# Etiological studies in complex diseases

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# Etiological studies in complex diseases

Etiologische studies en complexe ziektebeelden

(met een samenvatting in het Nederlands)

Proefschrift

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# CHAPTER 1

# GENERAL INTRODUCTION

## INTRODUCTION

Chronic diseases make up a substantial part of present-day morbidity and mortality. More often than not, a chronic disease can be better understood as a syndromal diagnosis. Rather than a disease with a specific cause, chronic diseases often consist of a correlated cluster of particular symptoms and signs (Pearce, 1992). In many cases, a chronic disease or syndrome is pathological in that it is incurable and often progressive in nature. Like other scientists, epidemiologists seek explanations for the occurrence of observable phenomena. In the context of epidemiology, this search is aimed at understanding why a particular health-related complication occurs; to determine its cause or etiology. When dealing with health risks among chronically ill patients, determination of cause and effect based on epidemiologic evidence is challenging, especially for long-term complications. That is because with complex chronic diseases, putative causes for health-related complications to a high degree occur simultaneously. For example, obesity, sedimentary lifestyle, high-caloric diet, and the use of blood-glucose lowering agents often occur together in patients with type 2 diabetes mellitus. Among patients with type 2 diabetes mellitus, an increased incidence of (specific types of) cancer has been established (Harding *et al*, 2015). However, determining which specific features of type 2 diabetes mellitus are causally linked to this increased incidence of cancer becomes troublesome under these circumstances (Johnson *et al*, 2012). Similarly, inflammation, the use of glucocorticoids, and the loss of mobility typically ensue at the same time in patients with multiple sclerosis (Christensen *et al*, 2012). For all these individual factors hypotheses have been formed that causally link them to an excess risk of venous thromboembolism. And while we might be limited in our abilities to search for answers, questions of cause and effect will relentlessly emerge, for both scientific and health policy reasons.

## ETIOLOGY BASED ON EPIDEMIOLOGIC EVIDENCE

The literal meaning of epidemiology (*epi* meaning 'upon', *demos* meaning 'people', *logos* meaning 'the study of') reads 'the study of what is among the people'. In accordance with this literal meaning, epidemiology was traditionally concerned with describing the frequency and distribution of disease and its putative risk factors among specific populations. This so-called *descriptive* epidemiology has fairly recently been complemented with *analytical* epidemiology, which is focused on studying associations between variables in a population with the aim of determining *causes* of health-related states (Charlton, 1996). As a relatively young discipline, the definition of epidemiology, its content and aims, has not fully crystallized and has notably broadened over the past 90 years. *A Dictionary of Epidemiology* currently defines epidemiology as the study of the occurrence and distribution of health-related events, states, and processes in specified populations, including the study of determinants influencing such processes, and the application of this knowledge to control relevant health problems. Moreover, it states that the primary "knowledge object" of epidemiology are *causes* of health-related events, states, and processes in groups and populations (Porta, 2014). By broadening its aims, from describing the distribution of group characteristics to determining the *etiology* of health-related events, epidemiology has moved into the realm of empirical science.

As a non-experimental (observational) form of empirical research, epidemiology is characterized by a lack of manipulation of the natural world. It utilizes data which is collected or routinely recorded without interference with the processes as they naturally occur to study associations between certain observable phenomena within specified populations. Strictly speaking, epidemiologic evidence therefore consists of observation statements; *e.g.* type 2 diabetes mellitus is associated with an excess risk of incidence for overall and a number of site-specific cancers (Harding *et al*, 2015; Tsilidis *et al*, 2015). However, a statistical association by itself does not indicate whether one causes the other (Savitz, 2003; Weisberg, 2010). Yet, causality remains the issue of ultimate interest. Observing a relationship between two events naturally raises the more substantial question as to why they are related. Therefore, most epidemiologic research ultimately has an explicit or implicit goal of contributing toward a broader causal inference (Savtiz, 2003). However, while causality lies at the core of meaningful scientific knowledge, the concept of causality is remarkably complex in philosophical terms and tests to assess causality are subject to special requirements. And although assessing the nature of an association by itself is already challenging, Savitz (2003, p.10) goes even further by describing the goal of epidemiologic research as "(…) the quantification of the causal relation between exposure and disease". He thereby follows Rothman (1986), who advocated against simple statistical hypothesis testing in favor of the estimation of causal effects as the main focus of epidemiology.

## Elimination of alternative explanations

Against this background, assessing the causal implications of observed associations in epidemiology is still subject of debate. Mainly, because the assessment of causality does not logically follow from epidemiologic evidence. In experimental studies exposure is assigned randomly and thus isolated from other exposures (Greenland, 1990). In doing so, the only alternative explanation for any observed difference between the exposed an unexposed group in sound experimental research is chance, or *random* variability. The likelihood that an observed difference in an experimental setting is attributable to chance can be calculated statistically and diminishes with increasing study size, effectively leaving the exposure of interest as the only significant independent variable. Conversely, in observational studies alternative explanations are not systematically remedied. As such, the difference observed between groups in non-experimental comparison studies is not *necessarily* attributable to the exposure of interest but could be the result of systematic, or *non-random*, variability.

From this contrast between experimental and observational studies it becomes clear that confidence in the elimination of alternative explanations is attained on theoretical grounds. This notion shows that the concept of causality has a theoretical foundation as well and cannot be directly observed or calculated. Consequently, to attain whether an observed association has causal implications depends solely on the configuration of the study design and its ability to exclude alternative explanations. In this perspective, experimental control and random exposure allocation are methodological concepts grounded in scientific theory that effectively exclude non-random distortion of exposure-effect relationships (Weisberg, 2010). For that reason, study results from experimental studies, although acquired through the same statistical methods, are interpreted differently from those attained by means of observational research. That is, although experimental drug trials also yield mere measures of association, these are considered to reflect a causal relation, while in observational research such conclusions cannot logically be drawn from the evidence. The attribution of

causality thereby is fundamentally tied to the design of a study.

It follows that when designing etiological studies, the exclusion of alternative explanations is the primary focus. Similar to experimental research, the interpretation of epidemiologic evidence and its causal implications depend on an assessment whether potential sources of bias have been sufficiently addressed in the study's design. As stated by Savitz (2003, p.20), "Causal inference in epidemiology is based on exclusion of non-causal explanations for observed associations". For both experimental and observational studies the solution to the problem regarding the necessity to exclude alternative explanations in order to draw causal conclusions is sought in the methodology. Therefore, the capability to draw causal conclusions in etiological studies composes an *a priori* theoretical challenge. But while experimental research contrives a standardized controlled environment in which the putative cause is the only significant independent variable, epidemiologic research is restricted to making comparisons between non-randomly constructed groups, thereby introducing non-random variability, or *bias*. As a result, epidemiologic studies seek other, non-interfering, ways to account for the effect of non-random variation. This inherent vulnerability of observational research causes the validity of non-randomized studies usually not to be doubted because of the fear of chance events (*i.e.* random variability), but because of the fear of potential bias and confounding resulting from non-random variability (Vandenbroucke, 2008).

## Assumption of interchangeability

To argue that an observed association has a causal nature the estimate should be derived from a comparison between groups that are interchangeable except for the exposure of interest. In experimental studies, randomized assignment of exposure generates interchangeable groups that, in the absence of intervention, would have identical health experiences (Savitz, 2003). Epidemiologic studies on the other hand make use of a counterfactual conceptualization of causality (Greenland & Robins, 1986). That is to say, to assess the influence of a particular factor, a comparison is made between what *has* happened and what *would have* happened if that factor was absent (Weisberg, 2010). However, with non-random allocation of intervention, the confidence in our ability to constitute groups at equal baseline risk of the health outcome of interest is lost. Still, to argue the effect estimate reflects a causal effect of exposure requires the assumption that the rate among the unexposed reflects what the rate among the exposed would have been in the absence of exposure (Savitz, 2003).

There are multiple ways in which a particular effect measure can be systematically distorted, most notably selection bias and confounding (Weisberg, 2010). Of particular relevance to observational studies, such non-random distortion of a comparison is a structural tendency that, unlike random variability, does not balance out. For the same reason, classical statistical methods cannot be relied on to account for non-random distortion. The origin of classical statistical techniques lies in an era in which scientific thought was dominated by positivism; a form of pure empiricism in which all risk of serious error should be avoided by focusing on what can be analyzed with mathematical precision (Weisberg, 2010). However, the concept of bias is directly linked to that of causality and therefore also has a theoretical, intangible nature. That is to say, the existence of bias can only be discussed by interpreting the data in light of some causal theory, as an alternative explanation for an observed link

between phenomena. Or as Weisberg (2010, p.25) puts it: "To assess whether an observed effect reflects causation or mere coincidence entails considerations beyond the purview of purely mathematical analysis".

Since the presence of non-random distortion in observational studies, unlike the effects of random variability, cannot be objectively calculated, it remains subject of debate in the interpretation of effect estimates. In other words, the judgement whether an observed effect can potentially be explained in alternative ways becomes a matter of subjective opinion. And as stated by Savitz (2003, p.23): "The list of alternative explanations is limited only by the imagination of critics (…)". So to reach an explicit judgement requires the formulation of causal schemes and a judgment on the theoretical ability of the study's design to adequately deal with all potential sources of bias considered relevant. It therefore also requires a subjective qualification as to the effect that potential biases could have had on the effect estimate. From this contemplation of study design in relation to the causal scheme held by the interpreter, statistical measures gain scientific meaning. Not surprisingly, multiple experts often come to radically different conclusions after examining the same body of evidence (Savitz, 2003).

### Current common practice

As the previous sections have illustrated, the elimination of bias can never be absolutely assured when experimental control is absent (Weisberg, 2010). Consequently, unlike in experimental settings, in epidemiology the judgement whether alternative explanations have been truly eliminated ultimately remains subjective, making statements regarding causality a matter of judgement as well. In line with the positivist attitude towards science, epidemiologists therefore in general shy away from drawing causal conclusions. In an attempt to isolate objective measurements from the subjective interpretation thereof, epidemiologists generally merely state measures of *association* to emphasize the observational nature of their research (Borer, 2013). As a result, current common practice in epidemiology entails that researchers provide a complete description of their methods and results with the goal of sharing as much of the information as possible that will assist in the interpretation by the investigators and others (Vandenbroucke *et al*, 2007). Such an approach gives the investigators the first opportunity to interpret the results in a formal discussion, but enables others to make their own assessments regarding the absence of alternative explanations and the probability that a reported association has causal implications (Savitz, 2003). This notion accentuates the complexity of the concept of causality and the ambiguity surrounding the capability for epidemiology to assess causal effects.

## ETIOLOGY AND COMPLEX DISEASES

When the assessment of causality in epidemiology depends upon the effective exclusion of non-causal explanations then non-randomized etiological studies of health-related conditions among patients with complex diseases are particularly strenuous. As depicted above, studying subjects in their natural environment, with all the associated biological and behavioral diversity, in general complicates any claim that alternative explanations have been adequately neutralized. In etiological studies among patients with chronic diseases,

the highly correlated cluster of particular signs and symptoms making up chronic diseases further complicates causal inferences regarding any particular sign or symptom and a specific health-related outcome. Even more so when the health-related condition under study is considered a long-term effect rather than an acute reaction. If causality can only be assessed by eliminating all potential sources of bias resulting from the non-random allocation of exposure then etiological studies are especially vulnerable when conducted in settings in which a multitude of health-related problems are conjoined. At the least, any claim that all alternative explanations have been ruled out cannot escape the criticism of subjective judgement. Unfortunately, these circumstances often also leave epidemiologic studies as the only viable means to seek answers to questions regarding etiology.

While the design of experimental studies is better equipped to assess causality, experiments are often not considered feasible for a variety of ethical and practical reasons. When dealing with questions regarding any long-term complication of certain aspects of a chronic disease, randomization of exposure is unethical and often impractical or unattainable when it pertains an inherent aspect of the chronic disease. When it relates to a manipulable factor connected to the chronic disease, like an adverse reaction to a particular drug used in the management of the disease, both ethical and financial constraints often exclude randomized controlled experiments (Johnson *et al*, 2012). In addition, when dealing with severe putative long-term health risks from manipulable factors, a sense of urgency to find answers to questions of causality from a health care policy perspective cannot be overlooked. Prospective trials can take years to set up and generate their first results years after initiation (Van Santvoort *et al*, 2008; Origin Trial Investigators, 2008). In addition, these experimental studies often have insufficient numbers of participants to accurately determine the occurrence of rare adverse events (Duijnhoven *et al*, 2013). These practical and ethical motives that hamper experimental research in these circumstances, rather than the somewhat forlorn attempts to turn inherent limitations of epidemiology into an "(…) inherent strength of studying free-living human populations" (Savitz, 2003, p.27), legitimize the use of observational research by default.

## Challenges to etiology in complex diseases

Etiological studies in complex diseases particularly test the abilities and inabilities to draw causal conclusions in observational research. Differentiating between a causal effect and effects resulting from selection bias or confounding is a particular challenge in circumstances where numerous putative causes come together, intricately entangled with one another. To do so requires an *a priori* conceptualization of the causal schemata involved for the health-related outcome of interest. As argued previously, such a causal scheme is not only required to define the exposure of interest, but is also a necessity for a meaningful *a priori* discussion of potential sources of bias (Hernán *et al*, 2002). In order to design an etiological study, aimed at assessing the causal implications of a particular exposure, this scheme should therefore not only contain the nature of the hypothesized link between the exposure of interest and the study outcome but also that of all assumed interfering dynamics. Ultimately, the causal implications of results are weighted in consideration of the study's design and its ability to exclude bias. Therefore, the potential sources of bias should also be identified beforehand.

Drawing up an *a priori* causal scheme in epidemiology is not straightforward. With its characteristic feature of studying free-living individuals in mind, all potential non-random

distortion resulting from imperfect interchangeability should be taken into account. In line with its observational nature, the concept of causality in epidemiology is often portrayed according to the *sufficient component cause* model formulated by Rothman (1976). This model conceptualizes a health-related condition not as the outcome of a single necessary cause, but rather as the outcome of a constellation of conditions that could jointly precipitate the event (Weisberg, 2010). Evidently, such a pattern of contributing factors grows increasingly complex when studying health-related outcomes in chronic syndromal disorders. Even more so in the case of long-term complications in chronically ill patients, where the increase in complexity not only stems from a diagnosis which itself is based on a cluster of symptoms, but also from the progressive nature of many chronic complex diseases. That is to say that the disease state of chronically ill patients should not be seen as stagnant, but rather as a dynamic process. In many cases, disease severity progresses over time, as does associated comorbidity, both of which lead to changes in the prescribed medications.

Problems of particular importance when designing an etiological study to assess long-term complications among chronically ill patients include the non-random distortion from imperfect interchangeability and exposure misclassification. As depicted above, both issues require a comprehensive causal scheme based on which relevant exposure windows and potential sources of bias can be identified. Against this proposed causal scheme, the establishment of the exposed group places the burden on investigators to identify the most suitable (interchangeable) unexposed reference group (Savitz, 2003). Since the interpretation of the epidemiologic evidence depends on a qualification of suitability of the counterfactual comparison, the mechanism by which groups are selected in non-randomized settings is deserving of thorough contemplation. Given the progressive nature of chronic diseases, identification of an interchangeable reference group is troublesome in etiological studies into long-term health-related complications of chronic diseases. The less suitable the counterfactual comparison, the more interfering dynamics from the constellation of relevant contributing conditions should be accounted for via statistical adjustment. Moreover, besides the problems related to the imperfect interchangeability, additional distortion of the exposure-effect relationship can arise as a result of exposure misclassification due to time-related biases (Suissa & Azoulay, 2012) or the inclusion of biologically implausible exposure windows (Stricker & Stijnen, 2010). Problems arising from exposure misclassification are of particular importance when it comes to health-related outcomes in the long-term.

All these issues need to be conceptualized *a priori* and addressed in the study design and methodology in order to allow an assessment regarding causality. Any such conclusions will, however, always be drawn with some reservation since the number of ways in which residual non-random distortion can be present increases when results are obtained from inherently 'noisy' environments. Truly accounting for all potential sources of bias might therefore be unattainable for etiological studies in complex diseases. As a result, an unambiguous answer to the question whether the measured association truly reflects the magnitude of the causal effect, in the words of Savitz (2003, p.23): "(…) will always be 'maybe' with the goal of making an accurate assessment of where the evidence fits within the wide spectrum that extends from the unattainable benchmarks of 'yes' or 'no' ". Whether in these conditions, observational studies can ever transcend its core descriptive nature will likely remain subject of debate.

## SCOPE AND OUTLINE OF THE THESIS

In this thesis, answers to etiological questions in complex environments will be sought by means of observational study. In light of the limitations of observational research discussed in the previous sections, the work concentrates on two particularly 'noisy' circumstances in which the isolation of any particular relationship between a tentative cause and a particular effect is troublesome. More specifically, this thesis focuses on two major questions:

- Why is the incidence of (particular types of) cancer higher in patients with type 2 diabetes mellitus?
- Why is the incidence of venous thromboembolism higher in patients with multiple sclerosis?

Although the relevant exposure and outcome vastly differ, similar obstacles to acquire knowledge, in the sense of answers to questions regarding etiology, surround these two questions. That is, for both issues, the study population consists of patients with a chronic, pathological and generally progressive disease, which yields a high degree of correlation between putative causes. These conditions hinder the isolated testing of the effect of any single risk factor on a particular health-related state. Moreover, in both instances, the health -related complication of interest is expected to occur in the long term, rather than as an acute effect of exposure to a specific determinant.

### Cancer risk and type 2 diabetes mellitus

The most commonly referred to theory to explain the higher incidence of (certain types of) cancer among patients with type 2 diabetes mellitus is the increased insulin signaling pathway (Khandekar *et al*, 2011). Type 2 diabetes mellitus is characterized by a progressive decrease in insulin sensitivity, leading to chronic compensatory hyperinsulinaemia (Tabak *et al*, 2009). A hyperinsulinaemic state is hypothesized to cause cancer cell proliferation through increased insulin signaling via the insulin-like growth factor-1 (IGF-1) receptor (Pollak, 2012). This mechanism is proposed as the main explanation for the increased cancer incidence observed in type 2 diabetic patients and has numerous implications for particular features associated with type 2 diabetes mellitus, such as obesity and the use of specific hypoglycaemic agents (*e.g.* exogenous insulin and insulin analogues). However, assessing the causal effect that can be attributed to specific risk factors is hindered by the presence of interfering dynamics and shared risk factors for both type 2 diabetes mellitus and (certain types of) cancer (Bianchini *et al*, 2002), as well as the high-degree of correlation between them; *e.g.* high-caloric diet, sedentary lifestyle, obesity, hyperinsulinaemia, hyperlipidemia, hyperglycaemia, and the use of hypoglycaemic agents (Ioannidis *et al*, 2009; Carey *et al*, 1996). Moreover, as time progresses, so does age and disease severity, leading to changes in the type of hypoglycaemic agents prescribed, as well as in body weight (UKPDS group, 1998). In addition, cumulative exposure to hypoglycaemic agents progresses, together with duration of obesity and associated comorbidity. Of additional importance for observational research is the fact that the etiology of cancer is considered to involve interplay between genetic and environmental factors and develops gradually over time (Croce, 2008). This requires the differentiation between induction, latency, and disease periods, and the subsequent definition of relevant exposure windows.

## Venous thromboembolism and multiple sclerosis

Various etiological explanations have been proposed with regard to the higher risk of venous thrombus formation observed in patients with multiple sclerosis (Christensen *et al*, 2012). As several other autoimmune diseases have also been associated with an increased risk of venous thromboembolism (Zoller *et al*, 2012), chronic systemic inflammation has been proposed as a potential explanation for this relatively high incidence in patients with auto-immune disorders in general. The use of anti-inflammatory medications, such as high-dose glucocorticoids, has also been suggested as a causal factor (Huerta *et al*, 2007). With multiple sclerosis, however, alternative explanations include the characteristic gradual loss in mobility and other disease-related comorbidity (Myhr *et al*, 2001; Christensen *et al*, 2012). Testing these proposed mechanisms side-by-side is frustrated by the high-degree of correlation between them, especially when considering venous thromboembolism as a long-term complication. More importantly, the etiology of venous thromboembolism is thought to consist of a constellation of hereditary and acquired risk factors, with shared accountability for its occurrence (Tapson, 2008). In addition, the diagnosis of venous thromboembolism might be delayed, as common symptoms and signs are ambiguous or might even be absent (Elliot *et al*, 2005).

## Thesis objectives

The primary focus of this thesis is the assessment of the influence of specific risk factors in the etiology of health-related complications among patients with complex chronic diseases. More specifically, this thesis concentrates on the issues related to the exclusion of alternative explanations for observed exposure-effect relationships in order to make causal inferences from epidemiologic evidence. Both methodological and empirical alternative explanations are investigated that could distort associations between a particular exposure and its presumed effect. The following issues related to the attribution of causality from epidemiologic evidence are investigated in more detail:

- The influence of changes in study design on observed exposure-effect relationships
- The use of cumulative exposure measures, as a tool to determine the nature of an observed exposure-effect relationship
- The use of comparisons between alternative explanations for an observed exposure-effect relationship
- The influence of alternative explanations on observed exposure-effect relationships
- The theoretical limitations of epidemiologic research to assess causality based on empirical evidence

## Outline of the thesis

In **Chapter 2** the incidence of several cancer types is determined among patients treated for type 2 diabetes mellitus, as well as among patients never treated for type 2 diabetes mellitus in the United Kingdom. In this chapter, focus primarily lies on the assessment of the total disease burden and not on providing explanations for the findings. **Chapter 2.1** focuses on trends in colorectal cancer incidence among men and women with and without type 2 diabetes mellitus, while in **Chapter 2.2** trends in incidence rates of all gastrointestinal cancer

types are described. **Chapter 2.3** concentrates on trends in the incidence of breast cancer among women with and without type 2 diabetes mellitus.



In **Chapter 3**, the influence of variations in study design on the risk estimates for different types of cancer associated with exogenous insulin use is studied among type 2 diabetic patients. In this methodological chapter, the significance of variations in risk estimates as a result of changes in study design for the interpretation of epidemiologic evidence is discussed. Explanations for variations in risk estimate caused by changes in study design might have a methodological origin, such as misclassification of exposure due to time-related biases, or might be related to the hypothesized causal scheme, such as the inclusion of biologically implausible exposure windows or reversed causation bias. In general, this chapter highlights the importance of interpreting results from epidemiologic studies in light of the study design and methodology used to generate them.

In **Chapter 4**, cumulative exposure measures are used to establish whether a biological gradient between exposure and outcome exists, often interpreted to signify the existence of a causal relationship. In **Chapter 4.1**, the effect of metformin use on survival among type 2 diabetic patients diagnosed with breast cancer is studied. Metformin increases insulin sensitivity, thereby lowering overall insulin levels, which, according to the increased IGF-1 signaling theory depicted above, could lead to a reduction in cancer cell proliferation in type 2 diabetic patients. In this sense, the hypothesis that metformin use has a protective effect on breast cancer mortality is a derivative of a broader theory. **Chapter 4.2** focuses on the association between cumulative exposure to insulin glargine and the risk of breast cancer in type 2 diabetic women newly treated with insulins in the United Kingdom. Insulin glargine has an increased affinity for the IGF-1 receptor, which might therefore be associated with an increased breast cancer risk as compared to other insulin types.

**Chapter 5** focuses on alternative explanations for the higher incidence rates of long-term complications in patients with complex diseases. **Chapter 5.1** is aimed at seeking a more universal explanation for the increased colorectal cancer incidence among type 2 diabetic patients. Here, focus lies on the use of indicators for hyperinsulinaemia, which is considered to be the ultimate causal factor in the increased IGF-1 signaling theory. In this respect, type 2 diabetes mellitus diagnosis and the use of hypoglycaemic agents will merely signify a decline in endogenous insulin production, but are not interpreted as a causal factor in the development of cancer by themselves. In **Chapter 5.2**, the three proposed explanations for the higher incidence of venous thromboembolism in multiple sclerosis patients – chronic inflammation, loss of mobility, and high-dose glucocorticoids – are tested concurrently. Taken together with the influence of other relevant risk factors, it is studied whether these three mechanisms can explain the increased incidence in venous thromboembolism seen in patients with multiple sclerosis.

**Chapter 6** provides a general discussion of the empirical evidence in light of the philosophical foundation of science and the concept of causation. This deliberation is aimed at the interpretation of epidemiologic evidence from a critical rationalist perspective. As such, it is determined what knowledge was gained by the research presented in this thesis, followed by recommendations on how to improve upon it.

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# CHAPTER 2

# SKETCHING THE LANDSCAPE:

# THE IMPACT ON PUBLIC HEALTH

## CHAPTER 2.1

# Trends in colorectal cancer incidence among patients with type 2 diabetes mellitus in the United Kingdom

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## SUMMARY

### Background

2.1

We aimed to assess age-standardized trends in colorectal cancer incidence among type 2 diabetic patients.

### Methods

Data were obtained from the Clinical Practice Research Datalink in the United Kingdom (1989-2012). All adult users of hypoglycaemic medications and a matched (1:1) non-diabetic comparison cohort were selected. Colorectal cancer cases were stratified by anatomical subsite (colon/rectum), current body mass-index (BMI), and history of obesity.

### Results

During circa 3.5 million person years 3,014 cases (2,106/908 colon/rectum) were recorded in the diabetic cohort and 2,622 cases (1,802/820 colon/rectum) in the non-diabetic cohort. Age-standardized rates were significantly higher among diabetic patients – 60.7 (95%CI 58.0-63.3) versus 54.6 (95%CI 52.3-56.9) per 100,000 person years – and remained so over time. In the diabetic population, colon cancer rates increased during follow-up, while rectal cancer rates declined. Specifically, colon cancer incidence was higher among male patients with type 2 diabetes mellitus. Among type 2 diabetic patients with a low current BMI and in those with a history of obesity colorectal cancer rates were higher.

### Conclusion

Our results indicate that targeted screening of type 2 diabetic patients should be considered. Among this group, male patients would be best suitable, as their risk for colon cancer appears to be the highest.

## INTRODUCTION

With an estimated 746,000 new cases in 2012, colorectal cancer is the third most common cancer in men worldwide. For women, colorectal cancer is the second most common cancer type, with 614,000 new cases in 2012 worldwide (Ferlay *et al*, 2015). In Europe, the incidence of colorectal cancer appears to have stabilized in both sexes in recent years from 60.5 per 100,000 men and 37.2 per 100,000 women in 2008 (Ferlay *et al*, 2010) to 59.0 per 100,000 men and 36.1 per 100,000 women in 2012 (Ferlay *et al*, 2013). Looking a bit further back in time, we see that after an initial rise in colorectal cancer incidence, rates have relatively stabilized in developed countries over the past three decades (Bejar *et al*, 2012; Cheng *et al*, 2011; Siesling *et al*, 2003; Thygesen *et al*, 2004). However, several studies have shown that trends over time differ by anatomical subsite, with a rise in colon cancer incidence, while rectal cancer incidence is gradually falling (Caldarella *et al*, 2013; Siesling *et al*, 2003; Thygesen *et al*, 2004).

In contrast, the incidence of type 2 diabetes mellitus has increased globally (Danaei *et al*, 2011). In the United Kingdom (UK), the number of type 2 diabetic patients has doubled over the past decade. Among men, the prevalence of type 2 diabetes mellitus increased from 3.7% in 1994 to 7.2% in 2005, and in women from 2.3% to 4.9% (Imkampe & Gulliford, 2011). Moreover, type 2 diabetes mellitus prevalence is expected to keep rising in the future (Whiting *et al*, 2011). Many studies have shown that type 2 diabetes mellitus is associated with an increased risk of colorectal cancer (Deng *et al*, 2012; Larsson *et al*, 2005), but absolute numbers regarding the incidence of colorectal cancer in the type 2 diabetic population are often not highlighted. Particularly, incidence rates among the diabetic population over time are largely missing.

Type 2 diabetes mellitus and colorectal cancer share important risk factors, including metabolic disturbances, obesity, lack of physical exercise, and a high-caloric diet (Bhaskaran *et al*, 2014; Kahn *et al*, 2001; Rampal *et al*, 2014). With the expected continued rise in type 2 diabetes mellitus prevalence, colorectal cancer incidence trends stratified by type 2 diabetes mellitus status are needed to achieve better understanding of the total disease burden. Since some studies have suggested that the association with obesity is stronger for colon cancer than rectal cancer (Bhaskaran *et al*, 2014), rates per anatomical subsite for type 2 diabetic patients may also be of value. Furthermore, national screening programs were rolled-out in the UK in 2006 and 2007 among patients aged 60 to 69 years old and later also among patients between the age of 70 and 74 (McClements *et al*, 2012). Although the efficacy of screening for colorectal cancer in reducing incidence rates in general has been demonstrated by pilot studies (Mandel *et al*, 2000), time trends in colorectal cancer rates among diabetic patients could provide insights into the impact of screening programs in this high-risk population.

The aim of our study was to assess age-standardized colorectal cancer incidence rates by type 2 diabetes mellitus prevalence, calendar period, and stratified by anatomical subsite and gender, using the data from general practitioners in the United Kingdom (UK) from 1989 to 2012. In addition, among type 2 diabetic patients, incidence rates were determined by previously reported risk factors for the development of colorectal cancer (*i.e.* age, gender, body-mass index, and insulin use) in order to identify high risk groups and opportunities for targeted screening.

## METHODS

2.1

### Source of data

For this study, data were obtained from the Clinical Practice Research Datalink (CPRD). It comprises electronic medical records from British general practitioners since 1987 (Herrett *et al*, 2015). Currently, CPRD includes approximately 7% of the total UK population. Data recorded in CPRD include demographic information, prescription details, clinical events, preventive care provided, specialist referrals, hospital admissions, and major outcomes. The accuracy and completeness of CPRD data have been well-validated in previous studies (Herrett *et al*, 2010; Khan *et al*, 2010). The protocol of this study was approved by CPRD's Independent Scientific Advisory Committee (protocol no: 13_050).

### Study population

To examine colorectal cancer rates across age and calendar time in the period 1989 to 2012 among a type 2 diabetic population and a non-diabetic population, we used a cohort of hypoglycaemic drug users (*i.e.* diabetic cohort) and a matched comparison cohort. The diabetic cohort comprised of all registered adult patients (≥18 years) with at least one prescription for a hypoglycaemic agent recorded in CPRD during up-to-standard follow-up, until 31 October 2013. At the date of the first prescription (*i.e.* index date), a reference patient without any past recorded prescriptions for hypoglycaemic agents was matched by sex, year of birth, and practice to each type 2 diabetic patient (1:1). Patients in the comparison cohort could transfer to the diabetic cohort if a prescription for a hypoglycaemic agent was recorded. At that prescription date the patient was censored as a reference and matched, as a diabetic patient, to a new reference.

Patients with a prescription for insulin on the index date without any concomitant prescriptions for non-insulin antidiabetic drugs (NIADs) were considered type 1 diabetic patients if (a) they had a recorded diagnosis for type 1 diabetes mellitus or (b) they were under 30 years of age on the prescription date. Patients meeting these criteria for type 1 diabetes mellitus were excluded. In addition, all subjects with a history of colorectal cancer prior to cohort entry were excluded. Lastly, diabetic patients without any subsequent prescription for hypoglycaemic agents (after the initial prescription recorded at baseline) were also excluded. If a patient was excluded, so was their matched counterpart.

### Study outcome

All patients were followed up from cohort entry until the occurrence of colorectal cancer, the patient's death, transfer out of practice, or end of data collection, whichever came first. The first medical record for colorectal cancer in CPRD after cohort entry was taken as the diagnosis date of a new case. Colorectal cancer cases are recorded in CPRD by anatomical region; *i.e.* colon or rectal cancer (see Table 2.1.A of the appendices for the list of medical codes). Clinical records from CPRD are found to be a valid measure to capture colorectal cancer occurrence as compared to the national cancer registries (Boggon *et al*, 2013).

### Data analysis

The study period was restricted to full calendar years, from 1 January 1989 to 31 December

2012, to assure full data availability in all time periods. Gender-specific age-adjusted incidence rates (IR) per 100,000 person years (py) were calculated for colorectal cancer in the diabetic cohort and the reference cohort, with rates adjusted to the European (EU-27) standard population of 2012 (http://ec.europa.eu/eurostat/data/database). Confidence intervals (CI) were calculated for standardized (Boyle & Parkin, 1991) and crude rates (Rothman & Boice, 1979). To assess secular trends, data were presented by calendar year period and age group. Age groups for standardization consisted of 5-year intervals, starting with '18 to 20 years' and ending with '85+ years'. For calendar year period, we constructed seven time period intervals (*i.e.* 1989-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008, 2009-2010, 2011-2012). Rates 1989-2000 were aggregated because of limited data availability. Age was determined per calendar year as the year difference with the year of birth.

Among the diabetic population, gender-specific IRs were determined over the entire follow-up period (1989-2012), stratified by the presence of specific risk factors (*i.e.* body-mass index, history of obesity, insulin use) using time-dependent modeling. For body-mass index (BMI), follow-up time per patient was divided into categories (*i.e*. <25, 25-30, 30-35, ≥35 kg/m², unknown) based on current measurements. That is, a patient's BMI was determined from medical records and was valid for 1 year, starting on the entry date. Thereafter, if no consecutive measurement was recorded within 1 year, a patient's BMI status was set to 'unknown' until a new BMI was recorded. In addition, we determined IRs separately for patients with and without a history of obesity. Here, patient time was marked as 'ever obese', starting on the entry date of a BMI record ≥30kg/m². Lastly, we produced IRs stratified by past insulin exposure (yes/no); *i.e*. follow-up was labeled as 'ever exposed to insulins', starting on the prescription date for any type of insulin.

Follow-up time for all patients was divided into periods with a variable length, depending on the occurrence of relevant events (*i.e.* new recording of BMI, prescription for insulin). Subsequently, IRs per category were produced as the number of events within each category divided by the total amount of follow-up time (*i.e.* the sum of all time periods within this category). Differences between IRs were determined by calculating incidence rate ratios with 95% CI and associated *p* values (Poole, 1987). In a sensitivity analysis, we excluded the first year of follow-up for all patients to address issues of diagnostic bias. All data management and statistical analyses were conducted using SAS 9.2 (SAS Institute Inc, Cary, NC, USA). All graphs were made with Microsoft Excel 2013 (Microsoft, WA, USA).

## RESULTS

A total of 329,726 type 2 diabetic patients and 329,726 reference patients with an index date before 1 January 2013 were included in the study population; see Figure 2.1.i of Appendices for a specified flow chart. During a total follow-up of approximately 3.5 million person years, 5,636 colorectal cancer cases occurred; 3,014 cases (2,106 colon and 908 rectal cancer) in the diabetic population - with a crude incidence rate of 167 per 100,000 py - and 2,622 cases (1,802 colon and 820 rectal cancer) in the reference population - with a crude incidence rate of 154 per 100,000 py. Baseline characteristics are shown in Table 2.1.1.

Table 2.1.2 shows that the age- and sex-standardized rate for colorectal cancer over the entire duration of follow-up was significantly higher among type 2 diabetic patients; 60.7 (95% CI 58.0-63.3) per 100,000 py versus 54.6 (95% CI 52.3-56.9) per 100,000 py ($p$<0.01).

**TABLE 2.1.1.** Baseline characteristics of the diabetic cohort and non-diabetic comparison cohort.

| | Type 2 diabetic cohort (n=329,726) | | Reference cohort (n=329,726) | |
|---|---|---|---|---|
| **Gender** | | | | |
| Male | 174,939 | (53.1) | 174,939 | (53.1) |
| | | | | |
| **Age (median, IQR)** | 63 | (52-73) | 63 | (52-73) |
| 18-40 | 29,128 | (8.8) | 29,128 | (8.8) |
| 40-60 | 107,708 | (32.7) | 107,708 | (32.7) |
| 60-80 | 156,473 | (47.5) | 156,473 | (47.5) |
| >80 | 36,417 | (11.0) | 36,417 | (11.0) |
| **Body mass-index*** | | | | |
| <20 | 2,869 | (0.9) | 4,152 | (1.3) |
| 20-25 | 26,001 | (7.9) | 21,846 | (6.6) |
| 25-30 | 61,457 | (18.6) | 28,294 | (8.6) |
| 30-35 | 52,087 | (15.8) | 13,593 | (4.1) |
| >35 | 45,742 | (13.9) | 6548 | (2.0) |
| Unknown | 141,570 | (42.9) | 255,293 | (77.4) |
| **Smoking*** | | | | |
| Current | 51,708 | (15.7) | 50,105 | (15.2) |
| Ex | 58,935 | (17.9) | 45,062 | (13.7) |
| Never | 107,123 | (32.5) | 107,176 | (32.5) |
| Unknown | 111,960 | (34.0) | 127,383 | (38.6) |
| **Alcohol use*** | | | | |
| Yes | 131,133 | (39.8) | 131,527 | (39.9) |
| No | 48,024 | (14.6) | 30,433 | (9.2) |
| Unknown | 150,569 | (45.7) | 167,766 | (50.9) |
| **Prior cancer†** | | | | |
| Yes | 27,526 | (8.3) | 27,558 | (8.4) |
| **Type of hypoglycaemic agent‡** | | | | |
| Insulin | 34,338 | (10.4) | - | |
| Metformin | 216,201 | (65.6) | - | |
| Sulfonylurea | 105,857 | (32.1) | - | |
| Thiazolidinediones | 7,683 | (2.3) | - | |
| Other oral hypoglycaemic drug | 5,272 | (1.6) | - | |

Abbreviations: IQR, interquartile range. *Based on the most recent medical record in the year prior to baseline. †Any cancer (excluding non-malignant melanomas) other than colorectal cancer. ‡Multiple prescriptions on the index date occurred. Of note: numbers between brackets display percentages, unless otherwise specified.

**TABLE 2.1.2.** Comparisons of age- and sex-standardized incidence rates (in events per 100,000 person years), stratified by anatomical subsite and gender, between patients with and without type 2 diabetes mellitus and between subgroups within the diabetic cohort.

| | | Diabetic cohort | | Reference cohort | | | | |
|---|---|---|---|---|---|---|---|---|
| **Entire study population** | | AS-IR | 95% CI | AS-IR | 95% CI | IRR | 95% CI | *p* value |
| *Colorectal cancer* | | 60.7 | (58.0-63.3) | 54.6 | (52.3-56.9) | 1.11 | (1.06-1.17) | <0.01 |
| Male | | 68.6 | (64.8-72.4) | 60.5 | (57.3-63.8) | 1.13 | (1.06-1.21) | <0.01 |
| Female | | 53.1 | (49.5-56.7) | 49.0 | (45.7-52.3) | 1.08 | (1.00-1.18) | 0.07 |
| *Colon cancer* | | 42.3 | (40.1-44.6) | 37.9 | (35.9-39.8) | 1.12 | (1.05-1.19) | <0.01 |
| Male | | 46.7 | (43.4-50.0) | 38.8 | (36.2-41.4) | 1.20 | (1.11-1.31) | <0.01 |
| Female | | 38.2 | (35.0-41.3) | 36.9 | (34.0-39.8) | 1.04 | (0.94-1.14) | 0.5 |
| *Rectal cancer* | | 18.3 | (17.0-19.7) | 16.8 | (15.5-18.0) | 1.09 | (0.99-1.20) | 0.08 |
| Male | | 21.9 | (20.0-23.8) | 21.7 | (19.8-23.6) | 1.01 | (0.90-1.13) | 0.9 |
| Female | | 14.9 | (13.1-16.8) | 12.1 | (10.5-13.7) | 1.23 | (1.04-1.46) | <0.01 |
| *Colorectal cancer* | | | | | | | | |
| Excluding the first year* | | 58.2 | (55.3-61.0) | 54.5 | (51.8-57.2) | 1.06 | (1.01-1.13) | 0.04 |
| **Among T2DM patients** | | | | | | | | |
| *Colorectal cancer* | | | | | | | | |
| Previous insulin use: | **yes**† | 54.7 | (45.8-63.7) | 61.2 | (58.3-64.0) | 0.89 | (0.76-1.05) | 0.16 |
| Record of obesity: | **yes**† | 65.1 | (59.7-70.5) | 58.0 | (55.2-60.9) | 1.12 | (1.05-1.21) | <0.01 |
| Male gender: | **yes**† | 75.7 | (66.5-84.9) | 64.4 | (60.1-68.7) | 1.18 | (1.07-1.29) | <0.01 |
| Female gender: | **yes**† | 55.0 | (48.9-61.1) | 52.0 | (47.9-56.0) | 1.06 | (0.94-1.19) | 0.37 |
| *Colon cancer* | | | | | | | | |
| BMI <25 kg/m$^2$: | **yes**† | 54.9 | (48.1-61.7) | 40.3 | (37.9-42.7) | 1.36 | (1.22-1.52) | <0.01 |
| *Rectal cancer* | | | | | | | | |
| BMI <25 kg/m$^2$: | **yes**† | 20.2 | (16.3-24.0) | 17.7 | (16.3-19.2) | 1.14 | (0.97-1.35) | 0.13 |

*Abbreviations*: AS-IR, age- and sex-standardized incidence rate in events per 100,000 person years; CI, confidence interval; IRR, incidence rate ratio; T2DM, type 2 diabetes mellitus; BMI, body mass-index. *Sensitivity analysis where the first year of follow-up was excluded for all patients. †Reference category included all remaining person time (*i.e.* not exposed or 'No'). Of note: when stratified to sex, incidence rates are age-standardized.

In particular, the standardized IR for colon cancer was higher among diabetic patients; 42.3 (95% CI 40.1-44.6) per 100,000 py versus 37.9 (95% CI 35.9-39.8) per 100,000 py ($p$<0.01). After stratification to sex and anatomical subsite, colon cancer incidence was higher among male patients with type 2 diabetes mellitus as compared to non-diabetic male patients; 46.7 per 100,000 py (95% CI 43.4-50.0) versus 38.8 (95% CI 36.2-41.4) per 100,000 py ($p$<0.01).

*Trends in incidence rates*

The time trends in the age- and sex-standardized IRs for colorectal cancer for the diabetic and reference population are shown in Figure 2.1.1. In general, rates among type 2 diabetic patients remained higher over time as compared to those observed in the reference population. From 2003 onwards, the IRs tended to slightly decline for both the diabetic and the reference cohort. When stratified by anatomical subsite, the rates of both colon and rectal cancer were in general higher among diabetic patients and remained so over time (Figure 2.1.2). Furthermore, the colon cancer rates appeared to increase in the diabetic population, whereas in the non-diabetic population colon cancer incidence tended to decline from 2007 onwards. Rectal cancer incidence declined slightly over the duration of follow-up in both cohorts. The stratified sex-standardized IRs stratified by subsite showed no clear pattern in colon or rectal cancer incidence among patients eligible for screening (age groups 60-69 and 70-79) in either the diabetic or reference cohort, however confidence intervals were large due to limited study power (see Appendices, Figure 2.1.ii and 2.1.iii).

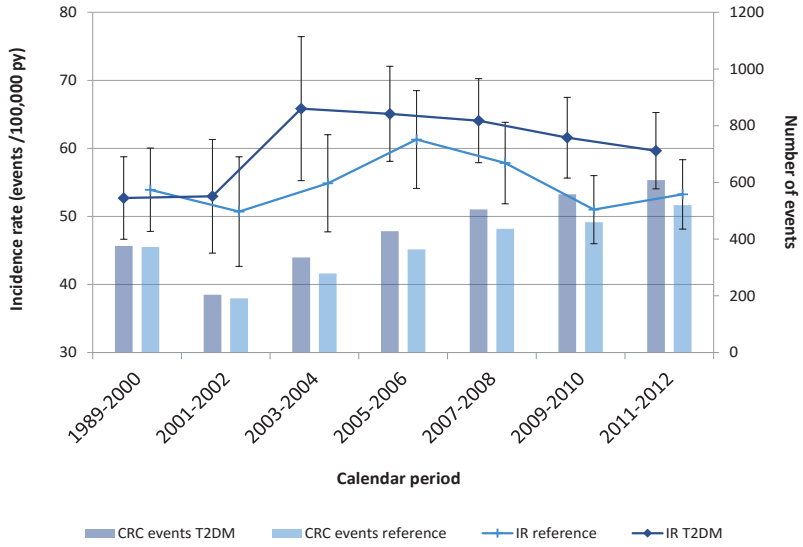
Figure 2.1.3 shows the crude IRs for colorectal cancer per age group for male and female patients separately. In both male and female patients, the rates per age group are higher in patients with type 2 diabetes mellitus, except for the highest age groups. For the diabetic male and female patients, incidence of colorectal cancer peaks between the age of 80 and 84 years. Among diabetic patients, stratification by current body mass-index showed different patterns per anatomical subsite. The adjusted IRs for colon cancer decreased with increasing current BMI, whereas no clear trend was observed for rectal cancer incidence (Figure 2.1.4). A significantly higher adjusted IR for colon cancer was observed among patients with a current BMI <25kg/m² (Table 2.1.2).

When stratified by history of obesity (*i.e.* a recorded BMI ≥30kg/m²), the age standardized IR for colorectal cancer was higher among diabetic male patients with a history of obesity than in those without. For female diabetic patients, no significant difference between patients with and without a history of obesity was found (Table 2.1.2; see Appendices, Figure 2.1.vi). The age- and sex-standardized IR for colorectal cancer appeared to be lower in patients ever prescribed any type of insulin, but with only 156 events among patients with a past prescription for insulin, the confidence interval was wide (Table 2.1.2).

## DISCUSSION

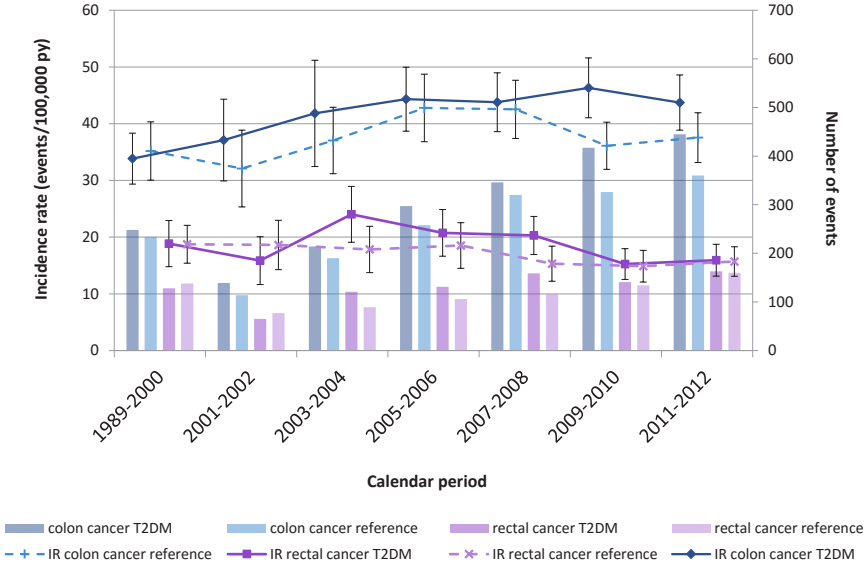
Overall, colorectal cancer incidence was consistently higher in patients with type 2 diabetes mellitus as compared to patients without diabetes in the United Kingdom since the year 2000. The difference between diabetic and non-diabetic patients was larger among males than among females, especially for colon cancer. In general, colorectal cancer incidence

**FIGURE 2.1.1.** Time trends in age-sex-standardized incidence rates (*line*) and number of events (*bar*) for colorectal cancer among type 2 diabetic and non-diabetic patients, by calendar period (1989-2012).



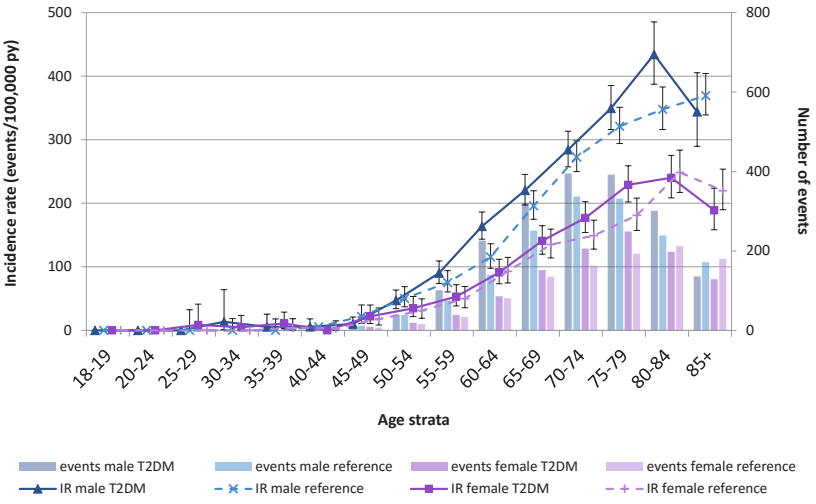

*Abbreviations:* IR, incidence rate; CRC, colorectal cancer; T2DM, type 2 diabetes mellitus; py, person years.

**FIGURE 2.1.2.** Time trends in age-sex-standardized incidence rates (*line*) and number of events (*bar*) for colon and rectal cancer among type 2 diabetic and non-diabetic reference patients, by calendar period (1989-2012).



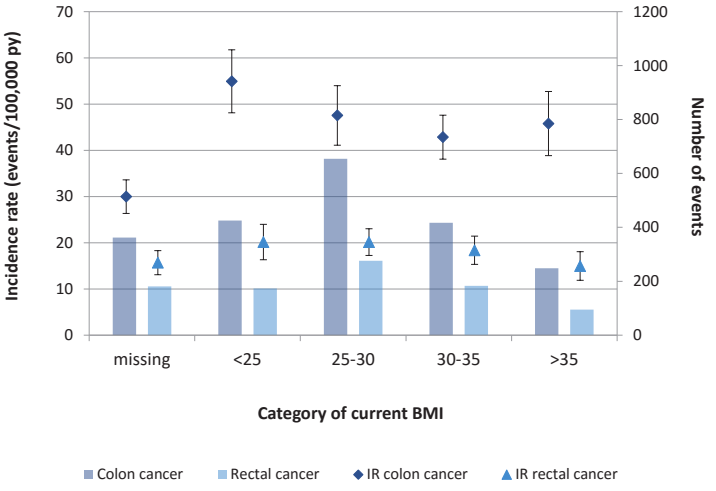

*Abbreviations:* IR, incidence rate; T2DM, Type 2 diabetes mellitus; py, person years.

**FIGURE 2.1.3.** Age-specific crude incidence rates (*line*) and number of events (*bar*) for colorectal cancer in diabetic patients and reference patients over the entire follow-up period (1989-2012), by gender.




*Abbreviations:* IR, incidence rate; T2DM, Type 2 diabetes mellitus; py, person years.

**FIGURE 2.1.4.** Age-sex-standardized incidence rates (*point estimate*) and number of recorded events (*bar*) for colon and rectal cancer among type 2 diabetic patients, by current body mass-index.




*Abbreviations:* IR, incidence rate; py, person years; BMI, body mass-index in kg/m$^2$.

declined from 2006 onward. However, when stratified by anatomical subsite, rates for colon cancer among diabetic patients appeared to increase over the duration of follow-up. In the non-diabetic population, a decline in colon cancer incidence was observed, starting in the year 2007. Rectal cancer incidence steadily decreased over time in both the diabetic and non-diabetic population.

Colorectal cancer incidence rates observed in our study are largely in agreement with those previously reported by others. The study by Ferlay *et al*. (2013) reported rates of 55.6 per 100,000 py for males and 36.7 per 100,000 py for females in the UK. These rates are comparable to those reported by Cancer Research UK (Cancer Research UK, 2014). In our study, the rates in the non-diabetic population were higher, even after standardization for age; 60.5 per 100,000 py among males and 49.0 per 100,000 py among females. This might be due to the study population consisting of patients registered to general practitioners and therefore, perhaps, in poorer physical condition as compared to the general population. Furthermore, the age distribution in our matched non-diabetic cohort was determined by the diabetic cohort. Therefore, our study population consisted mostly of patients aged 50 years and older (circa 80%). As a result, incidence rates in the higher age groups could be determined more accurately which may have resulted in a higher overall incidence rate, even after standardization. Moreover, since type 2 diabetes mellitus is more prevalent among males, the exclusion of diabetic patients from our comparison cohort may have influenced the incidence rate in the male population to a greater extent than the female population. Consequently, the incidence rate found in the male non-diabetic cohort may be an underestimation of the incidence rate in the general male CPRD population. Lastly, colorectal cancer rates vary across the regions in the UK (Quinn *et al*, 2005) and population coverage of CPRD primary data is relatively high in areas with higher colorectal cancer incidence rates (Herrett *et al*, 2015).

Our results appear to confirm earlier reports indicating that rectal cancer incidence has gradually fallen, while colon cancer incidence has generally risen (Caldarella *et al*, 2013; Siesling *et al*, 2003; Thygesen *et al*, 2004). We observed similar trends in both the diabetic and non-diabetic population. Overall, our results showed a decrease in colorectal cancer incidence from 2006 onwards. In particular, a decrease in colon cancer incidence can be observed among the non-diabetic population in the years 2007 to 2012, after the initiation of national screening programs. Conversely, among the diabetic population the decrease in overall colorectal cancer incidence after 2006 was primarily caused by a reduction in rectal cancer incidence, while a rising trend in colon cancer incidence persisted beyond 2006. The efficacy of screening in reducing colorectal cancer incidence, through detection and removal of premalignant adenomatous polyps, has been demonstrated (Mandel *et al*, 2000). Therefore, the observed decline in colon cancer rates among the non-diabetic population since 2006 may be attributed to these national screening programs. If so, the effect appears to be more pronounced for patients without type 2 diabetes mellitus, since we observed no obvious downward trends in colon cancer rates among the diabetic population after the initiation of screening. Unfortunately, we were unable to robustly estimate incidence rates over time for the age groups eligible for screening; 60-69 years starting in 2006 and 70-74 starting in 2007 (McClements *et al*, 2012). However, if replicated, these findings may have important consequences.

Among diabetic patients, our analyses by BMI showed that colorectal cancer incidence was highest among those with a low BMI (<25kg/m$^2$), and declined to the average

value with increasing BMI. In particular, this pattern was observed for colon cancer, where the adjusted rate (per 100,000 py) was substantially higher for patients with a current BMI under 25 kg/m² (54.9, 95% CI 48.1-61.8) as compared to the overall incidence rate of 42.3. For rectal cancer, the differences between BMI categories were smaller. Previous studies have reported the risk of colorectal cancer to increase with each 5-point incremental increase in BMI, particularly for colon cancer (Bhaskaran *et al*, 2014). This incongruity could be explained by the fact that weight loss is a key clinical symptom of active colon cancer (Mitchell *et al*, 2015). Since we stratified by current BMI – measured no longer than 1 year ago – the decline in rate with categories of increasing BMI confirms that at the time of diagnosis a patient may not suffer from overweight (anymore) (Mitchell *et al*, 2015). On the other hand, the incidence rate in diabetic patients with a history of obesity was significantly higher, particularly among males.

Life-style factors have been associated with the risk of colorectal cancer by numerous epidemiological studies (Harriss *et al*, 2009a; Harriss *et al*, 2009b; Huxley *et al*, 2009). These risk factors for colorectal cancer are also associated with type 2 diabetes mellitus (Bhaskaran *et al*, 2014; Kahn *et al*, 2001; Rampal *et al*, 2014), raising questions with regard to the causal pathways involved (Peeters *et al*, 2015). Postulated theories regarding relevant biological mechanisms focus primarily on hyperinsulinaemia caused by increased insulin resistance, which in turn may lead to elevated cell proliferation through increased activation of insulin-like growth factor-1 receptor (Giouleme *et al*, 2011). Although this study was not set out to answer questions regarding causality, our results show colorectal cancer incidence to be consistently higher among type 2 diabetic patients. Moreover, our results show that screening programs may not have the same impact in the diabetic population. This could in fact be linked to the same life-style factors associated with both type 2 diabetes mellitus and colorectal cancer. A recent study showed that patients with a high BMI, a smoking habit, lack of physical exercise, and high alcohol consumption are less likely to participate in screening programs (Blanks *et al*, 2015). In our study, this may be reflected by the small but steady increase in colon cancer incidence among type 2 diabetic patients that persisted beyond 2006.

Several limitations of our study should be mentioned. Firstly, because of the limited follow-up time and cancer events, we were unable to determine trends in incidence rates over time prior to 2001. Secondly, since the incidence rates found in our CPRD comparison cohort are higher than those reported for the general UK population, the difference in colorectal cancer incidence between type 2 diabetic patients and the general population may be larger than observed here. However, by selecting a reference population from general practices, we were able to correct for increased health care use and still observed significantly increased rates among diabetic patients. Thirdly, linkage with the UK cancer registry was not performed in this study. Case ascertainment for colorectal cancer in CPRD has been shown to be high (Boggon *et al*, 2013; Moller *et al*, 2011). Nonetheless, missing cases of colorectal cancer may have occurred, but most likely in a limited fashion. Moreover, misclassification of colon and rectal cancer cases should be considered. However, there is no reason to believe this misclassification would be different for diabetic and non-diabetic patients. Finally, due to limited study power, we were unable to further visualize any potential effects of national screening programs that were initiated during follow-up or trends in incidence rates among insulin users within the diabetic population.

In conclusion, our study confirmed the increased incidence of colorectal cancer in patients suffering from type 2 diabetes mellitus as compared to non-diabetic individuals, which was persistently higher since the year 2000. Furthermore, our results indicate that targeted screening is needed, as colon cancer incidence has not decreased among type 2 diabetic patients after the screening for colorectal cancer was introduced in the UK. For future screening, it may be worth studying whether male patients with type 2 diabetes mellitus are a suitable target group, as their risk for colon cancer appears to be the highest.

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**TABLE 2.1.A.** Medical codes for colon and rectal cancer identified for use in CPRD.

| Medcod* | Events† | Readterm‡ | Colon cancer | Rectal cancer |
|---|---|---|---|---|
| 1220 | 29,021 | Malignant neoplasm of colon | X | |
| 3811 | 5,229 | Malignant neoplasm of caecum | X | |
| 9118 | 4,727 | Colonic cancer | X | |
| 2815 | 4,200 | Malignant neoplasm of sigmoid colon | X | |
| 11628 | 3,458 | Cancer of bowel | X | |
| 28163 | 1,812 | Malignant neoplasm of colon NOS | X | |
| 10946 | 904 | Malignant neoplasm of ascending colon | X | |
| 6935 | 522 | Malignant neoplasm of transverse colon | X | |
| 22163 | 455 | Carcinoma of caecum | X | |
| 10864 | 404 | Malignant neoplasm of descending colon | X | |
| 9088 | 308 | Malignant neoplasm of hepatic flexure of colon | X | |
| 18619 | 214 | Malignant neoplasm of splenic flexure of colon | X | |
| 48231 | 40 | Malignant neoplasm of other specified sites of colon | X | |
| 93478 | 3 | Malignant neoplasm, overlapping lesion of colon | X | |
| 1800 | 16,833 | Malignant neoplasm of rectum | | X |
| 5901 | 7,165 | Rectal carcinoma | | X |
| 7219 | 1,869 | Carcinoma of rectum | | X |
| 27855 | 747 | Malignant neoplasm of rectosigmoid junction | | X |
| 35357 | 741 | Malignant neoplasm of rectum, rectosig. junction and anus | | X |

*Medical codes (Medcode) in the Clinical Practice Research Datalink (CPRD) correspond to Read-codes, which are the standard clinical terminology system used in General Practice in the United Kingdom. †List the total number of events (i.e. recordings of the specific medical code) within the CPRD. ‡Contains the description of the clinical event linked to the specific medical code.

**FIGURE 2.1.i.** Specified flow chart for patient selection of the diabetic and non-diabetic cohort. CRC, colorectal cancer; incidence density sampling (IDS), patients that become diabetic after contributing time to the reference cohort; T1DM, type 1 diabetes mellitus; $R_x$, prescription.

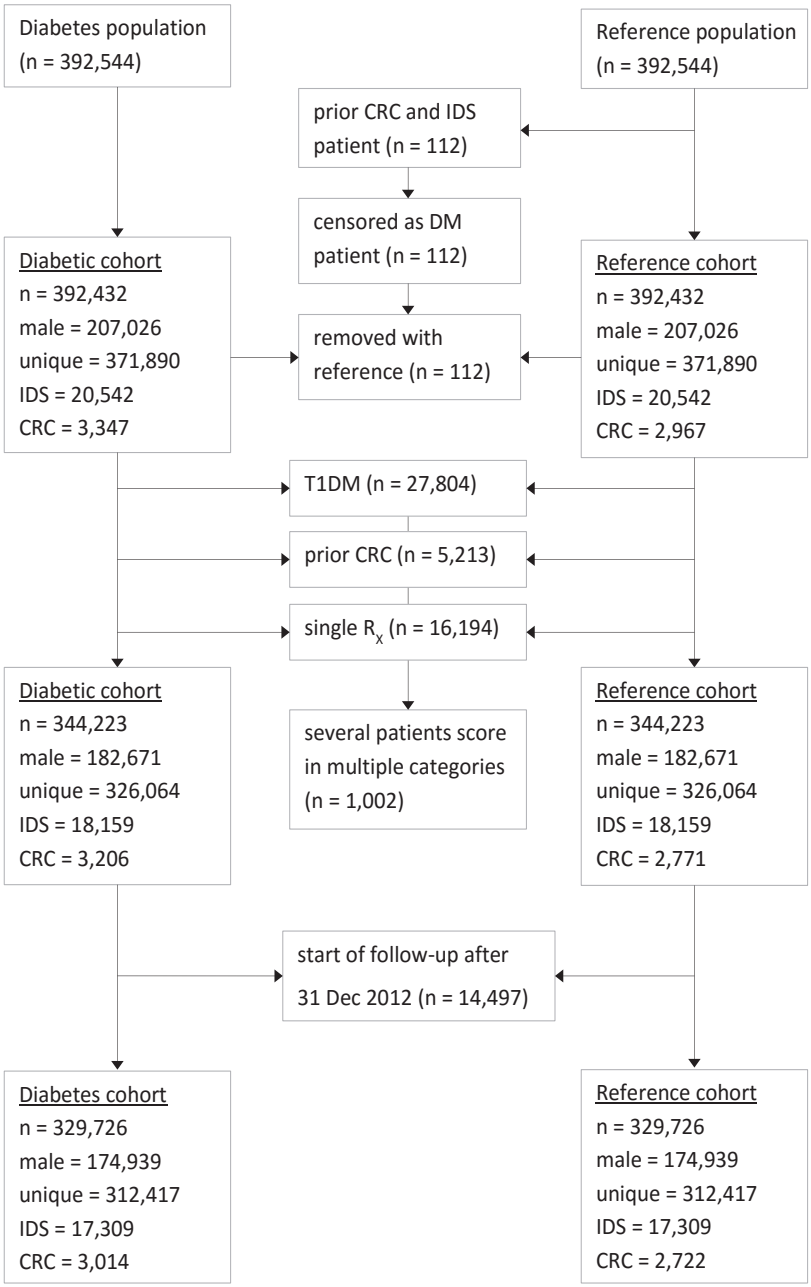
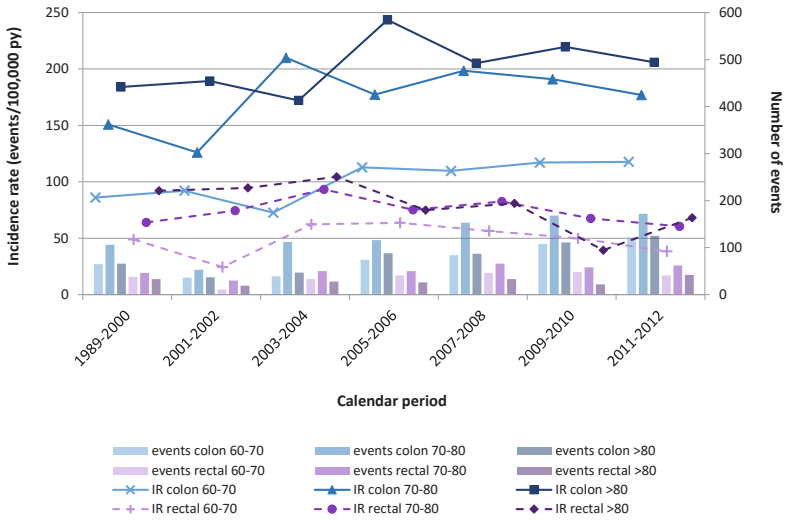
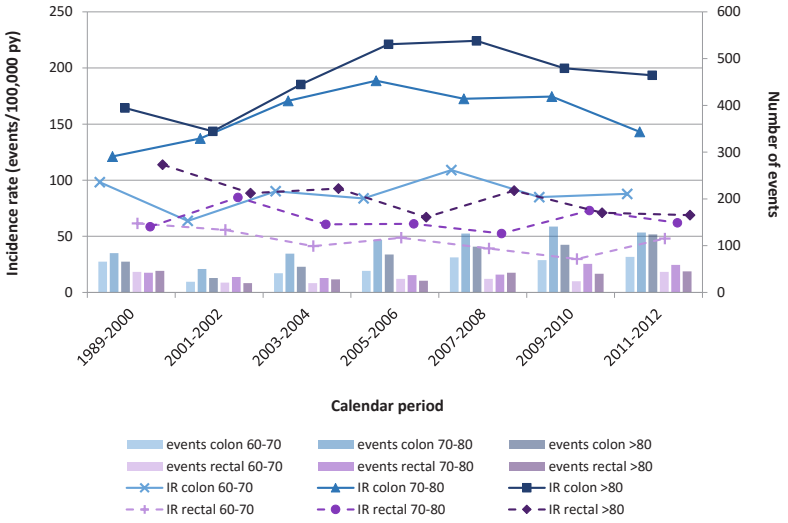


**FIGURE 2.1.ii.** Time trends in gender-standardized incidence rates (*line*) and number of events (*bar*) for colon and rectal cancer among diabetic patients, by age groups eligible for screening. Confidence intervals are intentionally omitted for visual purposes, but were generally wide.



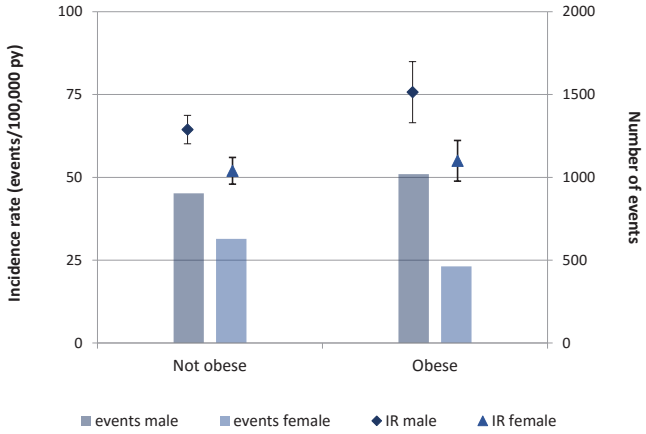

*Abbreviations:* py, person years; IR, incidence rate.

**FIGURE 2.1.iii.** Time trends in gender-standardized incidence rates (*line*) and number of events (*bar*) for colon and rectal cancer among reference patients, by age groups eligible for screening. Confidence intervals are intentionally omitted for visual purposes, but were generally wide.




*Abbreviations:* py, person years; IR, incidence rate.

**FIGURE 2.1.iv.** Age-standardized incidence rates (*point estimate*) and number of events (*bar*) for colorectal cancer, stratified by gender and history of obesity (>30kg/m²).




*Abbreviations:* IR, incidence rate; py, person years.

## CHAPTER 2.2

# Gastrointestinal cancer incidence in type 2 diabetes mellitus: results from a large retrospective population-based cohort study

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## SUMMARY

### Background

Patients with type 2 diabetes mellitus (T2DM) have been shown to have higher incidences of liver, pancreatic, and colorectal cancer compared to non-diabetic individuals. Current evidence is conflicting for other gastrointestinal (GI) cancers. Therefore, we aimed to determine incidence rates (IRs) of GI cancers in patients with and without T2DM.

### Methods

A cohort study was performed using the UK Clinical Practice Research Datalink (1988-2012). A cohort of hypoglycaemic drug users was matched at baseline to a non-diabetic cohort, by age, sex, and practice. Crude IRs and 95% confidence intervals (95% CI) of GI cancers per 100,000 person years were calculated stratified by age, sex, and calendar year.

### Results

333,438 T2DM and 333,438 non-diabetic individuals were analyzed. IRs of liver (IR 26, 95% CI 24-28 vs. 8.9, 95% CI 7.7-10), pancreatic (IR 65, 95% CI 62-69 vs. 31, 95% CI 28-34), and colon cancer (IR 119, 95% CI 114-124 vs. 109, 95% CI 104-114) were significantly higher in the diabetic compared to the non-diabetic cohort, whereas the IR of esophageal cancer was significantly lower (IR 41, 95% CI 39-44 vs. 47, 95% CI 44-51). Sex-specific IRs of colon cancer remained significantly higher in men with T2DM, and IRs of esophageal cancer remained significantly lower in women with T2DM.

### Conclusion

In study, T2DM patients were shown to have higher crude IRs of liver, pancreatic and colon cancer, but not of gastric, biliary, and rectal cancer. Moreover, the lower observed IRs of esophageal cancer in diabetic patients warrants further investigation.

## INTRODUCTION

There is a growing body of evidence on an increased risk of cancer in type 2 diabetic patients, including gastrointestinal (GI) malignancies (Giovannucci *et al*, 2010; Habib & Rojna, 2013; Jamal *et al*, 2009; Chiu *et al*, 2013; Peeters *et al*, 2015; Knapen *et al*, 2015; Starup-Linde *et al*, 2013). However, the data are conflicting for specific GI cancer sites, such as the upper gastrointestinal tract and biliary system. The strongest associations have been found for liver and pancreatic cancer, although ascertainment bias and reverse causality may have played an important role (Johnson *et al*, 2011; Nicolucci, 2011; Vigneri *et al*, 2009). Furthermore, age-sex stratified analyses have not always been reported, despite the demonstration of age- and sex-specific differences in cancer risk, with GI cancer occurring more frequently at a higher age and more frequently in men (Giovannucci *et al*, 2010).

Type 2 diabetic patients may have an increased risk of GI cancers through several common risk factors, such as older age, exposure to alcohol, smoking, a high caloric diet, lack of physical activity, and increased body mass-index (BMI) (Giovannucci *et al*, 2010). In addition, site-specific risk factors that are more prevalent among diabetic patients may play an important role. These include gastro-esophageal reflux disease in esophageal cancer, Helicobacter pylori infections in gastric cancer, gallstone formation in biliary tract cancer, and non-alcoholic fatty liver disease or cirrhosis in hepatocellular carcinoma (Lagergren *et al*, 1999; Bosetti *et al*, 2012; Lin *et al*, 2011; Hemminki *et al*, 2010).

The underlying biological mechanisms that may explain the association between type 2 diabetes mellitus and cancer have yet to be further unraveled. In general, three pathophysiological mechanisms have been proposed which act through metabolic, hormonal and inflammatory pathways, namely: hyperglycaemia/hyperinsulinaemia, insulin/insulin-like growth factor (IGF) axis and chronic inflammation. Hyperinsulinaemia stimulates IGF-1 production, which may subsequently promote tumor growth by induction of cell proliferation and inhibition of apoptosis. Hyperinsulinaemia is also the hallmark of insulin resistance, which in turn stimulates the release of pro-inflammatory cytokines causing a pro-inflammatory state (Giovannucci *et al*, 2010).

Most studies have reported relative measures of cancer risk with type 2 diabetes mellitus, thereby largely losing sight of the absolute numbers regarding the incidence of GI cancer in the diabetic population. To our knowledge population-based incidence rates of all subtypes of GI cancers in diabetic patients versus matched controls are unknown. Therefore, our aim was to determine incidence rates of GI malignancies for each site of the digestive tract in type 2 diabetic and non-diabetic individuals in the United Kingdom (UK).

## METHODS

### Data source

Data were obtained from the Clinical Practice Research Datalink (CPRD). The CPRD is an ongoing primary care database that comprises anonymized electronic medical records from British general practitioners since 1987, with coverage of over 11.3 million patients from 674 practices (Parkinson *et al*, 2007; Herrett *et al*, 2015). Currently, the population of active

patients represents 6.9% of the total UK population. CPRD records include demographic information, medication prescription details, clinical events, preventive care provided, diagnostic tests, specialist referrals, hospital admissions, and major outcomes (Herrett *et al*, 2015). The accuracy and completeness of CPRD data have been well-validated (Khan *et al*, 2010; Herrett *et al*, 2010). The protocol of this study was approved by CPRD's Independent Scientific Advisory Committee (Protocol 15_143).

## Study population

To examine GI cancer incidence rates (IRs) across anatomical subsite, age, sex, and calendar year among type 2 diabetic patients and non-diabetic individuals, we included a cohort of hypoglycaemic medication users (*i.e.* diabetic cohort) and a (1:1) matched reference cohort using incidence sampling techniques. The diabetic cohort consisted of all registered adult patients (aged 18+ years) with at least one prescription for a hypoglycaemic agent recorded in CPRD during valid data collection (January 1988-December 2012). The date of first prescription for a hypoglycaemic agent defined the start of follow-up (*i.e.* index date). Each diabetic patient was matched to a reference patient without any past prescriptions for hypoglycaemic agents, by sex, year of birth, and practice. Reference patients were assigned the same index date as their matched diabetic patient. Patients in the reference cohort could become diabetic patients if a prescription for a hypoglycaemic agent was recorded. At the prescription date the patient was censored as a reference and matched, as a diabetic patient, to a new reference. Non-diabetic reference subjects could have suffered from any other disease than diabetes mellitus or those mentioned as exclusion criteria below.

Patients with a prescription for insulin at the index date, without any concomitant prescriptions for non-insulin antidiabetic drugs (NIADs), were excluded if they were under 30 years of age at cohort entry. These patients were considered to have type 1 diabetes mellitus. Secondly, all subjects with a history of the cancer of interest prior to cohort entry (*e.g.* all subjects with a history of gastric cancer when investigating gastric cancer) were excluded. Furthermore, all metformin only users who had a history of polycystic ovary syndrome (PCOS) prior to cohort entry were excluded, as they are more likely to have received metformin as a treatment for PCOS, instead of type 2 diabetes mellitus. In addition, we excluded diabetic patients without any subsequent prescriptions for hypoglycaemic agents (after the initial prescription recorded at baseline). All matched individuals of excluded patients were excluded as well.

## Outcome

All study participants were followed up from the index date to a diagnosis of a GI malignancy, the end of data collection, the date of transfer out of the practice area, or death, whichever came first. The first medical record for a GI cancer in CPRD after cohort entry was taken as the diagnosis date of a new case. Subsites of cancer were classified according to their anatomical location; *i.e.* cancer of the esophagus, stomach, liver, gallbladder and extrahepatic bile ducts (biliary), pancreas, small intestines, colon, and rectum. A high level of validity for the recording of cancer in the CPRD has been previously reported (Dregan *et al*, 2012).

### Statistical analyses

To describe and compare both cohorts at baseline, we analyzed various lifestyle factors (*i.e.* smoking status, alcohol use, body mass-index), a diagnosis of various comorbidities ever before (*i.e.* gallstone disease, gastro-esophageal reflux disease, Helicobacter pylori infection, hypertension, inflammatory bowel disease, chronic liver disease, and chronic pancreatitis), use of medications during the past 6 months before start of follow-up (*i.e.* antihypertensives, aspirin, non-steroidal anti-inflammatory drugs, proton-pump inhibitors, and statins), and if a subject had a colonoscopy for colorectal cancer screening purposes during the year before the start of follow-up.

Overall, age-, sex-, and site-specific incidence rates (IR) per 100,000 person years (py) and incidence rate ratios (IRR) with 95% confidence intervals (CI) were calculated for GI cancers in the diabetic and reference cohort. IRRs were calculated by dividing the IR of the non-diabetic cohort by the IR of the type 2 diabetic cohort. Differences between IRs were tested for statistical significance using the normal-theory test ($\alpha < 0.05$) (Rosner, 2006). To assess secular trends, data were presented by age group and time period of cancer diagnosis. Age groups consisted of 5-year intervals, with the exception of those aged '18 through 29 years' (as cancer is rare among these patients) and ending with '85+ years'. Calendar time was broken down into six periods: 2001-2002, 2003-2004, 2005-2006, 2007-2008, 2009-2010, and 2011-2012. Time periods for 1988-2000 were not shown due to lower accuracy of CPRD data during that period. Due to a small number of small intestinal cancer cases, graphs for this cancer site are not shown as no reliable conclusions could be drawn. Furthermore, when the number of cases in a specific subgroup was less than six, data were not shown (suppressed) for reasons of patient privacy.

### Sensitivity analyses

To prevent possible detection bias after the diagnosis of type 2 diabetes mellitus and account for possible reverse causality, a sensitivity analysis was performed by excluding the first year of follow-up after the index date from the analysis for all patients and subsequently calculating subsite- and sex-specific IRs during the remaining follow-up period. All data management and statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

During more than 3.6 million person years of follow-up, 10,977 GI cancer cases were observed in 333,438 type 2 diabetic patients and 333,438 non-diabetic individuals. Baseline characteristics are presented in Table 2.2.1. Type 2 diabetic patients had on average a higher BMI, and a higher proportion was former smokers. Non-diabetic individuals were more often current smokers, and a higher proportion was classified as alcohol consumer. In addition, statistical significant differences were seen between the type 2 diabetic and non-diabetic cohort in the histories of various comorbidities (*e.g.* gallstone disease, gastro-esophageal reflux disease, hypertension) at baseline, use of medications (*e.g.* antihypertensives, aspirin, statins) during the 6 months before baseline, and colorectal cancer screening colonoscopy during the year before cohort entry.

**TABLE 2.2.1.** Baseline characteristics of the type 2 diabetic and non-diabetic cohorts

| Characteristic | Type 2 diabetic cohort (n = 333,438*) | Non-diabetic cohort (n = 333,438*) | *p* value |
|---|---|---|---|
| **Median age at start follow-up (years, IQR)** | 61.8 (52-73) | 61.8 (52-73) | |
| **Male (n, %)** | 183,297 (55) | 183,297 (55) | |
| **Type of hypoglycaemic agent† (n, %)** | | | |
| Metformin | 205,288 (61.6) | - | |
| Sulfonylureas | 105,273 (31.6) | - | |
| Thiazolidinediones | 7,632 (2.3) | - | |
| Meglitinides | 1,017 (0.3) | - | |
| DPP-4 inhibitors | 1,584 (0.5) | - | |
| GLP-1 analogues | 481 (0.1) | - | |
| Insulin | 49,340 (14.8) | - | |
| **BMI category (n, %)** | | | |
| <20 | 4,929 (1.5) | 13,357 (4.0) | |
| 20-24 | 45,379 (13.6) | 87,337 (26.2) | |
| 25-29 | 96,021 (28.8) | 95,728 (28.7) | |
| 30-34 | 73,749 (22.1) | 36,223 (10.9) | |
| ≥35 | 58,551 (17.6) | 14,601 (4.4) | |
| Unknown | 54,809 (16.4) | 86,192 (25.8) | <0.05 |
| **Smoking status (n, %)** | | | |
| Current | 69,225 (20.8) | 70,518 (21.1) | |
| Former | 68,672 (20.6) | 52,520 (15.8) | |
| Never | 147,391 (44.2) | 150,281 (45.1) | |
| Unknown | 48,150 (14.4) | 60,119 (18.0) | <0.05 |
| **Alcohol use (n, %)** | | | |
| Yes | 184,431 (55.3) | 198,074 (59.4) | |
| No | 72,026 (21.6) | 47,918 (14.4) | |
| Unknown | 76,981 (23.1) | 87,446 (26.2) | <0.05 |
| **Comorbidities (n, %)** | | | |
| Gallstone disease | 9,173 (2.8) | 5,737 (1.7) | <0.05 |
| Gastro-esophageal reflux disease | 29,463 (8.8) | 26,638 (8.0) | <0.05 |
| Helicobacter pylori infection | 3,756 (1.1) | 3,543 (1.1) | <0.05 |
| Hypertension | 146,486 (43.9) | 83,326 (25.0) | <0.05 |
| Inflammatory bowel disease | 3,090 (0.9) | 2,516 (0.7) | <0.05 |
| Chronic liver disease | 3,613 (1.1) | 1,190 (0.4) | <0.05 |
| Chronic pancreatitis | 1,419 (0.4) | 270 (0.1) | <0.05 |
| **Other drug-use† (n, %)** | | | |
| Antihypertensives | 192,086 (57.6) | 102,911 (30.9) | <0.05 |
| Acetylsalicylic acid | 92,558 (27.8) | 41,511 (12.4) | <0.05 |
| NSAIDs‡ | 44,265 (13.3) | 38,245 (11.5) | <0.05 |
| Proton-pump inhibitors | 53,164 (15.9) | 35,558 (10.7) | <0.05 |
| Statins | 130,666 (39.2) | 43,526 (13.0) | <0.05 |
| **Colorectal cancer screening (n, %)** | 2,903 (0.9) | 3,577 (1.1) | <0.05 |

*Abbreviations*: DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; NSAID, non-steroidal anti-inflammatory drugs; BMI, body mass-index in kg/m$^2$. *Based on analysis of any gastrointestinal cancer. †Multiple prescriptions on the index date occurred. ‡Non-steroidal anti-inflammatory drugs (excluding acetylsalicylic acid).

### Cancer incidence by cancer site

The IRs for any GI cancer and for liver, pancreatic and colon cancer were significantly higher in the diabetic cohort as compared to the reference cohort (Table 2.2.2). For any GI cancer, the IR in the diabetic cohort was 330 (95% CI 322-339) per 100,000 py, versus 276 (95% CI 268-284) in the reference cohort (IRR 1.20, $p<0.05$). For liver cancer, an IR of 26 (95% CI 24-28) per 100,000 py was found among type 2 diabetic patients, versus 8.9 (95% CI 7.7-10) in the non-diabetic cohort (IRR 2.87, $p<0.05$). The IR for pancreatic cancer in the diabetic cohort was 65 (95% CI 62-69) per 100,000 py, as compared to 31 (95% CI 28-34) among patients without diabetes mellitus (IRR 2.12, $p<0.05$). For colon cancer, the difference in IR between the diabetic and non-diabetic cohort was 119 (95% CI 114-124) versus 109 (95% CI 104-114) per 100,000 py, respectively (IRR 1.09, $p<0.05$). In contrast, the IR of esophageal cancer was significantly lower in the diabetic cohort as compared to the reference cohort: 41 (95% CI 39-44) versus 47 (95% CI 44-51) per 100,000 py. Among other GI cancer subsites no significant differences were observed. Similar results were found in a sensitivity analysis excluding the first year of follow-up [data not shown], except for the IR of pancreatic cancer in the diabetic cohort which declined to 48 (95% CI 45-52) per 100,000 py. However, the difference between the diabetic and reference cohort remained statistically significant.

### Cancer incidence by sex

Men with type 2 diabetes mellitus had significantly higher IRs of any GI, liver, pancreatic and colon cancer compared to male reference patients (Table 2.2.2). In women with type 2 diabetes mellitus, significantly higher IRs were observed for any GI, liver, and pancreatic cancer compared to female reference patients. The lower IRs for esophageal cancer in the diabetic cohort only remained statistically significant in women, although in general, males had higher IRs of esophageal cancer than females. Among the other GI cancer sites no significant differences in IRs between the diabetic and reference cohorts were found after stratifying by sex.

### Cancer incidence by age

Figure 2.2.1 shows the site-specific IRs of GI cancers stratified by 5-year age groups for the diabetic and reference cohorts. Amongst all cancer sites, IRs increased with increasing age for both populations. Differences in IR between the diabetic and reference cohort at increasing age were most pronounced in liver, pancreatic and colon cancer. For other GI cancer sites, IRs by age overlapped between the two cohorts. Age-specific IRs of gastrointestinal cancers did not differ evidently when stratified by sex [data not shown].

### Cancer incidence over time

Inicidence rates of any GI, liver, and pancreatic cancer in the diabetic cohort remained clearly elevated over time compared to the reference cohort (Figure 2.2.2). Moreover, IRs of liver cancer more than doubled in time in the diabetic cohort, while remaining stable in the reference cohort. Also, trends of increasing IRs for colon cancer were observed in both the diabetic and reference cohort. In contrast, IRs of pancreatic cancer declined slightly over time in both cohorts, while IRs of any GI, esophageal, gastric, and biliary cancer remained more or less stable. In addition, IRs of esophageal

**TABLE 2.2.2.** Overall, site- and sex-specific gastrointestinal cancer incidence rates in patients with and without type 2 diabetes mellitus.

| **Overall** | **Type 2 diabetic cohort** | | | | **Non-diabetic cohort** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Cancer site* | *Cases* | *PY* | *IR* | *95% CI* | *Cases* | *PY* | *IR* | *95% CI* | *IRR* | *95% CI* |
| Any gastrointestinal* | 6,165 | 1,866,547 | 330 | (322–339) | 4,812 | 1,744,473 | 276 | (268-284) | 1.20 | (1.15-1.24) |
| Esophagus* | 785 | 1,900,616 | 41 | (39-44) | 842 | 1,776,232 | 47 | (44-51) | 0.87 | (0.79-0.96) |
| Stomach | 520 | 1,900,968 | 27 | (25-30) | 497 | 1,776,437 | 28 | (26-31) | 0.98 | (0.86-1.11) |
| Small intestines | 36 | 1,902,494 | 1.9 | (1.4-2.6) | 33 | 1,777,819 | 1.9 | (1.3-2.6) | 1.02 | (0.64-1.63) |
| Liver* | 489 | 1,902,065 | 26 | (24-28) | 159 | 1,777,586 | 8.9 | (7.7-10) | 2.87 | (2.40-3.44) |
| Biliary | 232 | 1,902,096 | 12 | (11-14) | 202 | 1,777,298 | 11 | (9.9-13) | 1.07 | (0.89-1.30) |
| Pancreas* | 1,243 | 1,900,866 | 65 | (62-69) | 548 | 1,775,796 | 31 | (28-34) | 2.12 | (1.92-2.34) |
| Colon* | 2,243 | 1,882,327 | 119 | (114-124) | 1,920 | 1,759,228 | 109 | (104-114) | 1.09 | (1.03-1.16) |
| Rectum | 1,007 | 1,892,627 | 53 | (50-57) | 911 | 1,768,695 | 52 | (48-55) | 1.03 | (0.94-1.13) |
| **Men** | **Type 2 diabetic cohort** | | | | **Non-diabetic cohort** | | | | | |
| *Cancer site* | *Cases* | *PY* | *IR* | *95% CI* | *Cases* | *PY* | *IR* | *95% CI* | *IRR* | *95% CI* |
| Any gastrointestinal* | 3,959 | 1,024,487 | 386 | (375-399) | 3,005 | 932,751 | 322 | (311-334) | 1.20 | (1.14-1.26) |
| Esophagus | 593 | 1,044,940 | 57 | (52-62) | 600 | 951,374 | 63 | (58-68) | 0.90 | (0.80-1.01) |
| Stomach | 362 | 1,045,280 | 35 | (31-38) | 347 | 951,552 | 36 | (33-41) | 0.95 | (0.82-1.10) |
| Small intestines | 26 | 1,046,340 | 2.5 | (1.7-3.6) | 17 | 952,565 | 1.8 | (1.1-2.9) | 1.39 | (0.76-2.57) |
| Liver* | 386 | 1,045,964 | 37 | (33-41) | 106 | 952,328 | 11 | (9.2-13) | 3.32 | (2.67-4.11) |
| Biliary | 120 | 1,046,173 | 11 | (9.6-14) | 104 | 952,338 | 11 | (9.0-13) | 1.05 | (0.81-1.37) |
| Pancreas* | 666 | 1,045,411 | 64 | (59-69) | 296 | 951,393 | 31 | (28-35) | 2.05 | (1.79-2.35) |
| Colon* | 1,408 | 1,034,745 | 136 | (129-143) | 1,109 | 942,327 | 118 | (111-125) | 1.16 | (1.07-1.25) |
| Rectum | 676 | 1,039,851 | 65 | (60-70) | 632 | 946,575 | 67 | (62-72) | 0.97 | (0.87-1.09) |
| **Women** | **Type 2 diabetic cohort** | | | | **Non-diabetic cohort** | | | | | |
| *Cancer site* | *Cases* | *PY* | *IR* | *95% CI* | *Cases* | *PY* | *IR* | *95% CI* | *IRR* | *95% CI* |
| Any gastrointestinal* | 2,206 | 842,060 | 262 | (251-273) | 1,807 | 811,721 | 223 | (213-233) | 1.18 | (1.11-1.25) |
| Esophagus* | 192 | 855,676 | 22 | (19-26) | 242 | 824,858 | 29 | (26-33) | 0.76 | (0.63-0.92) |
| Stomach | 158 | 855,688 | 18 | (16-22) | 150 | 824,885 | 18 | (15-21) | 1.02 | (0.81-1.27) |
| Small intestines | 10 | 856,154 | 1.2 | (0.6-2.2) | 16 | 825,254 | 1.9 | (1.2-3.2) | 0.60 | (0.27-1.33) |
| Liver* | 103 | 856,101 | 12 | (9.9-15) | 53 | 825,258 | 6.4 | (4.9-8.4) | 1.87 | (1.35-2.61) |
| Biliary | 112 | 855,923 | 13 | (11-16) | 98 | 824,959 | 12 | (9.7-14) | 1.10 | (0.84-1.44) |
| Pancreas* | 577 | 855,455 | 67 | (62-73) | 252 | 824,403 | 31 | (27-35) | 2.21 | (1.90-2.56) |
| Colon | 835 | 847,581 | 99 | (92-105) | 811 | 816,901 | 99 | (93-106) | 0.99 | (0.90-1.09) |
| Rectum | 331 | 852,776 | 39 | (35-43) | 279 | 822,119 | 34 | (30-38) | 1.14 | (0.98-1.34) |

*Abbreviations:* PY, person years; IR, incidence rate per 100,000 person years; CI, confidence interval; IRR, incidence rate ratio. *Statistically significant, p<0.05.

**FIGURE 2.2.1**. Overall and site-specific GI cancer incidence rates stratified by 5-year age categories (x-axis). The y-axis indicates the incidence rate in number of events per 100,000 person years. GI: gastrointestinal, T2DM: Type 2 diabetes mellitus, IR: incidence rate. Black line: type 2 diabetic cohort, Grey line: reference cohort.

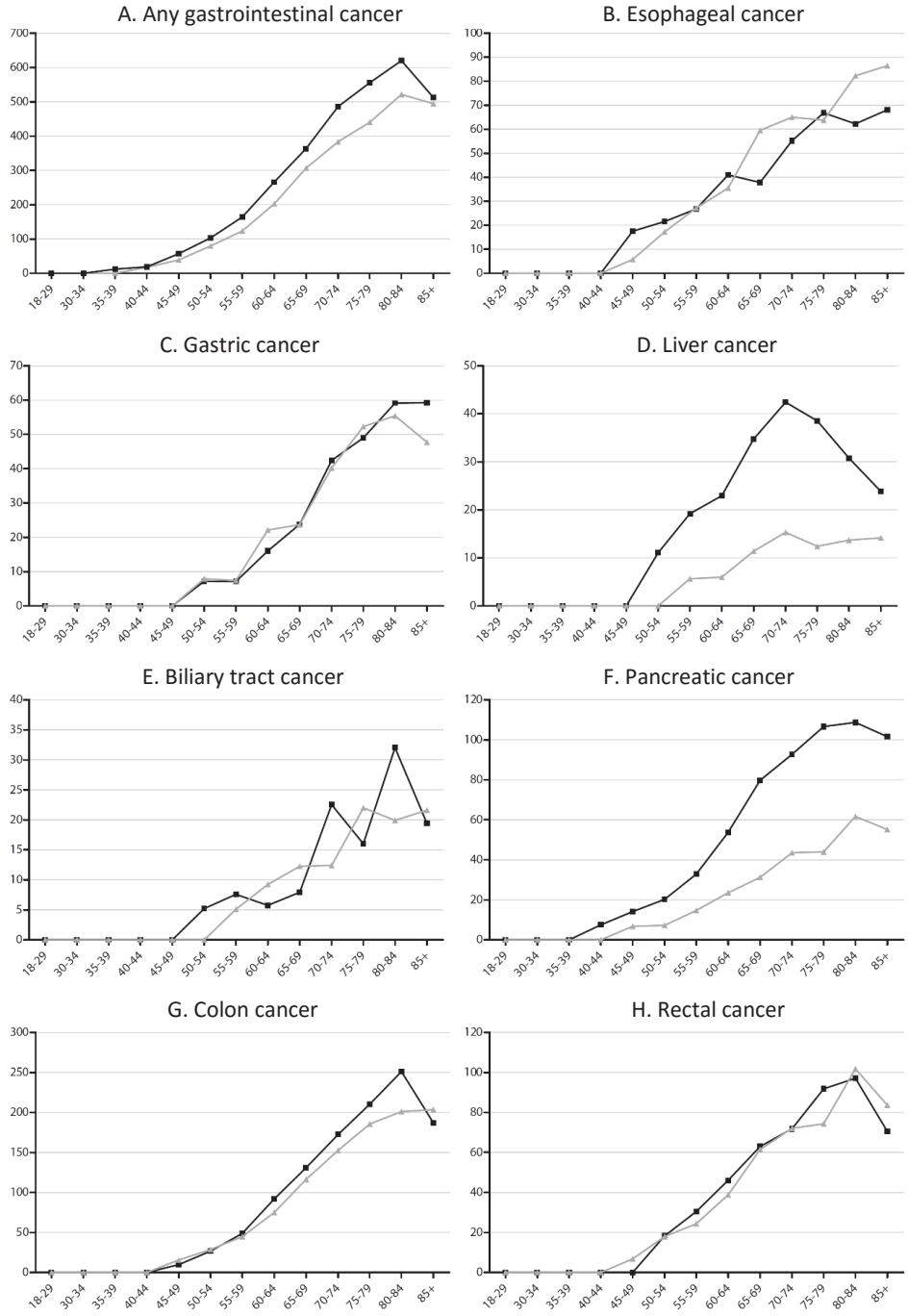
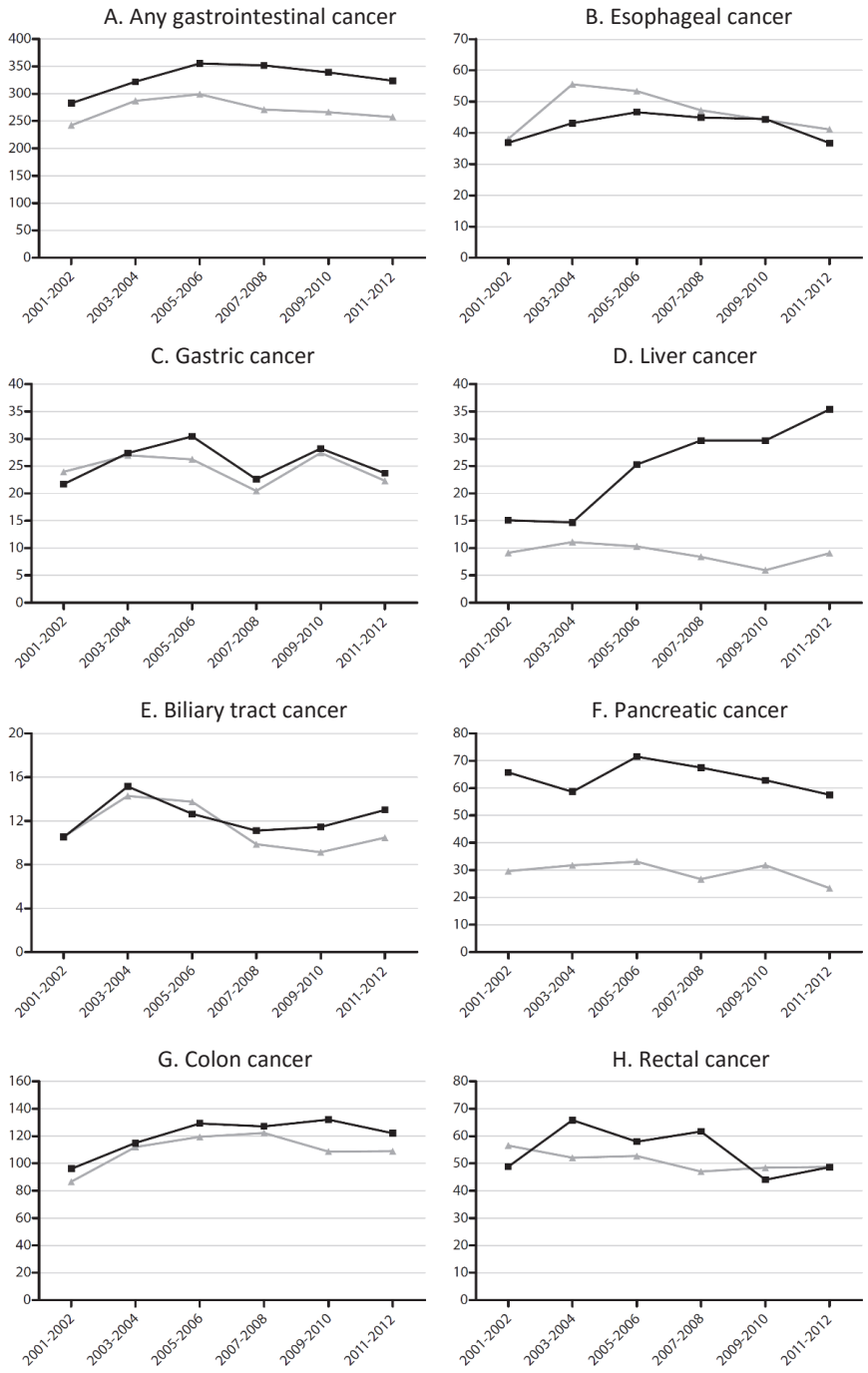


**FIGURE 2.2.2**. Time trends in any and site-specific IRs of GI cancer in the diabetic and non-diabetic cohort, by calendar period (2001-2012; x-axis). The y-axis indicates the IR in number of events per 100,000 person years. GI: gastrointestinal, IR: incidence rate. Black line: type 2 diabetic cohort, Grey line: reference cohort.



cancer differed only in the time periods 2003-2004 and 2005-2006 between the two cohorts, where IRs were higher in the reference cohort. For other GI cancer subsites no noteworthy differences in IRs were seen between the diabetic and reference cohorts over time.



## DISCUSSION

This study provides a comprehensive overview of IRs of GI cancers in people with and without type 2 diabetes mellitus using the CPRD database. Approximately one in every 300 type 2 diabetic patients in the UK developed a GI cancer yearly. In general, IRs of any GI cancer was higher in patients with type 2 diabetes mellitus as compared to non-diabetic individuals, as well as several site-specific cancers: liver cancer, with an IR of 26 per 100,000 person years, pancreatic cancer, with an IR of 65 per 100,000 person years, and colon cancer, with an IR of 119 per 100,000 person years. In contrast, patients with type 2 diabetes mellitus had a lower IR of esophageal cancer as compared to individuals without diabetes, however this difference was small, namely 6 esophageal cancers per 100,000 person years. In the diabetic cohort, IRs for any GI cancer and for liver, pancreatic, and colon cancer were clearly elevated in almost all age groups and time periods compared to the non-diabetic cohort. In addition, an increasing time trend was observed for liver cancer in the diabetic cohort, for colon cancer in both cohorts, whereas for pancreatic cancer a decreasing trend was observed in both cohorts.

A substantial number of studies have reported increased risks of liver, pancreatic, and colon cancer in patients with type 2 diabetes mellitus independent of other risk factors (Luo *et al*, 2013; Ogunleye *et al*, 2009; Larsson *et al*, 2005; Atchison *et al*, 2011; Deng *et al*, 2012; Elena *et al*, 2013; Li *et al*, 2011; Chen *et al*, 2015; Schlesinger *et al*, 2013). As a result, type 2 diabetes mellitus is considered as a risk factor for these cancer types (Giovannucci *et al*, 2010). Our results support this claim, especially for liver and pancreatic cancer where the differences in IRs were most pronounced. Furthermore, these differences became more apparent when stratified by age and time period. However, more recent studies have shown that part of the association might be affected by detection bias or reverse causation (Johnson *et al*, 2011; De Bruijn *et al*, 2014). To minimize these biases, a sensitivity analysis was performed, excluding the first year of follow-up after the index date. This, however, did not change the results in a notable way, except for a substantial, but non-significant decrease in the IR of pancreatic cancer in the diabetic cohort. This might suggest that reverse causality plays a role in the link between type 2 diabetes mellitus and pancreatic cancer.

Insulin is thought to be one of the major hormonal contributors to the diabetes-cancer link. Both the liver and the pancreas are, via the portal venous system, exposed to higher levels of endogenous insulin as compared to other organs, possibly leading to an increased risk of cancer (Giovannucci *et al*, 2010). Conversely, both liver and pancreatic cancers are known to impair glucose regulation and induce diabetes (Johnson *et al*, 2011; Li, 2012). Therefore, the association between type 2 diabetes mellitus and these cancers may very well be bidirectional. Of note, measurement of fasting blood glucose levels has been actively promoted in the past decade as part of the Quality and Outcomes Framework in the UK. This might have resulted in an increase in hypoglycaemic medication use among patients with undiagnosed early stage liver cancer, which could potentially explain the

increase in the incidence rate of liver cancer among type 2 diabetic patients since 2001.

As for colorectal cancer (CRC), a recent umbrella review showed that meta-analyses on the risk of CRC in type 2 diabetic patients are robust, reporting an absolute risk increase of around 30 percent (Tsilidis *et al*, 2015). More importantly, because of the sheer number of incident CRC cases worldwide, the growing number of type 2 diabetic patients, and the increasing time trend observed in this study, this might have an enormous impact on the world population and global health care systems. Furthermore, since CRC screening programs have been implemented or are at present being implemented in an increasing number of countries, more targeted and tailored screening of diabetic patients should be considered in the near future.



In contrast to the other gastrointestinal cancer sites, we observed a significantly lower IR of esophageal cancer in patients with type 2 diabetes mellitus as compared to non-diabetic individuals, although the observed difference was small (IR 41 vs. 47 per 100,000 py) and no longer that notable after stratification by sex. Lifestyle factors such as smoking and alcohol use are important risk factors for esophageal cancer, especially for squamous cell carcinoma (Steevens *et al*, 2010). At baseline these factors differed significantly between the diabetic and reference cohorts, the latter being more often current smokers and users of alcohol, which could explain the observed difference in esophageal cancer incidence. On the other hand, type 2 diabetic patients had a higher BMI as compared to non-diabetic individuals, predisposing them to a higher risk of gastro-esophageal reflux disease, reflux esophagitis, and subsequently Barrett's esophagus and adenocarcinoma of the esophagus (Huang *et al*, 2012; Mearin & Malagelada, 1995; Kamiya *et al*, 2009). Unfortunately, histologic subtypes of esophageal cancer could not be analyzed in this study. Indeed, it is known that the two main histologic subtypes of esophageal cancer (*i.e.* squamous cell carcinoma and adenocarcinoma) show marked epidemiological, pathogenic, and biological differences (Huang *et al*, 2012). For instance, the incidence of esophageal adenocarcinoma has increased in recent years, whereas the incidence of esophageal squamous cell carcinoma has markedly decreased (Botterweck *et al*, 2000). In general, a modestly increased risk of esophageal cancer in type 2 diabetic patients as compared to non-diabetic individuals has been observed (summary relative risk 1.30, 95% CI 1.12-1.50), although not remaining significant after stratification for sex (Huang *et al*, 2012).

The major strength of this study is the use of the CPRD, one of the world's largest population-based health databases. Containing health care data from approximately 7% of the UK population, the CPRD is considered to be representative of the UK general population in terms of age, sex, and ethnicity (Parkinson *et al*, 2007; Herrett *et al*, 2015). In addition, a high level of validity for the recording of cancer in the CPRD has been reported by previous studies, with cancer diagnosis being valid and accurate in over 90% of the cases (Dregan *et al*, 2012). However, potential ascertainment, or misclassification, bias cannot be ruled out, but is most likely to be non-differential. Furthermore, we reported the absolute number of cases for a large variety of site-specific GI cancers instead of relative risks, which adequately shows the differences in IRs between type 2 diabetic patients and non-diabetic patients.

The main limitation of this study is that the causal interpretation of these findings is restricted. Secondly, diabetes mellitus status was defined by the recorded prescription of hypoglycaemic agents. Therefore, misclassification of exposure, and thereby of diabetes

mellitus status, might have occurred since the derived prescription from the GP system may not have been dispensed by the pharmacy, or actually used by the subject. In general, diabetic patients require chronic medication for adequate glycaemic control, making it less likely for diabetic patients to be misclassified as non-diabetic. Conversely, potential non-diabetic patients with a single prescription for a hypoglycaemic agent were excluded in this study. Any resulting misclassification, if present, would have likely biased our results towards the null. Additionally, controls could have suffered from any other disease than diabetes mellitus or those mentioned in the exclusion criteria, which could have impacted their survival and therewith their chance of developing cancer. This might explain the somewhat lower total sum of person years included for the reference cohort. Furthermore, the observed IRs in the reference cohort were generally higher as compared to the age-standardized incidence rates (ASRs) of GI cancers reported for the UK general population (Forman *et al*, 2014). We calculated ASRs using the direct method according to the Segi-Doll world standard population to verify whether these were comparable to the ASRs reported for the UK (Segi *et al*, 1954; Doll *et al*, 1966). After age-standardization, ASRs of the reference cohort were generally in line with those reported for the UK in the tenth volume of the Cancer Incidence in Five Continents series, published by the International Agency for Research on Cancer and the International Association of Cancer Registries [data not shown] (Forman *et al*, 2014).

This large retrospective population-based cohort study shows that patients with type 2 diabetes mellitus have higher incidence rates for liver, pancreatic, and colon cancer as compared to non-diabetic individuals. Yearly, one in every 300 patients with type 2 diabetes mellitus developed a GI cancer. Furthermore, we found no differences in incidence rates between type 2 diabetic and non-diabetic individuals for gastric, biliary, and rectal cancer. Conversely, a slightly lower incidence rate was observed in type 2 diabetic patients for esophageal cancer. The results of this study underline the importance of clinical awareness for liver, pancreatic, and colon cancer in the type 2 diabetic population. In addition, the lower incidence rate of esophageal cancer observed in patients with type 2 diabetes mellitus warrants further investigation.

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## CHAPTER 2.3

# Trends in breast cancer incidence among patients with type 2 diabetes mellitus in the United Kingdom

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## SUMMARY

### Background

Type 2 diabetes mellitus has been associated with an increased breast cancer risk. Breast cancer incidence rates stratified by diabetes mellitus are, however, largely missing. This study assessed age-standardized trends in invasive breast cancer incidence by type 2 diabetes mellitus prevalence, age group, category of body mass-index (BMI) and insulin use.

### Methods

A population-based cohort study was conducted in the Clinical Practice Research Datalink (1989-2012), which contains primary care data from the United Kingdom. All adult women prescribed hypoglycaemic medications were selected and matched (1:1) by age and practice to a non-diabetic reference cohort. Age-standardized incidence rates (IRs) with 95% confidence intervals (CI) for primary invasive breast cancer were calculated per calendar year period, age group, BMI, and insulin use.

### Results

During approximately 1.6 million person years (py), 2,371 breast cancer cases were diagnosed in the diabetic cohort (n=147,998) and 2,252 in the reference cohort (n=147,998). No significant difference in age-standardized IRs per 100,000 py between the diabetic (150, 95% CI 143-157) and reference cohort (148, 95% CI 141-156) were observed. Apart from a temporary increase in IRs since the early 2000s among women aged 65-69 in both cohorts, no clear time trends were observed. Postmenopausal diabetic women with a BMI ≥35kg/$m^2$ had a significantly higher age-standardized IR than those with a BMI <25kg/$m^2$; 313 (95% CI 270-355) versus 421 (95% CI 372-470) per 100,000 py.

### Conclusion

Breast cancer incidence was comparable between diabetic and non-diabetic women. Within the diabetic cohort, age-standardized IRs for breast cancer were higher among women with severe obesity than in those who were not overweight. There is no clear indication for intensified screening for breast cancer among women with type 2 diabetes mellitus. However, among type 2 diabetic women, patients with obesity could be considered for targeted screening.

## INTRODUCTION

Type 2 diabetes mellitus and breast cancer are two major global health problems. Recent estimates indicate that 7.3% of women worldwide suffer from type 2 diabetes mellitus (Danaei *et al*, 2011) and 1.67 million women are diagnosed with breast cancer each year (Ferlay *et al*, 2014). For both diseases, incidence rates (IR) are rising among women (Danaei *et al*, 2011; Ferlay *et al*, 2013). Female breast cancer IRs have increased dramatically since the late-1970s in the United Kingdom (UK), with a 62%-increase (Cancer Research UK, 2016). However, between 2001-2012 these rates have relatively stabilized, with a total increase of approximately 6%, most of which occurred before 2005 (Cancer Research UK, 2016). The number of women with type 2 diabetes mellitus in the UK has doubled since 1994. Among women, the prevalence of type 2 diabetes mellitus increased from 2.3% to 4.9% (Imkampe & Gulliford, 2011). Moreover, the number of patients with type 2 diabetes mellitus is expected to keep rising in the future (Whiting *et al*, 2011).



Meta-analyses have reported a positive association between type 2 diabetes mellitus and the risk of breast cancer (Starup-Linde *et al*, 2013; Larsson *et al*, 2007). Possible explanations for this increased risk in type 2 diabetic patients include high blood glucose levels, hyperinsulinaemia, shared risk factors (Xue & Michels, 2007; Rose & Vona-Davis, 2012; Ozcan *et al*, 2004), or side-effects of exogenous insulin use (Karlstad *et al*, 2013; Bronsveld *et al*, 2015). The rise in type 2 diabetes mellitus incidence has been associated with ageing populations and changes in lifestyle patterns, such as high-caloric diet and decreased physical activity, resulting in obesity (WHO, 1994). Similar risk factors are involved in the etiology of breast cancer (Giovannucci *et al*, 2010, Renehan *et al*, 2008; Anderson *et al*, 2015). Also, the introduction of population-wide breast cancer screening programs might have affected the incidence of breast cancer over time (Peairs *et al*, 2011; Liao *et al*, 2011).

As the incidence of type 2 diabetes mellitus is increasing (Danaei *et al*, 2011) and several studies have reported an increased breast cancer risk associated with type 2 diabetes mellitus (Starup-Linde *et al*, 2013; Larsson *et al*, 2007), it is important, from a health care policy perspective, to estimate the absolute magnitude of this problem. Breast cancer incidence trends, stratified by type 2 diabetes mellitus status, would allow insight into the total disease burden behind the observed relationship between type 2 diabetes mellitus and breast cancer incidence. However, absolute numbers are largely missing. Incidence rates by body mass-index (BMI) category among type 2 diabetic patients may also be of value in this respect, since a high BMI is associated with an increased postmenopausal breast cancer risk (Renehan *et al*, 2008) and plays an important role in the development of type 2 diabetes mellitus (Kahn, 2001).

This study examined age-standardized trends (1989-2012) in breast cancer IRs in the UK, among women with type 2 diabetes mellitus as compared to women without diabetes mellitus by age category. Secondary aims were to determine IRs among categories of diabetes-related factors, namely BMI category and any insulin use (‘ever’ vs. ‘never’).

## METHODS

### Source of data

Data were obtained from the Clinical Practice Research Datalink (CPRD) (22). This healthcare database comprises electronic medical records from general practitioners since 1987 and represents approximately 7% of the UK population. Patients in the CPRD are broadly representative of the UK general population in terms of age, sex, and ethnicity (Herrett *et al*, 2015). The accuracy and completeness of CPRD data have been well validated in previous studies (Herrett *et al*, 2010; Khan *et al*, 2010). Data recorded in CPRD include demographic information, prescribed medication, clinical events, preventive care provided, specialist referrals, and hospital admissions. The CPRD's Independent Scientific Advisory Committee approved the protocol of this study (protocol no: 13_050).



### Study population, follow-up and case definition

To estimate breast cancer rates among type 2 diabetic and non-diabetic patients during 1989-2012, we used a cohort of prevalent and incident hypoglycaemic drug users (*i.e.* diabetic cohort) and a matched reference cohort. The diabetic cohort consisted of registered adult women (≥18 years) with at least 1 prescription for any hypoglycaemic agent recorded in CPRD during up-to-standard follow-up, until 31st of October 2013. The date of the first hypoglycaemic drug prescription during up-to-standard follow-up was taken as the date of cohort entry, though women might have used hypoglycaemic drugs prior to that date. The diabetic cohort was matched (1:1) by year of birth and practice to a reference cohort of women without any recorded prescriptions for hypoglycaemic agents. If a reference patient started using hypoglycaemic agents during follow-up, she was censored and categorized as a diabetic patient from that day forward. As a newly diagnosed diabetic patient, she was then matched to a new reference patient.

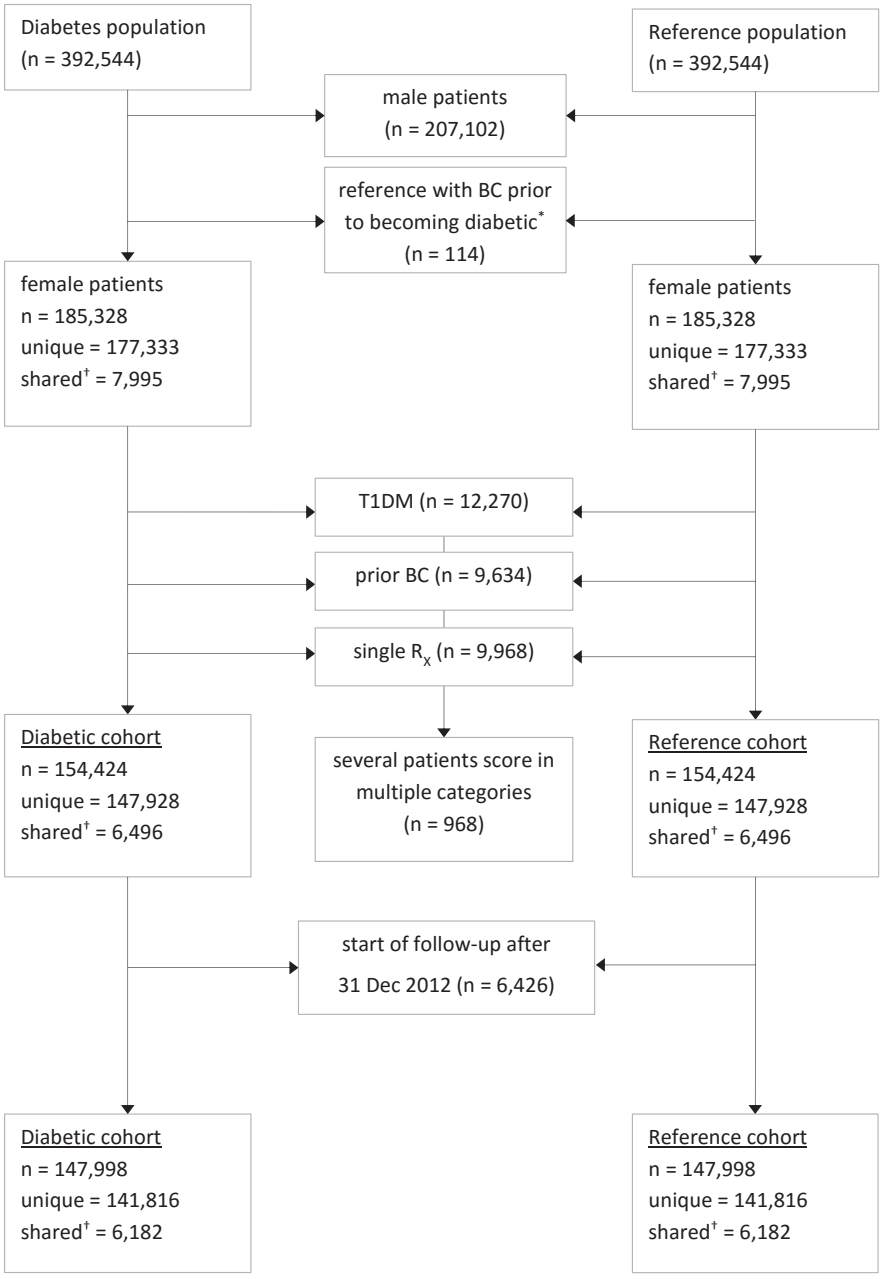
To select our final cohort, we excluded patients with type 1 diabetes mellitus. Women with a prescription for insulin on the index date without any concomitant prescriptions for non-insulin antidiabetic drugs (NIADs) were considered as having type 1 diabetes mellitus, if (a) they had a recorded diagnosis for type 1 diabetes mellitus or (b) they were under the age of 30 at cohort entry. In addition, women with a history of colorectal cancer prior to cohort entry and diabetic women without any subsequent prescriptions for hypoglycaemic agents after the initial prescription recorded at cohort entry were excluded. If a diabetic or reference patient met any of the exclusion criteria, the patient was excluded together with her matched counterpart (Figure 2.3.1).

All patients were followed up from cohort entry until the occurrence of breast cancer, the patient's death, transfer out of practice, or end of data collection, whichever came first. The first-ever diagnostic code for invasive breast cancer (see Appendix, Table 2.3.A) in CPRD after cohort entry was taken as the date of diagnosis. Medical records from CPRD are regarded as a valid measure to capture breast cancer occurrence (Boggon *et al*, 2013).

### Statistical analysis

For the diabetic and reference cohorts, IRs for primary invasive breast cancer were calculated and standardized for age using direct standardization by weighting all the strata according to

**FIGURE 2.3.1.** Specified flow chart for patient selection of the diabetic and non-diabetic comparison cohort.




*Abbreviations:* T1DM, type 1 diabetes mellitus; $R_x$, prescription.
*Female reference patients who would have transferred to the diabetic cohort during follow-up, if they would not have been diagnosed with breast cancer before the first recorded prescription for a hypoglycaemic agent. These were excluded as diabetic patients, together with their newly matched reference patient. †Women who started as a reference patient but became a diabetic patient at some point during follow-up.

the age distribution in the 2012 European (EU-27) standard population (Eurostat). All IRs are provided as the number of new breast cancer events per 100,000 py. Confidence intervals (CI) of the crude IRs (Rothman & Boice, 1979) and the age-standardized IRs were calculated (Boyle & Parkin, 1991). To assess secular trends, IRs are presented by calendar year period and age group. Age categories for standardization consisted of 5-year intervals, starting with '18 to 20 years' and ending with '85+ years'. We assessed IRs in age groups (*i.e.* <45, 45-54, 55-64, 65-69, 70-79, ≥80 years) over time. Rates for women <45 years are not presented separately as the numbers were too small. Additionally, IRs by screening age (*i.e.* 50-64, 65-69 years) were calculated. The IR per age group was standardized for age in 5-year intervals. For calendar year period, two-year intervals were created, where the years 1989-2000 were aggregated due to low data availability. Age was determined per calendar year as the year difference with the year of birth. An individual could therefore contribute to different age-groups in different calendar years.

For the diabetic cohort, age-standardized IRs for breast cancer were determined over the entire follow-up period (1989-2012), stratified by the presence of specific risk factors (*i.e.* BMI, insulin use). Stratification by BMI in the reference cohort was not done since for 76% of the women BMI was not available in the year prior to cohort entry. Follow-up time was labeled as 'never exposed to insulin' for as long as a patient did not receive a prescription for insulin. Once a prescription for (any type of) insulin was recorded in CPRD, the follow-up time from the prescription date onwards was labeled as 'ever exposed to insulin'. To evaluate the association between BMI and breast cancer risk among postmenopausal women (Bhaskaran et al, 2014), overall age-standardized IRs were stratified by BMI category (*i.e.* <25, ≥25 to <30, ≥30 to <35, ≥35 kg/m², unknown) for the diabetic cohort aged ≥55 years. BMI was determined time-dependently, where BMI was updated with each new recording at the date of measurement. If the last measurement was over 1 year old, BMI was labeled as 'unknown'.

Follow-up time for all women was divided into periods with variable length, depending on the occurrence of relevant events (*i.e.* new recording of BMI, prescription for insulin). Subsequently, IRs per category were produced as the number of events within each category, divided by the total amount of follow-up time; *i.e.* the sum of all time periods within this category. All IRs are provided as the number of new breast cancer events per 100,000 py. Differences between IRs were determined by calculating the incidence rate ratio (IRR) with 95% CI (Boyle & Parkin, 1991).

To exclude the influence of potential diagnostic bias (*i.e.* increased breast cancer screening around the time of initiation of treatment with hypoglycaemic medications) (Harding *et al*, 2014), we performed a sensitivity analysis, in which the first year of follow-up was excluded for all diabetic and non-diabetic women.

## RESULTS

### Patient characteristics

In total, 147,998 diabetic and 147,998 non-diabetic women were included in the study with a median age of 64 years at cohort entry (Table 2.3.1). Of the women with type 2 diabetes

mellitus, 11% was treated with insulin at cohort entry. In the diabetic cohort, 26% of the women were obese (BMI 30-35 kg/m²) and 31% severe obese (BMI ≥35 kg/m²), according to the most recent measurement in the year prior to cohort entry; in the reference cohort these proportions were 17% and 11%, respectively.



## Age-standardized incidence rates

During a total follow-up of circa 1.6 million py, 2,371 women were diagnosed with invasive breast cancer in the diabetic cohort (crude IR: 295/100,000 py) and 2,252 in the reference cohort (crude IR: 290/100,000 py). Overall, age-standardized breast cancer IRs per 100,000 py were not significantly different between the diabetic (150, 95% CI 143-157) and reference cohort (148, 95% CI 141-156) with an IRR of 1.01 (95% CI 0.94-1.08, $p>0.05$). We observed no clear time trends (Figure 2.3.2). The sensitivity analysis, in which the first year of follow-up was excluded, resulted in a lower age-standardized IR per 100,000 py for the diabetic cohort (140, 95% CI 132-148, n=141,902), but not for the reference cohort (148, 95% CI 140-157, n=141,902), IRR 0.94 (95% CI 0.87-1.02).

Age-specific IRs showed slightly different patterns with a constant rise by age for diabetic women (except for a drop at age 70-74 years) as compared to a flattening around the age of 64 years for the non-diabetic women (Figure 2.3.3). Incidence rates in diabetic women between 80-84 years and ≥85 years were significantly higher as compared to non-diabetic women; IRR 1.15 (95% CI 1.01-1.32, $p<0.05$) and IRR 1.25 (95% CI 1.08-1.44, $p<0.05$), respectively. Incidence rates per age category were reasonably stable over time (Figure 2.3.4). We observed a trend of increasing IRs of breast cancer in diabetic and non-diabetic women aged 65-69 years, since the early 2000s.

In diabetic women, IRs were higher in women over 80 years compared to non-diabetic women, which was significant in the periods 1989-2000 and 2007-2008. This is in line with the age-specific IRs presented in Figure 2.3.3. The observed IRs (per 100,000 py) in screened (50-64, 65-69 years) and non-screened age groups (<50, ≥70 years) were respectively: 288 (95% CI 265-311), 358 (95% CI 322-395) and 50 (95% CI 40-60), 362 (95% CI 342-382) in the diabetic women, and 296 (95% CI 272-320), 348 (95% CI 310-386) and 52 (95% CI 40-60), 339 (95% CI 320-358) in the non-diabetic women. The IRs between the diabetic and reference cohort in the screened and non-screened-age groups were not significantly different.

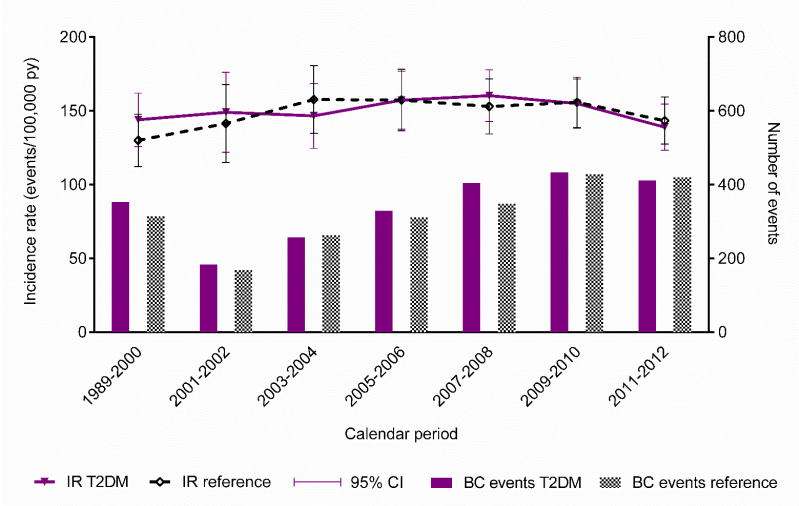
Among postmenopausal (≥55 years) diabetic women, age-standardized IRs for breast cancer (per 100,000 py) increased with increasing BMI (Figure 2.3.5). Breast cancer incidence was significantly higher among extreme obese diabetic women (BMI ≥35kg/m²) compared to diabetic women not overweight (BMI<25kg/m²); IRR 1.35 (95% CI 1.13-1.61, $p<0.05$). The IRR for women with a BMI between 30-35kg/m² compared to not overweight women was 1.17 (95% CI 0.99-1.38, $p>0.05$). Age-standardized IRs for diabetic women with missing BMI were comparable to those with a BMI <25kg/m². With regard to insulin use, the age-standardized IR (per 100,000 py) was similar between diabetic women ever-exposed to insulin (156, 95% CI 133-178) and in diabetic women never-exposed to insulin (153, 95%CI 145-160).

**TABLE 2.3.1.** Baseline characteristics of the diabetic and non-diabetic reference cohort.

| | Type 2 diabetic cohort (n=147,998) | | Reference cohort (n=147,998) | |
|---|---|---|---|---|
| **Age in years** (median, IQR) | 64 | (51-74) | 64 | (51-74) |
| | *n* | *%* | *n* | *%* |
| **Prior cancer*** | 10,034 | (6.8) | 10,058 | (6.8) |
| **BMI (kg/m²)†** | | | | |
| <20 | 1,578 | (1.9) | 2,804 | (7.9) |
| 20-25 | 10,627 | (13.1) | 11,487 | (32.3) |
| 25-30 | 22,321 | (27.5) | 11,439 | (32.2) |
| 30-35 | 21,398 | (26.3) | 6,050 | (17.0) |
| >35 | 25,343 | (31.2) | 3,779 | (10.6) |
| Unknown | 66,731 | (45.1) | 112,439 | (76.0) |
| **Smoking†** | | | | |
| Current | 20,318 | (21.2) | 20,599 | (22.1) |
| Ex | 19,046 | (19.9) | 15,847 | (17.0) |
| Never | 56,582 | (59.0) | 56,600 | (60.8) |
| Unknown | 52,052 | (35.2) | 54,952 | (37.1) |
| **Alcohol use†** | | | | |
| Yes | 49,092 | (63.2) | 54,953 | (74.6) |
| No | 28,645 | (36.8) | 18,697 | (25.4) |
| Unknown | 70,261 | (47.5) | 74,348 | (50.2) |
| **Type of hypoglycaemic drug‡** | | | | |
| Insulin | 15,773 | (10.7) | - | |
| Metformin | 98,259 | (66.4) | - | |
| Sulfonylurea | 45,208 | (30.5) | - | |
| Thiazolidinediones | 3,158 | (2.1) | - | |
| Other oral hypoglycaemic drugs | 2,251 | (1.5) | - | |

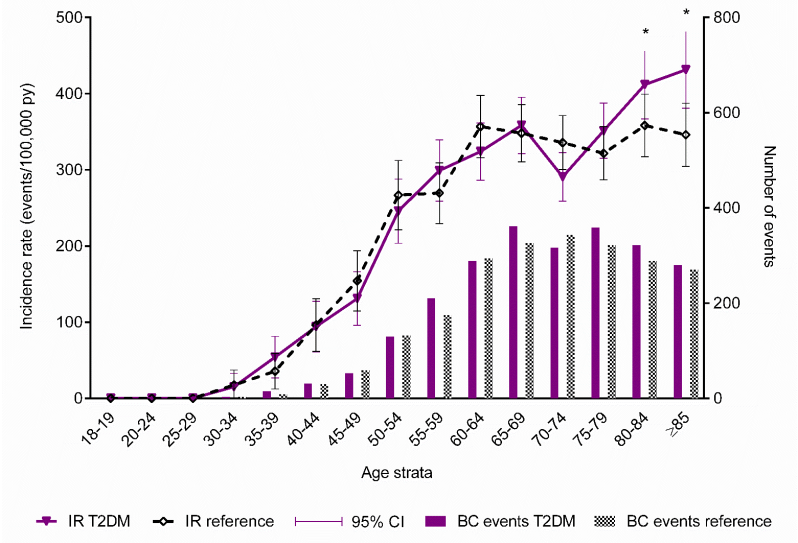
*Abbreviations:* IQR, interquartile range; BMI, body mass-index. *Any type, except non-melanoma skin cancer or breast cancer. †BMI, alcohol and smoking information is based on the most recent record in the year prior to baseline. ‡Several patients have multiple prescriptions on the index date.

**FIGURE 2.3.2.** Time trends in age-standardized incidence rates (line) for breast cancer and number of events (bar) among type 2 diabetic and non-diabetic women, by calendar year (1989-2012).




*Abbreviations:* IR, incidence rate; BC, breast cancer; T2DM, type 2 diabetes mellitus; py, person years; CI, confidence interval.

**FIGURE 2.3.3.** Age-specific crude incidence rates for breast cancer (line) and number of events (bar) in type 2 diabetic and non-diabetic women, over the entire follow-up period (1989-2012).




*Incidence rates of T2DM and reference patients differ significantly ($p$<0.05).

*Abbreviations:* IR, incidence rate; BC, breast cancer; T2DM, type 2 diabetes mellitus; py, person years; CI, confidence interval.

## DISCUSSION



Results from our study provide a detailed description of IRs for breast cancer among type 2 diabetic and non-diabetic women in the United Kingdom over the period 1989-2012. The IRs between the diabetic and reference cohort were similar. In both groups, overall and age specific rates for breast cancer have remained relatively stable between 2001 and 2012. Only among women aged 65-69 years, a steep increase in age-standardized incidence was observed between 2001 and 2006, after which IRs declined again. Age-specific IRs were comparable between the diabetic and reference cohort until the age of 64 years. However, for non-diabetic women breast cancer rates remained the same above the age of 64 years, while after a drop at age 70-74, the rates among diabetic women above 74 years of age continued to rise. Breast cancer IRs were higher among diabetic women with a high BMI as compared to those not overweight. No difference in IRs was observed between diabetic women treated with insulin as compared to diabetic women never treated with insulin.

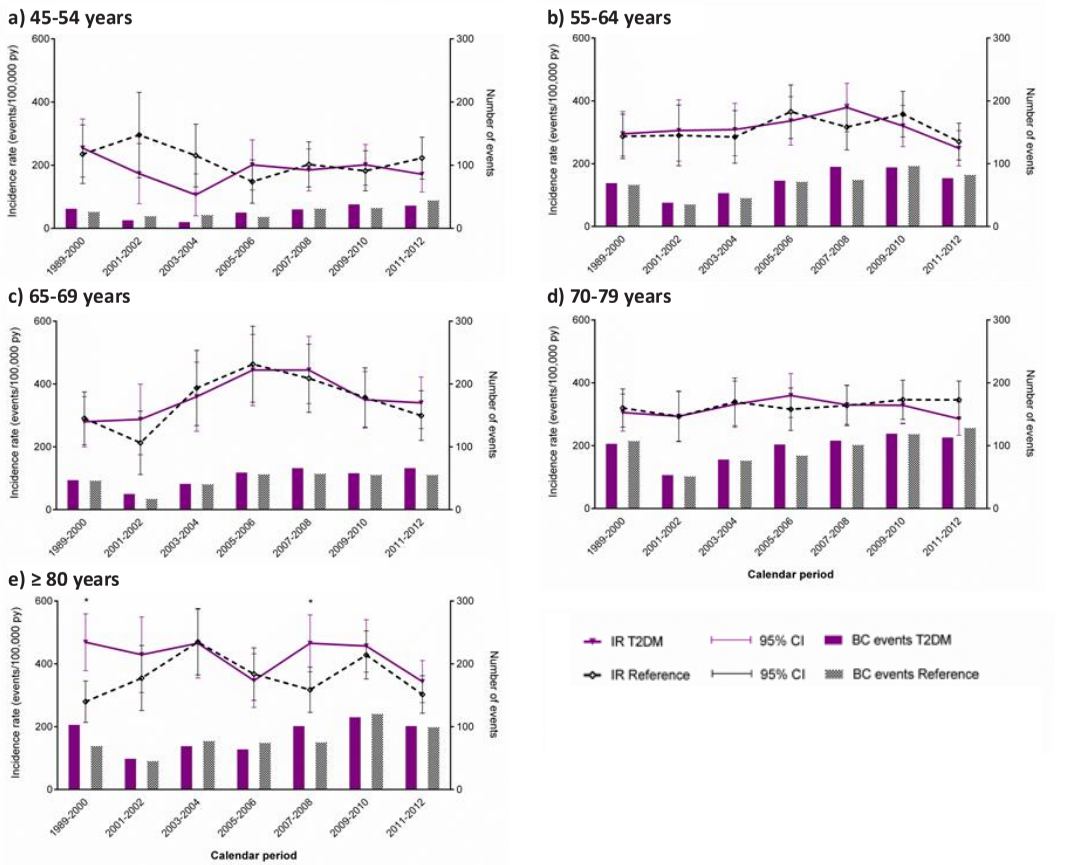
Meta-analyses of previous case-control and cohort studies (Starup-Linde *et al*, 2013; Larsson *et al*, 2007) have shown a positive association between type 2 diabetes mellitus and breast cancer risk. However, some studies included in these meta-analyses, with a large contribution to the pooled estimate, compared breast cancer risk in a cohort of diabetic women to IRs derived from national cancer registries. We estimated IRs in an age and practice-matched non-diabetic reference cohort, which could have resulted in an overall higher IR in the reference population. Another explanation for the observed discrepancy might be differences in diabetes mellitus ascertainment. We defined type 2 diabetic patients based on hypoglycaemic medication use while previous studies in the meta-analyses used hospital registries, health care databases, or questionnaires for diabetes mellitus diagnosis ascertainment. Studies that included only women hospitalized with overt symptoms of diabetes mellitus possibly selected patients who suffered from more advanced disease as compared to hypoglycaemic medication users in the CPRD. Then again, our definition of type 2 diabetic patients excluded women with diagnosed type 2 diabetes mellitus who were treated exclusively with diet and exercise.

If we compare our results with age-specific breast cancer IRs and time trends in the general population published by UK Cancer Research, our results are largely in agreement (Cancer Research UK, 2014). However, the overall age-standardized IR of our reference cohort was somewhat higher than that reported by the UK cancer registry (148 versus 125 per 100,000 py). This is hard to explain as 98% of the UK population is registered at a GP practice. However, the CPRD may not be representative of all practices in the UK based on the geographical location of contributing practices (Herrett *et al*, 2015). Underlying risk factors for breast cancer such as social status, hormone use, and reproductive history might have been different between our cohort and that of the cancer registry.

The Dutch Cancer Society also reported prevalence rates of diabetes mellitus among a sample of Dutch women visiting their GP and among women who were diagnosed with breast cancer (KWF kankerbestrijding, 2004). They found that diabetes mellitus prevalence rates were similar among women with breast cancer (35-64 years: 3%; ≥65 years: 13.4%) as compared to women without breast cancer (35-64 years: 3.1%; ≥65 years: 13.2%). These statistics are in line with our results.

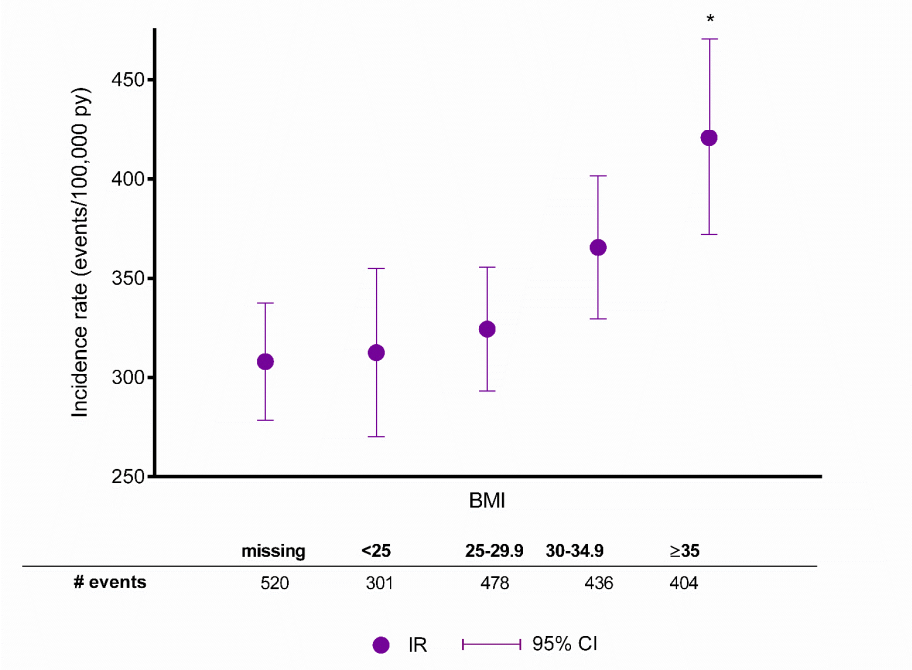
It is known that screening leads to an increase in the incidence of breast cancer

**FIGURE 2.3.4.** Time trends in incidence rates for breast cancer (line) and number of events (bar) in type 2 diabetic and non-diabetic women over 1989-2012, by age group.




*Incidence rates of T2DM and reference patients differ significantly ($p$<0.05). Rates are standardized for age in 5-year intervals within age groups.
*Abbreviations:* IR, incidence rate; BC, breast cancer; T2DM, type 2 diabetes mellitus; py, person years; CI, confidence interval.

**FIGURE 2.3.5.** Age-standardized incidence rates for breast cancer over the entire follow-up period (1989-2012) among type 2 diabetic women ≥ 55 years, by current BMI category.




*Significantly different compared to BMI<25

*Abbreviations:* BMI, body mass-index in gk/m²; py, person years; #, number; CI, confidence interval.

(Advisory Committee on Breast Cancer Screening, 2006; Independent UK Panel on Breast Cancer Screening, 2012). In the UK, breast cancer screening began in 1988 for women aged 50-64 years and was expanded to women aged 65-70 years in 2000. Although the increase in breast cancer IRs observed between 2001 and 2006 among diabetic and non-diabetic women aged 65-69 years can probably to a great extent be attributed to increased screening, this effect was only temporary. Since breast cancer screening for women aged 50-64 years started before our study period, we were unable to determine the effect of screening within this group. Women aged 70-74 years are not screened, which might explain the decreased age-specific IR in this group.

There may be a higher non-participation for screening among postmenopausal obese women compared to non-obese women, and possibly in particular those with diabetes mellitus (Hellman *et al*, 2015). We observed that IRs for breast cancer increased with increasing BMI among postmenopausal diabetic women. The association between high BMI and an increased breast cancer risk in postmenopausal women is well established (Renehan *et al*, 2008). Even though BMI among diabetic women is higher than among non-diabetic women in our study, we did not find an overall higher IR for breast cancer in the diabetic cohort. As screening leads to an increase in breast cancer incidence, and non-diabetic normal weight women are more likely to participate in screening programs (Hellman *et al*, 2015), this may possibly explain why we did not find an overall higher IR

among diabetic women as compared to non-diabetic women.

To our knowledge, we are the first to present comprehensive time-trend and age-specific data on breast cancer incidence rates among type 2 diabetic women. A large and accurate health care database was used in which clinical records are regarded as a valid measure to capture breast cancer incidence as compared to the National Cancer Registry (Boggon *et al*, 2013). However, this study also had limitations. First of all, we were unable to determine trends in incidence over time before 2001 because of the limited follow-up time and number of cancer events. For the same reason, we could not investigate trends over time among insulin users, with only 11% of the type 2 diabetic women treated with insulins. Secondly, potential diagnostic bias at the start of follow-up might be present, as the age-standardized IR for breast cancer among the diabetic cohort decreased from 150 to 140/100,000 py after elimination of the first year of follow-up. Finally, we could not match diabetic and non-diabetic women on BMI because of information asymmetry between the two cohorts. In addition, for the non-diabetic women we were unable to stratify IRs for BMI categories because the majority had no recently recorded BMI measure. Body mass-index is less frequently measured in non-diabetic (normal weight) women as the Quality and Outcomes Framework in the UK specifically rewards practices for the registration of BMI among patients with diabetes mellitus and among women with a BMI of over 30 kg/m$^2$. Therefore, we assumed that unmeasured BMI reflects normal BMI.

Based on our data we found no evidence that the incidence rates for breast cancer in women with type 2 diabetes mellitus is different from non-diabetic women. As such, there is no indication that points towards a need for intensified screening for breast cancer in women with type 2 diabetes mellitus. However, among type 2 diabetic women, the breast cancer incidence rate was higher in women with a high BMI (≥30kg/m$^2$). Therefore, type 2 diabetic women with obesity could be considered for targeted screening.

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## APPENDIX

**TABLE 2.3.A.** Medical codes for invasive breast cancer from CPRD used to identify new breast cancer events

| Medcode* | Events† | Readterm‡ |
|---|---|---|
| 3968 | 109444 | Malignant neoplasm of female breast |
| 348 | 51428 | Ca female breast |
| 9470 | 8511 | Malignant neoplasm of female breast NOS |
| 18608 | 1246 | Malig neop of bone, connective tissue, skin and breast |
| 9902 | 1072 | Carcinoma of bone, connective tissue, skin and breast |
| 12499 | 907 | [X]Malignant neoplasm of breast |
| 23399 | 749 | Malignant neoplasm of upper-outer quadrant of female breast |
| 26853 | 284 | Malignant neoplasm of nipple and areola of female breast |
| 12427 | 243 | [M]Lobular carcinoma NOS |
| 29826 | 215 | Malignant neoplasm of upper-inner quadrant of female breast |
| 31546 | 157 | Malignant neoplasm of central part of female breast |
| 42070 | 146 | Malignant neoplasm of lower-outer quadrant of female breast |
| 23380 | 133 | Malignant neoplasm of nipple of female breast |
| 39760 | 120 | [M]Infiltrating duct and lobular carcinoma |
| 45222 | 104 | Malignant neoplasm of lower-inner quadrant of female breast |
| 20685 | 77 | Malignant neoplasm of axillary tail of female breast |
| 42542 | 71 | [M]Paget's disease and infiltrating breast duct carcinoma |
| 30189 | 62 | [M]Intraductal papillary adenocarcinoma with invasion |
| 56715 | 59 | Malignant neoplasm of other site of female breast |
| 19389 | 50 | Malig neop of bone, connective tissue, skin and breast OS |
| 41011 | 48 | Malig neop of bone, connective tissue, skin and breast NOS |
| 38475 | 46 | Malignant neoplasm of other site of female breast NOS |
| 64686 | 27 | Malignant neoplasm of areola of female breast |
| 67701 | 26 | [M]Secretory breast carcinoma |
| 40359 | 25 | [M]Juvenile breast carcinoma |
| 59831 | 20 | Malignant neoplasm of nipple or areola of female breast NOS |
| 49148 | 18 | Malignant neoplasm, overlapping lesion of breast |
| 95057 | 2 | Malignant neoplasm of ectopic site of female breast |

*Medical codes (Medcode) in the Clinical Practice Research Datalink (CPRD) correspond to Read-codes, which are the standard clinical terminology system used in General Practice in the United Kingdom. †List the total number of events (i.e. recordings of the specific medical code) within the CPRD. ‡Contains the description of the clinical event linked to the specific medical code.

# CHAPTER 3

# THE IMPACT OF VARIABILITY IN STUDY DESIGN

## CHAPTER 3.1

# Insulin use and cancer risk: the effect of choices in study design on risk estimates

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## SUMMARY

### Background

Meta-analyses on the risk of cancer associated with insulin use generally pooled results from observational studies with differences in study designs, exposure definitions, and selection of the study population. This study was aimed to evaluate the influence of these different choices in study design on the risk estimates for cancer associated with insulin use.

### Methods

A series of case-control studies was conducted nested within 4 different study cohorts of hypoglycaemic drug users (≥40 years) in the Clinical Practice Research Datalink; (1) any users, (2) exclusive users, (3) inception cohorts, and (4) treatment stages. Cases were defined as patients with a first ever diagnosis of any cancer during follow-up, stratified by anatomical site; *i.e.* breast, prostate, colorectal, pancreatic, liver, and lung cancer. On the date of diagnosis (*i.e.* index date), 3 matched controls without a record of cancer from the same study cohort were selected. Additional variations in the case-control design regarded (1) the insulin exposure definition, (2) matching criteria, (3) cancer diagnosis date, and (4) a prescription threshold for exposure (*i.e.* immortal time). In all models, conditional logistic regression was used to estimate odds ratios (ORs) associated with insulin use as compared to metformin use.

### Results

In total, 1440 different case-control studies were conducted within a total study population of 232,952 patients. For any cancer, the effects of insulin ranged from a protective effect (OR 0.76, 95% CI 0.71-0.83) to a near 3-fold increased risk (OR 2.86, 95% CI 2.60-3.16). Especially the risk of pancreatic cancer associated with insulin use varied widely, from 0.47 (95% CI 0.25-0.89) to 5.13 (95% CI 3.23-8.13). Variations in matching by age did not markedly affect the range in ORs, nor exposure time window definitions or inclusion of immortal time. The backward shifting of the index date had the most profound effect on risk estimates for pancreatic and liver cancer.

### Conclusion

The risk estimates for any cancer associated with insulin use varied from a protective effect to a harmful effect, depending on the choices made in study design. Our results clearly question the justification of combining results from studies with different designs to provide an aggregate risk estimate.

## INTRODUCTION

Multiple meta-analyses have reported on the risk of cancer associated with insulin use for the treatment of type 2 diabetes mellitus (Bosetti *et al*, 2014; Bu *et al*, 2014; Chen *et al*, 2013; Colmers *et al*, 2012; Janghorbani *et al*, 2012; Karlstad *et al*, 2013; Nie *et al*, 2014; Singh *et al*, 2013a; Singh *et al*, 2013b; Singh *et al*, 2013c; Sun *et al*, 2014; Wang *et al*, 2013a; Wang *et al*, 2013b; Wu *et al*, 2015; Yin *et al*, 2014). Most meta-analyses concluded that insulin use was associated with an increased risk of (some sorts of) cancer among patients with type 2 diabetes (Bosetti *et al*, 2014; Bu *et al*, 2014; Colmers *et al*, 2012; Janghorbani *et al*, 2012; Karlstad *et al*, 2013; Singh *et al*, 2013c; Sun *et al*, 2014; Wang *et al*, 2013a; Wu *et al*, 2015; Yin *et al*, 2014). These meta-analyses generally pooled the results from observational studies. However, these observational studies often varied in their study designs, exposure definitions, and the selection of a study population. For example, some studies selected incident users (Campbell *et al*, 2010; Carstensen *et al*, 2012; Chang *et al*, 2012), while others included prevalent users of hypoglycaemic agents (starting follow-up after the start of treatment) (Kawaguchi *et al*, 2010; Li *et al*, 2011; Vinikoor *et al*, 2009). Also, some studies looked at associations with current insulin use only (*e.g.* a prescription in the past 3 months) (van Staa *et al*, 2012; Vinikoor *et al*, 2009), while others also considered past insulin use as relevant to the overall exposure (Campbell *et al*, 2010; Carstensen *et al*, 2012). Furthermore, studies defined the outcome of interest as the cancer diagnosis date (Mizuno *et al*, 2013), while others contemplated a latent period for cancer and subsequently shifted the diagnosis date backwards in time (Bodmer *et al*, 2012). One of the main assumptions when performing a meta-analysis is the presence of a certain degree of homogeneity in study design across the included studies in order to combine only similar pieces of evidence (Lau *et al*, 1998).

While variability between data sources is recognized as a source of heterogeneity in risk estimates (Bosetti *et al*, 2014; Voss *et al*, 2015), less is known about the influence of choices in study design. Researchers make a series of choices when designing an observational study, determining the study type and definitions of the study population, reference population, and relevant exposure time. However, it has been shown that variation in design choices influences the results, even within the same database (de Vries *et al*, 2006). Previous observational studies regarding cancer risks associated with insulin use also made very different design choices. The variation in the observed risk estimates as a direct result of differences in study design is, however, unknown. This information could be valuable, as it can shed light on the reliability of meta-analyses that combine results from studies with substantial heterogeneity in study design.

The objective of this study was to evaluate the variation in risk estimates that can be attributed to choices in study design. A series of studies with different design choices was conducted within the same data source in order to describe the range of risk estimates for cancer associated with insulin use in type 2 diabetic patients.

## METHODS

### Review of study design choices

A range of study designs was determined by reviewing previous observational studies that assessed the association between the use of hypoglycaemic agents and the occurrence of (a specific type of) cancer. Study designs were categorized based on (a) the selection of the study population (*i.e.* inclusion/exclusion criteria for eligible patients), (b) matching of the reference population, (c) relevant exposure window, and (d) relevant date of cancer occurrence (*i.e.* date of cancer diagnosis or taking into account a certain lag-time between start of disease and the diagnosis thereof). In addition, we determined whether immortal time bias was introduced by the design, as was the case in several studies concerning cancer risk and the use of hypoglycaemic agents (Suissa & Azoulay, 2012). Table 3.1.1 shows the variations in design features among these studies. This range of study design was then used to evaluate the influence variations in study design have on risk estimates within a single data source.

3.1

### Source of data

Data were obtained from the Clinical Practice Research Datalink (CPRD), which comprises electronic medical records from British general practitioners since 1987 (Herrett *et al*, 2015). The accuracy and completeness of CPRD data have been well-validated (Herrett *et al*, 2010; Khan *et al*, 2010). The data from CPRD have been the source for numerous epidemiologic studies, including studies on cancer risk associated with insulin use (Redaniel *et al*, 2012; van Staa *et al*, 2012; Yang *et al*, 2004). Currently, CPRD includes approximately 7% of the total UK population (Herrett *et al*, 2015). The period of valid data collection is dependent on the date at which a practice's data are considered up-to-standard. The protocol of this study was approved by CPRD's Independent Scientific Advisory Committee.

### Study population

The overall study population consisted of permanently registered patients (≥40 years), who received at least one prescription for any hypoglycaemic agent during the period of data collection. Patients with a diagnosis for any type of cancer (excluding non-melanoma skin cancer; NMSC) or type 1 diabetes mellitus prior to the start of follow-up were excluded. Start of data collection was derived from the practice's up-to-standard date or the patient's registration date, whichever date came last. End of data collection was the date of the patient's transfer out of the practice or death, study outcome, or the practice's last data collection, whichever date came first.

A series of case-control studies was conducted nested within four different study cohorts, where both cases and controls were selected from the same cohort. These cohorts were constructed using similar definitions as those used by previous studies: (1) a cohort of users of any hypoglycaemic medication (≥1 prescription recorded), including prevalent and incident users (*i.e.* full study population); (2) exclusive users of hypoglycaemic agents (*i.e.* metformin, sulfonylureas, glitazones, or insulin users) who did not switch between medication types at any time during follow-up; (3) inception cohorts of metformin and (any type of) insulin users (*i.e.* a first ever prescription for metformin or insulin at least one year

after the start of data collection); (4) treatment cohorts as defined by Currie *et al*. (2009). Start of follow-up was defined as the prescription date of the first eligible prescription, according to the cohort definition.

For the inception cohorts (cohort 3), patients starting in the metformin cohort (*i.e.* with a prescription for metformin at baseline) were transferred to the insulin cohort at the time an insulin prescription was recorded. From that date onwards, patient time in the metformin cohort was censored. The cohort following the definition of Currie *et al.* (2009) consisted of separate treatment cohorts of patients newly initiated on monotherapy with either metformin or sulphonylureas (preceded by a wash-in period of at least 6 months), patients newly switched from monotherapy with metformin or sulphonylureas to a regimen with both of these drugs, and patients previously treated with oral hypoglycaemic agents who were newly prescribed (any type of) insulin.



**TABLE 3.1.1.** Variations in study design of previously published studies, when translated to the nested-case control design variations applied.

| **Study designs** | **Case-control** | **Cohort** |
|---|---|---|
| | *Count* | *Count* |
| **Cohorts** | | |
| cohort 1: all users | 10 | 5 |
| cohort 2: exclusive users | 0 | 4 |
| cohort 3: new users | 3 | 11 |
| cohort 4: Currie *et al.** | 0 | 1 |
| **Matching** | | |
| none | 1 | 16 |
| same year | 8 | 2 |
| within 5 years | 4 | 3 |
| **Reset period†** | | |
| none | 11 | 20 |
| 1 year | 1 | 1 |
| 2 years | 1 | 0 |
| **Exposure** | | |
| past 3 months | 1 | 5 |
| past 12 months | 1 | 2 |
| past 24 months | 1 | 0 |
| ever before | 10 | 14 |
| **Immortal time** | | |
| none | 11 | 14 |
| 6 prescriptions | 0 | 5 |
| 12 prescriptions | 2 | 2 |

*Cohort definitions from Currie *et al.* (2009): cohort 1, metformin monotherapy; cohort 2, sulphonylureas monotherapy; cohort 3, metformin and sulfonylureas dual therapy; cohort 4, insulin therapy. †Cancer diagnosis date is shifted backward in time.

## Study outcome

Cases for the nested case-control analyses were patients with a first ever diagnosis of any cancer (except NMSC) during follow-up. In addition, case-control analyses were conducted for specific types of cancer; *i.e.* breast, prostate, colorectal, pancreatic, liver, and lung cancer. The date of diagnosis was used as the index date. On this date, three controls from the same study cohort, without a record of cancer, were matched on gender, year of birth, practice, and calendar time. When no controls could be found within the same practice, controls were selected from other practices. The index date of the controls corresponded to the cancer diagnosis date of the case.



## Variations in case-control study design

In addition to the variation in cohort definitions, we applied various changes in the case-control design, based on the design features identified from previous studies, related to (1) the insulin exposure definition, (2) matching criteria, (3) cancer diagnosis date, and (4) additional inclusion criteria regarding the required number of prescriptions (*i.e.* immortal time). Per study design feature we created a broader range of variations to amplify its effect:

1. The definition of current exposure was varied as at least one prescription for any type of insulin in the past 3, 12, or 24 months, or at any time before the index date.
2. We applied several different levels of tolerance to match controls by year of birth: matching on exact year of birth, within 5 years, and within 10 years.
3. The cancer diagnosis date was shifted backward by a reset period of 0, 1, 2, 3, 4, or 5 years. Here, controls were assigned the same backward shifted index date. Exposure to insulin was assessed on the new index date. If the backward shifted index date was prior to the start of follow-up (*i.e.* date of cohort entry), the matched case-control pair was excluded.
4. We applied additional inclusion criterion regarding number of required prescriptions; the study population was restricted to patients with at least 1, 6, 12, 18 or 24 prescriptions for hypoglycaemic agents, while both cases and controls were selected from the start date of data collection. These design variations inherently introduce immortal time in the study cohort (Suissa & Azoulay, 2012). A nested case-control design is said to adequately deal with immortal time bias (Levesque *et al*, 2010).

## Statistical analyses

In all models, conditional logistic regression was used to estimate odds ratios (ORs) for cancer risk associated with current insulin use, with metformin use as the reference category. Each case-control matching (with its inclusion, exclusion, and matching criteria) was repeated 10 times. Subsequently, non-parametric bootstrapping techniques were used to estimate 95% confidence intervals (CI) of the OR (*i.e.* the 2.5 and 97.5 percentile) for each study design. All models were adjusted for use of hypoglycaemic agents other than insulin or metformin by including a single variable indicating exposure (yes/no). Exposure to other hypoglycaemic agents was defined similarly to current insulin exposure.

Baseline tables were created to summarize basic characteristics of the patient population in each study cohort. Boxplots were used to describe the full range of (bootstrapped) ORs found per cancer type. We assessed the range of ORs per design feature, stratified by cancer type. In addition, boxplots were created stratified by cohort and design feature. Lastly, we selected those designs that have been used in several past studies

**TABLE 3.1.2.** Baseline patient characteristics and number of cancer cases by anatomical subsite during follow-up per study cohort.

| | | 1: All BGLD users (n=232,952) | | 2: Exclusive users (n=130,401) | | 3: Inception cohorts | | | | 4: Currie *et al.* (n=122,303) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Metformin (n=121,579) | | Insulin (n=22,936) | | | |
| **Age** | Mean (std) | 63.5 | (12.5) | 65.4 | (13.2) | 62.1 | (11.6) | 63.8 | (12.6) | 64.6 | (12.7) |
| | Median | 63 | | 66 | | 62 | | 65 | | 65 | |
| **Male** | | 129,858 | (55.7) | 70,706 | (54.2) | 68,113 | (56.0) | 12,874 | (56.1) | 67,039 | (54.8) |
| **Follow-up** | Mean (std) | 5.1 | (4.2) | 3.6 | (3.3) | 3.2 | (2.9) | 5.3 | (4.2) | 4.0 | (3.4) |
| | Median | 4.1 | | 2.6 | | 2.5 | | 4.3 | | 3.0 | |
| **BGLD history*** | Metformin | 0 | (0.0) | 9,475 | (7.3) | 0 | (0.0) | 13,849 | (60.4) | 35,054 | (28.7) |
| | Sulfonylureas | 0 | (0.0) | 11,056 | (8.5) | 0 | (0.0) | 16,757 | (73.1) | 36,203 | (29.6) |
| | Insulins | 0 | (0.0) | 2,705 | (2.1) | 4,428 | (3.6) | 0 | (0.0) | 0 | (0.0) |
| **BGLD baseline†** | Metformin | 159,232 | (68.4) | 84,207 | (64.6) | 121,579 | (100.0) | 290 | (1.3) | 64,323 | (52.6) |
| | Sulfonylureas | 65,476 | (28.1) | 25,837 | (19.8) | 0 | (0.0) | 96 | (0.4) | 13,807 | (11.3) |
| | Glitazones | 4,598 | (2.0) | 460 | (0.4) | 329 | (0.3) | 10 | (0.0) | 8 | (0.0) |
| | Insulins | 21,260 | (9.1) | 19,897 | (15.3) | 222 | (0.2) | 22,936 | (100.0) | 37,704 | (30.8) |
| **Cancer cases** | Any‡ | 17,437 | (7.5) | 8,675 | (6.7) | 8,410 | (6.9) | 2,663 | (11.6) | 10,167 | (8.3) |
| | Breast | 1,753 | (0.8) | 852 | (0.7) | 915 | (0.8) | 267 | (1.2) | 1,010 | (0.8) |
| | Prostate | 2,061 | (0.9) | 932 | (0.7) | 1,011 | (0.8) | 279 | (1.2) | 1,104 | (0.9) |
| | Colorectal | 2,104 | (0.9) | 1,036 | (0.8) | 1,040 | (0.9) | 301 | (1.3) | 1,208 | (1.0) |
| | Lung | 2,207 | (0.9) | 1,206 | (0.9) | 1,044 | (0.9) | 301 | (1.3) | 1,328 | (1.1) |
| | Pancreatic | 957 | (0.4) | 407 | (0.3) | 416 | (0.3) | 221 | (1.0) | 653 | (0.5) |
| | Liver | 335 | (0.1) | 165 | (0.1) | 140 | (0.1) | 70 | (0.3) | 211 | (0.2) |

*Abbreviations:* BGLD, blood glucose lowering drugs; std, standard deviation. *Any prescription prior to baseline. †Type of prescription at the start of follow-up. ‡Any malignant neoplasm, except non-melanoma skin cancer.

regarding cancer risk associated with insulin use. Although no exact replications were performed, an overview is provided of the variety in risk estimates that can be found with different study designs, similar to those used in prior studies, within the same data set.

All data management and statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). All graphs were made with Microsoft Excel 2013 (Microsoft, WA, USA).

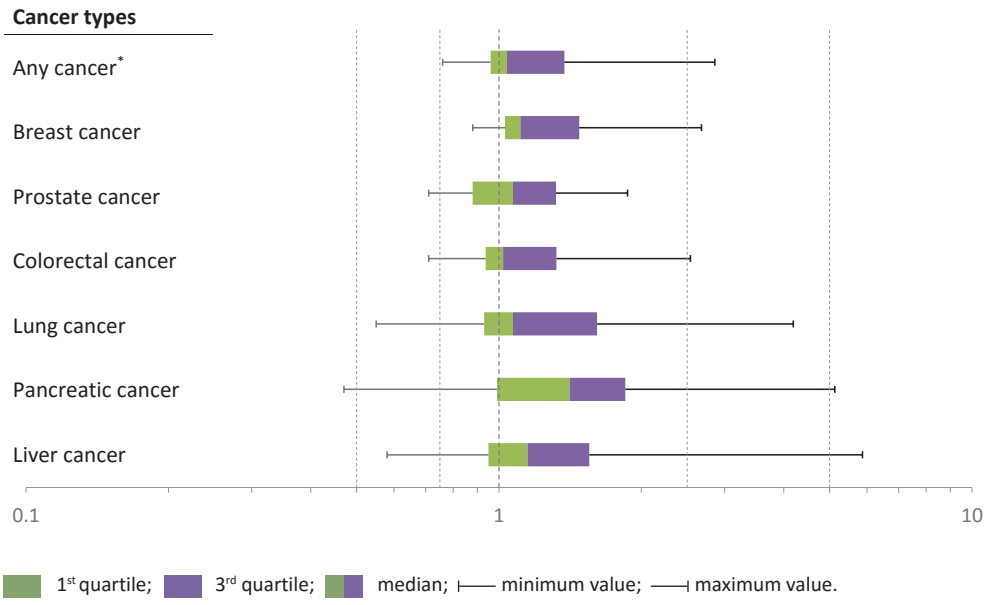


## RESULTS

Table 3.1.2 shows the baseline characteristics of patients by study cohort. In total, 232,952 users of hypoglycaemic agents were included in the full study population. The characteristics of the study population and the number of cancer cases differed between the study cohorts.

In total, 1,440 different design combinations were applied. Figure 3.1.1 shows boxplots that indicate the range of the point estimates for cancer risk associated with insulin use in case-control studies that applied these different designs. For any cancer, the effect of insulin use ranged from a protective effect (OR 0.76, 95% CI 0.71-0.83) to a near 3-fold increased risk (OR 2.86, 95% CI 2.60-3.16). The range of ORs increased for the less common cancer types. Especially the risk of pancreatic cancer associated with insulin use varied widely, from a minimum of 0.47 (95% CI 0.25-0.89) to a maximum of 5.13 (95% CI 3.23-8.13).

**FIGURE 3.1.1.** Boxplots showing the distribution of bootstrapped point estimates for cancer risk associated with insulin use derived from all variations in nested case-control studies conducted, per cancer type.




*Any cancer type, except non-melanoma skin cancer.

The range of ORs stratified by study design feature and cancer type is displayed in Figure 3.1.2. Exposure time window definitions had minimal effect on the range of ORs produced. The variability in ORs was high between study cohorts and differed among cancer types. Furthermore, a variable reset period (*i.e.* the backward shifting of the cancer diagnosis date) had an effect on the risk estimates for certain cancer outcomes, in particular pancreatic and liver cancer. For pancreatic cancer, the median OR observed on the index date was 2.20 (95% CI 1.67-3.38), as compared to 1.06 (95% CI 0.51-2.48) when the cancer diagnosis date was shifted back by 5 years. The same trend was observed for liver cancer. Immortal time incorporated in the underlying study cohort did not affect the risk estimates from the nested case-control series. Variations in matching by age did not markedly affect the range in ORs.



Figure 3.1.3 provides boxplots for ORs for any cancer (other than NMSC) per study cohort and variation of study design feature. The range of ORs was higher in the inception cohort (cohort 3) as compared to the other cohorts. Furthermore, the most notable effect of the implementation of a reset period was observed in the inception cohort. Conversely, a reset period appeared to have the opposite effect in cohorts that were constructed according to the definitions by Currie *et al.* (2009). When looking at the individual cancer types, the trends observed with variable reset periods were strongest for lung, prostate, and pancreatic cancer (see Figure 3.1.A, Appendices). Inclusion of immortal time did not result in variations in the effect estimates in any of the study cohorts for any type of cancer (Figure 3.1.3) or for any specific type of cancer [data not shown]. Neither age matching criteria nor variations in exposure time window did markedly affect the range in ORs (Figure 3.1.3 and Figure 3.1.A, Appendices).

In Figure 3.1.4, the risk estimates for any type of cancer associated with insulin use are displayed using similar designs as those used in previously published studies. A large heterogeneity in ORs was observed for the study designs previously used, ranging from a significant risk reduction (OR 0.89, 95% CI, 0.84-0.95) to a nearly 3-fold increased risk (OR 2.76, 95% CI 2.51-3.03). These patterns were fairly consistent for the specific cancer subtypes, with the exception of pancreatic cancer, where almost all studies found significantly increased risks (see Figure 3.1.B, Appendices).

## DISCUSSION

Our analyses showed that simple choices in study design can cause a huge variation in risk estimates within the same database. The risk estimates for any type of cancer associated with insulin use varied from a protective effect to a harmful effect, depending on the choices made in study design. Great heterogeneity in risk estimates was observed among nested case-control studies with designs comparable to those used to determine cancer risk associated with insulin use in previous studies.

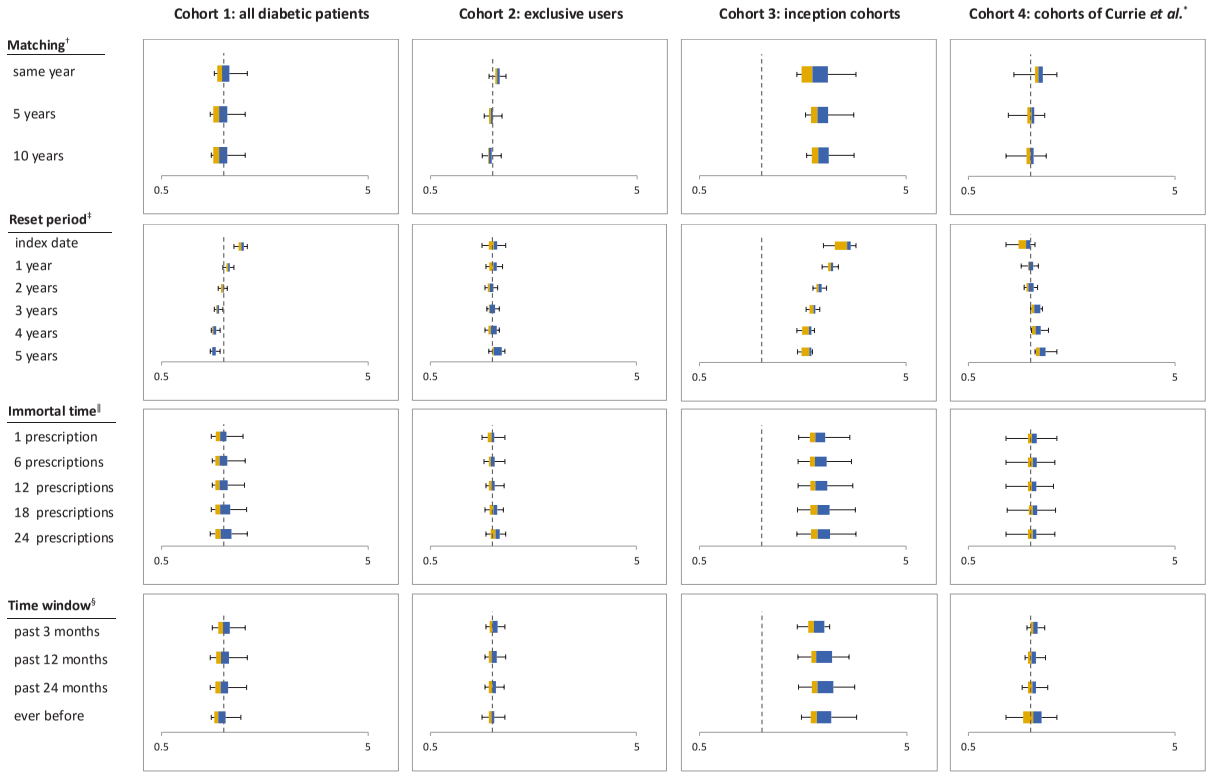
Numerous past observational studies have evaluated the risk of cancer associated with insulin use among type 2 diabetic patients (Bosetti *et al*, 2014; Bu *et al*, 2014; Chen *et al*, 2013; Colmers *et al*, 2012; Janghorbani *et al*, 2012; Karlstad *et al*, 2013; Nie *et al*, 2014; Singh *et al*, 2013a; Singh *et al*, 2013b; Singh *et al*, 2013c; Sun *et al*, 2014; Wang *et al*, 2013a; Wang *et al*, 2013b; Wu *et al*, 2015; Yin *et al*, 2014). Although these studies often

**FIGURE 3.1.2.** Boxplots showing the distribution of bootstrapped point estimates for cancer risk associated with insulin use derived from all variations in nested case-control studies conducted, stratified by study design feature and cancer type.


1st quartile; 3rd quartile; median; minimum value; maximum value.

*Exposure definition time window at the cancer diagnosis date. †Cohort definitions by Curry *et al*. (2009). ‡Reset period, cancer diagnosis date (*i.e*. index date) is shifted backward in time. §Additional inclusion criteria, related to number of insulin prescriptions. ‖Age matching of cases and controls.

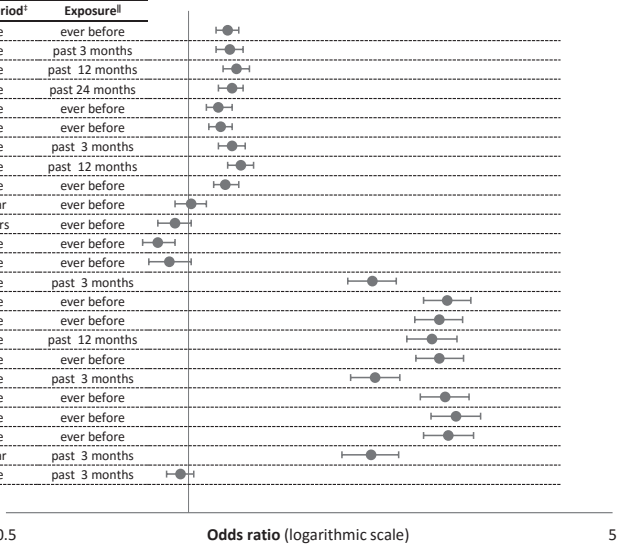
**FIGURE 3.1.3.** Boxplots showing the distribution of bootstrapped point estimates for the risk of any cancer (except non-melanoma skin cancer) associated with insulin use derived from all variations in nested case-control studies conducted, stratified by study cohort and study design feature.




*Cohort definitions by Curry *et al.* (2009). †Age matching of cases and controls. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. ‖Additional inclusion criteria, related to number of insulin prescriptions. §Exposure definition time window at the cancer diagnosis date.

**FIGURE 3.1.4.** Odds ratios and 95% confidence intervals for the risk of any cancer (except non-melanoma skin cancer) associated with insulin use determined by nested case-control analysis, applying different study designs previously used by others.

| Cohort | Matching* | Immortal time† | Reset period‡ | Exposure‖ |
|---|---|---|---|---|
| 1: all ADD users | same year | none | none | ever before |
| 1: all ADD users | 5 years | none | none | past 3 months |
| 1: all ADD users | 5 years | none | none | past 12 months |
| 1: all ADD users | 5 years | none | none | past 24 months |
| 1: all ADD users | 5 years | none | none | ever before |
| 1: all ADD users | 10 years | none | none | ever before |
| 1: all ADD users | 10 years | 6 prescriptions | none | past 3 months |
| 1: all ADD users | 10 years | 6 prescriptions | none | past 12 months |
| 1: all ADD users | 5 years | 12 prescriptions | none | ever before |
| 1: all ADD users | same year | none | 1 year | ever before |
| 1: all ADD users | same year | none | 2 years | ever before |
| 2: excl. users | 10 years | none | none | ever before |
| 2: excl. users | 10 years | 12 prescriptions | none | ever before |
| 3: incept. cohorts | same year | none | none | past 3 months |
| 3: incept. cohorts | same year | none | none | ever before |
| 3: incept. cohorts | 5 years | none | none | ever before |
| 3: incept. cohorts | 10 years | none | none | past 12 months |
| 3: incept. cohorts | 10 years | none | none | ever before |
| 3: incept. cohorts | 10 years | 6 prescriptions | none | past 3 months |
| 3: incept. cohorts | 10 years | 6 prescriptions | none | ever before |
| 3: incept. cohorts | same year | 12 prescriptions | none | ever before |
| 3: incept. cohorts | 10 years | 12 prescriptions | none | ever before |
| 3: incept. cohorts | 10 years | none | 1 year | past 3 months |
| 4: Currie *et al.*§ | 10 years | 6 prescriptions | none | past 3 months |




*Abbreviations:* ADD, antidiabetic drugs; excl., exclusive; incept., inception.
● point estimate, odds ratio; ⊢⊣ 95%-confidence interval. *Age matching of cases and controls. †Additional inclusion criteria, related to number of insulin prescriptions. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. Exposure definition time window at the cancer diagnosis date. §Cohort definitions by Curry *et al.* (2009).

reported a similar aim and seemingly tested the same hypothesis, a great deal of variety in the applied study designs exists. We replicated these design choices and found that they were associated with major heterogeneity in risk estimates within the same study population. Variability in adjustment for confounding and data quality between studies is often mentioned as a source of heterogeneity in meta-analyses of observational studies (Bosetti *et al*, 2014, Higgins & Green, 2011). However, our findings indicate that study design choices other than adjustment for confounding factors can also have profound effects. This raises questions with regard to the justification of meta-analyses without taking detailed study design choices into account.

Originally, meta-analyses were used to pool data from randomized trials and are, in that form, still considered to provide the highest level of evidence (Higgins & Green, 2011). Aggregation of data from independent studies increases study power, thereby enhancing the precision and accuracy of the pooled result (Naylor, 1997). However, this methodology presumes that observed differences between studies are primarily caused by chance. In experimental research, replication of a study has become common practice. Also within the field of genetic epidemiology, replication of a study in an independent sample is highly recommended in the case of 'hypothesis-free testing' (Cooper *et al*, 2002). In both instances, chance findings are considered probable.

Here, an important distinction between randomized controlled trials on the one hand and non-randomized observational studies on the other becomes apparent. In non-randomized studies, the validity is usually not doubted because of the fear of chance events. Instead, results from observational research (testing *a priori* hypotheses) is doubted due to a fear of potential bias and confounding (Vandenbroucke, 2008). Consequently, some researchers even advocate against simple replication of non-randomized studies, as it would also replicate the same problems. Vandenbroucke (2008) therefore recommends the use of different study designs and different methodologies to address potential flaws in prior studies. However, this recommendation directly affects our ability to perform meta-analyses of observational studies. In fact, when differences arise due to variations in study design, meaningful heterogeneity between studies is ignored when providing a pooled estimate (Naylor, 1997).



Rather than performing a meta-analysis, within non-randomized observational research it may be preferable to focus on robustness of observed associations; *i.e.* to evaluate and test the impact of variation in methods and design choices. Evaluation of robustness of a previously reported association provides insights into its behavior under different circumstances. In this perspective, also a test of heterogeneity – an elemental part of a meta-analysis – is not very useful, as it would only tell us if the reported association is robust or not. Ultimately, the interpretation of results from non-randomized studies has to take place in light of the various circumstances; *i.e.* the used study design. Often, it requires an in-depth evaluation of the design choices made to determine what specific hypothesized causal relationship was tested in an individual study and in what context. Moreover, we need to reason about the abilities and inabilities of individual designs to stringently test this underlying hypothesis.

An evaluation of prior observational studies within the same broad area of interest (*e.g.* insulin use and cancer risk) is a common starting point for all researchers in epidemiology. Researchers then usually design a different study to determine whether new empirical evidence is in line with the hypothesized causal relationship (*i.e.* whether the association behaves in a way that is predicted) or test alternative explanations for the results presented by others. A particularly convincing strategy is to perform a replication of the previous study (with an identical design) followed by an alternative design, to allow for direct comparison of the results. For example, Suissa & Azoulay (2011) replicated multiple studies with immortal time bias and showed that adjustment for immortal time significantly altered the results. Also, van Staa *et al*. (2012) provided evidence of reverse causation (*i.e.* protopathic bias) influencing the association between insulin use and cancer risk by using multiple study designs to determine patterns of risk. Similarly, in our study, insulin use was associated with an increased risk of pancreatic cancer when measured at the time of diagnosis. However, when the diagnosis date was shifted backwards in time, the excess risk disappeared. Unfortunately, replication of a prior study often proves impossible based on the information provided in the research dissemination (Ranopa *et al*, 2015).

When observational research is used to test a hypothesized potential healthcare risk, a logical first step would be to determine the most suitable study design. When designing an observational study, the hypothesized overall causal scheme should be scrutinized to logically deduce testable and specific conjectures (Savitz, 2003). Since no meaningful study can be performed without a hypothesized causal mechanism - we would simply not know

where to look - each new observational study with a different design provides a new piece of a complex puzzle. This process, where different studies within the same area of interest are performed, can be interpreted as 'dossier building'. In this respect, meta-analyses are not the right tool to provide an overview of the progress made in a certain 'dossier'. Instead, a systematic review might be more appropriate, where the progress within a 'dossier' can be presented through a discussion of individual studies, placed in their relevant context. This process could be greatly facilitated when researchers formulate a specific and detailed study aim and motivate their choices in study design.



Several limitations of this study should be mentioned. Firstly, this study was not set out to assess the risk of cancer associated with insulin use. We also did not aim to give a recommendation with regard to the 'best design' when studying cancer risks and insulin use. Secondly, we applied a broad range in study design variations. Not all the study variations may be equally likely to be used by researchers. However, all study design features included here were in fact varied in primary analyses of previous studies. Thirdly, we did not aim to fully replicate any of the previous studies (we did not have access to code lists and programs). Therefore, we were not able to directly compare our findings with those reported by others. Lastly, our results with regard to immortal time bias indicate that a nested case-control design adequately adjusts for this type of time-related bias, as has been described previously (Suissa & Azoulay, 2012). However, this does not mean that previous cohort studies were not affected by immortal time bias.

This study shows that choices made by researchers when designing an observational study can greatly influence the observed risk estimate. Our results call for acknowledgement of the influence and the implications of the use of different study designs when performing meta-analyses and clearly question the justification of combining results from studies with different designs to provide an aggregate risk estimate. To prevent unfounded pooling of results, researchers should more clearly state the context of their study by formulating specific and detailed study aims and motivate their choices in study design. Moreover, researchers should more closely work together to facilitate progress within a certain research area and allow future studies to improve upon previous ones. For that reason, study protocols, code lists, and programs should be published online.

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APPENDICES

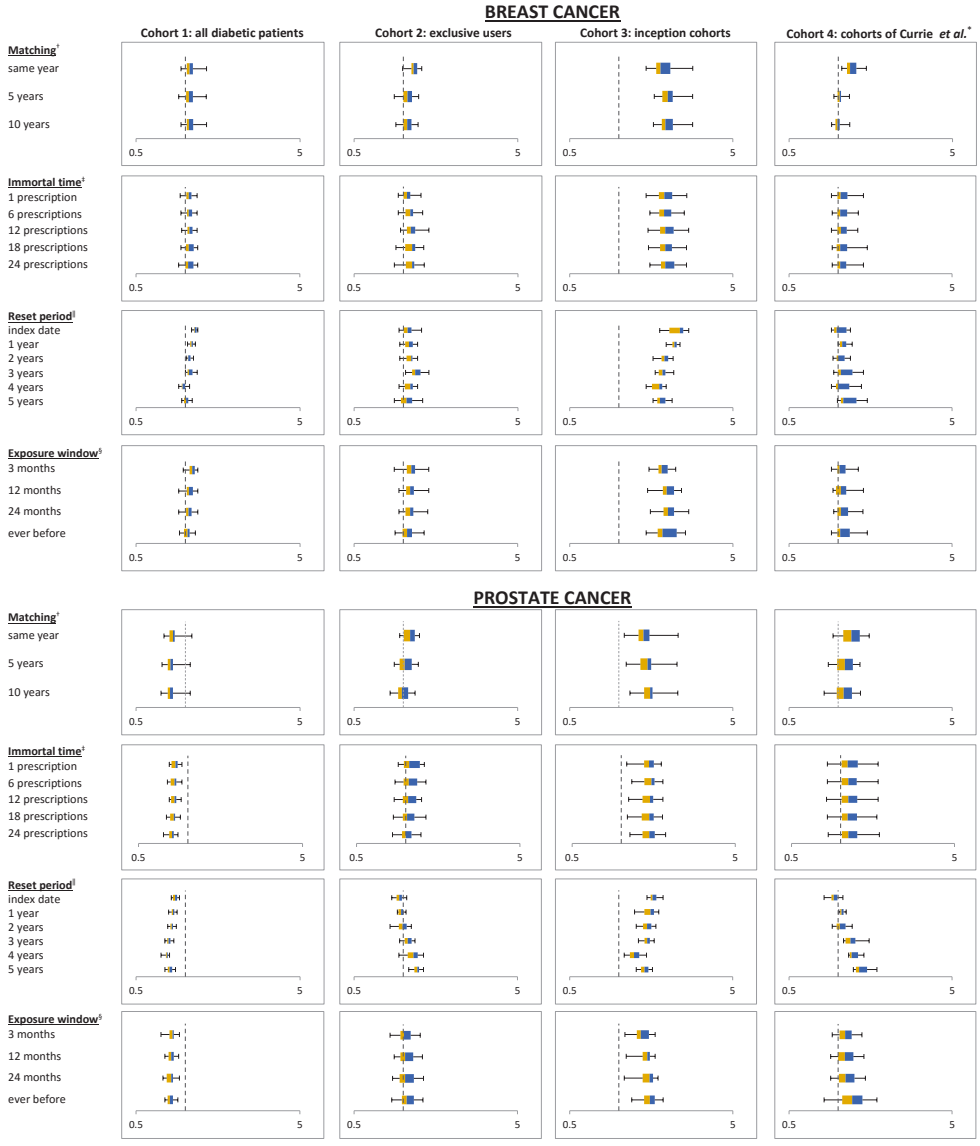
**FIGURE 3.1.A.** Boxplots showing the distribution of bootstrapped point estimates for the risk of cancer associated with insulin use derived from all variations in nested case-control studies conducted, stratified by study cohort, study design feature, and cancer subtype.


*Abbreviations:* BGLD, blood glucose lowering drugs; excl. excluding; incept, inception.

1st quartile; 3rd quartile; median; ⊢ minimum value; ⊣ maximum value.

*Cohort definitions by Curry *et al*. (2009). †Age matching of cases and controls. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. Exposure definition time window at the cancer diagnosis date.

**FIGURE 3.1.A.** *Continued*

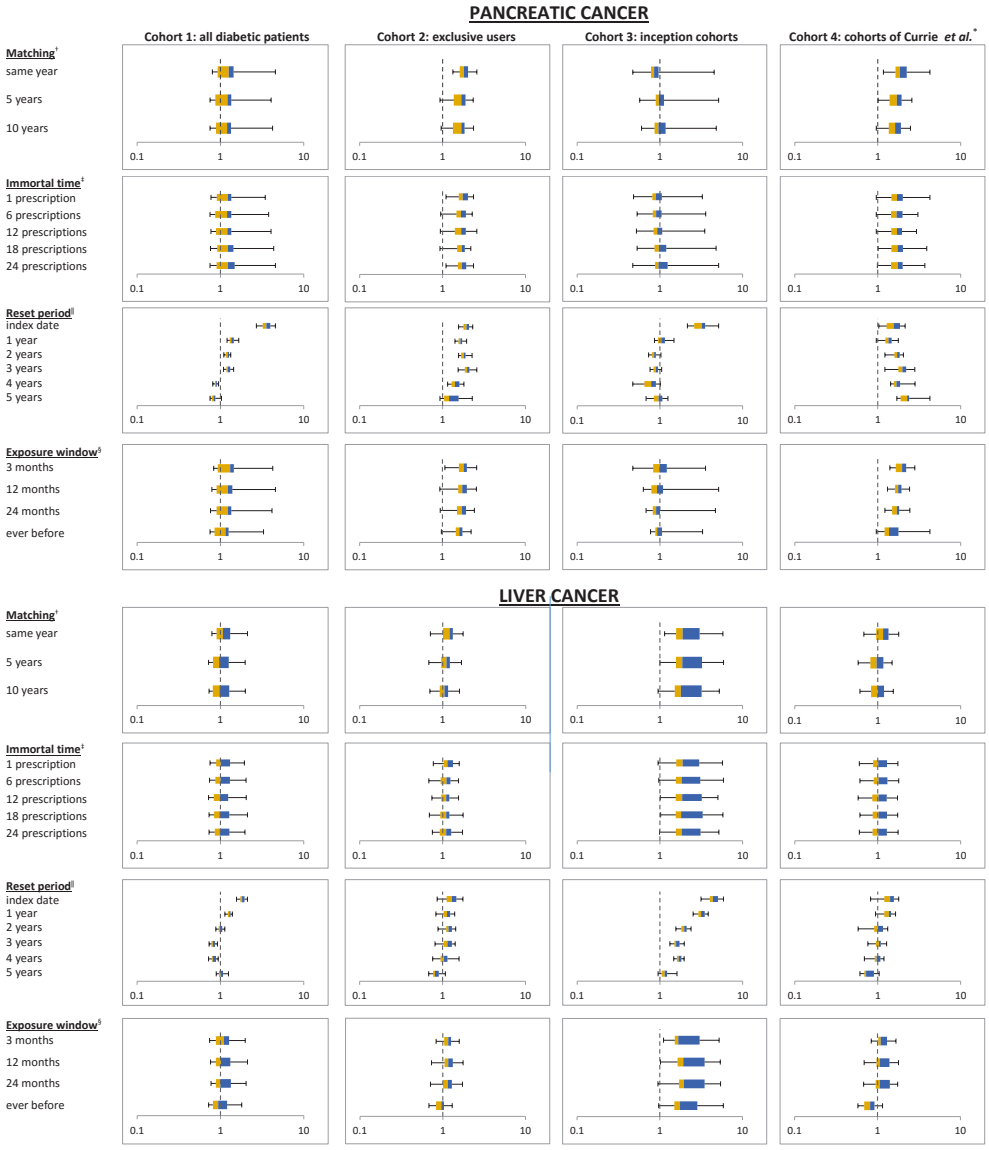



*Abbreviations:* BGLD, blood glucose lowering drugs; excl. excluding; incept, inception.

1st quartile; 3rd quartile; median; ⊢ minimum value; ⊣ maximum value.

*Cohort definitions by Curry *et al*. (2009). †Age matching of cases and controls. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. Exposure definition time window at the cancer diagnosis date.

**FIGURE 3.1.A.** *Continued*




*Abbreviations:* BGLD, blood glucose lowering drugs; excl. excluding; incept, inception.

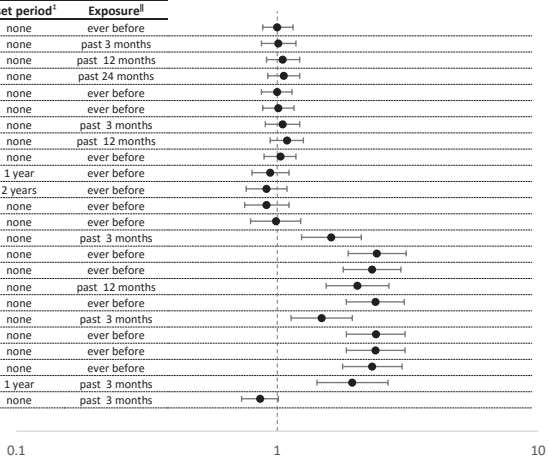
1st quartile; 3rd quartile; median; minimum value; maximum value.

*Cohort definitions by Curry *et al*. (2009). †Age matching of cases and controls. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. Exposure definition time window at the cancer diagnosis date.

**FIGURE 3.1.B.** Odds ratios and 95% confidence intervals for the risk of cancer associated with insulin use determined by nested case-control analysis, applying different study designs previously used by others, stratified by cancer subtype.

## COLORECTAL CANCER

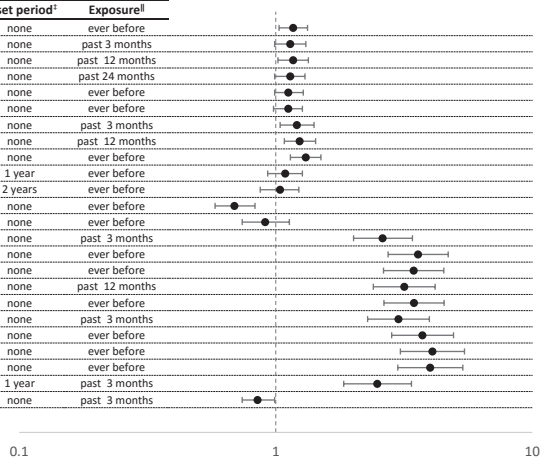
| Cohort | Matching* | Immortal time† | Reset period‡ | Exposure‖ |
|---|---|---|---|---|
| 1: all users | same year | none | none | ever before |
| 1: all users | 5 years | none | none | past 3 months |
| 1: all users | 5 years | none | none | past 12 months |
| 1: all users | 5 years | none | none | past 24 months |
| 1: all users | 5 years | none | none | ever before |
| 1: all users | 10 years | none | none | ever before |
| 1: all users | 10 years | 6 prescriptions | none | past 3 months |
| 1: all users | 10 years | 6 prescriptions | none | past 12 months |
| 1: all users | 5 years | 12 prescriptions | none | ever before |
| 1: all users | same year | none | 1 year | ever before |
| 1: all users | same year | none | 2 years | ever before |
| 2: excl. users | 10 years | none | none | ever before |
| 2: excl. users | 10 years | 12 prescriptions | none | ever before |
| 3: inception | same year | none | none | past 3 months |
| 3: inception | same year | none | none | ever before |
| 3: inception | 5 years | none | none | ever before |
| 3: inception | 10 years | none | none | past 12 months |
| 3: inception | 10 years | none | none | ever before |
| 3: inception | 10 years | 6 prescriptions | none | past 3 months |
| 3: inception | 10 years | 6 prescriptions | none | ever before |
| 3: inception | same year | 12 prescriptions | none | ever before |
| 3: inception | 10 years | 12 prescriptions | none | ever before |
| 3: inception | 10 years | none | 1 year | past 3 months |
| 4: Currie *et al.*§ | 10 years | 6 prescriptions | none | past 3 months |




*Abbreviations:* ADD, antidiabetic drugs; excl., exclusive; incept., inception.
● point estimate, odds ratio; ⊢⊣ 95%-confidence interval. *Age matching of cases and controls. †Additional inclusion criteria, related to number of insulin prescriptions. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. ‖Exposure definition time window at the cancer diagnosis date. §Cohort definitions by Curry *et al*. (2009).

## LUNG CANCER

| Cohort | Matching* | Immortal time† | Reset period‡ | Exposure‖ |
|---|---|---|---|---|
| 1: all users | same year | none | none | ever before |
| 1: all users | 5 years | none | none | past 3 months |
| 1: all users | 5 years | none | none | past 12 months |
| 1: all users | 5 years | none | none | past 24 months |
| 1: all users | 5 years | none | none | ever before |
| 1: all users | 10 years | none | none | ever before |
| 1: all users | 10 years | 6 prescriptions | none | past 3 months |
| 1: all users | 10 years | 6 prescriptions | none | past 12 months |
| 1: all users | 5 years | 12 prescriptions | none | ever before |
| 1: all users | same year | none | 1 year | ever before |
| 1: all users | same year | none | 2 years | ever before |
| 2: excl. users | 10 years | none | none | ever before |
| 2: excl. users | 10 years | 12 prescriptions | none | ever before |
| 3: inception | same year | none | none | past 3 months |
| 3: inception | same year | none | none | ever before |
| 3: inception | 5 years | none | none | ever before |
| 3: inception | 10 years | none | none | past 12 months |
| 3: inception | 10 years | none | none | ever before |
| 3: inception | 10 years | 6 prescriptions | none | past 3 months |
| 3: inception | 10 years | 6 prescriptions | none | ever before |
| 3: inception | same year | 12 prescriptions | none | ever before |
| 3: inception | 10 years | 12 prescriptions | none | ever before |
| 3: inception | 10 years | none | 1 year | past 3 months |
| 4: Currie *et al.*§ | 10 years | 6 prescriptions | none | past 3 months |

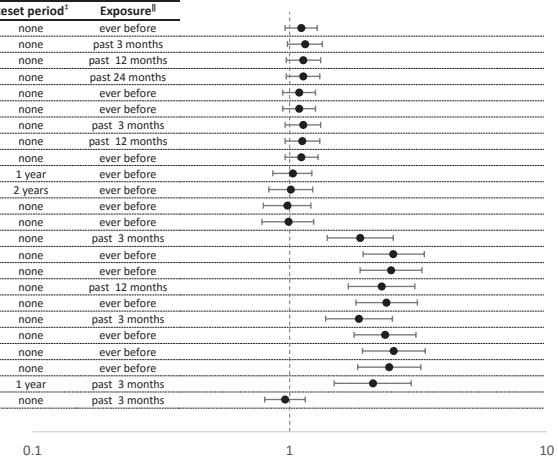



*Abbreviations:* ADD, antidiabetic drugs; excl., exclusive; incept., inception.
● point estimate, odds ratio; ⊢⊣ 95%-confidence interval. *Age matching of cases and controls. †Additional inclusion criteria, related to number of insulin prescriptions. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. ‖Exposure definition time window at the cancer diagnosis date. §Cohort definitions by Curry *et al*. (2009).

**FIGURE 3.1.B.** *Continued*

## BREAST CANCER

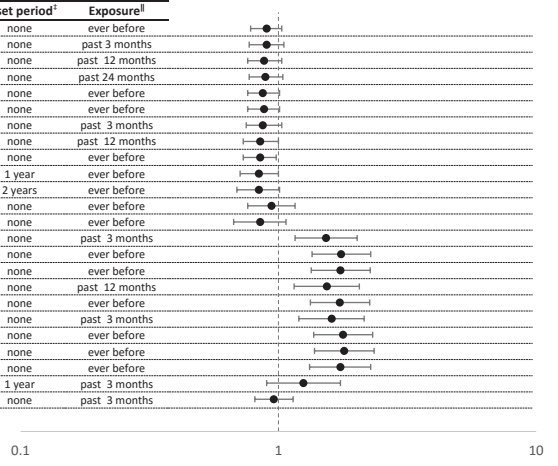
| Cohort | Matching* | Immortal time† | Reset period‡ | Exposure‖ |
|---|---|---|---|---|
| 1: all users | same year | none | none | ever before |
| 1: all users | 5 years | none | none | past 3 months |
| 1: all users | 5 years | none | none | past 12 months |
| 1: all users | 5 years | none | none | past 24 months |
| 1: all users | 5 years | none | none | ever before |
| 1: all users | 10 years | none | none | ever before |
| 1: all users | 10 years | 6 prescriptions | none | past 3 months |
| 1: all users | 10 years | 6 prescriptions | none | past 12 months |
| 1: all users | 5 years | 12 prescriptions | none | ever before |
| 1: all users | same year | none | 1 year | ever before |
| 1: all users | same year | none | 2 years | ever before |
| 2: excl. users | 10 years | none | none | ever before |
| 2: excl. users | 10 years | 12 prescriptions | none | ever before |
| 3: inception | same year | none | none | past 3 months |
| 3: inception | same year | none | none | ever before |
| 3: inception | 5 years | none | none | ever before |
| 3: inception | 10 years | none | none | past 12 months |
| 3: inception | 10 years | none | none | ever before |
| 3: inception | 10 years | 6 prescriptions | none | past 3 months |
| 3: inception | 10 years | 6 prescriptions | none | ever before |
| 3: inception | same year | 12 prescriptions | none | ever before |
| 3: inception | 10 years | 12 prescriptions | none | ever before |
| 3: inception | 10 years | none | 1 year | past 3 months |
| 4: Currie *et al.*§ | 10 years | 6 prescriptions | none | past 3 months |




*Abbreviations:* ADD, antidiabetic drugs; excl., exclusive; incept., inception.
● point estimate, odds ratio; ⊢⊣ 95%-confidence interval. *Age matching of cases and controls. †Additional inclusion criteria, related to number of insulin prescriptions. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. ‖Exposure definition time window at the cancer diagnosis date. §Cohort definitions by Curry *et al*. (2009).

## PROSTATE CANCER

| Cohort | Matching* | Immortal time† | Reset period‡ | Exposure‖ |
|---|---|---|---|---|
| 1: all users | same year | none | none | ever before |
| 1: all users | 5 years | none | none | past 3 months |
| 1: all users | 5 years | none | none | past 12 months |
| 1: all users | 5 years | none | none | past 24 months |
| 1: all users | 5 years | none | none | ever before |
| 1: all users | 10 years | none | none | ever before |
| 1: all users | 10 years | 6 prescriptions | none | past 3 months |
| 1: all users | 10 years | 6 prescriptions | none | past 12 months |
| 1: all users | 5 years | 12 prescriptions | none | ever before |
| 1: all users | same year | none | 1 year | ever before |
| 1: all users | same year | none | 2 years | ever before |
| 2: excl. users | 10 years | none | none | ever before |
| 2: excl. users | 10 years | 12 prescriptions | none | ever before |
| 3: inception | same year | none | none | past 3 months |
| 3: inception | same year | none | none | ever before |
| 3: inception | 5 years | none | none | ever before |
| 3: inception | 10 years | none | none | past 12 months |
| 3: inception | 10 years | none | none | ever before |
| 3: inception | 10 years | 6 prescriptions | none | past 3 months |
| 3: inception | 10 years | 6 prescriptions | none | ever before |
| 3: inception | same year | 12 prescriptions | none | ever before |
| 3: inception | 10 years | 12 prescriptions | none | ever before |
| 3: inception | 10 years | none | 1 year | past 3 months |
| 4: Currie *et al.*§ | 10 years | 6 prescriptions | none | past 3 months |



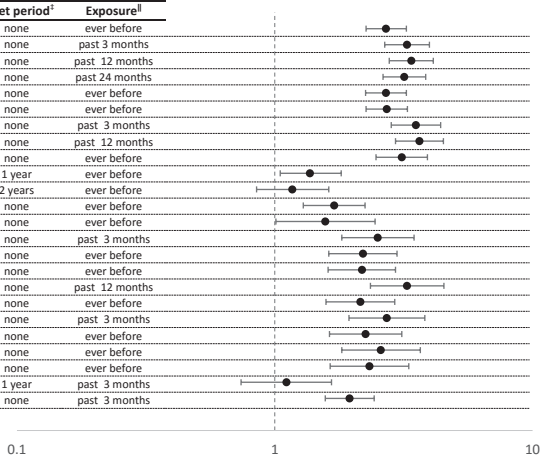

*Abbreviations:* ADD, antidiabetic drugs; excl., exclusive; incept., inception.
● point estimate, odds ratio; ⊢⊣ 95%-confidence interval. *Age matching of cases and controls. †Additional inclusion criteria, related to number of insulin prescriptions. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. ‖Exposure definition time window at the cancer diagnosis date. §Cohort definitions by Curry *et al*. (2009).

**FIGURE 3.1.B.** *Continued*



## PANCREATIC CANCER

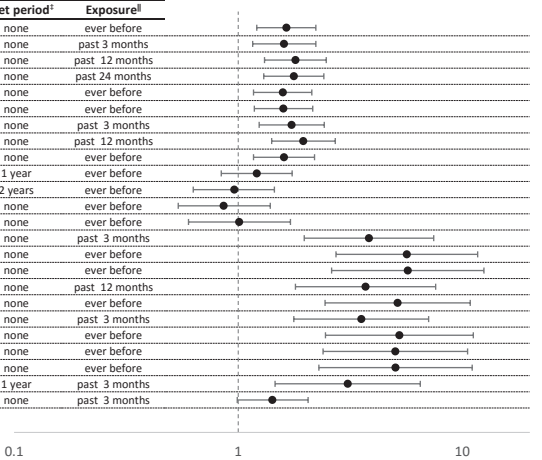
| Cohort | Matching* | Immortal time† | Reset period‡ | Exposure‖ |
|---|---|---|---|---|
| 1: all users | same year | none | none | ever before |
| 1: all users | 5 years | none | none | past 3 months |
| 1: all users | 5 years | none | none | past 12 months |
| 1: all users | 5 years | none | none | past 24 months |
| 1: all users | 5 years | none | none | ever before |
| 1: all users | 10 years | none | none | ever before |
| 1: all users | 10 years | 6 prescriptions | none | past 3 months |
| 1: all users | 10 years | 6 prescriptions | none | past 12 months |
| 1: all users | 5 years | 12 prescriptions | none | ever before |
| 1: all users | same year | none | 1 year | ever before |
| 1: all users | same year | none | 2 years | ever before |
| 2: excl. users | 10 years | none | none | ever before |
| 2: excl. users | 10 years | 12 prescriptions | none | ever before |
| 3: inception | same year | none | none | past 3 months |
| 3: inception | same year | none | none | ever before |
| 3: inception | 5 years | none | none | ever before |
| 3: inception | 10 years | none | none | past 12 months |
| 3: inception | 10 years | none | none | ever before |
| 3: inception | 10 years | 6 prescriptions | none | past 3 months |
| 3: inception | 10 years | 6 prescriptions | none | ever before |
| 3: inception | same year | 12 prescriptions | none | ever before |
| 3: inception | 10 years | 12 prescriptions | none | ever before |
| 3: inception | 10 years | none | 1 year | past 3 months |
| 4: Currie *et al.*§ | 10 years | 6 prescriptions | none | past 3 months |




*Abbreviations:* ADD, antidiabetic drugs; excl., exclusive; incept., inception.
● point estimate, odds ratio; ⊢⊣ 95%-confidence interval. *Age matching of cases and controls. †Additional inclusion criteria, related to number of insulin prescriptions. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. ‖Exposure definition time window at the cancer diagnosis date. §Cohort definitions by Curry *et al.* (2009).

## LIVER CANCER

| Cohort | Matching* | Immortal time† | Reset period‡ | Exposure‖ |
|---|---|---|---|---|
| 1: all users | same year | none | none | ever before |
| 1: all users | 5 years | none | none | past 3 months |
| 1: all users | 5 years | none | none | past 12 months |
| 1: all users | 5 years | none | none | past 24 months |
| 1: all users | 5 years | none | none | ever before |
| 1: all users | 10 years | none | none | ever before |
| 1: all users | 10 years | 6 prescriptions | none | past 3 months |
| 1: all users | 10 years | 6 prescriptions | none | past 12 months |
| 1: all users | 5 years | 12 prescriptions | none | ever before |
| 1: all users | same year | none | 1 year | ever before |
| 1: all users | same year | none | 2 years | ever before |
| 2: excl. users | 10 years | none | none | ever before |
| 2: excl. users | 10 years | 12 prescriptions | none | ever before |
| 3: inception | same year | none | none | past 3 months |
| 3: inception | same year | none | none | ever before |
| 3: inception | 5 years | none | none | ever before |
| 3: inception | 10 years | none | none | past 12 months |
| 3: inception | 10 years | none | none | ever before |
| 3: inception | 10 years | 6 prescriptions | none | past 3 months |
| 3: inception | 10 years | 6 prescriptions | none | ever before |
| 3: inception | same year | 12 prescriptions | none | ever before |
| 3: inception | 10 years | 12 prescriptions | none | ever before |
| 3: inception | 10 years | none | 1 year | past 3 months |
| 4: Currie *et al.*§ | 10 years | 6 prescriptions | none | past 3 months |




*Abbreviations:* ADD, antidiabetic drugs; excl., exclusive; incept., inception.
● point estimate, odds ratio; ⊢⊣ 95%-confidence interval. *Age matching of cases and controls. †Additional inclusion criteria, related to number of insulin prescriptions. ‡Reset period, cancer diagnosis date (*i.e.* index date) is shifted backward in time. ‖Exposure definition time window at the cancer diagnosis date. §Cohort definitions by Curry *et al*. (2009).

# CHAPTER 4

# TRENDS IN RISK ESTIMATES WITH CUMULATIVE EXPOSURE

## CHAPTER 4.1

# Use of metformin and survival of type 2 diabetic women with breast cancer

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## SUMMARY

### Background
This study was set out to determine whether metformin use influences survival in breast cancer patients treated with hypoglycaemic agents as compared to non-users.

### Methods
We used data from the Danish national registries (1996-2008) to identify adult female patients diagnosed with breast cancer who were prescribed hypoglycaemic medication. We performed multivariate Cox-proportional hazard regression to assess all-cause and breast cancer-specific mortality risks associated with metformin exposure. In a secondary analysis, we stratified use of metformin according to the cumulative number of prescriptions.

### Results
Of the 1,058 breast cancer patients 349 died during follow-up, with breast cancer listed as the primary cause of death for 152 cases. Compared to non-use, current metformin treatment was associated with a significant reduction in overall mortality (adjusted HR 0.74, 95% CI 0.58-0.96). For breast cancer-specific mortality, a non-significant risk reduction (adjusted HR 0.88, 95% CI 0.59-1.29) was observed, which became significant after stratification according to cumulative number of prescriptions. An increased risk of both overall and breast cancer-specific mortality was observed in the first 12 months after discontinuation of metformin.

### Conclusion
We observed a non-significant reduction in breast cancer-specific mortality associated with metformin exposure among breast cancer patients treated with hypoglycaemic agents. However, our findings suggest that long-term metformin use may have a beneficial effect on survival in patients with breast cancer. Further confirmation of these findings is needed.

## INTRODUCTION

Breast cancer patients with type 2 diabetes mellitus have a higher mortality risk as compared to their non-diabetic counterparts (Currie *et al*, 2012; Peairs *et al*, 2011; Redaniel *et al*, 2012; Renehan *et al*, 2012). Much discussion has recently focused on variations in mortality risk with specific types of hypoglycaemic medication. Metformin use has been associated with an improved cancer prognosis in observational studies (Bowker *et al*, 2010; Currie *et al*, 2012; Landman *et al*, 2010) and results from preclinical studies suggest that metformin reduces the growth of breast cancer cells (Alimova *et al*, 2009; Dowling *et al*, 2007; Zakikhani *et al*, 2006). As a relatively safe and inexpensive drug, metformin currently receives much attention as a potential adjuvant to standard breast cancer treatment.



Metformin could decrease breast cancer cell growth, either indirectly by reducing circulating insulin – with known mitogenic effects on breast cancer cell progression *in vitro* (Chappell *et al*, 2001) – or directly via activation of adenosine mono-phosphate kinase (Alimova *et al*, 2009; Dowling *et al*, 2007; Zakikhani *et al*, 2006; Zakikhani *et al*, 2010). Evidence of reduced tumor cell proliferation from clinical trials, however, is inconclusive (Bonanni *et al*, 2012; Hadad *et al*, 2011; Niraula *et al*, 2012). Moreover, due to the small numbers of patients, short follow-up periods, and use of surrogate endpoints, findings from clinical trials cannot easily be translated to daily practice.

In addition, results from observational studies on breast cancer survival in diabetic patients are conflicting. Although some, fairly small studies reported a beneficial effect of metformin use on survival (He *et al*, 2011; Jiralerspong *et al*, 2009), a recent large and well-designed study failed to show a significant reduction in breast cancer mortality in patients treated with metformin (Lega *et al*, 2013). However, the latter did report on a potential effect associated with cumulative duration of exposure, with a possible 9% reduction (HR=0.91, 95% CI 0.81-1.03) in breast-cancer specific mortality per additional year of cumulative use (Lega *et al*, 2013). In agreement with these findings, we hypothesized that duration of exposure should be taken into account when evaluating the potential inhibitory effect of metformin use.

The objective of this study was to determine the relationship between metformin use and all-cause and breast-cancer specific mortality in a cohort of patients diagnosed with breast cancer and treated with hypoglycaemic agents and to assess whether this association is dependent upon cumulative duration of exposure.

## METHODS

### Data source and population

Data for this study were obtained from nationwide health care registries in Denmark, which include hospital admission records, drug prescriptions, and causes of death for all inhabitants. Linkage of these computerized data is enabled through the use of a personal identification number. Data regarding migration and dates of birth and death are kept by The Ministry of the Interior. All hospital admission records from 1977 onwards are accessible through The National Hospital Discharge Register, which also holds all outpatient

visits to hospitals, clinics, and emergency rooms since 1995. The validity of registrations in the database is high and captures almost 100% of contacts (Mosbech *et al*, 1995). The National Pharmacological Database includes records of all prescription drugs dispensed by pharmacies from 1996 onwards (Kildemoes *et al*, 2011), including the type of medication (by ATC code) and dispensing dates (Pedersen, 2011).



For this study, a cohort was defined from all female patients (aged 18+ years) receiving treatment for diabetes mellitus who had a first ever diagnostic code for breast cancer (ICD-10 code C50) between 1997 and 2007 (Bazelier *et al*, 2012). Patients were required to have received at least two prescriptions for a non-insulin antidiabetic drug (NIAD) between 1996 and 2007, of which at least one was dispensed within the year prior to the breast cancer diagnosis. Patients were followed from the moment of breast cancer diagnosis onwards. All patients were required to have a minimum of one year of prescription data available prior to the start of follow-up. Patients with a diagnosis of cancer (except non-melanoma skin cancer) before the start of follow-up were excluded (n=191). Given the potential mitogenic effect of insulin (Osborne *et al*, 1976), patients were censored at the time of their first ever insulin prescription (ATC code A10A). Similarly, patients receiving insulin treatment before the start of follow-up were excluded (n=520). In a sensitivity analysis - adjusted for insulin use in a time-dependent manner, with insulin use defined as a prescription for insulin within the past 6 months - we tested the effect of censoring at the time of insulin treatment initiation on the outcome measure. In addition, patients receiving biguanide agents other than metformin (*i.e.* phenformin, buformin) were excluded (n=2).

### Drug exposure

Exposure to metformin was assessed both before and after breast cancer diagnosis. At the start of follow-up (breast cancer diagnosis), baseline metformin exposure was assessed as the number of prescriptions (ATC-code A10BA) in the year preceding the diagnosis. During follow-up, the cumulative number of metformin prescriptions was updated and assessed as a time-dependent variable.

For the time-dependent exposure measurement, time since breast cancer diagnosis was divided into 5-day intervals for each patient and exposure status was updated at the start of each (5-day) interval. Current exposure to metformin was defined as a prescription within 3 months prior to the start of an interval. Recent, past, and distant users received their last dispensing in respectively the 3–6 months, 6–12 months or >1 year before the start of an interval. These categories were mutually exclusive. A patient without a prescription for metformin ever before the start of an interval was considered a 'never user' until the time a metformin prescription was filled. To assess cumulative exposure, current use of metformin was stratified according to the total number of prescriptions since 1 year before the index date up to that point. Due to left-truncated data (prescription data was available from 1996 onwards), a proportion of the 'distant users' may have been misclassified as 'never users'. A sensitivity analysis was performed in which distant use of metformin was relabeled as never use.

### Follow-up and outcome

Participants were followed from the date of breast cancer diagnosis till the end of data collection (31 December 2007), emigration, the use of an insulin prescription, or the patient's death, whichever came first. Data on causes of death were deducted from the death certificate register. If breast cancer was listed as the primary cause of death, the outcome was labeled as a breast cancer-related death. All other deaths were labeled as breast cancer-unrelated. A sensitivity analysis was performed with breast cancer-unrelated deaths as the study outcome.



### Other covariates

Information regarding *a priori* risk factors for breast cancer prognosis was collected and incorporated in the analysis. The presence of risk factors was updated at the start of each 5-day interval and analyzed as time-dependent covariates. Age was included as a continuous variable. Other potential confounders included the Charlson Comorbidity Index - based on a history of chronic diseases, including amongst others cerebrovascular disease, congestive heart failure, and ischemic heart disease (Charlson *et al*, 1987) - and the use of concomitant medication: *i.e.* sulfonamides (A10BB and A10BC), thiazolidinediones (A10BG), other glucose-lowering agents (A10BF, alfa-glucoside inhibitors; A10BH, dipeptidyl peptidase 4 inhibitors), statins (C10AA), and hormone replacement therapy (G03FB). Exposure to comedication was defined as a prescription within the past 6 months. To adjust for variations in breast cancer treatment over time, the number of years between the start of the study period (January 1, 1997) and the date of breast cancer diagnosis was included as a continuous variable.

### Statistical analysis

Patient characteristics and risk factors for breast cancer prognosis available in the dataset were compared between patients treated with metformin at the time of breast cancer diagnosis and those receiving other non-insulin hypoglycaemic medications. Cox-proportional hazards models were used to evaluate the effect of current, past, and distant past exposure to metformin versus never use on all-cause and breast cancer-specific mortality. In a secondary analysis, current use of metformin was differentiated according to the cumulative number of prescriptions since 1 year before the index date. The association between metformin exposure and the study outcome was adjusted for all specified potential confounders in a time-dependent manner. All statistical analyses were performed with SAS (version 9.2).

## RESULTS

### Study population

The study cohort consisted of 1,058 subjects who were diagnosed with breast cancer and were dispensed at least one prescription for an hypoglycaemic agent in the year prior to the diagnosis, with a total follow-up of 2,971 person years. Table 4.1.1 shows the baseline characteristics of patients treated with metformin within the year prior to the breast cancer diagnoses compared to those who were not treated with metformin. Subjects treated with

metformin were younger and more likely to receive concomitant statin treatment. Patients not receiving metformin were primarily treated with sulfonylurea monotherapy. The cumulative number of metformin prescriptions within a year before breast cancer diagnosis varied between 1 and 27. Overall comorbidity was comparable; besides complications resulting from type 2 diabetes mellitus, the most common comorbidities in both groups were cerebrovascular disease, congestive heart failure, and ischemic heart disease. Insulin treatment was started earlier in patients not treated with metformin, resulting in a 51% reduction in follow-up time after censoring. For patients treated with metformin, follow-up time was reduced by 31%.



In total, 349 patients (33.0%) died within the study period. Of those, cancer was listed as the primary cause of death for 172 cases (49.3%) and for the vast majority of cancer deaths, patients died of breast cancer (n=152, 88.4%). After breast cancer, the most common cause of death was cardiovascular disease (n=74, 21.2%). Forty-four (12.6%) death certificate records did not specify cause of death.

### Effect of metformin use on all-cause mortality

As shown in Table 4.1.2, current use of metformin was associated with a significant reduction in all-cause mortality (adjusted HR 0.74, 95% CI, 0.58-0.96). Differentiation according to the number of prescription revealed that the reduction was most profound for the categories with the highest cumulative exposure. However, the differences between prescription categories failed to reach statistical significance for any direct comparison. Conversely, recent and past use were both associated with a significantly increased overall mortality risk, while no difference was observed between never use and distant use of metformin.

### Effect of metformin use on breast cancer-specific mortality

Table 4.1.3 shows a non-significant risk reduction in breast cancer-specific mortality was observed in association with current use of metformin as compared to non-use (adjusted HR 0.88, 95% CI 0.59-1.29). Differentiation according to the number of prescriptions showed a noticeable fluctuation in risk: while the lowest two categories were associated with a non-significant increased breast cancer mortality (adjusted HR 1.39, 95% CI 0.73-2.65 and adjusted HR 1.29, 95% CI 0.76-2.21, respectively), a decrease in risk was observed for a cumulative number of prescriptions between 21 and 30 and for the highest category (adjusted HR 0.20, 95% CI 0.05-0.84 and 0.38, 95% CI 0.13-1.09, respectively). Moreover, significant differences in breast cancer mortality risk were observed between the lower and higher exposure categories. The increased risk associated with recent and past use was more pronounced for breast cancer mortality than observed in the analysis on all-cause mortality. When the last metformin prescription was filled over 1 year ago, the risk was similar to patients never exposed to metformin.

### Sensitivity analyses

A sensitivity analysis with breast cancer-unrelated deaths (n=197) as the study outcome revealed that current metformin use was associated with a reduced mortality risk (adjusted HR 0.66, 95% CI 0.47-0.93). A non-significant increased risk was observed for recent and past use of metformin (adjusted HR 1.16, 95% CI 0.53-2.54 and adjusted HR

**TABLE 4.1.1.** Baseline characteristics of diabetic women with breast cancer, by metformin exposure in the year prior to diagnosis.

| Characteristic | Metformin (n=508) | | Non-metformin (n=550) | |
|---|---|---|---|---|
| **Follow-up (yrs)** | 1280.5 | | 1690.2 | |
| Median (IQR) | 1.8 | (0.8-3.8) | 2.6 | (0.9-4.4) |
| **Age at breast cancer diagnosis** | | | | |
| Median (IQR) | 68 | (60-76) | 76 | (67-82)* |
| By category (%) | | | | |
| <50 | 23 | (4.5) | 9 | (1.6)** |
| 50-60 | 94 | (18.5) | 41 | (7.5)** |
| 60-70 | 165 | (32.5) | 122 | (22.2)** |
| 70-80 | 152 | (29.9) | 189 | (34.4) |
| 80-90 | 70 | (13.8) | 160 | (29.1)** |
| 90+ | 4 | (0.8) | 29 | (5.3)** |
| **Year of breast cancer diagnosis** | | | | |
| <2000 | 75 | (14.8) | 179 | (32.5)** |
| 2000-2005 | 279 | (54.9) | 293 | (53.3) |
| >2005 | 154 | (30.3) | 78 | (14.2)** |
| **Charlson comorbidity index** | | | | |
| Median (IQR) | 3 | (3-4) | 3 | (3-5)* |
| By category (%) | | | | |
| 3 | 307 | (60.4) | 294 | (53.5)** |
| 4-5 | 139 | (27.4) | 184 | (33.5)** |
| 6-8 | 46 | (9.1) | 57 | (10.4) |
| ≥9 | 16 | (3.2) | 15 | (2.7) |
| **Medication in previous year** | | | | |
| Metformin | 508 | (100.0) | 0 | - |
| By number of $R_x$ | | | | |
| Median (IQR) | 6 | (4-8) | 0 | - |
| 1-5 | 219 | (43.1) | - | - |
| 6-10 | 216 | (42.5) | - | - |
| > 10 | 73 | (14.4) | - | - |
| SU-derivates | 288 | (56.7) | 541 | (98.4)** |
| Thiazolidinediones | 11 | (2.2) | 6 | (1.1) |
| Other NIAD† | 17 | (3.4) | 23 | (4.2) |
| HRT | 85 | (16.7) | 106 | (19.3) |
| Statins | 197 | (38.8) | 115 | (20.9)** |

*Abbreviations:* IQR, interquartile range; NIAD, non-insulin antidiabetic drug, HRT, hormone replacement therapy. *Statistically significant difference ($p$<0.05), based on Mann-Whitney-Wilcoxon test. **Statistically significant difference ($p$<0.05) between metformin and non-metformin groups, based on Chi-square test. †glucagon-like peptide-1 agonists, alpha-glucosidase inhibitors, meglitinides, and dipeptidyl peptidase 4-inhibitors.

1.59, 95% CI 0.73-3.45). Stratification according to the cumulative number of prescriptions showed a protective effect associated with all prescription categories for mortality by causes other than breast cancer, with no significant differences between categories. In addition, relabeling of distant use as never use of metformin did not affect the results as they are presented in Table 4.1.2 and 4.1.3 in any significant way. Likewise, the results of the sensitivity analysis regarding the effect of censoring at the time of insulin treatment initiation showed similar results [data not shown].

4.1

## DISCUSSION

In this study we found that current metformin use was associated with a significant reduction in overall mortality (adjusted HR 0.74, 95% CI 0.58-0.96), but not in breast cancer-specific mortality (adjusted HR 0.88, 95% CI 0.59-1.29). After stratification according to the cumulative number of prescriptions, the categories with the highest cumulative metformin use appeared to be associated with lower breast cancer mortality. Unexpectedly, a significant increase in both overall and breast cancer-specific mortality was observed between 3 and 12 months after the last metformin prescription.

The present findings are consistent with the results of a recent population-based study by Lega *et al*. (2013). Although their findings did not reach statistical significance, they reported a possible 9%-reduction in breast cancer-specific mortality per additional year of cumulative metformin use, suggesting the beneficial effect of metformin use may be dependent on duration of treatment. In agreement with this hypothesis, we observed an inverse relationship between the cumulative number of prescriptions and breast cancer-specific mortality, where the highest cumulative use appeared to be associated with a reduced risk. Moreover, our results coincide with regard to the specificity of this duration response effect; like Lega *et al* (2013), the effect of cumulative duration of use in our study was most pronounced for breast-cancer specific mortality.

A comparison with the results from previous observational studies concerning the effect of metformin use on survival in breast cancer patients is hindered by several limitations. In two relatively small observational studies, concerning specific breast cancer subtypes, metformin use was associated with improved breast cancer-specific survival (He *et al*, 2011) and with a lower risk of distant metastases (Bayraktar *et al*, 2012). However, exposure to hypoglycaemic agents in these two studies was defined by use at the time of diagnosis or by any use during follow-up, potentially introducing immortal time bias (Suissa & Azoulay, 2012).

Results from preclinical studies suggest that metformin may decrease breast cancer cell growth, either by reducing circulating insulin or through direct activation of AMPK (Alimova *et al*, 2009; Dowling *et al*, 2007; Zakikhani *et al*, 2006; Zakikhani *et al*, 2010). Small randomized controlled clinical trials indicate that metformin treatment causes significant reductions in surrogate endpoints, such as Ki-67 levels (Hadad *et al*, 2011; Niraula *et al*, 2012). Furthermore, the effect appeared to be modified by insulin resistance status (Bonanni *et al*, 2012; Hadad *et al*, 2011; Niraula *et al*, 2012), indicating that metformin may affect breast cancer prognosis mainly by lowering circulating insulin levels (Bonanni *et al*, 2012; Niraula *et al*,

**TABLE 4.1.2.** All-cause mortality associated with use of metformin in female diabetic breast cancer patients.

| | Incidence rate | | | Hazard ratios | | | |
|---|---|---|---|---|---|---|---|
| | *Events* | *py* | *IR* | *Age-adj.* | *(95% CI)* | *Fully adj.** | *(95% CI)* |
| **Never†** | 176 | 1,150.1 | (153.0) | 1 | Reference | 1 | Reference |
| **Current‡** | 112 | 1,457.0 | (76.9) | 0.72 | (0.56-0.93) | 0.74 | (0.58-0.96) |
| Number of prescriptions§ | | | | | | | |
| 1 to 5 | 19 | 169.3 | (112.2) | 0.99 | (0.62-1.60) | 1.09 | (0.67-1.76) |
| 6 to 10 | 26 | 263.1 | (98.8) | 0.90 | (0.59-1.37) | 0.86 | (0.56-1.31) |
| 11 to 20 | 31 | 420.8 | (73.7) | 0.71 | (0.48-1.06) | 0.68 | (0.46-1.02) |
| 21 to 30 | 14 | 243.2 | (57.6) | 0.56 | (0.32-0.97) | 0.55 | (0.31-0.96) |
| >30 | 22 | 360.5 | (61.0) | 0.55 | (0.35-0.87) | 0.64 | (0.40-1.05) |
| **Recent‖** | 18 | 86.1 | (209.1) | 1.91 | (1.17-3.13) | 1.75 | (1.07-2.88)** |
| **Past¶** | 14 | 56.4 | (248.2) | 1.96 | (1.13-3.39) | 1.85 | (1.07-3.21)** |
| **Distant#** | 29 | 221.0 | (131.2) | 0.99 | (0.67-1.47) | 0.98 | (0.65-1.46) |

*Abbreviations:* HR, hazard ratio; py, person years; IR, incidence rate in events per 1,000 person years; adj., adjusted; CI, confidence interval; ref., reference category. *Adjusted for all potential confounders (age, Charlson Comorbidity Index, number of years between January 1, 1997 and the date of breast cancer diagnosis, and use of concomitant medication during follow-up: metformin, sulfonylureas, thiazolidinediones, other hypoglycaemic agents, hormone replacement therapy, and statins in the past 6 months). †No prior recorded prescription for metformin. ‡Metformin prescription in past 3 months. §Total number of prescriptions for metformin since 1 year before breast cancer diagnosis. ‖Last metformin prescription within past 3 to 6 months. ¶Last metformin prescription within past 6 to 12 months. #Last metformin prescription more than 1 year ago. **Statistically significant difference ($p$<0.05) with current use of metformin, based on Wald test.

**TABLE 4.1.3.** Breast cancer-specific mortality associated with use of metformin in female diabetic breast cancer patients.

| | Incidence rate | | | Hazard ratios | | | |
|---|---|---|---|---|---|---|---|
| | *Events* | *py* | *IR* | *Age adj.* | *(95% CI)* | *Fully adj.** | *(95% CI)* |
| **Never**† | 75 | 1150.1 | (65.2) | 1 | Reference | 1 | Reference |
| **Current**‡ | 51 | 1457.0 | (35.0) | 0.83 | (0.57-1.22) | 0.88 | (0.59-1.29) |
| Number of prescriptions§ | | | | | | | |
| 1 to 5 | 11 | 169.3 | (65.0) | 1.32 | (0.69-2.49) | 1.39 | (0.73-2.65) |
| 6 to 10 | 18 | 263.1 | (68.4) | 1.44 | (0.85-2.45) | 1.29 | (0.76-2.21) |
| 11 to 20 | 16 | 420.8 | (38.1) | 0.92 | (0.53-1.61) | 0.87 | (0.49-1.53) |
| 21 to 30 | 2 | 243.2 | (8.2) | 0.20 | (0.05-0.81) | 0.20 | (0.05-0.84)** |
| >30 | 4 | 360.5 | (11.1) | 0.28 | (0.10-0.77) | 0.38 | (0.13-1.09)** |
| **Recent**‖ | 11 | 86.1 | (127.8) | 2.84 | (1.49-5.41) | 2.58 | (1.35-4.96)†† |
| **Past**¶ | 7 | 56.4 | (124.1) | 2.42 | (1.10-5.30) | 2.23 | (1.01-4.91)†† |
| **Distant**# | 8 | 221.0 | (36.2) | 0.72 | (0.34-1.50) | 0.79 | (0.38-1.67) |

*Abbreviations:* HR, hazard ratio; py, person years; IR, incidence rate in events per 1,000 person years; adj., adjusted; CI, confidence interval; ref., reference category. *Adjusted for all potential confounders (age, Charlson Comorbidity Index, number of years between January 1, 1997 and the date of breast cancer diagnosis, and use of concomitant medication during follow-up: sulfonylureas, thiazolidinediones, other hypoglycaemic agents, hormone replacement therapy, and statins in the past 6 months). †No prior recorded prescription for metformin. ‡Metformin prescription in past 3 months. §Total number of prescriptions for metformin since 1 year before breast cancer diagnosis. ‖Last metformin prescription within past 3 to 6 months. ¶Last metformin prescription within past 6 to 12 months. #Last metformin prescription more than 1 year ago. **Statistically significant difference ($p$<0.05) with 1 to 5 and 6 to 10 prescriptions for metformin, based on Wald test. ††Statistically significant difference ($p$<0.05) with current and distant past use of metformin, based on Wald test.

2012). Insulin has known mitogenic effects on mammary tissue and breast cancer cells *in vitro* (Chappell *et al*, 2001).

Nevertheless, our findings are not fully in line with the suggested mechanism of action. Patients receiving treatment with metformin in the year preceding the breast cancer diagnosis were significantly younger at the time of diagnosis. If metformin slows cell growth, age at diagnosis would expectedly be higher in patients treated with metformin. However, the inverse relationship between breast cancer mortality and the cumulative number of prescriptions suggests that the improved survival associated with metformin use is not simply caused by selection bias. This notion is supported by the observation that the relative risk of all-cause and breast cancer-unrelated mortality did not show significant differences between prescription categories. Based on these findings, we cannot rule out nor confirm that the significant protective effect for all-cause mortality is the result of selection bias, while the reduction in breast cancer-specific mortality for the upper categories of cumulative use is a causal effect of metformin exposure. However, the specificity of the observed duration-response relationship for breast cancer-specific mortality provides an indication that our findings did not result from a fundamental flaw in the study design. Moreover, as we used Cox proportional hazard analyses, the duration of follow-up is held constant within the comparison between categories of cumulative number of prescriptions.



We can only speculate on an explanation for the increase in breast cancer-specific mortality observed between 3 and 12 months after the last metformin prescription. Drawing from postulated theories regarding metformin's actions, no harmful effects of discontinuation can be expected. More peculiar, the pattern of increased mortality risk after metformin was discontinued seems to be restricted to breast cancer-specific mortality. An explanation for this finding could entail a reduction in medication burden or increased weight loss in late-stage cancer patients, leading to discontinuation of (some) hypoglycaemic medications. Metformin treatment may also be stopped out of caution to prevent lactic acidosis. In addition, hospital admission (or admission to a palliative care unit) of end-stage cancer patients may lead to non-observable prescription data. Lastly, active cancer may destabilize glucose metabolism, causing switches in hypoglycaemic medications that could result in protopathic bias. Unfortunately, we did not have sufficient information to test any of these alternative explanations.

Our study is subject to several limitations that should be taken into consideration when interpreting these findings. First of all, we did not perform a competing risk analysis. However, as metformin use was associated with a significant reduction in breast cancer-unrelated mortality, patients currently treated with metformin had a lower risk of being censored due to a competing risk. If anything, this may have biased our results towards an increased breast cancer-specific mortality associated with metformin use. As metformin users were younger at time of diagnosis, they may have had a better initial prognosis. However, since all analyses were adjusted for age at diagnosis, the consequential bias towards a protective effect of metformin should be minimal. Furthermore, the current cohort incorporated mainly postmenopausal women and our findings are only relevant to this particular patient population. Moreover, due to the definition of the inclusion criteria, the cohort comprised only individuals treated with NIADs who, if not treated with metformin, had to be treated with another NIAD. Consequently, confounding by indication may have influenced our results. Since metformin is a commonly used first-line drug, this

may have biased our results towards a protective effect (Suissa & Azoulay, 2012). However, an exaggerated protective effect stemming from this type of bias would not be influenced by cumulative use. Furthermore, cumulative number of prescriptions is a crude measure of duration of use, since time between prescriptions can vary between individuals. Lastly, we were unable to adjust for several potential confounders (*e.g.* tumor staging at the time of diagnosis, cancer treatment, body mass-index, smoking, social deprivation, menopause status), since these data were not available. However, a recent study found no difference in tumor stage or nuclear grade between diabetic patients treated with metformin and those treated with other hypoglycaemic agents (Jiralerspong *et al*, 2009). Based on these findings, we also do not expect cancer treatment to vary by concomitant use of specific NIADs.



Strengths of this study comprise the large number of patients included and the use of high quality data, which were objectively gathered from the population. By using pharmacy prescription data, we were able to assess metformin use as a time-dependent variable which prevents immortal time bias. In addition, by restricting the study population to patients treated with oral hypoglycaemic agents, we attempted to select relatively comparable patients with respect to disease burden. Moreover, data from death certificates allowed for differentiation between breast cancer deaths and deaths by other causes. Further, while overall mortality is expected to be susceptible to confounding by indication (*i.e.* metformin use may be a proxy for overall better health), cancer specific-mortality may be more robust. Lastly, our study incorporated a measure of duration of exposure (measured by number of prescriptions) to assess any duration-response effect.

In summary, our study provides further evidence that the duration of use is relevant when evaluating the clinical effect of metformin exposure in breast cancer treatment. However, unexpected findings with regard to an increased mortality after discontinuation of metformin necessitate additional confirmatory studies.

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## CHAPTER 4.2

# Insulin glargine use and breast cancer risk: associations with cumulative exposure

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## SUMMARY

### Background

This study was aimed to assess the risk of breast cancer associated with exposure to insulin glargine in women with type 2 diabetes mellitus and evaluate whether the pattern of risk concurs with the hypothesized trend of an increase in risk with longer duration of use, taking into account previous cumulative exposure to other types of insulin.

4.2

### Methods

We performed a retrospective cohort study (2002-2013) in the Clinical Practice Research Datalink among adult female patients with a first ever insulin prescription (n=12,468). Time-dependent exposure measures were used to assess associations with duration of use of (a) other insulin types before glargine was first prescribed (*i.e.* among switchers) and (b) of glargine during follow-up. Analyses were performed separately for insulin-naïve glargine users and patients switched to glargine. Cox proportional hazards models were used to derive *p*-trends, hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer associated with glargine use.

### Results

During 66,151 person years, 186 breast cancer cases occurred; 76 in glargine users (3.0/1,000 person years) and 110 in users of other insulins (2.7/1,000 person years). Among insulin-naïve women, no association with cumulative exposure to glargine was observed (*p* trend 0.91), even after ≥5 years (HR 1.06, 95% CI 0.48-2.33). Among switchers, a linear trend with years of prior exposure to other insulins was found (*p* trend 0.02). An increased risk was observed in glargine users with extensive (>3 years) past exposure to other insulins (HR 3.17, 95% CI 1.28-7.84). A non-significant trend with cumulative glargine exposure was found among switchers (*p* trend 0.24).

### Conclusion

Exposure to insulin glargine was not associated with an increased breast cancer risk in insulin-naïve patients. Exposure to other insulins prior to the start of glargine appears to be relevant when studying breast cancer risk associated with insulin glargine use.

## INTRODUCTION

In women, type 2 diabetes mellitus is associated with an increased risk of breast cancer (Larsson *et al*, 2007; Tsilidis *et al*, 2015). A number of observational studies emerged in 2009, that linked the use of long-acting insulin glargine to an increased cancer incidence among women with type 2 diabetes mellitus, in particular breast cancer (Colhoun *et al*, 2009; Hemkens *et al*, 2009; Jonasson *et al*, 2009). Since then, treatment with insulin glargine has been studied intensively for its possible association with an increased breast cancer risk (Bronsveld *et al*, 2015; Karlstad *et al*, 2013). However, observational studies among insulin users are complicated by the fact that all insulins to some extent act as a growth stimulating factor (Kurtzhals *et al*, 2000).



Human insulin acts as a growth promoting agent, stimulating breast cancer cell growth and inhibiting apoptosis *in vitro* (Osborne *et al*, 1976). Differences in mitogenic potency between human insulin and insulin analogues on breast cancer cells have been shown in vitro (Kurtzhals *et al*, 2000; Staiger *et al*, 2007). Insulin glargine specifically appears to have an increased mitogenic potency (Kurtzhals *et al*, 2000; Staiger *et al*, 2007), possibly related to its increased affinity for the insulin-like growth factor-1 receptor (Weinstein *et al*, 2009). Overall, results from cell studies indicate a cell growth stimulating effect, rather than a carcinogenic effect (Home, 2013). Consequently, the risk of breast cancer is expected to increase with longer duration of exposure.

However, the majority of epidemiologic studies conducted did not assess trends in breast cancer risk with duration of glargine use. Of the more detailed 'second-generation' studies, only five assessed cancer risk in relation to cumulative exposure (Fagot *et al*, 2013; Habel *et al*, 2013; Lim *et al*, 2014; Sturmer *et al*, 2013; Suissa *et al*, 2011). All of them evaluated duration of glargine use among insulin-naïve users separately and only 1 out of the 5 studies observed a significant increased breast cancer risk associated with high cumulative exposure to glargine (Habel *et al*, 2013). However, all studies lacked sufficient follow-up to robustly estimate cancer risks beyond 3 years of cumulative duration of use (Fagot *et al*, 2013; Habel *et al*, 2013; Lim *et al*, 2014; Sturmer *et al*, 2013; Suissa *et al*, 2011). Therefore, a further investigation into the risk of breast cancer associated with long-term glargine use (>3 years) remains necessary.

Of the two studies that included prevalent insulin users who switched to glargine, one observed an increased breast cancer risk after at least 5 years of cumulative glargine use (Suissa *et al*, 2011). The other did not find an association with duration of use, but was unable to study effects of long-term glargine use due to a median duration of glargine use of 1.2 years (Habel *et al*, 2013). Both studies determined exposure to glargine at baseline (*i.e.* intention-to-treat) and were limited by left-truncated data, which resulted in misclassification of duration of exposure and potential underestimation of past exposure to other insulins prior to the switch to glargine. Moreover, neither study was able to determine how the duration of non-glargine insulin use before cohort entry modifies the effect of glargine use.

The aim of our study was to assess the risk of breast cancer associated with exposure to insulin glargine in women with type 2 diabetes mellitus and evaluate whether the pattern of risk concurs with the hypothesized trend of an increase in risk with longer duration of use, taking into account previous cumulative exposure to other types of insulin.

## METHODS

### Source of data

Data were obtained from the Clinical Practice Research Datalink (CPRD), which comprises electronic medical records from British general practitioners since 1987 (Herrett *et al*, 2015). The accuracy and completeness of CPRD data have been well-validated in previous studies (Herrett *et al*, 2010). Currently, CPRD includes approximately 7% of the total UK population (Herrett *et al*, 2015). The protocol of this study was approved by CPRD's Independent Scientific Advisory Committee.

4.2

### Study population

For this retrospective cohort study, we used a 'new user' design with incident insulin users. All women (≥18 years) with at least one prescription for any type of insulin in CPRD during the inclusion period were eligible. To ensure a minimal follow-up period of approximately three years between cohort entry and the end of data collection (1 October 2013), the inclusion period stretched from 1 September 2002 - the marketing date for glargine in the United Kingdom - to 31 December 2010. The index date was defined as the date of the first recorded prescription for any type of insulin within the inclusion period. On the index date, all subjects were required to have at least 1 year of up-to-standard patient history in CPRD without any recorded history of insulin use to improve the validity of the 'new user' design.

Patients considered to have type 1 diabetes mellitus were excluded. These were patients without any use of non-insulin antidiabetic drugs (NIADs) in the year before cohort entry who (a) had a recorded diagnosis for type 1 diabetes mellitus on or prior to the index date, or (b) were under 30 years of age on the index date. Subjects with a history of breast cancer at baseline were also excluded. All subjects were followed from the index date until the outcome of interest, end of data collection, date of migration out of the CPRD population, or death, whichever came first.

### Exposure to insulins

We used a time-dependent design (Figure 4.2.1) to define exposure. For all patients, the follow-up period after the index date was divided into discrete 30-day intervals. Exposure to glargine ('any use') was then defined as a prescription for glargine on the start date or at any time before the start of each interval. Patients with a prescription for non-glargine insulin (*i.e*. any insulin type except insulin glargine) at cohort entry could become exposed during follow-up if a prescription for glargine was recorded (*i.e.* 'switchers'). Current exposure to glargine and non-glargine insulins was defined as a prescription on the start date or in the 3 months prior to the start of each 30-day interval.

In a stepwise manner, we added time-dependent cumulative measures for duration of use of: (a) glargine during follow-up; and (b) of non-glargine insulins before the initiation of glargine therapy. In a final model (c) we studied breast cancer risk associated with cumulative duration of glargine use separately among insulin-naïve glargine users and users of glargine with prior use of other insulin types (Figure 4.2.1). Cumulative duration of use calculations were based on the number of days of 'current exposure'. Duration of glargine

use during follow-up was determined at the start of each 30-day interval and classified as '0 - 1 years', '1 - 3 years', or '>3 years' (Figure 4.2.1a), based on the total number of days of current exposure to glargine. Consequently, cumulative exposure to glargine could only increase or remain stable over time.

In switchers, the cumulative number of days of past exposure to non-glargine insulins was calculated at the start date of glargine treatment. Here, we differentiated between insulin-naïve patients at the start of glargine treatment, and switchers with a cumulative duration of past exposure to non-glargine insulins of '0 - 3 years' and '>3 years' (Figure 4.2.1b).



In our final model, we performed separate analyses regarding associations with duration of glargine exposure during follow-up among insulin-naïve patients and prevalent insulin users switched to glargine (Figure 4.2.1c). In all models, we quantified the risk of breast cancer associated with glargine use as compared to 'never use' of glargine; as the study was performed among insulin users, person-time on glargine was compared to person-time on other insulins.

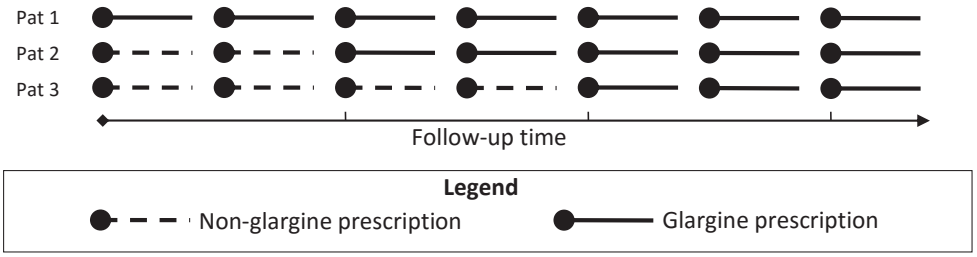
## Study outcome

All subjects were followed up for the occurrence of a first medical diagnosis for breast cancer in CPRD (see Appendices, Table 4.2.A for a list of the medical codes). Completeness of case ascertainment for breast cancer in CPRD is high as compared to the National Cancer Registry data (Boggon *et al*, 2013). A recent study found a concordance rate of 89.8% with cancer registries and a subsequent 6.4% of the records were in agreement with hospital records or death certificates (Boggon *et al*, 2013).

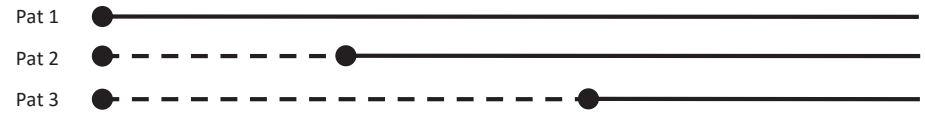
## Covariates

Models were adjusted for potential confounders in a time-dependent manner. Age was determined as the year difference between calendar year and year of birth at the start of each 30-day interval. A history of cancer other than breast cancer (or non-melanoma skin cancer) and oophorectomy was determined as a medical diagnosis at any time before the start of each interval. Smoking status (yes or no) and alcohol use (yes or no) were determined at cohort entry and subsequently updated during follow-up at the start of each interval. Current use of comedication (*i.e.* hormone replacement therapy, statins, metformin, sulfonylureas, and glitazones) was determined as a prescription in the past 180 days prior to the start of each interval. For body mass-index (BMI) and $HbA_{1C}$, the most recent record before the start of follow-up was used to classify patients at baseline. Subsequently, obesity (BMI ≥ 30 kg/m²) and increased $HbA_{1C}$ level (>75 mmol/mol) were determined based on the most recent measurement at the start of each interval. We used step-wise model building for adjustment for potential confounders. In Model 1, we adjusted for all potential confounders, while in Model 2 we performed additional adjustment for the number of years of past exposure to non-glargine insulins before the start of glargine as a continuous variable to adjust for the potential effect of past exposure to non-glargine insulins on the association between glargine use and breast cancer risk among switchers. We evaluated the linearity assumption by adding a squared term to the model, together with the continuous variable.

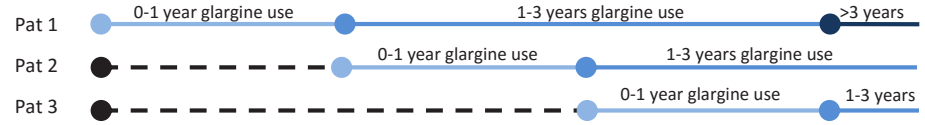
**FIGURE 4.2.1.** Schematic representation of exposure measures used. The basic time-dependent design considers a patient exposed from the first prescription of glargine onward. Cumulative exposure measures were included as a refinement, where patients are stratified by (a) cumulative exposure to insulin glargine during follow-up (blue), (b) duration of exposure to other insulin types before the start of glargine treatment (red), and (c) stratified to insulin-naïve starters of glargine and switchers.



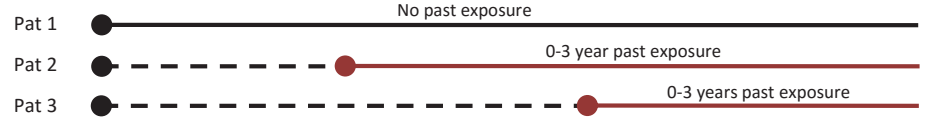

1. Time-dependent exposure to glargine ('ever use')




(a) Cumulative exposure to glargine during follow-up



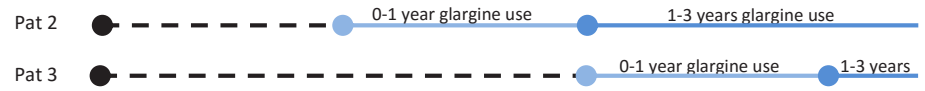

(b) Past cumulative exposure to non-glargine insulins




(c) Stratification by past use and cumulative use of glargine

Insulin-naïve glargine starters without past exposure to non-glargine insulins


Switchers with past exposure to non-glargine insulins



### Statistical analysis

Multivariate Cox proportional hazards models were used to estimate the hazard ratio (HR) and 95% confidence intervals (CI) of breast cancer associated with the use of glargine, with survival time in 30-day intervals as the time variable. For all models, 'never use' of glargine was used as the reference category. In addition to the analyses stratified by categories of cumulative duration of use, we performed *p*-trend analyses, where cumulative exposure to insulins was included as a continuous variable.

In a sensitivity analysis, an extended category of cumulative duration of glargine exposure was added - '0 to 1 years', '1 to 3 years', '3 to 5 years', and '>5 years' - when study power was sufficient. In a separate analysis, the cumulative duration-response effect was studied among glargine users independently. Here, patients started on glargine were censored at the time a different insulin type was prescribed. In this sensitivity analysis, the lowest category of cumulative exposure to glargine was taken as the reference. All data management and statistical analyses (PROC PHREG) were conducted using SAS 9.2 (SAS Institute Inc, Cary, NC, USA).



## RESULTS

For this study, we selected 12,468 female incident insulin users for our final study cohort (see Appendices, Figure 4.2.A). Baseline characteristics of new users of insulin who received a first prescription for glargine (n=3,858) or non-glargine insulins (n=8,610) are shown in Table 4.2.1. The median duration of follow-up was comparable between the glargine and non-glargine starters (5.0 versus 5.1 years), as was the median duration of insulin exposure during follow-up; 2.6 years of glargine use among glargine starters, and 3.0 years of non-glargine insulin use among non-glargine starters. Glargine starters were in general older at baseline (median age of 66 versus 61 years). Of the non-glargine starters, the majority received a first prescription for insulin aspart (44.6%) or human insulin (39.4%). Glargine starters in general received NIADs in the year prior to baseline (94.3%).

### Risk of breast cancer

During a total follow-up of 66,151 person years, 186 breast cancer cases occurred. Of these, 76 occurred in patients after exposure to glargine (3.0 per 1,000 person years), and 110 in patients never exposed to glargine (2.7 per 1,000 person years). In our model adjusted for potential confounders (Model 1), no discernible increase in breast cancer risk was associated with 'ever use' of glargine (HR 1.06, 95% CI 0.79-1.44), as compared to 'never use' (Table 4.2.2). When adjusted for years of exposure to other insulins before the start of glargine treatment (Model 2), no risk difference was observed (HR 0.98, 95% CI 0.72-1.35).

### Cumulative exposure measures

Stratification by cumulative duration of exposure to glargine during follow-up (Figure 4.2.1a, Table 4.2.2) did not show an association with breast cancer risk (*p* trend 0.83 in Model 1). Even when cumulative exposure to glargine of over 5 years was modelled as a separate category (sensitivity analysis), no significant difference in risk was observed (HR 1.26, 95% CI 0.64-2.47 in Model 1).

**TABLE 4.2.1.** Baseline characteristics of incident insulin users started on insulin glargine or non-glargine insulin.

| | Glargine cohort (n=3,858) | | Non-glargine cohort (n=8,610) | |
|---|---|---|---|---|
| **Follow-up (median, IQR)** | 5.0 | (3.0-7.5) | 5.1 | (3.1-7.9) |
| Maximum | 11.0 | | 11.2 | |
| **Age (median, IQR)** | 66 | (55-76) | 61 | (45-72) |
| <30 | 43 | (1.1) | 165 | (1.9) |
| 30-40 | 183 | (4.7) | 1,387 | (16.1) |
| 40-50 | 381 | (9.9) | 1,025 | (11.9) |
| 50-60 | 736 | (19.1) | 1,476 | (17.1) |
| 60-70 | 923 | (23.9) | 1,894 | (22.0) |
| 70-80 | 937 | (24.3) | 1,791 | (20.8) |
| >80 | 655 | (17.0) | 872 | (10.1) |
| **BMI (median, IQR)** | 30.4 | (26.0-35.3) | 30.4 | (25.9-35.4) |
| <20 | 125 | (3.2) | 337 | (3.9) |
| 20-25 | 589 | (15.3) | 1,326 | (15.4) |
| 25-30 | 1,085 | (28.1) | 2,137 | (24.8) |
| 30-35 | 962 | (24.9) | 2,054 | (23.9) |
| >35 | 962 | (24.9) | 2,086 | (24.2) |
| Missing | 135 | (3.5) | 670 | (7.8) |
| **$HbA_{1c}$ (median, IQR)** | 79.1 | (67.1-95.5) | 78.0 | (63.8-94.4) |
| <32 mmol/mol | 8 | (0.2) | 39 | (0.5) |
| 32-64 mmol/mol | 689 | (17.9) | 1,756 | (20.4) |
| 64-75 mmol/mol | 815 | (21.1) | 1,250 | (14.5) |
| >75 mmol/mol | 2,104 | 54.5) | 3,796 | (44.1) |
| Missing | 242 | (6.3) | 1,769 | (20.5) |
| **Smoking habit** | | | | |
| Non-smoker | 1,900 | (49.2) | 4,187 | (48.6) |
| Current smoker | 550 | (14.3) | 1,398 | (16.2) |
| Ex-smoker | 983 | (25.5) | 2,051 | (23.8) |
| Missing | 425 | (11.0) | 974 | (11.3) |
| **Medical diagnosis (ever before)** | | | | |
| Other cancer | 255 | (6.6) | 583 | (6.8) |
| Oophorectomy | 6 | (0.2) | 25 | (0.3) |
| **Type of insulin (at baseline)** | | | | |
| Glargine | 3,858 | (100.0) | 0 | (0.0) |
| Non-glargine | 288 | (7.5) | 8,610 | (100.0) |
| Human insulin | 12 | (0.3) | 3,394 | (39.4) |
| Aspart | 207 | (5.4) | 3,841 | (44.6) |
| Detemir | 1 | (0.0) | 1,186 | (13.8) |
| Glulisine | 32 | (0.8) | 16 | (0.2) |
| Lispro | 38 | (1.0) | 656 | (7.6) |
| Other insulins | 0 | (0.0) | 27 | (0.3) |
| **Medication use (year prior to index)** | | | | |
| NIADs | | | | |
| None | 220 | (5.7) | 2,093 | (24.3) |
| Metformin | 3,014 | (78.1) | 5,233 | (60.8) |
| Sulfonylureas | 3,158 | (81.9) | 5,174 | (60.1) |
| Glitazones | 1,331 | (34.5) | 2,101 | (24.4) |
| Other* | 518 | (13.4) | 826 | (9.6) |
| HRT | 286 | (7.4) | 594 | (6.9) |
| Statins | 2,701 | (70.0) | 4,722 | (54.8) |

*Abbreviations:* IQR, interquartile range; BMI, body mass-index in kg/m²; $HbA_{1C}$, glycated heamoglobine; mmol, millimol; NIAD, non-insulin antidiabetic drug; HRT, hormone replacement therapy. *Glinides, alpha-glucosidase inhibitors, glucogon-like peptide 1 agonists, dipeptidyl peptidase-4 inhibitors, guar gum.

Stratification by prior exposure to non-glargine insulins at the start of glargine treatment within the group of patients switched to glargine during follow-up (Figure 4.2.1b, Table 4.2.2) showed a linear trend with increasing years of past exposure (*p* trend 0.02 in Model 1). Here, a significant 3-fold increase in breast cancer risk was observed in switchers with a history of non-glargine insulin use of more than three years, as compared to women never exposed to glargine (HR 3.17, 95% CI 1.28-7.84).

Among insulin-naïve women (Figure 4.2.1c, Table 4.2.3), no increased breast cancer risk was associated with 'ever use' of glargine (HR 0.99, 95% CI 0.71-1.37). Also, no trend with cumulative duration of exposure to glargine was observed (*p* trend 0.91; HR 0.99, 95% CI 0.90-1.10, per additional year of exposure). After additional stratification (sensitivity analysis), no increased breast cancer risk was associated with ≥5 years of cumulative exposure to glargine (HR 1.06, 95% CI 0.48-2.33).



A slight, non-significantly increased breast cancer risk was associated with 'ever use' of glargine (HR 1.40, 95% CI 0.83-2.34) among insulin users switched to glargine. Moreover, a non-significant trend was observed with cumulative number of years of glargine exposure (*p* trend 0.24; HR 1.10, 95% CI 0.94-1.29, per additional year of exposure in Model 1). Adjustment for number of years of exposure to non-glargine insulins before the start of glargine treatment (Model 2) resulted in noticeable reductions in risk estimates. The hazard ratio for 'ever use' of glargine in switchers was reduced to 0.97 (95% CI 0.47-1.99), while the hazard ratio for the highest category of cumulative glargine exposure was reduced from 1.58 (95% CI 0.63-3.94) to 1.14 (95% CI 0.42-3.09) (Table 4.2.3). In addition, the non-significant trend with cumulative number of years of glargine exposure disappeared after adjustment for cumulative number of years of exposure to non-glargine insulins (*p* trend 0.99; HR 1.00, 95% CI 0.91-1.10, per additional year of exposure, Model 2). In this model, a significant trend was observed with number of years of prior non-glargine insulin exposure (*p* trend 0.02; HR 1.24, 95% CI 1.03-1.50, per additional year of exposure, Model 2) [data not shown]. Results from our sensitivity analysis that censored glargine insulin users if any other type of insulin was initiated also showed no trend with cumulative duration of use (see Appendices, Table 4.2.B).

## DISCUSSION

In this cohort study among women with type 2 diabetes mellitus newly started on insulin, glargine use was not associated with an increased risk of breast cancer after a median follow-up of five years as compared to use of other insulins. However, a difference in breast cancer risk between insulin-naïve new users of glargine and women who switched to glargine after having used other types of insulin was observed. More specifically, no association between glargine use (either in general or with cumulative use) and breast cancer risk was seen among insulin-naïve new users of glargine, even after five cumulative years of glargine exposure. In contrast, a non-significant increase in breast cancer risk was found among patients who switched to glargine, depending on the number of years of past insulin use. That is, a significant trend was observed for each additional year of non-glargine exposure before the start of glargine treatment in patients who switched to insulin glargine.

**TABLE 4.2.2.** Hazard ratios for breast cancer associated with the use of insulin glargine, stratified by cumulative duration of glargine use during follow-up and by cumulative exposure to other insulins before the initiation of glargine therapy.

| | Events | Person years | IR | Age adj. HR (95% CI) | | Model 1 HR (95% CI)* | | Model 2 HR (95% CI)† | |
|---|---|---|---|---|---|---|---|---|---|
| **Use of non-glargine insulins‡** | 110 | 40912.6 | (2.7) | 1 | Reference | 1 | Reference | 1 | Reference |
| **Ever use of glargine** | 76 | 25238.5 | (3.0) | 1.08 | (0.81-1.45) | 1.06 | (0.79-1.44) | 0.98 | (0.72-1.35) |
| *Cumulative glargine use§,‖* | | | | | | | | | |
| <1 year | 29 | 8621.0 | (3.4) | 1.11 | (0.74-1.68) | 1.15 | (0.76-1.76) | 1.05 | (0.68-1.62) |
| 1-3 years | 26 | 8994.3 | (2.9) | 1.02 | (0.66-1.56) | 0.99 | (0.64-1.53) | 0.91 | (0.58-1.42) |
| >3 years | 21 | 7623.1 | (2.8) | 1.12 | (0.69-1.81) | 1.05 | (0.64-1.70) | 0.99 | (0.61-1.62) |
| 3-5 years | 11 | 4550.0 | (2.4) | 0.97 | (0.52-1.81) | 0.91 | (0.48-1.71) | 0.86 | (0.45-1.61) |
| >5 years | 10 | 3073.1 | (3.3) | 1.37 | (0.70-2.67) | 1.26 | (0.64-2.47) | 1.22 | (0.62-2.39) |
| *Cumulative prior non-glargine use¶,#* | | | | | | | | | |
| None | 59 | 19923.3 | (3.0) | 1.03 | (0.75-1.41) | 0.99 | (0.71-1.37) | | |
| 0-3 years | 12 | 4628.8 | (2.6) | 1.08 | (0.59-1.96) | 1.14 | (0.62-2.07) | | |
| >3 years** | 5 | 686.4 | (7.3) | 3.11 | (1.26-7.67) | 3.17 | (1.28-7.84) | | |

*Abbreviations:* IR, incidence rate in events per 1,000 person years; HR, hazard ratio; adj., adjusted; CI, confidence interval. *Model 1, adjusted for potential confounders; *i.e.* obesity, smoking, alcohol use, $HbA_{1c}$>75 mmol/mol, history of oophorectomy or other cancer types, and use of metformin, sulfonylureas, glitazones, hormone replacement therapy, or statins. †Model 2, additional adjustment for number of years of exposure to other non-glargine insulin(s) at the start of glargine treatment as a continuous variable. ‡Person time without any history of insulin glargine use. §Cumulative number of years of current exposure to insulin glargine during follow-up. ¶Cumulative number of years of current exposure to non-glargine insulins before the start of glargine treatment. **Median past exposure 4.4 (interquartile range, 3.6-5.6 years; maximum, 10.3 years).

Linear trends based on the slope of continuous cumulative number of years of current exposure in Model 1:
‖ *p* trend=0.83, (HR=1.01, 95% CI 0.92-1.10), for cumulative current exposure to glargine
\# *p* trend=0.02, (HR=1.24, 95% CI 1.03-1.49), for cumulative current exposure to non-glargine insulin(s)

**TABLE 4.2.3.** Hazard ratios for breast cancer associated with the use of insulin glargine, among insulin naïve glargine users and glargine users with prior exposure to other insulins, stratified by categories of cumulative glargine use and adjusted for use of other insulins before the start of glargine.

| | Events | Person years | IR | Age adj. HR (95% CI) | | Model 1 HR (95% CI)* | | Model 2 HR (95% CI)† | |
|---|---|---|---|---|---|---|---|---|---|
| **Use of non-glargine insulin‡** | 110 | 40912.6 | (2.7) | 1 | Reference | 1 | Reference | 1 | Reference |
| **Ever use of glargine** | 76 | 25238.5 | (3.0) | 1.08 | (0.81-1.45) | 1.06 | (0.79-1.44) | 0.98 | (0.72-1.35) |
| *Insulin-naïve glargine users§* | 59 | 19923.3 | (3.0) | 1.02 | (0.74-1.41) | 0.99 | (0.71-1.37) | | |
| Cumulative glargine use‖ | | | | | | | | | |
| < 1 year | 23 | 6759.8 | (3.4) | 1.09 | (0.69-1.71) | 1.11 | (0.69-1.77) | | |
| 1-3 years | 20 | 7119.9 | (2.8) | 0.96 | (0.59-1.54) | 0.91 | (0.56-1.49) | | |
| >3 years | 16 | 6043.6 | (2.6) | 1.05 | (0.61-1.80) | 0.96 | (0.56-1.66) | | |
| 3-5 years | 9 | 3610.5 | (2.5) | 0.97 | (0.49-1.93) | 0.90 | (0.45-1.80) | | |
| >5 years | 7 | 2433.0 | (2.9) | 1.18 | (0.54-2.57) | 1.06 | (0.48-2.33) | | |
| Patients switched to glargine¶ | 17 | 5315.2 | (3.2) | 1.33 | (0.80-2.22) | 1.40 | (0.83-2.34) | 0.97 | (0.47-1.99) |
| Cumulative glargine use# | | | | | | | | | |
| < 1 year | 6 | 1861.2 | (3.2) | 1.24 | (0.55-2.83) | 1.32 | (0.58-3.03) | 0.84 | (0.31-2.31) |
| 1-3 years | 6 | 1874.4 | (3.2) | 1.31 | (0.57-2.98) | 1.36 | (0.59-3.10) | 0.89 | (0.33-2.37) |
| >3 years | 5 | 1579.6 | (3.2) | 1.59 | (0.64-3.95) | 1.58 | (0.63-3.94) | 1.14 | (0.42-3.09) |

*Abbreviations:* IR, incidence rate in events per 1,000 person years; HR, hazard ratio; adj., adjusted; CI, confidence interval. *Model 1, adjusted for potential confounders; *i.e.* obesity, smoking, alcohol use, $HbA_{1c}$>75 mmol/mol, history of oophorectomy or other cancer types, and use of metformin, sulfonylureas, glitazones, hormone replacement therapy, or statins. †Model 2, additional adjustment for number of years of exposure to other non-glargine insulin(s) at the start of glargine treatment as a continuous variable. ‡Person time without any history of insulin glargine use. §Patients without past cumulative exposure to other insulins at the start of glargine therapy. ¶Patients with past cumulative exposure to other insulins at the start of glargine therapy.

Linear trends based on the slope of continuous cumulative number of years of current exposure in Model 1:
‖ *p* trend=0.91, (HR=0.99, 95% CI 0.90-1.10), for cumulative years exposed to glargine.
\# *p* trend=0.24, (HR=1.10, 95% CI 0.94-1.29), for cumulative years exposed to glargine.

Our results regarding insulin-naïve patients (*i.e.* without prior exposure to other insulins), are in agreement with those from most observational studies that used cumulative exposure measures. Of previous studies, 4 out of 5 did not show an association between cumulative duration of glargine use and breast cancer risk among insulin-naïve starters of glargine (Fagot *et al*, 2013; Lim *et al*, 2014; Sturmer *et al*, 2013; Suissa *et al*, 2011). The study by Habel *et al*. (2013) did report an increased breast cancer risk associated with extended duration (≥2 years) of use (HR=1.6, 95% CI 1.0-2.8). This result might be a chance finding, since with a median duration of glargine use of 1.2 years they were unable to assess patterns of risk with longer duration of use. In fact, all previous studies among insulin-naïve glargine users were limited by insufficient study power to robustly estimate effects of long-standing (>3 years) glargine exposure. Moreover, our results are also in line with those from clinical trials among new users of insulin that consistently showed no increased cancer risk associated with glargine exposure (Home & Lagarenne, 2009; ORIGIN Trial Investigators, 2012; Rosenstock *et al*, 2009). The ORIGIN trial, with a median follow-up of 6.2 years, found no increased risk of breast cancer among patients assigned to glargine versus standard care. However, with only 28 breast cancer cases in both treatment arms, study power was limitied (ORIGIN Trial Investigators, 2012).

Among patients switched to glargine after having used other insulins, we observed a non-significant increase in risk associated with glargine use. This result is in line with that of Suissa *et al*. (2011) who reported a significant risk increase associated with glargine use after 5 years or more among switchers. However, the 2.7-fold risk increase (95% CI 1.1-6.5) found in their study is much larger than the one observed in our study. Conversely, the only other study that included switchers found no increased risk associated with duration of glargine use, but was limited by a relatively short follow-up for glargine users (median of 2.3 years) (Habel *et al*, 2013). In our study, we observed that breast cancer risk increased with each added year of non-glargine insulin use before the start of glargine (*i.e.* effect modification). Neither of the previous studies assessed the effect of past insulin exposure on the association between glargine use and breast cancer risk. Suissa *et al.* (2011) are thus far the only ones to acknowledge that duration of insulin use before the start of glargine should be taken into account. However, by matching on duration of past use, the potential effect of past exposure was not measured.

Extensive past use (≥3 years) of non-glargine insulins was associated with a 3-fold increase in breast cancer risk among patients switched to glargine. However, when considering latency periods and the longer duration of type 2 diabetes mellitus and the treatment thereof (Suissa & Azoulay, 2012), it is impossible to attribute this excess risk among switchers to a single factor (*i.e.* glargine use). Nonetheless, this result sheds some light on the dynamics linked to the apparent difference in breast cancer risk seen in insulin-naïve patients started on glargine and switchers with past exposure to other insulins. The importance of taken prior use of other insulins into account was demonstrated by the noticeable reduction in all risk estimates for breast cancer associated with glargine use after adjustment for the number of years previously exposed to other insulins.

Alternative explanations for the difference in breast cancer risk associated with glargine use between insulin-naïve starters of glargine and switchers may entail that total duration of insulin use (or diabetes duration), rather than exposure to any particular insulin type, is associated with an increased risk of breast cancer. In addition, patients switched

to glargine may differ from insulin-naïve patients started on glargine. If glargine is used as an add-on in prevalent insulin users with poorly controlled blood glucose levels, a possible variation in background risk might be introduced. Such a dynamic could lead to channeling and potential protopathic bias among switchers. This alternative hypothesis could be evaluated in future studies.

Major strengths of our study include the use of time-dependent measures of exposure based on prescription data to determine insulin use. This definition minimizes misclassification of exposure and more accurately reflects real exposure than time since the start of follow-up, as was used in previous studies. In addition, since only incident insulin users (≥1 year without any insulin use at baseline) were included, we had comprehensive information on insulin use for patients included in the cohort. In patients who switched to glargine during follow-up, we were able to determine the effect of past insulin use on the association between glargine use and breast cancer risk. To our knowledge, we are the first to assess this effect. Furthermore, we had three additional years of follow-up as compared to the study by Suissa *et al.* (2011) and were thereby able to determine breast cancer risk estimates for long-standing glargine use in insulin-naïve patients.



Several limitations of our study should be noted as well. First of all, the comparator consisted of all other (non-glargine) insulins. This category included both short- and long-acting insulins, resulting in a heterogeneous reference group. However, it can be regarded as a relevant reference group when you want to assess glargine associated risks versus the other treatment options available. On the other hand, this approach did not allow for direct comparisons between long-acting insulin types. Secondly, our models did not account for any additional effects of combined use of both glargine and non-glargine insulins. Ideally, combined use should be considered as a separate category. Also, we were unable to make direct comparisons between glargine and non-glargine insulin users with the same duration of exposure. Thirdly, we did not take latency into account, since follow-up time was insufficient to incorporate a sensible latency period for breast cancer. Fourthly, since the cohort was restricted to new users of insulin at cohort entry, study power to analyze effects with cumulative duration of use was limited for the group of switchers, hindering further stratification by strata of cumulative years of past non-glargine insulin use. Fifthly, since CPRD covers a dynamic patient population (*i.e.* circa 7% of the total UK population), patients who transfer to a general practitioner who does not provide data to CPRD are lost to follow-up. Lastly, when fitting several exposure models on the same data, focus should not be on individual significant results, but the total of analyses should be seen in perspective and interpreted together (Patel & Ioannidis, 2014).

In conclusion, exposure to insulin glargine did not appear to be associated with an increased breast cancer risk in insulin-naïve patients. Our results, however, do indicate an association between glargine use and breast cancer among patients previously treated with other insulins before the start of glargine. Glargine use in patients with extensive past exposure to other insulin types was associated with a 3-fold increased risk. Therefore, observational studies need to take past exposure to other insulins into account when studying breast cancer risk associated with glargine use. Future studies should consider whether this excess risk of breast cancer observed in patients switched to glargine is caused by protopathic bias.

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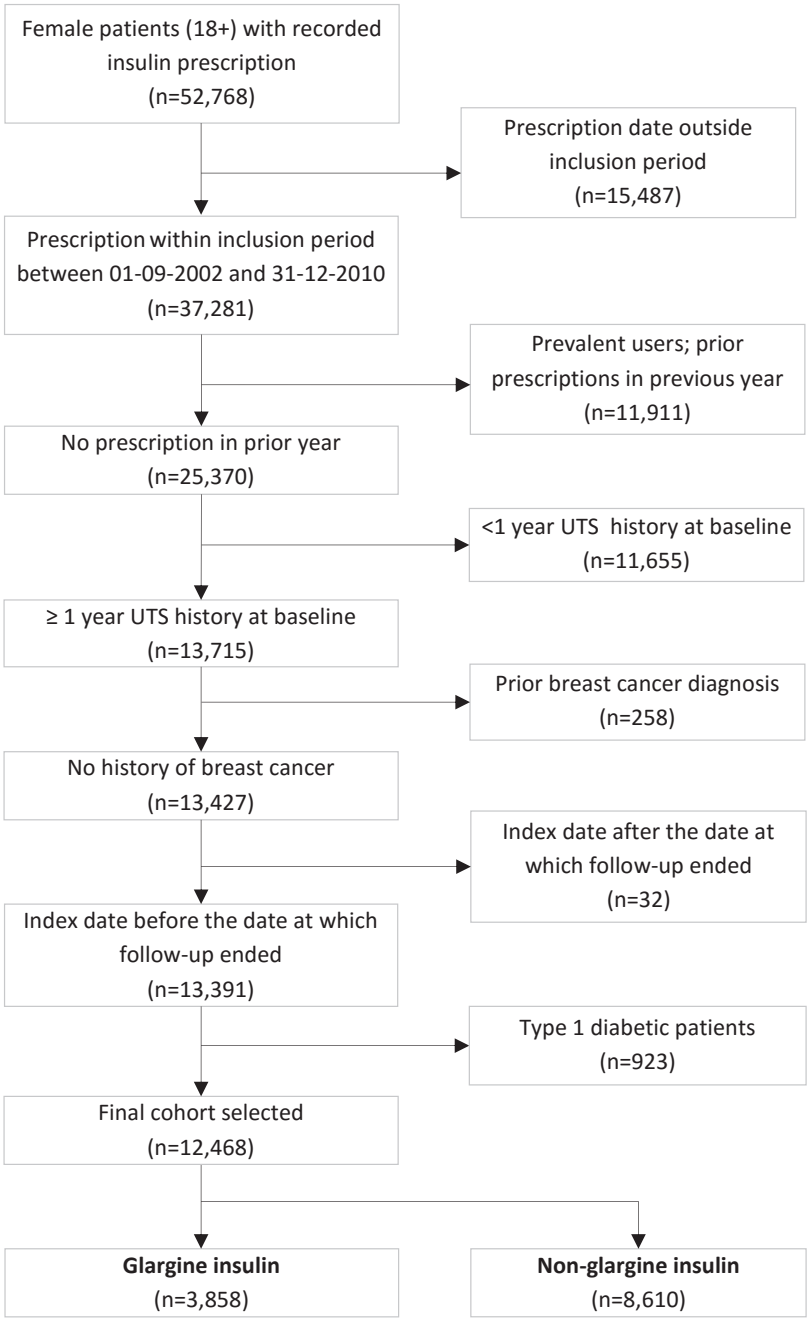
## APPENDICES

4.2

**FIGURE 4.2.A.** Flowchart of patient selection of the final study cohort of incident insulin users with type 2 diabetes with an initiation date after the marketing of insulin glargine in the United Kingdom in September 2001.




*Abbreviation*: UTS, up-to-standard.

**TABLE 4.2.A.** List of medical codes for breast cancer used to identify breast cancer events in CPRD medical records.

| Medcode | Readcode | Readterm |
|---|---|---|
| 348 | B34..11 | Ca female breast |
| 3968 | B34..00 | Malignant neoplasm of female breast |
| 9470 | B34z.00 | Malignant neoplasm of female breast NOS |
| 9902 | B3...11 | Carcinoma of bone, connective tissue, skin and breast |
| 12427 | BB9F.00 | [M]Lobular carcinoma NOS |
| 12480 | BB9K000 | [M]Paget's disease and intraductal carcinoma of breast |
| 12499 | Byu6.00 | [X]Malignant neoplasm of breast |
| 18608 | B3...00 | Malig neop of bone, connective tissue, skin and breast |
| 19389 | B3y..00 | Malig neop of bone, connective tissue, skin and breast OS |
| 20685 | B346.00 | Malignant neoplasm of axillary tail of female breast |
| 23380 | B340000 | Malignant neoplasm of nipple of female breast |
| 23399 | B344.00 | Malignant neoplasm of upper-outer quadrant of female breast |
| 26853 | B340.00 | Malignant neoplasm of nipple and areola of female breast |
| 29826 | B342.00 | Malignant neoplasm of upper-inner quadrant of female breast |
| 30189 | BB91000 | [M]Intraductal papillary adenocarcinoma with invasion |
| 31546 | B341.00 | Malignant neoplasm of central part of female breast |
| 38475 | B34yz00 | Malignant neoplasm of other site of female breast NOS |
| 39760 | BB91100 | [M]Infiltrating duct and lobular carcinoma |
| 40359 | BB94.00 | [M]Juvenile breast carcinoma |
| 41011 | B3z..00 | Malig neop of bone, connective tissue, skin and breast NOS |
| 42070 | B345.00 | Malignant neoplasm of lower-outer quadrant of female breast |
| 42542 | BB9K.00 | [M]Paget's disease and infiltrating breast duct carcinoma |
| 45222 | B343.00 | Malignant neoplasm of lower-inner quadrant of female breast |
| 49148 | B347.00 | Malignant neoplasm, overlapping lesion of breast |
| 56715 | B34y.00 | Malignant neoplasm of other site of female breast |
| 59831 | B340z00 | Malignant neoplasm of nipple or areola of female breast NOS |
| 64686 | B340100 | Malignant neoplasm of areola of female breast |
| 67701 | BB94.11 | [M]Secretory breast carcinoma |
| 95057 | B34y000 | Malignant neoplasm of ectopic site of female breast |

*Abbreviations:* CPRD, Clinical Practice Research Datalink; medcode, medical code that is linked to read codes and used in CPRD to identify medical diagnoses; read code, diagnostic code entered by general practitioners; Readterm, concise text description of medical diagnosis.

**TABLE 4.2.B.** Sensitivity analysis, with hazard ratios for breast cancer associated with the glargine, where patients were censored at the time a prescription for any other insulin type was recorded, stratified by cumulative exposure categories with short duration of use as the reference.

| | Events | Person years | IR | Age adj. HR (95% CI) | | Fully adj. HR (95% CI)* | |
|---|---|---|---|---|---|---|---|
| **Ever use glargine**† | 35 | 11350.6 | (3.1) | | | | |
| Cumulative use‡ | | | | | | | |
| <1 year | 12 | 4079.0 | (2.9) | 1 | Reference | 1 | Reference |
| 1-2 years | 10 | 2408.0 | (4.2) | 1.42 | (0.61-3.33) | 1.25 | (0.52-3.00) |
| 2-4 years | 6 | 2898.5 | (2.0) | 0.75 | (0.27-2.09) | 0.62 | (0.22-1.76) |
| >4 years | 7 | 1965.2 | (3.6) | 1.39 | (0.49-3.94) | 1.01 | (0.34-3.00) |

*Abbreviations:* IR, incidence rate in events per 1,000 person years; HR, hazard ratio; adj., adjusted; CI, confidence interval. *Adjusted for potential confounders; *i.e.* obesity, smoking, alcohol use, $HbA_{1c}$>75 mmol/mol, history of oophorectomy or other cancer types, and use of metformin, sulfonylureas, glitazones, hormone replacement therapy, or statins. †Person time without any history of insulin use other than glargine.

Linear trends based on the slope of continuous cumulative number of years of current exposure in a fully adjusted model:

‡ *p* trend=0.80, (HR=0.97, 95% CI 0.79-1.19), for cumulative current exposure to glargine.

# CHAPTER 5

# DISENTANGLING DISEASE PROGRESSION AND MEDICATION EFFECTS

## CHAPTER 5.1

# The risk of colorectal cancer in patients with type 2 diabetes mellitus: associations with treatment stage and obesity

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Frank de Vries, Marie L. De Bruin

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## SUMMARY

### Background
This study was set out to assess the risk of colorectal cancer associated with type 2 diabetes mellitus, as compared to a non-diabetic reference population, and to study additional associations with treatment stage and duration of obesity among type 2 diabetic patients.

### Methods
We conducted an observational population-based cohort study within the Clinical Practice Research Datalink (1987-2012). All patients (≥18 years) with at least one prescription for a hypoglycaemic agent (n=300,039) were matched (1:1) by birth year, sex, and practice to a comparison cohort without diabetes. Cox proportional hazards models were used to derive adjusted hazard ratios (HR) for colorectal cancer associated with type 2 diabetes mellitus. Within the diabetic cohort, associations of colorectal cancer with treatment stages and duration of obesity (body mass-index ≥ 30 kg/m²) were studied.

### Results
After a median follow-up of 4.5 years, 2,759 cases of colorectal cancer were observed among the diabetic study population. Type 2 diabetes mellitus was associated with a 1.3-fold increased risk of colorectal cancer (HR 1.26, 95% confidence interval [CI] 1.18-1.33). Among type 2 diabetic patients, no association was found with treatment stages. A trend of increased colorectal cancer risk was observed with longer duration of obesity. Risk of colorectal cancer was significantly increased for patients with recorded duration of obesity of 4 to 8 years (HR 1.19, 95% CI 1.06-1.34) and over 8 years (HR 1.28, 95% CI 1.11-1.49).

### Conclusion
Type 2 diabetes mellitus is associated with a moderately increased risk of colorectal cancer. Among type 2 diabetic patients, an increased risk was observed for patients who suffered from obesity for a total duration of 4 years or more.

## INTRODUCTION

Colorectal cancer is the third most common cancer in men and the second in women (Ferlay *et al*, 2010). Individuals with type 2 diabetes mellitus appear to have an increased risk of developing colorectal cancer as compared to their non-diabetic counterparts (Larsson *et al*, 2005). The global increase in prevalence of type 2 diabetes melitus, with an estimated total of 347 million adults suffering from type 2 diabetes in 2008 (Danaei *et al*, 2011), warrants further examination of the potential link between type 2 diabetes mellitus and colorectal cancer.

Observational cohort studies have found that colorectal cancer is more common in people with metabolic disturbances (Schoen *et al*, 1999; Stocks *et al*, 2011). Shared risk factors for colorectal cancer and type 2 diabetes mellitus include obesity, sedentary lifestyle, and high-caloric diet. Studies reported a fairly consistent, albeit moderate, increased risk of colorectal cancer associated with both type 2 diabetes mellitus (Larsson *et al*, 2005) and obesity (Larsson & Wolk, 2007). This may, at least in part, be due to a progressive decrease in insulin sensitivity in type 2 diabetic patients (Golay *et al*, 1986; Tabak *et al*, 2009), leading to chronic compensatory hyperinsulinaemia (Mitrakou *et al*, 1992; Reaven, 1988; Tabak *et al*, 2009). Hyperinsulinaemia was shown to have a strong association with increased body weight, in particular abdominal body fat (Carey *et al*, 1996). Mechanistically, insulin (both endogenic or exogenic) may promote colorectal carcinogenesis through a cross-talk with the insulin-like growth factor-1 (IGF-1) receptor, which stimulates proliferation and prolongs cell survival (Pollak *et al*, 2004). A stimulatory effect on cell growth of intestinal epithelial and colon cancer cells was shown in preclinical studies (Bjork *et al*, 1993; Koenuma *et al*, 1989). In addition, dietary hyperinsulinaemia was associated with increased tumor growth in *in vivo* experiments (Tran *et al*, 1996). Moreover, several nested case-control studies have found positive associations between increased blood insulin levels and colorectal cancer incidence (Ma *et al*, 1999; Ma *et al*, 2004).

If hyperinsulinaemia is considered a major causal factor for colorectal cancer incidence, hypotheses should focus on insulin resistance status (as the main cause of required hyperinsulinaemia) rather, than specific medications that increase insulin levels. The type(s) of hypoglycaemic agent(s) used could, however, be indicative of overall insulin resistance status, with more intensive treatment indicating higher overall insulin resistance.

Therefore, we firstly quantified the risk of colorectal cancer associated with type 2 diabetes mellitus as compared to a non-diabetic reference population. Secondly, among patients identified as having type 2 diabetes mellitus, we evaluated additional associations between colorectal cancer risk and treatment stage and duration of obesity as indicators of chronic hyperinsulinaemia.

## METHODS

### Data source

Data were obtained from the Clinical Practice Research Datalink (CPRD), which comprises electronic medical records from British general practitioners since 1987 (Parkinson *et al*, 2007). The accuracy and completeness of CPRD data have been well-validated in previous studies (Herrett *et al*, 2010; Khan *et al*, 2010). Currently, CPRD includes approximately 7% of the total UK population. The period of valid data collection varies between practices, depending on the date at which the data are considered up-to-standard. In April 2004, the Quality and Outcomes Framework (QOF) was implemented in the United Kingdom, which stimulates payments to general practices based on quality indicators that focus on specific aspects of care (*e.g.* registration of body mass-index). The protocol of this study was approved by CPRD's Independent Scientific Advisory Committee.



### Study design and population

For this retrospective cohort study, we identified men and women (≥18 years) treated with at least one prescription for a hypoglycaemic agent. The date of cohort entry was the date of the first recorded prescription for a hypoglycaemic agent during up-to-standard data collection. Subjects aged 30 years or younger with a first recorded prescription for insulin at cohort entry, without a concomitant prescription for a non-insulin antidiabetic drug (NIAD), were considered type 1 diabetic patients and excluded from the cohort. In addition, we excluded all patients with a diagnostic code for type 1 diabetes in CPRD prior to cohort entry.

At the date of cohort entry, a reference patient without any past recorded prescriptions for hypoglycaemic agents was matched to each subject in the diabetic cohort by sex, year of birth, and practice. The comparison cohort was selected using incidence density sampling; if a reference subject received a prescription for a hypoglycaemic agent during follow-up, this person was censored as a reference at that time and became a diabetic patient. A patient was excluded from the cohort if no suitable reference subject was found.

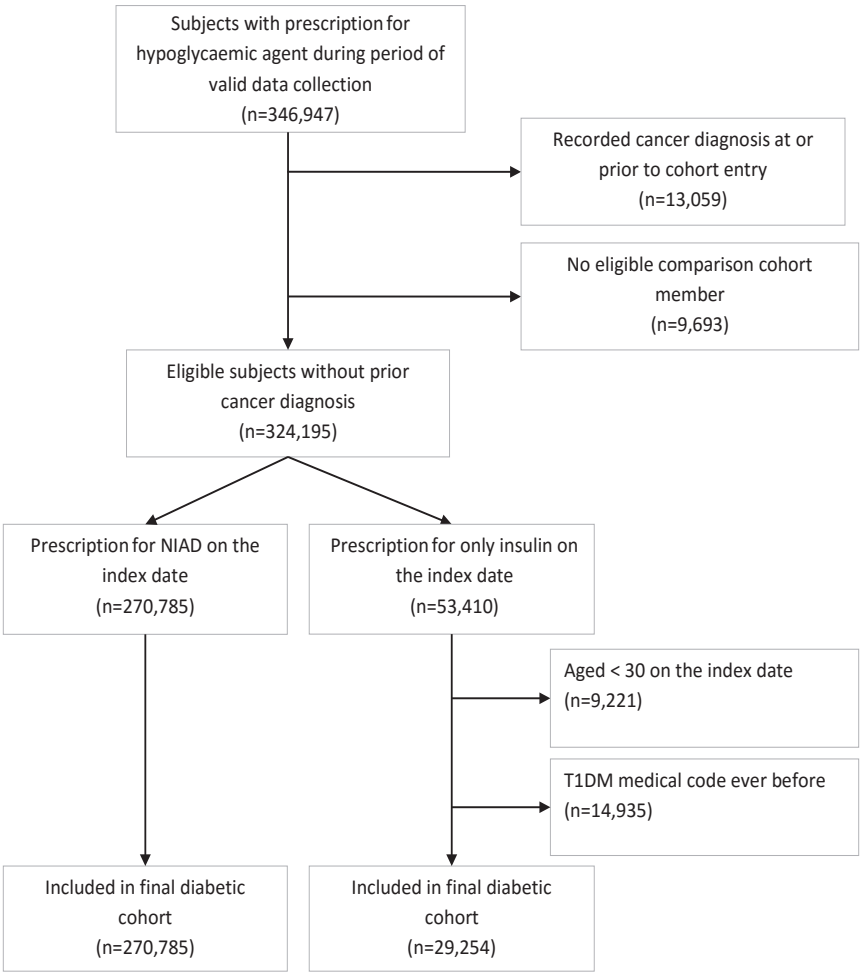
All participants were followed from the index date until the outcome of interest, end of data collection (December 2012), date of migration out of the CPRD population, or death, whichever came first. Patients with a history of any type of cancer prior to the index date (except non-melanoma skin cancer) were excluded together with their matched counterpart (see Figure 5.1.1).

### Exposure

Individual follow-up for all subjects was divided into fixed time periods of 90 days. In the primary analysis, patients treated with hypoglycaemic agents were considered type 2 diabetic patients and retained this status throughout follow-up (time-fixed).

Since factors influencing the degree of insulin resistance can change throughout the years, we evaluated two time-varying approaches to estimate the effect of insulin resistance on the colorectal cancer risk among type 2 diabetic patients. Firstly, a previously applied proxy indicator for type 2 diabetes mellitus severity was adapted (Bazelier *et al*,

**FIGURE 5.1.1.** Flow chart for patient inclusion and exclusion in the diabetes study cohort.




*Abbreviations:* NIAD, non-insulin antidiabetic drug; T1DM, type 1 diabetes mellitus.

2012), using prescribed antidiabetic medication to construct treatment stages. We used recent guidelines to define treatment stages, based on the step-wise approach in type 2 diabetes mellitus treatment (Inzucchi *et al*, 2012). Although guidelines have changed over time, the general medicinal approach has remained fairly consistent (Tiengo & Del Prato, 1988). We determined current exposure to hypoglycaemic agents time-dependently at the start of each 90-day interval as a prescription on the start date or in the 90 days before. The following classes of NIADs were defined: biguanides, sulfonylureas, glinides, thiazolidinesdiones, dipeptidyl peptidase-4 inhibitors, alpha-glucosidase inhibitors, glucagon-like peptide-1 agonists, and a separate category for all remaining NIADs. We constructed five mutually exclusive treatment stages, being (1) current use of a single NIAD, (2) simultaneous use of two or (3) more than two NIADs from different classes, (4) current use

of NIAD(s) combined with insulin, and (5) current insulin monotherapy. Treatment intensity (and hence treatment stage) may be reduced, as a result of reduced insulin resistance due to, for example, weight loss (Lloret-Linares *et al*, 2008), use of insulin sensitizers (Erdem *et al*, 2008), or lifestyle intervention (Houmard *et al*, 2004).

Secondly, we used body mass-index (BMI) as an indicator for insulin resistance, given its strong association with body fat content (Carey *et al*, 1996). Obesity (BMI ≥ 30kg/$m^2$) was then determined from BMI measurements recorded in CPRD. The most recent BMI measurement before the start of follow-up was used to determine obesity at baseline. Individuals without a recorded BMI prior to baseline were categorized as 'unknown BMI' at cohort entry. Subsequently, obesity status was updated time-dependently during follow-up, using the most recent measurement recorded at the start of each 90-day interval. The cumulative number of years with mapped obesity was then calculated at the start of each interval. A categorical variable was created with mutually exclusive duration categories, where person time with unknown BMI was included in the reference group (non-obese). We performed a sensitivity analysis to evaluate the influence of the potential difference in quality of data on BMI following the implementation of QOF in April 2004 (see statistical analyses).



### Study outcome

Patients were followed up for the occurrence of colorectal cancer, measured as a first medical record in CPRD (see Appendices, Table 5.1.A for a list of medical codes), stratified by anatomical region (*i.e.* distal colon, proximal colon, and rectal cancer).

### Potential confounders

Estimated risks were adjusted for patient characteristics, clinical conditions, or medications known or suggested to be associated with colorectal cancer and thus, able to confound the association between type 2 diabetes mellitus and colorectal cancer. Potential confounders determined at cohort entry were sex, alcohol consumption, and smoking status (Johnson *et al*, 2013). Age (as determined by year of birth), the presence of medical conditions (as a medical diagnosis ever before), and current drug use (as a prescription in the past 180 days) were assessed in a time-dependent manner and updated at the start of each 90-day interval. As a significant risk factor for colorectal cancer, inflammatory bowel disease (Johnson *et al*, 2013) was considered as a potential confounder. Comedication that was tested for confounding included opposed hormone replacement therapy, aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), statins, and bisphosphonates.

### Statistical analysis

Crude incidence rates were calculated as the number of events per 1,000 person years of observation. The relative risks and 95% confidence intervals (CI) of colorectal cancer were estimated by hazard ratios (HR) using time-dependent Cox proportional hazard models, with survival time in 90-day intervals as the time variable. The primary analyses compared the risk among patients with type 2 diabetes mellitus versus the comparison cohort. For the secondary analyses, the study population was restricted to the diabetic cohort. Here,

the risk of colorectal cancer associated with treatment stage and duration of obesity were estimated in two separate models. Both measures are intended to capture the patient's exposure to hyperinsulinaemia and were therefore not combined in a single model.

Relative risk estimates were adjusted for all potential confounders that generated a >5%-change in the beta-coefficient in an age-sex adjusted model (Greenland, 1989). For the primary analysis, BMI was not included in the model, as it was considered part of the exposure of interest. However, we did perform a sensitivity analysis of the primary model where duration of obesity was included. In a sensitivity analysis of the secondary outcomes, all patients on insulin monotherapy (stage 5) at cohort entry were excluded from the diabetic population. For a sensitivity analysis regarding the change in data quality caused by the implementation of QOF on the analysis concerning duration of obesity, the cohort was stratified to subjects with an index date before the introduction of QOF – here the end of follow-up was 31 March 2004 – and those with an index date equal to or later than 1 April 2004. Data management and statistical analyses were conducted using SAS 9.2 (SAS Institute Inc, Cary, NC, USA).



## RESULTS

### Patient characteristics

We followed 300,039 type 2 diabetic patients (median age 61 years, 52% male) for a median period of 4.5 years. The majority (80.1%) used a single NIAD at baseline (treatment stage 1), most often being metformin, followed by sulfonylureas. Compared to the matched non-diabetic reference population (median follow-up of 5 years), diabetic patients were more often obese (40.1% versus 14.8%) and used more statins and NSAIDs (Table 5.1.1).

### Risk of colorectal cancer

During the study period, 2,759 colorectal cancer events (1,941 colon cancer events and 819 rectal cancer events) were observed in the diabetic cohort (crude incidence rate 1.7 per 1,000 person years), as compared to 2,359 (1,625 colon cancer events and 737 rectal cancer events) in the reference population (crude incidence rate 1.3 per 1,000 person years). A moderate increased risk of colorectal cancer was found to be associated with type 2 diabetes mellitus (adjusted HR 1.26, 95% CI 1.18-1.33). No relevant differences in risk estimates were observed between the anatomical regions (Table 5.1.2). Adjustment for duration of obesity led to a marginal reduction in the risk estimate (HR 1.22, 95% CI 1.15-1.30).

Among patients with type 2 diabetes mellitus, no clear trend of increasing risk of colorectal cancer was observed with progressing treatment stages. Although the final two stages (combined NIAD/insulin therapy or insulin monotherapy) tended to be associated with a marginally increased risk of colorectal cancer as compared to stage 1 (NIAD monotherapy), none of the risk estimates reached statistical significance; adjusted HR 1.08, 95% CI 0.94-1.26 and adjusted HR 1.07, 95% CI 0.95-1.20 for stage 4 and 5, respectively (Table 5.1.3).

**TABLE 5.1.1.** Baseline characteristics of type 2 diabetic patients and non-diabetic reference patients.

| Characteristic | Diabetic cohort (n = 300,039) | | Non-diabetic cohort (n = 300,039) | |
|---|---|---|---|---|
| **Follow-up years** (total) | 1,668,354 | | 1,798,108 | |
| Mean (sd) | 5.6 | (4.5) | 6.0 | (4.7) |
| Median (IQR) | 4.5 | (1.9-8.4) | 5.0 | (2.2-8.9) |
| **Male** | 158,309 | (52.8) | 158,309 | (52.8) |
| **Age** (median - IQR) | 61 | (50-71) | 61 | (50-71) |
| 18-39 | 33,197 | (11.0) | 33,197 | (11.0) |
| 40-59 | 104,977 | (35.0) | 104,977 | (35.0) |
| 60-79 | 134,940 | (45.0) | 134,940 | (45.0) |
| 80+ | 26,925 | (9.0) | 26,925 | (9.0) |
| **BMI (median - IQR)** | 30.0 | (26.4-34.5) | 25.5 | (23.3-29.1) |
| Unknown | 57,481 | (19.2) | 77,766 | (25.9) |
| <20 | 3,866 | (1.3) | 12,577 | (4.2) |
| 20 – 24.9 | 36,556 | (12.2) | 80,107 | (26.7) |
| 25 – 29.9 | 81,865 | (27.3) | 85,165 | (28.4) |
| ≥30 | 120,271 | (40.1) | 44,424 | (14.8) |
| **Smoking** | | | | |
| Never | 151,013 | (50.3) | 154,126 | (51.4) |
| Current | 64,033 | (21.3) | 65,184 | (21.7) |
| Ex | 72,802 | (24.3) | 60,551 | (20.2) |
| Unknown | 12,191 | (4.1) | 20,178 | (6.7) |
| **Alcohol use** | | | | |
| No | 83,841 | (27.9) | 54,436 | (18.1) |
| Yes | 184,827 | (61.6) | 200,134 | (66.7) |
| Unknown | 31,371 | (10.5) | 45,469 | (15.2) |
| **History of disease**[*] | | | | |
| Inflammatory bowel disease | 1,075 | (0.4) | 1,173 | (0.4) |
| **Prescribed HGA**[†] | | | | |
| Metformin | 193,531 | (64.5) | - | (0.0) |
| Sulfonylureas | 95,923 | (32.0) | - | (0.0) |
| Glinides | 883 | (0.3) | - | (0.0) |
| Thiazolidinediones | 6,323 | (2.1) | - | (0.0) |
| Other NIADs[‡] | 3,696 | (1.2) | - | (0.0) |
| Insulin | 33,194 | (11.1) | - | (0.0) |
| **Treatment stage** | | | | |
| Stage 1: NIAD monotherapy | 240,288 | (80.1) | - | (0.0) |
| Stage 2: 2 NIAD classes combined | 24,144 | (8.0) | - | (0.0) |
| Stage 3: >2 NIAD classes combined | 2,413 | (0.8) | - | (0.0) |
| Stage 4: NIAD/insulin combined | 3,940 | (1.3) | - | (0.0) |
| Stage 5: insulin monotherapy | 29,254 | (9.8) | - | (0.0) |
| **Medication use**[§] | | | | |
| Hormone replacement therapy | 37,292 | (12.4) | 31,122 | (10.4) |
| NSAIDs | 75,609 | (25.2) | 30,983 | (10.3) |
| Bisphosphonates | 4,894 | (1.6) | 5,364 | (1.8) |
| Aspirin | 1,996 | (0.7) | 3,359 | (1.1) |
| Statins | 108,015 | (36.0) | 33,918 | (11.3) |

Data are no (%) of patients unless stated otherwise.

*Abbreviations:* IQR, interquartile range; BMI, body mass-index in kg/m²; HGA, hypoglycaemic agent; NIAD, non-insulin antidiabetic drug; NSAIDs, non-steroidal anti-inflammatory drugs. *Any time before the start of follow-up. †At the start of follow-up. ‡Guar gum (98 patients), dipeptidyl peptidase-4 inhibitors (1,218 patients), glucagon-like peptide-1 agonists (389 patients), alpha-glucosidase inhibitors (1,991 patients). §Any prescription within 180 days prior to the start of follow-up.

**TABLE 5.1.2.** Relative risk of colorectal cancer associated with type 2 diabetes mellitus as compared to a reference cohort of patients without type 2 diabetes, matched by age, gender, and practice.

| | T2DM cohort (n=300,039) | | Non-diabetic cohort (n=300,039) | | Age-sex adj. HR | | Fully adj. HR* | |
|---|---|---|---|---|---|---|---|---|
| | *Events* | *IR* | *Events* | *IR* | | | | |
| **Colorectal cancer** | 2,759 | (1.7) | 2,359 | (1.3) | 1.32 | (1.25-1.40) | 1.26 | (1.18-1.33) |
| Colon cancer† | 1,941 | (1.2) | 1,625 | (0.9) | 1.36 | (1.27-1.45) | 1.26 | (1.17-1.35) |
| Proximal | 319 | (0.2) | 258 | (0.1) | 1.42 | (1.21-1.68) | 1.29 | (1.08-1.54) |
| Distal | 255 | (0.2) | 203 | (0.1) | 1.42 | (1.18-1.70) | 1.31 | (1.07-1.60) |
| Unknown | 1,370 | (0.8) | 1,164 | (0.6) | 1.34 | (1.23-1.44) | 1.25 | (1.15-1.36) |
| Rectal cancer‡ | 819 | (0.5) | 737 | (0.4) | 1.25 | (1.13-1.38) | 1.24 | (1.12-1.38) |

*Abbreviations:* T2DM, type 2 diabetes mellitus; adj, adjusted; IR, incidence rate in events per 1,000 person years; HR, hazard ratio. *Model adjusted for age, sex, statin use in the previous 6 months, smoking, and alcohol consumption. †Some patients diagnosed with both distal and proximal colon cancer. ‡Some patients were diagnosed with both rectal cancer and colon cancer.



With regard to the risk of colorectal cancer associated with the duration of obesity, we observed a more pronounced trend, where the highest exposure categories conveyed the highest risk. An increased risk was observed for diabetic patients being obese for a cumulative duration of 4 to 8 years (adjusted HR 1.19, 95% CI, 1.06-1.34) and 8 years or more (adjusted HR 1.28, 95% CI, 1.11-1.49), as compared to non-obese diabetic patients (Table 5.1.3). The sensitivity analyses – where all patients receiving insulin monotherapy at baseline were excluded, regardless of age – showed similar results for all secondary analyses. In addition, the results from the stratified analyses of the follow-up period before and after the introduction of the QOF were comparable (see Appendices, Table 5.1.B and Table 5.1.C).

## DISCUSSION

In this population-based cohort study, type 2 diabetes mellitus was associated with a 1.3-fold increased risk of colorectal cancer. This finding concurs with that of a recent meta-analysis, which reported a similar moderately increased risk (HR 1.27; 95% CI, 1.21-1.36) (Starup-Linde *et al*, 2013), indicating our diabetic cohort is representative of type 2 diabetic patients. Within the diabetic cohort, stratification to treatment stages did not reveal any noticeable trends in colorectal cancer risk. In contrast, cumulative duration of obesity did appear to be associated with increased colorectal cancer incidence. More specifically, an increased risk was observed among type 2 diabetic patients that were obese for long periods of time (over 4 years) as compared to non-obese type 2 diabetic patients.

In our primary analyses, we did not adjust for obesity since we considered it a key causal factor for both type 2 diabetes mellitus and colorectal cancer. In this perspective, type 2 diabetes mellitus and colorectal cancer coincide but are not causally related to each other. However, type 2 diabetic patients are characterized by increased insulin resistance and are therefore exposed to hyperinsulinaemia, which in turn is regarded a key causal

**TABLE 5.1.3.** Relative risk of colorectal cancer among patients with type 2 diabetes according to treatment stage (*model 1*) and duration of obesity (*model 2*).

| | Events | Person years | Crude IR | Age-sex adj. HR (95% CI) | | Fully adj. HR* (95% CI) | |
|---|---|---|---|---|---|---|---|
| **Treatment stages (model 1)**†,‡ | | | | | | | |
| Stage 1: NIAD monotherapy | 1,423 | 850,518 | (1.7) | 1 (reference) | | 1 (reference) | |
| Stage 2: combitherapy 2 NIAD classes | 645 | 397,811 | (1.6) | 0.95 | (0.86-1.04) | 0.94 | (0.86-1.03) |
| Stage 3: combitherapy >2 NIAD classes | 136 | 85,192 | (1.6) | 1.03 | (0.86-1.23) | 1.01 | (0.85-1.21) |
| Stage 4: combitherapy NIAD and insulin | 209 | 122,455 | (1.7) | 1.10 | (0.95-1.28) | 1.08 | (0.94-1.26) |
| Stage 5: insulin monotherapy | 346 | 212,379 | (1.6) | 1.07 | (0.95-1.20) | 1.07 | (0.95-1.20) |
| **Duration of obesity (model 2)**§,‖ | | | | | | | |
| Non-obese¶ | 1,395 | 772,184 | (1.8) | 1 (reference) | | 1 (reference) | |
| < 1 year | 276 | 197,187 | (1.4) | 1.10 | (0.96-1.25) | 1.09 | (0.96-1.24) |
| 1 - 2 years | 192 | 146,809 | (1.3) | 1.05 | (0.90-1.23) | 1.03 | (0.88-1.20)# |
| 2 - 4 years | 298 | 212,123 | (1.4) | 1.09 | (0.96-1.24) | 1.07 | (0.94-1.22)# |
| 4 - 8 years | 383 | 230,495 | (1.7) | 1.21 | (1.08-1.36) | 1.19 | (1.06-1.34) |
| ≥ 8 years | 215 | 109,556 | (2.0) | 1.29 | (1.11-1.50) | 1.28 | (1.11-1.49) |

*Abbreviations:* IR, incidence rate in events per 1,000 person years; HR, hazard ratio; CI, confidence interval; adj, adjusted; NIAD, non-insulin antidiabetic drug. *Model adjusted for age, sex, smoking, alcohol consumption and statin use in the previous 6 months. †See Method section for detailed description. ‡*p* trend=0.14, based on the slope of stage as a continuous variable in a fully adjusted model (HR 1.02, 95% CI 0.99-1.05). §Cumulative duration of exposure to a BMI ≥ 30kg/m$^2$. ‖*p* trend=0.0008, based on the slope of continuous cumulative duration of obesity in years in a fully adjusted model (HR 1.02, 95% CI 1.01-1.03). ¶Included patient time with missing data on body mass-index. #Statistically significant difference ($p<0.05$) with ≥ 8 years cumulative duration of obesity.

factor (Pollak, 2012). Obesity is considered a major cause of insulin resistance and is highly associated with a hyperinsulinaemic state (Carey *et al*, 1996; Karam *et al*, 1963). Adjusting for obesity would therefore annul a key characteristic that links type 2 diabetes mellitus to an increased colorectal cancer risk. In our study, obesity (BMI ≥ 30kg/m$^2$) was indeed far more common among type 2 diabetic patients than among non-diabetic comparison subjects at baseline (40.1% vs. 14.8%).

With our adaptation of treatment stages (Bazelier *et al*, 2012), we made an effort to develop a tool that, in contrast to simple diabetes duration, accounted for variations in insulin needs but also allowed patients to regress in insulin resistance status; *e.g.* through weight loss (Lloret-Linares *et al*, 2008) or lifestyle intervention (Houmard *et al*, 2004). However, within the diabetic cohort, the risk of colorectal cancer did not appear to be associated with a specific treatment stage. The lack of association with colorectal cancer risk may, in part, be explained by the unknown level of endogenous insulin production. As β-cell functionality decreases over time (Kahn *et al*, 2014), an intensified treatment does not necessarily entail exposure to a higher overall insulin level, as it can also indicate further deterioration of endogenous insulin production. In addition, if indicative of insulin resistance, present treatment intensity refers to the current insulin resistance status and may not accurately reflect historical exposure to hyperinsulinaemia.



In a distinct attempt to stratify by total insulin requirement, we took BMI as a measure of insulin resistance resulting in hyperinsulinaemia (Carey *et al*, 1996; Karam *et al*, 1963). Although previous studies have shown a link between the risk of colorectal cancer and body weight (Larsson & Wolk, 2007), the potential link between duration of obesity and colorectal cancer risk is seldom studied. As ultimately the degree of insulin resistance determines the required overall insulin level, duration of obesity was thought to reflect both level and duration of exposure to hyperinsulinaemia. Stratification by cumulative duration of obesity showed that patients who suffered from obesity for an extended period of time (>4 years) had an increased risk of colorectal cancer as compared to patients without a history of obesity. These findings are in line with the observation of a growth promoting effect of dietary hyperinsulinaemia provided by preclinical studies (Tran *et al*, 1996). Although, insulin resistance is affected by other factors - for example by genetic predisposition, age, exercise, physical fitness, and diet (Kahn *et al*, 2001) - and can be significantly reduced without weight loss (Houmard *et al*, 2004), these factors are likely interrelated in daily practice. Therefore, the trend between duration of obesity and colorectal cancer risk observed here provides an indication that long-term exposure to hyperinsulinaemia increases the risk of colorectal cancer in type 2 diabetic patients.

We consider the use of multiple records for body mass-index during follow-up in a time-dependent manner a major strength of our study. Moreover, the testing of duration of obesity (time-dependently), instead of current BMI (or BMI at baseline), is a novel approach that, at least in theory, more accurately describes the total duration of exposure to high insulin dosages. The association found between duration of obesity and cancer risk is, in our opinion, therefore a valuable contribution to the research conducted in this field. In the diabetic cohort, on average 1.5 measurements per annum (IQR, 1.0-2.2) were recorded in CPRD during follow-up. For only 15.2% of the type 2 diabetic patients no BMI measurement was recorded during follow-up. The introduction of QOF led to an increase in the availability of BMI recordings (from 75.6% to 86.7%), but the average number of measurements per

year did not increase drastically; median of 1.4 (IQR 0.8-2.3) to 1.6 (IQR 1.1-2.3). Moreover, the introduction of the QOF did not have a notable impact on the observed risk estimates associated with duration of obesity. Other strengths include the large cohort, high data quality (Herrett *et al*, 2010), and the availability of comprehensive patient characteristics.

Several limitations of our study should also be noted. First of all, the rationale for this study relies on the assumption that obesity-driven compensatory hyperinsulinaemia, rather than the use of specific hypoglycaemic agents, is the key causal factor that links type 2 diabetes mellitus to colorectal cancer. Consequently, we considered previously reported differences in cancer risk associated with different types of hypoglycaemic agents (Karlstad *et al*, 2013) to be the result of confounding by indication. Moreover, biological mechanisms other than hyperinsulinaemia are considered relevant in the link between obesity and cancer (Louie *et al*, 2013). Secondly, we did not validate our study outcome (*e.g.* through linkage with other databases). However, CPRD morbidity records can be regarded as a valid measure to capture colorectal cancer occurrence (Boggon *et al*, 2013). Thirdly, left truncation of our data hindered our ability to determine past duration of obesity at baseline, as well as time since the initiation of treatment with hypoglycaemic agents. Given the existence of peripheral insulin resistance in pre-diabetic patients, this led to a skewed distribution with regard to past exposure to endogenous hyperinsulinaemia at baseline. In addition, we were unable to estimate the potential effect of reversed causation (*e.g.* protopathic bias). Fourthly, we included patient time with unknown BMI in the reference category for the analysis concerning duration of obesity. If anything, this may have biased our results towards the null. Fifthly, using a single prescription for a hypoglycaemic agent as the inclusion criterium for the diabetic cohort may have led to misclassification of patients. For our primary analysis, such misclassification would have biased our results towards the null, while in the secondary analyses the effect would be in the opposite direction. Residual confounding by unmeasured risk factors (*e.g.* physical activity, red meat and coffee consumption, high-caloric diet) may also have influenced our results, particularly in the primary analysis. Lastly, detection bias may have affected our results, leading to an overestimation in our primary analysis.

In summary, we observed a moderate, yet significant, 1.3-fold increased risk of colorectal cancer in patients treated for type 2 diabetes mellitus. Among type 2 diabetic patients, an additional 1.2 to 1.3-fold increased risk was observed for patients who suffered from obesity for a total duration of 4 years or more. This trend between cumulative duration of obesity and the risk of colorectal cancer provides an indication that long-term exposure to high levels of insulin increases the risk of colorectal cancer. Moreover, these findings signal the risk of colorectal cancer increases the longer a patient with type 2 diabetes mellitus remains obese. Future studies could determine whether the increased risk observed here is reversible through weight loss.

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APPENDICES

**TABLE 5.1.A.** Medical codes used to identify colon and rectal cancer diagnoses in the Clinical Practice Research Datalink.

| | | | Colon cancer | | | Rectal cancer |
|---|---|---|---|---|---|---|
| Medcode* | Events† | Readterm‡ | *Proximal* | *Distal* | *Unknown* | |
| 1220 | 29,021 | Malignant neoplasm of colon | | | X | |
| 3811 | 5,229 | Malignant neoplasm of caecum | X | | | |
| 9118 | 4,727 | Colonic cancer | | | X | |
| 2815 | 4,200 | Malignant neoplasm of sigmoid colon | | X | | |
| 11628 | 3,458 | Cancer of bowel | | | X | |
| 28163 | 1,812 | Malignant neoplasm of colon NOS | | | X | |
| 10946 | 904 | Malignant neoplasm of ascending colon | X | | | |
| 6935 | 522 | Malignant neoplasm of transverse colon | X | | | |
| 22163 | 455 | Carcinoma of caecum | X | | | |
| 10864 | 404 | Malignant neoplasm of descending colon | | X | | |
| 9088 | 308 | Malignant neoplasm of hepatic flexure of colon | X | | | |
| 18619 | 214 | Malignant neoplasm of splenic flexure of colon | X | | | |
| 48231 | 40 | Malignant neoplasm of other specified sites of colon | | | X | |
| 93478 | 3 | Malignant neoplasm, overlapping lesion of colon | | | X | |
| 1800 | 16,833 | Malignant neoplasm of rectum | | | | X |
| 5901 | 7,165 | Rectal carcinoma | | | | X |
| 7219 | 1,869 | Carcinoma of rectum | | | | X |
| 27855 | 747 | Malignant neoplasm of rectosigmoid junction | | | | X |
| 35357 | 741 | Malignant neoplasm of rectum, rectosigmoid junction and anus | | | | X |

*Medical codes (Medcode) in the Clinical Practice Research Datalink (CPRD) correspond to Read-codes, which are the standard clinical terminology system used in General Practice in the United Kingdom. †List the total number of events (*i.e.* recordings of the specific medical code) within the CPRD. ‡Contains the description of the clinical event linked to the specific medical code.

**TABLE 5.1.B.** Sensitivity analyses, relative risk of colorectal cancer according to treatment stage and duration of obesity, after exclusion of patients in treatment stage 5 (insulin monotherapy) at baseline (n=29,254).

| | Events | Person years | Crude IR | Age-sex adj. HR (95% CI) | | Fully adj.HR* (95% CI) | |
|---|---|---|---|---|---|---|---|
| **T2DM treatment stages†** | | | | | | | |
| Stage 1: NIAD monotherapy | 1,418 | 845,696 | (1.7) | 1 (reference) | | 1 (reference) | |
| Stage 2: combitherapy 2 NIAD classes | 643 | 396,245 | (1.6) | 0.94 | (0.86-1.04) | 0.94 | (0.85-1.03) |
| Stage 3: combitherapy >2 NIAD classes | 136 | 84,834 | (1.6) | 1.02 | (0.86-1.22) | 1.00 | (0.84-1.20) |
| Stage 4: combitherapy NIAD and insulin | 178 | 104,366 | (1.7) | 1.09 | (0.93-1.27) | 1.06 | (0.91-1.25) |
| Stage 5: insulin monotherapy | 147 | 72,168 | (2.0) | 1.11 | (0.94-1.31) | 1.10 | (0.93-1.31) |
| **Duration of obesity‡** | | | | | | | |
| Non-obese§ | 1,246 | 834,033 | (1.5) | 1 (reference) | | 1 (reference) | |
| < 1 year | 260 | 183,495 | (1.4) | 1.11 | (0.97-1.27) | 1.10 | (0.96-1.26) |
| 1 - 2 years | 179 | 136,528 | (1.3) | 1.04 | (0.89-1.22) | 1.03 | (0.88-1.21) |
| 2 - 4 years | 277 | 197,907 | (1.4) | 1.08 | (0.95-1.24) | 1.07 | (0.93-1.22) |
| 4 - 8 years | 355 | 214,604 | (1.7) | 1.21 | (1.07-1.36) | 1.19 | (1.05-1.34) |
| ≥ 8 years | 205 | 101,787 | (2.0) | 1.33 | (1.14-1.55) | 1.32 | (1.13-1.54) |

*Abbreviations:* IR, incidence rate in events per 1,000 person years; HR, hazard ratio; CI, confidence interval; adj, adjusted. *Model adjusted for age, sex, statin use in the previous 6 months, smoking, and alcohol consumption. †See Method section for detailed description. ‡Cumulative duration of exposure to a BMI ≥ 30kg/m$^2$. §Included patient time with missing data on body mass-index.

**TABLE 5.1.C.** Sensitivity analyses, cumulative duration of obesity (BMI ≥ 30kg/m²), stratified by year with regard to the implementation of the Quality and Outcomes Framework in April 2004.

| | Events | Person years | Crude IR | Age-sex adj. HR (95% CI) | | Fully adj. HR* (95% CI) | |
|---|---|---|---|---|---|---|---|
| **Period until 1 April 2004; n=137,064†** | | | | | | | |
| Non-obese‡ | 514 | 291,897 | (1.8) | 1 (reference) | | 1 (reference) | |
| < 1 year | 71 | 60,163 | (1.2) | 0.97 | (0.75-1.24) | 1.00 | (0.78-1.29) |
| 1 - 2 years | 43 | 40,704 | (1.1) | 0.86 | (0.63-1.18) | 0.90 | (0.65-1.23) |
| 2 - 4 years | 60 | 48,265 | (1.2) | 0.97 | (0.74-1.28) | 1.01 | (0.77-1.33) |
| 4 - 8 years | 53 | 31,329 | (1.7) | 1.26 | (0.95-1.68) | 1.32 | (0.99-1.76) |
| ≥ 8 years | 15 | 7,475 | (2.0) | 1.37 | (0.82-2.32) | 1.44 | (0.86-2.43) |
| **Period since 1 April 2004; n=162,870** | | | | | | | |
| Non-obese‡ | 389 | 234,872 | (1.7) | 1 (reference) | | 1 (reference) | |
| < 1 year | 128 | 101.933 | (1.3) | 1.07 | (0.87-1.31) | 1.06 | (0.87-1.31) |
| 1 - 2 years | 102 | 73,106 | (1.4) | 1.24 | (0.99-1.55) | 1.23 | (0.98-1.53) |
| 2 - 4 years | 117 | 95,391 | (1.2) | 1.06 | (0.86-1.32) | 1.05 | (0.85-1.30) |
| 4 - 8 years | 110 | 71,302 | (1.6) | 1.33 | (1.07-1.66) | 1.32 | (1.05-1.64) |
| ≥ 8 years | 13 | 5,233 | (2.5) | 1.70 | (0.97-2.96) | 1.67 | (0.96-2.92) |

*Abbreviations:* IR, incidence rate in events per 1,000 person years; HR, hazard ratio; CI, confidence interval; adj, adjusted; BMI, body mass index in kg/m². *Model adjusted for age, sex, statin use in the previous 6 months, smoking, and alcohol consumption. †Subjects where the start date equaled the end of follow-up were excluded (n=105). ‡Included patient time with missing data on BMI.

## CHAPTER 5.2

# The risk of venous thromboembolism in patients with multiple sclerosis: the Clinical Practice Research Datalink

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## SUMMARY

### Background

In patients with multiple sclerosis (MS), disability and auto-inflammatory processes may result in an increased risk of venous thromboembolism (VTE). We aimed to evaluate the risk of VTE associated with MS.

### Methods

We conducted an observational cohort study within the Clinical Practice Research Datalink (1987-2009) linked to the national registry of Hospitalizations (1997-2008). At the time of MS diagnosis, a comparison cohort (n=33,370) without a recorded MS diagnosis during the study period was matched (6:1) to the MS cohort (n=5,566) by birth year, sex, and practice. Subjects were followed from the index date until the occurrence of VTE, end of data collection, migration, or death, whichever came first. Cox proportional-hazards models were used to derive adjusted hazard ratios (aHR) and 95% confidence intervals (CI) for VTE associated with MS and VTE risk factors within the MS cohort. Time-dependent adjustments were made for age, comorbidity, and medication use.

### Results

Compared with the comparison cohort, a 2.6-fold increased risk of VTE was observed for MS patients (aHR 2.56, 95% CI 2.06-3.20). A prior VTE event, varicose veins, obesity and major trauma were found to be associated with an increased risk of VTE within the MS population. Moreover, the risk of VTE was increased in MS patients with recent records indicating immobility, spasticity or glucogorticoid use or disability.

### Conclusion

Patients with MS had an increased risk of VTE. Furthermore, our results provide evidence that this association is, at least partly, mediated through an increased prevalence of VTE risk factors in MS patients.

## INTRODUCTION

Multiple Sclerosis (MS) is a progressive neurodegenerative autoimmune disease, causing a gradual loss of mobility. The initial course of the disease is typically characterized by exacerbations followed by remissions (relapsing-remitting MS). However, within 15 years approximately a quarter of MS patients require a wheelchair (Myhr *et al*, 2001).

Various autoimmune diseases have been associated with an increased risk of VTE (Ramagopalan *et al*, 2011; Zoller *et al*, 2012). Even though MS comprises a local brain inflammation, disruption of the blood-brain barrier (Minagar & Alexander, 2003) and elevated levels of endothelial microparticles during MS relapses (Minagar *et al*, 2001) may lead to activation of coagulation pathways (Reitsma *et al*, 2012). Due to the gradual loss in mobility, multiple sclerosis may be an autoimmune disease particularly associated with VTE (Bovill & van der Vliet, 2011).



Previous studies that assessed the risk of VTE in MS patients have had several important limitations. Originally, it was reported that MS patients may be at a lower risk of developing VTE (Kaufman *et al*, 1988). It was hypothesized that a potential protective effect stemmed from muscle spasms – a common symptom in MS (Oreja-Guevara *et al*, 2013; Rizzo et al, 2004) – that may contribute to a more effective emptying of lower extremity veins. Interpretation of the results of this initial study is however hindered by the lack of a description of the comparison group. In contrast, a recent Danish study reported an elevated risk of VTE associated with MS, with a 2- to 3-fold increase in incidence rate among MS patients (Christensen *et al*, 2012). However, this study lacked potentially important information on the presence of VTE risk factors (*e.g.* body mass index, smoking behavior and immobilization) or potential protective factors (*e.g.* spasticity). Others also reported an increased incidence of VTE (Arpaia *et al*, 2010; Zoller *et al*, 2012) in MS patients, although these studies did not use individual matching to a comparison cohort. Moreover, previous studies were unable to evaluate the prevalence of important VTE risk factors in MS patients and, if any, used crude measures to assess whether the risk of VTE varied by disease severity.

Therefore, the aim of this study was to evaluate the risk of VTE associated with MS.

## METHODS

### Source of data

For this population-based matched cohort study, data were obtained from the Clinical Practice Research Datalink (CPRD), formerly known as the General Practice Research Database. It comprises electronic medical records from British general practitioners since 1987. In the United Kingdom (UK), general practitioners provide primary health care and are responsible for specialist referrals. As such, they play a central role in the health care system. Currently, medical records are being collected from more than 600 general practices for approximately 5 million active patients, who represent 7% of the total UK population. Data recorded in CPRD include demographic information, prescription details, clinical events (by medical code), preventive care provided, specialist referrals, hospital admissions, and major outcomes (Parkinson *et al*, 2007). The accuracy and completeness of these data have been

well-validated and documented (Herrett *et al*, 2010). For this study, patient data was linked individually to the national registry of hospitalizations – the Hospital Episode Statistics (HES) – for about 45% of the practices. This registry uses ICD-coding to record primary and secondary diagnoses.

## Study population

The study population comprised all men and women aged 18 years and older with at least one recorded diagnosis of MS during the period of CPRD or HES data collection, between 1987 and August 2009 and between April 1997 and March 2008, respectively. The index date was defined as the first ever MS diagnosis. At the time of the first recorded MS diagnosis, a comparison cohort was constructed by random selection of up to six reference patients, without any MS diagnosis recorded in the registries during the study period, matched to each MS patient by sex, year of birth, and practice.



Reference patients were assigned the same index date as their matched MS patient. All patients were followed from the index date until the occurrence of the study outcome, end of data collection, date of migration out of the CPRD population, or death, whichever came first. This study population has been described more extensively elsewhere (Bazelier *et al*, 2011).

## Study outcome

The primary outcome was a diagnosis of deep vein thrombosis (DVT) or pulmonary embolism (PE) in CPRD or HES. As a secondary outcome, we distinguished between DVT and PE, based on medical records. In a sensitivity analysis, we discriminated between probable and possible VTE events, using a definition previously validated in CPRD (Lawrenson *et al*, 2000). A probable event was defined as either a VTE diagnosis in both CPRD and HES within 6 successive months or a single diagnosis supported by at least one of the following: a) a prescription for warfarin or low-molecular weight heparin (LMWH), b) laboratory testing for these agents, c) evidence of attendance at a clinic for treatment with anticoagulants within 3 months of diagnosis or d) death within one month of diagnosis. All other VTE events were considered possible VTE events. For patients with multiple VTE diagnoses occurring in the registries during follow-up, the first date recorded was used as the study outcome.

## Covariates

The total follow-up for each patient was divided into 30-day intervals. Risk factors for VTE determined at baseline (*i.e.* MS diagnosis date) were sex, body mass-index (BMI) (Huerta *et al*, 2007), smoking status (Severinsen *et al*, 2009), and a history of VTE (Iorio *et al*, 2010). These factors were considered as time-fixed variables during follow-up. The presence of other risk factors for VTE was assessed at baseline and in a time-dependent manner during follow-up by reviewing the electronic medical records prior to baseline and at the start of each interval, respectively. These included age (Huerta *et al*, 2007), a history of a chronic disease (*i.e.* varicose veins (Huerta *et al*, 2007), inflammatory bowel disease (Grainge *et al*, 2010), COPD (Rizkallah *et al*, 2009), and rheumatoid arthritis (Matta *et al*, 2009)), a diagnosis of cancer (Huerta *et al*, 2007) in the previous year, evidence of major trauma in the previous 6 months (Sweetland *et al*, 2009), pregnancy (Sultan *et al*, 2012), and any

prescription for hormone replacement therapy or contraceptives (Huerta *et al*, 2007), non-steroidal anti-inflammatory drugs (NSAIDs) (Schmidt *et al*, 2011), or antibiotics as a proxy for acute bacterial infection (Clayton *et al*, 2011) in the previous 6 months. Pregnancy was determined in a time-dependent manner according to previous studies in CPRD (Devine *et al*, 2010), with a three-month post-partum period (Sultan *et al*, 2012). Major trauma was defined as major injury to head, neck, thorax, abdomen, hip/thigh, and knee/lower limb or major orthopaedic surgery (*i.e.* total knee and total hip replacements).

Furthermore, several MS related risk factors for VTE were considered as potential confounding factors. Spasticity was identified through the use of spasmolytic drugs (*i.e.* baclofen or tizanidine) or a diagnosis for spasticity in the previous 6 months (Oreja-Guevara *et al*, 2013; Rizzo *et al*, 2004). A definition of disability was adopted from a previous study concerning MS (Bazelier *et al*, 2011). This definition uses proxy indicators – *i.e.* home visits by a general practitioner, nursing care, and patients receiving residential care, living in a care home, or using a wheelchair or walking aid – to identify increased disability in the previous 6 months. Prescriptions for antidepressants and glucocorticoids in the previous 6 months were also considered potential confounders and regarded as proxies for disease severity (Byatt *et al*, 2011) and activity (Grainge *et al*, 2010), respectively. Using a strategy from a previous study in CPRD, average daily dose of glucocorticoids was determined from therapy records and free text analysis (Bazelier *et al*, 2011).



## Statistical analysis

Cox proportional hazards models were used to provide an estimate of the relative risk (HR; hazard ratio) of VTE in MS patients as compared to population-based reference cohort members. In addition, the cohort was stratified by sex to estimate the relative risk for male and female subjects separately. Furthermore, the relative risk of VTE was estimated for the first year after MS diagnosis by restricting the follow-up period to a maximum of one year following the index date. Calculations were adjusted for all potential confounders that changed the β-coefficient more than 1% as compared to an age- and sex-adjusted analysis. Missing data on baseline characteristics were treated as a separate category. In the main analysis, we did not adjust for MS-related risk factors (*i.e.* immobility, spasticity, and use of glucocorticoid and antidepressants). VTE events were stratified by type (DVT and PE) and by probability (probable and possible events). In a secondary analysis, we added MS-related risk factors that changed the β-coefficient more than 1% compared to an age- and sex-adjusted analysis in a stepwise manner to the model. Within the MS population, the relative risk of VTE associated with various risk factors was estimated. The proportional hazard assumption was tested and found to be justified, using Schoenfeld residuals and through inclusion of time-dependent covariates in the model. All data management and statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA).

## RESULTS

### Patient characteristics

Baseline characteristics of patients with MS (n=5,566) and matched reference patients without MS (n=33,370) are shown in Table 5.2.1. For both patients and reference cohort members the median age at the index date was 44 years and 70% were female. The median duration of follow-up was 4.5 years for the MS cohort and 5.0 years for the reference cohort, comprising a total follow-up of 31,036 and 201,673 person years, respectively. At baseline, MS patients had a lower BMI and were more prone to smoke. Prior VTE events were more common in the MS cohort (1.2%) as compared to the reference cohort (0.9%). Furthermore, the use of NSAIDs and antibiotics was more frequent among MS patients. Fewer women with MS were pregnant at the time of diagnosis.



### Risk of VTE

A total of 115 VTE events were observed in the MS population (crude incidence rate 3.7 per 1,000 person years), as compared to 284 in the reference cohort (crude incidence rate 1.4 per 1,000 person years). Table 5.2.2 shows the results from the primary multivariate Cox regression analysis. An increased risk of VTE was observed among MS patients as compared to reference patients without MS (HR 2.56, 95% CI 2.06-3.20). No difference was observed between the risk estimates for DVT and PE. The risk was higher for male than for female MS patients (HR 3.16, 95% CI 2.18-4.57 versus HR 2.28, 95% CI 1.73-3.00). Of note, determination of a gender difference was limited by the imprecise estimates. Furthermore, the relative risks of probable and possible VTE events were quite similar. There were 363 VTE events (259 DVT events) observed in CPRD and 88 events (66 DVT events) in HES, with 48 events registered in both databases within 180 days. Subsequent analyses included all VTE events. The risk of VTE was slightly lower in the first year after MS diagnosis (HR 1.99, 95% CI 1.16-3.41) as compared to the rest of the follow-up period (HR 2.62, 95% CI 2.05-3.35).

Table 5.2.3 shows that adjustment for MS-related risk factors for VTE led to a considerable attenuation of the association between MS and VTE. Recent records (within the past 6 months) indicating immobility, spasticity, or use of glucocorticoids or antidepressants were shown to be important confounding factors. Adjustment for these confounders lowered the relative risk to 1.79 (95% CI 1.38-2.31) as compared to the reference cohort.

Table 5.2.4 shows that within the MS population, a prior VTE event (HR 5.56, 95% CI 2.99-10.35) and a prior diagnosis for varicose veins (HR 5.93, 95% CI 2.15-16.38) were highly associated with the occurrence of VTE during follow-up. This was also apparent for patients who recently suffered major trauma (HR 3.17, 95% CI 1.28-7.86). Of MS-related risk factors, a recent record of spasticity (HR 2.59, 95% CI 1.72-3.91) or disability (HR 2.04, 95% CI 1.26-3.31) was associated with VTE. Furthermore, the risk of VTE was higher in patients who had recently been exposed to high-dose glucocorticoids (HR 2.27, 95% CI 1.20-4.31). No significant relationship was observed between the risk of VTE and MS disease duration (*i.e.* time since the index date).

**TABLE 5.2.1.** Baseline characteristics of MS patients and reference patients without MS matched by birth year, sex, and practice. Data are no (%) of patients unless stated otherwise.

| Characteristic | | MS cohort (%) | | Reference cohort (%) | |
|---|---|---|---|---|---|
| | | (n=5,566) | | (n=33,370) | |
| **Follow-up (years)** | Mean (sdev) | 5.7 | (4.7) | 6.0 | (4.8) |
| | Median (IQR) | 4.5 | (1.8-8.5) | 5.0 | (2.1-9.0) |
| **Sex** | Female | 3,897 | (70.0) | 23,366 | (70.0) |
| | Male | 1,669 | (30.0) | 10,004 | (30.0) |
| **Age** | 18-39 | 2,113 | (38.0) | 12,676 | (38.0) |
| | 40-59 | 2,682 | (48.2) | 16,088 | (48.2) |
| | 60+ | 771 | (13.9) | 4,606 | (13.8) |
| | Median (IQR) | 44 | (35-53) | 44 | (35-53) |
| **Body mass-index (kg/m²)** | Median (IQR) | 24.7 | (21.9-28.5) | 25.1 | (22.4-28.8) |
| | Unknown | 987 | (17.7) | 5,736 | (17.2) |
| **Smoking** | Never | 2,137 | (38.4) | 15,186 | (45.5) |
| | Current | 1,544 | (27.7) | 7,173 | (21.5) |
| | Ex | 815 | (14.6) | 4,290 | (12.9) |
| | Unknown | 1,070 | (19.2) | 6,721 | (20.1) |
| **Pregnancy*** | Pregnant† | 33 | (0.8) | 490 | (2.1) |
| **History of disease‡ (any time before)** | Cancer | 143 | (2.6) | 881 | (2.6) |
| | VTE | 68 | (1.2) | 308 | (0.9) |
| | COPD | 56 | (1.0) | 309 | (0.9) |
| | IBD | 46 | (0.8) | 191 | (0.6) |
| | Rheumatoid arthritis | 34 | (0.6) | 234 | (0.7) |
| | Heart Failure | 33 | (0.6) | 156 | (0.5) |
| | Varicose veins | 22 | (0.4) | 166 | (0.5) |
| **History of disease§ (previous 6 months)** | Major trauma | 33 | (0.6) | 106 | (0.3) |
| | Disability | 316 | (5.7) | 689 | (2.1) |
| | Spasticity | 224 | (4.0) | 169 | (0.1) |
| **Medication use§ (previous 6 months)** | Antibiotics | 1,132 | (20.3) | 6173 | (18.2) |
| | NSAIDs | 832 | (14.9) | 3,183 | (9.4) |
| | Statins | 237 | (4.3) | 1,106 | (3.3) |
| | Glucocorticoids | 329 | (5.9) | 522 | (1.6) |
| | Anticoagulants | 46 | (0.8) | 180 | (0.5) |
| | Contraceptives† | 362 | (9.2) | 2,239 | (9.6) |
| | HRT† | 307 | (7.9) | 1,739 | (7.4) |

*Abbreviations:* IQR, interquartile range; VTE, venous thromboembolism; IBD, inflammatory bowel disease; COPD, chronic obstructive pulmonary disease; NSAIDs, non-steroidal anti-inflammatory drugs; HRT, hormone replacement therapy.*See methods section for definitions. †Percentage of female population. ‡Diagnosis at any time prior to the start of follow-up. §Record within 6 months prior to the start of follow-up.

**TABLE 5.2.2.** Risk of VTE for patients with MS (n=5,566) as compared to patients without MS (n=33,370), matched by birth year, sex, and practice, by type of VTE.

| | | | Hazard ratios | | | |
|---|---|---|---|---|---|---|
| | VTE events | IR | Age-sex adj. (95% CI) | | Fully adj.* (95% CI) | |
| **No MS** | 284 | (1.4) | 1 (reference) | | 1 (reference) | |
| **MS** | | | | | | |
| VTE | 115 | (3.7) | 2.81 | (2.26-3.49) | 2.56 | (2.06-3.20) |
| Probable† | 83 | (2.7) | 2.85 | (2.21-3.68) | 2.59 | (2.00-3.36) |
| Possible† | 32 | (1.0) | 2.70 | (1.79-4.06) | 2.54 | (1.67-3.84) |
| DVT | 84 | (2.7) | 2.85 | (2.21-3.67) | 2.61 | (2.02-3.38) |
| PE | 31 | (1.0) | 2.70 | (1.79-4.10) | 2.46 | (1.61-3.75) |

*Abbreviations:* IR, incidence rate in events per 1,000 person years; HR, hazard ratio; CI, confidence interval; adj, adjusted; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism. *Adjusted for age, sex, and most recent record of body mass index before the index date; a history of venous thromboembolism, COPD, and varicose veins ever before; a history of major trauma (within the past 6 months) or cancer (within the past 12 months); pregnancy; and use of contraceptives, hormone replacement therapy, non-steroidal anti-inflammatory drugs, anticoagulants, and antibiotics in the previous six months. †See methods section for definitions.

**TABLE 5.2.3.** Stepwise adjustment of risk of VTE associated with MS as compared to patients without MS matched by birth year, sex, and practice.

| | | Hazard ratio | 95% CI |
|---|---|---|---|
| I | Age-sex adjusted | 2.81 | (2.26-3.49) |
| II | + Common risk factors* | 2.56 | (2.06-3.20) |
| III | + Use of corticosteroid† | 2.42 | (1.93-3.02) |
| IV | + Use of antidepressants† | 2.27 | (1.81-2.85) |
| V | + Disability‡ | 2.18 | (1.74-2.74) |
| VI | + Spasticity‡ | 1.79 | (1.38-2.31) |

*Abbreviations:* CI, confidence interval. *Adjustment similar to the fully adjusted model in Table 5.2.2. †Any prescription within the past 6 months. ‡Record within the past 6 months; see methods section for definitions.

## DISCUSSION

In this population-based matched cohort study, we found that MS was associated with a 2.6-fold increased risk of VTE. Within the MS cohort, the risk of VTE was increased in patients with a recent record of spasticity (2.6-fold), disability (2.0-fold), or who had recently been exposed to high-dose glucocorticoids (2.3-fold), as compared to MS patients without these risk factors. These MS-related factors appeared to be important confounders in the relationship between MS and VTE, as adjustment led to a noticeable reduction in the risk estimate. Expectedly, well-known VTE risk factors (*i.e.* prior VTE, varicose veins, recent major trauma, and high BMI) were also found to be associated with an increased risk of VTE in patients with MS.



Our results are largely in agreement with those reported in recent epidemiologic studies. A recent Danish study by Christensen *et al*. (2012) found a roughly twofold increase in the occurrence rate of DVT and PE in patients with MS. In contrast with our results, Christensen *et al*. found the risk to be the highest in the first year of follow-up for both DVT (adjusted incidence rate ratio; aIRR 3.02, 95% CI 2.14-4.26) and PE (aIRR 2.85, 95% CI 1.72-4.70). Of relevance, while our study included data from general practitioners, Christensen *et al*. relied on hospital admission data for a significant part of the study period (from 1977 to 1995). The first MS diagnosis was taken as the start of follow-up. As a consequence, the first year of follow-up may be considered as the year following the first recorded acute MS relapse for, at least, part of the study period. Another study that compared the risk of VTE in patients hospitalized for MS with a national incidence rate, also found the risk to be particularly elevated in the first year after hospitalization (Zoller *et al*, 2012).

Conversely, a study by Ramagopalan *et al*. (2011) among hospitalized patients did not observe the risk to be higher in the first 90 days of follow-up for patients admitted for MS as compared to patient hospitalized for other reasons. Instead, they found a consistent 2-fold elevated risk of VTE during the study period. Like other recent studies, our results are in disagreement with the early notion of Kaufman *et al*. (1988) that spasticity in MS patient may provide protection against VTE events. Contrarily to this hypothesis, we found muscle spasticity to be independently associated with a significant 2.6-fold increased risk of VTE among MS patients. All previous studies were limited by the lack of adjustment for important risk factors, especially those factors directly related to MS (*e.g.* immobilization). Although Christensen *et al*. (2012) stratified their analysis by cumulative number of MS-related hospitalizations, the relapsing-remitting nature of MS makes this measure of disease severity less suitable.

There are various explanations for the increased risk of VTE found in patients with MS. The pathophysiology of VTE is considered to comprise three interrelated factors ("Virchow's triad"); damage to the vessel wall, slowing down of the blood flow, and increased coagulability. It has been postulated that autoimmune diseases in general cause a hypercoagulable state due to inflammatory processes (Zoller *et al*, 2012). Studies have reported alterations in the coagulation and biochemical status in MS patients (Aksungar *et al*, 2008; Minagar *et al*, 2001). A relationship between disease activity and VTE has been described for inflammatory bowel disease (Grainge *et al*, 2010). Of note, acute exacerbations of autoimmune diseases are often treated with corticosteroids, which themselves have been associated with an increased risk of VTE (Huerta *et al*, 2007; Johannesdottir *et al*, 2013). We also found an

**TABLE 5.2.4.** The association between study covariates and VTE, in the population of MS patients (n=5,566).

| | | | | Age-sex adjusted | | Fully adjusted* | |
|---|---|---|---|---|---|---|---|
| **General risk factors** | | **VTE** | **IR** | *HR* | *(95% CI)* | *HR* | *(95% CI)* |
| *Smoking* | Never | 37 | 3.1 | 1 | (reference) | 1 | (reference) |
| | Current | 27 | 5.9 | 1.27 | (0.77-2.10) | 1.16 | (0.69-1.94) |
| | Past | 20 | 3.4 | 1.75 | (1.01-3.03) | 1.52 | (0.87-2.65) |
| | Unknown | 31 | 4.0 | 1.14 | (0.70-1.87) | 1.23 | (0.73-2.07) |
| *Body mass-index (kg/m²)* | <20 | 7 | 2.6 | 1.12 | (0.49-2.54) | 1.12 | (0.49-2.55) |
| | 20 - <25 | 31 | 2.7 | 1 | (reference) | 1 | (reference) |
| | 25 - <30 | 32 | 4.3 | 1.45 | (0.85-2.30) | 1.21 | (0.73-2.00) |
| | ≥30 | 31 | 6.4 | 2.32 | (1.41-3.81) | 1.73 | (1.04-2.90) |
| | Unknown | 14 | 3.1 | 0.96 | (0.51-1.82) | 0.79 | (0.40-1.57) |
| *History of VTE* | No | 100 | 3.3 | 1 | (reference) | 1 | (reference) |
| | Yes | 15 | 41.9 | 8.67 | (4.97-15.12) | 5.56 | (2.99-10.35) |
| *History of varicose veins* | No | 111 | 3.6 | 1 | (reference) | 1 | (reference) |
| | Yes | 4 | 29.0 | 7.50 | (2.76-20.42) | 5.93 | (2.15-16.38) |
| *Major trauma†* | No | 110 | 3.6 | 1 | ref. | 1 | ref. |
| *(previous 6 months)* | Yes | 5 | 18.6 | 3.86 | (1.56-9.51) | 3.17 | (1.28-7.86) |
| *Antibiotics* | No | 88 | 3.9 | 1 | (reference) | 1 | (reference) |
| *(previous 6 months)* | Yes | 27 | 3.3 | 1.51 | (1.02-2.21) | 0.98 | (0.65-1.48) |
| **MS-related risk factors** | | | | | | | |
| *MS duration (years since first diagnosis)* | <1 | 20 | 4.0 | 1 | (reference) | 1 | (reference) |
| | 1-5 | 45 | 3.2 | 0.77 | (0.45-1.31) | 0.83 | (0.49-1.42) |
| | >5 | 50 | 4.1 | 0.80 | (0.47-1.38) | 1.00 | (0.57-1.76) |
| *Disability†* | No | 92 | 3.2 | 1 | (reference) | 1 | (reference) |
| *(previous 6 months)* | Yes | 23 | 10.3 | 2.66 | (1.66-4.26) | 2.04 | (1.26-3.31) |
| *Spasticity†* | No | 77 | 2.9 | 1 | (reference) | 1 | (reference) |
| *(previous 6 months)* | Yes | 38 | 9.0 | 2.75 | (1.86-4.07) | 2.59 | (1.72-3.91) |
| *Glucocorticoids (previous 6 months)* | No | 97 | 3.4 | 1 | (reference) | 1 | (reference) |
| | Any | 18 | 7.0 | 2.36 | (1.42-3.91) | 2.02 | (1.20-3.41) |
| | Low‡ | 7 | 7.1 | 2.04 | (0.95-4.40) | 1.72 | (0.78-3.78) |
| | High§ | 11 | 7.0 | 2.62 | (1.40-4.92) | 2.27 | (1.20-4.31) |
| *Antidepressants* | No | 69 | 3.1 | 1 | (reference) | 1 | (reference) |
| *(previous 6 months)* | Yes | 46 | 5.3 | 1.77 | (1.21-2.59) | 1.31 | (0.88-1.94) |

*Abbreviations:* IR, incidence rate in events per 1,000 person years; HR, hazard ratio; CI, confidence interval. *Adjustment similar to the fully adjusted model in Table 5.2.2 in combination with MS-related risk factors: a record indicating disability or spasticity in the past 6 months; use of glucocorticoids or antidepressants in the previous 6 months. †See methods section for definitions. ‡<0.75 DDD. §≥0.75 DDD.

increased risk of VTE in MS patients who were recently exposed to high-dose glucocorticoid therapy as compared to unexposed MS patients. However, thus far any direct effect of glucocorticoids on coagulation remains controversial (van Zaane *et al*, 2010). The use of NSAIDs was considered as a potential confounder, as these agents have been associated with an increased risk of VTE (Schmidt *et al*, 2011) and exposure in MS patients may be higher to cope with symptomatic pain (Pollmann & Feneberg, 2008). However, inclusion of this variable in an age- and sex-adjusted model did lead to a change in the estimated β-coefficient of more than 1%.

In patients with severe MS, immobilization is likely to be an important factor leading to an increased risk of VTE. Immobilization in MS patients can result from the progressive neurodegeneration or from symptomatic spasticity (Oreja-Guevara *et al*, 2013; Rizzo *et al*, 2004). Muscle spasticity is a common symptom of MS that causes difficulty walking and a higher degree of disability (Rizzo *et al*, 2004). Consequently, spasticity may add to a patient's immobility and thereby further increases the risk of VTE. Lack of mobility is a well-known risk factor for VTE and causes venous stasis with resulting hypoxia and activation of coagulation pathways (Bovill & van der Vliet, 2011). We found MS-related immobility and spasticity were influential on the association between MS and VTE, as adjustment for these confounding factors led to a noticeable reduction in the risk estimate. Moreover, a 2.6-fold increased risk was observed for MS patients with a recent record for spasticity as compared to MS patients without such a record, while a recent record of disability was associated with a 2-fold increased risk. In addition, recent studies have shown that MS patients are more prone to endure fractures (Bazelier *et al*, 2011; Bazelier *et al*, 2012a; Bazelier *et al*, 2012b). However, in our study, recent major trauma did not appear to be an important confounder (<2% change in beta-coefficient) for the association between MS and VTE. It remains debatable whether the residual risk of VTE associated with MS after adjustment for all confounders is attributable to the autoimmune inflammatory processes underlying the disease or the result of residual confounding. Likewise, we cannot deduce from our results whether the use of glucocorticoids has a direct effect on coagulation or whether this should be regarded as a proxy for disease activity.

Major strengths of our study are the use of population-based data, personal matching of a reference cohort of patients without MS, large sample size, and long follow-up. In addition, we had detailed longitudinal information on all subjects with regard to outpatient diagnostic and prescription data, which allowed us to assess the presence of well-known risk factors for VTE in both the exposed and unexposed cohort. Information on home visits, nursing care, patient's residential care, and use of walking aids enabled us to identify patients with reduced mobility. As far as we know, our study is the first to assess the presence of VTE risk factors among MS patients and to assess whether the increased risk of VTE reported by previous studies is confounded by the presence of these risk factors. Lastly, we discriminated between probable and possible VTE events, following the recommendations depicted by Lawrenson *et al*. (2000).

There are, however, several important limitations of our study. As a result of the dynamic nature of CPRD and left truncation of the data, a proportion of the MS patients were likely to be prevalent cases. The mean age on the index date (*i.e.* first recorded diagnosis) was 44 years in our study, which is older than the typical age of MS onset (Bermel *et al*, 2010). Also, the uneven distribution of MS-related factors at baseline provides evidence of

the inclusion of prevalent MS patients in the MS cohort. Consequently, the disease duration calculated might be unreliable. This could explain the lack of association between MS duration and the risk of VTE. In addition, some misclassification of MS diagnosis may have occurred. However, since misclassifications of MS would only abate the actual exposure, this would cause the hazard ratios to be biased towards the null. Also, the partial linkage of practices to HES, may have resulted in selection bias, as linked practices likely contributed a larger amount of probable events. However, given the grouping of probable and possible events, we believe this selection bias had minor influence on the overall results. Furthermore, although we constructed a proxy indicator for disability, we did not have routinely collected information on the degree of disability in MS patients or on the course of their disease. As a result, we may not have been able to account for all confounding by disability, which may have led to an exaggeration of the residual risk of VTE after adjustment for immobility. In addition, lack of specificity in the criteria used to validate the study outcome, as well as the inclusion of secondary diagnoses to identify VTE-related hospitalizations, may have led to some misclassification of VTE events. If anything, this would have biased our results towards the null, thereby underestimating the risk of VTE associated with MS. Also, we lacked information with regard to the distribution of genetic predisposition to blood clotting disorders (*e.g.* Factor V Leiden). Conversely, as hereditary hypercoagulability is likely to occur independent of MS, we do not believe this has influenced our findings. Of note, we did not exclude patients treated with anticoagulants at baseline. However, the proportion of patients treated with anticoagulants was fairly similar for both cohorts, with a slightly higher prevalence in the MS cohort. Since treatment with anticoagulants reduces the risk of VTE, this choice in *a priori* study design may have led to an underestimation of the risk of VTE associated with MS. Lastly, we did not compare risks between MS patients with a record of disability or spasticity and reference patients without MS with a similar record. As a result, we cannot say if MS was associated with an additional increased risk within a subpopulation of patients who sustained disability or spasticity.



In conclusion, we observed a 2.5-fold increased risk of VTE among patients with MS. In addition, our results provide evidence that this association is, at least partly, mediated through an increased prevalence of disability, spasticity, and treatment with glucocorticoids in MS patients. Well-known risk factors (*i.e.* prior VTE, varicose veins, major trauma, obesity) also appeared to be associated with an increased risk of VTE among MS patients. Awareness of potential VTE symptoms is particularly important in MS patients with an increased degree of disability, spasticity, or who are treated with glucocorticoids.

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# CHAPTER 6

# GENERAL DISCUSSION

## INTRODUCTION

When we seek to understand why certain long-term complications occur in patients with progressive chronic diseases, observational epidemiologic research is often the first and perhaps the only approach in our pursuit of explanations. To explain something means to convert the unknown into the known, by making the seemingly unintelligible more intelligible (Evered, 1976). In other words, explanations invariably relate to knowledge on how particular phenomena can be explained in terms of the factors that cause them. In epidemiology, however, causal claims are seldom made explicit. Coming to an unambiguous verdict regarding the nature of an association is hampered in epidemiology mainly because of the inherently flawed comparisons that are made as a result of its non-experimental nature. That is, the observational basis leaves alternative explanations to a causal link between the exposure and outcome under investigation as viable options in epidemiologic studies. For this reason, epidemiology in general operates under the mantra 'correlation does not imply causation'. But however true this might be for epidemiology, measures of association between a specific cause and its presumed effect are of no worth to us without making causal inferences. This mantra, if strictly interpreted, would deem epidemiologic studies of little to no relevance to scientists.



In reality, while conscious of its limitations, conclusions regarding the causal nature of a particular exposure and a health-related outcome are regularly drawn based upon epidemiologic evidence. Such causal inferences are made by interpreting the body of epidemiologic evidence in light of potential alternative ways in which the findings could be explained. The justification of any causal conclusion in epidemiology then resides in our confidence in the effective exclusion of alternative explanations for a given association between a putative cause and a health-related outcome. Only if we believe that all alternative explanations are eliminated or deemed satisfactorily improbable, are we able to pass judgement regarding causation. This manner in which epidemiologic evidence is weighted has led to the criticism that, in the words of Charlton (1996, p.106): "(…) epidemiological attribution of causation is not a science but an activity more akin to the arguing of a case in law: based on evidence but not dictated by the evidence". More importantly, this implies that the indistinctive structure of epidemiologic evidence could make the field of epidemiology vulnerable to authoritarianism, where 'expert opinions' become decisive.

In order to improve upon this rather gloomy perspective requires a more in-depth analysis of the concept of causality and its general use in the interpretation of epidemiologic evidence. Central to this deliberation is the question: *What is good epidemiologic knowledge*? It will be argued that many of the controversies in epidemiology arise from conflicts between different interpretations of or approaches to 'good epidemiologic knowledge'. The question of what good knowledge constitutes can be considered to precede the formation of a scientific method. In other words, it is succeeded by the question: Which methods result in good epidemiologic knowledge? And what is most important, the true meaning of epidemiologic findings can only be established in light of the answers to these fundamental questions.

## Epistemological debate in epidemiology

The questions posed in the previous sections direct the discussion towards the theory of knowledge, or epistemology, reputed to be the most abstract form of philosophy. Science is driven by a search for the truth; by an intrinsic desire for knowledge. We want to understand the inner workings of the world that surrounds us through explanation. We do so by searching for structure; for general universal theories that explain empirical phenomena. The purpose of understanding what causes a certain phenomenon to occur is therefore first and foremost abstract truthfulness. But how should we search for the truth? And how do we know if we found it? While these epistemological questions might seem remote and of little relevance to scientific practice, they have important practical consequences for science and the way in which we interpret its findings (Popper, 1963). As a branch of epistemology, philosophy of science concentrates on the philosophical assumptions, foundations, and implications of science. It is thus aimed at determining what constitutes good knowledge and how we should approach scientific research in order to acquire it.



Like other scientists, epidemiologists want to understand why certain observable phenomena occur. The main questions of this thesis 'Why is the incidence of (particular types of) cancer higher in patients with type 2 diabetes mellitus?' and 'Why is the incidence of venous thromboembolism higher in patients with multiple sclerosis?' are aimed at determining the general relationships between certain determinants and disease. In other words, epidemiologists know that disease exists and seek explanations for its occurrence (Weed, 1986). A great deal of debate amongst epidemiologists on how to acquire good epidemiologic knowledge has transpired over the past decades. In many ways, this debate was aroused by the introduction of the epistemological position formulated by Karl Popper, termed 'critical rationalism'. Briefly, the debate can be described as a disagreement between two fundamentally different views on knowledge. One side holds a dogmatic attitude towards nature – the epistemological position that certainty in knowledge can be established, that nature is an open book, and truth can be revealed through careful observation – while the other side holds a critical attitude – the epistemological position that absolute certainty in knowledge is impossible and hence that any explanation will always remain conjectural and speculative (Popper, 1963).

Conditional on their epistemological position, each side has its own approach on how to conduct and interpret epidemiologic research; that is, how to seek answers to questions of cause and effect. The dogmatic attitude towards nature leads to a methodology aimed at the verification or justification of theories, while the critical attitude results in a methodology aimed at the refutation or falsification of theories. An essential difference between the two is the value that is attributed to logic. In short, logic is concerned with a special kind of thinking about thinking, namely the systematic study of arguments in which a conclusion is the logical consequence of given premises (Black, 1946). Through theoretical inquiry, logic enables a structuring of arguments. As stated by Hume (1743, T.xv): "The sole end of logic is to explain the principles and operations of our reasoning faculty, and the nature of our ideas". As such, the implications of our ideas can be teased out to determine conflicts that might arise between ideas and between our ideas and the empirical world. Logic has a central position in the refutationist approach to scientific discovery, while in the verificationist approach, observation is considered the ultimate source of all knowledge.

A lively debate between epidemiologists with a refutationist (Maclure, 1985; Weed, 1986; Charlton, 1996) and a verificationist (Susser 1986; Rothman 1976) perspective in the last decades of the past century did not result in a clear victory for either side. While verificationists hold that the refutationist approach, "(…) is not a working model founded on the realities of the epidemiologic enterprise" (Susser, 1986, p.712), refutationists argue that the verificationist approach "(…) led epidemiology to move away from scientific status" (Charlton, 1996). With both sides agreeing to disagree, epidemiology was left sort of at a cross-roads. Since then the debate, unfortunately, somewhat quieted down (Lucas & McMichael, 2005). And although this debate led to the contemplation of the refutationist position in epidemiology (Buck, 1975), epidemiologists in general did not reject their dogmatic attitude towards knowledge.

Meanwhile, the fundamental scientific problem for epidemiologists remains: how to propose and test causal hypotheses. A solution to this problem can only be found through an inquiry into the causal nature of epidemiologic hypotheses and our patterns of thought and their impact upon our methodological practices (Weed, 1986). This theoretical analysis will start with a brief discussion of the verificationist and the refutationist approach to scientific discovery. Here, the argument will be made that, although epidemiology has shifted towards the refutationist position, a traditional verificationist perspective still widely prevails. At the same time it will be argued that the refutationist approach leads to better knowledge of the empirical world and also to changes in the classification and structure of epidemiologic evidence. However, since the refutationist principles have not been systematically translated into epidemiological terms and concepts, an attempt to such a translation is required before turning to the interpretation of the empirical content of this thesis. This philosophical inquiry will show that the acceptance of a critical attitude (and thereby the rejection of the verificationist approach) has important practical implications for both the conduct of epidemiologic research and the interpretation of its results. The conceptual framework constructed in the first section of this discussion will subsequently be used to evaluate the empirical evidence presented in the foregone chapters.



## THE PROBLEM WITH VERIFICATIONISM

The verificationist (or inductivist) approach entails that through careful observation we can uncover nature's causal and material structure. It was born out of the positivist empiricist movement at the start of the 19th century which holds the assumption that only statements verifiable by direct observation or capable of logical or mathematical proof are cognitively meaningful (Chalmers, 1999). This philosophical system was fueled by an unparalleled epistemological optimism: "(…) a most optimistic view of man's power to discern truth and to acquire knowledge" (Popper, 1963, p.6). As a result, the verificationist approach firmly rejected any appeal to authority.

At the heart of this anti-authoritarian movement lies the doctrine that *truth is manifest*: the truth perhaps may be veiled, but it may reveal itself or can be unveiled by us. And since each man carried the sources of knowledge in himself – in his power of perception, which can be used for the careful observation of nature, or his power of intellectual intuition, which can be used to distinguish truth from falsehood – there was no

need for any man to appeal to authority in matters of truth (Popper, 1963). This optimistic epistemological position inspired the birth of modern science; as a methodology to search for truth through observation. The main approach to do so, according to the verificationists, is by reasoning *inductively:* to infer general rules from a limited number of observations.

According to the verificationist approach, *induction* is the logical foundation of science. Hence, scientific propositions are those deducible from true observation statements; those concerning facts which can in principle be ascertained by observation (Popper, 1963). Following this line of reasoning, science proceeds from observations to theory. Herein lies the assumption that facts can be observed objectively and, as such, constitute a solid basis for the formulation of scientific theories (Chalmers, 1999). That is, it assumes that nature is truthful and that we can properly read the book of nature by purging our minds of all anticipations, conjectures, guesses, or prejudices (Popper, 1963). Thus, this school of thought holds that scientific propositions are those propositions which can be *verified* based on observation statements. In line with this outlook, the more a theory has been proven to be 'true' by objective observation, the more scientific it becomes (Maclure, 1986).



The doctrine at the heart of the verificationist approach - that truth is manifest - is reflected by the methods that have been developed to determine causality. In 1843, John Stuart Mill formulated five methods of induction, as a guideline to *unveil* nature's causal structure (Mill, 1843). Later, Mill's work was the inspiration for the Bradford Hill criteria, which have been, and still are, widely used in epidemiology to determine causality (Hill, 1965; Rothman, 1986). Clearly, such an approach stems from the underlying assumptions that the recognition of facts precedes the formation of theories and that objective observation leads to the truth.

There are, however, two major problems with verificationism and its underlying doctrine that are of particular relevance to epidemiology. The first, and perhaps most fundamental, problem pertains to the epistemological position from which the verificationist approach is derived: the doctrine that truth is manifest. As a logical consequence of this doctrine, the concept of causality becomes something tangible; something directly observable or measurable. In other words, once the naked truth stands revealed before our eyes, we have the power to see it and know that it is truth (Popper, 1963). However, the history of science, and particularly of medicine, can provide us with numerous examples of erroneous beliefs that have been 'known to be true' for hundreds of years, such as blood-letting and spontaneous generation. The second problem is related to the logical fallacy embedded in verification, as a scientific approach to searching for the truth. Both these issues will be discussed in the following sections.

## The metaphysical nature of the concept of causality

Empirical knowledge refers to a theoretical or practical understanding of a subject; that is, to its causal structure. Knowledge of this kind is dictated by observable facts, the existence of which serves as vital input for the formation and testing of theories. However, by itself the mere existence of particular observable facts – such as surface temperatures on Mars or the incidence rate of cancer among type 2 diabetic patients – does not constitute scientific knowledge. Relevant scientific knowledge pertains to the general relationships

between observable facts. That is to say, it consists of theories of causality. From this perspective, the interesting part of new observations is whether they do or do not concur with our expectations. We thereby place new observations in a framework consisting of our expectations based on pre-existing knowledge. How we conceive these expectations – the nature of our knowledge and the origin of our projections – is the subject of relevance.

According to Hume, the human psychology has a propensity to pass from an 'impression' of a cause to an idea of its effects (or vice versa). In the words of Hume (1739, T1.4.1): "Nature, by an absolute and uncontroulable necessity has determin'd us to judge as well as to breathe and feel (…)". This propensity leads us mistakenly to believe in the existence of a necessary connection between a perceived cause and its effect. According to Hume, this belief is mistaken because empirically speaking, causality is no more than mere regularity, contiguity, and time order. He states that the idea of causation enables us to form beliefs about unobserved facts. To go beyond what we can observe directly, we create a necessary connection amongst objects in our mind, which permits us to project certain expectations. And although the objects themselves can be presented to sense, the perceived connection cannot. Hume argues that there is no object which implies the existence of any other if we consider these objects in themselves and never look beyond the ideas which we form of them. The impression of a necessary connection between them therefore is only to be found in the mind, where it occurs as an accompaniment to our causal inferences. According to Hume, it is from this impression that we derive the idea of necessity at the heart of our idea of causation (Hume, 1739, T1.3.14).



## Rejection of the idea of ultimate sources of knowledge

As Hume, Popper questioned the foundation of our knowledge and thereby the fanatic doctrine of verificationists that truth is manifest and that certainty in our knowledge is theoretically achievable. He argues that even though verificationists set out to free their epistemology from authority, they did not succeed in doing so. Instead, they could only replace one authority – that of Aristotle and the Bible – by another: an appeal to a new authority of the senses or the intellect (Popper, 1963). Observation and reason became the ultimate sources of knowledge, accompanied by a doctrine that nature itself is truthful; that truth is above human authority. Taken together, this train of thought leads to the conclusion that falsehood, or failure to see the truth, can only be explained by prejudices that have poisoned our minds. It was not knowledge, or the possession of truth, itself that needed to be explained (Popper, 1963). This doctrine, according to Popper, leads to authoritarianism simply because the truth is not manifest. Like the widespread belief in spontaneous generation – that life springs spontaneously from inanimate material, such as mice from river banks and maggots from dead flesh – that lasted for centuries, only to be conclusively dispelled during the 19$^{th}$ century on the basis of experimental evidence (Deichmann, 2012). If the truth is indeed manifest, how can it be that we collectively fail to see it for such a long time? Popper argues that since such failure, according to the verificationist doctrine, can only be explained by admitting that our senses and minds have been corrupted, this doctrine gives rise to an uncontrolled need for constant verification and justification of our knowledge by reasons capable of establishing it.

According to Popper, the solution to the failure of verificationists to free their epistemology from authority lies in the rejection of the idea of ultimate sources of knowledge.

Adhering to the dogmatic attitude of the manifest truth, or the truthfulness of nature, and our ability to discover the truth leads to a mistaken belief that certainty in knowledge can be established. Central to the rejection of the idea of ultimate sources of knowledge is therefore the acceptance that all theories and laws that make up our knowledge are metaphysical human constructs. As such, according to Popper, we must admit that even though our explanations are often inspired, our inspiration carries no authority (Popper, 1963). In other words, Popper argues that we do not draw our knowledge from nature but impose it upon nature. In agreement with Hume, Popper holds that we have instinctive expectations, or an inborn 'knowledge' prior to all observational experience. The most important of these expectations is the expectation to find a regularity. By observing and judging nature, we form ideas about what we see and forge connections between objects in our minds. However, this by no means implies that whatever we conceive must necessarily be true.



By questioning the foundation of our beliefs, Popper formulated a truly anti-authoritarian epistemological position: *critical rationalism*. However, in order to admit that our knowledge is fallible, without at the same time implying that it is all arbitrariness, a regulative mechanism is needed by which we can eliminate erroneous beliefs. That is, we need to attain the idea of objective truth as the standard which we may fall short of. But once we admit that our knowledge is intrinsically fallible – that all of us, singly and collectively, may and often do err – no fanaticism springs from attaining the idea of objective truth (Popper, 1963). Objective truth merely becomes a regulative principle that guides our search. This radically different epistemological position implies that we may seek for truth, but we may not know when we have found it. That is, "(…) all we can do is grope for the truth even though it be beyond our reach" (Popper, 1963, p.39). Concomitantly, since our ideas are fundamentally fallibile, we should allow our beliefs to be tested in order to determine whether they are fallacious. In our search for truth, the focus then no longer lies on the psychological aspect of how we arrived at our ideas or in attempts to verify their truthfulness, but solely on their testability.

## Logical criticism of induction

The logical *problem of induction* is perhaps the most commonly referred to criticism to the verificationist approach (Rothman, 1986). Inductive arguments proceed from a finite number of specific facts to a general conclusion. However, by proceeding from statements about *some* events to statements about *all* events of a particular kind, they go beyond what is contained in the premises. General statements regarding causality invariably go beyond the finite amount of observable evidence that is available to support them. For that reason, they can never be proven in the sense of being logically deduced from the evidence. Neither will any finite amount of observable evidence make conclusions drawn from inductive reasoning even more probable (Chalmers, 1999).

Hume has undoubtedly been most influential to point out the problem of induction. He states that "(…) all inferences from experience suppose, as their foundation, that the future will resemble the past, and that similar powers will be conjoined with similar sensible qualities" (Hume, 1748, E4.2.32). Any attempt to establish the validity of inductive reasoning by referring to the observations themselves will then inherently lead to a circular argument: "It is impossible, therefore, that any arguments from experience can prove this resemblance

of the past to the future, since all these arguments are founded on the supposition of that resemblance" (Hume, 1748, E4.2.32). Hume argues that: "In reality, all arguments from experience are founded on the similarity which we discover among natural objects, and by which we are induced to expect effects similar to those which we have found to follow from such objects" (Hume, 1748, E4.2.31). In his examination of the principle of human nature that gives this mighty authority to experience, he concludes that: "It seems evident that, if this conclusion were formed by reason, it would be as perfect at first, and upon one instance, as after ever so long a course of experience. But the case is far otherwise" (Hume, 1748, E4.2.31). And while Hume spent considerable effort trying to understand the human mind's propensity to accept causal statements based on inductive arguments, he ultimately concluded that "(…) it is not reasoning which engages us to suppose the past resembling the future, and to expect similar effects from causes which are, to appearance, similar" (Hume, 1748, E4.2.33).



For verificationists, the problem of induction is not necessarily considered to pose a problem. The logical fallacy contained in the inference of general rules from a finite number of observations can be dismissed since, according to their dogmatic doctrine, nature itself is truthful. The statements by Savitz (2003) and Rothman (1986) with regard to the quantification or estimation of causal effects as the primary goal of epidemiologic research, as referred to in general introduction to this thesis, are testament to the dismissal of the problem of induction. Perhaps such theoretical criticism is considered insignificant in light of the evident truth. With objective observation as the ultimate source of knowledge – that enables us to read the book of nature – any requirement that empirical statements should be logically deduced from (or dictated by) our observations is waivered. However, for critical rationalists, the problem of induction signifies the inherent uncertainty built into our knowledge and became illustrative of the misconception that lies at the foundation of the verificationist doctrine: that we draw our knowledge from nature.

Hume was particularly intrigued by the process of the mind to expect certain behavior based on past experience. For Popper, however, it did not matter how the human mind conceives its ideas, whether this be by unfounded inductive arguments based on past experience or from some sort of creative thought. For him the only thing that mattered was that the truth of any idea, any theory or law, no matter how many times it might be verified by particular instances, cannot in any sense be established from empirical evidence. And since our knowledge has no logical foundation, Popper argues that our knowledge consists merely of guesses, of hypotheses, rather than of final and certain truths. This, by necessity, leads to the concomitant realization that our attempts to find the truth are never final. Criticism and critical discussion are then the only way of getting nearer to the truth; through trial and error (Popper, 1963). Therefore, an entirely different question should be asked: How can we hope to detect and eliminate error?

## CRITICAL RATIONALISM

The question from the previous paragraph brings us to the approach central to the epistemological position of critical rationalism. That the acceptance by science of a law or a theory is tentative – as the laws and theories that make up our knowledge are inherently

conjectural – implies that we may reject them based on new empirical evidence. That is, *although we cannot prove a theory to be true, we can prove it to be false*: "Only the falsity of the theory can be inferred from empirical evidence, and this inference is a purely deductive one" (Popper, 1963, p.72). Deductive logic is a valid form of reasoning as it starts with general statements, regardless of how we arrived at them, and uses singular observation statements to reach a logical conclusion. Through logical deduction it is possible for new observations to serve as premises, in order to arrive at the falsity of universal laws and theories (Chalmers, 1999). As a logical prerequisite for this approach, tentative statements should be testable in order for them to be considered scientific. In other words, testability, or falsifiability, is the criterion of demarcation between science and pseudo-science: "(…) statements or systems of statements, in order to be ranked scientific, must be capable of conflicting with possible, or conceivable, observations" (Popper, 1963, p.51).



## Testability as the criterion of demarcation

The fundamental condition that a general statement should satisfy in order for it to be considered scientific, is that it must be *falsifiable*. In this respect, a scientific statement should clearly specify the conditions needed to prove it to be false. Refutationists (or falsificationists) demand that scientific hypotheses be falsifiable because it is only by ruling out a set of logically possible observations that a law or theory is informative. When general statements are unfalsifiable, the world can have any properties whatsoever, and can behave in any way whatsoever, without conflicting with the statement (Chalmers, 1999). So, a good scientific theory is falsifiable because it makes definite claims about the world.

The approach to scientific discovery, founded on the critical rationalist perspective on knowledge, then proceeds through trial and error. It involves the proposal of testable hypotheses, followed by deliberate and tenacious attempts to falsify them (Chalmers, 1999). Only by proposing theories that make definitive claims and testing them against the empirical evidence can we know which ideas about the world are false. However, the opposite should never be interpreted as evidence that proves a theory to be true. Refutationists thereby reject the doctrine of verificationists that we should accept a belief only if it can be justified by positive evidence; if it can be shown to be true or be highly probable of being true. As stated by Popper: "For us [falsificationists], therefore, science has nothing to do with the quest for certainty or probability or reliability. Conscious of our fallibility we are only interested in criticizing them and testing them, hoping to find out where we are mistaken; of learning from our mistakes; and, if we are lucky, of proceeding to better theories" (Popper, 1963, p.310).

## The evolution of knowledge

It follows from this perspective that the more a theory claims, the more potential opportunities there will be for showing that the world does not in fact behave in the way laid down by the theory and thus the more potential it has to be false. A theory that makes wide-ranging claims about the world is consequently highly falsifiable (Chalmers, 1999). This raises an interesting point of the critical rationalist perspective, namely that a hypothesis can be judged *a priori*, based on its empirical or informative content. That is to say, as Popper puts it: "We can know of a theory, even before it has been tested, that if it passes certain tests it will be better than some other theory" (Popper, 1963, p.294). This criterion of relative

progressiveness allows us to grade theories: it characterizes as preferable the theory which tells us more; the theory which contains the greater amount of empirical information or content; which has the greater explanatory and predictive power; which is logically stronger; and consequently which can be more severely tested by comparing predicted facts with observation (Popper, 1963). Empirical content, in a falsificationist sense, constitutes the class of all basic statements which contradict the theory and indicates the degree of falsifiability. For example, the statement 'all *Felidae* purr when petted' is preferable to 'all domesticated cats purr when petted' as the first statement tells us all the latter one does, and more. And while the second statement may at first sight appear to contain more detailed information, the first statement is preferable from a falsificationist point of view as it has a larger number of potential falsifiers.

Against this background, the growth of scientific knowledge can be better explained as an evolutionary process, in which inadequate theories are replaced by better ones. In brief, the empirical content of the better theory exceeds that of the previous one. As such, the better theory makes more precise predictions, and these more precise predictions stand up to more precise tests. It takes account of and explains more facts than the rival theory, has passed tests which the rival theory failed to pass, and suggests new tests not considered before the inception of the theory and passes these tests (Popper, 1963). Falsificationists therefore welcome the proposal of bold conjectures. It follows from a methodological conviction that only by means of such bold conjectures may we hope to discover interesting and relevant truth (Popper, 1963). As stated by Popper: "I can therefore gladly admit that falsificationists like myself much prefer an attempt to solve an interesting problem by a bold conjecture, even (and especially) if it soon turns out to be false, to any recital of a sequence of irrelevant truisms. We prefer this because we believe that this is the way in which we can learn from our mistakes; and that in finding that our conjecture was false, we shall have learnt much about the truth, and shall have got nearer to the truth." (Popper, 1963, p.313).

The idea that knowledge develops through the tentative acceptance of conjectures, brings us back to the epistemological aspect of the critical attitude. As mentioned previously, critical rationalists hold that we have an inborn propensity to look out for regularities. By means of this natural urge to find regularities, we project conjectural theories (by means of 'irrational' thought) upon the world and see whether they hold up to the test (by means of rational deduction). On an epistemological level, falsificationists therefore reject the doctrine that observation of facts precedes the formation of a theory. On the contrary, falsificationists argue that science proceeds from theory to observation and that, to use the words of Popper, "(…) the belief that we can start with pure observations alone, without anything in the nature of a theory, is absurd" (Popper, 1963, p.61). In other words, to establish significant facts about the world we need some guidance as to what kind of knowledge we are seeking or what problems we are trying to solve (Chalmers, 1999). The growth of knowledge then proceeds by the formation of tentative theories – this is true for scientific knowledge, as well as for individual beliefs – which are tested against the evidence and improved upon by the formation of better theories with more empirical content.

## A CRITICAL APPROACH TO EPIDEMIOLOGIC KNOWLEDGE

The previous sections focused on scientific discovery in general and were intended to highlight the philosophical problems related to the verificationist approach. In the following paragraphs these issues will be discussed for the field of epidemiology. The relevance of this discussion is proven by the still dominant dogmatic attitude towards knowledge among epidemiologists. The ongoing regular use of the Hill criteria to assess causality and the advocacy to appoint the quantification of causal effects as the main focus of epidemiology are but two examples. It is also this doctrine – that truth is manifest – that underlies the statement of Savitz (2003, p.23) that "(…) capturing causal relations with accuracy is tremendously challenging". In addition, the use of meta-analysis techniques in observational research, not aimed at eliminating random variability (or chance) but as a tool to prove consistency among results, is tantamount to an attempt to prove a causal relation based on positive evidence (or verification). More importantly, Savitz (2003) immediately proves the inherent vulnerability of this dogmatic epistemological position to authoritarianism by suggesting that when it comes to the interpretation of epidemiologic evidence: "An easier and perhaps more commonly used approach to assessing evidence is to rely on a summary judgment of experts, either individually or as a committee" (Savitz, 2003, p.30).



It can be considered surprising that, while the philosophical fallacies of the dogmatic attitude towards knowledge has led the scientific enterprise to adopt the critical rationalist perspective, epidemiology at large has resisted this change. Admittedly, statistical hypotheses testing has largely been implemented, but the idea that certain knowledge is obtainable has never been abandoned. Perhaps it have been the demands of health policy decision making to find a definitive answer to questions concerning public health that have prevented epidemiology to discard the verificationist doctrine (Charlton, 1996; Weed, 1986). The conviction that the impatient demands of health policy trump the methodological rigor of critical rationalism probably also underlies the objection that critical rationalism is not a working model for epidemiology. However, these pragmatic arguments would be hallow if the fundamental problem of verificationism was acknowledged. That is, such pragmatic arguments have the connotation that the objections against the dogmatic attitude – the manifest truth and the belief in the existence of ultimate sources of knowledge – are frivolous. In other words, the argument that the critical rationalist approach leads to better epidemiologic knowledge is not recognized.

The verificationist doctrine that observation is the ultimate source of knowledge might also have a naturally appeal to epidemiologists. Not only does it imply that epidemiologists can unveil nature's causal structure, which provides them with a sense of purpose, but epidemiology, by its very nature, might have to rely on these beliefs. The methodology of correcting and adjusting for all potential interfering dynamics in epidemiology – to exclude all alternative explanations in order to ultimately unveil the causal nature of an association – is dependent on the idea that we *can know the truth*. That is, these methodological techniques are firmly based on the doctrine that knowledge can be *secured*, as opposed to the critical outlook that our causal theories are speculative from which experimental research operates. It is exactly this difference in epistemological position based on which results generated by experimental research are attributed greater value (Greenland, 1990).

In connection to this, the implication of the critical rationalist approach that science progresses through trial and error – that explanations should be subjected to crucial tests – poses a problem for epidemiology. It requires an evident interpretation of results, in which the empirical evidence dictates a final verdict. Since alternative explanations are not systematically remedied, epidemiology faces a lacking potential to construct stringent tests, perhaps causing epidemiologists to feel they had to denounce the critical attitude for the fear of becoming irrelevant. After all, trial and error is hindered when the answer to the question whether a measured association reflects a causal effect, in the words of Savitz (2003, p.23), "(…) will always be 'maybe' with the goal of making an accurate assessment of where the evidence fits within the wide spectrum that extends from the unattainable benchmarks of 'yes' or 'no' ". However, regardless of the implications, the evident fallacies connected to the verificationist perspective necessitate a change in epistemological position, in which epidemiology can form no exception.



## A shift in epistemological foundation

Several epidemiologists have argued that epidemiology has largely neglected deductive logic (Maclure, 1985; Weed, 1986; Charlton, 1996). In other words, researchers within the field of epidemiology are reluctant to propose, test, and reject explanations in favor of better ones (Weed, 1986). Often, the argument revolves around the criticism that inductive reasoning lies outside the realm of logic, whilst deductive reasoning is logically sound. The underlying assumption here is that the *modus operandi* in epidemiology relies on inductive reasoning; that it is centered on verification of hypotheses. An argument can indeed be made that in epidemiology, the presentation of research and the interpretation of evidence often involves an appeal to induction. The emphasis on positive evidence from different research areas and consistency in results between studies on a particular subject, as arguments for the probability of an association being causal, constitute as two examples. However, it is not as often recognized that this appeal to inductive arguments originates from a more fundamental epistemological level; the doctrine that truth is manifest.

Inductive reasoning and verification can only be justified by assuming that the objective observation of absolute truths is possible. So, to change our approach to finding epidemiologic explanations from an appeal to inductive arguments to the use of deductive logic, we first need to change the underlying epistemological doctrine from a dogmatic attitude to a critical attitude towards epidemiologic knowledge. However, this change in outlook requires that we acknowledge that our explanations, regardless of our personal convictions, are fallible. And that only by allowing our theories to be subjected to potential refutation, we gain knowledge about the truth.

## Causality in critical epidemiology

Not surprisingly, the clash between verificationists and refutationists in epidemiology culminates when it comes to their views on causality. Verificationists believe causality to be a feature of nature, while falsificationist see it as a metaphysical human construct. As a result, verificationists have attempted to formulate objective criteria to infer causation from observations; to determine the 'nature' of an observed association. Such criteria for causal inference (like the Bradford Hill criteria), as well as conceptual frameworks of non-necessary component or sufficient causes, exemplify the verificationist attitude towards causation;

that causal connections can be unveiled by means of objective observation. On the other hand, refutationists reject the idea of the existence of any objective criterium to attribute causation – to establish truth – since the concept of causation is intangible. In other words, it cannot be observed empirically or deduced from observable facts. Refutationists argue that the concept of causation is merely a product of our imagination which cannot be proven, only criticized. These contrasting views on the concept of causation – as an objectively observable feature of associations on the one hand and as an intangible imaginary feature of objects conceived by the mind on the other – lead to differences in definitions and hence in the interpretation of empirical evidence.



According to verificationists, a consistent association, independent of specificity, can be considered as proof for the causal nature of that association. That is to say, it depends to a great extent on sheer weight of evidence – the massed number of studies – to attribute causation (Charlton, 1996). Moreover, based on the doctrine that truth is manifest, evidential fragments from other scientific disciplines can be used to bridge gaps in the available epidemiologic evidence. The attribution of causation from a verificationist perspective is thereby "(…) based on evidence but not dictated by the evidence" (Charlton, 1996, p.106). Consequently, this approach to a great extent must rely on *judgement* and, as such, the line between what constitutes as a causal relationship and what as a mere association is ultimately subjective. Arguments for the attribution of causation then always refer to empirical observations as *positive evidence* to proof the causal nature of an association. In other words, by referring to particular instances in which the 'veil was lifted' and the causal structure of nature was revealed to us. As a consequence, epidemiologic theories are supported by a network of linked evidence from numerous disciplines, where contradictory findings cannot do more than alter the balance of probability of causation (Charlton, 1996).

From a critical rationalist perspective, the concept of causation constitutes an idea, intangible and abstract. As such, cause and effect can be considered as conceptual definitions that refer to a relationship between objects. More specifically, these abstract terms refer to the ideas that were formed of objects and not to the objects themselves. As stated by Hume: "When the mind, therefore, passes from the idea or impression of one object to the idea or belief of another, it is not determined by reason, but by certain principles, which associate together the ideas of these objects, and unite them in the imagination. Had ideas no more union in the fancy than objects seem to have to the understanding, we could never draw any inference from causes to effects, nor repose belief in any matter of fact. The inference, therefore, depends solely on the union of ideas" (Hume, 1739, T1.3.6). As a consequence, from a critical rationalist point of view, the nature of this connection is determined *a priori* and independent of the empirical evidence. Explanations, in the sense of cause and effect relationships, then consist of *necessary* connections between objects. Only by creating a necessary connection, are we able to go beyond what is immediately present to the senses; to formulate predictions. This necessary connection – the concept of causation – is the determination of the thought to pass from causes to effects, and from effects to causes (Hume, 1739, T1.3.2). And since causation is an abstract concept, its component parts – cause and effect – are abstract concepts as well, where the former is inevitably followed by the latter.

*Causality versus association*

Verificationists claim that "arguments [regarding causation] that demand specificity are fallacious, if not absurd" (Susser, 1995, p.713). As an example, Susser (1995) argues that marital status, in the form of transition into widowhood, has been found to cause suicides, entry into psychiatric care, and cirrhosis of the liver. With this statement, Susser provides a clear example of an argument containing a reference to positive evidence to attribute causation that is subsequently used to define causation. In contrast, from a critical rationalist point of view, it seems that in this example causation is confused with association. In other words, losing a spouse might be *associated* with suicide, psychological instability, and alcoholism, but this does not provide us with any *explanation* for these associations; it is merely a description of observations. The interesting part of science is to seek explanations that tell us *why* certain phenomena are associated with each other. Explanations, according to the critical rationalist, can be understood only as general rules containing necessary connections between phenomena; only then are we able to make predictions about unobserved objects. If in this example in fact the exposure or cause is similar in all instances (which is already dubious), there must be other determinants that explains why people have different ways of coping with tragedy. Arguing against specificity of hypotheses – discouragement of thinking in terms of necessary causes – then seems like arguing against the search for explanations.



In this perspective, the common use of multi-causal models – the idea that health-related states are the result of a network, constellation, or web made up of determinants, components, or contributory causes – demonstrates the widespread acceptance of the dogmatic attitude towards knowledge and the verificationist approach to scientific discovery. According to some critical rationalists, epidemiology has merely devised less rigorous modes of reasoning by adopting multi-causal risk factor models (Charlton, 1996). Such a framework of component, sufficient, and necessary causes, when followed to its logical conclusion, would only lead us down the road towards post hoc adjustments to bold statements and ultimately to a mere description of the empirical world in terms of associations between phenomena, instead of explanations for them. For example, in this *sufficient component cause* framework measles virus is a necessary cause for measles, whereas lack of immunity to measles virus is considered a sufficient component cause (Rothman, 1995). However, while implying a similar causal scheme, general conjectures stating necessary connections that 'a first-time exposure to measles virus causes illness' and 'immunological memory after an initial measles virus infection effectively immunizes a person to future like infections' are preferable, as each statement by itself is simply falsifiable. Admittedly, when increasing amounts of intermediate steps in the proposed chain of causation are skipped or remain undefined, we might lose track of what constitutes a necessary cause. But in such instances, we should determine at what point in the chain of events specificity was lost – where we are mistaking – instead of settling for plain descriptions.

In a reductionist view of a chain of necessary connections, each event is the result of a *specific* antecedent event. For instance, when the first domino in a line of dominoes is toppled, it sets in motion a causal chain, where each domino is toppled by the antecedent domino. While strictly speaking, we only made the first domino topple, the perfect correlation between toppling the first domino and toppling all remaining dominoes leads us to say that by pushing over the first one, we *caused* the toppling of all dominoes. In a similar fashion, when we observe an association between cancer incidence and type 2 diabetes mellitus, we

seek an *explanation* for this observed association. Surely, type 2 diabetes mellitus is not a necessary cause of cancer, since the association is weak and non-specific. That is, in contrast to what verificationists might argue, a consistent association by itself does not serve as an explanation for the critical rationalist.

To truly explain the perceived connection, we need to build a chain of necessary causes (Charlton, 1996). Certainly, this might be easier in some cases than in others, depending on the transparency of the biological process and our ability to formulate and test meaningful causal hypotheses. Referring to the bedrock of epidemiology, for infectious diseases the chain of events often more resembles a line of dominoes, where the toppling of the first domino (*i.e.* exposure) causes the fall of the last domino (*i.e.* clinical presentation of illness). And even though we might not comprehend the entire chain of events in all its complexity, a high degree of correlation enables us to skip intermediate steps in our search for an explanation; a necessary connection. That is to say, if a general statement that first-time exposure to measles virus necessarily causes measles is not falsified by particular observations, there is no need for better explanations and perhaps no need to further reduce the causal scheme. However, in any case, such a reduction of the hypothesized causal pathway into its intermediate steps stimulates thinking in more general terms and enables the formation of more universal rules. As in the example of the dominoes, reduction leads us to see that what we in fact witness is the repetition of the same event over and over again, each time a domino in a line of dominoes falls. This reductionist view ultimately even stimulates the formulation of general laws of physics that would not have been thought of when observing the line as a holistic entity. In similar ways, the events associated with changes in marital status, as referred to by Susser, should be further explained by reducing them into their intermediate steps. Surely, nobody would argue that the loss of a spouse directly causes liver damage.



As we seek to explain certain phenomena, we seek reliable knowledge; a chain consisting of necessary causes with a high degree of specificity (Charlton, 1996). Counterarguments for a chain of necessary causes that appeal to the verificationist doctrine by referring to observation statements as positive proof – for example, that "(…) [in reality] causal effects are far more likely to be small and probably far more likely to be tiny" (Ioannidis, 2015, p.12) – do naturally not suffice for critical rationalists. By changing the epistemological position from which we approach epidemiology, the concept of causation automatically shifts from something that can be proven by means of observation to something fundamentally intangible that can only be disproven; from *something arguable* to *something not in need of any arguments*. According to the critical rationalist approach, we should therefore criticize and test our ideas of causal connections against objective truth by the critical use of empirical evidence. This means that we should allow our beliefs to be open to severe criticism in order to find out where we are mistaken and hopefully proceed to better theories.

## Testability of epidemiological hypotheses

When it comes to subjecting our ideas regarding cause and effect to severe scrutiny, experiments are the primary weapon in the scientist's toolbox. They enable the testing of a theory's predictions in a standardized controlled environment in which the putative cause is the only significant independent variable. As such, experimental research aims at formulating

a most rigorous test; a crucial attempt to refute a theory. Often, however, empirical explanations cannot be tested by experimentation for theoretical, moral, or economic reasons. In these circumstances, analytical epidemiology is often burdened with the task of providing tests for empirical explanations. However, due to its observational nature – with its inherent non-random distortion of comparisons as a result of imperfect interchangeability between groups – epidemiology has tremendous difficulty in constructing decisive tests, as alternative explanations can never be ruled out entirely. By not systematically excluding alternative explanations, the hypothetico-deductive process comes to a grinding halt.

Be that as it may, this inherent limitation does not justify the rejection of critical rationalism. Thus, the notion that untestable hypotheses cannot be labeled as scientific and thus do not contribute to scientific knowledge still stands. Consequently, whether epidemiologic explanations can be considered scientific then depends on the ability of observational research to contrive a decisive test in spite of these limitations. Admittedly, untestable statements in immediate practical terms might not necessarily be unscientific (Susser, 1986). However, it does matter whether a hypothesis has survived any crucial test. Thus, the content of conjectures that are theoretically testable but practically untestable and the implications that emanate from them, should not be given much consideration, except for the purpose of constructing a crucial test. Not until a theory has survived decisive tests, should it be tentatively accepted. But to devise such an evolutionary process in epidemiologic knowledge – one that progresses through trial and error – requires a rather extensive effort in theoretical development (Weed, 1986); it requires that we improve the scientific rigor of observational research and reason about its theoretical abilities and limitations.



## A priori deliberation

Without a doubt, analytical epidemiology faces a lacking potential to determine conclusively the falsehood of a tentative theory and therefore has a limited ability to discriminate between sense and nonsense; explaining the spiraling of epidemiologic explanations. In part, this is due to the appeal, out of necessity, to various forms of statistical adjustment for interfering dynamics. Such attempts only lead to an increase in complexity of the causal scheme involved and hence in the study's design to effectively account for all potential sources of bias. Recall from the general introduction to this thesis, that the existence of bias can only be discussed by interpreting the data in light of some causal theory. To do so requires an *a priori* conceptualization of the causal schemata involved which should not only contain the nature of the hypothesized link between the exposure of interest and the study outcome, but also that of all assumed interfering dynamics considered relevant.

From a critical rationalist perspective, however, the exemption from scrutiny of all other operationalized relationships in a statistical model might be problematic. Particularly because such practice operates under the assumption that all other modelled relationships are incontestable truths. In other words, it requires comprehensive *certain knowledge* of all relevant interfering dynamics in order to adjust for them. So, the assumption of certain knowledge reappears as a prerequisite for statistical adjustment that allows the decisive testing of any single hypothesized link. This in contrast to experimental research, which recognizes the *fundamental uncertainty of knowledge* with regard to interfering variables by resorting to randomization and controlled experiments. Simply because knowledge is far

from certain, the decisiveness of observational studies, as tests for empirical hypotheses, is undermined when the causal hypotheses for all alternative explanations included in the model are not also subjected to criticism. Configuring such an *a priori* model of all relevant factors may be tiresome (or perhaps even impossible), especially when considering long-term complications in complex diseases. However, it might very well be a necessity if we want to determine the true meaning of epidemiologic findings.

Another, more controllable, reason why epidemiological studies have proven to be less conclusive in testing explanations is the reluctance to *a priori* specify hypotheses. It could be argued that, in light of the intrinsic limitations of observational research highlighted in the previous sections, the refutationists approach to scientific discovery implied by the critical rationalist outlook should be adhered to even more stringently. To put it more plainly, while weak tests might not be able to falsify weak claims about the world, they might be able to test strong claims. Or in the words of Rothman (1986, p.23): "(...) vague hypotheses have only vague consequences that can be difficult to test". That is, epidemiologists should focus on increasing the falsifiability of their hypotheses by increasing the empirical content. In other words, by making bolder conjectures.



As depicted earlier, the content of a theory specifies what it takes to proof it to be false; it makes definite claims, or predictions, which can be tested against observations and hence specifies what tests are able to falsify it. By increasing the empirical content, tentative explanations make more detailed predictions and specify more specific tests and, therefore, have a higher degree of falsifiability. Recall that the empirical content of a theory is palpable *a priori* and as such, researchers should prefer those theories that contain the greater amount of empirical information. As the content of conjectures implies which decisive tests should be designed, the appropriateness of a study design to adequately test a conjecture can also be determined *a priori* and independent of the results. That is, while a hypothesis might make definite claims about the world that are testable in theory, it might not be practically possible to test them in the manner proposed or through observational research in general. By means of a critical evaluation of the tentative conjecture and its claims on the one hand, and the rigor of the proposed test on the other, an assessment can be made with regard to what can be expected in terms of knowledge gained from a particular study. When a theory prescribes a high degree of correlation between relevant determinants, a deliberation of this kind could even yield the *a priori* conclusion that the knowledge sought after is inaccessible, at least in the manner proposed.

Such an approach would entail that less emphasis is placed on testing of the null hypothesis – stating no difference or no association between phenomena – and instead would redirect the researchers' efforts towards specifying the actual, alternative hypothesis.

## Beyond simple statistical hypothesis testing

In current epidemiological practice, the implementation of deductive logic appears to be limited to statistical testing of the null hypothesis. However, essential accompanying parts of the critical attitude towards knowledge, that forms the foundation on which the appeal to deductive reasoning is based, have been neglected. In the end, refuting the hypothesis that no association of any kind exists between phenomena is not of interest to the scientist; it does not provide an explanation to a problem. More importantly, by focusing on this

less informative null hypothesis, researchers have largely overlooked their duty to specify their actual hypothesis: the primary reason why the study was conducted. Preferably, such a hypothesis should be bold, improbable, and informative and should yield testable predictions. Contrariwise, the opposing, alternative hypothesis to the null hypothesis, stating that some association exists, can hardly be qualified as a bold conjecture; it can only be refuted by a lack of any sort of association. Moreover, the conclusion that disproof of the null hypothesis is indirect proof that the alternative hypothesis is true, is a common misconception. In the end, such an argument would be based on verification rather than logically sound refutation. Instead, the only conclusion that can be drawn from refuting the null hypothesis is that the rather vague alternative hypothesis that '*some kind of association exists*' will be tentatively accepted. But, as stated by Maclure (1985, p.349): "simple statistical testing is petty science (…)" and "(…) mere existence [of an association] is of little interest to the scientist".



Epidemiologists are often hesitant to *a priori* stipulate their ideas regarding the nature of the association under study. Instead, reasons are given why a subject is considered to be of relevance, or references to other empirical evidence are provided to explain, or even justify, how the hypothesis was conceived; to clarify on what grounds the hypothesis is based. However, the origin of our ideas is irrelevant for the critical evaluation thereof, nor can the probability of the causal nature of an association be argued based on positive evidence. By losing sight of the essential part of scientific research to formulate specific testable conjectures, results are presented without accompanying predictions that enable the evaluation of the actual study hypothesis. In fact, this practice, at the very least, raises the suspicion that the study is conducted under the assumption that *all will become evident from the results*; that the truth will be unveiled for everyone to see and that once the naked truth stands revealed before us, we have the power to see it.

Only by *a priori* specifying the hypothesized causal pathway believed to be involved can results be interpreted in light of the expectations drawn from the assumption that the hypothesis is true. These *a priori* claims about the world are tantamount to the empirical content of a theory. Bold hypotheses, making wide-ranging claims, are highly falsifiable and therefore to be preferred over hypotheses with less empirical content, provided they are not yet falsified (Popper, 1963). It follows that general explanations, containing necessary causes, have a high amount of empirical content. The same holds for hypotheses specifying patterns over time, such as a biological gradient in the form of duration-response or dose-response predictions.

As an example, William Farr proposed the equivalent of a normal distribution as a 'general law' that governed epidemics based on data from earlier smallpox outbreaks. During an unfamiliar rinderpest epidemic, Farr predicted, based on his theory, that the number of deaths would soon decline (Susser, 1995). As such, Farr openly put his epidemiologic theory – with bold, wide-ranging definite claims – to the test by clearly stating the conditions for his theory to be refuted. Similarly, the field of astronomy out of necessity has to rely on observational research to test theories. While keeping in mind that the complexity of the subject matter is of a completely different magnitude, theories of physics can also only be tested due to the very precise predictions that emanate from them. A recent example is the first direct detection of gravitational waves in September 2015, already predicted by Albert Einstein in 1916 based on his field equations of general relativity (Abbot *et al*, 2016). Of note,

here a clear example is provided of how theory precedes observation and how the content of a theory specifies new crucial tests. These examples in which observational research was able to construct decisive tests indicate the importance of strong *a priori* predictions and could be seen as a hopeful sign for the field of epidemiology after all.

## CRITICAL DISCUSSION OF EMPIRICAL FINDINGS

Etiological studies on long-term complications in patients with complex diseases face many methodological challenges. At large, the validity of results from observational studies is threatened by the non-randomized attribution of exposure, resulting in potential bias and confounding (Vandenbroucke, 2008). Beside this inherent limitation of observational research, the quest for knowledge with regard to these kind of etiological issues is further complicated by the complex and heterogeneous character of the conditions themselves. When it comes to etiological studies regarding cancer incidence in people with type 2 diabetes mellitus or the incidence of venous thromboembolism in patients with multiple sclerosis, the question as to how best to characterize the relationships between these conditions is far from simple. Multiple hypotheses have been posed as explanations for the observed link between type 2 diabetes mellitus and the incidence of (certain types of) cancer on the one hand and that between venous thromboembolism and multiple sclerosis on the other. However, since any epidemiologic study on these subjects is plagued by ample alternative explanations for their findings, decisive answers remain elusive.



This final paragraph discusses the different stages of etiological studies and the methodological challenges that should be considered when investigating long-term complications associated with complex chronic diseases. More specifically, attention will be paid to the function of an *a priori* deliberation of the causal scheme in designing an etiological study and ways in which to improve the testability of epidemiologic hypotheses. To aid in this discussion, the relevance of the notion that science proceeds from theory to observation should be emphasized. That is, the verificationist view that we can start with pure objective observation is simply illogical since we would not know where to look for relevant facts without a tentative theory guiding our search. Perhaps in no field of scientific research the absurdity of the impression that science starts with pure observation becomes more evident than in epidemiology. To illustrate this point, just imagine the process of designing an observational study without anything in the nature of a theory. This notion raises an interesting perspective on the interpretation of epidemiologic studies, even if they were not strictly carried out from a critical rationalist point of view. A critical evaluation of the Bradford Hill criteria for causal inference (Hill, 1965) will serve as an illustration of the difference in the interpretation of epidemiologic studies between verificationists and refutationists.

### A first toe in the water

Etiological studies are set out to gain understanding why particular health-related complications occur. That is, to seek explanations for the occurrence of known diseases. Numerous epidemiologic studies have consistently shown an excess incidence of overall and many site-specific cancers among type 2 diabetic patients as compared to the non-diabetic

population, including gastrointestinal cancers and breast cancer (Tsilidis *et al*, 2015; Johnson *et al*, 2012). Descriptive epidemiologic studies are considered the first foray into a new area of inquiry (Grimes & Schulz, 2002). As such, they provide observational statements regarding the distribution of variables, such as the occurrence of disease, among a population, without much regard to explanations for these statements in terms of cause and effect (Porta, 2014). However, as mentioned above, a tentative theory always guides our search for relevant facts. Hence, even purely descriptive studies are carried out with some causal hypothesis in mind. For example, in **Chapter 2.1** and **Chapter 2.3** we described colorectal and breast cancer incidence rates among type 2 diabetic patients, stratified by categories of current body mass-index and not by the color of their eyes. We did so because body mass-index has been linked to both colorectal and breast cancer and obesity is considered to play a pivotal role in linking type 2 diabetes mellitus to the excess risk of these and other cancers (Renehan *et al*, 2008; Roberts *et al*, 2010). Similarly, time trends were described as to assess whether the implementation of national screening programs had a notable effect on the incidence rates for these types of cancer. Again, following an (unpronounced) hypothesis that the intervention of screening influences cancer diagnosis.



In this respect, the statement by Grimes & Schulz (2002, p.147) that descriptive studies are a useful tool to "(…) develop hypotheses about cause" might be better interpreted as a first crude measurement to see whether further exploration of the hypothesized causal link is warranted. That is, from a critical rationalist perspective the distinction between descriptive and analytical studies becomes somewhat ambiguous, where the difference only pertains to the efforts made to refute a tentative theory. In that regard, descriptive studies also constitute the starting point of our search for answers to etiological questions, as a first attempt to test out our intuitions. It follows that there can be no such thing as a 'hypothesis-generating study'. While observations can, and often do, stimulate the need for further explanation, even this occurs only when they conflict with expectations based on some tentative theory already in place (Weed, 1986).

In **Chapter 2.2**, for example, we observed an unexpected lower incidence rate for esophageal cancer among type 2 diabetic patients, as compared to non-diabetic individuals. The link between body weight and type 2 diabetes mellitus was thought to predispose type 2 diabetic patients to a higher risk of gastro-esophageal reflux disease, reflux esophagitis, and subsequently Barrett's esophagus and adenocarcinoma of the esophagus (Mearin & Malagelada, 1995). Now, as stated by Popper (1965, p.327): "(…) it is always possible to produce a theory to fit any given set of explicanda". For any new explanation not to be *ad hoc*, Popper argues that it should be independently testable. In other words, apart from explaining all the observable facts which the new theory was designed to explain, it must have new and testable consequences (Popper, 1965).

## Choices in study design in analytical epidemiology

At the heart of an analytical study in epidemiology lies an etiological question as to why a certain health-related event occurs. The proposed explanation then contains a causal scheme consisting of the independent variable that is believed to cause alteration in the specified dependent variable and all potential sources of distortion of the effect of this putative cause resulting from the imperfect interchangeability inherent to observational studies. Ideally, predictions can be deduced from this causal scheme that allow for a comparison

of the study hypothesis to the objective truth, being the empirical evidence. From a critical rationalist perspective, it follows that formulating these predictions should be the focal point of each study proposal, as the choices in study design are based on these predictions. In doing so, the observed risk estimates immediately gain meaning, as an impartial referee that distinguishes false beliefs from tentative truths. That is to say, any discussion regarding a study's validity should be solely based on the suitability of the proposed study design to test the predictions originating from the proposed causal hypothesis. Such an *a priori* discussion is then purely theoretical and should ensue independent of the results.

*Conception of causal hypotheses*

Exemplary for the inductivist approach, researchers in epidemiology often primarily provide reasons to explain how they *conceived* a hypothesis. Not seldom are these introduced not until the formal discussion, usually to argue the causal implications of the study results. They thereby follow the Hill criteria of plausibility, analogy, experiment, and coherence, intended by Hill as features that can help determine whether an observed association has a causal nature by either referring to empirical evidence from other disciplines or referring to 'known' effects of other, comparable, determinants (Hill, 1965). Characteristic of inductivism, these criteria thus invoke a network of linked *positive evidence* from numerous disciplines based on which the probability of causation is *argued*. However, these criteria in their original form, as set forth by Hume (1739, T1.3.15), should be interpreted as a psychological theory rather than a philosophical (or scientific) one. That is to say, they try to give a causal explanation of a psychological fact: why we believe in certain regularities, laws of nature, or causation (Popper, 1963). And although our belief in causation, in the words of Hume (1748, E4.2.32), "(…) surely is a step or progress of the mind, which wants to be explained", it has no relevance for the conduct of scientific research. In other words, there are *no good reasons* that *a priori* can justify a hypothesis. Not on the basis of analogy, plausibility, nor coherence can it be argued that a belief is 'true'. For that reason, researchers should spend less time attempting to explain how they conceived a hypothesis and more time specifying how they envision the relationship between certain observable phenomena.

*Causal schemes and predictions*

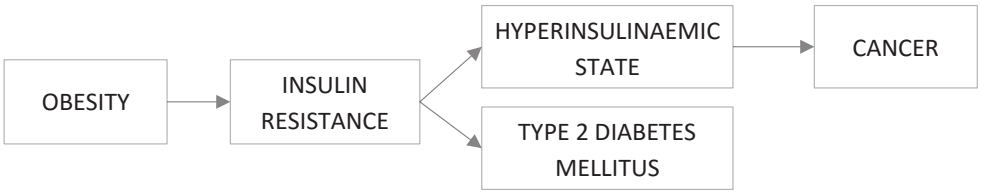
Any etiological study revolves around a proposed causal scheme that specifies in detail how the relationship between the putative cause and its presumed effect is understood. For example, the studies presented in **Chapter 4.1**, **Chapter 4.2**, and **Chapter 5.1** were conducted under the assumption that the relationship between cause and effect followed a dose-response curve. That is, in **Chapter 4.1** metformin use was expected to lower circulating insulin and insulin-like growth factor-1 levels (Pawelczyk *et al*, 2004). Thereby, the use of metformin was thought to reduce cancer cell proliferation by tempering the increased signaling via the insulin-like growth factor-1 (IGF-1) receptor (Pollak, 2012). Consequently, a reduction in breast cancer mortality was predicted with longer duration of metformin exposure. Similar reasoning in **Chapter 4.2** predicted that breast cancer risk would increase with duration of exposure to the insulin analogue glargine as a result of its increased affinity for the insulin-like growth factor-1 receptor as compared to human insulin (Weinstein *et al,* 2009). These fairly specific predictions would then allow for a comparison with the empirical evidence to reach a logical conclusion regarding the rejection or tentative acceptation of these hypotheses, were they tested in a randomized controlled experiment. In non-

randomized studies – in particular those concerning long-term complication among patients with complex syndromal disorders – the number of potential alternative explanations due to the imperfect interchangeability is extensive, if not infinite (Ioannidis *et al*, 2014). Under these circumstances, the *a priori* causal framework already suggests that epistemological humility regarding the findings is unavoidable (Bofetta *et al*, 2008).

In **Chapter 5.1** the focus shifted from hypotheses regarding the causal implications of the use of paticular hypoglycaemic agents, to more general explanations for the increased incidence of (certain types of) cancer among type 2 diabetic patients. By reducing the causal scheme linking type 2 diabetes mellitus to an excess incidence of cancer into its component parts, it becomes clear that the level of insulin required to reach euglycaemia is determined by insulin resistance status. In other words, if hyperinsulinaemia is the driving force behind the increased risk of cancer, then the use of specific medications that increase insulin levels is of little interest. In the end, it does not matter *how* insulin levels were achieved, only the *total amount* of insulin exposure is considered relevant. From this perspective, the use of insulin-raising medications is simply indicative of a decrease in β-cell functionality and reduced endogenous insulin production (Kahn *et al*, 2014). Moreover, although increased insulin resistance is characteristic for type 2 diabetes mellitus, it is not a synonym for type 2 diabetes mellitus. Hence, patients not suffering from overt diabetes mellitus can also be at risk of exposure to hyperinsulinaemia (Festa *et al*, 2006). From this causal interpretation it follows that type 2 diabetes mellitus and (certain types of) cancer coincide – where overt type 2 diabetes mellitus is merely a point in time along a path of greater metabolic disturbances – but are not causally related to each other (Figure 6.1). The focus therefore should lie on the much broader link between insulin resistance status and cancer risk. One of the main determinants for decreased insulin sensitivity is the amount of abdominal body fat (Carey *et al*, 1996) and a link between body mass-index and several different kinds of cancer, including colon cancer, has been observed (Bashkaran *et al*, 2014). However, given the concomitant hypothesis that insulin acts as a growth factor (Pollak, 2012), interest lies primarily in the effect of cumulative duration of exposure to hyperinsulinaemia.



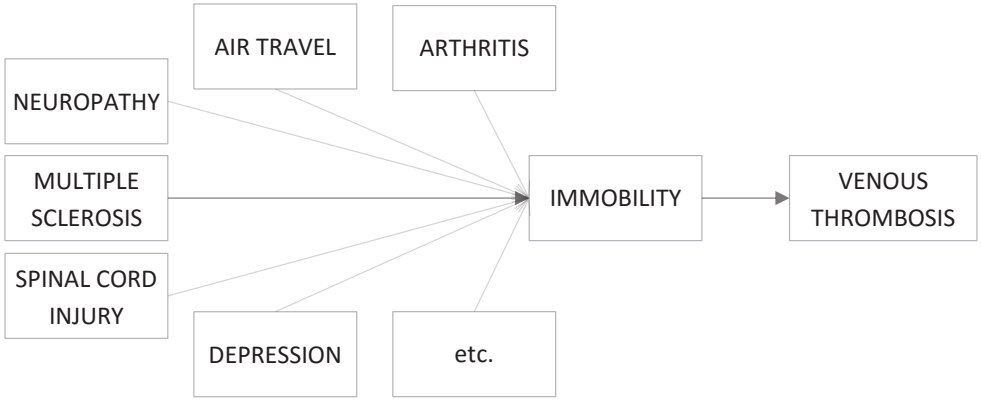
**FIGURE 6.1.** Schematic representation of the causal scheme linking obesity to an increased risk of cancer, in which no direct causal link exists between type 2 diabetes mellitus and cancer.




Similarly, in **Chapter 5.2** reduction of the causal structure led to the hypothesis that in multiple sclerosis patients the conglomeration of risk factors for venous thromboembolism, rather than the disease itself, causes the risk of deep venous thrombosis and pulmonary embolism to be increased. Patients with multiple sclerosis suffer from a gradual loss in mobility (Myhr *et al*, 2001). Multiple sclerosis should then be characterized as an indirect factor in the causal scheme. The increased risk of venous thromboembolism observed in multiple sclerosis patients was thought to be the direct result of hypoxia and activation

of coagulation pathways due to immobility (Bovill & van der Vliet, 2011). While this might seem trivial, describing and analyzing complex phenomena in terms of their fundamental constituents can help in the formulation of ideas regarding necessary causes and broader patterns that might not have been thought of when regarding them in holistic terms. More importantly, it could assist in finding caveats in our theory and in determining if and were specificity might have been lost. For example, multiple sclerosis is not the only source of immobility and immobility not the sole cause of venous thromboembolism (Figure 6.2).

**FIGURE 6.2.** Schematic representation of the causal scheme linking multiple sclerosis to venous thromboembolism, in which no direct causal link is considered between the two conditions.






*Design choices*

Choices made in the design of an etiological study, from a critical rationalist perspective, should be motivated as the most critical test to compare predictions originating from the *a priori* hypothesized causal scheme to the empirical evidence (*i.e.* objective truth). A discussion concerning the suitability of a design to stringently test the proposed relationship is therefore a purely theoretical one, which could transpire *a priori* and independent of the results. Ideally, researchers would explain every part of the study design in terms of causality. What do they expect? What can prevent the study result to be decisive? What can be done to counter such a distortion? That is, in order for a study to truly have meaning, investigators should ultimately have the confidence *a priori* that once the results are in, they will surmount to an impartial, clear, and logical conclusion regarding the truthfulness of their hypothesis.

Unfortunately, the inherent limitations of observational research might prevent such confidence in the final judgement regarding the hypothesis under study. Alternative explanations are abundant and accounting for all of them might be nearly impossible. The acceptation that comparisons from observational research are invariably vulnerable to non-random distortion has led to the custom of using different study designs to gather more insight into the reliability of answers to certain etiological questions. The generally large study populations in epidemiology make the effect of random variability negligible. For these reasons, simple replication of studies – aimed to further reduce the probability of chance findings – is not considered sensible in most instances. When it comes to non-random distortion, replication of a study does not alleviate our concerns. Therefore, different study

designs and methodologies are recommended to address potential flaws in prior studies (Vandenbroucke, 2007). However, this would imply that, at least from a critical rationalist perspective, we ought to contemplate the possibility that studies using different designs then address different research questions, regardless of their seemingly similar overall aims. That is to say, researchers within the same general field – for example, the risk of cancer associated with insulin use – who make different choices in study design, might actually test different conjectures, based on different causal schemes, with different accompanying predictions.

In **Chapter 3.1** we described the range in risk estimates of certain site-specific cancers that were associated with the use of insulin from 1,440 nested case-control studies with different study designs. We observed that simple realistic choices in study design can cause a huge variation in risk estimates within the same source population of patients. Dependent on the choices made, the risk estimates for any cancer associated with insulin use varied from a protective to a harmful effect. Not seldom are results from studies like these, with a great amount of heterogeneity in study design, combined in a meta-analysis to yield an aggregate measure, where differences in results between studies are interpreted as random variability (Colmers *et al*, 2012). However, the variation in risk estimates described in **Chapter 3.1** is the result of deliberate changes in study design. When placed in the theoretical framework posed in the previous sections of this discussion, it becomes questionable whether in fact the same hypothesis can be tested in such different ways. That is to say, while study aims are often indistinctly formulated, the choices made in the study design are far from arbitrary; they must be motivated by some more specific aim.

From the above it follows that when *a priori* design choices differ, so does the meaning of the study results. Consequently, it should be acknowledged that different studies provide answers to different questions. For example, in **Chapter 3.1**, insulin use was associated with an increased risk of pancreatic and liver cancer when measured at the time of diagnosis. However, when the diagnosis date was shifted backwards in time by 5 years, no risk difference was observed. From its design it can be assumed that the latter study theorizes a latency period in which cancer is already present but not yet diagnosed, otherwise the backward shifting of the cancer diagnosis date would be ludicrous. As such, the former study tested whether patients diagnosed with cancer are more often treated with insulin – making the result vulnerable to potential reverse causality (Johnson *et al*, 2012) – while the latter tested if insulin use was more common around the hypothesized time of cancer cell development, thereby considering the possibility of reverse causality. Taken together, these results appear to falsify the hypothesis that insulin use causes pancreatic or liver cancer, while they do not falsify the antithesis that (undiagnosed) pancreatic or liver cancer causes the use of insulin. Such a conclusion can only be drawn when interpreting these results in light of the choices made in study design. For that reason, we advocated against the use of meta-analysis techniques in favor of a process more akin to 'dossier building', where the progress within a 'dossier' can be presented through a discussion of individual studies placed in their relevant context. Such a process, however, would be greatly facilitated by researchers attributing more effort towards an *a priori* elaboration of the proposed causal scheme, based on which the choices in study design are motivated and predictions are made.

## Empirical content of explanations

The fundamental fallibility of knowledge dictates that it is simply unachievable, on theoretical grounds, to state with certainty that all alternative explanations for a given association have been eliminated in an epidemiologic study. Nor can we hope to reach any degree of probability that we succeeded in doing so. No matter how carefully constructed an observational study might be, the number of ways in which we can be deceived is limitless. Not surprisingly, do theories that survived tests in observational research often fail when put to the test in experimental settings (Ioannidis *et al*, 2013).

At least part of a solution is to question the empirical content of our hypotheses and find ways in which to increase it. Following the critical rationalist approach, by increasing the empirical content, tentative explanations make more detailed predictions and specify more specific tests and therefore have a higher degree of falsifiability. In other words, we should not aim to be right, but aim to be wrong. A bold statement, depicting a necessary cause is testable, perhaps even in noisy circumstances. We might not necessarily need a randomized controlled experiment to test a hypothesis that direct contact with an open flame leads to skin burns. In similar ways, the hypothesis that thalidomide use during the start of pregnancy causes phocomelia (Mellin & Katzenstein, 1962) could be adequately tested because of the very specific effect – phocomelia is a very rare congenital disorder (Bermejo-Sánchez *et al*, 2011) – and a very specific relevant exposure time window. Thus, the hypothesis that thalidomide causes phocomelia has a high degree of empirical content, making the prediction highly improbable, and yet it was not falsified when tested against the empirical evidence. Following this line of reasoning, hypotheses predicting rare acute effects related to specific exposures would be best suitable for testing by means of observational research, as the high amount of empirical content at least reduces (but not eliminates) the potential of alternative hypotheses able to explain the same phenomenon while not being refuted by the presented empirical evidence.



### *Strength, specificity, and consistency*

As discussed at length in the previous sections, the concept of causality refers to an intangible relationship between ideas. As we seek to explain phenomena in terms of cause and effect, the search is aimed at strong, consistent, and specific rules. A satisfying explanation is one that has survived the most stringent tests implied by its content. When we reason in probabilities (or average risks) it reflects our ignorance of hidden causal determinants (Rothman, 1986). The better we are able to predict certain effects based on our hypothesis, the closer we are to explaining it, and the stronger, more consistent, and more specific an association between the putative cause and the effect becomes. One could argue that only when our predictions fully coincide with the empirical evidence – as would be the most stringent test thinkable – can we say our hypothesis fully satisfies our definition of an explanation. Until then, any hypothesis would not suffice as an explanation in scientific terms, as countless observations remain that would falsify any statement of a necessary connection; *e.g.* "My aunt smoked her whole life and never got lung cancer". In this perspective, strength, specificity, and consistency of an association can be thought of as regulative features in the *a priori* specification of hypotheses, where each new explanation makes more and better predictions than previous explanations. In doing so, the empirical content of a theory increases by asserting to explain more than a rival theory does; constituting a crucial test of competing non-null causal hypotheses (Rothman, 1986).

Consequently, better explanations result in *stronger*, *more consistent*, and *more specific* associations between the tentative cause and its presumed effect.

Thus, the use of specificity, strength, and consistency of associations as criteria to argue *a posteriori* the probability of a causal connection (Hill, 1965) is tantamount to an incorrect interpretation of the scientific process. These features merely serve as an indicator of whether a better explanation is needed. It can be assumed that each study in analytical epidemiology seeks etiological explanations and therefore predicts a strong association. When the association instead is very weak – which certainly might be more common (Susser, 1986; Ioannidis, 2005) – we can only conclude that, although the hypothesis that no association exists between the specific determinant and a given health-related condition might be rejected, we are still a long way apart from an *explanation* for it.



*The use of cumulative exposure measures*

Another approach to increase the empirical content of *a priori* hypotheses is by focusing on comparisons of risk estimates by means of pattern predictions, like a biological gradient. Even in situations where reasoning in probabilities is unavoidable, pattern predictions still enable a further specification of the hypothesis' predictions. For both verificationists and refutationist the determination of patterns with cumulative exposure is considered valuable. From a verificationist point of view, the presence of a biological gradient is one of the most convincing arguments for a causal association, as causal connections in general simply reveal themselves in this fashion (Hill, 1965). In contrast, the refutationist position interprets the inclusion of more specific predictions of this kind as increasing the empirical content and hence the testability of a conjecture. Interpreted in that way, such a prediction is made *a priori* as it is already specified in the study design, before any result was generated.

However, the presence of a biological gradient is not simply a universally applicable criterium, as Hill described, but dependent on the hypothesized causal scheme; the content of the conjecture. In many cases a biological gradient might be predicted, but hypotheses can very well state the absence of a biological gradient (*e.g.* in severe immunosuppressed patients, any exposure to a pathogen has the same detrimental effect as high dose exposure) or an inverse pattern (*e.g.* with allergen immunotherapy, hypersensitivity decreases with cumulative exposure), rendering them equally testable, without meeting the Hill criterion. Consequently, any type of pattern prediction (*i.e.* comparisons of risk estimates) – whether it be over time, after cessation, with cumulative exposure, with average daily dosage, or some other independent variable – increases the empirical content of the conjecture. As such, like predictions imply new tests and make any conjecture more falsifiable. But most importantly, it should be recognized that such a pattern is an integral part of the hypothesis' *a priori* predictions.

In **Chapter 4.1**, the cumulative number of metformin prescriptions – as an indicator of duration of metformin exposure – was used to assess whether the observed trend in breast cancer mortality concurred with the predicted risk reduction with duration of metformin use in diabetic women diagnosed with breast cancer. In addition, cumulative exposure measures were extensively used in **Chapter 4.2** to determine whether the predicted trend of an increase in breast cancer risk with duration of exposure to insulin glargine was indeed observed in patients newly started on insulins. Also, in **Chapter 5.1** the hypothesized link

between obesity and colorectal cancer risk was expected to follow a duration-response curve. Here, cumulative duration of obesity was taken as the relevant exposure, as it was considered to reflect a hyperinsulinaemic state. In a similar manner, some of the variations in study design that were applied in **Chapter 3.1**, when taken together, could serve to increase the empirical content of hypotheses by making pattern predictions. For example, a hypothesis of reverse causality when considering insulin use and pancreatic cancer incidence would predict a decrease in risk estimate when the cancer diagnosis date was shifted backwards in time. In all these instances, the testability of the underlying hypothesis was improved by increasing the empirical content, making more specific predictions that imply more specific tests.

In summary, inductivist criteria for causal inference, like the ones proposed by Bradford Hill, from a refutationist perspective, can be better understood as a cookie-cutter solution for conceiving and formulating hypotheses in the field of epidemiology (Table 6.1). Interpreted in this way, these inductivist criteria do provide some guidance in improving the empirical content and hence the testability of a hypothesis, albeit rather restrictive. But since these standardized criteria stem from a dogmatic attitude that causal associations present themselves according to particular patterns that can be objectively determined through careful observation, they clearly do not acknowledge the limitless imagination of the mind to propose any type of connection between any sorts of phenomena. From a refutationist perspective, it is precisely this creative process that should be encouraged; to specify the matter of the proposed explanation; to increase its empirical content; whether it be by comparison to other explanations, by predicting a biological gradient, or any other pattern.



*Step-wise adjustment*

Another approach to seek explanations for an observed excess risk of disease is by applying step-wise adjustment for confounders, each with a distinct causal hypothesis underlying its effect on the putative causal connection under study. In **Chapter 5.2**, we hypothesized that the excess risk of deep venous thrombosis and pulmonary embolism in patients with multiple sclerosis was primarily caused by the immobility as a result of neurodegenerative processes and muscle spasticity (Oreja-Guevara *et al*, 2013; Rizzo *et al*, 2004). Multiple indicators were used to determine disease severity and activity, as well as direct measures of spasticity and immobility. Step-wise adjustment for these indicators led to a marked reduction in the risk estimate of venous thromboembolism associated with multiple sclerosis. Although a residual risk still remained, these results come close to falsifying a theory of a direct causal link between multiple sclerosis and venous thromboembolism. However, when making such statements, concurring events should naturally always be considered: for example, when immobility is always accompanied by chronic inflammation, then there is no way of differentiating between the two and neither hypothesis can be effectively rejected.

## CONTRIBUTIONS TO EMPIRICAL KNOWLEDGE

The theoretical discussion of the previous paragraphs has made clear that the interpretation of epidemiologic research is far from straightforward. The same holds true for the empirical

**TABLE 6.1.** A verificationist and refutationist interpretation of the Bradford Hill criteria for causal inference.

| Hill criteria | Verificationist interpretation | Refutationist interpretation |
|---|---|---|
| *Strength of an association* | The stronger an association, the more likely it is to be causal. However, a weak association is not by itself falsifying (Hill, 1965; Susser, 1986). | Regulative feature in the *a priori* specification of hypotheses and indicator of whether a better explanation is required. |
| *Consistency of an observed association in different places, circumstances, and times* | When similar results can be reached in quite different ways, it indicates a causal connection (Hill, 1965). Consistency in results is the most powerful verification available (Susser, 1986). | Regulative feature in the *a priori* specification of hypotheses and indicator of whether a better explanation is required. |
| *Specificity of an association* | If an association is limited to a specific exposure and to a particular effect, then clearly that is a strong argument in favor of causation (Hill, 1965). Specificity is an affirmative criterion which adds plausibility to a causal claim, but if absent does not detract from it (Susser, 1986). | Regulative feature in the *a priori* specification of hypotheses and indicator of whether a better explanation is required. |
| *Temporality of an association* | It should be determined what is the cause and what the effect, where the cause should precede the effect (Hill, 1965). | Logically contained within the *a priori* hypothesis, as the theoretical concepts of cause and effect, where the putative cause by necessity precedes the effect |
| *Biological gradient* | If the association is one which can reveal a biological gradient, or dose-response curve, then this can be perceived as clear evidence for a causal connection (Hill, 1965) | One of many possible *a priori* pattern predictions that increases the empirical content of an *a priori* hypothesis, thereby increasing its falsifiability. |
| *Plausibility* | The existence of a biologically plausible mechanism could indicate a causal connection. However, this depends upon the biological knowledge of the day (Hill, 1965). | Attempt to argue the probability of causation based on positive evidence. Serves as an explanation why a hypothesis was conceived; no relevance for the conduct of scientific research. |
| *Coherence* | The cause-and-effect interpretation of the association should not seriously conflict with generally known facts (Hill, 1965). Incoherence shifts the balance toward rejection (Susser, 1986). | Attempt to argue the probability of causation based on positive evidence. Serves as an explanation why a hypothesis was conceived; no relevance for the conduct of scientific research. |
| *Experiment* | Occasionally it is possible to appeal to experimental, or semi-experimental, evidence, which could provide the strongest support for the causation hypothesis (Hill, 1965). | Attempt to argue the probability of causation based on positive evidence. Serves as an explanation why a hypothesis was conceived; no relevance for the conduct of scientific research. |
| *Analogy* | In some circumstances it would be fair to judge by analogy. If some cause produces a certain effect, then similar causes could produce similar effects (Hill, 1965). | Attempt to argue the probability of causation based on positive evidence. Serves as an explanation why a hypothesis was conceived; no relevance for the conduct of scientific research. |

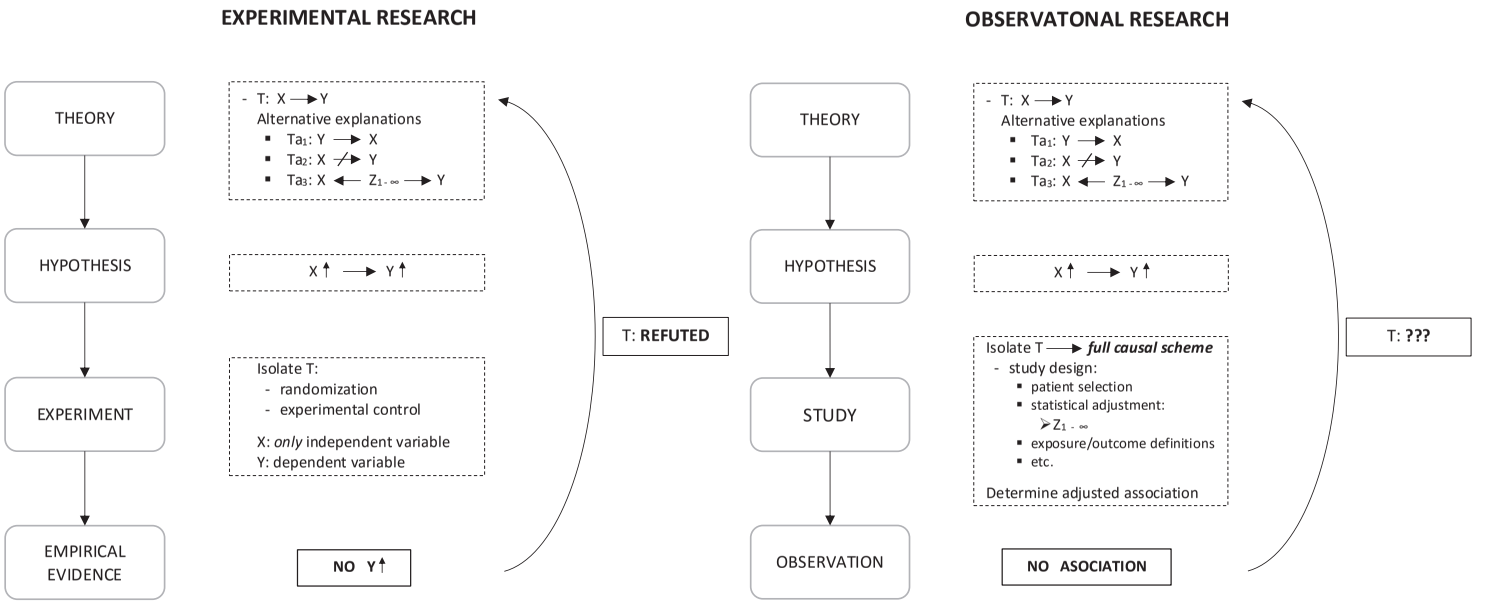
studies presented in this thesis. A common theoretical structure on the basis of which to determine the value of epidemiologic evidence is largely missing. As has been argued, the result-based interpretation of evidence is problematic as it reflects a dogmatic attitude towards knowledge. A critical interpretation would not be *based* on evidence but *dictated* by it (Charlton, 1996). The influence of the choices in study design on the acquired risk estimate observed in **Chapter 3.1** shows the importance of *a priori* decisions. The methods by which we gather our evidence logically determine what evidence is acquired. The choices made in our methods are subsequently preceded by the theories that we hold and the questions that we ask. The results from **Chapter 3.1** thus signify the importance to motivate our *a priori* choices, as they relate to the way in which we search for evidence. Even when these choices might be based on epidemiologic intuition rather than reason, we should aim to put these intuitions into words as it enables us to better interpret the findings that we gather. This notion, while more of a theoretical nature, has important implications for the conduct of future research and a better understanding of epidemiologic evidence.



In **Chapter 4.1**, **Chapter 4.2**, and **Chapter 5.1**, the use of cumulative exposure measures signifies the increased empirical content of the underlying hypotheses. Although concise, in our research papers we tried to specify the predictions that our hypotheses generated in order to compare them with the empirical findings. The empirical evidence in **Chapter 4.1** and **Chapter 5.1** indeed concurred with the predicted patterns in risk estimates, where, respectively, the risk of breast cancer mortality decreased with cumulative use of metformin and the risk of colorectal cancer increased with cumulative duration of obesity. These findings would imply, if alternative explanations were effectively eliminated by the study's design, that the underlying hypotheses – that metformin use reduces breast cancer mortality and that long-term exposure to hyperinsulinaemia increases the risk of colorectal cancer – would be tentatively accepted. Similarly, the lack of agreement between predictions and the empirical evidence in **Chapter 4.2** would lead us to reject the hypothesis that cumulative exposure to glargine causes breast cancer, at least to a higher degree than human insulin does. Unfortunately, however, such conclusions do not logically follow from the evidence. As became apparent from the formal discussion of these results, we are unable to claim, on theoretical grounds, that all alternative explanations have been successfully eliminated from the equation. Consequently, the hypothetico-deductive process is corrupted and therefore such conclusions cannot logically be justified (Figure 6.3).

In **Chapter 5.1**, a comparison of two different approaches to test the same hypothesis was presented. To operationalize insulin resistance status among type 2 diabetic patients we compared the use of a treatment stage algorithm to duration of obesity. With the use of treatment stages, the underlying assumption was that previously reported differences in cancer risk associated with different types of hypoglycaemic agents (Karlstad *et al*, 2013) were the result of confounding by indication. As such, this model was intended to falsify the hypothesis that a causal connection between cancer risk and the use of particular types of hypoglycaemic agents exists. However, our predictions did not match the results and hence we failed in providing an alternative explanation for this hypothesis. With duration of obesity, on the other hand, we formulated a more direct measure of insulin resistance status and hence of hyperinsulinaemia. Of note, this proposed alternative explanation is independently testable as, apart from explaining the association between type 2 diabetes mellitus and colorectal cancer, it has new and testable consequences. In other words, a fairly narrow theory regarding the increased risk of colorectal cancer in type 2 diabetic patients

**FIGURE 6.3.** Schematic representation of the hypothetico-deductive process in experimental research and the failing of this logical system in observational research due to the lack of systematic elimination of alternative explanations (Ta), hindering the isolated testing of any single tentative causal relationship. As a result, the empirical evidence is not conclusive with regard to the refutation or tentative acceptation of a causal theory (T). In order to claim any hypothesis was tested in isolation, the study design should account for all influence of relevant interfering causal patterns thought to exist. Hence, observational studies require detailed and comprehensive causal schemes not only of the theory under scrutiny but of all relevant causal relationships ($Z_{1-\infty}$). Such a condition is theoretically out of reach, since it is built upon the assumption that certain knowledge is attainable, as a prerequisite for full statistical adjustment.



caused by the use of particular hypoglycaemic agents was contested by a broader alternative hypothesis, with wider, more general claims. Based on their empirical content, it follows from a refutationist perspective that the latter theory is to be preferred, would it survive severe scrutiny.

The predicted pattern was indeed observed in our empirical data. Ideally, these findings should lead to the tentative acceptation of the proposed *explanation*, constituting a causal link between duration of obesity and colorectal cancer risk, for the observed *association* between type 2 diabetes mellitus and an increased colorectal cancer risk. However, given the potential non-random distortion, other explanations could still involve the use of sulfonylureas and, in particular, exogenous insulin, as both are associated with an increase in body weight (UKPDS group, 1998). As such, duration of obesity may potentially be correlated with the types of hypoglycaemic medications used. The proposed explanation that a causal link between duration of obesity – or more specifically duration of exposure to hyperinsulinaemia – and colorectal cancer risk will manifest itself as an association between the type of hypoglycaemic agent prescribed and colorectal cancer risk then can also be reversed, rendering our results inconclusive.



Different aspects of the relationship between body mass-index and the incidence of colorectal cancer were studied in **Chapter 2.1** and **Chapter 5.1**. When it comes to current body mass-index (as measured no less than 12 months ago), type 2 diabetic patients in the lowest category appeared to have the highest incidence for colorectal cancer. Conversely, stratified by cumulative number of years with obesity, the risk of colorectal cancer among type 2 diabetic patients appeared to increase with each incremental year. These findings raise interesting questions with regard to the 'adjustment for body mass-index' often applied in epidemiologic research studying colorectal cancer risk. How is body weight thought to be causally linked to colorectal cancer? And how should this relationship then be operationalized? If hyperinsulinaemia is considered to have a growth-promoting effect then clearly duration of obesity is preferable over current body mass-index or body mass-index at cohort entry; the latter two would merely signify the height of insulin levels at the time of measurement. As choices in the adjustment of risk estimates are also made in light of some causal theory, definitions of covariates require an equally thorough motivation, which should be consistent with the hypothesis under scrutiny.

In **Chapter 5.2** we aimed to determine the cause for the increased risk of venous thromboembolism observed in multiple sclerosis patients. Two alternative hypotheses were tested: one that attributed this excess risk to the chronic inflammation present in these patients, the other to an increased immobility due to neurodegenerative processes and muscle spasticity. Once again, a broader alternative to a narrow explanation was introduced, with more empirical content: chronic inflammation or immobility as the cause of an increased risk of venous thromboembolism, as opposed to a causal link between multiple sclerosis itself and venous thromboembolism. In fact, predictions from both alternative hypotheses were not contradicted by the evidence, as the risk of venous thromboembolism was increased in multiple sclerosis patients recently treated with glucocorticoids as well as in patients with a recent record of disability or spasticity. According to a verificationist point of view, these findings would not contradict each other, as both could well be a component in the causal constellation. Conversely, for a critical rationalist, this finding indicates that the causal scheme should be further reduced in order to find an explanation containing a

necessary cause able to outperform any rival explanation. However, it should be noted that the impossibility to differentiate between these, and other, rival explanations could have been known beforehand. To name a few: glucocorticoids are almost exclusively prescribed for acute inflammation (Johannesdottir *et al*, 2013), acute inflammation in multiple sclerosis is associated with a decrease in mobility (Myhr *et al*, 2001), and immobility is associated with a higher risk of fractures (Bazelier *et al*, 2012) and might very well lead to a lack of vitamin D (Prabhala *et al*, 2000), all of which have been associated with an increased risk of venous thromboembolism (Brøndum-Jacobsen *et al*, 2013; Bovill & van der Vliet, 2011; Johannesdottir *et al*, 2013; Dahl *et al*, 2000).

The problem of alternative explanations arises both from a verificationist as from a critical rationalist perspective. However, verificationists still have hope that once all relevant distorting factors are known, ultimately the ability arises to fully adjust for them and what will remain is the naked truth. Whether a true causal connection is indeed *unveiled* then depends on expert knowledge on the full-scale causal structure involved and a ruling with regard to the probability that alternative explanations were effectively eliminated. Conversely, critical rationalists have abandoned this hope because of the inherent fallibility of human knowledge which negates any claim that adjustment for all non-random distortion has been achieved. In epidemiology, empirical findings therefore cannot serve their purpose; that of objective truth as the standard which we may fall short of. Consequently, epidemiologic studies cannot be considered to constitute a critical test to determine the falsehood of an epidemiologic hypothesis, nor does failure to refute a hypothesis necessarily lead to the tentative acceptation of it.



What is left then is to conclude that, from a critical rationalist point of view, our findings constitute observation statements and ultimately hold little meaning in terms of etiology, or cause and effect, because the methods that were used are unfit to stringently test our ideas of causation. That is to say, while we cannot escape dealing with causality in epidemiology, as causal theories guide our observations (Popper, 1963) and our approach to dealing with non-random distortion (Weisberg, 2010), epidemiologic studies might not lead us nearer to the truth. Notwithstanding, observation statements can still be considered useful from a health care policy perspective. For example, our results have contributed to the identification of high-risk patient groups when it comes to the incidence of colorectal cancer, breast cancer, and deep venous thrombosis. These observations can lead to targeted screening programs and increased awareness of symptoms related to these conditions, which advances the early detection and treatment thereof. Such practical recommendations can be made in the absence of well-tested theories of causation, as long as the proposed intervention has been well tested and has survived these crucial tests.

Ultimately, it becomes clear that, for a critical rationalist, we must choose our battles with nature carefully, when resorting to observational research. There might be situations in which such accurate predictions can be made that non-random distortion has to have had limited influence. Once again referring to astronomy, Einstein's formulas render predictions as accurate as the bending of light by gravity; something which was not considered possible under Newtonian physics. This example also once more demonstrates how theory precedes observation and that the empirical content of a theory implies new ways in which it can be tested and proven to be false. Proposing bold epidemiologic explanations that contain improbable but accurate predictions can likewise be considered an important step towards

improving the scientific status of epidemiologic research. As depicted earlier, this train of thought leads us to conclude that epidemiologic theories predicting rare acute effects of specific exposures would be best suitable for stringent testing. However, in the case of long-term effects, particularly with regard to cancer epidemiology, in the words of Bofetta *et al* (2008, p.993): "(…) epidemiologists should practice some epistemological modesty when interpreting and presenting their findings".

## FINAL CONSIDERATIONS



There is a subtle but fundamental difference between questions in health care policy and questions in science. Practical decisions in health care policy regarding the most efficient allocation of resources do not require well-tested theories of causality. Empirical statements containing observations allow for the identification of patient groups who suffer the highest incidence of disease. As such, this kind of information can greatly facilitate the allocation of resources to patients that would benefit most from certain interventions. To this extent, health care policy strategies aimed at reducing certain observed increased risks only require the proposed interventions to be scientifically well-tested and not proven to be ineffective towards this end. Observational research in this respect serves a practical purpose in health care policy: to determine how best to implement scientifically well-tested interventions. However, when we seek answers to scientific questions, to questions regarding cause and effect, stringent demands are raised on what constitutes solid empirical evidence. In other words, empirical statements from observational research might be sufficiently compelling to answer questions of health care policy but are to be considered insufficient for a scientifically valid answer to questions of causality. Central to this distinction between the value of observational research in health care policy as opposed to its value in scientific research is the inability to draw logical conclusions from the empirical evidence generated by observational studies. Scientific knowledge progresses through the application of the rules of logic. In order for epidemiology to make a valuable contribution to scientific knowledge, the theoretical problems surrounding the lack of decisiveness of its results need to be addressed. To put it differently, we need to improve the logic of epidemiologic discovery.

In particular when it comes to etiological studies in patients with complex diseases, we might be caught between a rock and a hard place. On the one hand, we are unable to construct crucial tests to eliminate faulty causal hypotheses, while on the other hand, our ignorance of the hopelessly complex biological processes prevent the formation of sharp epidemiologic hypotheses with distinct and testable predictions. These fundamental problems perhaps have been the main reasons why it is so hard to abandon a dogmatic attitude towards knowledge in the field of epidemiology. In these circumstances it is only natural to have a strong preference to simply present objective measures of association and refrain from further interpretation, leaving the evaluation to 'experts' who "(…) will be able to accurately evaluate the strength and clarity of the epidemiologic evidence" (Savitz, 2003, p.13). However, this confidence, how comforting it may seem, is simply misplaced, because the judgement of a causal theory's refutation or survival does not lie in the hands of individuals but can only be determined through a logical comparison of a theory's predictions to the empirical evidence. Perhaps conscious of this vulnerability to

authoritarianism, inductivists have searched for 'objective' criteria for causal inference, like the ones proposed by Sir Austin Bradford Hill. These attempts are nonetheless forlorn as these criteria are built upon the same erroneous dogmatic attitude towards knowledge. When empirical evidence does not constitute objective crucial tests but instead consists of observation statements, causal conclusions are unattainable since they are not based on logic but rather on subjective judgement. The same holds true for any hopes that observational research would be able to quantify causal relationships between exposure and disease. In the end, there is no escaping the obvious fact that objective observation is a myth and that the truth cannot be unequivocally revealed to us.

Once we accept the simple truth that our knowledge is not drawn from nature but imposed upon it, we cannot but conclude that our empirical knowledge is irreducibly fallible. However, accepting the fallibility of our knowledge – as ideas of cause and effect originating from our imagination – immediately calls for ways to critically test our hypotheses to find out where we are mistaken. Particularly, when it comes to explaining complex health-related conditions, we must acknowledge that we are punching far above our weight. Randomized and controlled experiments fully accept the limitations of our understanding and allow for the testing of causal hypotheses regardless of this uncertainty. However, when we revert to observational research, the fallible nature of our knowledge raises the fundamental question: can observational studies serve as crucial tests for epidemiologic hypotheses? That is to say, the study design should take into account all potential sources of non-random distortion in order for the study to serve as a crucial test. But can we know for certain that all alternative explanations have been excluded? While no easy answers to these questions are foreseeable, a matter of such importance cannot escape our investigation.

When we are unable to proof the falsity of hypotheses, not only will our knowledge concerning the etiology of disease seize to advance, it also creates a vulnerability to a spiraling of unfounded epidemiologic explanations. To find out if, where, and how we might be able to improve our knowledge requires the invigoration of the scientific principles in analytical epidemiology and the revival of our imagination as the source from which we draw our hypotheses. In other words, we must determine what faculties an observational study requires for the result to serve as the final objective referee; that we will succumb when our ideas are tested and our predictions fail to concur with the empirical evidence, or will tentatively accept an idea knowing it has survived the most stringent test. Simultaneously, the value of epidemiologic hypotheses should be increased by making bolder statements, containing more precise predictions and necessary causes. Changing the epistemological perspective from a dogmatic attitude to a critical attitude towards knowledge stimulates the formation of such bolder conjectures, while simultaneously demanding decisive ways to test them. A broader discussion on these issues should be reignited among epidemiologists, even if such a discussion ultimately leads to the conclusion that some of the etiological questions we have might never be answered. And although it remains doubtful whether epidemiology can escape its observational nature, in the end, to know what we do not know can be considered knowledge in itself. Or in the words of Hume (1748, E4.2.32): "Can I do better than propose the difficulty to the public, even though, perhaps, I have small hopes of obtaining a solution? We shall at least, by this means, be sensible of our ignorance, if we do not augment our knowledge".

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# CHAPTER 7

SUMMARY

## 1. INTRODUCTION

The incidence of a number of site-specific cancers is increased among patients with type 2 diabetes mellitus (T2DM) as compared to non-diabetic patients. Similarly, the incidence of venous thromboembolism (VTE) is higher in patients with multiple sclerosis (MS) when compared to patients without MS. These observation statements are said to be of a descriptive nature in that they merely state the frequency and distribution of disease among specific populations. That is to say, an association is observed between T2DM and (several types of) cancer on the one hand, and between VTE and MS on the other. However, merely knowing that a disease occurs in certain patient groups is not the issue of ultimate interest. Observing a relationship between two health-related events naturally raises the more substantial question as to why they are related. In other words, we want to know whether the observed association has a causal nature.



When it comes to these long-term health-related complications among patients with a complex chronic disease, epidemiologic studies are generally the only available means to determine their causes, or etiology. There are, however, theoretical limitations to take into account when we resort to observational research to determine causality. **Chapter 1** provides a brief introduction to the difficulties surrounding the assessment of causality based on epidemiologic evidence. In brief, these difficulties originate from the lack of confidence in the design of observational studies to eliminate all alternative explanations: explanations other than a causal connection between the exposure and the outcome. This first chapter indicates that the difference observed between groups in non-experimental comparison studies is not necessarily attributable to the variable under study but could be the result of systematic, or non-random, variability.

Since the conclusion that an observed association has a causal nature is based on a process of excluding all alternative explanations, etiological studies in complex diseases are particularly strenuous. That is to say, distinguishing between a causal effect and effects resulting from imperfect comparisons becomes increasingly difficult in situations where numerous putative causes come together. As for the association between T2DM and (certain types of) cancer, the most commonly referred to explanation is that insulin, at high levels (hyperinsulinaemia), acts as a growth factor that could stimulate cancer cell growth. Type 2 diabetes mellitus is characterized by chronic hyperinsulinaemia. However, associations have also been reported between cancer risk and obesity, high-caloric diet, sedentary lifestyle, and hyperlipidemia, all of which are also highly correlated with T2DM. Similarly, VTE is associated with auto-immune diseases, anti-inflammatory drug use, inflammation, and immobility. All these features are conjoined in patients with MS. Assessing the causal effect of any specific feature is hindered by these shared risk factors, as well as the high degree of correlation between them.

This thesis concentrates on the assessment of the influence of specific risk factors in the etiology of (particular types of) cancer in patients with T2DM and in the etiology of VTE in patients with MS. More specifically, this thesis concentrates on the issues related to the exclusion of alternative explanations for observed exposure-effect relationships in order to facilitate the assessment of causality from epidemiologic evidence. The influence of both methodological and of empirical alternative explanations is investigated, as well as the use of trend analysis with cumulative exposure as a tool to determine the nature of a particular exposure-effect relationship.

## 2. SKETCHING THE LANDSCAPE

**Chapter 2** consists of three descriptive epidemiologic studies on the trends in incidence rates of certain site-specific cancers among T2DM patients over time. In **Chapter 2.1**, trends in colorectal cancer (CRC) incidence rates (IR) were assessed among a large cohort of T2DM patients and a matched non-diabetic reference cohort in the United Kingdom (UK), using British electronic health care registries (1989-2012). Among adult patients with T2DM, age-sex-standardized CRC rates were significantly higher as compared to non-diabetic patients: 60.7 per 100,000 person years (py) (95% confidence interval [CI] 58.0-63.3) versus 54.6 (95%CI 52.3-56.9). Trends over time showed the rate of CRC to be consistently higher among T2DM patients. Furthermore, colon cancer rates in patients with T2DM appeared to increase over the duration of follow-up and were highest among males. The initiation of national screening programs in the UK in 2006/2007 did not appear to have an effect on the rate of colon cancer among T2DM patients. Therefore, we suggested targeted screening of male patients with T2DM, as they were shown to be at the highest risk.



Subsequently, in **Chapter 2.2**, trends in crude incidence rates for other gastro-intestinal cancers were determined within the same study population. Overall, one in every 300 patients with T2DM in the UK developed a type of gastrointestinal cancer each year. Aside from CRC, the incidence rates of liver and pancreatic cancer were significantly higher among patients with T2DM. The incidence rate for liver cancer among T2DM patients was 26 per 100,000 py (95% CI, 24-28), as compared to 8.9 (95% CI, 7.7-10) among non-diabetic patients. Moreover, the incidence rate of liver cancer steeply increased between 2001 and 2012 among T2DM patients. For pancreatic cancer, the incidence rate among T2DM patients was double that of non-diabetic patients: 65 per 100,000 py (95% CI 62-69) versus 31 (95% CI 28-34). Both pancreatic and liver cancer are known to impair glucose regulation and induce diabetes symptoms. The link between T2DM and these cancer types might therefore very well be bidirectional. No difference in incidence rate was observed for gastric and biliary tract cancer, while the incidence of esophageal cancer was lower among patients with T2DM.

Previous studies also have reported an association between T2DM and breast cancer in women. In **Chapter 2.3**, age-standardized trends in invasive breast cancer incidence were assessed in women with T2DM from the same UK study population. No significant difference was observed between the incidence rate for breast cancer among women with and without T2DM: 150 per 100,000 py (95% CI 143-157) versus 148 (95% CI 141-156), respectively. However, among postmenopausal T2DM women with a current body mass-index (BMI) ≥35 kg/m$^2$, the age-standardized rate for breast cancer was significantly higher than among those with a BMI <25 kg/m$^2$; 421 per 100,000 py (95% CI 372-470) versus 313 (95% CI 270-355). Postmenopausal T2DM women with obesity could therefore be considered for targeted breast cancer screening.

## 3. THE IMPACT OF VARIABLITY IN STUDY DESIGN

**Chapter 3.1** focuses on the influence of choices in study design on the risk estimates for (specific types of) cancer associated with insulin use in patients with T2DM. A series of 1,440 different case-control studies was conducted within the same study population, using British electronic health care registries. Variations in study design choices were related

to the definition of insulin exposure, selection criteria of the study population, and the definition of cancer events. Our findings showed a wide range of risk estimates, where the risk of any cancer associated with insulin use ranged from a protective effect (odds ratio [OR] 0.76, 95% CI 0.71-0.83) to a near 3-fold increased risk (OR 2.86, 95% CI 2.60-3.16). In particular, the risk of pancreatic cancer associated with insulin use varied widely, from an OR of 0.47 (95% CI 0.25-0.89) to 5.13 (95% CI 3.23-8.13). For pancreatic and liver cancer, the risk estimates associated with insulin use markedly decreased when the cancer diagnosis date was shifted backwards in time. Such an approach takes into account a lag-time between cancer development and cancer diagnosis and is aimed to counter reverse causation bias, where yet undiagnosed pancreatic or liver cancer causes metabolic disturbances and the use of insulin instead of the other way around.

The findings from **Chapter 3.1** raise questions regarding the justification of meta-analyses without taking detailed study design choices into account. When differences in risk estimates arise due to variations in study design, meaningful heterogeneity between studies is ignored when an aggregate risk estimate is calculated. We therefore suggested an alternative approach to sum up the progress made within a certain field of investigation, such as the risk of (certain types of) cancer associated with insulin use. Instead of a meta-analysis of individual studies, each new observational study with a different design should be interpreted as a new piece of a complex puzzle. Consequently, a systematic review that places each individual study in its relevant context, based on the specific choices made in the study design, would be more appropriate. Such an approach would be more akin to dossier building, a process that could be greatly facilitated when researchers would clearly motivate their choices in study design.



## 4. TRENDS IN RISK ESTIMATES WITH CUMULATIVE EXPOSURE

In **Chapter 4** trends with cumulative duration of exposure were assessed. A biological gradient, so to say, is often interpreted as an indication of a causal connection between the exposure and outcome. Both studies presented in this chapter are related to breast cancer among T2DM patients. **Chapter 4.1** focuses on the potential beneficial effect of metformin use on survival in breast cancer patients. Metformin could decrease breast cancer cell growth, either indirectly by reducing circulating insulin levels or directly via activation of enzymes that play a role in cellular energy homeostasis. Using data from Danish health care registries (1996-2008), 1,058 T2DM women with breast cancer were identified, of whom 349 died during follow-up. In 152 deaths breast cancer was listed as the primary cause of death. Current use of metformin was associated with a reduction in both overall mortality (hazard ratio [HR] 0.74, 95% CI 0.58-0.96) and breast cancer-specific mortality (HR 0.88, 95% CI 0.59-1.29). For breast cancer-specific mortality, a trend was observed with cumulative number of prescriptions for metformin, where patients with >20 prescriptions had a significantly reduced risk. However, other, unpredicted patterns, such as a peculiar increase in breast cancer-specific mortality after discontinuation of metformin, indicate that non-causal alternative explanations should not be ruled out; for example, healthy user bias, where diabetic breast cancer patients on metformin are generally in better physical health.

**Chapter 4.2** concentrates on a comparison of breast cancer occurrence between patients using different types of insulin. Preclinical cell studies have reported an increased breast cancer cell growth stimulating effect associated with the insulin analogue glargine, as opposed to other types of insulin. To assess whether the use of insulin glargine causes an

increase in breast cancer incidence, we selected a cohort of 12,468 incident insulin users, utilizing British electronic health care data (2002-2013). Exposure to insulin glargine and non-glargine insulins was determined time-dependently during follow-up. We differentiated between patients started on insulin glargine (insulin-naïve users) and patients started on other types of insulins (switchers). Among insulin-naïve users, no association between breast cancer risk and exposure to insulin glargine was observed. Moreover, there appeared to be no association with cumulative exposure to insulin glargine (*p* trend 0.91), even after ≥5 years (HR 1.06, 95% CI 0.48-2.33). However, among switchers, a linear trend was observed with the number of years of past exposure to other insulin types (*p* trend 0.02), while a non-significant trend with cumulative exposure to insulin glargine was found (*p* trend 0.24). As such, our observations do not concur with the predicted increase in breast cancer risk with cumulative exposure to insulin glargine, at least in insulin-naïve users. Furthermore, these results show the importance of accounting for exposure to all types of insulin when studying the effect of insulin glargine use on breast cancer risk.



## 5. DISENTANGLING DISEASE PROGRESSION AND MEDICATION EFFECTS

In **Chapter 5**, a different approach is taken to assess the etiology of long-term complications in patients with complex diseases. Here, the focus shifts towards broader explanations that call into question whether there actually is a direct link between T2DM and CRC or between VTE and MS. Obesity is highly associated with hyperinsulinemia and T2DM. If hyperinsulinaemia causes an increase in CRC risk, then the observed association between overt T2DM and CRC risk would be of a non-causal nature. **Chapter 5.1** focuses on the relationship between insulin resistance status, as the main cause of hyperinsulinaemia, and CRC risk among T2DM patients. A large cohort of T2DM patients and a non-diabetic reference cohort was selected, using British electronic health care registries. In this study population, T2DM was associated with a 1.3-fold increased risk of CRC (HR 1.26, 95% CI 1.18-1.33). Firstly, we constructed five treatments stages based on the types of hypoglycaemic medications used to gauge T2DM disease severity, but found no association between treatment stage and CRC risk among T2DM patients. Secondly, we calculated the cumulative number of years suffered from obesity (BMI ≥30kg/$m^2$) per individual T2DM patient. A trend of increased CRC risk was observed with longer duration of obesity, where patients with over 8 years of obesity had a 1.3-fold increase in CRC risk (HR 1.28, 95% CI 1.11-1.49). These findings provide an indication that long-term exposure to hyperinsulinaemia increases the risk of CRC in T2DM patients rather than the use of any specific type of hypoglycaemic agent.

Similarly, in **Chapter 5.2** we approached the issue of the increased risk of VTE in patient with MS under the assumption that VTE risk factors accumulate in MS patients, such as the gradual loss of mobility that is characteristic of this complex neurodegenerative autoimmune disease. We used data from British health care registries to select a cohort of MS patients (n=5,566) and a comparison cohort of patients without MS (n=33,370) and found a 2.6-fold increased risk of VTE in patient with MS (HR 2.56, 95% CI 2.06-3.20). Several risk factors, such as a prior VTE, varicose veins, obesity, and major trauma, were found to be associated with an increased risk of VTE within the MS population. In addition, the risk of VTE was indeed increased in MS patients with a recent record indicating immobility, spasticity, glucocorticoid use, or disability. As such, our results provide evidence that the association between VTE and MS is, at least partly, mediated through an increased prevalence of VTE risk factors in patients with MS.

## 6. GENERAL CONCLUSION

In **Chapter 6** the causal implications of the empirical findings of this thesis are discussed. This chapter starts with a theoretical investigation into whether epidemiologic evidence can contribute to our understanding of why certain health-related conditions occur. Based on a deliberation of the foundation of knowledge and the abstract concept of causality, the controversies surrounding the attribution of causality from epidemiologic evidence are put into perspective. Central to this discussion is the question: what is good epidemiologic knowledge? The chapter adopts a critical attitude towards some of the current practices in epidemiology and provides arguments for important theoretical limitations of epidemiology as a means to assess causality. This deliberation also brings forth several recommendations that might improve the ability of epidemiologic research to find answers to questions of causality. Ultimately, a framework is proposed that provides some guidance to what knowledge can and cannot be gained from epidemiologic research that is subsequently used to place the empirical findings of this thesis into perspective with regard to their contribution to our general understanding.

# CHAPTER 8

# NEDERLANDSE SAMENVATTING

## 1. INTRODUCTIE

De incidentie van een aantal specifieke vormen van kanker is hoger bij patiënten met type 2 diabetes mellitus (T2DM) dan bij patiënten zonder T2DM. Bij patiënten met multiple sclerosis (MS) wordt een verhoogde incidentie van veneuze thrombo-embolie (VTE) waargenomen ten opzichte van mensen zonder MS. Beide observaties zeggen iets over de frequentie en verdeling van ziekte in bepaalde patiëntpopulaties en zijn daarom descriptief van aard. Dergelijke waarneembare feiten bevatten kennis over het bestaan van een associatie tussen twee fenomenen, zoals tussen T2DM en bepaalde kankersoorten en tussen MS en VTE. Een waargenomen associatie tussen twee ziektebeelden roept vervolgens een meer substantiële vraag op: waarom zijn twee verschijnselen met elkaar geassocieerd? Wat we uiteindelijk willen weten is of er een causaal verband bestaat tussen een ziektebeeld en specifieke patiëntkenmerken.

Het in kaart brengen van de oorzaken, of etiologie, van langetermijncomplicaties bij patiënten met chronische, complexe ziektebeelden gebeurt bij uitstek op basis van resultaten van epidemiologische studies. De methodologie van epidemiologisch onderzoek maakt het mogelijk om op basis van routinematig verzamelde gegevens een beeld te vormen van de factoren die geassocieerd zijn met het ontstaan van bepaalde ziektebeelden. Daarmee is epidemiologisch onderzoek in veel gevallen de eerste en mogelijk enige manier om greep te krijgen op de complextiteit rondom het ontstaan van langetermijncomplicaties bij chronische aandoeningen. De praktische relevantie van observationeel onderzoek kan echter de theoretische beperkingen van dergelijk onderzoek bij de bepaling van causaliteit niet wegnemen. In **Hoofdstuk 1** wordt verder aandacht besteed aan deze theoretische beperkingen. Centraal staat de theoretische onmogelijkheid om binnen de context van observationeel onderzoek alternatieve verklaringen voor een waargenomen verband uit te sluiten; verklaringen anders dan een causale relatie tussen de waargenomen blootstelling en het waargenomen effect. De belangrijkste beperking van non-experimenteel (lees observationeel) onderzoek wordt samengevat in het onvergmogen om een waargenomen verschil in ziekte-incidente tussen twee groepen noodzakelijkerwijs toe te schrijven aan een verschil in blootstelling aan een specifieke factor in plaats van aan systematische, of non-random, variabiliteit.

De conclusie dat een waargenomen verband een causale oorzaak heeft, is gebaseerd op de uitsluiting van mogelijke andere, niet-causale verklaringen. Langetermijncomplicaties bij patiënten met complexe chronische aandoeningen manifesteren zich tegen een achtergrond die bestaat uit een veelvoud aan mogelijke oorzaken. Dit bemoeilijkt elke poging om causale verbanden te onderscheiden van effecten die het gevolg zijn van een imperfecte vergelijking tussen groepen. Met betrekking tot het waargenomen samengaan van T2DM en (bepaalde vormen van) kanker, luidt de meest aangehaalde verklaring dat een (zeer) hoge insulinespiegel (hyperinsulinemie) zou kunnen leiden tot de stimulering van groei van kankercellen. Hyperinsulinemie is karakteristiek voor T2DM. Tegelijkertijd worden echter associaties waargenomen tussen kankerrisico en obesitas, hoogcalorische voeding, hyperlipidemie en een inactieve levensstijl. Op vergelijkbare wijze wordt VTE geassocieerd met auto-immuunziekten, het gebruik van anti-inflammatoire middelen, ontsteking en immobiliteit. Al deze potentiële oorzaken van VTE vallen samen wanneer het patiënten met MS betreft. Het bepalen of er een causaal verband bestaat tussen VTE en een specieke risicofactor in het bijzonder wordt gecompliceerd door de hoge mate van correlatie tussen deze factoren.

Dit proefschrift richt zich op het bepalen van de invloed van specifieke risicofactoren

in de etiologie van een aantal specifieke vormen van kanker bij patiënten met T2DM en in de etiologie van VTE bij patiënten met MS. Centraal staan de mogelijkheden om causaliteit te bepalen op basis van epidemiologisch onderzoek door middel van het uitsluiten van alternatieve verklaringen voor een waargenomen verband. Hierbij worden alternatieve verklaringen van zowel methodologische als empirische aard onderzocht, evenals het gebruik van eenheden voor cumulatieve blootstelling als middel om de aard van een specifieke blootstelling-respons relatie te bepalen.

## 2. BESCHRIJVING VAN HET PROBLEEMGEBIED

**Hoofdstuk 2** bestaat uit drie descriptieve epidemiologische onderzoeken naar de incidentie van bepaalde vormen van kanker bij patiënten met T2DM. In **Hoofdstuk 2.1** zijn de incidentiecijfers van colorectaal kanker (CRK) bepaald over de tijd voor een groot cohort van T2DM patiënten en een op leeftijd, geslacht en huisartsenpraktijk geselecteerd referentiecohort bestaande uit patiënten zonder diabetes mellitus (1989-2012). Data werden verkregen uit elektronische gezondheidszorg databanken in het Verenigd Koninkrijk (VK). Het voor leeftijd en geslacht gestandaardiseerde CRK incidentiecijfer bij patiënten met T2DM was significant hoger dan dat bij niet-diabeten: 60,7 (95% betrouwbaarheidsinterval [BI] 58,0-63,3) versus 54,6 (95% BI 52,3-56,9) per 100.000 persoonsjaren (pj). Over de tijd bleken de incidentiecijfers voor CRK consistent hoger te liggen bij T2DM patiënten. Verder lieten de colonkanker incidentiecijfers over de tijd een stijgende trend zien bij T2DM patiënten, waarbij de incidentie onder mannen het hoogst was. Landelijke screening voor CRK werden in 2006/2007 geïntroduceerd in het VK, maar had geen eenduidig effect op de geobserveerde incidentie van colonkanker bij patiënten met T2DM. Aangezien mannelijke T2DM patiënten het hoogste risico lijken te lopen, hebben wij gerichte CRK screening van deze groep aanbevolen.

Vervolgens werden in **Hoofdstuk 2.2** de incidentiecijfers van overige gastro-intestinale kankersoorten over de tijd bepaald in dezelfde patiëntpopulatie. Over het algemeen werd jaarlijks bij 1 op de 300 patiënten met T2DM een vorm van gastro-intestinale kanker gediagnosticeerd in het VK. Naast CRK waren de incidentiecijfers van alvleesklier- en leverkanker significant hoger onder patiënten met T2DM. Leverkankerincidentie bij T2DM patiënten was 26 per 100.000 pj (95% BI, 24-28), vergeleken met 8,9 per 100.000 pj (95% BI, 7,7-10) onder niet-diabeten. Verder nam de incidentie van leverkanker in het T2DM cohort sterk toe gedurende de periode 2001-2012. Voor alvleesklierkanker was het incidentiecijfer onder patiënten met T2DM tweemaal zo hoog als dat bij niet-diabeten: 65 per 100.000 pj (95% BI 62-69) ten opzichte van 31 per 100.000 pj (95% BI 28-34). Zowel alvleesklier- als leverkanker kunnen leiden tot een verstoring van de bloedglucosespiegels en daarmee tot het ontstaan van symptomen van diabetes mellitus. Vandaar dat het verband tussen deze specifieke kankersoorten en T2DM mogelijk beide richtingen op kan gaan. Verder werd geen verschil in incidentie van maag- en galwegkanker vastgesteld, terwijl een lagere incidentie voor slokdarmkanker werd waargenomen bij patiënten met T2DM.

Eerdere onderzoeken hebben naast gastro-intestinale kankersoorten ook een verband gevonden tussen T2DM en borstkanker bij vrouwen. In **Hoofdstuk 2.3** zijn voor leeftijd gestandaardiseerde incidentiecijfers van invasieve borstkanker bepaald over de tijd onder vrouwen met T2DM afkomstig uit dezelfde Britse studiepopulatie. Er werd geen

significant verschil in borstkankerincidentie waargenomen tussen vrouwen met en zonder T2DM: 150 (95% BI 143-157) versus 148 (95% BI 141-156) per 100.000 pj. Daarentegen bleek onder postmenopauzale vrouwen met T2DM en een body mass-index (BMI) ≥35 kg/m$^2$ het incidentiecijfer van borstkanker significant hoger te liggen dan dat onder vrouwen met T2DM en een BMI <25 kg/m$^2$: 421 (95% BI 372-470) versus 313 (95% BI 270-355) per 100.000 pj. Gerichte borstkankerscreening bij postmenopauzale vrouwen met T2DM die lijden aan obesitas zou daarom kunnen worden overwogen.

## 3. DE INVLOED VAN VARIATIE IN STUDIEOPZET

In **Hoofdstuk 3.1** werd gekeken naar het risico op (specifieke vormen van) kanker bij het gebruik van insuline binnen een groep patiënten met T2DM en de invloed die verschillen in keuzes met betrekking tot het ontwerp van een epidemiologisch onderzoek hebben op de gevonden risicoschatting. Hiervoor werd een serie van 1440 verschillende patiënt-controle onderzoeken uitgevoerd binnen dezelfde studiepopulatie met behulp van elektronische patiëntgegevens uit het VK. De verschillen in keuzes voor de studieopzet waren gerelateerd aan de definitie van blootstelling aan insuline, selectiecriteria voor de patiëntselectie en de definitie van kankerdiagnose. Onze resultaten gaven een grote variëteit aan risicoschattingen weer, waarbij het risico op kanker dat geassocieerd was met insulinegebruik varieerde van een beschermend effect (odds ratio [OR] 0,76, 95% BI 0,71-0,83) tot een bijna 3 maal verhoogd risico (OR 2,86, 95% BI 2,60-3,16). In het bijzonder bleek het risico op alvleesklierkanker sterk te veranderen – van een OR van 0,47 (95% BI 0,25-0,89) tot een OR van 5,13 (95% BI 3,23-8,13) – afhankelijk van de gemaakte keuzes voor het studieontwerp. Bij zowel alvleesklier- als leverkanker nam het risico dat geassocieerd was met insulinegebruik sterk af indien de kankerdiagnosedatum terug in de tijd werd verschoven. Door het terugplaatsen van de diagnosedatum wordt rekening gehouden met een latentietijd tussen het ontstaan van kanker en de uiteindelijke diagnose ervan. Dit heeft tot doel om een omgekeerd causaal verband, waarbij nog niet gediagnosticeerde alvleesklier- of leverkanker juist leidt tot de verstoring van bloedglucosespiegels en daarmee tot het gebruik van insuline, uit te sluiten.

De bevindingen in **Hoofdstuk 3.1** trekken de rechtvaardiging van het veelvuldige gebruik van meta-analyse technieken in de epidemiologie in twijfel. Bij het uitvoeren van een meta-analyse worden de resultaten van observationele onderzoeken gecombineerd om een geaggregeerde risicoschatting te presenteren. Dergelijke technieken houden echter geen rekening met de verschillen in keuzes voor het ontwerp van deze individuele studies. Door het aggregeren van resultaten wordt betekenisvolle heterogeniteit onbenoemd gelaten. Om die reden stelden wij een alternatieve aanpak voor om de voortgang binnen een bepaald onderzoeksgebied – zoals het risico op (bepaalde soorten) kanker bij het gebruik van insuline – samen te vatten. In plaats van het uitvoeren van een meta-analyse van individuele studies zou ieder observationeel onderzoek met een andere studieopzet moeten worden beschouwd als een nieuw onderdeel van een complexe puzzel. Een systematische review zou daarom wellicht een beter passende aanpak zijn, waarbij ieder individueel onderzoek in de relevante context wordt geplaatst op basis van de keuzes die gemaakt zijn voor het studieontwerp. Een dergelijk proces zou erg gebaat zijn bij een heldere motivatie van onderzoekers waarom ze gekozen hebben voor een bepaalde studieopzet.

## 4. EENHEDEN VOOR CUMULATIEVE BLOOTSTELLING

In **Hoofdstuk 4** zijn eenheden voor cumulatieve blootstelling gebruikt om trends in de daaraan gerelateerde risicoschattingen te bepalen. Een verband tussen cumulatieve blootstelling en het risico op een bepaalde gezondheidsgerelateerde uitkomst wordt ook wel een biologische gradiënt genoemd en wordt veelal geïnterpreteerd als een indicatie voor een causaal verband. Beide onderzoeken in dit hoofdstuk hebben betrekking op borstkanker bij patiënten met T2DM. **Hoofdstuk 4.1** richt zich op het eventueel gunstige effect dat metforminegebruik kan hebben op de overlevingkans van patiënten met borstkanker. Op basis van preklinische studies zou metformine de borstkankercelgroei kunnen remmen via activatie van enzymen die een rol spelen bij de cellulaire energiehuishouding. Daarnaast zou metformine een indirect effect op borstkankercelgroei kunnen hebben door het verlagen van de insulinespiegel. Met behulp van data uit Deense gezondheidszorgdatabases (1996-2008) werd een cohort van 1058 vrouwen met invasieve borstkanker en T2DM geïdentificeerd, van wie er in totaal 349 overleden gedurende de periode van follow-up. In 152 gevallen werd borstkanker als de primaire doodsoorzaak genoemd. Metforminegebruik was geassocieerd met een significante verlaging van algemene mortaliteit (hazard ratio [HR] 0,74, 95% CI 0,58-0,96) en een niet-significante verlaging van borstkanker-specifieke mortaliteit (HR 0,88, 95% CI 0,59-1,29). Daarentegen werd voor borstkanker-specifieke mortaliteit wel een trend waargenomen met het cumulatieve aantal prescripties voor metformine, waarbij patiënten met >20 prescripties een significant verlaagd risico hadden. Er werden echter ook onverwachte patronen waargenomen, zoals een toename in mortaliteit na het stoppen met metforminegebruik. Dit zou kunnen duiden op een non-causale alternatieve verklaring, zoals een vertekening die kan optreden wanneer metformine voornamelijk wordt voorgeschreven aan patiënten die relatief gezien in een betere fysieke toestand verkeren.

In **Hoofdstuk 4.2** werd het risico op borstkanker bepaald bij patiënten die behandeld werden met verschillende soorten insuline. Hierbij ging de aandacht in het bijzonder uit naar het borstkankerrisico van patiënten die de behandeld werden met de insuline-analoog glargine. Preklinische studies hebben een verschil waargenomen tussen het effect dat insuline glargine had op borstkankercelgroei enerzijds en het effect van andere, non-glargine insulines anderzijds. Door een verhoogde affiniteit voor de insuline-achtige groeifactor-1 receptor, zou insuline glargine borstkankercelgroei in hogere mate stimuleren dan andere soorten insuline. Om te bepalen of het gebruik van insuline glargine leidt tot een verhoogd risico op borstkanker werd een cohort van 12.468 incidente insulinegebruikers geselecteerd met behulp van elektronische patiëntgegevens uit het VK (2002-2013). Blootstelling aan insuline glargine en non-glargine insulines werd tijdsafhankelijk bepaald gedurende follow-up. Daarbij werd een onderschied gemaakt tussen patiënten die gestart zijn op insuline glargine (insuline-naïeve gebruikers) en patiënten die tijdens follow-up wisselden naar insuline glargine nadat ze eerst andere soorten insuline hadden gebruikt (switchers). Bij insuline-naïeve gebruikers werd geen associatie gevonden tussen blootstelling aan insuline glargine en borstkankerrisico. Bovendien werd er geen verband waargenomen met cumulatieve blootstelling (*p* trend 0,91), zelfs niet na ≥5 jaar cumulatief gebruik (HR 1,06, 95% BI 0,48-2,33). Daarentegen werd bij switchers een lineaire trend vastgesteld met het cumulatief aantal jaar aan eerdere blootstelling aan andere soorten insuline voor de start van insuline glargine (*p* trend 0,02) en een non-significante trend met cumulatieve

blootstelling aan insuline glargine zelf (*p* trend 0,24). Concluderend, kwamen onze observaties niet overeen met het verwachte patroon van een verhoging in borstkankerrisico met toenemend cumulatief gebruik van insuline glargine. Daarnaast tonen deze resultaten het belang aan van het bepalen van de blootstelling aan alle soorten insuline wanneer het effect van insuline glargine op borstkankerrisico bestudeerd wordt in de epidemiologie.

## 5. ONDERSCHEIDEN VAN ZIEKTEPROGRESSIE EN DE INVLOED VAN MEDICATIE

In **Hoofdstuk 5** werd een andere benadering gekozen voor het bepalen van de etiologie van langetermijncomplicaties bij complexe ziektebeelden. Hierbij ging de aandacht uit naar meer algemene verklaringen die in twijfel trekken of er in wezen sprake is van een direct verband tussen T2DM en CRK enerzijds en MS en VTE anderzijds. Zo wordt T2DM gekenmerkt door insulineresistentie met de daaraan verbonden hyperinsulinemie, maar T2DM is geen synoniem voor hyperinsulinemie. Obesitas is een belangrijke oorzaak van insulineresistentie en daardoor sterk geasssocieerd met hyperinsulinemie. Indien hyperinsulinemie inderdaad de verklarende factor is voor de hogere incidentie van CRK bij T2DM patiënten, is het waargenomen verband tussen gediagnosticeerde T2DM en CRK enkel een non-causaal bijproduct van deze causale relatie. **Hoofdstuk 5.1** richt zich op de relatie tussen insulineresistentie en het risico op CRK bij patiënten met T2DM. Hiervoor werd een groot cohort van T2DM patiënten en een referentiecohort bestaande uit niet-diabeten geselecteerd met behulp van elektronische patiëntgegevens uit het VK. In deze studiepopulatie bleek T2DM geassocieerd te zijn met een 1,3 maal verhoogd risico op CRK (HR 1,26, 95% BI 1,18-1,33). Als maat voor T2DM ziekteprogressie – en daarmee mogelijk ook de mate van insulineresistentie – werden vijf behandelstadia gedefinieerd op basis van het type bloedglucoseverlagend middel dat werd voorgeschreven. Binnen patiënten met T2DM werd geen verband waargenomen tussen behandelstadium en het risico op CRK. In een andere, onafhankelijke, benadering werd het cumulatief aantal jaren met obesitas (BMI ≥30kg/m$^2$) berekend per individuele T2DM patiënt. Er werd een significante correlatie gevonden tussen het cumulatief aantal jaren met obesitas en het risico op CRK, waarbij patiënten die meer dan 8 jaar aan obesitas leden een 1,3 maal verhoogd risico op CRK hadden (HR 1,28, 95% BI 1,11-1,49). Deze bevinding zou gezien kunnen worden als een indicatie dat langdurige blootstelling aan hyperinsulinemie leidt tot een verhoogd risico op CRK in patiënten met T2DM.

Op eenzelfde wijze werd in **Hoofdstuk 5.2** het in eerdere onderzoeken waargenomen verband tussen VTE en MS benaderd vanuit de gedachte dat risicofactoren voor VTE accumuleren in patiënten met MS, zoals de kenmerkende graduele afname in mobiliteit. Om dit te onderzoeken werden elektronische gegevens uit gezondheidszorg databanken in het VK gebruikt om een cohort van MS patiënten (n=5566) en een referentiecohort bestaande uit patiënten zonder MS (n=33.370) te selecteren. In deze studiepopulatie werd MS geassocieerd met een 2,6 maal verhoogd risico op VTE (HR 2,56, 95% CI 2,06-3,20). Binnen de groep MS patiënten werd een significant verband waargenomen tussen het risico op VTE en de volgende risicofactoren: een eerdere VTE, spataderen, obesitas en een recent trauma. Daarnaast werd een verhoogd risico op VTE gevonden bij MS patiënten voor wie recent was vastgesteld dat ze immobiel, spastisch of invalide waren of glucocorticoïden

gebruikten. Deze bevindingen tonen daarmee aan dat de associatie tussen VTE en MS voor een belangrijk deel verklaard kan worden door een hogere prevalentie van VTE risicofactoren in patiënten met MS.

## 6. ALGEMENE CONCLUSIE

**Hoofdstuk 6** tenslotte, bestaat uit een theoretische uiteenzetting die zal leiden tot een antwoord op de vraag: wat is goede epidemiologische kennis? Een kentheoretische beschouwing van het concept causaliteit en de aard en oorsprong van kennis vormen de basis voor een kritische analyse van de werkwijze binnen de epidemiologie. In het bijzonder gaat deze discussie in op de controverse rondom het bepalen van causaliteit op basis van non-experimenteel (lees observationeel) onderzoek. De belangrijkste theoretische beperkingen van de methodologie van observationeel onderzoek om causale verbanden te toetsen komen in dit hoofdstuk aan de orde. Tevens leidt deze deliberatie tot enkele aanbevelingen die de waarde van epidemiologisch onderzoek met betrekking tot het bepalen van de etiologie van ziekten zouden kunnen verbeteren. Uiteindelijk resulteert dit hoofdstuk in een theoretisch kader op basis waarvan de mogelijkheden en beperkingen van observationeel onderzoek inzichtelijk worden. De empirische bevindingen uit de eerdere hoofdstukken worden vervolgens besproken in het licht van dit theoretisch kader.

# APPENDICES

# *APPENDIX A*

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Many others have made significant contributions to this work. In particular, I want to acknowledge the support of Marloes Bazelier, who might not have been officially part of my supervising team, but in reality rarely missed a meeting. She always made herself available to me whenever I ran into difficulties. For this and for her joyful personality I owe her much gratitude. There are many others who have influenced my work and to whom I want to express my appreciation. In case I forget to mention some of them, I apologize in advance. Naturally, I owe tribute to the people who were so kind to work with me on the various research projects. And even though I crossed swords with some of them on occasion, their comments have substantially strengthened the content of this book. First of all, my thanks are due to Anssi Auvinen, Mark de Groot, Marjanka Schmidt, Tjeerd van Staa, Bernard Uitdehaag, and Peter Vestergaard. For taking the time and making the effort to read and comment on my drafts and for the many ideas these people shared with me, I at least owe them these kind words. Then special thanks are owed to Heleen Bronsveld and Roy de Jong, with whom I had the pleasure to work with and who in vain tried to show me how to conduct research in a more orderly fashion. Of course, I want to acknowledge the support provided by the people of the CARING research team who I have not mentioned already: Anna, Irene, Jakob, Jari, Kari, Morten, Nils, Øystein, Vidar, and Zoltan. Our project meetings

in Utrecht, Oslo, Helsinki, and London were generally a lovely combination of thinking and drinking.

While at times I wondered, in the course of my research, whether my efforts would result in a valuable contribution to our general understanding of things, my friends and colleagues often provided much needed distraction that lifted my spirits. It is therefore that I not halfheartedly want to acknowledge the contribution that they made, perhaps even unknowingly, to this book. I am indebted to my colleagues at the division of pharmacoepidemiology and clinical pharmacology in general, and to the ones I now consider to be my friends in particular. For nearly four years, Maarten, Niels, Ruben, Sander, and I shared stories, thoughts, interests, trips, gossip, anger, frustration, bad jokes, drinks, heartbreak, and countless other things. It is not often that people forced upon you, so to say, are the kind you would also mingle with out of free will. Our many and at times heated debates, in bars, at lunchtime, during Friday drinks, near the coffee machine, or via text messaging, have bolstered my critical attitude towards my ideas, for which I am grateful. In this respect, I also owe thanks to Hamid for the delightful philosophical dialogues we had. Special thanks are due to my good friends Felix and Daniël, with whom I spent the larger part of my student years and who were willing to stand beside me during the public defense of my work. Lastly, I want to express my gratitude to my family, who supported me through these years, both emotionally and financially. To my mother, for always being there for me; my father, for always looking out for me; my sister, for being a worthy adversary; and my brother, for being such a tremendous help in the preparation of this book.

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# *APPENDIX B*

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# *APPENDIX C*

## LIST OF PUBLICATIONS

### The risk of venous thromboembolism in patients with multiple sclerosis: the Clinical Practice Research Datalink

- Peeters PJHL, Bazelier MT, Uitdehaag BMJ, Leufkens HGM, De Bruin ML, de Vries F
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### Insulin glargine use and breast cancer risk: associations with cumulative exposure

- Peeters PJHL, Bazelier MT, Leufkens HGM, Auvinen A, van Staa TP, de Vries F, De Bruin ML
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- *Current Drug Safety*. 2013; **8**(5): 357-363

## SUBMITTED OR IN PREPARATION

### Insulin use and cancer risk: the effect of choices in study design on risk estimates

- Peeters PJHL, Bazelier, MT, Leufkens HGM, de Vries F, De Bruin ML, van Staa TP

### Trends in breast cancer incidence among patients with type 2 diabetes mellitus in the United Kingdom

- Bronsveld HK, Peeters PJHL, de Groot MCH, de Boer A, Schmidt MK, De Bruin ML

### Trends in colorectal cancer incidence among patients with type 2 diabetes mellitus in the United Kingdom

- Peeters PJHL, Bronsveld HK, de Groot MCH, Leufkens HGM, de Vries F, De Bruin ML

### Gastrointestinal cancer incidence in type 2 diabetes mellitus: results from a large retrospective population-based cohort study

- de Jong RGPJ, Peeters PJHL, Burden AM, De Bruin ML, Haak HR, Masclee AAM, de Vries F, Janssen-Heijnen MLG

# *APPENDIX D*

## ABOUT THE AUTHOR

Paul Peeters (Heerlen, 3 January 1983) received his secondary education (VWO) at the Bernardinus College in Heerlen. Afterwards, he studied pharmacy at the Utrecht University from 2002 to 2011 and followed extracurricular courses in business economics at the Tilburg University from 2003 to 2005. During his Master studies, he conducted a research project in pharmacoepidemiology at the PHARMO Institute. In 2011, he graduated as a pharmacist from the Utrecht University. Shortly after, he started his PhD at the Division of Pharmacoepidemiology and Clinical Pharmacology.