C. Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis. BMJ. 2014;28;349:g6979.
13. Prokopakis E, Vardouniotis A, Kawauchi H et al. The pathophysiology of the hygiene hypothesis. Int J Pediatr Otorhinolaryngol. 2013 Jul;77:1065-71.
14. Kuo CH, Kuo HF, Huang CH, Yang SN, Lee MS, Hung CH. Early life exposure to antibiotics and the risk of childhood allergic diseases: an update from the perspective of the hygiene hypothesis. J Microbiol Immunol Infect. 2013 Oct;46:320-9.
15. Stokholm J, Schjørring S, Eskildsen CE et al. Antibiotic use during pregnancy alters the commensal vaginal microbiota. Clin Microbiol Infect. 2013 (Epub ahead of print).
16. Keski-Nisula L, Kyynäräinen HR, Kärkkäinen U, Karhukorpi J, Heinonen S, Pekkanen J. Maternal intrapartum antibiotics and decreased vertical transmission of Lactobacillus to neonates during birth. Acta Paediatr. 2013 May;102:480-5.
17. Suissa S.The case-time-control design. Epidemiology 1995;6: 248-53.
18. Hernández-Díaz S, Hernán MA, Meyer K, Werler MM, Mitchell AA. Case-crossover and case-time-control designs in birth defects epidemiology. Am J Epidemiol. 2003 Aug 15;158:385-91.
19. Kawada T. Prevalence of asthma and atopic dermatitis in children with special emphasis on birth order. Pediatr Allergy Immunol. 2012 Dec;23:795.

20. Offerhaus PM, de Jonge A, van der Pal-de Bruin KM, Hukkelhoven CW, Scheepers PL, Lagro-Janssen AL. Change in primary midwife-led care in the Netherlands in 2000-2008: a descriptive study of caesarean sections and other interventions among 789,795 low risk births. Midwifery. 2014;30:560-6.
21. Visser ST, Schuiling-Veninga CC, Bos JH, de Jong-van den Berg LT, Postma MJ. The population-based prescription database IADB.nl: its development, usefulness in outcome research and challenges. Expert Rev Pharmacoecon Outcomes Res. 2013 Jun;13:285-92.
22. Schirm E, Tobi H, de Jong-van den Berg LT. Identifying parents in pharmacy data: a tool for the continuous monitoring of drug exposure to unborn toddlers. J Clin Epidemiol. 2004;57:737-41.
23. Mulder B. Schuiling-Veninga CC, Bos JH, de Vries TW, Hak E. Acid-suppressive drug use in pregnancy and the toddler's asthma risk: a crossover, case-control study. J Allergy Clin Immunol. 2013 Dec;132:1438-40.
24. Hak E, Mulder B, Schuiling-Veninga CC, de Vries TW, Jick SS. Use of acid-suppressive drugs in pregnancy and the risk of childhood asthma: bidirectional crossover study using the general practice research database. Drug Saf. 2013 Nov;36:1097-104.
25. Smith GD. Assessing Intrauterine Influences on Offspring Health Outcomes: Can Epidemiological Studies Yield Robust Findings?. Basic & Clinical Pharmacology & Toxicology, 2008;102:245–56.
26. NHG (Dutch General Practitioner Guidelines). https://www.nhg.org/standaarden/volledig/nhgstandaard-astma-bij-kinderen. Last accessed 8th August 2013.
27. Vines SK, Farrington CP. Within-subject exposure dependency in case-crossover studies. Stat Med. 2001 Oct 30;20:3039-49.
28. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. Clinical &Experimental Allergy. 1993;23:941–48.
29. Koster ES, Van der Ent CK, Uiterwaal CS, Verheij TJ, Raaijmakers JA, Maitland-van der Zee AH. Asthma medication use in infancy: determinants related to prescription of drug therapy. Fam Pract. 2011;28:377-84.
30. Kummeling I, Thijs C. Reverse causation and confounding-by-indication: do they or do they not explain the association between childhood antibiotic treatment and subsequent development of respiratory illness? Clin Exp Allergy. 2008;38:1249-1251.
31. Semic-Jusufagic A, Belgrave D, Pickles A, Telcian AG, Bakhsoliani E et al. Assessing the association of early life antibiotic prescription with asthma exacerbations, impaired antiviral immunity, and genetic variants in 17q21: a population-based birth cohort study. Lancet Respir Med. 2014;2:621-30.
32. Bracken MB, Belanger K, Cookson WO, Triche E, Christiani DC, Leaderer BP. Genetic and perinatal risk factors for asthma onset and severity: a review and theoretical analysis. Epidemiol Rev. 2002;24:176-189.
33. Ter Horst PG, Bos HJ, de Jong-van de Berg LT, Wilffert B. In utero exposure to antidepressants and the use of drugs for pulmonary diseases in children. Eur J Clin Pharmacol. 2013 Mar;69:541-7.
34. Martel MJ, Rey E, Beauchesne MF et al. Control and severity of asthma during pregnancy are associated with asthma incidence in offspring: two-stage case-control study. Eur Respir J. 2009 Sep;34:579-87.
35. Vanderweele TJ, Arah OA. Bias formulas for sensitivity analysis of unmeasured confounding for general outcomes, treatments and confounders. Epidemiology. 2011 Jan;22:42-52.
36. Dzhindzhikhashvili MS, Joks R, Smith-Norowitz T et al. Doxycycline suppresses Chlamydia pneumoniae-mediated increases in ongoing immunoglobulin E and interleukin-4 responses by peripheral blood mononuclear cells of patients with allergic asthma. J Antimicrob Chemother. 2013;68:2363-8.

37. Joks R, Smith-Norowitz T, Nowakowski M, Bluth MH, Durkin HG. Tetracycline-mediated IgE isotype-specific suppression of ongoing human and murine IgE responses in vivo and murine memory IgE responses induced in vitro. Int Immunol. 2010;22:281-8.
38. Smith-Norowitz TA, Bluth MH, Drew H et al. Effect of minocycline and doxycycline on IgE responses. Ann Allergy Asthma Immunol. 2002;89:172-9.
39. Su W, Wan Q, Han L et al. Doxycycline exerts multiple anti-allergy effects to attenuate murine allergic conjunctivitis and systemic anaphylaxis. Biochem Pharmacol. 2014;91:359-68.
40. Scirica CV, Gold DR, Ryan L, Albulkerim H, Celedón JC et al. Predictors of cord blood IgE levels in children at risk for asthma and atopy. J Allergy Clin Immunol. 2007;119:81-8.
41. Raz R, Colodner R, Rohana Y et al. Effectiveness of estriol-containing vaginal pessaries and nitrofurantoin macrocrystal therapy in the prevention of recurrent urinary tract infection of postmenopausal women. Clin Infect Dis. 2003;36:1362-8.

APPENDIX 7.1

# Adjusting the case-sibling analysis for potential time-trends

Potential time-trends in the underlying cohort can influence results of the case-sibling study, especially since exposure periods were compared within the same person i.e. exposure periods where per definition always different periods in time [17,18]. To control for potential time trends in exposure frequencies, we designed a method akin the case-time-control design [17]. In this design we divided the odds ratio obtained with the case-sibling analysis with a "time-trend" odds ratio. This time-trend odds ratio was obtained by comparing exposure frequencies in two different pregnancies (case and control window) within the same mother that resulted both in children without asthma (derived from the control group of the case-control analysis), figure 1. In total 3,503 children without asthma and 3,503 siblings also without asthma could be detected for the time-trend analysis.

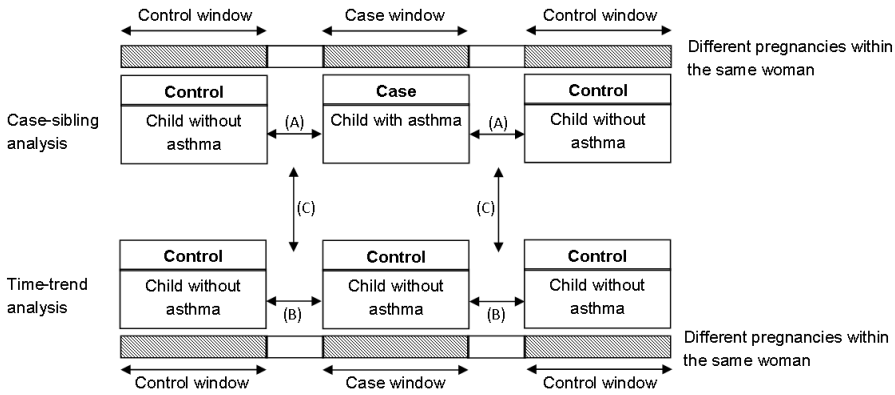


**Figure 7.A1.** Schematic overview of the case-sibling analysis and the adjustment for potential time-trends. The case-sibling odds ratio after adjustments for potential time-trends (C) is the case-sibling odds ratio (A) divided by the time-trend odds ratio (B).

We assigned the case and control windows in the time-trend analysis in such a way that this was similar to the distribution of the birth sequence in the case-sibling analysis. Since 66% of the cases in the case-sibling analysis were born after their control sibling (table 1), 66% of the pregnancies that resembled case-windows in the time-trend analysis occurred after the pregnancy that resembled a control window. By comparing exposure frequencies between different pregnancies within mothers that both resulted in children without asthma and by using a similar distribution in birth sequence as the case-sibling analysis, the odds ratio will be an estimate of the exposure frequency in the underlying cohort, a so called "time-trend" odds ratio. By dividing the odds ratio obtained with the case-sibling analysis with a "time-trend" odds ratio, results will be adjusted for potential

time trends in the underlying cohort. Confidence intervals for the time-trend adjusted odds ratios were computed by bootstrapping.

APPENDIX 7.2

# Sensitivity analysis unmeasured confounding

Since data on smoking, infections and IgE levels during pregnancy are not available in the IADB.nl, we used a simplified sensitivity analysis proposed by VanderWeele et al. to assess the impact of a binary unmeasured confounder or several unmeasured confounders combined on the association between third trimester antibiotic use and childhood asthma in the case-sibling analysis. Assumptions made with this method are (1) that relationships between the outcome and the unmeasured confounder and between the exposure and unmeasured confounder are the same across different levels of measured confounders and (2) that there is no three-way interaction between exposure, outcome and the unmeasured confounder.

For this study, we assumed that the prevalence of the binary unmeasured confounder ranges from 5% to 15% in the unexposed group and varied the association of this confounder with antibiotic use (ORxu) and asthma of the child (ORyu).

The following notations will be used in the formulas to obtain the bias term for the unmeasured confounder:

x Binary treatment status/exposure
y Binary outcome
u Unobserved binary confounder
c Observed confounders
p Prevalence

Formula for the prevalence of the unmeasured confounder among the exposed:

$$p(u|x=1) = \frac{ORxu \times p(u|x=0)}{1 - p(u|x=0) + ORxu \times p(u|x=0)}$$

Formula for the bias factor according to VanderWeele and Arah's approach

$$bias = \frac{1 + (ORyu - 1)p(u|x=1)}{1 + (ORyu - 1)p(u|x=0)}$$

**Table 7.A1.** Sensitivity analysis (according to the approach of VanderWeele and Arah) of an unmeasured confounder on the child's asthma risk after exposure to antibiotics in third trimester of pregnancy using a ORyu of 2.

| aOR (95% CI) | $p(u\|x=0)$ | *ORxu* | *Bias* | *ORyx ● cu (95%CI)* |
|---|---|---|---|---|
| 1.37 (1.02-1.83) | 0.050 | 1.25 | 1,01 | 1,35 (1.01-1.81) |
| 1.37 (1.02-1.83) | 0.050 | 1.50 | 1,02 | 1,34 (1.00-1.79) |
| 1.37 (1.02-1.83) | 0.050 | 1.75 | 1,03 | 1,33 (0.99-1.77) |
| 1.37 (1.02-1.83) | 0.050 | 2.00 | 1,04 | 1,31 (0.98-1.75) |
| 1.37 (1.02-1.83) | 0.050 | 3.00 | 1.08 | 1.27 (0.94-1.69) |
| 1.37 (1.02-1.83) | 0.075 | 1.25 | 1,02 | 1,35 (1.00-1.80) |
| 1.37 (1.02-1.83) | 0.075 | 1.50 | 1,03 | 1,33 (0.99-1.77) |
| 1.37 (1.02-1.83) | 0.075 | 1.75 | 1,05 | 1,31 (0.98-1.75) |
| 1.37 (1.02-1.83) | 0.075 | 2.00 | 1,06 | 1,29 (0.96-1.73) |
| 1.37 (1.02-1.83) | 0.075 | 3.00 | 1.11 | 1.23 (0.92-1.65) |
| 1.37 (1.02-1.83) | 0.100 | 1.25 | 1,02 | 1,34 (1.00-1.79) |
| 1.37 (1.02-1.83) | 0.100 | 1.50 | 1,04 | 1,32 (0.98-1.76) |
| 1.37 (1.02-1.83) | 0.100 | 1.75 | 1,06 | 1,30 (0.96-1.72) |
| 1.37 (1.02-1.83) | 0.100 | 2.00 | 1,07 | 1,28 (0.95-1.70) |
| 1.37 (1.02-1.83) | 0.100 | 3.00 | 1.14 | 1.21 (0.90-1.61 |
| 1.37 (1.02-1.83) | 0.125 | 1.25 | 1,02 | 1,34 (1.00-1.79) |
| 1.37 (1.02-1.83) | 0.125 | 1.50 | 1,05 | 1,31 (0.98-1.75) |
| 1.37 (1.02-1.83) | 0.125 | 1.75 | 1,07 | 1,28 (0.96-1.72) |
| 1.37 (1.02-1.83) | 0.125 | 2.00 | 1,09 | 1,26 (0.94-1.68) |
| 1.37 (1.02-1.83) | 0.125 | 3.00 | 1.16 | 1.19 (0.88-1.58) |

Table A1 shows the influence of a binary unmeasured confounder that increases the risk of asthma in the offspring 2 times (ORyu). To bias the effect estimate from 1.00 to 1.37, the prevalence of this confounder among exposed pregnant women should be 5.1 times higher than the prevalence among unexposed women (assuming a prevalence of 0.10 in unexposed women). The prevalence of this confounder among exposed women would then be 51% vs. 10% among unexposed women.

If the prevalence of the binary confounder is 2 times higher than the prevalence among unexposed women (assuming a prevalence of 0.10 in unexposed women), the OR between this confounder and asthma in the offspring (ORyu) should be 6.9 to bias the effect estimate from 1.00 to 1.37.

**Table 7.A2.** Sensitivity analysis of an unmeasured confounder on the child's asthma risk after exposure to antibiotics in third trimester of pregnancy using a p(u,x=0) of 0,1.

| aOR (95% CI) | $p(u\|x=1)$* | *ORyu* | *Bias* | *ORyx ● cu (95%CI)* |
|---|---|---|---|---|
| **1.37 (1.02-1.83)** | 0.125 | 1.5 | 1,01 | 1,35 (1.01-1.81) |
| **1.37 (1.02-1.83)** | 0.125 | 2 | 1,02 | 1,34 (1.00-1.79) |
| **1.37 (1.02-1.83)** | 0.125 | 3 | 1,04 | 1,32 (0.98-1.76) |
| **1.37 (1.02-1.83)** | 0.125 | 4 | 1,06 | 1,30 (0.96-1.73) |
| **1.37 (1.02-1.83)** | 0.150 | 1.5 | 1,02 | 1,34 (1.00-1.79) |
| **1.37 (1.02-1.83)** | 0.150 | 2 | 1,05 | 1,31 (0.95-1.75) |
| **1.37 (1.02-1.83)** | 0.150 | 3 | 1,08 | 1,26 (0.94-1.69) |
| **1.37 (1.02-1.83)** | 0.150 | 4 | 1,12 | 1,23 (0.91-1.64) |
| **1.37 (1.02-1.83)** | 0.175 | 1.5 | 1,04 | 1,32 (0.98-1.77) |
| **1.37 (1.02-1.83)** | 0.175 | 2 | 1,07 | 1,28 (0.95-1.71) |
| **1.37 (1.02-1.83)** | 0.175 | 3 | 1,13 | 1,22 (0.91-1.63) |
| **1.37 (1.02-1.83)** | 0.175 | 4 | 1,17 | 1,17 (0.87-1.56) |
| **1.37 (1.02-1.83)** | 0.200 | 1.5 | 1,05 | 1,31 (0.97-1.75) |
| **1.37 (1.02-1.83)** | 0.200 | 2 | 1,09 | 1,26 (0.94-1.68) |
| **1.37 (1.02-1.83)** | 0.200 | 3 | 1,17 | 1,17 (0.87-1.57) |
| **1.37 (1.02-1.83)** | 0.200 | 4 | 1,23 | 1,11 (0.83-1.49) |
| **1.37 (1.02-1.83)** | 0.250 | 1.5 | 1,07 | 1,28 (0.95-1.71) |
| **1.37 (1.02-1.83)** | 0.250 | 2 | 1,14 | 1,21 (0.90-1.61) |
| **1.37 (1.02-1.83)** | 0.250 | 3 | 1,25 | 1,10 (0.82-1.46) |
| **1.37 (1.02-1.83)** | 0.250 | 4 | 1,35 | 1,02 (0.76-1.36) |

* $p(u|x=0)$ = 0.10

APPENDIX 7.3

# Distribution of covariates between exposed and unexposed in case-sibling analysis

| | Case-sibling analysis | | | Case-control analysis | | |
|---|---|---|---|---|---|---|
| | n (%) | n (%) | | n (%) | n (%) | |
| | Exposed (N=575) | Unexposed (N=1881) | P-value | Exposed (N=4969) | Unexposed (N=21309) | P-value |
| ***Child characteristics*** | | | | | | |
| Male gender | 336 (58.4) | 1044 (55.5) | 0.215 | 2533 (51.9) | 10,647 (49.8) | 0.009 |
| Birth order: First born | 263 (45.7) | 965 (51.3) | 0.020 | | | |
| Use of ABs before index date | 424 (73.7) | 1146 (60.9) | <0.001 | 2742 (56.1) | 9754 (45.6) | <0.001 |
| ***Mother characteristics*** | | | | | | |
| Mean age in years | 29.9 | 29.8 | 0.575 | 29.8 | 30.1 | <0.001 |
| Use of medication for atopic diseases during pregnancy | 213 (37.0) | 421 (22.4) | <0.001 | 1566 (31.5) | 3913 (18.4) | <0.001 |
| Asthma medication | 59 (10.4) | 96 (5.1) | <0.001 | 376 (7.6) | 623 (2.9) | <0.001 |
| Drugs for atopic dermatitis | 71 (12.3) | 162 (8.6) | 0.007 | 592 (11.9) | 1749 (8.2) | <0.001 |
| Drugs for rhinitis | 124 (21.6) | 228 (12.1) | 0.000 | 882 (17.8) | 2055 (9.6) | <0.001 |
| Use of acid suppressive drugs during pregnancy (ATC A02B) | 34 (5.9) | 43 (2.3) | <0.001 | 34 (5.9) | 43 (2.3) | <0.001 |
| Use of insulin during pregnancy | 4 (0.7) | 14 (0.7) | 0.905 | 4 (0.7) | 14 (0.7) | 0.905 |
| Use of antidepressants during pregnancy | 17 (3.0) | 40 (2.1) | 0.247 | 17 (3.0) | 40 (2.1) | 0.247 |
| ***Father characteristics*** | | | | | | |
| Data present* | 903 | 835 | | 3220 | 12950 | |
| Use of medication for atopic diseases during pregnancy | 115 (27.3) | 297 (22.6) | 0.045 | 812 (25.2) | 2883 (22.3) | <0.001 |
| Asthma medication | 29 (6.9) | 94 (7.1) | 0.862 | 188 (5.8) | 658 (5.1) | 0.084 |
| Drugs for atopic dermatitis | 66 (15.7) | 130 (9.9) | 0.001 | 390 (12.1) | 1349 (10.4) | 0.005 |
| Drugs for rhinitis | 44 (10.5) | 130 (9.9) | 0.730 | 382 (11.9) | 1405 (10.8) | 0.101 |

CHAPTER 8

# Systematic comparison of effect estimates from case-only designs with traditional observational study designs

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This chapter is based on the published manuscript:
Pouwels KB, Mulder B, Hak E. Moderate concordance was found between case-only and parallel group designs in systematic comparison. *J Clin Epidemiol* 2015 [in press].

## Abstract

### Background

It is common to compare estimates from case-only designs and cohort or case-control studies to gain an indication about potential unmeasured confounding. We aimed to evaluate the concordance of effect estimates between case-only and case-control or cohort studies, and to identify predictors of discrepancy.

### Methods

MEDLINE and EMBASE databases were searched through 31 June 2013. Studies that used both a case-only (case-crossover or self-controlled case-series) and a parallel group design (cohort or case-control) were identified. Spearman correlation coefficient was used to evaluate the concordance between designs. Z-scores were used to assess whether differences in the effect estimates were common, using an absolute threshold value of 1.96. A prediction model was built to identify predictors of discrepancies.

### Results

The search identified 1,367 articles of which 53 were included for analysis. In total 519 comparisons were made. The correlation coefficient between case-only versus parallel group studies was 0.64 ($p<.001$). In 221 of the 519 comparisons (43%) the difference between both study designs was larger than the predetermined threshold. The following predictors of discrepancy were found: intermittent exposure, rare event, acute outcome, length of hazard period, type of case-only design and sample size (c-statistic of 0.783).

### Conclusions

The concordance between effect estimates of case-only and parallel group designs is moderate. Such discrepancies could be predicted by failure to meet assumptions of case-only designs.

## Introduction

One of the most important and challenging tasks in epidemiology is identifying appropriate comparison groups. Randomization is the best way to guarantee that comparison groups of patients will have similar characteristics. If the exposure of interest is allocated randomly and if the trial is large enough with no substantial dropout, risks of health outcomes will be similar, except for the potential benefits and risks associated with the exposure. However, often it is unethical or not feasible to perform a randomized controlled trial and observational studies may guide decision making.

To obtain valid effect estimates from observational data, it is crucial to measure and adjust for all relevant confounders. However, information on important potential confounders is often lacking in routine health care data [1-4]. Even when the effect estimates are adjusted for measured potential confounders, biased estimates might be obtained, especially when difficult to measure patient characteristics are expected to confound the association.

Case-only designs, such as the self-controlled case-series [5-7], case-crossover design [8,9], and sequence symmetry analysis [10-13], have been developed to overcome the problem of identifying a comparable control group and have become more popular in recent years. The underlying idea of such designs is that patients can serve as their own controls. The main advantage of such self-controlled comparisons is that it reduces confounding by factors that are stable over time, including characteristics that are often not available to a researcher, such as chronic use of nonprescription drugs, health behaviors, tendency to seek professional care, occupation, etcetera [14].

Given this theoretical advantage of case-only studies, it is increasingly common to compare the results of more traditional parallel group observational study designs, such as cohort and case-control studies, with case-only designs to gain an indication of the potential influence of unmeasured confounders. Although a difference in results between both types of designs may indeed indicate the influence of unmeasured time-invariant confounding, other reasons may underlie such a difference, such as selection of a different study sample [15], different impact of measurement error [14-16], time-variant confounding or failure to meet important assumptions of the case-only design. For example, a difference may be caused by studying the effect of chronic exposures, insidious outcomes with a long induction period, or recurrent events that are not independent [17]. As case-only designs are often applied in situations where at least one assumption is not met but not all assumptions are equally important, it would

be relevant to empirically evaluate how frequent case-only designs and parallel group designs would give discrepant results and whether there are strong predictors of such discrepancies.

For the current study, articles that made a comparison between the two most common case-only designs, the self-controlled case-series and case-crossover design, and the more traditional parallel group designs, the case-control and cohort design, were systematically identified and reviewed. The primary objective was to evaluate the concordance between case-only and parallel group designs. As secondary objective we evaluated whether there are certain study characteristics that predict a difference in effect estimates between case-only and the more traditional parallel group designs.

## Methods

### Article selection from literature databases

We searched the MEDLINE and EMBASE database from inception till 31 June 2013 to identify all articles that used both a case-crossover or self-controlled case-series and a case-control or cohort design to evaluate the effect of a medical or behavioral intervention. Only articles with identical exposure, outcome and risk-period definitions for both types of designs were included. We used the following keyword terms: "case-crossover" or "case crossover" or "case cross-over" or "self-controlled case series" or "self controlled case series" or "self-controlled case-series" or "case series analysis" or ("self control" AND "case series"). We excluded reviews, methodological studies, studies using simulated data, studies without an intervention (e.g. influence of COPD exacerbations or infections), and studies without a comparison between a case-only and a parallel group design and studies on environmental exposures. We included only studies that used the same data source for both study designs to avoid discrepancies that are due to data source heterogeneity instead of differences in study design. Retrieved citations were exported to Refworks (ProQuest, Ann Arbor, Michigan). Title and abstract screening was performed including all possibly relevant studies for further review. The full text of all remaining studies were independently retrieved and reviewed for eligibility by two reviewers (KP, BM). Disagreement was solved by consensus in all cases.

### Data collection

For each included study we subsequently extracted the following study characteristics: first author, journal, year of publication, study designs, outcome variable, exposure, and type of intervention (drug, vaccination, behavioral, or other medical interventions).

In addition, we extracted the following data for the prediction part of the current study: whether an intermittent exposure (interventions were classified as intermittent if it was a brief exposure such as vaccination or typically applied intermittently such physical exercise) , a rare event (if less than 10% of the patients experienced the event during follow-up, it was considered a rare event) and an acute outcome (acute outcomes included events that are acute and have a clear onset, e.g. motor vehicle crashes or acute liver injury) was investigated; the length of the hazard period; whether adjustment for time-variant confounding was performed; whether a washout period was included, whether a safety or effectiveness question was evaluated; and the sample size of the cohort or case-control study.

#### Exposure-outcome associations

In studies that assessed multiple exposures, outcomes and/or hazard periods, the associations that were evaluated as primary analysis of the case-only design (as indicated by the authors) were used. In case a primary analysis could not be distinguished, all evaluated associations were included. The data extraction was independently performed by two reviewers (KP, BM). Disagreement was solved by consensus in all cases.

#### Concordance in effect estimates between study designs

To evaluate the concordance between the results obtained with case-crossover or self-controlled case-series analyses with those from cohort or case-control studies, we performed two analyses. First, we evaluated the Spearman correlation coefficient to determine the association between effect estimates (OR/HR/RR) of case-only and parallel group studies.

Second, we evaluated whether the difference in the effect estimates between case-only and parallel group studies exceeded a predetermined threshold value of the z-score ($> |1.96|$) when comparing both types of designs, as previously done by Ioannidis et al [18]. It should be noted that because we only included studies that made a comparison between a case-only and a parallel group design using the same dataset, observations are not independent. Hence, an absolute value of the z-score greater than 1.96 does not indicate a difference beyond chance [19], but it is an indicator how large the difference is between both types of designs that takes into account the precision of the estimates.

#### Prediction of discrepant results

In secondary analyses, we evaluated whether the odds of discrepant results were dependent on certain study characteristics. This was done by performing a multivariable

logistic regression with a binary variable (yes/no) indicating whether the absolute value of the z-score was greater than 1.96. As potential predictors the following variables were included: intermittent exposure (yes/no); rare event (yes/no); acute outcome (yes/no); length of hazard period (≤1 day, 2-31 days, ≥32 days, variable); case-only design (self-controlled case-series/case-crossover); other design (cohort/case-control); adjustment for time-variant confounding (yes/no); safety study (yes/no); use of a washout period (yes/no); sample size traditional study design (≤10.000, >10.000 and ≤100.000, >100.000).

These data were extracted from the included studies by two reviewers (KP, BM). Disagreement was again solved by consensus. An automated backward predictor selection procedure was performed based on the Akaike information criterion (AIC). The performance of the final model was assessed with the c-statistic and its 95% confidence intervals (CI). Analyses were conducted using the statistical software R (version 3.0.2). All p-values are two-sided.

**Sensitivity analysis**

The study of Madigan et al contributed multiple comparisons to our analyses. That study evaluated several drug-outcome pairs across different databases. In our primary analyses, we extracted the database-specific estimates, since substantial heterogeneity was observed between databases in the study of Madigan et al [20]. As such, the contribution of the study of Madigan et al was very large with 281 comparisons (54% of the total number of comparisons) [20].

In sensitivity analyses, we repeated our analyses using the random effects meta-analysis effect estimates for the same drug-outcome pairs across different databases from the study of Madigan et al [20], instead of using database-specific estimates. For example, a meta-analysis effect estimate of the effect of angiotensin-coverting enzyme inhibitors on angioedema was obtained by summarizing the effect estimates across the 10 different databases into a single estimate using a random effects model [20]. This was done for both the self-controlled case-series and the cohort design, after which the concordance between meta-analysis estimates were compared for each drug-outcome pair. As a consequence the influence of the study of Madigan et al became less strong (50/288 = 17% of the total number of comparisons) [20].

Because the Z-score, as a measure of discrepancy between study designs, is vulnerable to correlations between compared datasets, we defined in a second sensitivity analysis discrepant results as comparisons in which the effect estimate of the case-only study was at least 50% larger or smaller than the natural logarithm of the effect estimate of the parallel group design [18]. Obviously, this definition does not take into account

the precision of the results and it should be noted that several analyses had very wide confidence intervals. To prevent that the selection of predictors would be strongly influenced by underpowered comparisons, we excluded comparisons where one of the designs had wide confidence intervals (natural logarithm of upper confidence limit – natural logarithm of lower confidence limit > 1.5).

In a third sensitivity analysis, we repeated the prediction model using post-estimation global shrinkage of regression coefficients to gain an indication about potential overfitting.

## Results

The search identified 1367 unique articles, of which 324 were considered potentially eligible for inclusion based on title and abstract screening. After reading the full texts 53 articles were included for analysis (Figure 8.1 and Table 8.A1), of which 17 articles compared a self-controlled case-series design with a parallel group design, 34 articles compared a case-crossover with a parallel group design, one article compared a self-controlled case-series with both a cohort and case-control design, and one article compared both case-only designs with both a cohort and case-control designs. Some studies contributed to our analyses with various comparisons, such as the study of Madigan et al [20].

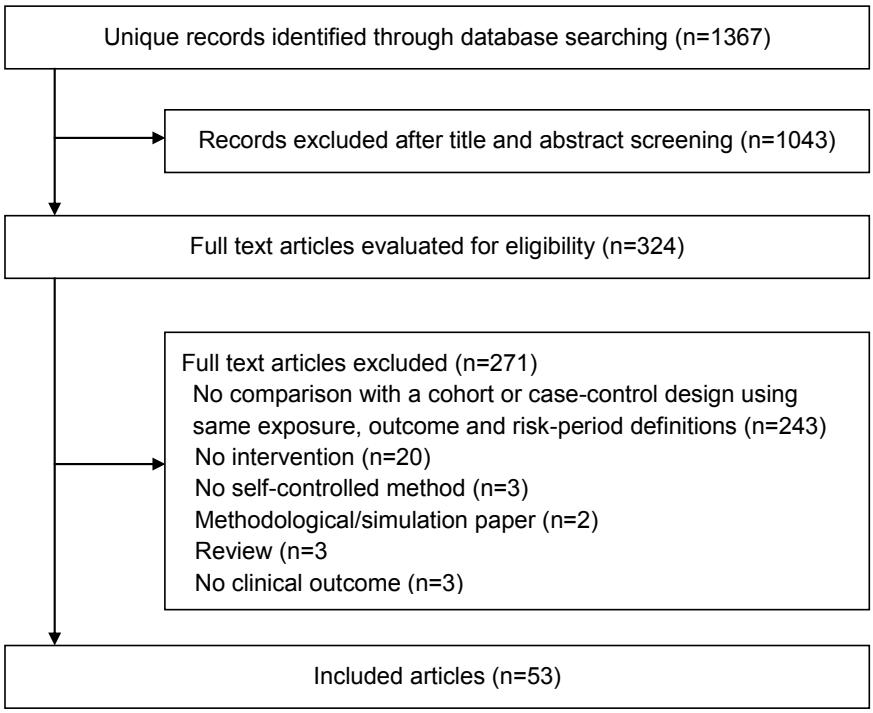


**Figure 8.1.** Flow diagram for study selection

In total 519 comparisons were made between case-only and parallel group designs. Of these, 463 evaluated the effect of a drug; 13 assessed the effect of a behavioral intervention; 33 evaluated the effect of vaccination, while 10 articles evaluated other medical interventions (e.g. chiropractic visits). The correlation coefficient between the treatment effect in case-only and parallel group studies was 0.64 ($p<.001$). A scatter plot visualizing the correlation between the effect estimates in both types of designs is shown in the appendix (Figure 8.B1). In 307 of 519 comparisons (59%), the case-only study showed a stronger effect size (effect estimate further away from 1) than the parallel group study. In 131 of 519 comparisons (25%) were qualitatively different in the way that one design had a point estimate above one while the other design had a point estimate below one. In 221 of the 519 comparisons (43%) there was a discrepancy between case-only and the parallel group design when using the z-score to define discrepancies (Table 8.1). Discrepancies with parallel group designs were more frequently seen for the self-controlled case-series than for the case-crossover design (52% vs 23%).

Subsequently, we assessed whether the odds of discrepant results were dependent on certain study characteristics. Univariate associations are shown in the appendix (Table 8.C1). Backward selection based on the AIC resulted in a final model including the following predictors: intermittent exposure, rare event, acute outcome, length of hazard period, type of case-only design and sample size of the traditional study design (Table 8.2). This model appeared accurate with a high discriminative value (c-statistic of 0.783; 95% CI 0.744-0.821).

**Table 8.1.** Frequency of discrepancies among case-only designs and cohort/case-control designs.

| Comparison | No (%) of discrepancies * | No (%) of discrepancies *,# | No (%) of discrepancies ¥ |
|---|---|---|---|
| Case-only vs cohort/case-control (n=519; n=288#; n=440¥) | 221 (43) | 78 (27) | 329 (75) |
| Case-only vs cohort (n=372; n=141#; n=348¥) | 195 (52) | 52 (37) | 291 (84) |
| Case-only vs case-control (n=147; n=92¥) | 26 (18) | 26 (18) | 38 (41) |
| Self-controlled case-series vs cohort/case-control (n=347; n=116#; n=319¥) | 182 (52) | 39 (34) | 268 (84) |
| Self-controlled case-series vs cohort (n=305; n=74#; n=290¥) | 174 (57) | 31 (42) | 255 (88) |
| Self-controlled case-series vs case-control (n=42; n=29¥) | 8 (19) | 8 (19) | 13 (45) |
| Case-crossover vs cohort/case-control (n=172; n=121¥) | 39 (23) | 39 (23) | 61 (50) |
| Case-crossover vs cohort (n=67; n=58¥) | 21 (31) | 21 (31) | 36 (62) |
| Case-crossover vs case-control (n=105; n=63¥) | 18 (17) | 18 (17) | 25 (40) |

* Discrepancies were characterized by an absolute value of the z-score >1.96

# Using random effects meta-analyses for the same drug-outcome pairs across different databases from the study of Madigan et al.

¥ Discrepancies were characterized by the natural logarithm of the effect estimate of the case-only design being ≥50% larger or smaller than the natural logarithm of the effect estimate of the parallel group design.

In accordance with the assumptions of the case-only designs, an intermittent/short-term exposure, a rare outcome and an acute outcome all predicted lower odds of discrepant results between case-only designs and parallel group designs (Table 2). A variable length of the hazard period and a larger sample size were associated with higher odds of discrepant results. The use of self-controlled case-series was associated with a lower odds of discrepant results compared with use of the case-crossover design. This may seem counterintuitive given the higher percentage of discrepancies for self-controlled case-series (52 vs 23%), however, this could be explained by certain characteristics (other predictors in the model) of the comparisons of the study of Madigan et al [20]. Excluding that study resulted in a lower percentage of discrepancies for the self-controlled case-series than for the case-crossover design (17% vs 23%).

**Sensitivity analyses**

The proportion of discrepancies decreased substantially when random effects meta-analyses for the same drug-outcome pairs across different databases from the study of Madigan et al [20]. were used instead of separate estimates for each different database (from 43% to 27%, Table 8.1). A prediction model based on these data differed from the model developed on the data including all database-specific estimates.

In contrast to that other model the following predictors were not included: intermittent exposure and whether the outcome was a rare event (Table 8.2).

This model had a comparable c-statistic of 0.782 (95% CI 0.720-0.843). The associations of the selected predictors with the odds of discrepant results differed in magnitude compared to the other model, but the directions were the same.

After excluding studies with very wide confidence intervals, when defining discrepant results based on a 50% difference between estimates on the natural logarithm scale, the proportion of discrepancies increased substantially. Using this definition, 329 of 440 (75%) comparisons would be labeled as discrepant. The prediction model, using database-specific estimates of the study of Madigan et al [20], also changed. This model had a c-statistic of 0.824 (95% CI 0.778-0.869). Since precision is ignored using this outcome and underpowered comparisons were excluded, the sample size of the parallel group design, although included as a potential predictor, was not selected as a predictor in the final model (Table 8.2).

Moreover, whether the outcome was a rare event was not included as a predictor, while using a wash out period and the design of the parallel group designs were in contrast to the main analysis included in the final model. Including a washout period

**Table 8.2.** Association between predictors and discrepant results between case-only design and parallel group designs using multivariate logistic regression.

| Predictor | OR (95% CI) *, # | OR (95% CI) *, § | OR (95% CI) ¥ |
|---|---|---|---|
| Intermittent/short-term exposure, yes vs no | 0.65 (0.40-1.05) | - | 0.31 (0.17-0.56) |
| Rare event, yes vs no | 0.40 (0.22-0.73) | - | - |
| Acute outcome, yes vs no | 0.34 (0.15-0.73) | 0.03 (0.00-0.21) | 0.17 (0.01-0.85) |
| Hazard period | | | |
| ≤1 day | Ref. | Ref. | Ref. |
| 2-31 days | 0.65 (0.16-2.92) | 0.75 (0.19-3.29) | 0.56 (0.10-2.95) |
| ≥32 days | 1.87 (0.48-8.27) | 2.09 (0.55-9.20) | 1.47 (0.21-10.33) |
| Variable | 9.66 (2.01-54.07) | 12.67 (2.53-73.44) | 2.27 (0.31-14.81) |
| Case-only design (SCCS vs CCO) | 0.29 (0.10-0.74) | 0.20 (0.06-0.56) | 0.27 (0.08-0.81) |
| Sample size | | | |
| ≤10.000 | Ref. | Ref. | - |
| >10.000 and ≤100.000 | 6.80 (2.33-22.23) | 8.69 (3.08-27.36) | - |
| >100.000 | 4.87 (1.53-17.44) | 3.88 (1.27-13.35) | - |
| Parallel group design (cohort vs case-control) | - | - | 3.24 (1.70-6.27) |
| Wash-out period, yes vs no | - | - | 0.38 (0.12-1.07) |

* Discrepancies were characterized by the absolute value of the z-score >1.96.
# Primary analysis (n= 519 comparisons).
§ Using random effects meta-analyses for the same drug-outcome pairs across different databases from the study of Madigan et al (n=288 comparisons).
¥ Discrepancies were characterized by the natural logarithm of the effect estimate of the case-only design being ≥50% larger or smaller than the natural logarithm of the effect estimate of the parallel group design (n=440 comparisons).

was associated with reduced odds of discrepant results, while more discrepancies were found when comparing with cohort studies than with case-control studies. Of note, if we ignored the influence of sample size in the primary analysis, using a washout period was also included as a predictor in the final model, while the rest of the model remained qualitatively the same.

Similar results were obtained when post-estimation shrinkage of regression coefficients was applied (shrinkage factor 0.9), suggesting that overfitting was not a substantial problem.

## Discussion

A moderate correlation in the effect estimates of case-only and the more traditional parallel group designs was found. As correlation does not necessarily imply similar effect sizes, it was also evaluated whether case-only studies tended to show stronger effect estimates. We indeed observed that case-only designs showed more often stronger effect estimates than the parallel group designs. In fact, in 43% of the comparisons, the difference between the study designs was beyond the predetermined threshold value of the z-score. As the Z-score as a measure of discrepancy between studies is vulnerable to correlations between compared datasets, we also applied a method suggested by Ioannidis et al [18], taking a threshold of ≥50% difference in the natural logarithm of the effect estimate between case-only and the parallel group design, and this analysis confirmed our results. Moreover, such large discrepancies can not be fully explained by differences in statistical properties of the applied statistical analysis, e.g. difference between conditional and logistic regression analysis, alone.

Case-only designs are predominantly applied because based on subject matter knowledge a-priori the investigators have concerns about potential for unmeasured confounding in traditional parallel group study designs. Therefore, a difference in effect estimates is often regarded as evidence of the presence of unmeasured confounding, while concordance between both types of designs is regarded as evidence that unmeasured confounding does not play an important role. However, if important assumptions of case-only designs are not met a difference can occur even without the presence of unmeasured confounding.

In this study we found that failure to meet important assumptions of the case-crossover and/or self-controlled case-series was associated with increased odds of discrepant results between effect estimates of case-only and the traditional parallel group designs.

Evaluating an intermittent or short-term exposure, a rare outcome and an acute outcome were associated with reduced odds of discrepant results. These predictors are all requirements of the case-crossover design [17]. Having a rare outcome (or independent recurrent events) and an acute outcome are also assumptions of the self-controlled case-series. When applying standard self-controlled case-series, dependences between events

may reintroduce bias due to unmeasured time-invariant confounders [21]. Having an acute outcome is important, since misclassification bias may occur when studying insidious outcomes for which it is difficult to determine the time of onset. Analyzing an acute outcome and the choice of the length of the hazard period were the only predictors (besides the type of case-only design) that were included in the final model in both the primary analysis and the two different sensitivity analyses, suggesting that those are the factors that certainly should be taken into account when considering to apply a case-only design. Using long hazard periods and studying insidious outcomes increases the likelihood of time-varying confounding and misclassification, which may be problematic when applying case-only designs, as both types of biases have a stronger impact on case-only designs than on traditional parallel group designs [22,23].

**Strengths and weaknesses**

This is the first study that systematically compared case-only with the more traditional parallel group study designs using published empirical data. An important strength of our study is the large sample size. We included only studies that used the same data source and similar criteria for both study designs, thereby avoiding discrepancies that are due to data source heterogeneity or for example outcome definitions instead of differences in study design [20]. In addition, this is the first study that evaluated whether discrepancies between case-only and parallel group designs can be predicted by certain study characteristics. It appeared that even without knowledge about potential unmeasured confounding, a good degree of discrimination between comparisons with and without discrepancies could be achieved.

This study has some potential limitations. Discrepancies may arise when different effect measures are used for the different designs, which could results in differences solely due to non-collapsibility of one of the effect measures. However, in almost all comparisons the same effect measure was used and/or a rare outcome was evaluated, a situation in which non-collapsibility is less relevant. The systematic comparison between case-only and parallel group studies is based on published articles. It is possible that investigators though they applied both types of designs, only published either the case-only or parallel group design. In case of discrepant findings, it is possible that researchers decide to publish only the design that is congruent with their hypothesis. If this would be the case, our comparison would have overestimated the concordance of case-only and parallel group designs. On the other hand, a comparison between both types of designs is more likely to be performed when there are concerns about unmeasured confounders, thereby decreasing the likelihood of concordance. Nevertheless, we observed that discrepancies could be reasonably predicted by a model without information about potential time-

invariant unmeasured confounding, suggesting that other factors may play an important role. Although we included several assumptions of the case-only designs as potential predictor variables of a discrepancy between case-only and parallel group designs, we did not include all assumptions, as this may be difficult to assess without having the original data at hand. For example, without those data it is difficult to assess for all studies whether there are exposure time-trends or if and to what degree events influence the probability of future exposure. Nevertheless, our final model based on all data had a good degree of discrimination based on few characteristics.

**Comparison with prior studies**

Several reviews about case-only designs are available in the literature that provided theoretical comparisons or evaluated reporting related to case-only designs [24-26]. Two of those reviews concluded that (reporting of) methodological standards of case-only studies should be improved [24-26]. Nordmann et al regarded conditional logistic regression as the only correct model for case-crossover studies and conditional Poisson regression as the only correct model for self-controlled case series [24-26]. However, it is well known that conditional logistic regression can be performed using a stratified Cox's proportional hazards model, with strata representing the matched sets [27]. Similarly, a Cox's stratified proportional hazards model and a conditional logistic regression model both produce the same effect estimates and variances in self-controlled case-series as a conditional Poisson regression model, if each event is treated like a separate individual and recurrent events are independent of each other [27,28]. Given the fact that both case-only models can be analyzed using different kind of statistical analyses that give similar or identical results, we did not include the type of statistical analyses as a predictor of discrepancies between case-only and parallel group designs.

The large study of Madigan et al contributed a large proportion of the comparisons between self-controlled case-series and cohort designs to our study [20]. In that study a higher percentage of discrepancies was found than in our study. Part of the higher percentage of discrepancies in that study may be due to the fact that that study was not vulnerable to publication bias. In addition, this may be due to the characteristics of certain drug-outcome pairs evaluated in the study of Madigan et al [20]. For example, they evaluated several drugs that are often used chronically (e.g. bisphosphonates, beta blockers and tricyclic antidepressants), outcomes that may alter the probability of further exposure (e.g. studying well-known side effects such as angiotensin converting enzyme inhibitors – angioedema and warfarin-bleeding) and outcomes that may alter the short-term mortality probability (e.g. acute myocardial infarction). Such factors increase the risk of bias in standard self-controlled case-series. Indeed, studying an exposure that is

not used intermittently predicted higher odds of discrepancies between case-only and parallel group designs in the current study.

**Conclusion**

The correlation between effect estimates of case-only and parallel group designs is moderate, and discrepancies are very common. Such discrepancies could be predicted by failure to meet assumptions of case-only designs. Especially the choice of outcome, acute vs not acute, and the choice of the length of the hazard period(s) appeared to be important predictors, stressing the need for evaluating the robustness of the results to the choice of the length of the hazard period in sensitivity analysis. It is important that researchers are aware that there may be other causes of discrepancies or agreement between both types of designs than the presence or absence of time-invariant unmeasured confounding.

## References


1. Groenwold RH, Hoes AW, Nichol KL, Hak E. Quantifying the potential role of unmeasured confounders: the example of influenza vaccination. *Int J Epidemiol* 2008;37:1422-9.
2. Strom BL. Overview of automated databases in pharmacoepidemiology. In: Pharmacoepidemiology, 5th ed. Malden (MA): Wiley 2012. p158-62.
3. Pouwels KB, Hak E. Re: "a prospective study of statin drug use and lower urinary tract symptoms in older men". *Am J Epidemiol* 2014;179:927.
4. Pouwels KB, Kalkman GA, Schagen D, Visser ST, Hak E. Is combined use of SSRIs and NSAIDs associated with an increased risk of starting peptic ulcer treatment? *Br J Clin Pharmacol* 2014;78:192-3.
5. Farrington P, Pugh S, Colville A, 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.
6. Farrington CP. Relative incidence estimation from case series for vaccine safety evaluation. *Biometrics* 1995;51:228-35.
7. Whitaker HJ, Farrington CP, Spiessens B, Musonda P. Tutorial in biostatistics: the self-controlled case series method. *Stat Med* 2006;25:1768-97.
8. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. *Am J Epidemiol* 1991;133:144-53.
9. Pouwels KB, Bos JH, Hak E. ACE inhibitors and urinary tract infections. *Epidemiology* 2014;25:466-7.
10. Pouwels KB, Visser ST, Bos HJ, Hak E. Angiotensin-Converting Enzyme Inhibitor Treatment and the Development of Urinary Tract Infections: A Prescription Sequence Symmetry Analysis. *Drug Saf* 2013;36:1079-86.
11. Garrison SR, Dormuth CR, Morrow RL, Carney GA, Khan KM. Nocturnal leg cramps and prescription use that precedes them: a sequence symmetry analysis. *Arch Intern Med* 2012;172:120-6.
12. van Boven JF, de Jong-van den Berg LT, Vegter S. Inhaled corticosteroids and the occurrence of oral candidiasis: a prescription sequence symmetry analysis. *Drug Saf* 2013;36:231-6.
13. Hallas J. Evidence of depression provoked by cardiovascular medication: a prescription sequence symmetry analysis. *Epidemiology* 1996;7:478-84.
14. Maclure M, Fireman B, Nelson JC, et al. When should case-only designs be used for safety monitoring of medical products? *Pharmacoepidemiol Drug Saf* 2012;21Suppl 1: 50-61.
15. Maclure M. 'Why me?' versus 'why now?'--differences between operational hypotheses in case-control versus case-crossover studies. *Pharmacoepidemiol Drug Saf* 2007;16:850-3.
16. Greenland S. The effect of misclassification in matched-pair case-control studies. *Am J Epidemiol* 1982;116:402-6.
17. Maclure M, Mittleman MA. Should we use a case-crossover design? *Annu Rev Public Health* 2000;21:193-221.
18. Ioannidis JP, Haidich AB, Pappa M, et al. Comparison of evidence of treatment effects in randomized and nonrandomized studies. *JAMA* 2001;286:821-30.
19. Altman DG, Bland JM. Interaction revisited: the difference between two estimates. *BMJ* 2003;326:219.
20. Madigan D, Ryan PB, Schuemie M, et al. Evaluating the impact of database heterogeneity on observational study results. *Am J Epidemiol* 2013;178:645-51.
21. Farrington C, Hocine MN. Within-individual dependence in self-controlled case series models for recurrent events. *Journal of the Royal Statistical Society: Series C (Applied Statistics)* 2010;59:457-75.

22. Greenland S. Confounding and exposure trends in case-crossover and case-time-control designs. *Epidemiology* 1996;7:231-9.
23. Frisell T, Oberg S, Kuja-Halkola R, Sjolander A. Sibling comparison designs: bias from non-shared confounders and measurement error.
24. Nordmann S, Biard L, Ravaud P, Esposito-Farese M, Tubach F. Case-only designs in pharmacoepidemiology: a systematic review. *PLoS One* 2012;7:e49444.
25. Consiglio GP, Cadarette SM. Systematic review of the case-crossover design in pharmacoepidemiology. *J Popul ther Clin Pharmacol* 2012;19:e139-40.
26. Hallas J, Pottegard A. Use of self-controlled designs in pharmacoepidemiology. *J Intern Med* 2014;275:581-9.
27. Xu S, Gargiullo P, Mullooly J, McClure D, Hambidge SJ, Glanz J. Fitting parametric and semi-parametric conditional Poisson regression models with Cox's partial likelihood in self-controlled case series and matched cohort studies. *J Data Sci* 2010;8:349-60.
28. The self controlled case series website. http://statistics.open.ac.uk/sccs. 2015.

# APPENDIX 8.A.

**Table 8.A1.** Characteristics of included studies

| Author, year | Journal | **Self-controlled design** | **Traditional design** | **Exposure** | **Outcome** |
|---|---|---|---|---|---|
| Tseng, 2013 [1] | Clin Infect Dis | Self-controlled case-series | Cohort | Tetanus-diphtheria-acellular pertussis vaccination | Anaphylaxis and generalized reaction, medically attended inflammation or allergic events, paralytic syndromes, brachial neuritis, Guillain-Barre syndrome, cranial nerve disorders, or composite of meningitis, encephalitis, and encephalopathy |
| Berry, 2013 [1 | Osteoporos Int | Case-crossover | Case-control | Loop or thiazide diuretics | Hip fracture |
| Ramsay, 2013 [3] | BMC Medical Research Methodology | Self-controlled case-series | Cohort | Proton pump inhibitors | Pneunomia |

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|---|---|---|---|---|---|
| Madigan, 2013 [4] | Am J Epidemiol | Self-controlled case-series | Cohort | Angiotensin-converting enzyme inhibitors, amphotericin B, antibiotics, antiepileptics, benzodiazepines, beta blockers, bisphosphonates, tricyclic antidepressants, warfarin, and typical antipsychotics | Aplastic anemia, acute liver injury, acute myocardial infarction, angioedema, acute renal failure, bleeding, hip fracture, mortality after myocardial infarction, and upper gastrointestinal ulcer hospitalization |
| Galeotti, 2013 [5] | Eur J Epidemiol | Self-controlled case-series | Case-control | Influenza vaccination | Guillain-Barre syndrome |
| Ye, 2013 [6] | Epidemiology | Case-crossover | Case-control | Alcohol use | Injury |
| Andrews, 2012 [7] | Vaccine | Self-controlled case-series | Cohort | Measles-mumps-rubella vaccination | Thrombocytopenic purpura |
| Fang, 2012 [8] | N Engl J Med | Case-crossover | Cohort | Receiving a diagnosis of cancer | Suicide and cardiovascular death |
| Lee, 2012 [9] | J Clin Epidemiol | Case-crossover | Case-control | Hepatoxic drugs | Liver injury |
| Sun, 2012 [10] | JAMA | Self-controlled case-series | Cohort | DTaP-IPV-Hib vaccination | Febrile seizures |
| Douglas, 2012 [11] | BMJ | Self-controlled case-series | Cohort | Proton pump inhibitors | Myocardial infarction |

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|---|---|---|---|---|---|
| De Wals, 2012 [12] | JAMA | Self-controlled case-series | Cohort | H1N1 influenza vaccination | Guillain-Barre syndrome |
| Ravera, 2012 [13] | Eur J Epidemiol | Case-crossover | Case-control | Medication use | Motor vehicle accidents |
| Pariente, 2012 [14] | Arch Intern Med | Self-controlled case-series | Cohort | Antipsychotic agents | Myocardial infarction |
| Nicholas, 2012 [15] | J Clin Epidemiol | Self-controlled case-series and case-crossover | Cohort and case-control | Thiazolidinediones, and liver enzyme-inducing anticonvulsants | Heart failure and fracture |
| Kuhnert, 2012 [16] | Vaccine | Self-controlled case-series | Case-control | Vaccination | Sudden infant death syndrome |
| Hambidge, 2012 [17] | Pediatrics | Self-controlled case-series | Case-control | Trivalent inactivated influenza vaccination | Sickle cell crisis |
| Lee, 2011 [18] | PLoS One | Case-crossover | Case-control | Radix bupleuri containing products | Liver injury |
| Gallo, 2011 [19] | Infect Dis Obstet Gynecol | Case-crossover | Cohort | Condom use | Bacterial vaginosis |
| Gribbin, 2011 [20] | Drugs Aging | Self-controlled case-series | Case-control | Serotonin-norepinephrine reuptake inhibitors | Falls |
| Risselada, 2011 [21] | J Thromb Haemost | Case-crossover | Case-control | Platelet aggregation inhibitors, vitamin K antagonists | Subarachnoid hemorrhage |
| Schelleman, 2010 [22] | Clin Pharmacol Ther | Case-crossover | Case-control | Anti-infectives | Severe hypoglycaemia |
| Fosbol, 2010 [23] | Circ Cardiovasc Qual Outcomes | Case-crossover | Cohort | Nonsteroidal anti-inflammatory drugs | Cardiovascular death, coronary dearth or nonfatal myocardial infarction, and fatal/non-fatal stroke |

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|---|---|---|---|---|---|
| Park, 2010 [24] | J Korean Med Sci | Case-crossover | Case-control | Contact with aseptic meningitis patients and contact with hand-foot-mouth disease patients | Aseptic meningitis and hand-foot-mouth disease |
| Olesen, 2010 [25] | Pharmacoepidemiol Drug Saf | Case-crossover | Cohort | Antiepileptic drugs | Suicide |
| Dall, 2009 [26] | Clin Gastroenterol Hepatol | Case-crossover | Case-control | Selective serotonin reuptake inhibitors, and tricyclic antidepressants | Serious upper gastrointestinal bleeding |
| Rifkin, 2009 [27] | Contraception | Case-crossover | Cohort | Hormonal contraceptives | Bacterial vaginosis |
| Zambon, 2009 [28] | Drug Saf | Case-crossover | Case-control | Macrolide, and fluoroquinolone antibacterials | Ventricular arrhythmia and cardiac arrest |
| Gislason, 2009 [29] | Arch Intern Med | Case-crossover | Cohort | Nonsteroidal anti-inflammatory drugs | Death, heart failure hospitalization, and acute myocardial infarction |
| Schelleman, 2008 [30] | Clin Pharmacol Ther | Case-crossover | Case-control | Warfarin with fluoroquinolones, sulphonamides, or azole antifungals | Hospitalization for gastrointestinal bleeding |
| Cassidy, 2008 [31] | Spine | Case-crossover | Case-control | Chiropractic visits, and primary care physician visits | Vertebrobasilar stroke |
| Hocine, 2007 [32] | Vaccine | Self-controlled case-series | Case-control | Hepatitis B vaccination | Central nervous system demyelinating events |

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| Hebert, 2007 [33] | Pharmacoepidemiol Drug Saf | Case-crossover | Case-control | Benzodiazepines | Motor vehicle crashes |
| Hugonnet, 2007 [34] | Am J Epidemiol | Case-crossover | Cohort | Nurse staffing level | Nosocomial infections |
| Gislason, 2006 [35] | Circulation | Case-crossover | Cohort | Selective cyclooxygenase-2 inhibitors, and nonselective nonsteroidal anti-inflammatory drugs | Death and rehospitalisation for acute myocardial infarction |
| Cohen, 2005 [36] | Acta Derm Venereol | Case-crossover | Case-control | Drug exposure | Psoriasis vulgaris |
| Tata, 2005 [37] | Aliment Pharmacol Ther | Self-controlled case-series | Case-control | Selective serotonin reuptake inhibitors and nonsteroidal anti-inflammatory drugs | Gastrointestinal bleeding |
| Warner, 2005 [38] | Am J Epidemiol | Case-crossover | Cohort | Condom use | Sexually transmitted infections |
| Tata, 2005 [39] | Heart | Self-controlled case-series | Case-control | Selective serotonin reuptake inhibitors and tricyclic antidepressants | Acute myocardial infarction |
| Spurling, 2005 [40] | Ann Fam Med | Case-crossover | Case-control | Alcohol consumption | Injury |
| Corrao, 2005 [41] | J Clin Epidemiol | Case-crossover | Case-control | Short-acting inhaled beta-2-agonists | Mortality from chronic obstructive pulmonary disease |
| Hagel, 2005 [42] | BMJ | Case-crossover | Case-control | Helmet use | Head injury |
| Hernandez-Diaz, 2003 [43] | Am J Epidemiol | Case-crossover | Case-control | Folic acid antagonists | Cardiovascular defects |

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|---|---|---|---|---|---|
| Hubbard, 2003 [44] | Am J Epidemiol | Self-controlled case-series | Case-control | Selective serotonin reuptake inhibitors and tricyclic antidepressants | Hip fracture |
| Vinson, 2003 [45] | J Stud Alcohol | Case-crossover | Case-control | Drinking | Intentional injury |
| Viboud, 2001 [46] | J Clin Epidemiol | Case-crossover | Case-control | Drug exposures | Severe cutaneous adverse reactions |
| Murphy, 2001 [47] | N Engl J Med | Self-controlled case-series | Case-control | Rotavirus vaccination | Intussusception |
| Fann, 2000 [48] | J Neurol Neurosurg Psychiatry | Case-crossover | Case-control | Vigorous physical activity | Subarachnoid haemorrhage |
| Hallqvist, 2000 [49] | Am J Epidemiol | Case-crossover | Case-control | Heavy physical exertion | Myocardial infarction |
| Dixon, 1997 [50] | Epidemiology | Case-crossover | Case-control | Insecticides or insect repellents | Haemorrhagic fever with renal syndrome |
| Farrington, 1996 [51] | Am J Epidemiol | Self-controlled case-series | Cohort and case-control | Measles-mumps-rubella vaccination | Febrile convulsion |
| Sturkenboom, 1995 [52] | J Clin Epidemiol | Case-crossover | Cohort | Acitretin | Vulvo-vaginal candidiasis |
| Willich, 1993 [53] | N Engl J Med | Case-crossover | Case-control | Physical exertion | Acute myocardial infarction |

## References


1. HF Tseng, LS Sy, L Qian, SM Marcy, LA Jackson, J Glanz, et al. Safety of a tetanus-diphtheria-acellular pertussis vaccine when used off-label in an elderly population. Clin Infect Dis 2013;56:315-321.
2. SD Berry, Y Zhu, H Choi, DP Kiel, Y Zhang. Diuretic initiation and the acute risk of hip fracture. Osteoporos Int 2013;24:689-695.
3. EN Ramsay, NL Pratt, P Ryan, EE Roughead. Proton pump inhibitors and the risk of pneumonia: a comparison of cohort and self-controlled case series designs. BMC Med Res Methodol 2013;13:82.
4. D Madigan, PB Ryan, M Schuemie, PE Stang, JM Overhage, AG Hartzema, et al. Evaluating the impact of database heterogeneity on observational study results. Am J Epidemiol 2013;178:645-651.
5. F Galeotti, M Massari, R D'Alessandro, E Beghi, A Chio, G Logroscino, et al. Risk of Guillain-Barre syndrome after 2010-2011 influenza vaccination. Eur J Epidemiol 2013;28:433-444.
6. Y Ye, JC Bond, CJ Cherpitel, G Borges, M Monteiro, K Vallance. Evaluating recall bias in a case-crossover design estimating risk of injury related to alcohol: data from six countries. Drug Alcohol Rev 2013;32:512-518.
7. N Andrews, J Stowe, E Miller, H Svanstrom, K Johansen, J Bonhoeffer, et al. A collaborative approach to investigating the risk of thrombocytopenic purpura after measles-mumps-rubella vaccination in England and Denmark. Vaccine 2012;30:3042-3046.
8. F Fang, K Fall, MA Mittleman, P Sparen, W Ye, HO Adami, et al. Suicide and cardiovascular death after a cancer diagnosis. N Engl J Med 2012;366:1310-1318.
9. CH Lee, JD Wang, PC Chen, Health Data Analysis in Taiwan (hDATa) Research Group. Case-crossover design: an alternative strategy for detecting drug-induced liver injury. J Clin Epidemiol 2012;65:560-567.
10. Y Sun, J Christensen, A Hviid, J Li, P Vedsted, J Olsen, et al. Risk of febrile seizures and epilepsy after vaccination with diphtheria, tetanus, acellular pertussis, inactivated poliovirus, and Haemophilus influenzae type B. JAMA 2012;307:823-831.
11. IJ Douglas, SJW Evans, AD Hingorani, AM Grosso, A Timmis, H Hemingway, et al. Clopidogrel and interaction with proton pump inhibitors: Comparison between cohort and within person study designs. BMJ 2012;345:e4388.
12. P De Wals, G Deceuninck, E Toth, N Boulianne, D Brunet, R- Boucher, et al. Risk of Guillain-Barre syndrome following H1N1 influenza vaccination in Quebec. JAMA 2012;308:175-181.
13. S Ravera, N Van Rein, JJ De Gier, LTW De Jong-Van Den Berg. A comparison of pharmacoepidemiological study designs in medication use and traffic safety research. Eur J Epidemiol 2012;27:473-481.
14. A Pariente, A Fourrier-Reglat, T Ducruet, P Farrington, S- Beland, J- Dartigues, et al. Antipsychotic use and myocardial infarction in older patients with treated dementia. Arch Intern Med 2012;172:648-653.
15. JM Nicholas, AP Grieve, MC Gulliford. Within-person study designs had lower precision and greater susceptibility to bias because of trends in exposure than cohort and nested case-control designs. J Clin Epidemiol 2012;65:384-393.
16. R Kuhnert, M Schlaud, C Poethko-Muller, M Vennemann, P Fleming, PS Blair, et al. Reanalyses of case-control studies examining the temporal association between sudden infant death syndrome and vaccination. Vaccine 2012;30:2349-2356.
17. SJ Hambidge, C Ross, J Glanz, D McClure, MF Daley, S Xu, et al. Trivalent inactivated influenza vaccine is not associated with sickle cell crises in children. Pediatrics 2012;129:e54-9.

18. CH Lee, JD Wang, PC Chen. Risk of Liver Injury Associated with Chinese Herbal Products Containing Radix bupleuri in 639, 779 Patients with Hepatitis B Virus Infection. PLoS ONE 2011;6:e16064
19. MF Gallo, L Warner, CC King, JD Sobel, RS Klein, S Cu-Uvin, et al. Association between semen exposure and incident bacterial vaginosis. Infect Dis Obstet Gynecol 2011;2011:842652.
20. J Gribbin, R Hubbard, J Gladman, C Smith, S Lewis. Serotonin-norepinephrine reuptake inhibitor antidepressants and the risk of falls in older people: case-control and case-series analysis of a large UK primary care database. Drugs Aging 2011;28:895-902.
21. R Risselada, H Straatman, F van Kooten, DW Dippel, A van der Lugt, WJ Niessen, et al. Platelet aggregation inhibitors, vitamin K antagonists and risk of subarachnoid haemorrhage. J Thromb Haemost 2011;9:517-523.
22. H Schelleman, WB Bilker, CM Brensinger, F Wan, S Hennessy. Anti-infectives and the risk of severe hypoglycemia in users of glipizide or glyburide. Clin Pharmacol Ther 2010;88:214-222.
23. EL Fosbol, F Folke, S Jacobsen, JN Rasmussen, R Sorensen, TK Schramm, et al. Cause-specific cardiovascular risk associated with nonsteroidal antiinflammatory drugs among healthy individuals. Circ Cardiovasc Qual Outcomes 2010;3:395-405.
24. SK Park, B Park, M Ki, H Kim, K Lee, C Jung, et al. Transmission of seasonal outbreak of childhood enteroviral aseptic meningitis and hand-foot-mouth disease. J Korean Med Sci 2010;25:677-683.
25. JB Olesen, PR Hansen, J Erdal, SZ Abildstrom, P Weeke, EL Fosbol, et al. Antiepileptic drugs and risk of suicide: a nationwide study. Pharmacoepidemiol Drug Saf 2010;19:518-524.
26. M Dall, OB Schaffalitzky de Muckadell, AT Lassen, JM Hansen, J Hallas. An association between selective serotonin reuptake inhibitor use and serious upper gastrointestinal bleeding. Clin Gastroenterol Hepatol 2009;7:1314-1321.
27. SB Rifkin, MR Smith, RM Brotman, RM Gindi, EJ Erbelding. Hormonal contraception and risk of bacterial vaginosis diagnosis in an observational study of women attending STD clinics in Baltimore, MD. Contraception 2009;80:63-67.
28. A Zambon, H Polo Friz, P Contiero, G Corrao. Effect of macrolide and fluoroquinolone antibacterials on the risk of ventricular arrhythmia and cardiac arrest: an observational study in Italy using case-control, case-crossover and case-time-control designs. Drug Saf 2009;32:159-167.
29. GH Gislason, JN Rasmussen, SZ Abildstrom, TK Schramm, ML Hansen, EL Fosbol, et al. Increased mortality and cardiovascular morbidity associated with use of nonsteroidal anti-inflammatory drugs in chronic heart failure. Arch Intern Med 2009;169:141-149.
30. H Schelleman, WB Bilker, CM Brensinger, X Han, SE Kimmel, S Hennessy. Warfarin with fluoroquinolones, sulfonamides, or azole antifungals: interactions and the risk of hospitalization for gastrointestinal bleeding. Clin Pharmacol Ther 2008;84:581-588.
31. JD Cassidy, E Boyle, P Cote, Y He, S Hogg-Johnson, FL Silver, et al. Risk of vertebrobasilar stroke and chiropractic care: results of a population-based case-control and case-crossover study. Spine (Phila Pa.1976) 2008;33:S176-83.
32. MN Hocine, CP Farrington, E Touze, HJ Whitaker, A Fourrier, T Moreau, et al. Hepatitis B vaccination and first central nervous system demyelinating events: reanalysis of a case-control study using the self-controlled case series method. Vaccine 2007;25:5938-5943.
33. C Hebert, JA Delaney, B Hemmelgarn, LE Levesque, S Suissa. Benzodiazepines and elderly drivers: a comparison of pharmacoepidemiological study designs. Pharmacoepidemiol Drug Saf 2007;16:845-849.
34. S Hugonnet, A Villaveces, D Pittet. Nurse staffing level and nosocomial infections: empirical evaluation of the case-crossover and case-time-control designs. Am J Epidemiol 2007;165:1321-1327.
35. GH Gislason, S Jacobsen, JN Rasmussen, S Rasmussen, P Buch, J Friberg, et al. Risk of death or reinfarction associated with the use of selective cyclooxygenase-2 inhibitors and nonselective

nonsteroidal antiinflammatory drugs after acute myocardial infarction. Circulation 2006;113:2906-2913.

36. AD Cohen, DY Bonneh, H Reuveni, DA Vardy, L Naggan, S Halevy. Drug exposure and psoriasis vulgaris: case-control and case-crossover studies. Acta Derm Venereol 2005;85:299-303.
37. LJ Tata, PJ Fortun, RB Hubbard, L Smeeth, CJ Hawkey, CJ Smith, et al. Does concurrent prescription of selective serotonin reuptake inhibitors and non-steroidal anti-inflammatory drugs substantially increase the risk of upper gastrointestinal bleeding? Aliment Pharmacol Ther 2005;22:175-181.
38. L Warner, M Macaluso, HD Austin, DK Kleinbaum, L Artz, ME Fleenor, et al. Application of the case-crossover design to reduce unmeasured confounding in studies of condom effectiveness. Am J Epidemiol 2005;161:765-773.
39. LJ Tata, J West, C Smith, P Farrington, T Card, L Smeeth, et al. General population based study of the impact of tricyclic and selective serotonin reuptake inhibitor antidepressants on the risk of acute myocardial infarction. Heart 2005;91:465-471.
40. MC Spurling, DC Vinson. Alcohol-related injuries: evidence for the prevention paradox. Ann Fam Med 2005;3:47-52.
41. G Corrao, A Zambon, S Faini, V Bagnardi, O Leoni, S Suissa. Short-acting inhaled beta-2-agonists increased the mortality from chronic obstructive pulmonary disease in observational designs. J Clin Epidemiol 2005;58:92-97.
42. BE Hagel, IB Pless, C Goulet, RW Platt, Y Robitaille. Effectiveness of helmets in skiers and snowboarders: case-control and case crossover study. BMJ 2005;330:281.
43. S Hernandez-Diaz, MA Hernan, K Meyer, MM Werler, AA Mitchell. Case-crossover and case-time-control designs in birth defects epidemiology. Am J Epidemiol 2003;158:385-391.
44. R Hubbard, P Farrington, C Smith, L Smeeth, A Tattersfield. Exposure to tricyclic and selective serotonin reuptake inhibitor antidepressants and the risk of hip fracture. Am J Epidemiol 2003;158:77-84.
45. DC Vinson, G Borges, CJ Cherpitel. The risk of intentional injury with acute and chronic alcohol exposures: a case-control and case-crossover study. J Stud Alcohol 2003;64:350-357.
46. C Viboud, PY Boelle, J Kelly, A Auquier, J Schlingmann, JC Roujeau, et al. Comparison of the statistical efficiency of case-crossover and case-control designs: application to severe cutaneous adverse reactions. J Clin Epidemiol 2001;54:1218-1227.
47. TV Murphy, PM Gargiullo, MS Massoudi, DB Nelson, AO Jumaan, CA Okoro, et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001;344:564-572.
48. JR Fann, WA Kukull, WJ Katon, WT Longstreth Jr. Physical activity and subarachnoid haemorrhage: a population based case-control study. J Neurol Neurosurg Psychiatry 2000;69:768-772.
49. J Hallqvist, J Moller, A Ahlbom, F Diderichsen, C Reuterwall, U De Faire. Does heavy physical exertion trigger myocardial infarction? A case-crossover analysis nested in a population-based case-referent study. Am J Epidemiol 2000;151:459-467.
50. KE Dixon. A comparison of case-crossover and case-control designs in a study of risk factors for hemorrhagic fever with renal syndrome. Epidemiology 1997;8:243-246.
51. CP Farrington, J Nash, E Miller. Case series analysis of adverse reactions to vaccines: a comparative evaluation. Am J Epidemiol 1996;143:1165-1173.
52. MC Sturkenboom, A Middelbeek, LT de Jong van den Berg, PB van den Berg, BH Stricker, H Wesseling. Vulvo-vaginal candidiasis associated with acitretin. J Clin Epidemiol 1995;48:991-997.
53. SN Willich, M Lewis, H Lowel, H- Arntz, F Schubert, R Schroder. Physical exertion as a trigger of acute myocardial infarction. N Engl J Med 1993;329:1684-1690.

# APPENDIX 8.B.

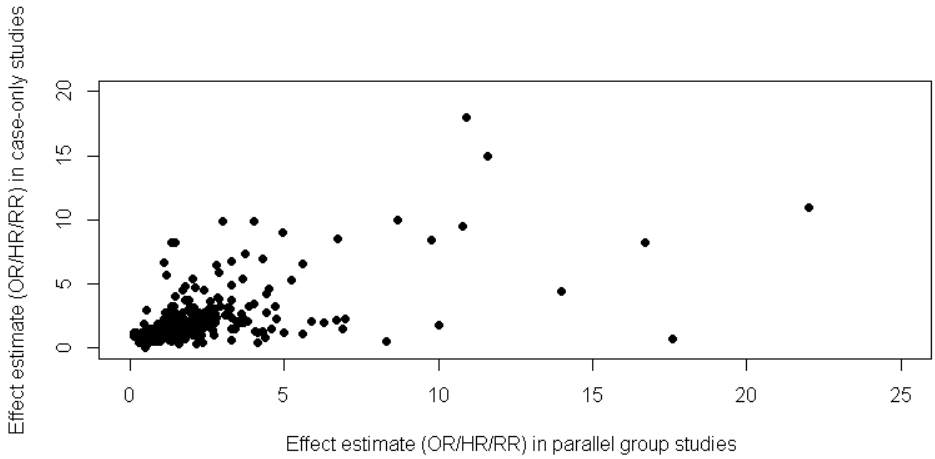


**Figure 8.B1.** Scatterplot of the correlation between effect estimates from case-only designs and those from parallel group designs.

# APPENDIX 8.C.

**Table 8.C1** Univariate association between predictors and discrepant results between case-only design and cohort or case-control designs.

| **Predictor** | **OR (95% CI)** |
|---|---|
| Intermittent/short-term exposure, yes vs no | 0.28 (0.19-0.41) |
| Rare event, yes vs no | 0.51 (0.31-0.84) |
| Acute outcome, yes vs no | 0.22 (0.10-0.45) |
| Hazard period | |
| ≤1 day | Ref. |
| 2-31 days | 1.30 (0.45-4.70) |
| ≥32 days | 2.89 (0.93-10.99) |
| Variable | 9.92 (3.75-34.26) |
| Case-only design (SCCS vs CCO) | 3.76 (2.51-5.75) |
| Parallel group design (cohort vs case-control) | 5.13 (3.25-8.35) |
| Adjustment for time-varying confounding, yes vs no | 0.37 (0.23-0.59) |
| Safety study, yes vs no | 2.99 (0.44-58.77) |
| Washout period, yes vs no | 0.31 (0.20-0.46) |
| Sample size | |
| ≤10.000 | Ref. |
| >10.000 and ≤100.000 | 6.43 (2.73-16.64) |
| >100.000 | 8.48 (4.21-19.55) |

CHAPTER 9

# General discussion

The studies presented in this thesis focused on confounding in observational studies. Several designs and analytical methods to quantify confounding were evaluated using single-study and systematic comparisons. We focused on various self-controlled designs and compared our findings with randomized designs as well as more traditional observational designs such as the cohort and case-control design.

In **Chapter 2** we evaluated whether the reporting of confounding in observational cohort and case-control studies improved after the publication of the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) statement using a systematic before-after comparison. In total, 174 articles published before and 220 articles published after the publication of the STROBE statement in high-impact general medical and epidemiological journals were included. Out of 8 pre-specified essential items in the reporting of confounding, on average only four were reported in both periods. Results were similar for journals that published and/or endorsed the STROBE statement in their Instruction for Authors and journals that did neither. Hence, research is needed into the development and evaluation of alternative strategies to improve the quality of reporting and adherence to reporting guidelines.

The effect of pravastatin and fosinopril, an angiotensin converting enzyme (ACE) inhibitor, on urinary tract infections was assessed in a post-hoc analysis of a randomized controlled trial (**Chapter 3**). Intention-to-treat analyses showed that pravastatin was associated with a reduced total number of urinary tract infections, but not with first urinary tract infections. Fosinopril was in contrast associated with an increased occurrence of first urinary tract infections.

Subsequently, we evaluated the effect of ACE inhibitors on the risk of first urinary tract infections using both a prescription sequence symmetry analysis (**Chapter 4**) and a case-crossover design (**Chapter 5**). Using both designs ACEi therapy initiation was associated with an increased risk of developing first urinary tract infections, even after adjustment for several time-varying confounders. Despite the similarities between the case-crossover design and the prescription sequence symmetry analysis, the latter design led to slightly lower effect estimates than the case-crossover design and the results from the randomized design discussed in **chapter 3**.

In **Chapter 6** we re-evaluated a previously published cohort study without adjustments for potential confounders that evaluated the association of combined use of selective serotonin reuptake inhibitors (SSRIs) and nonsteroidal anti-inflammatory drugs (NSAIDs) with the risk of starting peptic ulcer treatment. To evaluate whether the strong synergistically increased risk found in that other study is likely due to confounding we applied a prescription sequence symmetry design (**Chapter 6**). Using the prescription

sequence symmetry design, the effect estimate of concurrent use of SSRIs and NSAIDs did not exceed the effect estimate of NSAIDs alone, suggesting that at least part of the previously reported association between combined use of SSRIs with NSAIDs and peptic ulcer treatment might be attributed to unmeasured or residual confounding. Furthermore, this study shows that when limited data on potential confounders are available, applying a prescription sequence symmetry design can have more value than applying a traditional cohort design.

Given the ongoing debate about whether antibiotic use during pregnancy increases the risk of asthma development in the offspring, we assessed whether this increased risk is likely causal or due to confounding. We made a comparison between a case-control, case-sibling, a time-trend-adjusted case-sibling design and a maternal-paternal comparison and applied a quantitative bias analysis to evaluate the potential role of confounding (**Chapter 7**). In both the case-control, case-sibling and time-trend-adjusted case-sibling design exposure to antibiotics in the third trimester of pregnancy was associated with a small increased risk of asthma in preschool children. However, quantitative bias analysis showed that time-varying confounding could not be excluded as an explanation of the statistically significant findings.

Finally, we made a systematic comparison between effect estimates of the two most common case-only designs, the self-controlled case-series and case-crossover design, and the more traditional case-control and cohort design. A predictive model was built to assess whether discrepancies between both types of designs could be predicted by failure to meet assumptions of the case-only designs (**Chapter 8**). The concordance between effect estimates of case-only and cohort or case-control designs appeared to be moderate, and discrepancies beyond chance were very common. Such discrepancies could be predicted by failure to meet important assumptions of case-only designs. Hence, researchers should be aware that there may be other causes of discrepancies or agreement between both types of designs than the presence or absence of time-invariant unmeasured confounding.

In this final chapter we place the main findings in context and provide future perspectives.

## Selection strategies for measured confounders

Confounding is mixing or confusing the effect of the exposure of interest with the effects of other variables leading to bias [1]. Understanding and adjusting for confounding in observational intervention research is central to address causality when an association is observed [2]. To be considered a potential confounder, a variable must have an association

with the exposure and a causal relationship with the outcome (or be a surrogate measure of a cause) in the study population. In addition, the variable should not be affected by the exposure [3]. These are traditional requirements for variables to be considered potential confounders, however, there is no general consensus on how to identify and select confounders in research practice.

Some form of confounding selection procedure is required, especially when data are sparse [4]. Further, in any case, inclusion of non-confounders can introduce bias and reduce precision [5-7]. Traditionally, confounders are selected based on different quantitative strategies, including change in effect estimate criterion, univariate associations of the variable with the exposure and the outcome, or stepwise regression modelling [3,8]. More recently, other data-driven selection methods such as high-dimensional propensity score analysis or other exposure models and regularization methods have been proposed as potential alternative methods for confounder selection [4].

A problem with all these purely data-driven variable selection methods is the risk that bias and/or unnecessary large variance is introduced by selecting intermediates, colliders or instrumental variables [9,10-12]. The inclusion of colliders and instrumental variables is especially problematic if it introduces or amplifies uncontrolled unmeasured confounding [9]. A frequently proposed solution is to explicitly define the causal structure and relationships between variables based on subject knowledge [12-14]. A commonly proposed strategy is to summarize and communicate the assumed causal structure using causal diagrams, or directed acyclic graphs (DAG) [15,16]. A causal diagram for a confounder U of the relationship between exposure X and outcome Y would look like Figure 9.1.

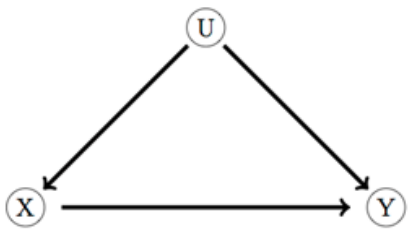


**Figure 9.1.** Causal diagram of the relationship between exposure X and outcome Y and confounder U.

Such causal diagrams, often much more complicated, are sometimes used to summarize and communicate the causal structure assumed by the researchers and can be used to identify the minimal sufficient adjustment sets for estimating the total effect of X on Y [15,17]. In theory, causal diagrams are essential for valid selection of confounders for adjustment. Such diagrams can help to identify and remove instrumental variables,

intermediates and any other variable influenced by exposure or outcome, leaving only the confounders for which one needs to adjust to obtain unconfounded effect estimates [15]. However, the usefulness of such diagrams has been criticized by researchers because often it is unknown how all variables interact with each other and causal diagrams quickly become too complicated to understand when one believes that hundreds of variables can act as (proxies for) confounders [18]. Hence, causal diagrams are useful for summarizing and communicating simple causal structures with relatively few variables, but seem to be less useful when sufficient prior knowledge is lacking as is often the case when many variables might be involved. In these latter situation one may, dependent on whether there are concerns about for example bias amplification, want to use confounder selection methods that are less vulnerable to bias amplification, such as regularization regression. *Regardless of the chosen method, it is important to report which method is used to enable the reader to identify potential problems that the selection method may have introduced.* Hence, it is worrying that both the reasons why potential confounders are selected for analysis and the rationale for their inclusion in the final model are reported infrequently as observed in the study described in **Chapter 2**.

### Controlling unmeasured confounding

In the paragraph above, we evaluated methods that can be very useful when there is a large number of measured covariates that may act as (proxy for) confounders. However, often there are several potential confounders that are not measured or captured in, for example, an administrative health care database. Moreover, even with high-dimensional propensity scores and shrinkage methods using hundreds of variables, unmeasured confounders may be uncorrelated with measured covariates. For example, smoking history is often absent in healthcare claims databases and is not likely to be proxied well by information available from claims [19]. Self-controlled or case-only designs have lately been developed to overcome the problem of unmeasured confounding in observational intervention studies. The general idea behind these designs is that patients can serve as their own controls, thereby minimizing confounding by factors that are stable over time. Consequently, these designs are especially useful when applied to evaluate short-term effects, as the likelihood that most factors will be (approximately) stable over time is in these situations generally relatively high. Several (variations on) self-controlled designs have been developed over the years, including case-crossover [20], case-time-control [21], fixed-effects case-time-control [22], case-case-time-control [23,24], prescription sequence symmetry [25], exposure-crossover [26], and self-controlled case-series designs [27]. In this thesis we focused on the prescription sequence symmetry, case-crossover, case-sibling and self-controlled case-series design.

**Prescription sequence symmetry analysis**

The prescription sequence symmetry analysis (PSSA) was first proposed by Hallas as a screening tool for adverse drug reactions [25]. This design is often regarded as a simplified version of a case-crossover [20] or self-controlled case-series design [27]. In contrast to those designs, the PSSA does not base the effect estimate on comparisons within patients, but within patient populations (i.e. between different patients using the same drug). Only patients that both use the exposure drug of interest and the outcome drug of interest within a certain period of each other are selected for analysis. Hence, by selecting only patients that initiate the drugs of interest, all included patients have an indication for the exposure drug, thereby limiting confounding by indication.

The PSSA method has several limitations and requires several assumptions. An obvious limitation of the PSSA is that there is a limit to the number of drugs that can serve as a valid proxy for acute clinical outcomes. Further, especially when using short time-windows, which reduces the probability that results are affected by several potential biases as explained below, it is crucial that the timing of drug prescribing is close to the timing of the actual clinical event to prevent misclassification. Hence we examined short-term adverse or beneficial effects which require immediate drug treatment.

In **chapter 4 and 5** we used certain antibiotics as a proxy for acute urinary tract infections, which would likely not result in severe misclassification of the timing of the outcome as there will be generally limited time between the onset of symptoms and the prescription of antibiotics. Although it is theoretically possible that increased contact with the general practitioner after angiotensin-converting enzyme inhibitors initiation explains part of the increased risk, the absence of an effect for beta-blocker initiation (sequence ratio of 1.01) suggests that this is not the case. The problem of finding valid drug-proxies can be solved by applying sequence symmetry analysis with outcome events instead of outcome drugs [28,29].

In the PSSA, patients that use the outcome drug both before and after initiation of the exposure drug are simply categorized as patients that use the outcome drug before the exposure drug and information about outcome drug use after exposure drug initiation is ignored. In contrast, a self-controlled case-series design would also incorporate the information after exposure drug initiation of the same patient. Hence, as explained in **Chapter 5**, the PSSA will result in effect estimates that are lower (closer to the null when evaluating an increased risk, a stronger protective effect when evaluating a protective effect) in comparison with a self-controlled case-series or a case-crossover design that does not have the same limitation. Nevertheless, we found similar results when applying the prescription sequence symmetry analysis to study the effect of ACE-inhibitors on the occurrence of urinary tract infections as in a randomized design (**Chapter 3 and 4**).

In **chapters 4 and 5**, we restricted the analysis to patients that initiated the outcome drug within 28 days of exposure drug initiation in an attempt to limit possible confounding by factors that are related to the timing of prescribing (e.g. the closer to the date of exposure initiation the higher the disease severity). The use of relatively short time-windows may have resulted in a relatively small difference between the PSSA and case-crossover design (**Chapter 5**). When using longer time-windows, e.g. one year as commonly applied [30-34], the influence of ignoring information after exposure initiation in patients that initiate the outcome drug in the before-period may be much larger (due to a higher probability that the outcome also occurs in the after-period). Consequently, when screening for unknown drug-related adverse effects, one needs to take into account that the PSSA method may result in more false negatives than self-controlled case-series designs such as the hierarchical Bayesian self-controlled case-series model [35]. Although the hierarchical Bayesian self-controlled case-series model still has to be empirically evaluated, it is a method that seems to be an interesting screening tool as it was specifically designed for estimating the effects of many drugs on many outcomes [35].

As explained in **Chapter 4 and 5**, using sequence symmetry analysis to evaluate adverse effects that are already known by prescribers [28,31,36-38] may result in artificially increased risks. This latter phenomenon is also observed with self-controlled case-series when exposure prescribing is outcome dependent. With well-known adverse effects, physicians may postpone prescribing a drug that further increases the risk of a recently experienced event. For the self-controlled case-series, a solution often used in the setting of temporary contraindication for vaccination is to incorporate a 'pre-vaccination risk' period that is not used to calculate the baseline outcome incidence [39]. Although a 'pre-exposure' period could be excluded from the at risk period in PSSA, one should be aware that this may introduce bias due to confounding by disease severity and potential time-trends by moving the before period further away from exposure initiation than the after period. Therefore, careful consideration of study periods is essential to limit up or downward bias.

Although confounding by indication is limited by including only patients that receive the exposure drug of interest, confounding by factors that are related to both the outcome and the timing of prescribing of the exposure is still possible. When there are concerns that such time-varying confounding may play a role (e.g. disease progression) it is important to use relatively narrow time-windows with all self-controlled methods. This is especially relevant for the PSSA design, as no adjustment for such confounders can be made via regression techniques as in real self-controlled methods (**Chapter 5**). Therefore, in our own studies we limited the time-windows to 28 days for all prescription sequence symmetry analyses, thereby minimizing confounding by factors

that are (nearly) stable around the moment of exposure initiation (**Chapters 4, 5 and 6**). Another reason why the use of relatively narrow time-windows can result in less biased effect estimates is that trends in prescribing of exposure drug and/or outcome drug can influence the results [25,40]. The effects of potential time-trends can be limited by using narrow time-windows (**Chapters 4, 5 and 6**), because the prescribing practice will generally not change drastically over very short time periods. Given the vulnerability to several biases when using relatively long time-windows, such as the commonly applied 1-year time-windows, an important recommendation would be to restrict the analysis to patients that initiate both drugs of interest within a relatively short-time period. This recommendation is also more in agreement with theory and recommendations of self-controlled designs such as the case-crossover study [20,40].

Although the prescription sequence symmetry design has, as other observational designs, some important limitations as discussed in chapters 4-6 and above, it also has important strengths. Due to the simplicity of the design it can be used to screen Big Data databases without encountering scaling issues. Moreover, when limited data on potential confounders are available, applying a prescription sequence symmetry design can have more value than performing a cohort study and neglecting potential unmeasured confounding. In **Chapter 6**, the PSSA indicated that at least part of the association of concomitant use of non-steroidal anti-inflammatory drugs (NSAIDs) and selective serotonin reuptake inhibitors (SSRIs) with peptic ulcer drug treatment may be due to unmeasured confounding in a previously published cohort study. Nevertheless, given the ever increasing computer power and recent developments in the self-controlled case-series method [35,41-48] that address much of the limitations encountered by the PSSA and/or standard self-controlled case-series, future research may additionally be focused on the self-controlled case-series methodology.

#### Case-crossover design

The case-crossover design was developed by Maclure to study the effect of transient effect on the risk of acute outcomes [20]. With this self-controlled design, the probability of exposure in the period just before the outcome event (hazard period) is compared with the probability of exposure in control period(s). Because patients (cases) serve as their own control, confounding by time-invariant characteristics, including unmeasured characteristics, are eliminated.

We evaluated the case-crossover design in both a single-study comparison (**Chapter 5**) and a systematic comparison (**Chapter 8**). The effect of angiotensin-converting enzyme inhibitors on urinary tract infections was similar using a case-crossover design as in a randomized setup (**Chapter 3 and 5**), suggesting that, with similar study populations,

exposure and outcome definitions, the case-crossover design appeared to pertain similar results as randomized controlled trials. Inevitably, there are situations in which a case-crossover design will less likely provide valid effect estimates than other designs. In **chapter 8**, we found that differences between case-only designs, including the case-crossover design, and case-control and/or cohort studies could be predicted by failure to meet important assumptions [49] of the case-only design. Although this finding does not necessarily mean that the case-only study is wrong and the cohort/case-control study right, it implies that researchers should at least be aware that discrepancies between both types of designs may thus be caused by wrong application of the case-only or case-crossover design. Predictors of discrepancies included evaluation of chronic exposures, common events, insidious events and the use of relatively long hazard periods. In **chapter 8** we did not include all assumptions of the case-crossover as potential predictors, as for some it was difficult to assess whether the assumption was met without having the original data at hand. Here, we will discuss more in depth some important remaining limitations of the case-crossover design that should be considered when considering whether a case-crossover design would be an appropriate study design for a specific causal research question.

Because the case-crossover design is a unidirectional design, with the control period always preceding the hazard period, the design is vulnerable to time-trends in exposure [20,21,50-52]. Several solutions exist to this potential problem, including an effective and simple solution: using short hazard and control periods without long periods between the hazard and control periods similar to the PSSA design. However, sometimes this is not possible due to possible carry-over effects or when it takes a while before the effect of the drug is noticeable [50]. Carry-over effects occur when the exposure in the control periods is not independent of the outcome event [49]. The PSSA design is not vulnerable to carry-over effects as within population instead of within patients comparisons are made. To solve the problem of potential time-trends in exposure different options have been proposed: the case-time-control design that uses crossover in control patients to assess and adjust for potential time-trends in exposure [21]; the case-case-time-control that uses future cases as present controls to adjust for exposure time-trend bias [23]; and a bidirectional case-crossover design that samples control windows before and/or after the outcome event [53,54]. With this latter method control periods should not be selected as a function of event times in order to get unbiased results using standard conditional logistic regression [49,53-56]. Hence, symmetrically sampling of control periods before and after the hazard period within individuals should preferably be avoided, although the related bias tends to be small [56]. When there is a linear trend in exposure a bidirectional case-crossover design will remove bias due to the time trend. An important assumption of this method is that the outcome does not alter the probability

of future exposure. When this assumption does not hold, alternative strategies need to be adopted to address time-trends in exposure.

The case-time-control design would be a valid alternative in such situations [21]. However, since this method uses crossover in controls to adjust for time-trends, bias may be introduced when the exposure trend is different among controls than among cases [57]. If controls would be different from cases with regard to exposure trends, one could use future cases as present controls using the case-case-time-control design [23]. A problem with this latter approach is that on the one hand future cases should not be too distant in time to eliminate to capture non-linear time-trends, while on the other hand there must be sufficient time between the event and the person-time sampled from the future case to ensure that the exposure is independent of the future event [23]. Moreover, the method may be inefficient as only patients that can be matched to a future case will contribute to the analysis. As those future cases need to be identified, follow-up must be longer than with a conventional case-time-control or case-crossover design. Finally, selection bias may be introduced, because individuals cannot become future cases unless they survive until they experience the outcome event [40].

Although the case-crossover design eliminates confounding by factors that are stable over time [20], the design is still vulnerable to confounding by time-varying factors. When factors that vary between the hazard and control period(s) are measured, a multivariate conditional logistic model can be used to adjust for these time-varying factors as we did in **chapter 5**. Although a recent review suggested that this is the only correct multivariate model for case-crossover designs [58], a stratified Cox's proportional hazards model with strata representing the matched sets is equally valid [59]. However, one should mind that applying conditional logistic regression or a stratified Cox's proportional hazards model to a case-crossover design with multiple control periods can induce bias if exposures at different time points within individuals are not statistically independent [60].

When a selection procedure for inclusion of potential confounders is necessary, earlier mentioned traditional methods (e.g. change in effect estimate criterion) or regularization methods such as Lasso can be used [61,62].

When applied to the right research question, the case-crossover design can be a very useful research design and provide less biased effect estimates than alternative designs, though empirical comparisons with golden standard RCTs are virtually lacking. Especially when examining transient effects of intermittent exposures on acute outcomes in presence of several important time-invariant confounders, the case-crossover design is a very valuable study design. In **chapter 5** we found similar effect estimates using a case-crossover design as in a randomized design, despite evaluating the effect of angiotensin-converting

enzyme inhibitors, which are often used more chronically. This suggests that even if some assumptions are not entirely met, still similar results can be obtained as in randomized settings. However, empirical studies and statistical simulations are needed to evaluate how vulnerable the design is to violation of the different assumptions. Such simulations would be especially interesting given the observation that discrepancies between case-only and cohort/case-control studies in empirical studies could be predicted by failure to meet assumptions of the case-only designs (**Chapter 8**).

#### Self-controlled case-series

The self-controlled case-series was developed by Farrington to evaluate the influence of transient exposures on acute outcomes [27]. However, the method has also been applied to evaluate long-term effects [63] and recently an exposure-adjusted self-controlled case-series method was proposed that was developed to evaluate the long-term effect of accumulated exposure [48]. However, with long follow-up the potential for bias due to time-varying confounding may become large. When large amounts of potential time-varying confounders are available, self-controlled case-series designs can be implemented with regularization methods [48,64].

The original self-controlled case-series design is similar to a bidirectional case-crossover design in the sense that person-time is sampled from both before as after the index date. In addition, in both designs patients serve as their own control, thereby minimizing confounding by factors that are (nearly) stable over time. Moreover, both methods have similar assumptions and limitations. The full-stratum [53] and time-stratified [65] bidirectional case-crossover design are basically versions of the self-controlled case-series designs [66,67]. The most notable difference is that with the bidirectional case-crossover design the index date is the outcome event [53], while the self-controlled case-series is exposure-indexed [27,66].

A necessary condition of the case-crossover design is that the exposure distribution is stationary, i.e. there is no time-trend bias [60]. It would be intuitive to assume that a necessary condition of the self-controlled case-series would be that the outcome distribution is stationary. However, variations in the baseline incidence can be allowed for in the model by incorporating age or time effects [66].

The standard self-controlled case-series has four main assumptions. The most important assumptions, similar to the PSSA method, is that the probability of exposure is not affected by the occurrence of an outcome event [66]. Obviously, this is also an important assumption of the bidirectional case-crossover design [53]. One solution to overcome the problem of outcome dependent exposure is redefining the observation period

as starting from exposure and continuing to the end of the observation period. This approach is only possible when the post-exposure risk period is not indefinite and is not valid for multiple exposure, because bias for only one exposure but not for subsequent exposures is corrected [45,66,68]. Furthermore, selection bias may be introduced by excluding cases for which the event occurred before exposure. In addition, this will result in a loss of power and less ability to adjust for time-varying confounders as unvaccinated cases will also be dropped [45]. As mentioned earlier, with temporary dependencies, a solution often used in the setting of vaccinations is to incorporate a 'pre-vaccination risk' period that is not used to calculate the baseline outcome incidence [39]. When the outcome-dependency is long, another solution exist that can also be used to analyse multiple exposures. In this pseudo-likelihood approach the observation period is remained the same as in standard self-controlled case-series, but only exposures experienced prior to the event are used to estimate the relative incidence [42]. The data are subsequently analysed as if there could be no subsequent exposures [42]. When in fact such exposures do occur, those exposure periods are incorporated into the baseline period and event counts during this period are adjusted to the number of events that would have been observed when there was no exposure in this period using Horvitz-Thompson-like estimators [42]. This method can be applied to transient exposures and rare non-recurrent events. The method is only valid when the risk returns to the baseline level at the end of the risk period [42].

Second, because the statistical model is derived from a Poisson cohort model [66,67], analysis of frequent non-recurrent or unique events is not valid. Rare non-recurrent events can be analysed using the self-controlled case-series, because the probability of observing more than one event per individual (given that at least one is observed) tends to be zero [27,66,67]. In addition, clustered recurrent events should also not be analysed using the self-controlled case-series design. However, if events clusters in episodes, but the episodes can be assumed independent, clustered events within a certain time-period can be grouped. When this is not appropriate only first events can be used, provided that the initiating event is rare [66]. More recently, two variations of the self-controlled case-series were developed to allow for dependence of events [43,47].

Third, only time-invariant confounders that act multiplicatively on the baseline incidence are cancelled out [67]. This assumption must also hold for modelled time-varying exposures of interest. For the exposure and measured covariates it can be tested whether these assumptions are not violated using methods similar to those used for the proportional hazards model [67].

Fourth, the observation periods should be independent of event times [67]. Thus, individuals must generally remain observable after an event occurs. This may be

problematic when the outcome of interest is death or strongly related to death [67,69]. When the exposure of interest does not appreciably affect the risk of death besides through the effect on the outcome, the method of Farrington *et al.* can be applied [69,70]. When death is the outcome of interest, similar methods as described above that address event-dependent exposure can be used [42,66]. Nevertheless, in practice, the self-controlled case-series method is generally robust to failure to meet the assumption that observation periods are independent of event times [67].

In **chapter 8**, the majority of comparison between case-only and cohort or case-control designs consisted of comparisons between self-controlled case-series and cohort designs. This was largely due to the high number of such comparisons in the study of Madigan *et al.* [71]. Although the authors also reported comparisons with the case-crossover design in the supplementary files of that paper, we did not include those comparisons as we detected an error in their data and results. Though we communicated with the authors of this study and they were going to look further into this issue, at the time of writing this issue has not been solved yet. Nevertheless that study may have contributed to the relatively high number of discrepancies in **chapter 8**. For example, several associations were included that potentially suffered from bias due to outcomes that alter the exposure probability, without using a modified version of the self-controlled case-series that could address this limitation [71]. Similarly, a study that concluded that within-person study designs, including the self-controlled case-series, may have greater susceptibility to bias, applied the self-controlled case-series in an incorrect manner [72]. They censored exposure at the outcome which is not valid, when the self-controlled case-series would have been applied in a correct manner it is likely that different conclusion would have been reached [73]. Hence, whenever applying the self-controlled case-series design to a research question, one needs to carefully consider whether the assumptions of the design will be met. When the assumptions are met, the self-controlled design can be a very valuable design, especially since all measured and unmeasured multiplicative time-invariant confounders are adjusted for by design.

#### Case-sibling design

Although sibling discordance studies were already applied in the 19$^{th}$ century, related designs such as the case-sibling design applied in **chapter 7** are increasingly being used in epidemiology [74]. In the context of prenatal exposures, the advantage of the case-sibling design is that both the case and the control sibling share the same mother and household and share genetic and time-invariant environmental factors that may differ when using a conventional case-control or cohort study [74]. Although the design is not self-controlled in the sense that children are compared with themselves, a comparison is made between pregnancies of the same mother. A major advantage over twin studies

is that twins always have the same prenatal exposure, while siblings can be differentially exposed during pregnancy. Although a contrast is often made between a case-sibling and a cohort or case-control design to gain an indication about unmeasured confounding, **chapter 7** shows that this is not always as easy as it seems. As described by Frisell *et al.*, agreement between the case-sibling design and cohort or case-control study does not necessarily imply absence of unmeasured time-invariant confounding and disagreement does not necessarily imply presence of unmeasured time-invariant confounding [75]. Confounding by factors that are not perfectly shared by siblings or measurement error of exposure can result in biased estimates and ultimately wrong conclusions [75]. Estimates of within-pair estimates are more susceptible to bias due to non-shared confounder than estimates from unpaired analyses [75]. As only case-sibling pairs that are differently exposed contribute to the analysis, a selection for pairs that differ on non-shared causes of the exposure is made. As non-differential (random) measurement error is not shared by siblings, it is likely that among the discordant pairs more individuals will be misclassified on exposure than in the general population [75]. Consequently, when no other biases than random measurement error play a role, the effect estimate will be closer to the null in a case-sibling design when compared with cases and controls from the general population. Indeed, the greater vulnerability to misclassification applies to all (self)-matched designs [76,77].

In **chapter 7**, we discussed another potential limitation of the case-sibling design when there is a natural ordering in the occurrence of cases. For example, firstborn children are at higher risk of developing asthma than their later born siblings [78] and it is therefore likely that the birth sequence is unevenly distributed between case and control siblings. This may result in bias when there is a trend in exposure. In **chapter 7** we applied a method akin to the case-time-control design that adjusts for such potential time-trends. Although for this application the adjustment did not substantially alter the results, because there was no substantial time-trend in the use of antibiotics during pregnancy, it may remove substantial bias when there is a strong time-trend and a natural ordering in the occurrence of cases. Such ordering of cases may occur in various association studies using case-sibling design [79,80]. Although such studies often adjust for birth order, this may not completely remove potential time trends in exposure. An important limitation of using the trend in controls may be that the trend may be different among control pregnancies, especially if mothers of first-born cases decide to stop with a potential harmful exposure during subsequent pregnancies.

Although a case-sibling design on its own does not proof causality, partly due to the limitations described above, it can provide an important contribution to our understanding of associations. Combined with other designs that may provide more information about potential confounding, such as the maternal-paternal comparison

applied in **chapter 7**, the increasing use of the case-sibling is an important step forward in the field of pregnancy outcomes, and the evidence base should be enlarged in the coming years.

#### Quantitative bias analysis

The potential impact of unmeasured confounders, misclassification and/or selection bias can also be estimated using quantitative bias analysis [2,81]. Methods of bias analysis have been well known for decades and are endorsed for widespread use [81]. Nevertheless, we found in **chapter 2** that quantitative bias analysis is rarely applied. When the exposure is not randomly assigned, as in all observational studies, the comparison between groups provides a probability that the outcome distribution is attributable to chance as opposed to the combined effects of exposure and systematic error [2]. Thus to infer causality, an educated guess about the impact of systematic errors is needed.

Between 2010 and 2012, 85% of published articles on case-control or cohort studies on interventions with a hypothesized beneficial effect added a *qualitative* comment about the likelihood of unmeasured confounding (**chapter 2**). However, studies on anchoring and adjustment heuristic suggest that people tend to make adjustments that are insufficient to capture the true impact of the bias [2]. Moreover, an understanding of overconfidence heuristic suggests that people will tend to be overconfident about results [2]. Consequently, qualitative comments on the likelihood and impact of bias are expected to be insufficient. Quantitative bias analysis, informed by internal validation studies or carefully selected external information, can potentially overcome this problem [2,81].

Arguments often posed against quantitative bias analysis are that it is difficult to assign valid values to bias parameters and that the methods are too complex to apply. However, frequentist approaches assume that all bias parameters are set to values that induce no systematic error. It is almost certain that these assigned values are not the true values (no unmeasured confounding, no selection bias and no measurement error) [81]. When different values may seem valid, one can evaluate the sensitivity of the results to variation in the assigned values by assigning a probability distribution or ranging the values using multidimensional bias analysis. The obtained information can also guide the direction of future research. Although Bayesian and probabilistic methods may indeed be complex to apply for the inexperienced researcher, simple bias analysis methods can be easily applied (**chapter 7**) [2].

In light of the increasing use of Big Data [82-84] leading to effect estimates with very narrow confidence intervals, quantitative bias analysis will become increasingly important to prevent policy actions based on overconfidence in the results. For example, based on conventional meta-analyses that found that residential electromagnetic fields were associated with an increased risk of childhood leukaemia, some stakeholders argued that it was necessary to undertake action and relocate of power lines [81]. However, using probabilistic bias analysis it was shown that there was much more uncertainty in the estimates than captured by the conventional confidence intervals [85]. The uncertainty intervals could easily include the null, indicating that costly policy action was not supported by the available data [85].

## Alternative methods to address unmeasured confounding

Although the different self-controlled methods as applied in this thesis work have been proven to be useful to address unmeasured confounding, there are some situations in which the assumptions of the methods are likely violated, and other approaches are needed. Two developments are shortly mentioned below: p-value calibration and the instrumental variable design.

### P-value calibration

Recently, a p-value calibration approach was proposed to take into account that conventional confidence intervals and p-values only take into account random error and ignore selection bias, measurement error and residual or unmeasured confounding [86]. With this method, p-values are calibrated using the observed null distribution for negative controls (drug-outcome pairs for which no causal relationship is assumed). A major problem with this method is that it only works well when negative control drugs really have no causal effect and when the influence of various sources of systematic error is similar for the negative controls as for the association of interest. This is always a potential problem when using negative or positive controls to gain an indication about potential unmeasured confounding. Since several negative controls are needed for calibration, p-value calibration may be difficult to apply in practice without failing to meet crucial assumptions of the method. Therefore, p-value calibration may suffer from a similar problem as conventional methods to calculate confidence intervals and p-values in observational studies: it is likely that the assumptions for the method do not hold. Therefore we would recommend to use quantitative bias analysis to estimate the susceptibility of the results to unmeasured confounding and other forms of systematic error whenever such analysis is productive [81].

**Instrumental variable design**

As mentioned under the heading 'selection strategies for measured confounders' (near)-instrumental variables can cause amplification of bias due to unmeasured confounding when wrongly selected as confounders in the final model. However, instrumental variables can in various situations be very helpful tools to address unmeasured confounding. Instrumental variable analysis can be applied when a variable can be identified that 1) is positively correlated with exposure (nonzero average causal effect), 2) is independent of unmeasured confounders conditional on covariates (random assignment), and 3) affects the outcomes only through its effect on exposure (exclusion restriction). The instrumental variable can be used to extract variation in exposure that is free of unmeasured confounding and subsequently use this variation in exposure to estimate the casual effect of the exposure [87]. However, in practice, it is often difficult to identify variables that fulfil all three requirements. The potential instrumental variable should have a relatively strong association with the exposure, because instrumental variable analysis with a weak instrument is sensitive to slight departures from being a valid instrument [87,88]. Hence, when there is some correlation between exposure and an unmeasured confounder, ordinary least squares regression may be less biased then an instrumental variables analysis with a weak instrument that has a weaker correlation with the unmeasured confounder [88]. However, even when a variable can be considered a valid instrument according to the three requirements listed above, two additional assumptions have to be met for valid analysis: the monotonicity assumption, although not necessary for all instrumental variable analyses [89], and the stable unit treatment value assumption (SUTVA). Suppose we have a binary instrumental variable Z and a binary exposure X. The monotonicity assumption requires that there are no subjects who are defiers, i.e. persons that are always exposed opposite of what would be expected on the level of the instrumental variable (no subject i with . Although this assumption will often not be violated, the model only estimates the average treatment effect for compliers . The effect estimate does not inform about the effect of the treatment among always takers or never takers , unless it can be realistically assumed that there is no heterogeneity in the treatment effects [87]. The SUTVA assumes that potential outcomes for each person are unrelated to the treatment status of other individuals (no interference). It is important to note that this assumption is also generally assumed for other methods than instrumental variable analysis and does not necessarily bias the estimation but changes the interpretation of the effect estimates [87]. When this is not taken into account one may misinterpret what is being estimated [90,91].

When all assumptions are met, instrumental variable analysis can be a useful tool to overcome bias due to unmeasured confounding. In practice it will be often difficult to identify a good instrumental variable candidate and to fulfil all assumptions. Hence, whenever performing an instrumental variable analysis, it is important to report about

the likelihood each assumption is met [92,93] and ideally perform sensitivity analysis to estimate the potential impact of violation of the assumptions.

## Conclusion

In observational research, measuring and adjusting for confounding is crucial to evaluate whether an observed association is possibly causal or biased. Hence, confounding needs to be addressed in the design or analysis phase of each observational study that is intended to study causal associations. In this thesis, we mainly focused on different designs to adjust for measured and unmeasured potential confounding. Various self-controlled methods have been developed to partly overcome the problem of time-invariant unmeasured confounding. When applying such methods it is important to verify whether assumptions are likely met or whether another design might be more applicable to the research question. Quantitative bias analysis with bias parameters based on internal or external validation data can always be applied, regardless of the design and analytical choices. We would like to encourage researchers to further empirically test and modify existing self-controlled methods to partly overcome their main limitations and to develop novel techniques that further reduce or better quantify confounding in observational research.

## References


1. Rothman KJ. Epidemiology: an introduction. Oxford University Press; 2012.
2. Lash TL, Fox MP, Fink AK. Applying quantitative bias analysis to epidemiologic data. Springer Science & Business Media; 2011.
3. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. Lippincott Williams & Wilkins; 2008.
4. Greenland S, Pearce N. Statistical foundations for model-based adjustments. Annu Rev Public Health 2015 Mar 18;36:89-108.
5. Cole SR, Platt RW, Schisterman EF, Chu H, Westreich D, Richardson D, et al. Illustrating bias due to conditioning on a collider. Int J Epidemiol 2010 Apr;39(2):417-420.
6. Myers JA, Rassen JA, Gagne JJ, Huybrechts KF, Schneeweiss S, Rothman KJ, et al. Effects of adjusting for instrumental variables on bias and precision of effect estimates. Am J Epidemiol 2011 Dec 1;174(11):1213-1222.
7. Greenland S. Invited commentary: variable selection versus shrinkage in the control of multiple confounders. Am J Epidemiol 2008 Mar 1;167(5):523-9; discussion 530-1.
8. Groenwold RH, Van Deursen AM, Hoes AW, Hak E. Poor quality of reporting confounding bias in observational intervention studies: a systematic review. Ann Epidemiol 2008 Oct;18(10):746-751.
9. Pearl J. Invited commentary: understanding bias amplification. Am J Epidemiol 2011 Dec 1;174(11):1223-7; discussion pg 1228-9.
10. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 2009 Jul;20(4):488-495.
11. Greenland S. Quantifying biases in causal models: classical confounding vs collider-stratification bias. Epidemiology 2003 May;14(3):300-306.
12. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 2009 Jul;20(4):488-495.
13. Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. Am J Epidemiol 2002 Jan 15;155(2):176-184.
14. Robins JM. Data, design, and background knowledge in etiologic inference. Epidemiology 2001 May;12(3):313-320.
15. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology 1999 Jan;10(1):37-48.
16. VanderWeele TJ, Hernan MA, Robins JM. Causal directed acyclic graphs and the direction of unmeasured confounding bias. Epidemiology 2008 Sep;19(5):720-728.
17. Textor J, Hardt J, Knuppel S. DAGitty: a graphical tool for analyzing causal diagrams. Epidemiology 2011 Sep;22(5):745.
18. Myers JA, Rassen JA, Gagne JJ, Huybrechts KF, Schneeweiss S, Rothman KJ, et al. Myers et al. Respond to "Understanding Bias Amplification". Am J Epidemiol 2011:kwr353.
19. Franklin JM, Eddings W, Glynn RJ, Schneeweiss S. Regularized regression versus the high-dimensional propensity score for confounding adjustment in secondary database analyses. Am J Epidemiol 2015 [in press].
20. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. Am J Epidemiol 1991 Jan 15;133(2):144-153.
21 Suissa S. The case-time-control design. Epidemiology 1995 May;6(3):248-253.
22. Allison PD, Christakis NA. FIXED-EFFECTS METHODS FOR THE ANALYSIS OF NONREPEATED EVENTS. Sociological methodology 2006;36(1):155-172.

23. Wang S, Linkletter C, Maclure M, Dore D, Mor V, Buka S, et al. Future cases as present controls to adjust for exposure trend bias in case-only studies. Epidemiology 2011 Jul;22(4):568-574.
24. Wang SV, Gagne JJ, Glynn RJ, Schneeweiss S. Case-crossover studies of therapeutics: design approaches to addressing time-varying prognosis in elderly populations. Epidemiology 2013 May;24(3):375-378.
25. Hallas J. Evidence of depression provoked by cardiovascular medication: a prescription sequence symmetry analysis. Epidemiology 1996 Sep;7(5):478-484.
26. Redelmeier DA. The exposure-crossover design is a new method for studying sustained changes in recurrent events. J Clin Epidemiol 2013 Sep;66(9):955-963.
27. Farrington CP. Relative incidence estimation from case series for vaccine safety evaluation. Biometrics 1995 Mar;51(1):228-235.
28. Wahab IA, Pratt NL, Wiese MD, Kalisch LM, Roughead EE. The validity of sequence symmetry analysis (SSA) for adverse drug reaction signal detection. Pharmacoepidemiol Drug Saf 2013 May;22(5):496-502.
29. Caughey GE, Roughead EE, Pratt N, Killer G, Gilbert AL. Stroke risk and NSAIDs: an Australian population-based study. Med J Aust 2011 Nov 7;195(9):525-529.
30 Garrison SR, Dormuth CR, Morrow RL, Carney GA, Khan KM. Nocturnal leg cramps and prescription use that precedes them: a sequence symmetry analysis. Arch Intern Med 2012 Jan 23;172(2):120-126.
31. van Boven JF, de Jong-van den Berg LT, Vegter S. Inhaled corticosteroids and the occurrence of oral candidiasis: a prescription sequence symmetry analysis. Drug Saf 2013 Apr;36(4):231-236.
32. Pratt N, Andersen M, Bergman U, Choi NK, Gerhard T, Huang C, et al. Multi-country rapid adverse drug event assessment: the Asian Pharmacoepidemiology Network (AsPEN) antipsychotic and acute hyperglycaemia study. Pharmacoepidemiol Drug Saf 2013 Sep;22(9):915-924.
33. Lai EC, Hsieh CY, Kao Yang YH, Lin SJ. Detecting potential adverse reactions of sulpiride in schizophrenic patients by prescription sequence symmetry analysis. PLoS One 2014 Feb 27;9(2):e89795.
34. Kalisch Ellett LM, Pratt NL, Barratt JD, Rowett D, Roughead EE. Risk of medication-associated initiation of oxybutynin in elderly men and women. J Am Geriatr Soc 2014 Apr;62(4):690-695.
35. Moghaddass R, Madigan D. The factorized self-controlled case series method: An approach for estimating the effects of many drugs on many outcomes. 2015; Available at: http://web.mit.edu/rudin/www/MoghaddassRuMa15.pdf. Accessed 04/28, 2015.
36. Vegter S, de Jong-van den Berg LT. Misdiagnosis and mistreatment of a common side-effect--angiotensin-converting enzyme inhibitor-induced cough. Br J Clin Pharmacol 2010 Feb;69(2):200-203.
37. Vegter S, de Boer P, van Dijk KW, Visser S, de Jong-van den Berg LT. The effects of antitussive treatment of ACE inhibitor-induced cough on therapy compliance: a prescription sequence symmetry analysis. Drug Saf 2013 Jun;36(6):435-439.
38. Pratt N, Chan EW, Choi NK, Kimura M, Kimura T, Kubota K, et al. Prescription sequence symmetry analysis: assessing risk, temporality, and consistency for adverse drug reactions across datasets in five countries. Pharmacoepidemiol Drug Saf 2015 Apr 22.
39. Weldeselassie YG, Whitaker HJ, Farrington CP. Use of the self-controlled case-series method in vaccine safety studies: review and recommendations for best practice. Epidemiol Infect 2011 Dec;139(12):1805-1817.
40. Hallas J, Pottegard A. Use of self-controlled designs in pharmacoepidemiology. J Intern Med 2014 Jun;275(6):581-589.
41. Kuhnert R, Hecker H, Poethko-Muller C, Schlaud M, Vennemann M, Whitaker HJ, et al. A modified self-controlled case series method to examine association between multidose vaccinations and death. Stat Med 2011 Mar 15;30(6):666-677.

42. Farrington CP, Whitaker HJ, Hocine MN. Case series analysis for censored, perturbed, or curtailed post-event exposures. Biostatistics 2009 Jan;10(1):3-16.
43. Farrington C, Hocine MN. Within-individual dependence in self-controlled case series models for recurrent events. Journal of the Royal Statistical Society: Series C (Applied Statistics) 2010;59(3):457-475.
44. Simpson SE. Self-controlled methods for postmarketing drug safety surveillance in large-scale longitudinal data 2011.
45. Hua W, Sun G, Dodd CN, Romio SA, Whitaker HJ, Izurieta HS, et al. A simulation study to compare three self-controlled case series approaches: correction for violation of assumption and evaluation of bias. Pharmacoepidemiol Drug Saf 2013 Aug;22(8):819-825.
46. Escolano S, Hill C, Tubert-Bitter P. A new self-controlled case series method for analyzing spontaneous reports of adverse events after vaccination. Am J Epidemiol 2013 Nov 1;178(9):1496-1504.
47. Simpson SE. A positive event dependence model for self-controlled case series with applications in postmarketing surveillance. Biometrics 2013 Mar;69(1):128-136.
48. Schuemie MJ, Trifiro G, Coloma PM, Ryan PB, Madigan D. Detecting adverse drug reactions following long-term exposure in longitudinal observational data: The exposure-adjusted self-controlled case series. Stat Methods Med Res 2014 Mar 31.
49. Mittleman MA, Mostofsky E. Exchangeability in the case-crossover design. Int J Epidemiol 2014 Oct;43(5):1645-1655.
50. Maclure M, Mittleman MA. Should we use a case-crossover design? Annu Rev Public Health 2000;21:193-221.
51. Maclure M, Fireman B, Nelson JC, Hua W, Shoaibi A, Paredes A, et al. When should case-only designs be used for safety monitoring of medical products? Pharmacoepidemiol Drug Saf 2012 Jan;21 Suppl 1:50-61.
52. Wang SV, Schneeweiss S, Maclure M, Gagne JJ. "First-wave" bias when conducting active safety monitoring of newly marketed medications with outcome-indexed self-controlled designs. Am J Epidemiol 2014 Sep 15;180(6):636-644.
53. Navidi W. Bidirectional case-crossover designs for exposures with time trends. Biometrics 1998 Jun;54(2):596-605.
54. Navidi W, Weinhandl E. Risk set sampling for case-crossover designs. Epidemiology 2002 Jan;13(1):100-105.
55. Janes H, Sheppard L, Lumley T. Case-crossover analyses of air pollution exposure data: referent selection strategies and their implications for bias. Epidemiology 2005 Nov;16(6):717-726.
56. Janes H, Sheppard L, Lumley T. Overlap bias in the case-crossover design, with application to air pollution exposures. Stat Med 2005 Jan 30;24(2):285-300.
57. Greenland S. Confounding and exposure trends in case-crossover and case-time-control designs. Epidemiology 1996 May;7(3):231-239.
58. Nordmann S, Biard L, Ravaud P, Esposito-Farese M, Tubach F. Case-only designs in pharmacoepidemiology: a systematic review. PLoS One 2012;7(11):e49444.
59. Xu S, Gargiullo P, Mullooly J, McClure D, Hambidge SJ, Glanz J. Fitting parametric and semi-parametric conditional Poisson regression models with Cox's partial likelihood in self-controlled case series and matched cohort studies. J Data Sci 2010;8:349-360.
60. Vines SK, Farrington CP. Within-subject exposure dependency in case-crossover studies. Stat Med 2001 Oct 30;20(20):3039-3049.
61. Avalos M, Grandvalet Y, Adroher ND, Orriols L, Lagarde E. Analysis of multiple exposures in the case-crossover design via sparse conditional likelihood. Stat Med 2012 Sep 20;31(21):2290-2302.

62. Avalos M, Orriols L, Pouyes H, Grandvalet Y, Thiessard F, Lagarde E, et al. Variable selection on large case-crossover data: application to a registry-based study of prescription drugs and road traffic crashes. Pharmacoepidemiol Drug Saf 2014 Feb;23(2):140-151.
63. Farrington CP, Miller E, Taylor B. MMR and autism: further evidence against a causal association. Vaccine 2001 Jun 14;19(27):3632-3635.
64. Simpson SE, Madigan D, Zorych I, Schuemie MJ, Ryan PB, Suchard MA. Multiple self-controlled case series for large-scale longitudinal observational databases. Biometrics 2013 Dec;69(4):893-902.
65. Lumley T, Levy D. Bias in the case–crossover design: implications for studies of air pollution. Environmetrics 2000;11(6):689-704.
66. Whitaker HJ, Farrington CP, Spiessens B, Musonda P. Tutorial in biostatistics: the self-controlled case series method. Stat Med 2006 May 30;25(10):1768-1797.
67. Farrington C, Whitaker H. Semiparametric analysis of case series data. Journal of the Royal Statistical Society: Series C (Applied Statistics) 2006;55(5):553-594.
68. Hua W, Sun G, Dodd CN, Romio SA, Whitaker HJ, Izurieta HS, et al. A simulation study to compare three self-controlled case series approaches: correction for violation of assumption and evaluation of bias. Pharmacoepidemiol Drug Saf 2013 Aug;22(8):819-825.
69. Farrington P, Whitaker H. Mortality and the self-controlled case series method: letter to the editor. Pharmacoepidemiol Drug Saf 2012 Aug;21(8):906; author reply 907.
70. Farrington CP, Anaya-Izquierdo K, Whitaker HJ, Hocine MN, Douglas I, Smeeth L. Self-controlled case series analysis with event-dependent observation periods. Journal of the American Statistical Association 2011;106(494).
71. Madigan D, Ryan PB, Schuemie M, Stang PE, Overhage JM, Hartzema AG, et al. Evaluating the impact of database heterogeneity on observational study results. Am J Epidemiol 2013 Aug 15;178(4):645-651.
72. Nicholas JM, Grieve AP, Gulliford MC. Within-person study designs had lower precision and greater susceptibility to bias because of trends in exposure than cohort and nested case-control designs. J Clin Epidemiol 2012 Apr;65(4):384-393.
73. Farrington P. Censoring on outcome is not valid in self-controlled case series studies. J Clin Epidemiol 2013 Dec;66(12):1428-1429.
74. Donovan SJ, Susser E. Commentary: Advent of sibling designs. Int J Epidemiol 2011 Apr;40(2):345-349.
75. Frisell T, Oberg S, Kuja-Halkola R, Sjolander A. Sibling comparison designs: bias from non-shared confounders and measurement error. Epidemiology 2012 Sep;23(5):713-720.
76. Greenland S. Confounding and exposure trends in case-crossover and case-time-control designs. Epidemiology 1996 May;7(3):231-239.
77. Greenland S. The effect of misclassification in matched-pair case-control studies. Am J Epidemiol 1982 Aug;116(2):402-406.
78. Kawada T. Prevalence of asthma and atopic dermatitis in children with special emphasis on birth order. Pediatr Allergy Immunol 2012 Dec;23(8):795; author reply 796.
79. Nielsen PR, Mortensen PB, Dalman C, Henriksen TB, Pedersen MG, Pedersen CB, et al. Fetal growth and schizophrenia: a nested case-control and case-sibling study. Schizophr Bull 2013 Nov;39(6):1337-1342.
80. Alves JG, Figueiroa JN, Meneses J, Alves GV. Breastfeeding protects against type 1 diabetes mellitus: a case-sibling study. Breastfeed Med 2012 Feb;7(1):25-28.
81. Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, Greenland S. Good practices for quantitative bias analysis. Int J Epidemiol 2014 Dec;43(6):1969-1985.

82. Mooney SJ, Westreich DJ, El-Sayed AM. Commentary: epidemiology in the era of big data. Epidemiology 2015 May;26(3):390-394.
83. Kaplan RM, Chambers DA, Glasgow RE. Big data and large sample size: a cautionary note on the potential for bias. Clin Transl Sci 2014 Aug;7(4):342-346.
84. Khoury MJ, Ioannidis JP. Medicine. Big data meets public health. Science 2014 Nov 28;346(6213):1054-1055.
85. Greenland S. Multiple-bias modelling for analysis of observational data. Journal of the Royal Statistical Society: Series A (Statistics in Society) 2005;168(2):267-306.
86. Schuemie MJ, Ryan PB, DuMouchel W, Suchard MA, Madigan D. Interpreting observational studies: why empirical calibration is needed to correct p-values. Stat Med 2014 Jan 30;33(2):209-218.
87. Baiocchi M, Cheng J, Small DS. Instrumental variable methods for causal inference. Stat Med 2014 Jun;33(13);2297-2340.
88. Small D, Rosenbaum P. War and wages: the strength of instrumental variables and their sensitivity to unobserved biases. J Am Stat Assoc 2008 Sep;103(483):924-933.
89. Swanson SA, Miller M, Robins JM, Hernan MA. Definition and evaluation of the monotonicity conidition for preference-based instruments. Epidemiology 2015;26(3):414-420.
90. Hudgens M, Halloran M. Towards causal inference with inference. J Am Stat Assoc 2008 Jun; 103(482):832-842.
91. Sobel M. What do randomized studies of housing mobility demonstrate? Causal inference in the face of interference. J Am Stat Assoc 2006 Dec;101(476):1398-1407.
92. Brookhart M, Rassen J, Schneeweiss S. Instrumental variable methods in comparative safety and effectiveness research. Pharmacoepidemiol Drug Saf 2010 Jun;19(6):537-554.
93. Davies N, Smith G, Windmeijer F, Martin R. Issues in the reporting and conduct of instrumental variable studies: a systematic review. Epidemiology 2013 May;24(3):363-369.

# Summary

In the absence of randomized controlled trials, the best available evidence for decision-making on interventions will often come from observational studies. However, observational studies that evaluate the effects of interventions are prone to confounding bias. Given the vulnerability of observational studies to confounding, complete and transparent reporting on confounding is necessary to enable readers to assess the validity of study findings. Therefore, the first aim of this thesis is to assess whether the reporting of confounding improved in articles published after the publication of the STROBE guideline compared with articles published before the guideline was introduced.

One of the more novel developments with regard to the control of confounding in observational studies is the application of a self-controlled or case-only design. Empirical comparisons of such study designs with each other, with more traditional observational designs and with randomized controlled trials are scarce. Hence, the second aim of this thesis is to apply and empirically compare various available self-controlled designs to quantify and control for confounding with other designs.

In **chapter 2** the reporting of confounding in observational cohort and case-control studies on interventions before and after the publication of a widely endorsed guideline for observational studies (STROBE) is presented. The median of reported number of items (range 1-8) was similar before and after the publication of the STROBE statement (median 4, interquartile range [IQR] 3-5 vs median 4, IQR 4-5), although the distribution shifted somewhat to the right ($p<0.001$). Results were similar for journals that published the STROBE statement, endorsed the guideline in their author instructions or required the submission of a completed STROBE checklist. Our study showed that although the quality of reporting about confounding improved in certain aspects, the overall quality remains suboptimal. Research is needed into the development and evaluation of strategies to improve the quality of reporting and adherence to reporting guidelines.

In **chapter 3** the effect of pravastatin on recurrent urinary tract infections is evaluated in a post-hoc analysis of a randomized controlled trial (PREVEND-IT). In addition, the effect of fosinopril, an angiotensin-converting enzyme inhibitor (ACEi), on acute urinary tract infections is assessed. Intention-to-treat analyses showed that pravastatin was associated with a reduced total number of urinary tract infection antibiotic prescriptions (relative risk, 0.43; 95% CI: 0.21–0.88) and occurrence of second urinary tract infection antibiotic prescriptions [hazard ratio (HR) 0.25; 95% CI: 0.08–0.77]. No significant effect on occurrence of first urinary tract infection antibiotic prescriptions was found (HR 0.83; 95% CI: 0.57–1.20). Fosinopril was associated with an increased

occurrence of first urinary tract infection antibiotic prescriptions (HR 1.82; 95% CI: 1.16–2.88). Combination therapy with fosinopril and pravastatin did not significantly influence the number of urinary tract infection antibiotic prescriptions. This study suggests that pravastatin can reduce the occurrence of recurrent urinary tract infections and fosinopril may induce first urinary tract infections. Larger studies are needed to confirm these findings.

In **chapter 4** the association of ACEi with the risk of acute urinary tract infections is assessed using a prescription sequence symmetry analysis. A total of 101 (63%) patients started ACEi therapy first followed by nitrofurantoin treatment (a proxy for urinary tract infections), while 60 (37%) patients started nitrofurantoin treatment first, which corresponds to an adjusted sequence ratio (ASR) of 1.68 (95% CI: 1.21-2.36). No association was found between β-blockers and nitrofurantoin treatment (ASR 1.01, 95%CI: 0.74-1.38). To conclude, a significant excess of patients received urinary tract infection antibiotic prescriptions following the first month after ACEi initiation. This prescription sequence asymmetry agrees with the trial findings and suggests that ACEi initiation increases the risk of developing urinary tract infections.

In **chapter 5** the association between ACEi and urinary tract infections is further evaluated using a case-crossover design. Of included patients, 276 patients were only exposed to ACEi during the risk window and 150 patients only during the control window (adjusted OR 1.74; 95% CI 1.42-2.13). When using similar criteria as in the prescription sequence symmetry analysis, the case-crossover estimates were slightly higher (adjusted OR 2.09, 95% CI 1.68-2.61). These findings suggest that ACEi use increases the risk of developing first urinary tract infections. Despite the similarities between the case-crossover design and the PSSA, the PSSA led to slightly lower effect estimates than the case-crossover design and the post-hoc analysis of the randomized trial.

In **chapter 6** the association of combined use of selective serotonin reuptake inhibitors (SSRIs) and nonsteroidal anti-inflammatory drugs (NSAIDs) with the risk of starting peptic ulcer treatment is evaluated using a prescription sequence symmetry design. A comparison is made with a previously published cohort study that used the same database but did not adjust for potential confounders. In contrast to the previous cohort study (RR 12.4; 95% CI: 3.2-48.0), combined use of SSRIs with NSAIDs was not associated with a higher risk of peptic ulcer treatment than NSAIDs alone (ASR 1.48; 95% CI: 0.90-2.49 vs ASR 2.50; 95% CI: 2.27-2.76). Our findings indicate that at least part of the previously reported association between combined use of SSRIs with NSAIDs and peptic ulcer initiation might be attributed to unmeasured or residual confounding.

In **chapter 7** the association between antibiotic use during pregnancy and the development of asthma in preschool children is analysed using different confounding-minimizing designs, including a case-control and case-sibling design. Both the case-sibling and case-control analysis yielded similar increased risks of asthma in preschool children when antibiotics were used in the third trimester of pregnancy (aOR 1.37; 95%CI: 1.02-1.83 and aOR 1.40; 95%CI: 1.15-1.47). Time trend analyses showed that results were not influenced by a time trend in antibiotic exposure. Significant increased risks of asthma in preschool children after exposure to antibiotics in any trimester of pregnancy was observed in the conventional case-control analysis only (aOR 1.46;95%CI 1.34-1.59). In conclusion, exposure to antibiotics in the third trimester of pregnancy appeared to be associated with a small increased risk of asthma in preschool children. This association appeared not to be influenced by time-invariant confounders or time trends in antibiotic exposure.

In **chapter 8** the concordance between case-only and parallel group designs in empirical studies is evaluated in a systematic way. In addition, predictors of discrepancies between both types of designs are identified. The correlation coefficient between the intervention effect in case-only versus parallel group designs was mediocre 0.64 ($p<.001$). In 221 of the 519 comparisons (43%) the difference between both study designs was beyond what would be expected by chance alone. The following predictors of discrepancy were found: intermittent exposure, rare event, acute outcome, length of hazard period, type of case-only design and sample size of the traditional study design. We found that the concordance between effect estimates of case-only and cohort or case-control design is moderate, and discrepancies beyond chance are very common. Such discrepancies could be predicted by failure to meet important assumptions of case-only designs.

To conclude, this thesis evaluated whether the reporting of confounding improved in articles published after the publication of the STROBE guideline compared with articles published before that guideline. In addition we applied and empirically compared various available self-controlled designs with other designs that were used to control for confounding. Based on the conclusions of the studies we recommend to focus on development and evaluation of strategies to improve the quality of reporting and adherence to reporting guidelines rather than adding another guideline to the steadily increasing pile of reporting guidelines; to apply self-controlled designs when there are concerns about unmeasured confounders and the research question seems appropriate for such a design; to verify whether assumptions are likely met when applying a study design and in case of uncertainty about this to apply sensitivity analyses. Finally, self-controlled methods should be empirically tested and modified to overcome their main limitations such as vulnerability for time trends and to develop new methodologies that further reduce or better quantify confounding in observational intervention research.

# Samenvatting

Vaak komt het bewijs waarop beslissingen over interventies zijn gebaseerd uit observationeel onderzoek. Een veelvoorkomend probleem in dergelijk niet-gerandomiseerd onderzoek is *confounding*. *Confounding* vindt plaats wanneer een factor het causale verband tussen een determinant en de uitkomst verstoord. Indien niet voor dergelijke factoren (*confounders*) gecorrigeerd wordt is de kans groot dat er verkeerde conclusies worden getrokken.

Omdat *confounding* veel voorkomt in observationele studies is het noodzakelijk volledige informatie te geven over hoe er is omgegaan met eventuele *confounding* om lezers de mogelijkheid te bieden de validiteit van de onderzoeksbevindingen adequaat te beoordelen. Derhalve is een belangrijk doel van deze thesis te evalueren of de verslaglegging met betrekking tot *confounding* verbeterde na de publicatie van de *STROBE* richtlijn. Deze richtlijn, die door steeds meer wetenschappelijke tijdschriften wordt aangeraden, is speciaal ontworpen om de verslaglegging in observationeel onderzoek te verbeteren.

Een recente ontwikkeling omtrent het controleren van *confounding* in observationeel onderzoek is het toepassen van designs waarbij patiënten als hun eigen controle fungeren (*self-controlled designs*). Onderzoeken waarbij empirische vergelijkingen tussen *self-controlled designs* of vergelijkingen met meer traditionele parallel groep designs en gerandomiseerde onderzoeken worden gemaakt zijn schaars. Een tweede belangrijk doel van deze thesis is het toepassen en vergelijken van verschillende *self-controlled designs* met andere designs die gebruikt worden om voor *confounding* te controleren.

In **hoofdstuk 2** wordt de verslaglegging met betrekking tot *confounding* voor en na de publicatie van *STROBE* richtlijn vergeleken. Het aantal gerapporteerde items (maximaal 8 items) was vergelijkbaar voor en na de publicatie van de richtlijn (mediaan 4, interkwartielafstand (IKA) 3-5 vs. mediaan 4, IKA 4-5), ook al verschoof de verdeling wel enigszins naar rechts ($p<0.001$). De resultaten waren vergelijkbaar voor wetenschappelijke tijdschriften die de STROBE richtlijn publiceerden, het gebruik van de richtlijn aanmoedigden in de instructies voor auteurs, of vereisten dat de auteurs een checklist meestuurden waarin ze aangaven op welke pagina ze voldaan hadden aan de individuele items van de richtlijn. Onze studie liet zien dat ondanks dat de kwaliteit van de verslaglegging met betrekking tot *confounding* in sommige aspecten verbeterde, de algehele kwaliteit nog flink kan worden verbeterd. Meer onderzoek is nodig naar de ontwikkeling en evaluatie van strategieën om de kwaliteit van de verslaglegging en met name de naleving van de richtlijnen te verbeteren.

In **hoofdstuk 3** wordt het effect van pravastatine op (recidiverende) urineweginfecties geëvalueerd middels een post-hoc analyse van een gerandomiseerde studie (PREVEND-IT). Daarnaast wordt in dit hoofdstuk het effect van fosinopril, een *angiotensine-converting-enzyme* (ACE-)remmer, op urineweginfecties onderzocht. Pravastatine was geassocieerd met een verminderd risico op urineweginfecties (relatief risico, 0.43; 95% CI: 0.21-0.88) en op tweede, oftewel recidiverende, urineweginfecties (hazard ratio (HR) 0.25; 95% CI: 0.08-0.77). Geen significant effect werd gevonden voor eerste urineweginfecties (HR 0.83; 95% CI: 0.57-1.20). Fosinopril was geassocieerd met een verhoogd risico op eerste urineweginfecties (HR 1.82; 95% CI: 1.16-2.88). Combinatietherapie met fosinopril en pravastatine had geen significant effect op het risico op urineweginfecties. Deze resultaten suggereren dat pravastatine het risico op recidiverende urineweginfecties kan verlagen, terwijl fosinopril mogelijk het risico op eerste urineweginfecties verhoogd. Echter, vanwege de beperkte omvang van de studie zijn er grotere studies nodig om te kijken of deze resultaten kunnen worden bevestigd.

In **hoofdstuk 4** wordt de associatie tussen ACE-remmers en het risico op urineweginfecties geëvalueerd met behulp van een *prescriptie sequence symmetry analysis*. In totaal startten er 101 (63%) patiënten met ACE-remmers voordat zij met nitrofurantoïne behandeling (een proxy voor urineweginfecties) startten, terwijl 60 (37%) patiënten juist beide geneesmiddelen in de omgekeerde volgorde startten. Na correctie voor mogelijke trends in het voorschrijven van een van beide middelen werd een gecorrigeerde sequence ratio (SR) van 1.68 (95% CI: 1.21-2.36) gevonden. Een dergelijke associatie werd niet gevonden tussen β-blokkers en nitrofurantoïne (SR 1.01; 95% CI: 0.74-1.38). Deze resultaten suggereren, net als de post-hoc analyse van de gerandomiseerde studie, dat het starten van ACE-remmer therapie het risico op het ontwikkelen van urineweginfecties (tijdelijk) verhoogt.

In **hoofdstuk 5** wordt de associatie tussen ACE-remmers en urineweginfecties verder onderzocht met behulp van een *case-crossover* design. Van de geïncludeerde patiënten gebruikten er 276 ACE-remmers alleen tijdens het risico-interval en 150 patiënten alleen tijdens het controle-interval (gecorrigeerde OR 1.74; 95% CI: 1.42-2.13). Wanneer de in- en exclusie criteria gelijk werden gesteld met de *prescription sequence symmetry analysis* werd een iets hoger risico gevonden (gecorrigeerde OR 2.09; 95% CI: 1.68-2.61). Deze bevindingen suggereren dat ACE-remmers het risico op urineweginfecties (tijdelijk) verhogen. Ondanks de overeenkomsten tussen het *case-crossover* design en de *prescriptie sequence symmetry analysis*, leidde dit laatste design tot lagere effect schattingen dan het *case-crossover* design en de post-hoc analyse van het gerandomiseerde onderzoek.

In **hoofdstuk 6** wordt de associatie tussen gecombineerd gebruik van selectieve serotonine heropname remmers (SSRIs) en niet-steroïde prostaglandine-synthetase-remmers (NSAIDs) en het risico op het starten van maagzweer behandeling onderzocht middels een *presciption sequence symmetry analysis*. Een vergelijking wordt gemaakt met een eerder gepubliceerde cohort studie die gebruik maakte van dezelfde database maar niet corrigeerde voor potentiële *confounders*. In tegenstelling tot de bevindingen van de eerdere cohort studie (RR 12.4; 95% CI: 3.2-48.0) was combinatietherapie van SSRIs met NSAIDs niet geassocieerd met een hoger risico op maagzweer behandeling dan behandeling met NSAIDs alleen (SR 1.48; 95% CI: 0.90-2.49 vs. SR 2.50; 95% CI: 2.27-2.76). Onze bevindingen geven aan dat tenminste een deel van de eerder gepubliceerde associatie tussen gecombineerd gebruik van SSRIs met NSAIDs en het starten van maagzweer behandeling kan worden verklaard door ongemeten *confounding*.

In **hoofdstuk 7** wordt de associatie tussen antibiotica gebruik tijdens de zwangerschap en ontwikkeling van astma in jonge kinderen geanalyseerd middels verschillende designs: een *case-control* (patiënt-controle) en een *case-sibling* design. Beide designs gaven vergelijkbare resultaten voor antibiotica gebruik tijdens het derde trimester van de zwangerschap (OR 1.37; 95% CI: 1.02-1.83 en OR 1.40; 95% CI: 1.15-1.47). Vergelijkbare resultaten werden verkregen wanneer met het *case-sibling* design tevens werd gecorrigeerd voor potentiële trends in het voorschrijven van beide geneesmiddelen. Wanneer de zwangerschap als een geheel werd geanalyseerd werd er alleen met het traditionele *case-control* design een verhoogd risico gevonden (OR 1.46; 95% CI: 1.34-1.59). Deze resultaten duiden er op dat antibiotica gebruik tijdens het derde trimester van de zwangerschap is geassocieerd met een klein verhoogd risico op astma in het kind.

In **hoofdstuk 8** wordt de overeenstemming tussen gepubliceerde *self-controlled* en parallel groep interventieonderzoeken geanalyseerd op een systematische manier. Daarnaast wordt er gekeken of er variabelen zijn die kunnen voorspellen of deze twee type designs verschillende resultaten geven. De correlatiecoëfficiënt tussen beide designs was middelmatig 0.64 ($p<.001$). In 221 van de 519 vergelijkingen (43%) was het verschil groter dan de vooraf vastgestelde drempelwaarde. Aan de hand van de volgende binaire en categorische variabelen kon worden voorspeld of resultaten overeenstemden: kortdurende of onderbroken interventie, zeldzame uitkomst, acute uitkomst, de lengte van het risico-interval, het type self-controlled design en de steekproefomvang. Er werd dus in deze studie gevonden dat de overeenstemming tussen *self-controlled* en parallel groep designs middelmatig is. Discrepanties konden worden voorspeld aan de hand van variabelen die aangaven of er werd voldaan aan belangrijke assumpties van *self-controlled* designs, zoals het analyseren van een acute uitkomst.

Concluderend is er in deze thesis onderzocht of de verslaglegging met betrekking tot *confounding* is verbeterd na de publicatie van de STROBE richtlijn. Tevens hebben we verscheidene *self-controlled* designs toegepast en vergeleken met designs die op een andere manier corrigeren voor *confounding*. Gebaseerd op de conclusies van de verschillende studies uit deze thesis raden we aan om onderzoek te concentreren op de ontwikkeling en evaluatie van strategieën om de kwaliteit van de verslaglegging en naleving van de richtlijnen te verbeteren in plaats van het toevoegen van weer een nieuwe richtlijn aan de steeds maar stijgende stapel; om *self-controlled* designs toe te passen wanneer er zorgen zijn over ongemeten *confounders* en de onderzoeksvraag geschikt voor een dergelijk design lijkt te zijn; om te verifiëren of er wordt voldaan aan belangrijke assumpties wanneer een studie design wordt toegepast en het toepassen van sensitiviteitsanalyses in het geval van onzekerheid. Tenslotte adviseren we *self-controlled* designs empirisch te testen en door te ontwikkelen om de belangrijkste beperkingen te overwinnen en om nieuwe methodes te ontwikkelen die beter kunnen corrigeren voor *confounding* in observationeel interventieonderzoek.

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# List of publications

Citations: 120 (September 2015). H-index: 6, based on Google Scholar.

**Pouwels KB**, Van Grootheest K. **The rosiglitazone decision process at FDA and EMA. What should we learn?** *Int J Risk Saf Med* 2012;24(2):73-80.

**Pouwels KB**, Visser ST, Hak E. **Effect of pravastatin and fosinopril on recurrent urinary tract infections.** *J Antimicrob Chemother* 2013;68(3):708-14.

De Vries FM, Denig P, **Pouwels KB**, Postma MJ, Hak E. **Primary prevention of major cardiovascular and cerebrovascular events with statins in diabetic patients: a meta-analysis.** *Drugs* 2012;72(18):2365-73.

De Boer PT, **Pouwels KB**, Cox JM, Hak E, Wilschut JC, Postma MJ. **Cost-effectiveness of vaccination of the elderly against herpes zoster in The Netherlands.** *Vaccine* 2013;31(9):1276-83.

**Pouwels KB**, Hak E, van der Ende A, Christensen H, van den Dobbelsteen GP, Postma MJ. **Cost-effectiveness of vaccination against meningococcal B among Dutch infants: Crucial impact of changes in incidence.** *Hum Vaccin Immunother* 2013;9(5):1129-38.

Hepkema H, **Pouwels KB**, van der Ende A, Westra TA, Postma MJ. **Meningococcal serogroup A, C, $W_{135}$ and Y conjugated vaccine: a cost-effectiveness analysis in the Netherlands.** *PLoS One* 2013;8(5):e65036.

**Pouwels KB**, Visser ST, Bos HJ, Hak E. **Angiotensin-converting enzyme inhibitor treatment and the development of urinary tract infections: a prescription sequence symmetry analysis.** *Drug Saf* 2013;36(11):1079-86.

Meijboom MJ, **Pouwels KB**, Luytjes W, Postma MJ, Hak E. **RSV vaccine in development: assessing the potential cost-effectiveness in the Dutch elderly population.** *Vaccine* 2013;31(52):6254-60.

**Pouwels KB**, Kalkman GA, Schagen D, Visser ST, Hak E. **Is combined use of SSRIs and NSAIDs associated with an increased risk of starting peptic ulcer treatment?** *Br J Clin Pharmacol* 2014;78(1):192-3.

**Pouwels KB**, Hak E. **Re:"a prospective study of statin drug use and lower urinary tract symptoms in older men".** *Am J Epidemiol* 2014;179(7):927.

**Pouwels KB**, Bos HJ, Hak E. **ACE inhibitors and urinary tract infections.** *Epidemiology* 2014;25(3):466-7.

**Pouwels KB**, Widyakusuma NN, Groenwold RHH, Hak E. **Quality of reporting of confounding before and after the STROBE guideline.** *J Cin Epidemiol* 2015. doi: 10.1016/j.jclinepi.2015.08.009 [in press].

**Pouwels KB**, Mulder B, Hak E. **Moderate concordance was found between case-only and parallel group designs in systematic comparison.** *J Clin Epidemiol* 2015 [in press].

Mulder B, **Pouwels KB**, Schuiling-Veninga CCM, Bos HJ, de Vries TW, Jick SS, Hak E. **Antibiotic use during pregnancy and the development of asthma in preschool children: the influence of confounding.** *Submitted*

**Pouwels KB**, Voorham J, Hak E, Denig P. **Identification of major cardiovascular events in patients with diabetes using primary care morbidity data and drug prescription data.** *Submitted*

Setiawan D, Luttjeboer J, **Pouwels KB**, Wilschut JC, Postma MJ. **Immunogenicity and safety of human papillomavirus (HPV) vaccines in Asian populations**. *Submitted*

**Pouwels KB**, Widyakusuma NN, Bos HJ, Hak E. **Association between statins and infections among patients with drug-treated type 2 diabetes**. *Submitted*

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# Curriculum Vitae

Koen Pouwels (1986) was born in Hardenberg, the Netherlands. After finishing secondary school at OSG Erasmus in Almelo, he moved to Groningen. He started studying Life Science & Technology at the University of Groningen and at the same time started rowing at the national level. He won several medals at national championships and competed at the World Rowing U23 Championships in the single sculls. After obtaining his bachelor degree, he joined the master program of Medical Pharmaceutical Sciences. He wrote his master thesis about the cost-effectiveness of vaccination of the elderly against respiratory syncytial virus under supervision of Prof. M.J. Postma at the department of PharmacoEpidemiology & PharmacoEconomics. In June 2011, after graduating, he started with a four-year PhD project at this department on methodological aspects of confounding and self-controlled designs under supervision of Prof. E. Hak. The studies performed as part of this project are presented in this thesis. Meanwhile Koen continued his cooperation with Prof. M.J. Postma and performed several cost-effectiveness studies for various vaccines. He received several nominations and awards for his work and presented his work at various (inter)national congresses. In July 2015, he started working as a mathematical modeller/health economist at Public Health England on a project about antimicrobial resistance.