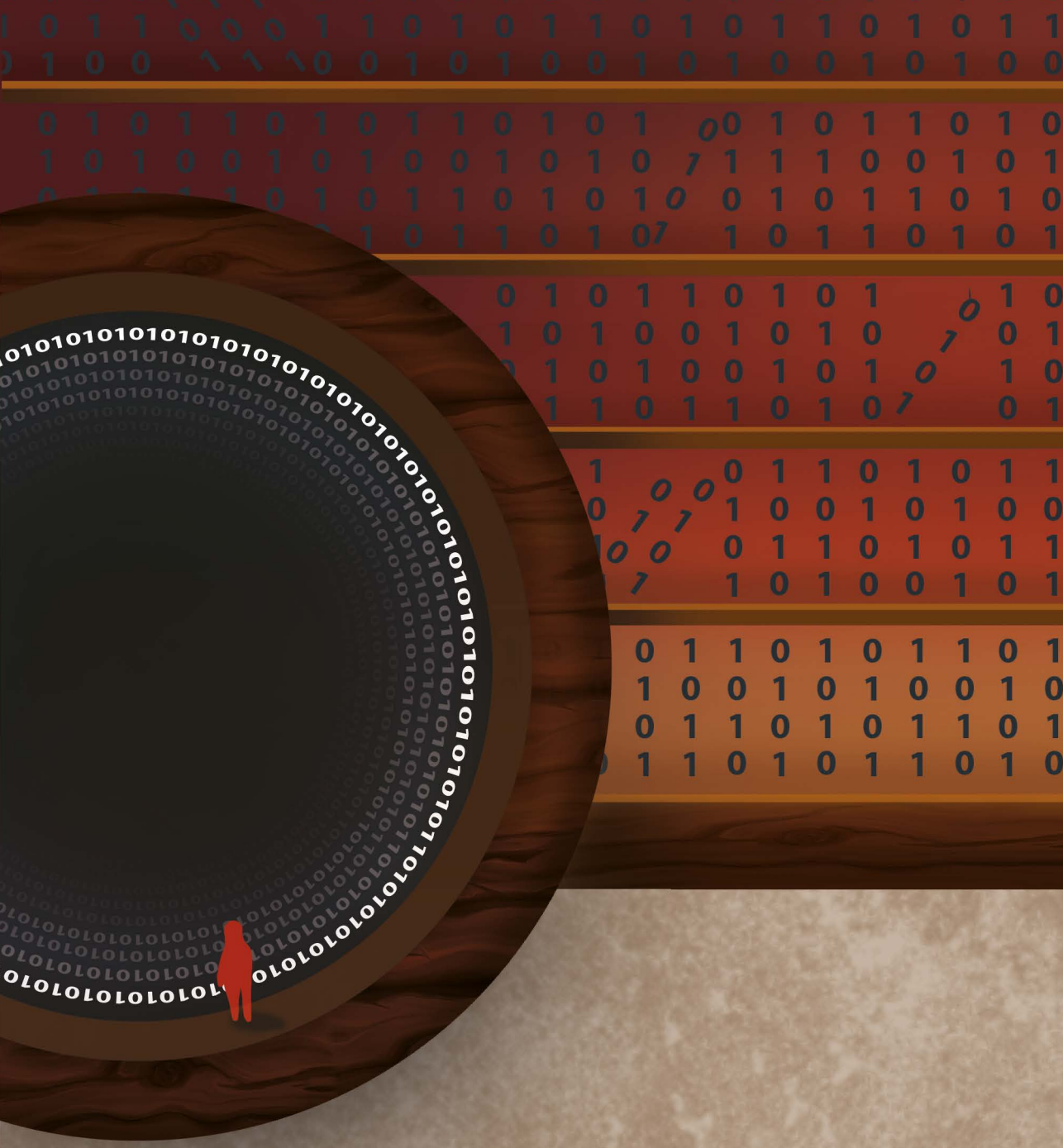
Type 2 diabetes mellitus and gastrointestinal cancer



Type 2 diabetes mellitus and gastrointestinal cancer : Disease, drugs, or distortion?

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Colofon

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Type 2 diabetes mellitus and gastrointestinal cancer:

Disease, drugs, or distortion?

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Chapter 1

General introduction

1. General introduction

Type 2 diabetes mellitus (T2DM) and gastrointestinal (GI) cancer are increasing global health problems. For both entities, the incidence and prevalence are still rising.[1] Cancer and diabetes are diagnosed within the same individual more frequently than would be expected to occur by chance, even after adjusting for confounding factors.[1] After several decades of research, it is still under debate whether T2DM truly is a risk factor for the development of GI cancer or whether T2DM is more of a 'risk indicator' of pathophysiological mechanisms that increase the chance of developing cancer. Furthermore, for clinicians the prevention of disease complications is an important goal of disease management. If stringent management of T2DM would convey a clinically relevant reduction of GI cancer risk, this may have important implications for clinical practice. In this thesis multiple aspects of the complex association between T2DM and GI cancer are analysed, with a special focus on the impact of anti-diabetic drugs (ADDs), glycaemic control, and methodological issues.

1.1 Gastrointestinal cancer

GI cancer comprises all malignancies of the digestive tract and its related digestive organs, including the oesophagus, stomach, liver, biliary tract, gallbladder, pancreas, small intestine, large intestine, rectum and anus. Symptoms for each cancer type relate to the affected organ and may include bowel or ductal (mechanical) obstruction (e.g. dysphagia, ileus, or jaundice), abnormal bleeding and blood loss, metabolic derangements with weight loss and malnutrition and other associated problems.

In the Netherlands, the most commonly diagnosed GI cancer is colorectal cancer, followed by pancreatic cancer and oesophageal cancer. In 2015 the highest ever observed incidence of GI cancer so far was reported (Crude Rate (CR): 142 per 100,000 persons per year).[2] Almost two-thirds of the diagnosed GI cancers concerned colorectal cancer (CR 92 per 100,000 persons per year).[2] Up to 2015, the incidence of GI cancer has increased in particular for colorectal cancer, oesophageal cancer, and pancreatic cancer. This contrasts with a decline by almost one third of the incidence of gastric cancer.

GI cancers are complex, multifactorial diseases for which various risk factors have been identified. Non-modifiable risk factors include high age and male sex, which are also the most important risk factors for GI cancer in general. Other risk factors include race (e.g. liver cancer occurs more frequently in Asians, and colorectal cancer is seen more frequently in blacks)[3], and personal and family medical history of specific cancers.[4] Apart from certain occupational exposures[5], environmental factors, and certain infections (e.g. liver fluke for cholangiocarcinoma), there are also some modifiable risk factors. These

are largely related to obesity, dietary habits and lifestyle choices (e.g. physical inactivity, smoking, alcohol use).[6-8] These modifiable factors influence the risk of GI cancer in varying degrees.

1.2 Type 2 diabetes mellitus

Diabetes mellitus is an umbrella term for a heterogeneous group of chronic, metabolic diseases characterized by increased blood glucose levels (hyperglycaemia), resulting from a deficient endogenous insulin production by the pancreas, insulin resistance, or both.[9,10] The number of cases and the prevalence of diabetes have been rising over the past decades, coinciding with the global obesity epidemic. As a result, diabetes has become a leading threat to global public health.[9]

In 2014, worldwide, an estimated 422 million adults were suffering from diabetes, compared to 108 million in 1980.[9] Between 1980 and 2014, the global prevalence has nearly doubled from 4.7% to 8.5%.[9] Separate estimates of diabetes prevalence for type 1 diabetes mellitus and T2DM do not exist, as laboratory tests to distinguish between these subtypes are not always available. However, approximately 90-95% of diabetes mellitus cases are comprised of individuals with T2DM.[10]

The diagnosis of T2DM is based on one of four abnormalities: a glycated haemoglobin (HbA1c) level ≥6.5% (48 mmol/mol), a fasting plasma glucose level ≥7.0 mmol/L, a random elevated blood glucose level with symptoms (polyuria, polydipsia, nocturia, blurred vision, or weight loss), or an abnormal oral glucose tolerance test (OGTT).[10,11] Although specific aetiologies for T2DM are unknown, it is thought to result from the presence of insulin resistance (the body's inability to effectively use insulin). The risk of T2DM is determined by a combination of genetic and metabolic factors such as ethnicity, family history of diabetes, history of gestational diabetes, older age, overweight and obesity, unhealthy diet, physical inactivity and smoking.[9] T2DM may go undiagnosed for multiple years until complications (such as retinopathy, nephropathy, or neuropathy) occur.[9] As there is yet no cure for T2DM, treatment is mainly focused on effective management of blood glucose levels through lifestyle modifications and the use of ADDs, and on the prevention of long-term complications. Multiple pharmacologic agents are available for lowering the blood glucose level in patients with T2DM. These drugs either focus on lowering peripheral insulin resistance and gluconeogenesis (metformin, thiazolidinediones) or on increasing the endogenous insulin output by the pancreas (sulfonylureas, glinides). To date, the Dutch and international guidelines advise the use of metformin as a first-line drug treatment when lifestyle advice such as dietary modification, physical exercise, and weight reduction fail to maintain glucose levels within normal range.[10,11] Subsequently, when T2DM progresses and glycaemic control is

inadequate, other ADDs or exogenous insulin may be added to the therapeutic drug regimen.

1.3 Diabetes and gastrointestinal cancer

T2DM has currently been associated with some, but not all, GI cancers. Substantial heterogeneity exists between results of observational studies, and at present high quality evidence is only available for associations between T2DM and colorectal cancer (15-30% increased risk) and intrahepatic cholangiocarcinoma (doubled risk).[12] In addition, T2DM has also been associated with an increased risk of liver and pancreatic cancer, but methodological aspects have so far precluded to draw firm conclusions.[12,13]
The biological relationship between T2DM and GI cancer is yet poorly understood.[1,14] It is currently thought that the relationship may not be entirely attributable to the direct effects of T2DM, such as hyperglycaemia, which effects will be further discussed in section 1.5.[1,15] Instead, T2DM may be a marker of an altered cancer risk due to changes in underlying metabolic conditions, such as insulin resistance, hyperinsulinaemia or inflammation.[1] Insulin resistance, hyperinsulinaemia (either endogenous due to insulin resistance, or exogenous due to administered insulin or insulin secretagogues) and elevated levels of Insulin-like Growth Factor-1 (IGF-1) reduce apoptosis and increase cell proliferation in target cells, leading to tumour development.[16-19] Insulin itself is also known to have mitogenic properties[20], and, in particular, both the liver and the pancreas are exposed to high levels of endogenously produced insulin through the portal venous system.[21] Alternatively, the relationship may be due to the sharing of common predisposing conditions, such as obesity, lack of physical activity, or a high-caloric diet.[1]
In addition, there is an added level of complexity in the association between T2DM and pancreatic cancer, as both diseases involve the same organ. While some studies suggest that diabetes can be an early manifestation of pancreatic cancer, other studies point in the direction of diabetes as an aetiological factor.[22-26] Lastly, associations have been found between drugs used in the treatment of T2DM and GI cancer risk, complicating the evaluation of the association between T2DM and GI cancer. This will be further discussed in section 1.4.

1.4 Anti-diabetic drugs and gastrointestinal cancer

Several drugs used in the treatment of T2DM have been associated with either a decreased or increased (GI) cancer risk. Below, the evidence regarding the associations between ADDs and GI cancer risk are summarised.
Substantial in-vitro and in-vivo evidence has suggested that metformin has anti-cancer properties.[27] Metformin has been shown to interact with several metabolic pathways,

such as the LKB1/AMPK pathway, that are often disrupted in sporadic cancers.[28,29] AMPK activation by metformin leads to inhibition of mammalian target of rapamycin (mTOR) signaling, which regulates protein synthesis, cell growth and cell proliferation.[30] Besides, metformin exerts systemic effects that could be responsible for its chemoprotective effects, such as a reduction in signaling molecules (glucose, insulin, and IGF-1), modification of inflammatory processes, or enhancement of the immune response.[20,31,32]
The use of metformin has been predominantly associated with a decreased risk of GI cancers in observational studies. Although an increased risk of colorectal cancer has also been reported.[33,34] In 2005, Evans et al. first highlighted an association between the use of metformin and reduced cancer risk in T2DM patients.[35] Since then, numerous observational studies have reported varying degrees of a protective effect of metformin for a variety of GI cancer types including liver, colorectal, pancreatic, gastric and oesophageal cancer.[36] In contrast, data from meta-analyses of randomized controlled trials (11 RCTs with 398 cancers during 51,681 person-years, comparing metformin with active glucose-lowering therapy or placebo/usual care) have not supported metformin's protective role on cancer development.[37,38] Furthermore, it has been shown that time-related biases have greatly impacted the results regarding the use of metformin and risk of multiple GI cancer types.[39] This methodological aspect will be further discussed in section 1.6. Based on the evidence mentioned above, the debate on metformin's anti-cancer effect is still ongoing.
Sulfonylureas (SUs) and exogenous administered insulin may increase the risk of GI cancer by increasing the levels of circulating insulin, which has direct and indirect mitogenic properties.[40] Insulin may directly stimulate proliferation of tumour cells by binding to the insulin receptor A. Indirect effects of insulin include promoting the synthesis of insulin-like growth factor (IGF)-1 in hepatocytes and a decrease of hepatic synthesis of IGF binding protein 1 and 2, resulting in a relative increase in free circulating IGF-1.
A meta-analysis of 33 RCTs, involving 26,022 T2DM patients and comparing the risk of cancer between users of SUs and users of other ADDs, did not show a significant difference in cancer risk (Odds Ratio (OR) 0.93, 95% CI 0.77-1.12). However, significant heterogeneity was observed (I^2=30%, p=0.05) and incomplete follow-up in each RCT was high.[41] Furthermore, adjusted analyses of pooled data from observational studies (15 cohort studies and 11 case-control studies involving over 1.8 million T2DM patients and 48,201 cancer events) comparing the use of SUs with the use of other ADDs did not show a significant difference in cancer risk (adjusted Hazard Ratio (HR) 1.05, 95% CI 0.96-1.15 and adjusted OR 1.13, 95% CI 0.93-1.17 respectively for cohort and case-control studies).[41] However, observational studies included in the analyses generally had a moderate to high risk of bias and also showed marked heterogeneity.

Regarding exogenous insulins, no significant differences in GI cancer risk have been observed in RCTs comparing the use of insulin glargine with other types of insulin or non-insulin ADDs.[42,43] In subsequent meta-analyses of observational studies, an increased risk of pancreatic and colorectal cancer has been found when comparing the use of insulin to the use of non-insulin antidiabetic drugs.[44] In contrast, lower risks of various GI cancers have been observed when comparing the use of insulin glargine to the use of non-glargine insulin.[44,45] Yet, important limitations have been noted for observational studies on the risk of cancer with the use of insulin, such as marked heterogeneity between studies, limited confounder adjustment, and short follow-up periods.

In vitro and in vivo studies have shown that thiazolidinediones (TZDs) possess anti-cancer properties in gastrointestinal cancer cells through various intracellular signalling pathways, resulting in cell growth arrest, induction of apoptosis, and inhibition of cell invasion.[46] A meta-analysis of RCTs has also shown a reduced risk of bowel cancer in users of rosiglitazone versus either placebo or other types of ADDs (OR 0.63, 95% CI 0.41-0.96, p=0.03), but not for pioglitazone or TZDs overall.[47] In addition, a decreased risk of both colorectal cancer (RR 0.93, 95% CI 0.90-0.97, 6 cohort studies) and liver cancer (RR 0.65, 95% CI 0.48–0.89, 4 cohort studies) has been observed in a meta-analysis of observational studies wherein use of TZDs was compared to no use of TZDs.[48]

Lastly, incretin-based drugs (glucagon-like peptide-1 receptor agonists (GLP-1RAs), such as exanatide en liraglutide, and dipeptidyl peptidase-4 (DPP-4) inhibitors, such as saxagliptin or sitagliptin) have been associated with an increased risk pancreatic cancer.[44,49,50] This concern has arisen from the association between incretins and pancreatitis that was found in adverse-event database studies.[51,52] Subsequent observational studies have so far produced conflicting results, with most recent and methodologically most accurate studies showing no evidence of an increased risk of pancreatic cancer in users of incretins compared to either sulfonylureas or other non-insulin ADDs.[53-56]

1.5 Hyperglycaemia and gastrointestinal cancer

In patients with T2DM, the risk of long-term complications is partially related to the degree of glycaemic control over time.[57] It is therefore interesting to explore whether the level of glycaemic control also influences the risk of GI cancer, especially as cancer cells are highly dependent on glycolysis for energy, resulting in a high requirement for glucose to generate adenosine triphosphate (ATP; also known as the Warburg effect).[58] Based on observational studies, high blood glucose levels or inadequate glycaemic

control are thought to impact cancer risk.[59,60] However, the evidence for an association between various measures of hyperglycaemia in patients with T2DM and cancer risk has been inconsistent. In a meta-analysis of clinical trials, cancer risk did not seem to be affected by the level of glycaemic control (i.e. randomisation to either standard or intensive glycaemic control).[61] In contrast, in a meta-analysis of observational studies, HbA1c levels in the diabetic range were associated with an increased risk of GI cancers.[62] Additionally, another meta-analysis of epidemiological studies has shown an increased risk of colorectal and pancreatic cancer in groups with the highest compared to those with the lowest ranges of markers of glycaemia (e.g. insulin, glycated haemoglobin [HbA1c], fasting blood glucose).[63] However, the studies used in meta-analyses all utilized individual or mean markers of glycaemia at the start of follow-up. Therefore, these measurements do not capture the cumulative effects of long-term hyperglycaemia over time which could cause considerable confounding.

1.6 Methodological considerations

Confounding

Evaluating the independent effects of shared risk factors, drug treatments for comorbid conditions, the use of various ADDs, and the possible effects of T2DM on the risk of GI cancer is challenging.[13] Results of observational studies may have been confounded due to incomplete or incorrect adjustment for shared risk factors for T2DM and GI cancer. Also, residual confounding may be present due to unmeasured confounding variables. For example, many patients with T2DM are overweight or obese, which is partially related to an unhealthy diet or physical inactivity.[64] These are also established risk factors for most GI-cancers.[65] On top of that, most T2DM patients receive drug treatments for comorbid conditions and will receive various ADDs in order to lower the blood glucose concentration.

Bias

The influence of bias on the association between T2DM and GI cancer is an important issue that must be taken into consideration. First, detection bias may arise as patients who are diagnosed with T2DM often experience an increased level of medical surveillance as compared to patients without a diagnosis of T2DM.[66] This results in an increased likelihood of a cancer being detected in the T2DM population. Second, protopathic bias is a type of bias that is exemplified by pancreatic cancer, in which symptoms of T2DM are the initial presentation of the growing malignancy.[67]

Many observational studies on the use of metformin and risk of incident cancer have been criticized for confounding and several types of bias.[39,68] These biases mainly revolve around two axes. The first being the classification of observed person time to different exposure categories, and the second being the implicit effect of the natural history of T2DM, both of which can be described as time-related biases.[13] As recently described by Klil-Drori et al.[13] these biases come down to this:

"…by incorrectly classifying various observational periods as periods of metformin use, one can easily change the likelihood of a cancer event, and thus create a spurious protective association (immortal time bias). Furthermore, when metformin use is compared with the use of antidiabetic drugs that are typically introduced at a later stage of T2DM (without accounting for the duration of disease), the progression of diabetes can confound any association between drug use and cancer (time-lag bias or confounding by indication)."

Moreover, observational studies in which these biases were accounted for revealed no associations between metformin use and specific cancer types.[69-71]

1.7 Aims and outline

Based on the existing data and literature, the association between T2DM and GI cancer is complex, not consistently reported, and subject to various methodological issues and biases, such as confounding and detection bias. Therefore, additional research is necessary to fill this knowledge gap. Studies on the association between T2DM and cancer often focus on cancer in general, or on highly prevalent cancers, such as colorectal cancer, or cancers for which strong associations have previously been described in the literature, such as liver and pancreatic cancer. Evidence for an association between other types of GI cancer (oesophageal cancer, gastric cancer) is less pronounced.

The studies in this thesis focus on the general association between T2DM and all GI cancer types, and diabetes-related factors that may influence this association while taking methodological challenges into account.

The main objectives of the studies described in this thesis are:

I. To evaluate the association between T2DM and different GI cancer types, and to what extent this association is explained by bias;
II. To evaluate the association between the use of anti-diabetic drugs and the risk of GI cancers, specifically the use of metformin and incretins;
III. To evaluate the association between the level of hyperglycaemia over time and the risk of GI cancers.

First, in **Part I** of this thesis (**Chapter 2, 3 and 4**), we aim to evaluate the incidence and risk of all GI cancers and its sub-sites in patients with T2DM compared to non-diabetic individuals. Two observational studies are presented using different prospective data sources. In **Chapter 2** the incidence rates of GI cancers are determined in a British cohort of anti-diabetic drug users and matched controls obtained from the Clinical Practice Research Datalink (CPRD) in the United Kingdom. In **Chapter 3** the incidence and risk of GI cancer is assessed in a Dutch cohort of anti-diabetic drug users and matched controls, using the linked database of the Eindhoven region of the Netherlands Cancer Registry and PHARMO Database Network (E-NCR-PHARMO database). We hypothesize that patients with T2DM may have higher incidence rates and risks of most, but possibly not all, GI cancers. Furthermore, the effects of detection bias/reverse causality on GI cancer risk are explored in these studies. Since our aim is to come to more definite conclusions on the association between T2DM and GI cancer for clinicians in The Netherlands, it is important to evaluate whether the results from UK-data can be extrapolated to the Dutch situation. Therefore, **Chapter 4** is dedicated to the representativeness of the CPRD database for the Dutch population by comparing both the age- and the sex distribution of the CPRD database with the total Dutch population.

Part II focuses on the impact of two anti-diabetic drugs on the risk of GI cancer and pancreatitis in patients with T2DM, using the linked E-NCR-PHARMO database and CPRD as data sources. Previous studies on the use of metformin and GI cancer have shown decreased risks of GI cancers in metformin users, but these studies are often affected by various (time-related) biases. In contrast, high quality studies applying time-varying exposure definitions of metformin did not confirm the suggested protective effects of metformin use on GI cancer risk. In **Chapter 5** our aim is to assess whether T2DM patients using metformin are at a decreased risk of developing GI cancer compared to T2DM patients not using metformin, using a time-varying exposure definition of anti-diabetic drug use. On the one hand, we hypothesize on a decreased risk of GI cancers in users of metformin based on the biological evidence. On the other hand, based on the latest observational evidence, we expect to find no differences in the risk of GI cancers between users of metformin and other non-insulin anti-diabetic drugs. In **Chapter 6** the risk of pancreatitis with the use of incretin-based anti-diabetic drugs is investigated by using the CPRD database. Pancreatitis is a debilitating disease that may convey an increased risk of pancreatic cancer over time. Post-marketing reports of incretin-based drugs have shown that incretins may cause pancreatitis. In addition, in **Chapter 7** the possible effect of selection bias in a study that focused on the risk of kidney cancer with use of metformin is discussed.

In **Part III** (**Chapter 8**) the aim is to investigate whether hyperglycaemia over time is associated with an increased risk of GI cancer in T2DM patients, using a previously described HbA1c-based measure of glycaemia: glycaemic burden (amount of time spend above a predefined HbA1c threshold during follow-up). Previous evidence points towards a higher risk of cancer with higher levels of glycaemia, but this is based on research using limited definitions of hyperglycaemia, such as high versus low HbA1c at the start of follow-up or mean HbA1c during the year before diagnosis of cancer. We hypothesized that patients with higher levels of glycaemic burden are at increased risk of developing GI cancer compared to T2DM who did not accumulate any glycaemic burden over time.

Finally, in the general discussion (**Chapter 9**) the main findings, methodological considerations, study limitations, and practical and clinical implications of the results presented in this thesis are discussed.

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Chapter 2

Gastrointestinal cancer incidence in type 2 diabetes mellitus; results from a large population-based cohort study in the UK

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Abstract

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 all 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 antidiabetic 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 versus 8.9, 95% CI 7.7-10), pancreatic (IR 65, 95% CI 62-69 versus 31, 95% CI 28-34), and colon cancer (IR 119, 95% CI 114-124 versus 109, 95% CI 104-114) were significantly higher in the diabetic compared to the non-diabetic cohort, whereas the IR of oesophageal cancer was significantly lower (IR 41, 95% CI 39-44 versus 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 this 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 oesophageal 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.[1-7] 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.[8-10] 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.[1]

Type 2 diabetic patients may have an increased risk of GI cancers through several common risk factors, such an older age, exposure to alcohol, smoking, a high caloric diet, lack of physical activity, and increased body mass index (BMI).[1] In addition, site-specific risk factors that are more prevalent among diabetic patients may play an important role. These include gastro-oesophageal reflux disease in oesophageal cancer, Helicobacter pylori infections in gastric cancer, gallstone formation in biliary tract cancer, and non-alcoholic fatty liver disease or cirrhosis in hepatocellular carcinoma.[11-14]

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.[1]

Most studies have reported relative measures of risk of cancer with diabetes, without a focus on 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).

Materials and methods

Data source

Data were obtained from the UK 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.[15,16] 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.[16] The accuracy and completeness of CPRD data have been well-validated.[17,18] 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 anatomic subsite, age, sex, and calendar year among type 2 diabetic patients and non-diabetic individuals, we included a cohort of antidiabetic drug (ADD) users (diabetic cohort) and a (1:1) matched reference cohort using incidence sampling technique (Supplementary Figure S2.1). The diabetic cohort consisted of all registered adult patients (aged 18+ years) with at least one prescription for an ADD recorded in CPRD during valid data collection (January 1988-December 2012). The date of first ADD prescription defined start of follow-up (index date). Each diabetic patient was matched to a reference patient without any past recorded prescriptions for ADDs 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 an ADD prescription 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 a concomitant prescription for a non-insulin ADD, were excluded if (a) they had a recorded diagnosis for type 1 diabetes mellitus or (b) they were under 30 years of age at cohort entry. These patients were considered having type 1 diabetes mellitus. Secondly, all subjects with a history of the cancer of interest prior to cohort entry (i.e. 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 receive metformin as a treatment for PCOS, instead of type 2 diabetes mellitus. In addition, we excluded diabetic patients

without any subsequent prescriptions for an ADD (after the initial prescription recorded at baseline). All matched individuals of excluded subjects 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 extra-hepatic 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.[19]

Statistical analyses

To describe and compare both cohorts at baseline, we analyzed various lifestyle factors (smoking status, alcohol use, body mass index), a diagnosis of various comorbidities ever before (gallstone disease, gastro-esophageal reflux disease (GERD), Helicobacter pylori infection, hypertension, inflammatory bowel disease (IBD), chronic liver disease, and chronic pancreatitis), use of drugs during the past 6 months before start of follow-up (antihypertensives, aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), proton-pump inhibitors, and statins), and if a subject had a colonoscopy for colorectal cancer screening purposes during the year before 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 ($\alpha<0.05$).[20] 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 in 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 database 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 2.1. 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 had used alcohol. 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 drugs (e.g. antihypertensives, aspirin, statins) during the 6 months before baseline, and colorectal cancer screening colonoscopy during the year before cohort entry.

Cancer incidence by cancer site

The IRs of any GI cancer (IR 330, 95% CI 322–339 vs. 276, 95% CI 268–284 per 100.000 py; IRR 1.20, 95% CI 1.15-1.24), liver (IR 26, 95% CI 24-28 vs. 8.9, 95% CI 7.7-10; IRR 2.87, 95% CI 2.40-3.44), pancreatic (IR 65, 95% CI 62-69 vs. 31, 95% CI 28-34; IRR 2.12, 95% CI 1.92-2.34), and colon cancer (IR 119, 95% CI 114-124 vs. 109, 95% CI 104-114; IRR 1.09, 95% CI 1.03-1.16) were significantly higher ($p<0.05$) in the diabetic cohort compared to the reference cohort (Table 2.2). In contrast, the IR of esophageal cancer was significantly lower in the diabetic cohort compared to reference cohort (IR 41, 95% CI 39-44 vs. 47, 95% CI 44-51; IRR 0.87, 95% CI 0.79-0.96, $p<0.05$). Among the other subsites of GI cancer no significant differences in IRs between the diabetic and reference cohorts were seen. Similar results were found in a sensitivity analysis excluding 1 year of follow-up after the index date, except for pancreatic cancer in the diabetic cohort which declined to an IR of 48, 95% CI 45-52 (data not shown). However, the difference in IRs for pancreatic cancer between the diabetic and reference cohort remained statistically significant.

Table 2.1 Baseline characteristics of the type 2 diabetic and non-diabetic cohorts.

Characteristic	Type 2 diabetic cohort (n=333,438[a])	Non-diabetic cohort (n=333,438[a])	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 antidiabetic drug[b] (n, %)			
Metformin	205,288 (61.6)		
Sulfonylureas	105,273 (31.6)		
Thiazolidinediones	7,632 (2.3)		
Meglitinides	1,017 (0.3)		
DPP4-inhibitors	1,584 (0.5)		
GLP-1 analogues	481 (0.1)		
Insulin	49,340 (14.8)		
Body mass index (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[b] (n, %)			
Antihypertensives	192,086 (57.6)	102,911 (30.9)	<0.05
Aspirin	92,558 (27.8)	41,511 (12.4)	<0.05
NSAIDs[c]	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

[a] Based on analysis of any gastrointestinal cancer; [b] Multiple prescriptions on the index date occurred; [c] Non-steroidal anti-inflammatory drugs (excluding apirin).

Table 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[a]	6165	1,866,547	330	322–339	4812	1,744,473	276	268-284	1.20	1.15-1.24
Oesophagus[a]	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[a]	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[a]	1243	1,900,866	65	62-69	548	1,775,796	31	28-34	2.12	1.92-2.34
Colon[a]	2243	1,882,327	119	114-124	1920	1,759,228	109	104-114	1.09	1.03-1.16
Rectum	1007	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[a]	3959	1,024,487	386	375-399	3005	932,751	322	311-334	1.20	1.14-1.26
Oesophagus	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[a]	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[a]	666	1,045,411	64	59-69	296	951,393	31	28-35	2.05	1.79-2.35
Colon[a]	1408	1,034,745	136	129-143	1109	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[a]	2206	842,060	262	251-273	1807	811,721	223	213-233	1.18	1.11-1.25
Oesophagus[a]	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[a]	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[a]	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

[a] Statistically significant, $p<0.05$. PY: person-years; IR: incidence rate per 100,000 person-years; CI: confidence interval: IRR: incidence rate ratio.

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). 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 2.1 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 between the diabetic and reference cohort for IR 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

IRs of any GI, liver, and pancreatic cancer in the diabetic cohort remained clearly elevated over time compared to the reference cohort (Figure 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 cancer differed only in the time periods 2003-2004 and 2005-2006 between the two cohorts, being 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.

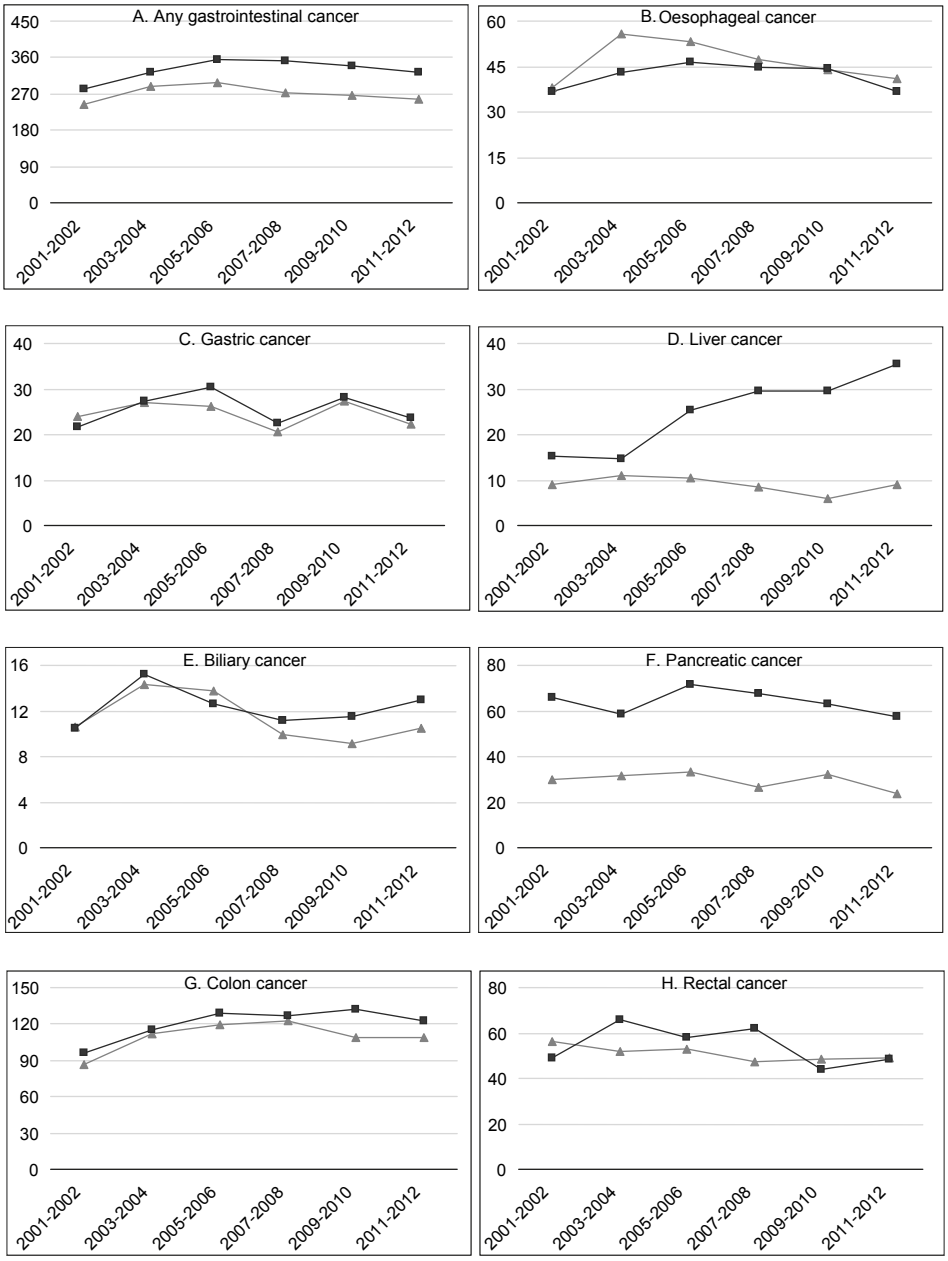

Figure 2.1 Overall and site-specific GI cancer incidence rates stratified by 5-year age categories (x-axis). The y-axis indicates the incidence rate in number of events per 100,000 person-years. GI: gastrointestinal, T2DM: Type 2 diabetes mellitus, IR: incidence rate. Black line: type 2 diabetic cohort, Grey line: reference cohort.

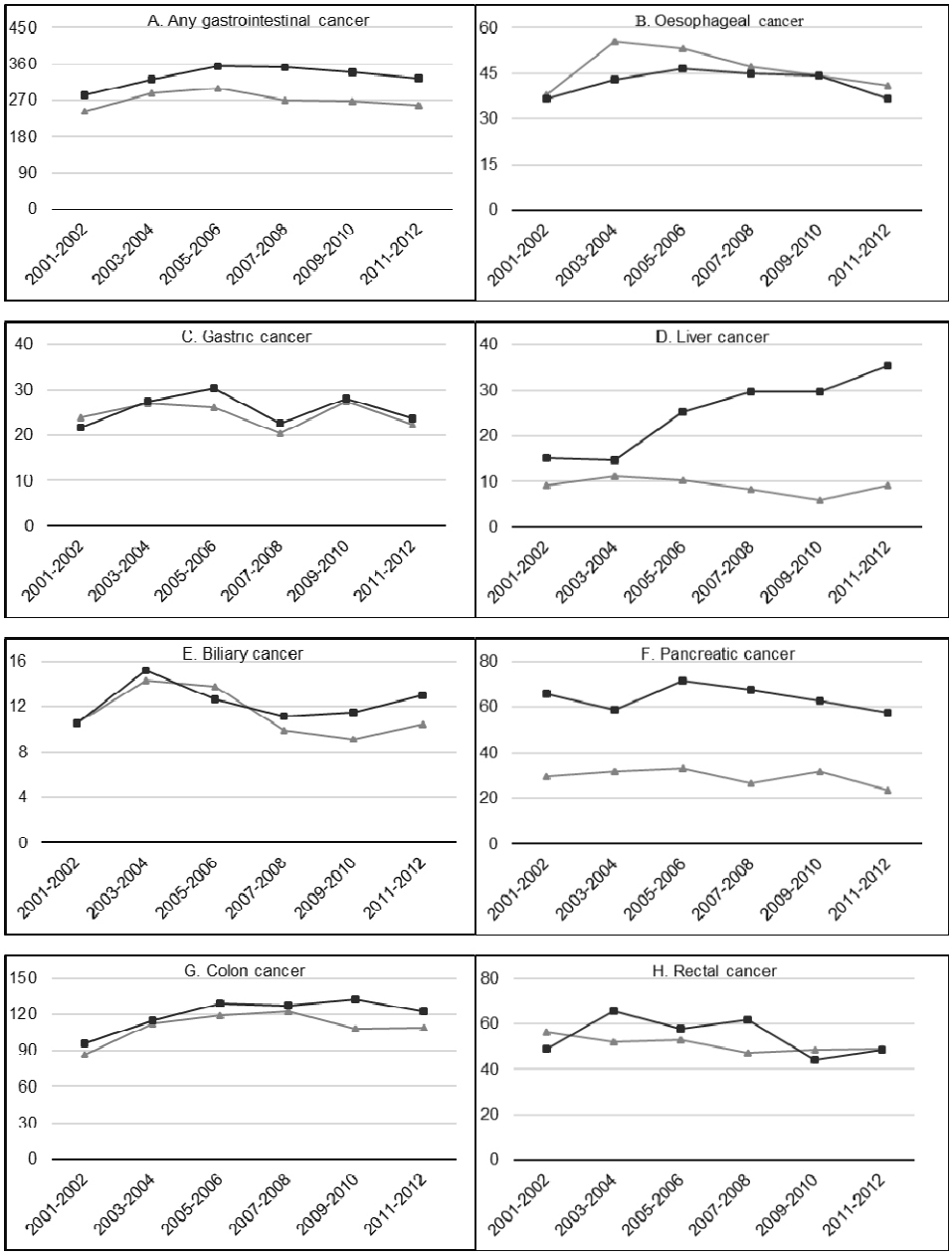

Figure 2.2 Time trends in any and site-specific IRs of GI cancer in the diabetic and non-diabetic cohort, by calendar period (2001-2012; x-axis). The y-axis indicates the IR in number of events per 100,000 person-years. GI: gastrointestinal, IR: incidence rate. Black line: type 2 diabetic cohort, Grey line: reference cohort.

Discussion

This study provides a comprehensive overview of IRs of GI cancers in people with and without diabetes mellitus using the CPRD database. Yearly, approximately one in every 300 type 2 diabetic patients in the UK developed a GI cancer. In general, IRs of any GI, liver, pancreatic, and colon cancer were higher in diabetic patients compared to non-diabetic individuals, with an IR of 26 per 100,000 person-years for liver cancer, an IR of 65 per 100,000 person-years for pancreatic cancer, and an IR of 119 for colon cancer in the diabetic population. In contrast, patients with type 2 diabetes mellitus had lower IRs of esophageal cancer 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, 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.[21–29] As a result, type 2 diabetes mellitus is considered as a risk factor for these cancer types.[1] 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.[8,30] To minimize these biases, a sensitivity analysis was performed, excluding the first year of follow-up after the index date, which did not change the results, 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 pancreatic cancer.

Insulin is thought to be one of the major hormonal contributors to the diabetes-cancer link.[1] On the one hand, both the liver and the pancreas are exposed to higher levels of endogenous insulin compared to other organs via the portal venous system, possibly leading to an increased risk of cancer.[1] On the other hand, both liver and pancreatic cancers are known to impair glucose regulation and induce diabetes as well.[8,31] Therefore, the association between type 2 diabetes mellitus and these cancers may very well be bidirectional.

As for colorectal cancer (CRC), a recent umbrella review of meta-analyses showed that meta-analyses reporting an increased risk of CRC in diabetics are robust, showing an absolute risk increase of around 30 percent.[32] More importantly, because of the sheer

number of incident CRC cases worldwide, the growing number of type 2 diabetics, 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 diabetics 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 compared to non-diabetic individuals, although the observed difference was small (IR 41 vs. 47 per 100,000 py) and did not differ much after stratification by sex. Lifestyle factors such as smoking and alcohol use are important risk factors for esophageal cancer, especially for squamous cell carcinoma.[33] 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 IRs. On the other hand, type 2 diabetic patients had a higher BMI 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.[34-36] 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 (squamous cell carcinoma and adenocarcinoma) show marked epidemiological, pathogenic, and biological differences.[34] For instance, the incidence of esophageal adenocarcinoma has increased in recent years, whereas the incidence of esophageal squamous cell carcinoma has markedly decreased.[37] In general, a modestly increased risk of esophageal cancer in type 2 diabetic patients (summary relative risk 1.30, 95% CI: 1.12-1.50) compared to non-diabetic individuals has been observed, although not remaining significant after stratification for sex.[34]

The major strength of this study is the use of the CPRD, one of the world's largest population-based databases. The CPRD contains approximately 7% of the UK population, and is representative of the UK general population in terms of age, sex, and ethnicity.[15,16] In addition, a high level of validity for the recording of cancer in the CPRD has been previously reported, with cancer diagnosis being valid and accurate more than 90% of the time.[19] However, potential ascertainment or misclassification bias could not be ruled out. Furthermore, we reported the absolute number of cases and IRs of GI cancers instead of relative risks, to adequately show the difference in IRs between both populations.

The main limitation of this study is that causal interpretation of the findings is restricted. Secondly, diabetic status was defined by the recorded prescription of ADDs. Therefore, misclassification of exposure, and thereby diabetic 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. However, most diabetic patients require chronic medication for adequate glycaemic control, making misclassification less likely for those being prescribed drugs on a regular basis. Also, it is possible that type 2 diabetic patients not treated with ADDs or undetected diabetes mellitus were included in the reference population. This could have biased the results by diminishing the difference in IRs between cohorts. Additionally, controls could have suffered from any other disease than diabetes mellitus or those mentioned as exclusion criteria. This could have impacted their survival and therewith their chance of developing cancer. This might explain the somewhat lower total person-years of follow-up in controls. Furthermore, it is possible that the results are confounded, as we could not take into account any risk factors (e.g. smoking, consumption of alcohol, obesity, drug use, and comorbidities) that might contribute to a higher cancer rate in diabetic patients. Also, type 2 diabetic patients and controls were matched on general practice, but residual confounding by socio-economic status could still be present. In addition, the observed IRs in the reference cohort were generally higher compared to age-standardized incidence rates (ASRs) of GI cancers in the general population of the UK (38). We calculated ASRs using the direct method according to the Segi-Doll world standard population to verify whether IRs were comparable to previously reported ASRs in the UK.[39,40] After age-standardization, ASRs of the reference cohort were in line with ASRs in the UK as reported 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).[38]

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 compared to non-diabetic individuals. In general, one in every 300 type 2 diabetic patients developed a GI cancer every year. Furthermore, we found no differences in IRs between type 2 diabetic and non-diabetic individuals for gastric, biliary, and rectal cancer. Conversely, slightly lower IRs were 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 observed IRs of esophageal cancer in diabetic patients warrants further investigation.

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Supplementary figure

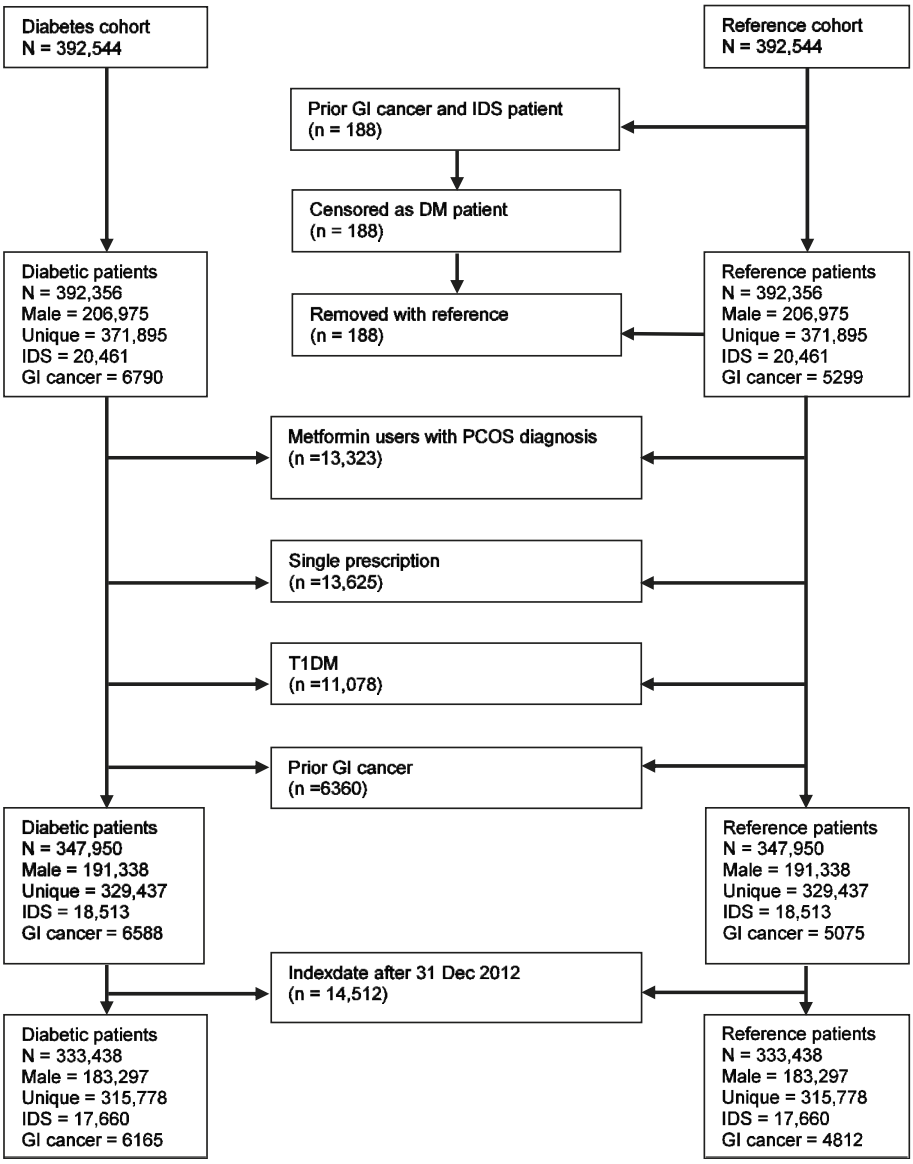

Figure S2.1 Study flow chart of eligible patients in the diabetic and non-diabetic reference cohort. GI: gastrointestinal cancer; IDS: incidence density sampling, i.e. patients that become diabetic after attributing time to the reference cohort.

Chapter 3

Impact of detection bias on the risk of gastrointestinal cancer and its subsites in type 2 diabetes mellitus

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Abstract

Background

Type 2 diabetes mellitus (T2DM) may be a risk factor for gastrointestinal (GI) cancers, but variations in study designs of observational studies may have yielded biased results due to detection bias. Furthermore, differences in risk for GI cancer subsites have not been extensively evaluated. We aimed to determine the risk of GI cancer and its subsites in patients with T2DM and how it is affected by detection bias.

Methods

A matched cohort study was performed using the NCR-PHARMO database. New users of ≥1 non-insulin anti-diabetic drug during 1998-2011 were matched with non-diabetic controls by year of birth, sex, and time between database entry and index. Cox regression analyses were performed with and without lag-period to estimate hazard ratios (HRs) for GI cancer and its subsites. Covariables included age, sex, use of other drugs and history of hospitalisation.

Results

An increased risk of GI cancer was observed in T2DM patients (HR 1.5, 95% confidence interval [CI] 1.3-1.7) compared with controls, which was attenuated in the 1-year lagged analysis (HR 1.4, 95% CI 1.2-1.7). Stratified by subsite, statistically significant increased risks of pancreatic (HR 4.7, 95% CI 3.1-7.2), extrahepatic bile duct (HR 4.2, 95% CI 1.5-11.8) and distal colon cancer (HR 1.5, 95% CI 1.1-2.1) were found, which remained statistically significantly increased in the lagged analysis.

Conclusions

T2DM patients had a 40% increased risk of GI cancer. Increased GI cancer risks tended to be weaker when reducing detection bias by applying a 1-year lag-period. Future observational studies should therefore include sensitivity analyses in which this bias is minimised.

Background

Gastrointestinal (GI) cancers, encompassing malignancies of the gut, from the oesophagus till the anus; including the liver, gallbladder, extrahepatic bile ducts and the pancreas, are among the most common and lethal malignant neoplasms. In 2015, almost 25% of the total cancer incidence, and a third of the total cancer mortality in the Netherlands was due to a GI cancer.[1] Furthermore, data from the Netherlands Cancer Registry (NCR) indicate incidences of these cancers are rising.[1]

Previous studies using NCR data have shown a higher prevalence of type 2 diabetes mellitus (T2DM) in patients with various GI cancers.[2,3] Indeed, a growing body of evidence suggests that T2DM may be a risk factor for the development of GI cancers (Table 3.1).[4-13] The strongest associations have been described for liver and pancreatic cancer, with both a two-fold increased risk.[14,15] In addition, a 15%-30% increased risk has been reported for colorectal cancer.[16-18] With 830,000 individuals living in the Netherlands with diabetes mellitus in 2011 (of which ±90% with T2DM), diabetes mellitus poses a highly prevalent and potentially modifiable risk factor for GI cancer development.[19] There has been much discussion about whether previously reported associations in observational studies present an underlying biological mechanism between T2DM and cancer or represent detection bias or even reverse causality. These biases could have been the result of a diagnostic (protopathic) bias, i.e. an increased odds of detecting cancer shortly after the onset of diabetes, or by specific GI cancers inducing disturbances in glucose homoeostasis.[20,21] To address this form of methodological bias, a lag time between disease onset and the start of follow-up for cancer outcomes can be considered.[22]

Furthermore, epidemiologic studies have shown that risk factors of GI cancer may vary within specific GI cancer anatomic subsites or histologic subtypes.[23,24] For instance, different risk factors have been identified for oesophageal squamous cell carcinoma and adenocarcinoma, and also for proximal and distal gastric cancer.[23] Up to now, data on subsite-specific risks of GI cancer in patients with T2DM are limited.[25]

Therefore, our primary aim was to determine the overall risk of GI cancer in patients with T2DM, and explore the effects of detection bias/reverse causality on the association between T2DM and risk of GI cancer. Second, we stratified these analyses for specific GI cancer subsites/subtypes.

Table 3.1 Overview of GI cancer risk in patients with type 2 diabetes mellitus in meta-analyses of cohort studies.

Author (Ref.)	Oesophageal cancer	Gastric cancer	Colorectal cancer	Liver cancer	Bile duct cancer	Pancreatic cancer
Larsson *et al.* 2005[14]			RR 1.30 (1.20-1.40)			
Huang *et al.* 2011[7]	SRR 1.30 (1.12-1.50)					
Ge *et al.* 2011[8]		SRR 1.09 (0.98-1.22)				
Ren *et al.* 2011[11]					GB: SRR 1.52 (1.26-1.84)	
Ben *et al.* 2011[12]						SRR 1.95 (1.66-2.28)
Jiang *et al.* 2012[10]			SRR 1.27 (1.21-1.34)			
Jing *et al.* 2012[9]				ICC: SRR 1.97 (1.57-2.46)	ECC: SRR 1.63 (1.29-2.05)	
Wang *et al.* 2012[13]				HCC: SRR 2.31 (1.87-2.84)		
Deng *et al.* 2012[15]			RR 1.26 (1.20-1.31)			
Wu *et al.* 2013[6]			RR 1.22 (1.19-1.26)			

ECC: extrahepatic cholangiocarcioma; HCC: hepatocellular carcionoma; HR: hazard ratio; GB: gallbladder; ICC: intrahepatic cholangiocarcinoma; Ref: reference number; RR: relative risk; SRR: summary relative risk.

Methods

Data source

Data for this population-based cohort study were obtained from the PHARMO Database Network and linked at the individual patient level to the Eindhoven area of the NCR (E-NCR-PHARMO database). The construct and validity of the linked database have been described elsewhere.[26] Data from the Eindhoven area of the NCR, maintained by the Netherlands Comprehensive Cancer Organisation, cover a demographic region with approximately 2.4 million inhabitants (±15% of the Dutch population) and no academic hospitals. Trained registration clerks actively collect data on diagnosis, patient characteristics, staging and initial treatment from hospital medical records. Vital status is obtained by linkage to Dutch municipal records.

The PHARMO Database Network is a large, patient-centric data network including linked observational databases designed for drug safety and outcomes research. For this study the Out-patient (community) Pharmacy Database was used, which contains longitudinal drug dispensing records, and included information on dispensing date, dose descriptions

and amount dispensed. All drugs are coded according to their Anatomical Therapeutic Chemical/Defined Daily Dose Classification code.[27] Both the NCR and the PHARMO Database Network are recognized as high-quality data sources for (pharmaco) epidemiological research that have collected information in overlapping regions in the Netherlands for a period of over 10 years.[26]

Population and study design

We selected all individuals aged 30 years and older who received at least one anti-diabetic drug (ADD) prescription (ATC code 'A10A' or 'A10B') in the E-NCR-PHARMO database between 1 January 1998 and 31 December 2011. These subjects were classified as potential T2DM patients and the first prescription for an ADD defined their start of follow-up (index date). A random sample of subjects who never received ADDs during the study period was extracted from the database and classified as non-diabetic controls (Figure 3.1).

Next, non-diabetic controls were matched to a T2DM patient by year of birth, sex and the time between database entry and the index date (±90 days). Non-diabetic controls were assigned the same index date as their matched T2DM patients. For T2DM patients with more than one matched control the most optimal control was selected based on highest similarity of matching parameters, yielding a 1:1 matched cohort.

Potential T2DM patients who initiated ADD treatment with insulin or an insulin analogue (ATC code 'A10A') were excluded to minimize the amount of people with type 1 diabetes mellitus being misclassified as T2DM. All study subjects with a history of GI cancer before the index date were excluded. Furthermore, we excluded all prevalent ADD-users, i.e. T2DM patients without a minimum of 1 year of ADD-free follow-up in the NCR-PHARMO database before the index date. In addition, all individuals matched to excluded subjects were excluded as well. Individuals were followed from the index date until the first occurrence of a GI cancer, death from any cause, migration out of the PHARMO catchment area or end of data collection, whichever came first.

Outcomes

GI cancers were classified according to the International Classification of Diseases of oncology.[28] These included 'any GI cancer' (C15-26, excluding anal cancer), oesophageal cancer (C15), gastric cancer (C16), small intestinal cancer (C17), colon cancer (C18), rectal cancer (C19-20), hepatic cancer (C22), biliary tract cancer (C23: gallbladder, and C24: extrahepatic bile duct cancer) and pancreatic cancer (C25). In addition, stratified analyses were performed by sublocalisation of GI cancer sites (see Supplementary Table S3.1 for sublocalisations). For the site-specific analyses, subjects were followed until the

first-occurrence of the site-specific GI cancer event, despite other types of GI cancers occurring during follow-up.

Covariables

Both time-fixed and time-dependent covariables were considered as confounders based on the existing literature. As time-fixed covariables sex and the number of hospitalisations before the index date (hospitalisation categories 0 or 1) were considered. Time-dependent covariables were determined at the start of every 90-day time-period and included: age, the duration of diabetes in years (time since first recorded NIAD prescription), the use of other drugs known to impact GI cancer risk in the 90 days before the start of each interval (statins, aspirin, non-aspirin non-steroidal anti-inflammatory drugs, proton pump inhibitors (PPIs), bisphosphonates, tamoxifen and oral contraceptives). In addition, the use of Helicobacter pylori eradication therapy was used as a proxy-indicator for Helicobacter pylori infection (see Supplementary Table S3.2 for ATC codes).

Statistical analyses

Differences in demographic and clinical characteristics at baseline between T2DM patients and matched controls were compared using chi-squared test for categorical variables and Student's t-test for continuous variables. Incidence rates (IR) of GI cancer for every (sub)group were calculated by dividing the number of GI cancer events by the total amount of person-years of follow-up (for the IR of GI cancers by tumour stage see Supplementary Table S3.3).

Cox proportional hazards analysis was used to estimate the hazard ratio (HR) and 95% confidence intervals (CIs) of GI cancer in T2DM patients versus matched controls. Stratified analyses were performed by sex, for specific GI cancer sites and for subsites of specific GI cancer sites. Covariables were entered into the final model if they changed the beta coefficient of the primary exposure variable by more than 5%. Detection bias after the onset of T2DM was reduced by repeating the overall analyses with a lag-period of 1 year. The lag-period implied censoring a subject on the date of cancer diagnosis if the cancer occurred during the first year of follow-up.

Sensitivity analysis

To further explore the effects of reducing detection bias, we preformed sensitivity analyses in which we repeated the 1-year lagged analyses as described previously with a lag-period of three years instead. All data management and analyses were performed with SAS software version 9.4 (SAS Institute, Cary, NC).

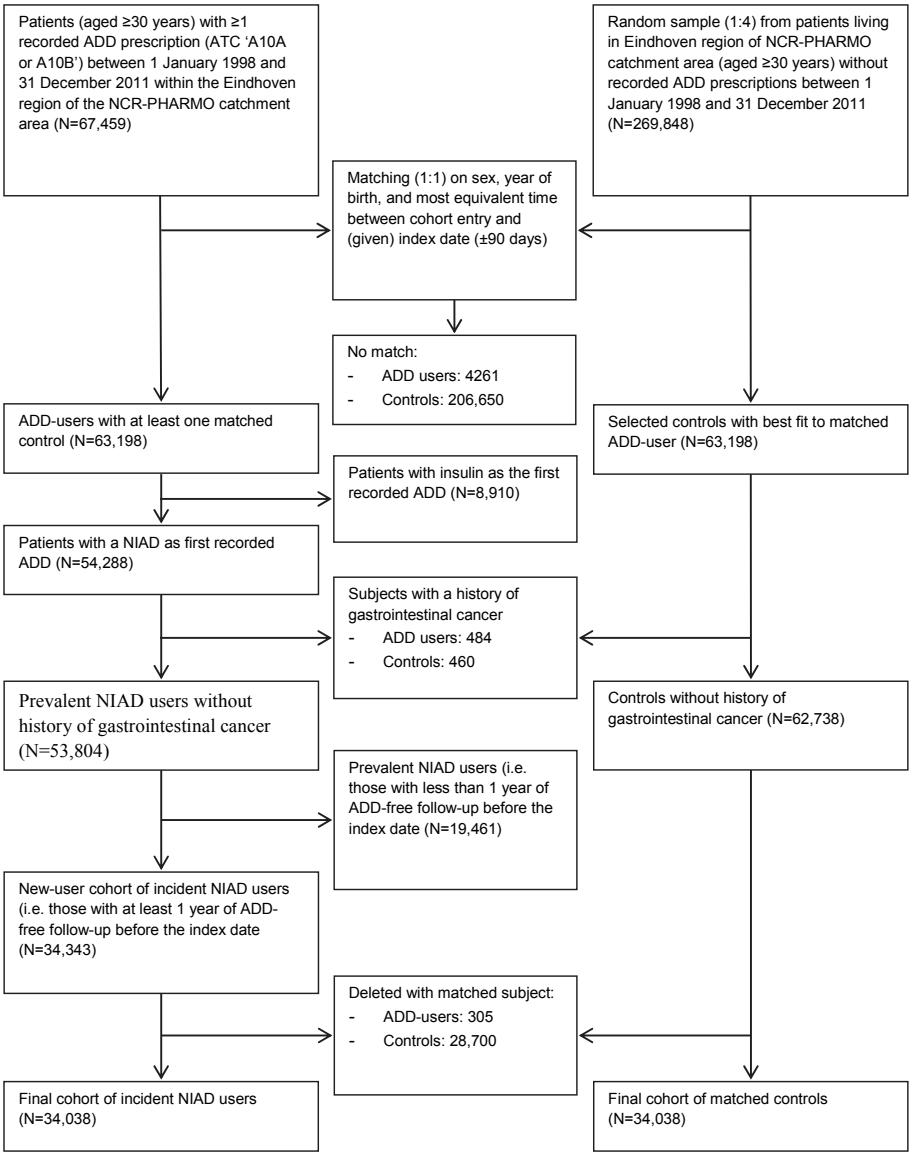

Figure 3.1 Flow-chart of study population. ADD: anti-diabetic drug; ATC: Anatomical Therapeutic Classification; NCR: Netherlands Cancer Registration; NIAD: Non-Insulin Anti-diabetic Drug.

Results

Baseline characteristics

In both T2DM patients and non-diabetic controls, the mean age at baseline was 63.9 years, and 51% of subjects were males (Table 3.2). There were no differences seen between the age and sex distribution at baseline. After at least 1 year of ADD-free follow-up, most incident ADD users initiated treatment with metformin (73.8%) and/or sulfonylureas (28.0%). Statistically significant differences were observed for the use of various other drugs during the 90 days before the start of follow-up, with the largest differences seen for prior use of statins, antihypertensives and PPIs. In addition, T2DM patients were more often hospitalised before the index date (51.7% versus 38.5%).

Table 3.2 Baseline characteristics of type 2 diabetic patients and matched non-diabetic controls.

Characteristic	Type 2 diabetic (n = 34,038)		Non-diabetic (n = 34,038)		p-value[a]
Age (years; Mean, SD)	63.9	12.6	63.9	12.6	1.00
Sex (n, % male)	17,343	51	17,343	51	1.00
Use of anti-diabetic drugs[b] (n, %)					
Metformin	25,115	73.8			
Sulfonylureas	9,536	28.0			
Thiazolidinediones	388	1.1			
Meglitinides	38	0.1			
Incretins	66	0.2			
Use of other drugs (n, %)					
Anti-hypertensives	20,667	60.7	9,495	27.9	<0.01
Aspirin	6,156	18.1	3,080	9.1	<0.01
Bisphosphonates	1,112	3.3	812	2.4	<0.01
H. pylori eradication therapy	40	0.1	24	0.1	0.05
Non-aspirin NSAIDs	5,171	15.2	3,324	9.8	<0.01
Proton pump inhibitors	6,795	20.0	3,268	9.6	<0.01
Statins	13,396	39.4	4,529	13.3	<0.01
History of hospitalisations (n, %)					
0 hospitalisations	16,450	48.3	20,932	61.5	<0.01
≥1 hospitalisations	17,588	51.7	13,106	38.5	

[a] p-value based on student's T-test for continuous variables and Chi-squared test for categorical variables. [b] During 90-days before the index date. H. pylori: helicobacter pylori; NSAIDs: non-steroidal anti-inflammatory drugs.

Risk of GI cancer overall

Generally, an increased risk of GI cancer was observed in patients with T2DM compared with non-diabetic controls (Adj. HR 1.5, 95% CI 1.3-1.7; Table 3.3), which remained statistically significant increased when applying a 1-year lag-period (Adj. HR 1.4,

95% CI 1.2-1.7). After stratification by GI cancer subsite, we observed a 4-fold increased risk of hepato-pancreatico-biliary (HPB) cancer (Adj. HR 4.4, 95% 3.0-6.4), but not for upper and lower GI cancer (Adj. HR 1.1, 95% CI 0.77-1.5 and Adj. HR 1.2, 95% CI 0.99-1.4, respectively). In the analysis that reduced detection bias (i.e. with the addition of a 1-year lag-period), a slightly attenuated risk of HPB cancer was seen (Adj. HR 4.0, 95% CI 2.4-6.7). When stratifying the analyses by sex, statistically significant increased risks of overall GI cancer and of lower GI cancer were seen in the 1-year lagged analyses in men (Adj. HR 1.6, 95% CI 1.2-1.9, and Adj. HR 1.3, 95% CI 1.0-1.8 respectively), but not in women (Table 3.4). Also, the increased risk of HBP cancer was more pronounced in men than in women.

Risk of specific GI cancer sites

After we had broken down our analyses by GI cancer site, we observed a statistically significant increased risk of colon cancer (Adj. HR 1.4, 95% CI 1.1-1.7), pancreatic cancer (Adj. HR 4.7, 95% CI 3.1-7.2) and biliary tract cancer (Adj. HR 3.5, 95% CI 1.4-8.4) in patients with T2DM compared with non-diabetic controls (Table 3.3). The latter two remained significantly increased in the 1-year lagged analysis (Adj. HR 3.6, 95% CI 2.0-6.5 and Adj. HR 4.2, 95% CI 1.3-13.1, respectively). However, no statistically significantly increased risk of pancreatic or biliary tract cancer was seen in the sensitivity analyses (Adj. HR 2.0, 95% CI 0.96-4.2 and Adj HR 8.1, 95% CI 0.95-68.8 respectively). In the sex-specific analyses, the increased risk of colon cancer confined to men, and the risk of pancreatic cancer was more pronounced in men (Table 3.4).

Risk of GI cancer subsites/subtypes

After stratifying the specific GI cancer sites by sublocalisation and subtype (Table 3.5), an increased risk in patients with T2DM was found for extra-hepatic bile duct cancer (Adj. HR 4.2, 95% CI 1.5-11.8), and for distal colon cancer (HR 1.5, 95% 1.1-2.1), both of which remained statistically significantly raised after removal of detection bias. No significant differences were observed for other subsites of GI cancer. Also, we did not observe any significant differences for histologic subtypes of oesophageal cancer.

Table 3.3 Risk of GI cancer in patients with type 2 diabetes mellitus and matched non-diabetic controls, by specific GI cancer site.

					Age-sex adjusted				Fully adjusted			
	Non-diabetic		Type 2 diabetic		Overall		1-year lagged		Overall		1-year lagged	
	Events	IR[a]	Events	IR[a]	HR	95% CI	HR	95% CI	HR[b]	95% CI	HR[b]	95% CI
Any GI cancer	351	252	583	408	1.7*	1.5-1.9	1.7*	1.4-2.0	1.5*	1.3-1.7	1.4*	1.2-1.7
By cancer subsite												
Upper GI cancer	71	51	96	67	1.4*	1.0-1.9	1.7*	1.2-2.5	1.1	0.77-1.5	1.3	0.90-2.0
Oesophageal cancer	25	18	41	29	1.7*	1.0-2.8	2.3*	1.3-4.1	1.3[c]	0.74-2.2	1.6[c]	0.86-3.1
Gastric cancer	46	33	50	35	1.1	0.75-1.7	1.4	0.86-2.2	0.88	0.57-1.4	1.1	0.7-1.8
Small intestinal cancer	<5	1	6	4	2.9	0.58-14.3	1.5	0.2-8.7	¥	¥	¥	¥
Lower GI cancer	241	173	330	231	1.4*	1.2-1.7	1.4*	1.2-1.7	1.2	0.99-1.4	1.1	0.93-1.4
Colon cancer	168	120	253	176	1.6*	1.3-1.9	1.5*	1.2-1.9	1.4*	1.1-1.7	1.2	0.96-1.6
Rectal cancer	77	55	87	61	1.1	0.83-1.5	1.2	0.88-1.8	0.88	0.63-1.2	0.99	0.68-1.4
HPB cancer	39	28	156	109	4.0*	2.8-5.7	4.1*	2.5-6.5	4.4*	3.0-6.4	4.0*	2.4-6.7
Liver cancer	<5	1	15	10	7.4*	1.7-32.4	-	-	¥	¥	¥	¥
Biliary tract cancer	7	5	20	14	2.9*	1.2-6.7	3.5*	1.2-10.7	3.5[d,]*	1.4-8.4	4.2[d,]*	1.3-13.1
Pancreatic cancer	30	21	122	85	4.1*	2.7-6.1	3.5*	2.0-6.0	4.7*	3.1-7.2	3.6*	2.0-6.5

[a] per 100,000 person-years; [b] Adjusted for age, sex, use of statins, proton pump inhibitors, anti-hypertensives 90 days prior to start of each time-interval. [c] Additionally adjusted for history of hospitalization; [d] Adjusted only for use of statins 90 days prior to start of each interval; * statistically significant with $p < 0.05$. ¥ Fully-adjusted analysis not possible due to insufficient events for additional covariate adjustments. CI: confidence interval; GI: gastrointestinal; HPB: hepato-pancreatico-biliary; HR: hazard ratio; IR: incidence rate.

Table 3.4 Sex-specific risk of GI cancer in patients with type 2 diabetes mellitus and matched non-diabetic controls, by specific GI cancer site.

	Non-diabetic		Type 2 diabetic		Age-sex adjusted				Fully adjusted			
					Overall		1-year lagged		Overall		1-year lagged	
	Events	IR[a]	Events	IR[a]	HR	95% CI	HR	95% CI	HR[b]	95% CI	HR[b]	95% CI
Men												
Any GI cancer	203	284	344	470	1.7*	1.5 - 2.1	1.8*	1.5 - 2.2	1.6*	1.3 - 1.9	1.6*	1.2 - 1.9
By cancer site												
Upper GI cancer	52	73	74	101	1.5*	1.0 - 2.1	1.9*	1.2 - 2.9	1.0	0.70 - 1.5	1.4	0.85 - 2.1
Oesophageal cancer	18	25	34	46	2.0*	1.1 - 3.5	3.2*	1.6 - 6.5	1.3[c]	0.71 - 2.4	2.1[c]	0.98 - 4.5
Gastric cancer	36	50	37	50	1.1	0.66 - 1.7	1.2	0.70 - 2.0	0.77	0.47 - 1.3	0.90	0.50 - 1.6
Small intestinal cancer	0	-	<5	5	-	-	-	-	-	-	-	-
Lower GI cancer	131	183	185	253	1.5*	1.2 - 1.8	1.5*	1.2 - 1.9	1.3*	1.0 - 1.7	1.3*	1.0 - 1.8
Colon cancer	85	119	139	189	1.7*	1.3 - 2.2	1.6*	1.2 - 2.2	1.6*	1.2 - 2.2	1.4*	1.0 - 2.0
Rectal cancer	49	68	54	73	1.1	0.75 - 1.6	1.3	0.87 - 2.2	0.91	0.59 - 1.4	1.2	0.72 - 1.9
HPB cancer	20	28	84	115	4.3*	2.6 - 6.9	4.5*	2.3 - 9.0	4.8*	2.9 - 8.1	4.5*	2.2 - 9.4
Liver cancer	0	-	10	14	-	-	-	-	-	-	-	-
Biliary tract cancer	<5	6	8	11	2.1*	0.62 - 6.9	2.7	0.51 - 13.8	3.0	0.86 - 10.2	4.0	0.75 - 21.4
Pancreatic cancer	16	22	67	91	4.2*	2.5 - 7.3	4.2*	2.0 - 9.1	5.0*	2.8 - 8.8	4.3*	1.9 - 9.8
Women												
Any GI cancer	148	218	239	343	1.6*	1.3 - 2.0	1.5*	1.2 - 1.9	1.4*	1.1 - 1.8	1.2	0.92 - 1.6
By cancer site												
Upper GI cancer	19	28	22	32	1.2	0.64 - 2.8	1.3	0.65 - 2.6	1.3	0.67 - 2.6	1.3	0.61 - 2.9
Oesophageal cancer	7	10	7	10	1.0	0.35 - 2.9	0.83	0.25 - 2.7	1.3	0.41 - 4.2	0.80	0.21 - 3.0
Gastric cancer	10	15	13	19	1.4	0.60 - 3.1	2.1	0.79 - 5.7	1.4	0.55 - 3.4	2.0	0.67 - 5.8
Small intestinal cancer	<5	3	<5	3	0.95	0.13 - 6.8	0.48	0.04 - 5.3	¥	¥	¥	¥
Lower GI cancer	110	162	145	208	1.3*	1.0 - 1.7	1.3	0.94 - 1.7	1.0	0.79 - 1.4	0.91	0.66 - 1.3
Colon cancer	80	122	114	163	1.4*	1.1 - 1.9	1.3	0.95 - 1.9	1.1	0.82 - 1.5	1.0	0.70 - 1.5
Rectal cancer	28	41	33	47	1.2	0.72 - 2.0	1.1	0.62 - 1.9	0.83	0.48 - 1.5	0.73	0.39 - 1.4
HPB cancer	19	28	72	103	3.7*	2.3 - 6.2	3.6*	1.9 - 7.1	4.0*	2.3 - 6.9	3.6*	1.8 - 7.4
Liver cancer	<5	3	5	7	2.5	0.48 - 12.7	-	-	¥	¥	¥	¥
Biliary tract cancer	<5	4	12	17	3.9*	1.1 - 13.9	4.4	0.96 - 20.6	4.2[d,]*	1.1 - 15.6	4.5[d,]*	0.93 - 22.0
Pancreatic cancer	14	21	55	78	3.9*	2.2 - 7.0	2.9*	1.3 - 6.1	4.5*	2.4 - 8.3	3.0*	1.3 -6 .9

[a] per 100,000 person-years; [b] Adjusted for age, use of statins, proton pump inhibitors, anti-hypertensives 90 days prior to start of each time-interval. [c] Additionally adjusted for history of hospitalization; [d] Adjusted only for use of statins 90 days prior to start of each interval; * statistically significant with p<0.05; [¥] Fully-adjusted analysis not possible due to insufficient events for additional covariate adjustments. CI: confidence interval; GI: gastrointestinal; HPB: hepato-pancreatico-biliary; HR: hazard ratio; IR: incidence rate.

Table 3.5 Risk of GI cancer in patients with type 2 diabetes mellitus and matched non-diabetic controls, by GI cancer subsite.

					Age-sex adjusted				Fully adjusted			
	Non-diabetic		Type 2 diabetic		Overall		1-year lagged		Overall		1-year lagged	
Cancer site	Events	IR[a]	Events	IR[a]	HR	95% CI	HR	95% CI	HR[b]	95% CI	HR[b]	95% CI
Oesophageal cancer												
By cancer subsite												
Upper/middle oesophageal cancer	<5	2	7	5	2.3	0.59 - 8.8	1.9	0.49 - 7.8	¥	¥	¥	¥
Lower oesophageal cancer	21	15	32	22	1.6	0.92 - 2.8	2.5*	1.3 - 5.0	1.2[c]	0.68 - 2.2	1.9[c]	0.92 - 4.0
By histologic subtype												
Squamous cell carcinoma	7	5	10	7	1.4	0.54 - 3.7	1.6	0.52 - 4.9	¥	¥	¥	¥
Adenocarcinoma	17	12	29	20	1.8	0.99 - 3.3	2.7*	1.3 - 5.6	1.3	0.68 - 2.4	1.9	0.86 - 4.1
Gastric cancer												
By cancer subsite												
Proximal gastric cancer	20	14	21	15	1.1	0.59 - 2.0	1.2	0.59 - 2.4	0.89	0.46 - 1.7	0.97	0.45 - 2.1
Distal gastric cancer	10	7	11	8	1.2	0.51 - 2.9	1.8	0.68 - 4.6	¥	¥	¥	¥
Biliary tract cancer												
By cancer subsite												
Gallbladder cancer	<5	1	<5	3	2.0	0.36 - 10.9	2.0	0.18 - 21.6	¥	¥	¥	¥
Extrahepatic bile duct cancer	5	4	16	11	3.2*	1.2 - 8.7	4.1*	1.2 - 14.4	4.2[d,]*	1.5 - 11.8	5.5[d,]*	1.5 - 20.0
Colon cancer												
By cancer subsite												
Proximal colon cancer	90	64	136	95	1.6*	1.2 - 2.0	1.3	0.98 - 1.8	1.3	0.98 - 1.8	1.1	0.75 - 1.5
Distal colon cancer	71	51	112	78	1.6*	1.2 - 2.2	1.8*	1.3 - 2.5	1.5*	1.1 - 2.0	1.5*	1.1 - 2.2

[a] per 100,000 person-years; [b] Adjusted for age, sex, use of statins, proton pump inhibitors, anti-hypertensives 90 days prior to start of each time-interval. [c] Additionally adjusted for history of hospitalization; [d] Adjusted only for use of statins 90 days prior to each interval; * statistically significant with p<0.05. ¥ Fully-adjusted analysis not possible due to insufficient events for additional covariate adjustments. CI: confidence interval; GI: gastrointestinal; HR: hazard ratio; IR: incidence rate.

Discussion

We observed a 50% increased risk of GI cancer in patients with T2DM compared with non-diabetic controls. However, after accounting for potential detection bias this dropped to a 40% increased risk. The overall increased risk in T2DM patients was explained by a four-fold increased risk of HPB cancers, which was driven by pancreatic cancer (five-fold increase) and biliary tract cancer (four-fold increase). The risk of HPB and pancreatic cancer, but not biliary tract cancer, was attenuated following adjustment to minimize detection bias.

While several pathways have been proposed, including insulin resistance and fat-induced chronic inflammation[5,29], the precise biological mechanisms by which T2DM increases the risk of GI cancer remains unclear. Insulin may promote carcinogenesis through the insulin receptor and insulin-like growth factor receptor (IGF-R), which are overexpressed on various types of tumour cells.[30] Binding of these receptors by insulin activates the mTOR signalling pathway (mammalian target of rapamycin signalling pathway), resulting in abnormal cell proliferation, inhibition of apoptosis, angiogenesis and carcinogenesis.[31] Hyperinsulinaemia may also predispose to carcinogenesis by indirectly increasing the production of IGF-1 via the liver, and by increasing the amount of bioavailable IGF-1 by decreasing the level of IGF-binding proteins.[29]

The results of this study add to the current evidence from observational studies. In their meta-analyses of cohort studies, Ben et al.[14] found a two-fold increased risk of pancreatic cancer in newly diagnosed T2DM patients, and Ren et al.[13] observed a 1.4-fold increased risk of extrahepatic biliary tract cancer. However, the potential for reverse causality is a primary concern for these cancers, as both can induce hyperglycaemia or frank diabetes.[32] Our results may still be affected by an unknown degree of protopathic bias (reverse causality), as a 1-year lag-period may not be enough to exclude the effects of these cancers on the development of T2DM symptoms. Indeed, when increasing the lag-period to 3 years, no statistically significantly increased risks of pancreatic cancer and biliary tract cancer between T2DM patients and controls were observed (Adj. HR 2.0, 95% CI 0.96-4.8 and Adj. HR 8.1, 95% CI 0.95-68.8, respectively). However, this could also be explained by a lack of statistical power. Nonetheless, an increased risk of pancreatic cancer with longstanding T2DM (10 years) has been reported in the literature, suggesting that diabetes might still be a risk factor for pancreatic cancer development.[14]

An interesting finding in this study was the difference in risk between genders and distal and proximal colon cancer. We identified that men, but not women, with T2DM were at an increased risk of colon cancer. Varying differences in the risk of colorectal cancer have been reported in men and women with T2DM[25,33-35], and large meta-analyses of

observational studies have reported moderate (20-30%) increased risks of colorectal cancer in both men and women.[8,16,17,36-38] With regards to colon cancer, three meta-analyses have reported increased risks of both proximal and distal colon cancer in patients with T2DM, with stronger risk estimates for proximal colon cancer.[16,37,38] However, differences in observed risks could result from variations in the definitions of proximal and distal colon cancer in the literature as it cannot always be defined from which part of the colon a tumour has originated.

In contrast to meta-analyses of cohort studies, we did not find a statistically significant increased risk of liver cancer in patients with T2DM. Wang et al.[15] reported a relative risk of 2.4 (95% CI 1.7-3.6) for hepatocellular carcinoma in T2DM patients, combining results from seven cohort studies. The most likely reason we could not replicate these findings is because of a lack of statistical power for this cancer site. Similarly, we did not find an increased risk of specific upper GI cancer sites like oesophageal, gastric, and small intestinal cancer in our cohort. Moreover, when all sites were combined we also did not identify an increased risk of upper GI cancer. This adds to the current literature for the risk of upper GI cancers, such as oesophageal and gastric cancer, in patients with T2DM.[6,39-42]

Our study has a number of limitations worth mentioning. First, we were not able to correct for several important general and cancer-specific risk factors, including obesity, smoking status, alcohol use, physical inactivity and high-caloric diet, which could have confounded the results. The majority of T2DM patients are obese, and obesity has been shown to be associated with and increased risk of GI cancers.[43] Moreover, visceral or abdominal fat is more metabolically active and therefore potentially more harmful than fat distributed at the hips.[31] Second, due to the relatively small size of the population and the matched design, a lack of statistical power existed for cancer sites, such as liver cancer and small intestinal cancer. This also resulted in a limited ability to statistically adjust for confounders in a multivariate analysis for subsites of GI cancer. Although we acknowledge that propensity score adjustment would be an effective strategy to further reduce residual confounding and limit the number of covariates in the multivariate model[22], it cannot overcome the unmeasured confounding in the data source and therefore this strategy was not applied. Third, the subsite-specific analyses were of an exploratory nature rather than a hypothesis-testing one. Fourth, T2DM patients were identified based on the use of anti-diabetic drugs, leading to potential misclassification of diet-controlled T2DM patients as controls. Also, included patients were required to have at least one drug prescription via their community pharmacy. Patients not registered at a pharmacy were therefore not included. Consequently, the control group may be sicker than the general population, which may have resulted in an elevated risk of GI cancer in this group. Ultimately, this would bias the risk ratio towards the null, yet

we observed a statistically significant association between T2DM and GI cancer sites. Finally, a causal relationship between T2DM and GI cancer cannot be proven in the present study. T2DM may function as a proxy indicator of several pathophysiologic mechanisms that, in turn, may promote cancer growth, such as insulin resistance, hyperglycaemia, hyperinsulinaemia, chronic inflammation and increase hormone levels.

The strengths of this study are provided by the use of the population-based linked E-NCR-PHARMO database, which guarantees a high level of cancer ascertainment and longitudinal information on drug exposure during follow-up. This prevents an overestimation of the number of (false positive) cancers, which may occur in studies using an insurance claims database or data from general practitioners without linking to some form of cancer registry or pathology database. In addition, the longitudinal nature of the PHARMO database provides reliable information on confounding drug exposures during follow-up; such as statins, non-steroidal anti-inflammatory drugs and PPIs.

In conclusion, following an adjustment for potential detection bias, T2DM was associated with a 40% increased risk of GI cancer, and a four-fold increased risk of pancreatic and biliary tract cancer. In particular, the strong associations found for HPB cancers and pancreatic cancer may be partly caused by an increased detection of these cancers in the first years after the onset of T2DM. Future studies investigating associations between T2DM and GI cancer should therefore always include a sensitivity analysis in which detection bias or reverse causality are kept to a minimum by including one or multiple years of lag-time.

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Supplementary tables

Table S3.1 Sublocalisations of specific gastrointestinal cancer sites included in the analysis.

Cancer site	Sublocalisation
Any GI cancer (C15-C26)	Upper GI cancer (C15-17)
	Lower GI cancer (C18-20)
	Hepato-Pancreatico-biliary (HPB) GI cancer (C22-25)
Oesophageal cancer (C15)	Upper and middle third oesophageal cancer (C15.0, C15.3, C15.4)
	Lower third oesophageal cancer (C15.5)
	Oesophageal squamous cell carcinoma (ICD-O code 8070/2)
	Oesophageal adenocarcinoma (ICD-O code 8140, 8144, 8260, 8480/1, 8490)
Gastric cancer (C16)	Proximal gastric cancer (C16.0/2)
	Distal gastric cancer (C16.3/4)
Liver cancer (C22)	Hepatocellular carcinoma (C22.0)
	Intrahepatic bile duct cancer (C22.1)
Biliary tract cancer (C23-24)	Gallbladder cancer (C23)
	Extrahepatic bile duct cancer (C24)
Pancreatic cancer (C25)	Proximal pancreatic cancer (C25.0)
	Distal pancreatic cancer (C25.1/2)
Colorectal cancer (C18-20)	Colon cancer (C18)
	Rectal cancer (C19-20)
Colon cancer	Proximal colon cancer (C18.1/5)
	Distal colon cancer (C18.6/7)

NOTE: cancers for which a specific sublocalisation could not be determined were categorized as unspecified/NOS.

Table S3.2 ATC codes for confounder drugs.

Drug	ATC	Description
Anti-hypertensives	C02*	Antihypertensives
	C03*	Diuretics
	C07*	Beta blocking agents
	C08*	Calcium channel blockers
	C09*	Agents acting on the renin-angiotensin system
Aspirin	A01AD05	acetylsalicylic acid
	B01AC06	acetylsalicylic acid
	N02BA01	acetylsalicylic acid
	N02BA01	acetylsalicylic acid
	N02BA01	acetylsalicylic acid
	M01BA03	acetylsalicylic acid and corticosteroids
	N02BA51	acetylsalicylic acid, combinations excl. psycholeptics
	B01AC56	acetylsalicylic acid, combinations with proton pump inhibitors
	N02BA71	acetylsalicylic acid, combinations with psycholeptics
	C10BX08	atorvastatin and acetylsalicylic acid
	C10BX06	atorvastatin, acetylsalicylic acid and ramipril
	C10BX02	pravastatin and acetylsalicylic acid
	C10BX05	rosuvastatin and acetylsalicylic acid
	C10BX01	simvastatin and acetylsalicylic acid
	C10BX04	simvastatin, acetylsalicylic acid and ramipril
Bisphosphonates	M05BA*	Bisphosphonates
	M05BB*	Bisphosphonates, combinations
Helicobacter pylori eradication therapy	A02BD*	Combinations for eradication of Helicobacter pylori
Non-steroidal anti-inflammatory drugs (excluding aspirin)	A01AD02	Benzydamine
	C01EB03	Indometacin
	C01EB16	Ibuprofen
	G02CC*	Antiinflammatory products for vaginal administration
	L01XX33	Celecoxib
	M01A*	Anti-inflammatory and antirheumatic products, non-steroids
	R02AX*	Flurbiprofen, Ibuprofen
	S01BC*	Anti-inflammatory agents, non-steroids
	S01CC*	Anti-inflammatory agents, non-steroids and anti-infectives in combination
Oral contraceptives	G03A*	Hormonal contraceptives for systemic use
Proton pump inhibitors	A02BC*	Proton pump inhibitors
	A02BD*	Combinations for eradication of Helicobacter pylori
	M01AE52	Naproxen and esomeprazole
Tamoxifen	L02BA01	Tamoxifen

Table S3.3 Incidence and stage distribution of gastrointestinal cancers by site in patients with and without diabetes mellitus type 2.

	Stage I				Stage II				Stage III				Stage IV				Stage missing/unknown			
	Non-DM		DM		Non-DM		DM		Non-DM		DM		Non-DM		DM		Non-DM		DM	
Cancer site (Events Non-DM/DM)	**Events (%)**	**IR***	**Events (%)**	**IR***	**Events (%)**	**IR***	**Events (%)**	**IR***	**Events (%)**	**IR***	**Events (%)**	**IR***	**Events (%)**	**IR***	**Events (%)**	**IR***	**Events (%)**	**IR***	**Events (%)**	**IR***
Gastrointestinal cancer overall (N=351/583)	50 (14)	36	76 (13)	53	81 (23)	58	123 (21)	86	67 (19)	48	113 (19)	79	97 (28)	70	139 (24)	97	56 (16)	40	132 (23)	92
Oesophageal cancer (N=25/41)	<5 (8)	1	5 (12)	3	<5 (8)	1	<5 (7)	2	<5 (16)	3	<5 (10)	3	8 (32)	6	13 (32)	9	9 (36)	6	16 (39)	11
Gastric cancer (N=46/50)	7 (15)	5	7 (14)	5	7 (15)	5	6 (12)	4	<5 (4)	1	7 (14)	5	21 (46)	15	17 (34)	12	9 (20)	6	13 (26)	9
Small intestinal cancer (N=<5/6)	-	-	<5 (33)	1	-	-	-	-	-	-	<5 (17)	1	<5 (50)	1	<5 (17)	1	<5 (50)	1	<5 (33)	1
Liver cancer (N=<5/15)	-	-	-	-	-	-	<5 (13)	1	-	-	6 (40)	4	<5 (100)	1	-	-	-	-	7 (47)	5
Biliary tract cancer (N=7/20)	<5 (14)	1	<5 (5)	1	-	-	<5 (20)	3	<5 (14)	1	<5 (15)	2	<5 (43)	2	<5 (15)	2	<5 (29)	1	9 (45)	6
Pancreatic cancer (N=30/122)	-	-	7 (6)	5	<5 (10)	2	9 (7)	6	<5 (3)	1	8 (7)	6	13 (43)	9	39 (32)	27	13 (43)	9	59 (48)	41
Colorectal cancer (N=241/333)	40 (17)	29	55 (17)	38	69 (29)	49	100 (30)	70	59 (24)	42	85 (26)	59	50 (21)	36	67 (20)	47	23 (10)	16	26 (8)	18
Colon cancer (N=168/253)	30 (18)	21	38 (15)	27	52 (31)	37	84 (33)	59	34 (20)	24	62 (25)	43	34 (20)	24	49 (19)	34	18 (11)	13	20 (8)	14
Rectal cancer (N=77/87)	12 (16)	9	20 (23)	14	19 (25)	14	17 (20)	12	25 (32)	18	24 (28)	17	16 (21)	11	19 (22)	13	5 (6)	4	7 (8)	5

* Incidence rate per 100,000 person-years of follow-up. DM: diabetes mellitus; IR: incidence rate; Non-DM: non-diabetes mellitus.

Chapter 4

Brief report: Comparability of the age and sex distribution of the UK Clinical Practice Research Datalink and the total Dutch population

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Abstract

Purpose

The UK Clinical Practice Research Datalink (CPRD) is increasingly being used by Dutch researchers in epidemiology and pharmacoepidemiology. It is however unclear if the UK CPRD is representative of the Dutch population and whether study results would apply to the Dutch population. Therefore, as first step, our objective was to compare the age and sex distribution of the CPRD with the total Dutch population.

Methods

As a measure of representativeness, the age and sex distribution of the UK CPRD were visually and numerically compared with Dutch census data from the StatLine database of the Dutch National Bureau of Statistics in 2011.

Results

The age distribution of men and women in the CPRD population was comparable to the Dutch male and female population. Differences of more than 10% only occurred in older age categories (75+ in men and 80+ in women).

Conclusions

Results from observational studies that have used CPRD data are applicable to the Dutch population, and a useful resource for decision making in the Netherlands. Nevertheless, differences in drug exposure likelihood between countries should be kept in mind, as these could still cause variations in the actual population studied, thereby decreasing its generalizability.

Introduction

The United Kingdom (UK) Clinical Practice Research Datalink (CPRD) is one of the world's largest primary care databases and is frequently used for post-authorisation safety studies, pharmaco-epidemiology, and disease epidemiology.[1-5] Examples include the evaluation of side effects of dopamine agonists2 or diabetes drugs[3,4], and the epidemiology of fractures.[5]

From a global perspective, the healthcare system in the Netherlands, a small country not far from the UK, is largely comparable to that of the UK's National Health Service (NHS); i.e. everyone has equal access to medical care regardless of income or socioeconomic status. In both countries, the general practitioner (GP) is the gatekeeper of the public healthcare system, meaning patients cannot refer themselves to secondary or tertiary care without the GP's approval. These conditions are key for conducting population-based pharmaco-epidemiological studies.

Although the Dutch public healthcare system has excellent conditions for establishing a large primary care database for pharmaco-epidemiological research that is comparable to the CPRD in terms of sample size, this has not yet occurred. Smaller primary care databases do exist (e.g. Netherlands Information Network of General Practice (LINH), Integrated Primary Care Information (IPCI), PHARMO Database Network); however these are generally restricted by a limited set of medical codes (approximately 1000 different "International Classification of Primary Care" codes versus over 100 000 READ codes in the CPRD), few validation studies, considerable smaller sample size (e.g. 350 000 in LINH and 1.5 million in IPCI versus over 11 million in CPRD in 2011), and limited access to routinely collected lifestyle data, such as tobacco use, alcohol consumption, and socioeconomic status.[6] Furthermore, claims that these data are representative for the total Dutch population are seldom supported by published figures.[7,8] For these reasons, an increasing number of CPRD studies are being conducted by researchers from the Netherlands and are financially supported by Dutch universities and funding agencies such as ZonMw and NWO. A recent study showed that the CPRD is representative of the total UK population with respect to age and sex and covers 6.9% of the UK population.[1,9]

A wide range of diagnoses in the CPRD have been validated in a number of studies, and data quality are further enhanced by NHS annual reward and incentive programme that details GP practice achievement results, the Quality and Outcomes Framework (QOF). The QOF awards GPs for regular recording of detailed data on a wide range of diseases. As a result, the CPRD contains millions of recordings for measurements such as blood pressure, cholesterol values, and lung function. In addition, the strength of CPRD's data partially explains why officials of the Food and Drug Administration in the United States

perform studies using the CPRD database for drug safety monitoring and regulatory decision making.[10-12]

Although there are many similarities between healthcare systems of the UK and the Netherlands, it is unclear whether results from CPRD studies would apply to the Dutch population. Therefore, our objective was to compare the age and sex distribution of the CPRD to the total Dutch population.

Methods

Using the same sample of data from the previously published CPRD data resource profile, the age and sex distribution of the CPRD primary care data on 27 March 2011 were visually and numerically compared with UK and Dutch census data in 2011.[1] The CPRD (formerly known as Value Added Medical Products, and later General Practice Research Database[13,14]) harnesses data from UK's general practices and produces a primary care dataset since 1987. Through the years, it has become one of the largest databases of longitudinal medical records from primary care in the world, with coverage of over 11.3 million patients from 674 practices. To date, 4.4 million active (alive, currently registered) patients meet quality criteria (approximately 6.9% of the UK population), who are broadly representative of the UK general population in terms of age, sex, and ethnicity. For this study, visual comparison was performed by inspecting the overlap between the respective lines in a graph (Figure 4.1). An additional comparison calculated the differences between proportional distributions of 5-year age groups of CPRD data versus Dutch census data in 2011. We are not aware of any objective methods to define representativeness of patients in a research database compared to a country's total population. We therefore described the absolute and proportional differences between the age and sex distributions of CPRD and Dutch Census data in order to leave this to the reader, and made a subjective decision to consider an age-sex specific difference of <10% representative (Table 4.1). Numbers for computing sex-stratified age categories of the total Dutch population in March 2011 were obtained from the StatLine database of the Dutch National Bureau of Statistics (www.cbs.nl).

Results

In general, the age distribution of men and women in the CPRD population was comparable to the Dutch male and female population (Figure 4.1).

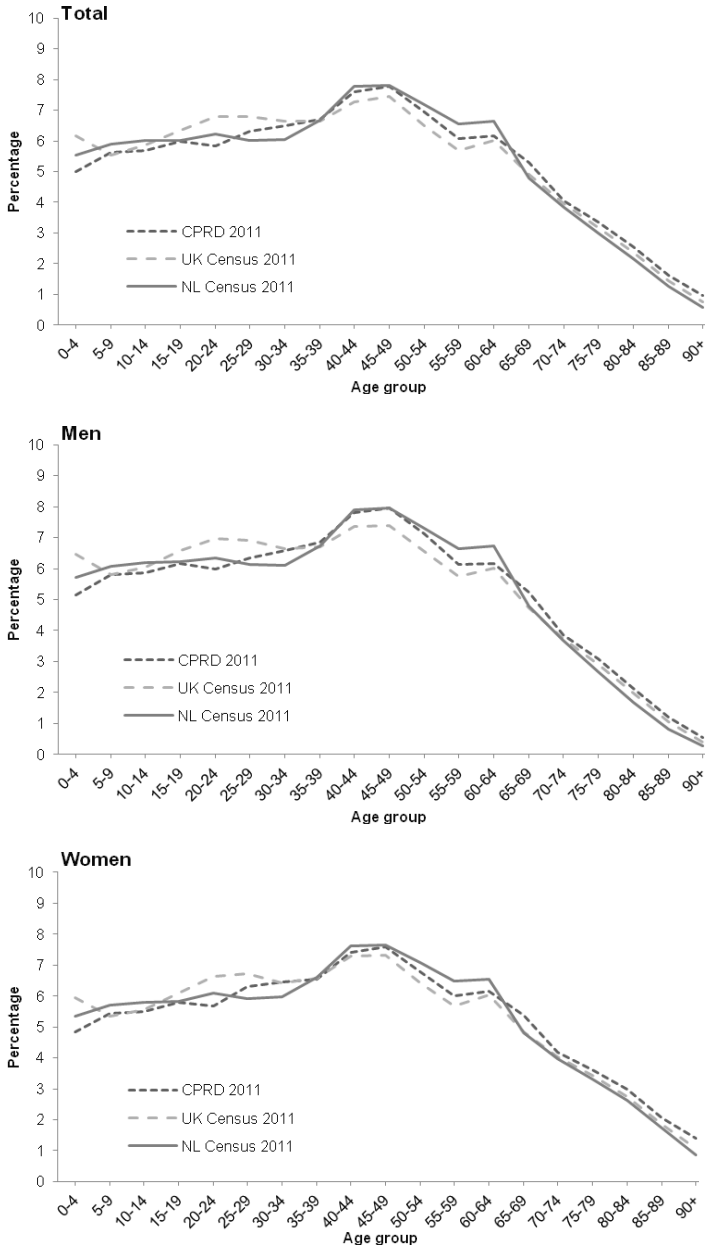

Figure 4.1 Age distribution of the CPRD primary care data on 27 March 2011 compared with UK and NL Census data from 2011, in both men and women (top panel), men only (middle panel) and women only (bottom panel). These data are based on a one-million patient sample of CPRD. Adapted from Herrett et al. Int. J Epidemiol 2015.

Overall, the percentage men and women in the CPRD in 2011 was the same as in the Dutch population (49.5% men, 50.5% women). The additional comparison based on calculating the differences between proportional distributions of 5-year age groups showed that differences of more than 10% occurred only in older age categories, starting from 75+ in men, and 80+ in women (Table 4.1).

Discussion

In this study, we showed that the age and sex distribution of the CPRD was visually and numerically comparable to that of the total Dutch population in 2011.

To our knowledge, this is the first study to directly compare the age and sex distribution of the CPRD to the total Dutch population. Apart from LINH, a national data network compiling electronic medical records (EMR) of 92 primary care practices with 211 GPs and over 350 000 patients, we are not aware of any Dutch EMR database with data from GPs, which has published data on its representativeness according to the total Dutch population. The IPCI database is a longitudinal primary care database maintained by the department of Medical Informatics of the Erasmus Medical Centre in Rotterdam. In published papers of studies using the IPCI database it is frequently stated that the database is comparable to the total Dutch population in terms of age and sex.[15,16] However, we could not verify this claim in a (peer-reviewed) publication. In a report of the Netherlands Institute for Health Services Research (NIVEL), which maintains the LINH database, it is shown that LINH is generally comparable to the total Dutch population, with a slight underrepresentation of women of 75 years and older.[8] However, age- and sex-stratified proportions were calculated as compared to the total LINH population and not as compared to the total LINH population stratified by sex, making direct comparison with our results difficult.

Our calculations of the differences between proportional distributions of 5-year age groups show that the CPRD is comparable to the Dutch population in terms of age and sex up to age 75 in men and age 80 in women. However, in Figure 4.1 it can be clearly seen that the age distribution of CPRD and Dutch census data almost overlap in these higher age groups. The large differences for higher age groups seen in Table 4.1 may be a spurious finding, because the calculations were based on very small proportions of the total population. Of note, some difference is also seen between CPRD and the UK population in Figure 4.1. Men and to a lesser extent women aged between 20 and 35 years of age are underrepresented in CPRD, which has been attributed to the fact that these individuals probably do not register with a GP.[9]

Table 4.1 Differences between proportional age distributions of CPRD and the total Dutch population by sex.

Age group	Total				Men				Women			
	CPRD	NL Census	Δ[a]	Δ%[b]	CPRD	NL Census	Δ[a]	Δ%[b]	CPRD	NL Census	Δ[a]	Δ%[b]
0-4	4.99	5.54	-0.55	-9.94	5.15	5.72	-0.57	-9.98	4.82	5.35	-0.53	-9.90
5-9	5.61	5.89	-0.28	-4.75	5.80	6.09	-0.29	-4.74	5.43	5.70	-0.27	-4.77
10-14	5.68	6.00	-0.32	-5.38	5.87	6.20	-0.33	-5.32	5.49	5.80	-0.31	-5.43
15-19	5.99	6.03	-0.04	-0.65	6.18	6.23	-0.05	-0.80	5.80	5.83	-0.03	-0.50
20-24	5.83	6.23	-0.40	-6.44	5.98	6.35	-0.37	-5.89	5.68	6.10	-0.42	-7.00
25-29	6.32	6.02	0.30	4.96	6.34	6.13	0.21	3.41	6.31	5.92	0.39	6.54
30-34	6.51	6.03	0.47	7.83	6.57	6.11	0.46	7.65	6.44	5.96	0.48	8.01
35-39	6.69	6.67	0.01	0.20	6.84	6.74	0.10	1.62	6.53	6.61	-0.08	-1.22
40-44	7.59	7.77	-0.17	-2.22	7.80	7.91	-0.11	-1.42	7.39	7.62	-0.23	-3.03
45-49	7.77	7.79	-0.02	-0.31	7.97	7.95	0.02	0.22	7.58	7.64	-0.06	-0.86
50-54	6.95	7.19	-0.24	-3.41	7.12	7.30	-0.18	-2.44	6.78	7.09	-0.31	-4.38
55-59	6.06	6.56	-0.49	-7.54	6.14	6.64	-0.50	-7.62	5.99	6.47	-0.48	-7.46
60-64	6.15	6.64	-0.48	-7.24	6.16	6.72	-0.56	-8.30	6.15	6.55	-0.40	-6.18
65-69	5.30	4.79	0.51	10.64	5.23	4.79	0.44	9.18	5.37	4.79	0.58	12.08
70-74	4.03	3.83	0.20	5.27	3.87	3.67	0.20	5.31	4.19	3.98	0.21	5.23
75-79	3.36	3.00	0.36	11.90	3.09	2.66	0.43	15.98	3.62	3.33	0.29	8.71
80-84	2.56	2.17	0.39	18.00	2.15	1.70	0.45	26.28	2.97	2.64	0.33	12.80
85-89	1.64	1.28	0.36	28.27	1.20	0.82	0.38	47.05	2.07	1.73	0.34	19.62
90+	0.98	0.57	0.41	70.52	0.55	0.27	0.28	102.20	1.40	0.87	0.53	60.92

[a] Absolute difference;, [b] Percentage difference between CPRD versus NL Census data (CPRD minus NL Census, divided by NL Census), with >10% regarded as different.
CPRD: Clinical Practice Research Datalink; NL: Netherlands; Δ difference.

Several strengths have to be noted for this study. First, UK CPRD data were compared to data from the StatLine database of the Dutch National Bureau of Statistics, a reliable source of population-based information, regulated by national and European codes and laws.[17] Second, by calculating the differences between proportional distributions in all age groups, we demonstrated that the distributions were not only visually, but also numerically comparable. Last, by showing the overall representativeness of the CPRD database of another population besides the UK population, CPRD may be used as rich data source for healthcare policymakers outside the UK.

There are also several limitations to this comparison. First, there are no objective methods to define representativeness of patients in a research database compared to a country's total population. To overcome this, we gave the reader insight into the various ways of comparing these data. Furthermore, the CPRD population was compared to the total Dutch population in terms of age and sex only. Therefore, we cannot rule out differences in for instance ethnicity, socioeconomic class, or lifestyle, which may in turn impact disease prevalence, exposure to important risk factors, or the degree of health care seeking behaviour. Based on the report of the OECD health indicators, the UK population has higher rates of tobacco and alcohol consumption, and especially obesity among adults, compared to the Dutch population.[18] Although the results of our study imply that the UK CPRD may also be representative of the total Dutch population, information on a specific population of drug users is ultimately necessary to know whether results from pharmaco-epidemiological studies are transferrable to one's own region. As of yet, we have not looked into the comparability of various subpopulations in CPRD and The Netherlands. In addition, relative risks of disease outcomes found in CPRD could be extrapolated to the Dutch population, incidence rates or absolute risks cannot.

In conclusion, this study showed that the age and sex distribution of CPRD were generally comparable to that of the total Dutch population. Results from observational studies that have used CPRD data are applicable to the total Dutch population (similar to how relative risks from randomized clinical trials apply to their demarcated population), and a useful resource for decision making in the Netherlands. Nevertheless, differences in drug exposure likelihood between countries should be kept in mind, as these could still cause variations in the actual population studied, thereby decreasing its generalizability. In addition, the results of this study may encourage scientists from other countries with similar healthcare systems to perform comparable studies of CPRD representativeness.

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Chapter 5

No decreased risk of gastrointestinal cancers in users of metformin in the Netherlands; a time-varying analysis of metformin exposure

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Abstract

Background

Previous studies on metformin use and gastrointestinal (GI) cancer risk have yielded inconclusive results on metformin's chemoprotective effects. We aimed to evaluate GI cancer risk in users of metformin in The Netherlands using a time-varying approach in a large population-based database.

Methods

A cohort study was performed using the E-NCR-PHARMO database. Patients using ≥1 non-insulin antidiabetic drug (NIAD) during 1998 to 2011 were included (N=57,621). Exposure to NIADs was modelled time-varyingly. Cox regression analysis estimated HRs of GI cancers in current metformin users versus current users of other NIADs. Covariables included age, sex, drugs known to impact cancer risk, history of hospitalization, and starting year of follow-up. A sensitivity analysis was performed, applying a new-user design.

Results

Current use of metformin was not associated with a decreased risk of GI cancer [HR, 0.97; 95% confidence interval (CI), 0.82-1.15] or specific GI cancer sites. The sensitivity analysis yielded comparable results. No decreasing trends were observed with increasing cumulative dose of metformin [HR 1.05, 95% CI, 0.85-1.28; HR 0.89, 95% CI, 0.73-1.10; HR 0.96, 95% CI, 0.77-1.19 for dose tertiles low (<405 g), medium (405-998 g), and high (≥999 g)]. In contrast, an increased risk of pancreatic cancer was found in current users of metformin plus insulin (HR, 4.90; 95% CI, 2.64-9.10).

Conclusions

In conclusion, no decreased risk of GI cancer was found in current metformin users compared with current users of other NIADs. Variations in the exposure definition of metformin use may be one of the explanations of previously found reduced cancer risks in metformin users.

Introduction

Metformin is an antidiabetic drug (ADD) that is widely used as the preferred first-line treatment for hyperglycaemia in type 2 diabetes mellitus (T2DM). The Dutch guideline for the treatment of T2DM advises metformin as first-line treatment as well, beside lifestyle advice such as dietary modification, physical exercise, and weight reduction.[1]
Metformin not only effectively lowers the blood glucose concentration through inhibition of gluconeogenesis and glycogenolysis in the liver, but is also known to decrease insulin resistance and hyperinsulinaemia through the insulin/IGF-1 signalling pathway.[2,3] Because insulin resistance is known to be a risk factor for cancer development, metformin may have a role in chemoprevention of cancer.[4-6] Other ways through which metformin may reduce cancer risk are: (1) direct activation of AMP-activated protein kinase (AMPK) signalling, which leads to inhibition of the mTOR signalling pathway, and subsequently to reduced cell proliferation, protein synthesis, and tumour angiogenesis[4]; (2) metformin may have anti-inflammatory effects on malignant cells and may inhibit malignant stem cells, which are important in cancer initiation, recurrence, and resistance to chemotherapies.[7]
Observational studies have shown reduced risks of up to 64% for colorectal cancer, 94% for liver cancer, and 85% for pancreatic cancer in patients with T2DM using metformin.[8-15] However, the validity of the reported risk reductions in observational studies may be limited due to methodological issues, such as confounding by indication, prevalent user bias, and time-related biases.[16-18] Moreover, recent studies that have used a time-varying approach of metformin exposure could not confirm the lower risk of several cancers with use of metformin.[19,20] Although metformin may contain antineoplastic properties based on the aforementioned in vitro evidence, this effect may not be clinically relevant and therefore not visible when applying an optimal exposure definition of metformin use in an observational design.
The aim of our study was to evaluate the risk of gastrointestinal (GI) cancers in patients with T2DM using metformin applying a time-varying approach to ADD exposure, and to show differences between a prevalent user design and a new-user design.

Materials and methods

Data source

Data for this population-based cohort study were obtained from the PHARMO Database Network and linked at the individual patient level to the Eindhoven area of the

Netherlands Cancer Registry (E-NCR-PHARMO database). The construct and validity of the linked database have been described elsewhere.[21] The Eindhoven area of the NCR, maintained by the Netherlands Comprehensive Cancer Organisation (NCCO), covers a demographic region with approximately 2.4 million inhabitants (±15% of the Dutch population). Trained registration clerks actively collect data on newly diagnosed cancers, patient characteristics, staging, and initial treatment from hospital medical records. Vital status is obtained by linkage to Dutch municipal records.
The PHARMO Database Network is a large, patient-centric data network including linked observational databases designed for drug safety and outcomes research. For this study, the Out-patient (community) Pharmacy Database was used, which contains longitudinal drug dispensing records, and included information on dispensing date, dose descriptions, and amount dispensed. All drugs are coded according to their Anatomical Therapeutic Chemical/Defined Daily Dose Classification (ATC/DDD) code.[22] Both the NCR and the PHARMO Database Network are recognized as high-quality data sources for (pharmaco-)epidemiological research that have collected information in overlapping regions in the Netherlands for a period of over 10 years.[21]

Study design and population

We conducted a cohort study of all adult patients aged ≥30 years with at least one drug dispensing for an ADD [ATC codes "A10A" insulins, or "A10B" non-insulin antidiabetic drugs (NIAD)] in the E-NCR-PHARMO region between January 1, 1998, and December 31, 2011 (Figure 5.1). The date of first recorded ADD defined the index date. We restricted the cohort to patients aged ≥30 years at the time of their first recorded prescription, as GI cancer rarely occurs before that age and to reduce misclassification by including type 1 diabetic patients. Patients for whom the first recorded ADD was insulin (ATC code "A10A") were excluded as they were more likely to have type 1 diabetes mellitus. Since coverage of the PHARMO database has gradually increased over time, there is a small chance that some prevalent T2DM patients were excluded, as patients could have entered the database at a later stage of their disease. Patients diagnosed with any type of GI cancer before the index date were excluded.

Exposure classification

Follow-up time for all subjects was divided into fixed 90-day time intervals in order to model drug exposure over time in a time-varying way. Exposure to metformin and non-metformin NIADs (other NIADs) was defined at the beginning of every 90-day interval. If a patient received a metformin or other NIAD prescription in the 90-days prior to the start of an interval, they were classified as a "current user" of that drug, otherwise they

were classified as a "past user." All patients were classified as "current user" of either metformin or a non-metformin other NIAD at each time interval, but they could move between current and past use throughout follow-up. The cumulative dose of metformin was calculated at each current metformin use interval by summation of the total dose of each metformin prescription during the previous current use intervals. The whole sample median value was used to impute missing values of the recorded dose per tablet and for missing and/or extreme values of the amount of tablets dispensed. Cumulative dose at the end of follow-up was stratified by tertiles of cumulative metformin dose and classified as low (<405 g), medium (405–998 g), and high (≥999 g) cumulative dose.

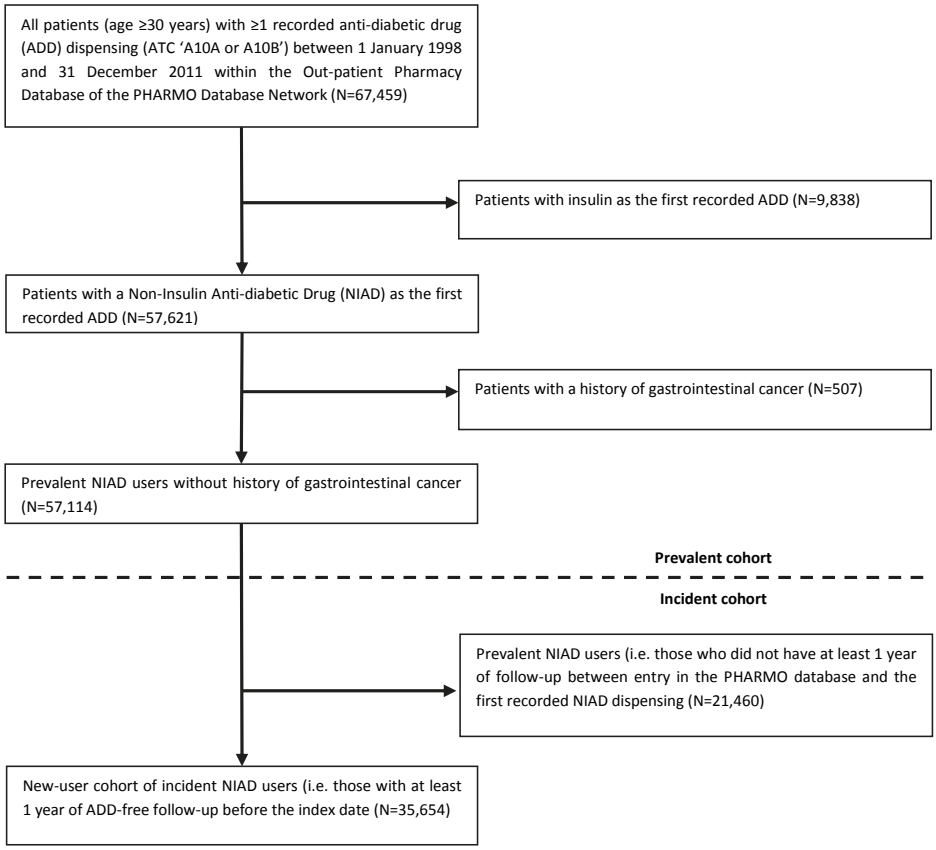

Figure 5.1 Flowchart of study population.

Outcomes

All patients were followed from the index date until a first ever diagnosis of a GI cancer, death from any cause, end of registration within the PHARMO catchment area, or end of

data collection (December 31, 2011), whichever came first. GI cancers were classified according to the International Classification of Diseases of oncology.[23] These included "any GI cancer" (C15-26), oesophageal cancer (C15), gastric cancer (C16), small intestinal cancer (C17), colorectal cancer (CRC, C18-C20), hepatic cancer (C22), biliary tract cancer (C23: gallbladder, and C24: extrahepatic bile duct cancer), and pancreatic cancer (C25).

Covariables

A number of covariables were considered as confounders based on the current literature. As time-fixed covariables sex and history of hospitalization prior to the index date (hospitalization categories 0 or ≥1) were considered. Time-dependent covariables were determined at the start of every 90-day time period and included age, the duration of diabetes in years (time since first recorded NIAD dispensing), and the use of other drugs known to impact GI cancer risk in the 90 days prior to the start of each interval [statins, aspirin, non-aspirin non-steroidal anti-inflammatory drugs (NSAID), proton pump inhibitors, bisphosphonates, tamoxifen, oral contraceptives, and insulin). In addition, the use of helicobacter pylori (H. pylori) eradication therapy was used as proxy-indicator for H. pylori infection. Also, the year of start of follow-up was included as covariable as the index date of current metformin users and current users of other NIADs differed significantly at baseline (Table 5.1).

Statistical analysis

Differences in demographic characteristics between current users of metformin and current users of other NIADs at baseline were compared using the Mann–Whitney U test for continuous variables and the χ^2 test for categorical variables.

Incidence rates per 100,000 person-years of follow-up were calculated by dividing the number of events by the total amount of person-years of follow-up. Overall and site-specific HRs and 95% confidence intervals (CI) of GI cancer in current users of metformin versus current users of other NIADs were calculated using time-varying Cox proportional hazards models. Stratified analyses were performed by sex, and by stratifying current metformin use by treatment stage and tertiles of cumulative dose. Subgroups of current metformin use by treatment stage included metformin monotherapy, metformin plus a sulfonylurea (SU) derivative, metformin plus another (non-SU) NIAD, and metformin plus insulin (regardless of other NIAD use). Potential confounders were entered into the regression models if they independently changed the β-coefficient for current metformin use by at least 5% in a univariate analysis.

Table 5.1 Baseline characteristics of current users of metformin or other NIADs.

Characteristic	Current metformin users n=37,215		Current other NIADs users n=19,899		p-value[a]
Age (mean, SD)	63.5	12.7	67.0	12.9	< 0.01
Sex (n, % male)	18,151	48.8	9,353	47.0	< 0.01
Year of index date (mean, SD)	2006	3.4	2002	3.4	< 0.01
Anti-diabetic drug use (n, %)[b]					
Metformin	37,215	100.0	0	0.0	< 0.01
Sulfonylureas	4,621	12.4	19,166	96.3	< 0.01
Thiazolidinediones	357	1.0	632	3.2	< 0.01
Meglitinides	9	0.0	54	0.3	< 0.01
Incretins	71	0.2	53	0.3	0.06
Use of other drugs (n, %)[c]					
Anti-hypertensives	21,653	58.2	10,246	51.5	< 0.01
Aspirin	6,326	17.0	3,102	15.6	< 0.01
Bisphosphonates	922	2.5	549	2.8	0.04
H. pylori eradication therapy	41	0.1	9	0.1	0.01
Non-aspirin NSAIDs	4,832	13.0	2,630	13.2	0.43
Proton pump inhibitors	6,478	17.4	2,702	13.6	< 0.01
Statins	14,898	40.0	4,408	22.2	< 0.01
History of hospitalization (n, %)					
0 hospitalisations	22,621	60.8	14,310	71.9	
≥1 hospitalisations	14,594	39.2	5,589	28.1	<0.01

[a] p-value based on Mann-Whitney U test for continuous variables and Chi-squared test for categorical variables. [b] At the start of follow-up (t0), [c] During 90 days before the index date. Abbreviations: H. pylori, helicobacter pylori; NIADs, non-insulin anti-antidiabetic drugs; NSAIDs, non-steroidal anti-inflammatory drugs.

Sensitivity analyses

Sensitivity analyses with a new-user design were performed to account for prevalent user bias. The main analyses were repeated with an inception cohort of incident NIAD users only (Figure 5.1). To create an inception cohort of incident NIAD users, we excluded all prevalent NIAD users, i.e., those who did not have at least 1 year of follow-up between entry in the PHARMO database and the first recorded NIAD dispensing. Data management and statistical analyses were conducted using SAS 9.4 software.

Results

Baseline characteristics

At the start of follow-up, 37,215 T2DM patients were current metformin users and 19,899 were current users of other NIADs (Table 5.1). Current metformin users were on average younger (mean age, 63.5 vs. 67.0 years, p<0.01) and more often males (48.8% vs. 47.0%, p<0.01) compared with current other NIAD users. The year of start of follow-

up was more recent for current metformin users than current other NIAD users (mean, 2006 vs. 2002, p<0.01). Most diabetic patients started follow-up either on metformin monotherapy or on SU (96.3% of current other NIAD users). Furthermore, current metformin users used more other drugs besides ADDs as compared with other NIAD users, such as statins (40.0% vs. 22.2%, p<0.01), aspirin (17.0% vs. 15.6%, p<0.01), anti-hypertensives (58.2% vs. 51.5%, p<0.01), and proton pump inhibitors (17.4% vs. 13.6%, p<0.01). Current metformin users were being hospitalized prior to the index date more often (39.2% vs. 28.1%, p<0.01).

GI cancer overall

During more than 280,000 person-years of follow-up (mean, 4.9 years per person), 1,076 GI cancers were observed (IR, 381 per 100,000 person-years). No statistically significant decreased risk of GI cancer was observed in current metformin users compared with current other NIAD users (fully adjusted HR 0.97; 95% CI, 0.82-1.15; Table 5.2). Stratified analyses of subgroups of current metformin use by treatment stage and tertiles of cumulative dose did not reveal a decreased risk of GI cancer. Furthermore, the sensitivity analysis and stratified analysis by sex yielded similar results (Table 5.2 and Supplementary Table S5.1, respectively).

GI cancer sites

In the site-specific analyses, no significant differences in HRs of GI cancers were observed in current metformin users versus current other NIAD users (Table 5.3). However, a statistically significant increased HR of pancreatic cancer was observed in the subgroup of current users of metformin plus insulin (fully adjusted HR 4.90; 95% CI, 2.64-9.10) and in female current metformin users (fully adjusted HR 1.95; 95% CI, 1.01-3.76; Supplementary Table S5.2). Furthermore, there were no trends with increasing cumulative dose of metformin. In addition, the new-user design did not show statistically significant decreased HRs of GI cancer sites in current metformin users compared with current other NIAD users (Table 5.4), whereas increased HRs of pancreatic cancer with current use of metformin plus a SU derivative and metformin plus insulin remained (fully adjustedHR1.98; 95% CI, 1.10-3.59 and fully adjusted HR 10.26; 95% CI, 4.96-21.22, respectively).

Table 5.2 Hazard ratios of gastrointestinal cancer overall in current metformin users compared with current other NIAD users.

Exposure category	Prevalent-user design						New-user design					
	Events (N=1,076)	IR	HR[a]	95% CI	HR[b]	95% CI	Events (N=612)	IR	HR[a]	95% CI	HR[b]	95% CI
Current other NIADs[c]	214	457	Ref.	-	Ref.	-	120	556	Ref.	-	Ref.	-
Current metformin	624	376	0.96	0.81-1.13	0.97	0.82-1.15	361	391	0.91	0.73-1.14	0.79	0.59-1.06
Stratified by treatment stage[d]												
Metformin only	277	341	0,83	0.69-1.01	0.89	0.73-1.07	208	359	0.81	0.63-1.03	0.75	0.55-1.04
Metformin + SU	269	432	1,07	0.89-1.29	1.07	0.89-1.29	117	450	1.06	0.81-1.39	0.85	0.60-1.21
Metformin + other NIADs	15	247	0,74	0.43-1.26	0.75	0.46-1.28	11	320	0.97	0.52-1.82	0.79	0.34-1.84
Metformin + insulin	63	379	1,15	0.86-1.54	1.06	0.78-1.43	25	496	1.57*	1.00-2.47	0.74	0.36-1.51
Stratified by cumulative dose[e]												
Low	201	376	0.93	0.76-1.14	1.05	0.85-1.28	143	429	0.95	0.74-1.23	0.78	0.54-1.11
Medium	196	343	0.88	0.72-1.08	0.89	0.73-1.10	109	324	0.76	0.58-1.01	0.73	0.51-1.04
High	227	408	1.10	0.90-1.36	0.96	0.77-1.19	109	430	1.10	0.82-1.47	0.91	0.62-1.34
Past metformin	194	361	0.95	0.77-1.16	0.87	0.70-1.07	115	397	0.95	0.72-1.25	0.74	0.52-1.06
Past other NIADs	44	281	0.66*	0.48-0.92	0.61*	0.44-0.84	16	199	0.41*	0.24-0.69	0.40*	0.21-0.76

[a] Age and sex adjusted. [b] Adjusted for age, sex, use of statins, insulin, history of hospitalization, duration of diabetes, and year of start of follow-up. [c] Excluding metformin. [d] Fully adjusted model not adjusted for insulin use. [e] Low: <405 g; Medium: 405-999 g; High: ≥999 g. * Statistically significant with p<0.05. CI: confidence interval; HR: hazard ratio; IR: incidence rate per 100,000 person-years; NIADs: non-insulin anti-diabetic drugs; SU: sulfonylureas.

Table 5.3 Site-specific hazard ratios of gastrointestinal cancer in current metformin users compared with current other NIAD users (Prevalent cohort).

Cancer site	Oesophagus (N = 84)			Stomach (N = 108)			Liver (N = 27)			Biliary tract (N = 40)			Pancreas (N = 175)			Colorectum (N = 637)		
Exposure category	**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**	
Current other NIADs[b]	16	Ref.		22	Ref.		<5	Ref.		9	Ref.		32	Ref.		132	Ref.	
Current metformin	48	0.90	0.48-1.67	61	1.06	0.63-1.80	19	2.07	0.58-7.43	20	1.36	0.59-3.17	105	1.11	0.72-1.71	368	0.89	0.71-1.10
Stratified by treatment stage[c]																		
Metformin only	24	0.95	0.48-1.87	20	0.74	0.39-1.41	13	3.42	0.92-12.76	10	1.61	0.62-4.15	40	0.73	0.44-1.12	165	0.86	0.67-1.10
Metformin + SU	21	1.19	0.61-2.34	35	1.42	0.81-2.49	5	1.53	0.36-6.59	10	1.99	0.80-4.99	42	1.59	0.98-2.57	158	0.93	0.73-1.18
Metformin + other NIADs	0	-	-	<5	1.14	0.26-4.98	0	-	-	0	-	-	<5	1.72	0.59-4.96	9	0.68	0.34-1.36
Metformin + insulin	<5	0.69	0.19-2.45	<5	0.66	0.22-2.00	<5	1.35	0.13-13.64	0	-	-	19	4.90*	2.64-9.10	36	0.83	0.56-1.23
Stratified by cumulative dose[d]																		
Low	14	0.83	0.40-1.75	20	1.29	0.68-2.43	5	1.66	0.39-7.14	8	1.44	0.54-3.83	55	1.29	0.81-2.07	97	0.86	0.65-1.14
Medium	13	0.72	0.33-1.57	18	0.94	0.49-1.80	6	2.00	0.47-8.50	6	1.18	0.40-3.47	26	0.81	0.47-1.42	123	0.89	0.69-1.15
High	21	1.26	0.60-2.65	23	0.96	0.49-1.88	8	2.91	0.69-12.24	6	1.49	0.48-4.59	24	1.10	0.58-2.06	148	0.91	0.69-1.20
Past metformin	15	1.03	0.48-2.20	20	0.96	0.50-1.85	<5	1.07	0.21-5.54	11	2.00	0.78-5.09	35	0.98	0.58-1.67	110	0.77	0.59-1.01
Past other NIADs	5	1.13	0.41-3.12	5	0.68	0.26-1.82	<5	2.18	0.36-13.16	0	-	-	<5	0.26*	0.08-0.86	27	0.59*	0.39-0.89

[a]Adjusted for age, sex, use of statins (oesophageal cancer, gastric cancer, biliary tract cancer, pancreatic cancer), proton pump inhibitors (oesophageal cancer, gastric cancer), aspirin (gastric cancer, pancreatic cancer), anti-hypertensives (oesophageal cancer, pancreatic cancer), helicobacter pylori eradication therapy (gastric cancer), insulin (oesophageal cancer, gastric cancer, pancreatic cancer, colorectal cancer), history of hospitalization (oesophageal cancer, gastric cancer, liver cancer, biliary tract cancer, pancreatic cancer, colorectal cancer), duration of diabetes (gastric cancer, pancreatic cancer, colorectal cancer), and year of start of follow-up (gastric cancer, pancreatic cancer, colorectal cancer). [b]Excluding metformin. [c]Not adjusted for insulin use. [d]Low: <405 g; Medium: 405-999 g; High: ≥999 g. *Statistically significant with p<0.05. CI: confidence interval; HR: hazard ratio; NIADs: non-insulin anti-diabetic drugs; SU: sulfonylureas.

Table 5.4 Site-specific hazard ratios of gastrointestinal cancer in current metformin users compared with current other NIAD users (new-user design).

Cancer site	Oesophagus (N=43)			Stomach (N=53)			Liver (N=15)			Biliary tract (N=20)			Pancreas (N=127)			Colorectum (N=352)		
Exposure category	**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**	
Current other NIADs[b]	6	Ref.		10	Ref.		<5	Ref.		<5	Ref.		23	Ref.		75	Ref.	
Current metformin	29	1.59	0.61-4.14	28	0.90	0.41-1.97	11	1.44	0.29-7.27	9	1.12	0.32-3.94	79	1.14	0.68-1.91	201	0.78	0.58-1.05
Stratified by treatment stage[c]																		
Metformin only	20	2.07	0.79-5.40	15	0.79	0.33-1.88	7	1.93	0.38-9.84	6	1.34	0.36-5.00	35	0.71	0.40-1.26	121	0.75	0.54-1.03
Metformin + SU	9	2.18	0.75-6.31	11	1.15	0.47-2.84	4	2.37	0.42-13.45	<5	1.54	0.33-7.09	26	1.98*	1.10-3.59	65	0.84	0.59-1.19
Metformin + other NIADs	0	-	-	<5	1.11	0.14-8.95	0	-	-	0	-	-	<5	2.74	0.92-8.18	6	0.77	0.33-1.80
Metformin + insulin	0	-	-	<5	0.66	0.08-5.47	0	-	-	0	-	-	14	10.26*	4.96-21.22	9	0.72	0.35-1.48
Stratified by cumulative dose[d]																		
Low	9	1.35	0.46-3.96	13	1.29	0.53-3.13	<5	0.80	0.11-6.01	<5	1.20	0.29-4.98	46	1.23	0.71-2.13	67	0.77	0.54-1.10
Medium	7	1.16	0.36-3.72	5	0.44	0.15-1.35	<5	1.60	0.26-9.71	<5	1.32	0.31-5.70	19	0.88	0.46-1.71	67	0.72	0.50-1.02
High	13	3.32*	1.10-10.06	10	0.99	0.36-2.72	5	2.75	0.43-17.53	<5	0.53	0.05-5.24	14	1.34	0.60-2.96	67	0.89	0.61-1.31
Past metformin	7	1.46	0.46-4.65	13	1.23	0.50-3.01	<5	0.43	0.04-5.12	7	2.32	0.63-8.60	24	0.94	0.50-1.77	65	0.79	0.55-1.13
Past other NIADs	<5	0.64	0.08-5.36	<5	0.57	0.12-2.63	<5	1.42	0.13-15.83	0	-	-	<5	0.12*	0.02-0.90	11	0.41*	0.22-0.78

[a] Adjusted for age, sex, use of statins (oesophageal cancer, gastric cancer, biliary tract cancer, pancreatic cancer), proton pump inhibitors (oesophageal cancer, gastric cancer), aspirin (gastric cancer, pancreatic cancer), anti-hypertensives (oesophageal cancer, pancreatic cancer), helicobacter pylori eradication therapy (gastric cancer), insulin (oesophageal cancer, gastric cancer, pancreatic cancer, colorectal cancer), history of hospitalization (oesophageal cancer, gastric cancer, liver cancer, biliary tract cancer, pancreatic cancer, colorectal cancer), duration of diabetes (gastric cancer, pancreatic cancer, colorectal cancer), and year of start of follow-up (gastric cancer, pancreatic cancer, colorectal cancer). [b] Excluding metformin. [c] Not adjusted for insulin use. [d] Low: <405 g; Medium: 405-999 g; High: ≥999 g. * Statistically significant with p<0.05. CI: confidence interval; HR: hazard ratio; NIADs: non-insulin anti-diabetic drugs; SU: sulfonylureas.

Discussion

In this population-based cohort study, in which we used a time-varying approach to determine metformin exposure in diabetic patients, no reduced risk of GI cancer was found when comparing current use of metformin with current use of other NIADs. In addition, results from the sensitivity analysis, in which a new-user design was applied, did not significantly differ from the main analyses with a prevalent cohort of NIAD users. The risk of pancreatic cancer was increased in female current users of metformin, and in current users of metformin combined with insulin compared with current other NIAD users in both the main and sensitivity analysis.

The results of this study add to the evidence of recently published observational studies on the effect of metformin and (GI) cancer risk.[20,24-31] These studies showed no statistically significant reductions in GI cancer risk in users of metformin compared with users of other NIADs. Furthermore, these studies meet methodological standards due to a time-varying definition of exposure to metformin and because metformin exposure in this study minimizes exposure misclassification and time-related bias. In addition, the results of our study will support future meta-analyses on the risk of GI cancer with use of metformin, and will help draw a firmer conclusion on metformin's chemoprotective effects.

The observed increased risk of pancreatic cancer in current users of metformin plus insulin and plus a SU derivative might be explained by the potential mitogenic effects of insulin and SU, as insulin secretagogues. A recent meta-analysis of observational studies reported an increased risk of pancreatic cancer with use of insulin versus NIADs.[32] However, the authors advised cautious interpretation of their results as they had identified various methodological issues such as confounding by indication and time-related bias in multiple included studies.[32] Bodmer and colleagues have reported an almost doubled risk of pancreatic cancer in users of SU (Adjusted OR 1.90; 95% CI, 1.32-2.74).[10]. However, also with respect to SU, studies on cancer risk have reported contrasting results.[33] In addition, the increased risk of pancreatic cancer in these subgroups of current metformin use may be explained by protopathic bias. It is possible that SU or insulins were added to metformin treatment as a result of disturbances in glucose homeostasis by an emerging pancreatic cancer.

Meta-analyses of observational studies on metformin and cancer risk have presented mixed results for various GI cancers, possibly due to the high heterogeneity among included studies (e. g., in definition of T2DM, type of database, geographic region). [33-40] Meta-analyses on metformin and cancer risk often combine results of observational studies with different types of exposure definitions to metformin, which potentially cause varying amounts of exposure misclassification and time-related bias. Future meta-

analyses on the risk of cancer with use of metformin would benefit from an in-depth description of possible biases and confounding in all included studies, and by performing stratified analyses including only studies with a low level of confounding and bias. In fact, Gandini and colleagues performed a systematic review and meta-analysis of observational studies on metformin and cancer risk with emphasis on studies controlling for confounding by body mass index (BMI) and for time-related biases.[41] Of the 47 included studies, only 18 were deemed to not have time-related biases. Regarding GI cancer risk, only the risk of colorectal cancer remained slightly decreased when analysing studies without time-related bias [N=3; summary relative risk (SRR), 0.92; 95% CI, 0.85-0.98]. Albeit, this decreased risk was not observed when analysing studies that adjusted for BMI (N=6). For liver and pancreatic cancer, no statistically significant decreased risks were found (SRR 0.77, 95% CI, 0.38-1.55 and 0.65, 95% CI, 0.39-1.08, respectively).

Certain limitations of our study merit discussion. First, it is possible that the results are not without any residual confounding due to our inability to correct for lifestyle factors (e.g., obesity, alcohol use, smoking status, and physical activity), diabetes severity (HbA1c), dietary habits, and the presence of unmeasured comorbidities (e.g., gastro-oesophageal reflux disease, chronic liver disease, or chronic pancreatitis). Second, a lack of statistical power existed for some cancer sites, such as liver cancer, and biliary tract cancer, especially in the sensitivity analyses wherein a new-user cohort was used. This resulted in a limited ability to statistically adjust for confounders in the multivariate analyses. Third, confounding by indication could have influenced the results, which we tried to minimize by including a cohort of ADD users only. Metformin is prescribed more readily to obese diabetic patients, as it may contribute to weight loss. In turn, obesity and its proxy indicator, high BMI, are closely linked to GI cancer risk.[42] Furthermore, although we compared metformin use with the use of other NIADs, the majority of other NIAD users was comprised of SU users with or without other NIADs (excluding metformin). Fourth, most GI cancers take decades to form, and the average follow-up time per person was 4.9 years. It is possible that the null results found in our study may be explained by the fact that most cancers were already present when patients started using metformin. Yet, we also do not know if metformin use may be able to slow down tumour progression, thereby delaying its diagnosis. Lastly, statistically significant inverse associations were found for GI cancer risk in past other NIAD users. The reasons, however, for becoming a past other NIAD user may vary greatly (e.g., start of insulin monotherapy or missing data due to a patient switching to a pharmacy outside the PHARMO catchment area). Therefore, the group of "past other NIAD use" is a very heterogeneous group, and no valid conclusions can be drawn from the point estimates in this group.

One of the major strengths of this study was the availability of complete and longitudinal drug dispensing data from PHARMO Database Network, which allowed us to model drug exposure during follow-up in a time-varying way. Furthermore, these drug dispensing data are derived directly from community pharmacies in the overlapping E-NCR-PHARMO region, with each dispensing being either picked up by the patient or directly delivered to the patient's address. Therefore, these data come very close to actual drug intake by the patient. In addition, cancer data from the NCR are known to contain high-quality data over a wide range of cancers and cancer characteristics, which guarantees a high level of cancer ascertainment. Furthermore, to account for prevalent user bias, we repeated the analyses in incident NIAD users. Inclusion of prevalent users in the main analyses could potentially introduce two biases. First, prevalent users probably have a survival benefit over incident users, as they are survivors of the early phase of therapy and make up a "survivor cohort" that generally consists of healthier patients. Secondly, prevalent drug use might alter the levels of risk factors (e.g., obesity, insulin resistance) over time, causing these risk factors to lose their confounding effect.[17]

In summary, we found that in current metformin users, the risks of GI cancer were not significantly different from current other NIAD users. Our data add to the evidence of recent publications and highlight that methodological standards for drug exposure definitions should be met in observational studies. Future meta-analyses will benefit from an in-depth description of possible (time-related) biases and confounding factors in all included studies, and by performing stratified analyses by studies with a low level of confounding and bias.

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Table S5.1 Hazard ratio of gastrointestinal cancer overall in current metformin users compared with current other NIAD users, by sex.

	Prevalent-user design						New-user design					
Exposure category	**Events**	**IR**	**HR[a]**	**95% CI**	**HR[b]**	**95% CI**	**Events**	**IR**	**HR[a]**	**95% CI**	**HR[b]**	**95% CI**
Men												
Current other NIADs[c]	123	554	Ref.	-	Ref.	-	63	585	Ref.	-	Ref.	-
Current metformin	377	453	0.92	0.74-1.15	0.94	0.75-1.18	221	455	0.98	0.72-1.34	1.01	0.74-1.38
Past metformin	101	405	0.84	0.64-1.11	0.81	0.61-1.08	67	479	1.05	0.72-1.51	0.96	0.66-1.40
Past other NIADs	24	328	0.64*	0.41-0.99	0.60*	0.38-0.93	9	239	0.47*	0.23-0.94	0.42*	0.21-0.85
Women												
Current other NIADs[c]	91	370	Ref.	-	Ref.	-	57	527	Ref.	-	Ref.	-
Current metformin	247	298	0.98	0.76-1.27	0.98	0.76-1.27	140	320	0.82	0.58-1.14	0.82	0.59-1.15
Past metformin	93	324	1.08	0.79-1.46	0.93	0.68-1.27	48	320	0.83	0.55-1.24	0.77	0.51-1.17
Past other NIADs	20	241	0.70	0.43-1.13	0.62	0.38-1.00	7	164	0.35*	0.16-0.76	0.33*	0.15-0.72

[a]Age adjusted. [b]Adjusted for age, sex, use of statins, insulin, history of hospitalization, duration of diabetes, and year of start of follow-up. [c]Excluding metformin. *Statistically significant with p<0.05. CI: confidence interval; HR: hazard ratio; IR: incidence rate per 100,000 person-years; NIADs: non-insulin anti-diabetic drugs.

Table S5.2 Site-specific hazard ratios of gastrointestinal cancer in current metformin users compared with current other NIAD users, by sex

Cancer site	Oesophagus (N=84)			Stomach (N=108)			Liver (N=27)			Biliary tract (N=40)			Pancreas (N=175)			Colorectum (N=637)		
Exposure category	**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**		**N**	**HR[a] (95% CI)**	
Men																		
Current other NIADs[b]	14	Ref.		13	Ref.		<5	Ref.		5	Ref.		19	Ref.		7 0	Ref.	
Current metformin	40	0.88	0.45-1.74	34	0.82	0.41-1.63	14	2.36	0.48-11.51	8	1.01	0.30-3.42	57	0.88	0.49-1.55	221	0.93	0.69-1.25
Past metformin	11	0.95	0.40-2.25	11	0.91	0.38-2.15	<5	0.58	0.05-6.82	6	2.17	0.60-7.89	18	0.75	0.37-1.53	56	0.77	0.53-1.13
Past other NIADs	<5	0.84	0.24-2.96	3	0.76	0.21-2.72	<5	1.79	0.16-20.09	0	-	-	<5	0.42	0.12-1.45	14	0.60	0.34-1.07
Women																		
Current other NIADs[b]	<5	Ref.		9	Ref.		<5	Ref.		<5	Ref.		13	Ref.		62	Ref.	
Current metformin	8	1.07	0.22-5.32	27	1.41	0.63-3.16	5	1.55	0.17-14.03	12	1.76	0.53-5.83	48	1.95	1.01-3.76	147	0.82	0.60-1.13
Past metformin	<5	1.38	0.23-8.14	9	0.99	0.36-2.69	<5	1.78	0.15-20.62	5	1.84	0.47-7.30	17	1.82	0.84-3.95	13	0.75	0.50-1.11
Past other NIADs	<5	2.39	0.32-17.51	<5	0.60	0.13-2.83	<5	2.76	0.17-44.55	0	-	-	0	-	-	54	0.57	0.31-1.05

[a] Adjusted for age, use of statins (oesophageal cancer, gastric cancer, biliary tract cancer, pancreatic cancer), proton pump inhibitors (oesophageal cancer, gastric cancer), aspirin (gastric cancer, pancreatic cancer), anti-hypertensives (oesophageal cancer, pancreatic cancer), helicobacter pylori eradication therapy (gastric cancer), insulin (oesophageal cancer, gastric cancer, pancreatic cancer, colorectal cancer), history of hospitalization (oesophageal cancer, gastric cancer, liver cancer, biliary tract cancer, pancreatic cancer, colorectal cancer), duration of diabetes (gastric cancer, pancreatic cancer, colorectal cancer), and year of start of follow-up (gastric cancer, pancreatic cancer, colorectal cancer). [b]Excluding metformin. *Statistically significant with p<0.05. CI: confidence interval; HR: hazard ratio; NIADs: non-insulin anti-diabetic drugs.

Chapter 6

Use of incretin agents and risk of acute and chronic pancreatitis: A population-based cohort study

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Abstract

Aim

To determine the association between the use of incretin agents (dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 receptor agonists) for the treatment of type 2 diabetes mellitus (T2DM) and the risk of any, acute and chronic pancreatitis.

Research design and methods

A population-based cohort study was conducted using data from the UK Clinical Practice Research Datalink (CPRD 2007-2012). A total of 182,428 adult patients with ≥1 non-insulin antidiabetic drug (NIAD) prescription were matched to control subjects without diabetes. Cox regression was used to estimate adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) of pancreatitis in incretin-users (N=28,370) compared with controls and with other NIAD users. Adjustments were made for lifestyle, disease and drug history. In a sensitivity analysis, a new-user design was used.

Results

Current incretin users had a 1.5-fold increased risk of any pancreatitis compared with NIAD users (adjusted HR 1.47, 95% CI 1.06-2.04). In incident current incretin users the risk of any and acute pancreatitis was increased 2.1- and 2.0-fold compared with NIAD users (adjusted HR 2.12, 95% CI 1.31-3.43 and adjusted HR 1.96, 95% CI 1.13-3.41), whereas there was no increased risk found for chronic pancreatitis.

Conclusions

Incretin use was associated with an increased risk of any pancreatitis. Moreover, risk of any and acute pancreatitis was higher when applying a new-user design. We were not able to detect an association with chronic pancreatitis, but the number in this subgroup was small.

Introduction

Type 2 diabetes mellitus (T2DM) has become a major threat to human health. Almost 90% of patients with T2DM fail to achieve target values for glucose, lipids and blood pressure while treated with non-insulin antidiabetic drugs (NIADs) or insulin.[1] Incretin agents or incretin-based therapies (glucagon-like peptide-1 receptor agonists [GLP-1RAs], such as exenatide or liraglutide, and dipeptidyl peptidase-4 [DPP-4] inhibitors, such as saxagliptin, linagliptin, vildagliptin or sitagliptin) are new therapeutic agents for the treatment of T2DM. Incretin-based therapies have an antihyperglycaemic effect, while promoting weight loss with a minimal risk of hypoglycaemia.[2] Yet, in recent years, evidence has become available that pancreatitis might be an important side effect.[3]

The glucagon-like peptide 1 (GLP-1) receptors are expressed in pancreatic islet β-cells as well as other cell types. They are directly stimulated by GLP-1RAs and indirectly stimulated by DPP-4 inhibitors through the increase in the body's GLP-1 concentration by inhibition of DPP-4.[4] GLP-1 receptor stimulation may lead to overgrowth of the cells that cover the smaller ducts, resulting in hyperplasia, an increase in pancreatic weight, duct occlusion, back pressure and ultimately acute or chronic pancreatic inflammation.[5-7] Pancreatitis is a serious condition, often leading to hospitalization, diminished quality of life and even death.[8] Furthermore, there is a spectrum of pancreatitis, often starting with one attack of pancreatitis, which leads to recurrent pancreatitis in some patients (±20%-30%) and progresses to chronic pancreatitis in others (±10%).[8,9]

Recent literature shows limited and conflicting evidence for an association between incretin-based therapy and risk of acute pancreatitis.[10] Spontaneous adverse event reporting systems have detected cases of pancreatitis in incretin users.[11] One observational study found that current use of sitagliptin or exenatide was significantly associated with risk of hospitalization for acute pancreatitis[12]; however, a systematic review and meta-analysis, including 9 studies, with >1.3 million individuals and an average follow-up of 0.7 to 1.4 years, found that incretin-based therapy did not increase the risk of pancreatitis.[13] Multiple observational studies have assessed the association between incretin-based therapy and pancreatitis.[14-17] Given the controversy, the European Medicines Agency and the US Food and Drug Administration have called for additional studies.[18-20]

Furthermore, in contrast to the risk of acute pancreatitis, the risk of chronic pancreatitis with incretin use has not been investigated in an observational setting. The aim of the present study, therefore, was to evaluate the association between incretin use and the risk of any, acute and chronic pancreatitis in a population-based cohort study.

Research design and methods

Data for this study were obtained from the UK Clinical Practice Research Datalink (CPRD; www.CPRD.com), previously known as the General Practice Research Database. The CPRD contains computerized medical records of 625 primary care practices in the UK, representing 6.9% of the population.[21] The data recorded in the CPRD include demographic information, prescription details, clinical events, preventive care provided, specialist referrals, hospital admissions and major outcomes since 1987. Previous studies using CPRD data have shown a high validity concerning wide ranges of diseases, including acute and chronic pancreatitis.[15,22,23]

We conducted a population-based cohort study, largely according to methods that have been described previously.[24] All patients aged ≥18 years at start of follow-up, with ≥1 NIAD prescription during the period of valid data collection, were included in the study population. The study period started on June 13, 2007 (date of first recorded prescription of an incretin in CPRD) and ended on August 31, 2012. The index date was defined as the date of first NIAD prescription after the practice had started to contribute data delivery to CPRD.

Each NIAD user was matched by sex, year of birth (within 5 years) and practice to one control patient who had never received prescriptions of NIADs or insulin during follow-up. The index date of each control patient was set to the index date of his/her matched NIAD user.

For NIAD users, follow-up time was divided into intervals based on their NIAD (and incretin) prescriptions; that is, for every prescription, a new interval was created. Exposure to an NIAD was defined as follows: after a washout period of 90 days, an interval was classified as "past NIAD use," until the end of follow-up or a new prescription of an antidiabetic drug, whichever came first. Otherwise an interval was classified as "current NIAD use." For control patients, the follow-up was divided into 90-day intervals. Each patient was followed from the index date up to the end of data collection, the date of transfer out of the practice area, the patient's death, or the earliest record of any, acute or chronic pancreatitis; that is, the outcome of interest, whichever came first.

NIAD users could move between current and past exposure over time. Current NIAD use was further stratified by the exposure status to incretin-based therapy and other non-incretin NIADs. Incretin use was further stratified by current GLP-1RA use and DPP-4 inhibitor use. Current, recent and past incretin use were defined as GLP-1RA/ DPP-4 inhibitor use 0 to 90, 91 to 180 and >180 days prior to start of an interval, respectively. Patients could move between current, recent and past use. To evaluate the effect of cumulative exposure to incretin-based therapy, a duration of incretin use analysis was

performed. Current use was stratified by the number of incretin prescriptions ever before (in the UK, a single incretin prescription is generally issued every 28 days in case of chronic use). The following incretin-based therapy was recorded in the CPRD and included in this study: exenatide and liraglutide (GLP-1RAs) and sitagliptin, vildagliptin, saxagliptin and linagliptin (DPP-4 inhibitors).

Any, acute and chronic pancreatitis were classified by the use of read codes that were reviewed by a gastroenterologist (Y.K.). The group "any pancreatitis" included read codes for acute and chronic pancreatitis, as well as read codes for pancreatitis not otherwise specified. For the outcome "any pancreatitis," all patients with a history of pancreatitis, either acute or chronic, were excluded. For acute pancreatitis, all patients with a history of acute pancreatitis were excluded, and for chronic pancreatitis, all patients with a history of chronic pancreatitis were excluded (Figure 6.1). For all studied outcomes, patients with polycystic ovaries or polycystic ovarian syndrome prior to start of follow-up were excluded because metformin may be used as a treatment for these conditions (Figure 6.1).

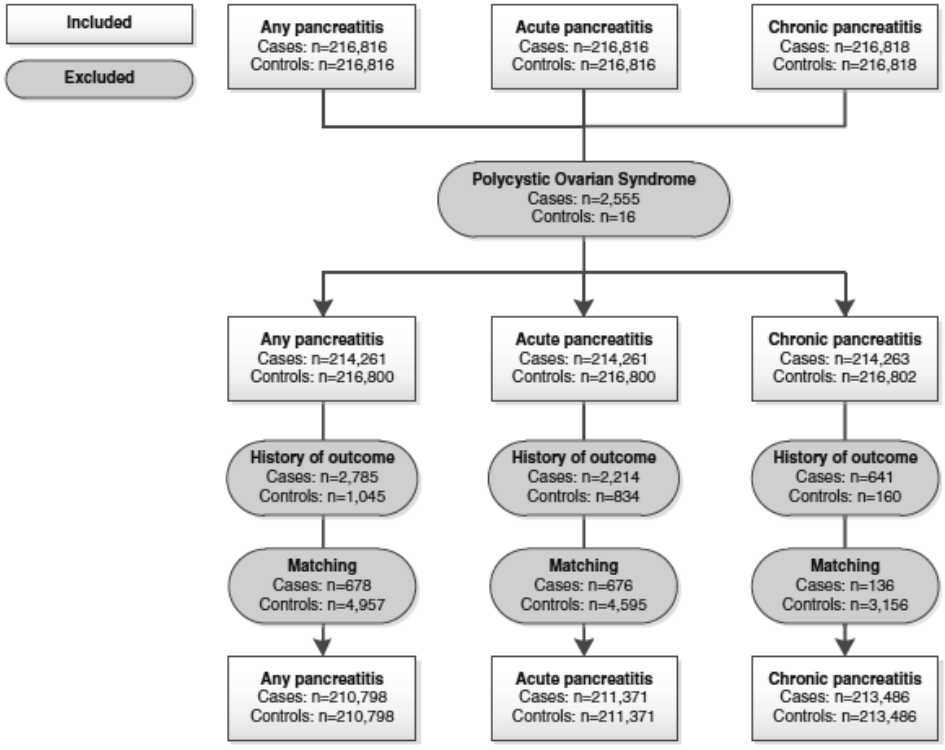

Figure 6.1 Study flow chart, stratified by study outcome.

The presence of potential confounders was assessed by reviewing the computerized medical records for any evidence of these risk factors before the start of an interval. The following potential confounders were considered to be general risk factors and were determined at baseline: sex, body mass index (BMI), smoking status and alcohol use. Other confounders considered in the present study were determined time-dependently (i.e. at the start of each new interval): age, gallstones/endoscopic retrograde cholangiopancreatography procedure or alcoholism.[25-28] Alcoholism was defined as history of specific drugs used to treat alcoholism or a diagnosis of alcoholism. In addition, the following drug prescriptions 6 months prior to the start of an interval were considered to be potential confounders: paracetamol; antibiotics (co-trimoxazole / macrolides / tetracyclines); angiotensin-converting enzyme (ACE) inhibitors; loop diuretics; statins; proton pump inhibitors; and systemic glucocorticoids.[29-31] The following potential confounders for disease severity were considered time-dependently: a history of retinopathy; neuropathy; and the most recent glycated haemoglobin (HbA1c) value in the year preceding the start of an interval.[30-32]

We estimated the adjusted hazard ratio (HR) of any, acute and chronic pancreatitis among current NIAD users vs controls and among current incretin users vs other NIAD users using time-varying Cox proportional hazards regression (SAS 9.2, PHREG procedure). Potential confounders and indicators of disease severity were included in the final model if they independently changed the β coefficient for the exposure of interest by at least 5%, or when a consensus about inclusion existed within the team of researchers, supported by clinical evidence from the literature. A sensitivity analysis repeated the main analysis in a "new-user" design, in which only patients who had started NIADs after June 13, 2007 were included.[33] To be more detailed, we excluded every patient with a NIAD prescription before June 13, 2007, therefore, patients were only included in the new user design if their record was available in the database for ≥1 year and patients who were not receiving any NIADs in the period from 1987 to June 13, 2007. An additional sensitivity analysis was performed to exclude all controls with an HbA1c measurement >7% at baseline, because the HbA1c level might indicate that these controls are actually T2DM patients. Furthermore, an extra sensitivity analysis was performed to compare current incretin use with current thiazolidinedione (TZD) use because TZD users might also be an appropriate comparison group. We also performed an extra sensitivity analysis to investigate the association between current incretin use and chronic pancreatitis when all patients with a history of both acute and chronic pancreatitis were excluded.

This study protocol was approved by the Independent Scientific Advisory Committee for Medicines and Healthcare Products Regulatory Agency database research by protocol number 14_036R5.

Results

Study population

The study population for any pancreatitis consisted of 28,370 incretin users and 182,428 NIAD users, who were matched with 210,798 controls without diabetes (Figure 6.1). For acute pancreatitis we included 211,371 controls without diabetes and for chronic pancreatitis 213,486 controls (Figure 6.1). The mean duration of follow-up was 4.1 years for incretin users, 3.3 years for other NIAD users and 3.3 years for controls without diabetes. The mean duration of actual incretin use was 1.2 years. Among incretin users, 43.7% of all patients were women, and the mean age at index was 58.1 years. At baseline, the average age of incretin users was 4 years younger than users of other NIADs, and incretin users had a higher body mass index (BMI). The severity of the underlying diabetes mellitus was higher among incretin users compared with other NIAD users, as their most recently recorded mean HbA1c measurement in the past 12 months was 8.7% higher. Besides exposure to ACE inhibitors, statins or various antidiabetic drug classes, there were no remarkable differences in history of comorbidities with incretin users vs other NIAD users at baseline (Table 6.1).

Incretin use and risk of pancreatitis compared with controls

Table 6.2 shows that as compared with control subjects without diabetes, current incretin users had a doubled risk of any pancreatitis (adjusted HR 2.01, 95% CI 1.42-2.83). The risk of developing acute pancreatitis was increased 1.6-fold (adjusted HR 1.60, 95% CI 1.09-2.35), while the risk of developing chronic pancreatitis was increased almost 6-fold (adjusted HR 5.82, 95% CI 2.77-12.23). DPP-4 inhibitor users had a higher risk of any pancreatitis than GLP-1RA users (adjusted HR 2.21, 95% CI 1.53-3.20 vs adjusted HR 1.23, 95% CI 0.62-2.43). Furthermore, we observed a 4.6-fold increased risk of any pancreatitis in the youngest age group (18-59 years). The elevated risks were partly explained by the underlying disease: patients with T2DM had a 1.4-fold increased risk of any pancreatitis as compared with controls without diabetes (adjusted HR 1.41, 95% CI 1.18-1.68).

Table 6.1 Baseline characteristics of incretin users, other NIAD users and non-diabetic controls for the outcome any pancreatitis.

Characteristic	Incretin users (n=28370)		Other NIAD (n=182428)		Controles (n=210798)	
	n	%	n	%	n	%
Women	12,410	(43.7)	86,000	(47.1)	98,410	(46.7)
Mean (s.d.), duration of follow-up, years	4.05	(1.5)	3.3	(1.8)	3.3	(1.8)
Age						
Mean (s.d.), at index date, years	58.1	(11.8)	62.4	(14.9)	61.8	(14.6)
18-49	6,746	(23.8)	35,585	(19.5)	42,331	(20.1)
50-59	8,319	(29.3)	34,764	(19.1)	43,083	(20.4)
60-69	8,359	(29.5)	47,650	(26.1)	56,009	(26.6)
>70	4,946	(17.4)	64,429	(35.3)	69,375	(32.9)
BMI at index date						
Mean (s.d.) BMI at index date, kg/m^2	33.6	(7.1)	31.1	(6.5)	26.8	(5.1)
<25.0 kg/m^2	2,180	(7.7)	26,648	(14.6)	72,236	(34.3)
25.0-29.9 kg/m^1	7,243	(25.5)	59,489	(32.6)	74,047	(35.1)
30.0-34.9 kg/m^2	8,462	(29.8)	50,309	(27.6)	29,927	(14.2)
>35.0 kg/m^2	10,293	(36.3)	41,014	(22.5)	12,095	(5.7)
Missing	192	(0.7)	4,968	(2.7)	22,493	(10.7)
Smoking status						
Never	13,897	(49.0)	90,786	(49.8)	110,907	(52.6)
Current	5,935	(20.9)	35,823	(19.6)	43,821	(20.8)
Ex	8,505	(30.0)	54,780	(30.0)	50,490	(24.0)
Missing	33	(0.1)	1,039	(0.6)	5,580	(2.6)
Alcohol use						
Yes	19,297	(28.6)	118,957	(29.0)	38,090	(18.1)
No	8,107	(68.0)	52,935	(65.2)	148,979	(70.7)
Missing	966	(3.4)	10,536	(5.8)	23,729	(11.3)
Alcoholism	533	(1.9)	3,961	(2.2)	4,105	(1.9)
History of comorbidities						
Gallstones	1,465	(5.2)	9,031	(5.0)	6,455	(3.1)
ERCP	162	(0.6)	1,302	(0.7)	897	(0.4)
Retinopathy	3,768	(13.3)	22,184	(12.2)	758	(0.4)
Neuropathy	2,128	(7.5)	14,047	(7.7)	2,492	(1.2)

Table 6.1 (continued)

Characteristic	Incretin users (n=28370)		Other NIAD (n=182428)		Controles (n=210798)	
	n	%	n	%	n	%
Drug use within 6 months						
Metformin	15,099	(53.2)	67,087	(36.8)	n/a	
Sulphonylurea derivatives	8,156	(28.7)	31,812	(17.4)	n/a	
Thiazolidinediones	5,481	(19.3)	13,899	(7.6)	n/a	
Insulin	2,219	(7.8)	19,283	(10.6)	n/a	
Paracetamol	7,170	(25.3)	47,502	(26.0)	38,093	(18.1)
ACE inhibitors	10,826	(38.2)	64,848	(35.5)	30,733	(14.6)
Loop diuretics	2,588	(9.1)	20,809	(11.4)	10,330	(4.9)
Statins	17,114	(60.3)	98,909	(54.2)	44,297	(21.0)
Proton pump inhibitors	5,891	(20.8)	37,850	(20.7)	31,998	(15.2)
Systemic glucocorticoids	1,086	(3.8)	9,387	(5.1)	6,791	(3.2)
HbA1c						
<6%	435	(1.5)	6,490	(3.5)	2,381	(1.1)
6%-6.9	3,345	(11.7)	26,968	(14.7)	2,122	(1.0)
7%-7.9%	5,623	(19.7)	32,869	(17.9)	438	(0.2)
8%-8.9%	3,403	(11.9)	16,986	(9.3)	222	(0.1)
≥9%	5,135	(18.0)	22,084	(12.0)	238	(0.1)
Missing	10,598	(37.1)	78,213	(42.6)	206,748	(97.5)

Values are n, (%) unless otherwise stated. ACE: angiotensin-converting-enzyme; BMI: body mass index; ERCP: endoscopic retrograde cholangioprancreaticography; HbA1c: glycosylated hemoglobin type A1c; NIAD: non-insulin antidiabetic drug; s.d.: standard deviation.

Table 6.2 Risk of pancreatitis in incretin users compared with controls, stratified by age, sex and type of NIAD.

	Any pancreatitis			Acute pancreatitis			Chronic pancreatitis		
	Events (n=797)	IR, per 1000 person-years	Fully adjusted HR[1] (95% CI)	Events (n=640)	IR, per 1000 person-years	Fully adjusted HR[1] (95% CI)	Events (n=196)	IR, per 1000 person-years	Fully adjusted HR[1] (95% CI)
NIAD exposure									
No NIAD use	287	0.40	Reference	246	0.34	Reference	36	0.05	Reference
Past NIAD use	37	0.35	0.88 (0.62-1.25)	25	0.23	0.64 (0.42-0.97)	17	0.16	4.59 (2.55-8.28)
Current NIAD use	473	0.78	1.44 (1.21-1.72)	369	0.61	1.21 (1.00-1.47)	143	0.23	5.06 (3.37-7.61)
Type of NIAD									
Non-incretin	422	0.76	1.41 (1.18-1.68)	329	0.59	1.19 (0.98-1.45)	130	0.23	5.00 (3.32-7.54)
Any incretin	51	1.02	1.88 (1.36-2.61)	40	0.80	1.52 (1.06-2.19)	13	0.26	5.94 (3.01-11.73)
Incretin recency									
Past	-[2]	0.58	0.99 (0.37-2.69)	-[2]	0.29	0.51 (0.13-2.08)	-[2]	0.43	6.39 (1.92-21.32)
Recent	-[2]	1.49	2.59 (0.83-8.15)	-[2]	1.98	3.56 (1.31-9.67)	-[3]	-[3]	-[3]
Current	44	1.07	2.01 (1.42-2.83)	34	0.83	1.60 (1.09-2.35)	10	0.24	5.82 (2.77-12.23)
Type of incretin									
Current GLP-1RA	9	0.81	1.23 (0.62-2.43)	8	0.72	1.25 (0.60-2.58)	-[2]	0.09	2.56 (0.34-19.20)
Current DPP-4 inhibitor	35	1.19	2.21 (1.53-3.20)	26	0.88	1.78 (1.16-2.72)	9	0.30	6.96 (3.24-14.95)
Sex[4]									
Men	26	1.11	2.06 (1.31-3.22)	19	0.81	1.49 (0.89-2.50)	6	0.25	6.14 (2.36-15.97)
Women	18	1.03	1.96 (1.15-3.33)	15	0.85	1.76 (0.98-3.15)	-[2]	0.22	5.26 (1.62-17.11)
Age[5]									
18-49 years	10	1.49	4.58 (1.95-10.75)	7	1.04	2.84 (1.07-7.59)	-[2]	0.44	14.34 (3.08-66.74)
50-59 years	14	1.26	5.55 (2.70-11.42)	13	1.17	5.34 (2.45-11.65)	-[2]	0.09	2.28 (0.26-20.02)
60-69 years	11	0.83	1.07 (0.55-2.11)	8	0.59	0.89 (0.41-1.94)	-[2]	0.22	2.60 (0.67-10.14)
≥70 years	9	0.93	1.37 (0.68-2.75)	6	0.62	0.92 (0.40-2.13)	-[2]	0.31	9.41 (2.51-35.20)

[1] Adjusted for age, gender, BMI, smoking status, alcohol use, history of alcoholism, neuropathy, retinopathy, gallstones/endoscopic retrograde cholangiopancreaticography procedures and the use of ACE inhibitors, loop diuretics or proton pump inhibitors in the previous 6 months; [2] Number of events in the group was <5; [3] No estimation of IR or HR possible because there were zero events in group; [4] Compared with controls of the same gender; [5] Compared with controls of the same age category. CI: confidence interval; DPP-4I: dipeptidyl peptidase-4 inhibitor; GLP-1RA: glucagon-like peptide-1 receptor agonist; HR: hazard ratio; IR: incidence rate; NIAD: non-insuline antidiabetic drug.

Incretin use and risk of pancreatitis compared with other NIAD use

To reduce confounding by indication, incretin users were compared with users of other NIADs (Table 6.3). Results showed a statistically significant 1.5-fold increased risk of any pancreatitis among current incretin users (HR 1.47, 95% CI 1.06-2.04), while no statistically significant association was found for the acute and chronic pancreatitis group (HR 1.42, 95% CI 0.98-2.06 and HR 0.87, 95% CI 0.45-1.69, respectively). The statistical adjustment for proxy indicators of disease severity and general risk factors did not substantially change the associations (Table S6.2). Similar to the results in Table 6.2, the risk of pancreatitis was higher among younger patients (age 18-59 years), those with a BMI <25 kg/m2, or DPP-4 inhibitor users compared with other NIAD users. No trend was observed in the duration-of-use analysis regarding the risk of pancreatitis.

Sensitivity analysis

Table 6.4 shows a sensitivity analysis with a new-user design, in which the cohort was restricted to starters of NIADs (including patients using incretin-based therapy). A statistically significant 2-fold risk of any pancreatitis was found in current incretin users vs other NIAD users (adjusted HR 2.12, 95% CI 1.31-3.43). This was mainly explained by the risk of acute pancreatitis (adjusted HR 1.96, 95% CI 1.13-3.41). The risk of acute and any pancreatitis was highest in patients who had been prescribed up to 150 to 270 days of incretin-based therapy (5-9 prescriptions), whereas there was no significant elevated risk with short (<5 prescriptions) and long-term use (≥10 prescriptions). In the extra sensitivity analysis in which controls with an HbA1c >7% at baseline were excluded, we found that current incretin use was still associated with any pancreatitis (adjusted HR 2.01, 95% CI 1.42-2.83). In the extra sensitivity analysis in which TZD users were used as a comparison group for the incretin users, we found that current incretin use was still associated with any pancreatitis (adjusted HR 1.59, 95% CI 1.05-2.41). In the sensitivity analysis in which all patients with a history of both acute and chronic pancreatitis were excluded, we found that current incretin use was still associated with chronic pancreatitis (adjusted HR 4.73, 95% CI 2.97-7.54).

Table 6.3 Risk of pancreatitis in incretin users compared with other NIAD users.

	Any pancreatitis			Acute pancreatitis			Chronic pancreatitis		
	Events (n=510)	IR, per 1000 person-years	Fully adjusted HR[1] (95% CI)	Events (n=394)	IR, per 1000 person-years	Fully adjusted HR[2] (95% CI)	Events (n=160)	IR, per 1000 person-years	Fully adjusted HR[3] (95% CI)
NIAD exposure									
Current NIAD use	422	0.76	Reference	329	0.59	Reference	130	0.23	Reference
Past NIAD use	37	0.35	0.50 (0.34-0.73)	25	0.26	0.44 (0.29-0.50)	17	0.16	0.65 (0.37-1.13)
Any incretin	51	1.02	1.35 (0.99-1.85)	40	0.26	1.36 (0.96-1.93)	13	0.26	0.90 (0.50-1.64)
Incretin recency									
Past	-[4]	0.58	0.67 (0.25-1.80)	-[4]	0.29	0.47 (0.12-1.89)	-[4]	0.43	1.04 (0.33-3.33)
Recent	-[4]	1.49	1.74 (0.55-5.47)	-[4]	1.98	3.23 (1.19-8.73)	-[5]	-[5]	-[5]
Current	44	1.07	1.47 (1.06-2.04)	34	0.83	1.42 (0.98-2.06)	10	0.24	0.87 (0.45-1.69)
Type of incretin									
Current GLP-1RA	9	0.81	1.08 (0.56-2.10)	8	0.72	1.11 (0.54-2.26)	-[4]	0.09	0.29 (1.04-2.12)
Current DPP-4 inhibitor	35	1.19	1.68 (1.18-2.39)	26	0.88	1.59 (1.05-2.40)	9	0.30	1.14 (0.57-2.28)
Sex[6]									
Men	26	1.11	1.42 (0.93-2.19)	19	0.81	1.32 (0.81-2.17)	6	0.25	0.81 (0.35-1.91)
Women	18	1.02	1.56 (0.93-2.60)	15	0.85	1.56 (0.89-2.74)	-[4]	0.22	0.97 (0.34-2.78)
Age[7]									
18-49 years	10	1.49	2.86 (1.36-6.02)	7	1.04	2.53 (1.07-5.99)	-[4]	0.44	1.69 (0.49-5.88)
50-59 years	14	1.26	2.12 (1.13-3.97)	13	1.17	2.41 (1.25-4.65)	-[4]	0.09	0.22 (0.03-1.65)
60-69 years	11	0.81	1.03 (0.54-1.97)	8	0.59	0.95 (0.45-2.01)	-[4]	0.22	0.92 (0.27-3.17)
≥70 years	9	0.93	1.05 (0.52-2.09)	6	0.62	0.92 (0.40-2.12)	-[4]	0.31	1.36 (0.41-4.53)
BMI									
<25 kg/m^2	7	2.58	3.65 (1.71-7.77)	-[4]	1.46	2.71 (0.97-7.58)	5	1.82	6.08 (2.45-15.08)
25-30 kg/m^2	5	0.5	0.72 (0.29-1.74)	-[4]	0.30	0.63 (0.20-1,98)	-[4]	0.20	0.63 (0.16-2.59)
30-35 kg/m^2	12	0.96	1.32 (0.73-2.36)	11	0.88	1.55 (0.82-2.91)	-[4]	0.11	0.32 (0.19-0.52)
≥35.0 kg/m^2	20	1.27	1.70 (1.07-2.69)	16	1.02	1.53 (0.89-2.63)	-[5]	-[5]	-[5]
Number of prescriptions									
0-6	21	1.30	1.75 (1.12-2.74)	17	1.05	1.84 (1.11-3.03)	-[4]	0.25	0.84 (0.31-2.29)
7-12	7	0.67	0.92 (0.43-1.95)	6	0.57	0.99 (0.44-2.23)	-[4]	0.19	0.71 (0.17-2.88)
≥13	16	1.11	1.50 (0.90-2.50)	11	0.76	1.27 (0.69-2.36)	-[4]	0.27	1.03 (0.37-2.83)

[1] Adjusted for age, gender, HbA1c, BMI, smoking status, alcohol use, history of alcoholism, neuropathy, retinopathy, gallstones/endoscopic retrograde cholangiopancreatography procedures and the use of ACE inhibitors, loop diuretics or proton pump inhibitors in the previous 6 months; [2] Adjusted for age, gender, HbA1c, BMI, smoking status, alcohol use, history of alcoholism, retinopathy, neuropathy, gallstones/endoscopic retrograde cholangiopancreatography procedure, and the use of proton pump inhibitors in the previous 6 months; [3] Adjusted for age, gender, HbA1c, alcohol use, history of alcoholism and the use of ACE inhibitors or loop diuretics in the previous 6 months; [4] Number of events in the grpup was <5; [5] No estimation of IR or HR possible because there were zero events in group; [6] Compared with controls of the same gender; 7 Compared with controls in the same age category. adj: adjusted; CI: confidence interval; DPP-4I: dipeptidyl peptidase-4 inhibitor; GLP-1RA: glucagon-like peptide-1 receptor agonist; HR: hazard ratio; IR: incidence rate, NIAD: non-insulin anti diabetic drug.

Table 6.4 Risk of pancreatitis in incretin users compared with other NIAD users, by type of pancreatitis, new user design (sensitivity analysis).

	Any pancreatitis			Acute pancreatitis			Chronic pancreatitis		
	Events (n=186)	IR, per 1000 person-years	Fully adjusted HR[1] (95% CI)	Events (n=143)	IR, per 1000 person-years	Fully adjusted HR[2] (95% CI)	Events (n=60)	IR, per 1000 person-years	Fully adjusted HR[3] (95% CI)
NIAD exposure									
Current NIAD use	151	0.73	Reference	1.18	0.57	Reference	47	0.23	Reference
Past NIAD use	13	0.43	0.73 (0.41-1.30)	8	0.26	0.58 (0.28-1.20)	8	0.26	1.13 (0.52-2.43)
Any incretin	22	1.26	1.96 (1.23-3.13)	17	0.97	1.87 (1.11-3.17)	5	0.28	1.32 (0.52-3.37)
Incretin recency									
Past	-[4]	0.51	0.75 (0.10-5.37)	-[4]	0.51	0.93 (0.13-6.70)	-[4]	0.38	1.75 (0.24-12.82)
Recent	-[4]	1.53	2.25 (0.31-16.16)	-[4]	1.52	2.75 (0.38-19.78)	-[5]	-[5]	-[5]
Current	20	1.34	2.12 (1.31-3.43)	15	1.00	1.96 (1.13-3.41)	-[4]	0.27	1.24 (0.44-3.50)
Number of prescriptions									
<5	6	1.25	2.15 (0.94-4.93)	-[4]	0.83	1.96 (0.71-5.39)	-[5]	-[5]	-[5]
5-9	7	1.71	3.06 (1.41-6.61)	6	1.46	3.44 (1.49-7.93)	-[5]	-[5]	-[5]
≥10	7	1.17	2.11 (0.97-4.58)	5	0.83	1.97 (0.79-4.92)	-[5]	-[5]	-[5]

[1] Adjusted for age, gender, alcohol use, history of alcoholism, neuropathy, retinopathy, smoking status, gallstones/endoscopic retrograde cholangiopancreatography procedures and the use of ACE inhibitors, loop diuretics, paracetamol, systemic glucocorticoids or proton pump inhibitors in the previous 6 months; [2] Adjusted for age, gender, alcohol use, history of alcoholism, smoking status, gallstones/endoscopic retrograde cholangiopancreatography procedure, and the use of ACE-inhibitors and loop diuretics or proton pump inhibitors in the previous 6 months; [3] Adjusted for age, gender, alcohol use and history of alcoholism; [4] Number of events in the group was <5; [5] No estimation of IR or HR possible because there were zero events in group. adj: adjusted; CI: confidence interval; HR: hazard ratio; IR: incidence rate, NIAD: non-insulin anti diabetic drug.

Discussion

The present study found a 1.5-fold statistically significant increased risk of any pancreatitis with current use of incretin-based therapy vs. other NIAD use. The risk of acute pancreatitis was 1.4-fold greater in current incretin users vs. other NIAD users, but this did not reach statistical significance. Furthermore, we were not able to detect an association between chronic pancreatitis and incretin use, but numbers in this subgroup were small. Interestingly, the increased risk of acute pancreatitis remained statistically significant in current users of DPP-4 inhibitors only, suggesting that differences in the pharmacodynamics properties of these agents are important for the incretin–pancreatitis link.

The present results are not consistent with the results of the studies by Elashoff et al.[3], Singh et al.[12] and Roshanov and Dennis[34] regarding the risk of acute pancreatitis with incretin use. In a case–control study, Singh et al. found that current use of sitagliptin or exenatide 30 days before the study outcome vs non-use was significantly associated with hospitalization for acute pancreatitis (odds ratio 2.24, 95% CI 1.36-3.68).[12] Elashoff et al. showed that pancreatitis was significantly more often reported among patients treated with sitagliptin or exenatide as compared with users of other antidiabetic therapies; however, that study only provided hypothesis-generating evidence as it was based on data from the US Food and Drug Administration's spontaneous adverse event reporting system.[3] The meta-analysis of large randomized clinical trials by Roshanov and Dennis found an 82% increase in the odds ratio of acute pancreatitis with the use of incretin-based therapy as compared with usual care (95% CI 1.17-2.82).[34]

Several previous studies have shown results consistent with the present findings regarding the risk of acute pancreatitis with incretin use, identifying no statistically significant increased risk of acute pancreatitis for incretin use.[13-15] A meta-analysis of 6 cohort and 2 case–control studies found no effect on the occurrence of acute pancreatitis (odds ratio 1.03, 95% CI 0.87-1.20).[13] That meta-analysis included a previous CPRD cohort study by Faillie et al., finding no effect on acute pancreatitis occurrence.[15] Furthermore, a large cohort study (n=1,532,513, mean follow-up 2.3 years) which included data from the CPRD did not find an association between current use of incretin-based drugs and acute pancreatitis.[35] A large systematic review and meta-analysis of randomized and non-randomized studies did not suggest an increased risk of acute pancreatitis with the use of incretin-based therapy.[14] In both the SAVOR (n=16 492, median follow-up 2.1 years) and EXAMINE (n=5380, median follow-up 18 months) cardiovascular outcome trials the cases of acute and chronic pancreatitis were similar in the saxagliptin and alogliptin arms as compared with the comparator agent arm.[36,37] The results of observational studies regarding the risk of pancreatitis remain conflicting. We

therefore advise regulatory agencies to consider using observational studies to learn about the methodological factors that influence the aetiology of pancreatitis risk in people with T2DM using incretin-based therapy, rather than confirming whether an association is truly present.[38]

The evidence regarding chronic pancreatitis is scarce and mainly based on in vitro and animal studies.[39-41] Other studies that did find cases of chronic pancreatitis in users of incretin-based therapy were most often post-marketing reports or reports in patients with T2DM aged ≥40 years with a history of a cardiovascular disease.[42] We are the first to report on the risk of chronic pancreatitis in an observational setting, finding no indication that patients with T2DM using incretin-based therapy were more prone to develop chronic pancreatitis. The results should be interpreted with caution, because the number of cases was small and follow-up time might have been too short; most acute pancreatitis events in randomized controlled trials occurred between 6 and 24 months after treatment initiation.[10] Furthermore, we were not able to confirm data from the literature showing a higher risk of chronic pancreatitis among men.[9] It is important to note that chronic pancreatitis is a serious disease, causing significant morbidity and mortality. Two to three decades after diagnosis of chronic pancreatitis, there is a mortality rate of 50%, and thus such patients have shorter survival times than the average population.[8] We have only started to learn about the association between incretin use and chronic pancreatitis, and hope future studies will investigate this in more detail.

In contrast to the study by Li et al., but consistent with the study by Roshanov and Dennis, we found that DPP-4 inhibitor users had a higher risk of any pancreatitis compared with GLP-1RA users.[14,34] There are key pharmacological differences between DPP-4 inhibitors and GLP-1RAs, such as the effect on HbA1c reduction (−0.6% to −1.9% for GLP-1RAs vs. −0.5% to −0.8% for DPP-4 inhibitors) and body weight (reduced for GLP-1RA but neutral for DPP-4 inhibitors).[43] Clinical data suggest that GLP-1RAs improve β-cell function, whereas the effects of DPP-4 inhibitors are less clear.[43] The different effects on β-cell function might contribute to the difference in risk of pancreatitis, but this is very speculative and more studies are needed to investigate this further.

The potential biological mechanisms of incretin agents promoting or enhancing pancreatitis are supported by limited indirect evidence. In animal models, three GLP-1-induced pathways have been proposed; proliferation in b-cells, inhibition of b-cells, and enhanced differentiation of adult stem cells in the ductal pancreatic epithelium. This could lead to chronic pancreatic damage, inflammation of pancreatic acinar and ductal cells, increased formation of dysplastic pancreatic intraepithelial neoplasia (PanIN) lesions and an increase in pancreatic weight.[3,5-7,12,15,19,20,44,45] Furthermore, duct cell proliferation and PanIN lesions might lead to duct occlusion, which could cause back

pressure in the pancreas, stressing the acinar cells to release digestive enzymes with the resulting chronic pancreatitis fostering further development of PanINs and duct cell proliferation.[7,41] By activating both above-mentioned pathways, incretin agents could promote acute pancreatitis and chronic pancreatitis.[7,41] Additionally, it was hypothesized that an incretin-based therapy-induced pancreatitis would mostly occur soon after initiating treatment with these agents (<5 prescriptions); however, based on the duration-of-use analysis it is also possible that a delayed onset of pancreatitis is induced by incretin-based therapy through underlying (cumulative) pathophysiological mechanisms, such as duct cell proliferation leading to inflammation. In the duration-of-use analysis of the prevalent cohort, pancreatitis risk was highest in patients who had been prescribed <7 prescriptions, while in the incident analysis pancreatitis risk was highest in patients who had been prescribed up to 5 to 9 months of incretin-based therapy.[46] The information provided from the duration-of-use analysis should be interpreted with caution because of the small number of events.

It is important to note several limitations of this observational study. True causality cannot be provided. Furthermore, it is likely that our observed associations are not without residual confounding and there might also be residual confounding as a result of adjustment for imperfect variables, such as the missing variables. Residual confounding might also be present because incretin-based therapy is less likely to be prescribed to patients with T2DM who consume alcohol, smoke or have a lower socio-economic status. This could have led to an underestimation in the results; however, it can also be proposed that incretin-based therapy is more likely to be prescribed to alcoholics with T2DM. It is known that alcoholics are more likely to experience hypoglycaemia, causing physicians to be more likely, in turn, to prescribe incretin agents rather than sulphonylurea derivatives. This could have led to overestimation in the results. Moreover, we were not able to correct for the amount of physical exercise. Hypertriglyceridemia, which is indirectly related to a lack of physical exercise, appears to increase the risk of pancreatitis, especially among overweight people.[47] Incretin users might be less physically active than non-incretin users, which could lead to an overestimation of our effect. Also, incretin-based therapy may be prescribed earlier to people with a higher BMI because of the promotion of weight loss and to people with a history of a cardiovascular disease because of the cardiovascular benefits of such therapy.[2] Furthermore, diagnostic bias may have influenced the results. As a result of early warnings of the possible side effects of incretin-based therapy by regulatory agencies, diabetes specialists are likely to have been vigilant for the occurrence of pancreatitis when first prescribing incretin-based therapy. This could have led to overestimation in the results. Lastly, the read codes used in this study for acute, chronic and any pancreatitis have not been validated, therefore, there might be some

misclassification. We expect the misclassification to be non-differential, resulting in an underestimation of the relationship between incretin-based therapy and pancreatitis, which might have led to restricted statistical power.

The present study also has a number of strengths. We were able to adjust statistically for several potentially important confounders, including age, HbA1c, alcoholism and drug use. Also, we were able to show the effect of confounding by indication on the risk of pancreatitis. Furthermore, CPRD data are collected prospectively, eliminating the risk of recall bias. In addition, this study gives the first insights into the risk of chronic pancreatitis in users of incretin-based therapy.

In conclusion, in this first study to report on all types of pancreatitis, it was found that incretin use was associated with an increased risk of any type of pancreatitis, but not with acute or chronic pancreatitis in patients with T2DM; however, the risk of any and acute pancreatitis was higher among users of DPP-4 inhibitors and incident incretin users. Observational studies that assessed the risk of pancreatitis in incretin-based therapy had conflicting results. The complex relationship, methodological challenges and relatively small numbers of exposed patients in published research suggest that we should probably learn more about the methodological factors that influence the aetiology of incretin-induced pancreatitis, rather than to confirm whether an association is truly present.[38]

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Table S6.1 Age- and sex-adjusted hazard rates of pancreatitis in incretin users compared with controls.

	Any pancreatitis			Acute pancreatitis			Chronic pancreatitis		
	Events (n=797)	IR (/1000 py)	Age-sex adj. HR (95% CI)	Events (n=640)	IR (/1000 py)	Age-sex adj. HR (95% CI)	Events (n=196)	IR (/1000 py)	Age-sex adj. HR (95% CI)
NIAD exposure									
No NIAD use	287	0.40	Reference	246	0.34	Reference	36	0.05	Reference
Past NIAD use	37	0.35	0.91 (0.64-1.28)	25	0.23	0.71 (0.47-1.07)	17	0.16	3.15 (1.76-5.62)
Current NIAD use	473	0.78	1.95 (1.69-2.26)	369	0.61	1.78 (1.51-2.09)	143	0.23	4.68 (3.25-6.76)
By type of NIAD									
Non-incretin	422	0.76	1.88 (1.62-2.19)	329	0.59	1.71 (1.45-2.02)	130	0.23	4.65 (3.21-6.74)
Any incretin	51	1.02	2.80 (2.07-3.79)	40	0.80	2.52 (1.80-3.54)	13	0.26	5.02 (2.64-9.54)
By incretin recency									
Past	-[d]	0.58	1.59 (0.59-4.26)	-[d]	0.29	0.91 (0.23-3.65)	-[d]	0.43	6.62 (2.03-21.59)
Recent	-[d]	1.49	4.10 (1.31-12.80)	-[d]	1.98	6.30 (2.35-16.93)	-[c]	-[c]	-[c]
Current	44	1.07	2.94 (2.13-4.05)	34	0.83	2.61 (1.82-3.76)	10	0.24	4.69 (2.31-9.51)
By type of incretin									
Current GLP-1RA	9	0.81	2.32 (1.19-4.51)	26	0.88	2.74 (1.82-4.12)	-[d]	0.09	1.70 (0.23-12.40)
Current DPP-4I	35	1.19	3.22 (2.26-4.59)	8	0.72	2.39 (1.18-4.85)	9	0.30	5.97 (2.85-12.47)
By sex[a]									
Males	26	1.11	2.84 (1.87-4.33)	19	0.81	2.37 (1.46-3.85)	6	0.25	4.75 (1.90-11.88)
Females	18	1.03	3.08 (1.87-5.09)	15	0.85	2.99 (1.73-5.17)	-[d]	0.22	4.66 (1.53-14.17)
By age (years)[b]									
18-49	10	1.49	7.47 (3.54-15.77)	7	1.04	5.88 (2.47-14.03)	-[d]	0.44	10.05 (2.37-42.64)
50-59	14	1.26	5.65 (2.97-10.75)	13	1.17	7.11 (3.56-14.23)	-[d]	0.09	1.45 (0.18-11.82)
60-69	11	0.81	1.93 (1.03-3.64)	8	0.59	1.68 (0.80-3.51)	-[d]	0.22	3.59 (1.00-12.87)
≥70	9	0.93	1.87 (0.95-3.68)	6	0.62	1.36 (0.60-3.09)	-[d]	0.31	7.98 (2.22-28.71)

[a] Compared to controls of the same gender; [b] Compared to controls in the same age category; [c] No estimation of IR or HR possible due to zero number of events in group; [d] Number of events in the group are <5. adj: adjusted; CI: confidence interval; HR: hazard ratio; IR: incidence rate; NIAD: non-insulin antidiabetic drug; PY: person years.

Table S6.2 Age- and sex-adjusted hazard rates of pancreatitis in incretin users compared with other NIAD-users.

	Any Pancreatitis			Acute pancreatitis			Chronic pancreatitis		
	Events (n=510)	IR (/1000 py)	Age/Gender HR (95% CI)	Events (n=394)	IR (/1000 py)	Age/Gender HR (95% CI)	Events (n=160)	IR (/1000 py)	Age/Gender HR (95% CI)
NIAD exposure									
Current NIAD use	422	0.76	Reference	329	0.59	Reference	130	0.23	Reference
Past NIAD use	37	0.35	0.48 (0.34-0.67)	25	0.26	0.41 (0.27-0.61)	17	0.16	0.67 (0.40-1.12)
Any incretin	51	1.02	1.48 (1.10-2.01)	40	0.26	1.46 (1.04-2.05)	13	0.26	1.04 (0.58-1.88)
By incretin recency									
Past	-[d]	0.58	0.85 (0.32-2.29)	-[d]	0.29	0.53 (0.13-2.15)	-[d]	0.43	1.37 (0.43-4.35)
Recent	-[d]	1.49	2.18 (0.70-6.78)	-[d]	1.98	3.63 (1.35-9.76)	-[c]	-[c]	-[c]
Current	44	1.07	1.55 (1.13-2.14)	34	0.83	1.50 (1.05-2.17)	10	0.24	0.98 (0.51-1.88)
By type of incretin									
Current GLP-1ra use	9	0.81	1.08 (0.56-2.10)	8	0.72	1.32 (0.65-2.68)	-[d]	0.09	0.35 (0.05-2.54)
Current DPP-4I use	35	1.19	1.68 (1.18-2.39)	26	0.88	1.61 (1.07-2.42)	9	0.30	1.24 (0.62-2.47)
By sex[a]									
Males	26	1.11	1.50 (0.99-2.28)	19	0.81	1.40 (0.86-2.27)	6	0.25	0.90 (0.39-2.09)
Females	18	1.02	1.63 (0.99-2.68)	15	0.85	1.66 (0.96-2.88)	-[d]	0.22	1.13 (0.40-3.21)
By age (years)[b]									
18-49	10	1.49	2.64 (1.30-5.37)	7	1.04	2.22 (0.96-5.11)	-[d]	0.44	1.42 (0.42-4.85)
50-59	14	1.26	2.05 (1.12-3.75)	13	1.17	2.41 (1.28-4.55)	-[d]	0.09	0.24 (0.03-1.77)
60-69	11	0.81	1.19 (0.63-2.24)	8	0.59	1.05 (0.50-2.21)	-[d]	0.22	1.24 (0.36-4.19)
≥70	9	0.93	1.16 (0.59-2.29)	6	0.62	1.01 (0.44-2.30)	-[d]	0.31	1.57 (0.48-5.19)
By BMI									
<25 kg/m2	7	2.58	3.67 (1.73-7.78)	-[d]	1.46	2.63 (0.98-7.08)	5	1.82	6.61 (2.67-16.34)
25-30 kg/m2	5	0.5	0.71 (0.29-1.72)	-[d]	0.30	0.54 (0.17-1.68)	-[d]	0.20	0.68 (0.17-2.76)
30-35 kg/m2	12	0.96	1.39 (0.78-2.48)	11	0.88	1.60 (0.87-2.94)	-[d]	0.11	0.34 (0.21-0.56)
≥35.0 kg/m2	20	1.27	1.88 (1.19-2.97)	16	1.02	1.88 (1.12-3.13	-[c]	-[c]	-[c]
By number of prescriptions									
0-6	21	1.30	1.65 (1.00-2.72)	17	1.05	1.68 (0.96-2.94)	-[d]	0.25	1.01 (0.37-2.73)
7-12	7	0.67	1.52 (0.85-2.72)	6	0.57	1.65 (0.85-3.22)	-[d]	0.19	0.76 (0.19-3.08)
≥13	16	1.11	1.49 (0.89-2.48)	11	0.76	1.27 (0.70-2.28)	-[d]	0.27	1.10 (0.40-3.03)

[a] Compared to other NIAD-users of the same gender; [b] Compared to other NAID-users in the same age category; [c] No estimation of IR or HR possible due to zero number of events in group; [d] Number of events in the group are <5. adj: adjusted; CI: confidence interval; HR: hazard ratio; IR: incidence rate; NIAD: non-insulin antidiabetic drug; PY: person years.

Chapter 7

Letter to the Editor: Comments on "Use of metformin and risk of kidney cancer in patients with type 2 diabetes", Chin-Hsiao Tseng, Eur J Cancer 2016;52:19-25

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European Journal of Cancer 2016;61:157-8

Dear Editor,

We have read with great interest the article by Chin-Hsiao Tseng, Use of metformin and risk of kidney cancer in patients with type 2 diabetes, which appeared in the European Journal of Cancer issue of January 2016.[1] We have noted, however, that the results of this study may have been affected by selection bias.

Using the Taiwanese National Health Insurance reimbursement database, Tseng performed a retrospective population-based cohort study of incident type 2 diabetic patients who either received metformin as the first antidiabetic drug (ever users of metformin) or other antidiabetic drugs as first treatment without receiving metformin during follow-up (never users of metformin). To estimate the risk of kidney cancer, the author performed a Cox regression analysis and adjusted for imbalance between baseline characteristics by applying inverse probability treatment weighting of the estimated propensity scores (PSs). Subsequently, a hazard ratio (95% confidence interval) of 0.279 (0.254-0.307) was estimated for the risk of kidney cancer in ever users of metformin compared to never users. Unfortunately, the PS approach incorporated in the statistical model cannot completely correct for any selection bias that may have occurred during the formation of the study population. For example, the history of any cancer excluding kidney cancer was 1.4-fold higher in never users of metformin (26.88%) versus ever users of metformin (19.32%).

Based on the methodology section of the paper and the results in Table 2, we believe a significant selection bias may have occurred during the allocation process of individuals to the treatment group of never users of metformin. Patients using other antidiabetic drugs before they start with metformin were excluded from the study population (n=200,785). Therefore, possible follow-up time designated for the group of never users of metformin is wrongfully excluded from the analysis. This can also be seen in Table 2, where the amount of follow-up time in the never users of metformin is much shorter than that in the ever users of metformin (433,005.63 vs. 1,144,982.82 person-years). In addition, this selection bias might partly explain the significant baseline differences as seen in Table 1.

Within the scientific community, there is ongoing debate concerning the protective effect of metformin on cancer development in patients with type 2 diabetes mellitus, with studies showing conflicting results for various cancers. Earlier studies concluded metformin could decrease cancer risk but were afflicted by time-related biases, such as immortal time bias. More recent studies that used methods to avoid these biases reported no effect of metformin use on cancer incidence.[2,3] Based on this, we are currently not convinced that metformin has a clinically relevant protective effect on cancer development. Costly trials based on methodologically inaccurate studies should

also not be encouraged. Therefore, we kindly ask Dr. C.-H. Tseng to reanalyze the risk of kidney cancer in users of metformin compared to non-users of metformin without the currently potential selection bias.

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Chapter 8

High glycaemic burden increases risk of gastrointestinal cancer in patients with type 2 diabetes mellitus; a population-based cohort study in the Netherlands

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In preparation for submission

Abstract

Background

High levels of glycosylated haemoglobin (HbA1c) are associated with an increased gastrointestinal (GI) cancer risk in type 2 diabetic (T2DM) patients. Most studies use single, time-fixed measures of HbA1c as determinant in regression models. We aimed to explore whether multiple high levels of HbA1c over time impacts GI cancer risk in T2DM patients using a novel measure of hyperglycaemia called glycaemic burden (GB).

Methods

A cohort study was performed using the linked database of the Eindhoven area of the Netherlands Cancer Registry (E-NCR) and PHARMO Database Network. All incident patients aged ≥30 years using ≥1 non-insulin anti-diabetic drugs (NIADs) were included. All HbA1c measurements recorded between the first NIAD prescription and the end of follow-up were used to calculate GB. GB was defined based on the extent and duration that HbA1c values exceeded a threshold of 7% (53 mmol/mol) and was expressed as glycaemic burden years (GBY). The association between GBY and GI cancer was analysed using Cox regression, with GBY entered in the regression model as a time-dependent categorical variable (no burden [0 GBY], >0-1.0 GBY, and >1.0 GBY).

Results

285 GI cancers were observed during >60,000 person-years of follow-up. Compared to patients with >0-1.0 GBY, patients without glycaemic burden had a decreased risk of GI cancer (HR 0.71, 95% CI 0.53-0.96), but patients with >1.0 GBY did not have a further increased risk of GI cancer (HR 1.15, 95% CI 0.84-1.57). For hepato-pancreatico-biliary tract (HPB) cancer, however, increased levels of GBY were associated with an increased risk of cancer.

Conclusions

Increased levels of glycaemic burden were associated with an increased risk of GI cancer, especially for HPB cancers. However residual confounding and selection bias may play an important role. Future studies are warranted to further investigate the clinical value of glycaemic burden as determinant of cancer risk.

Introduction

Hyperglycaemia, one of the hallmarks of type 2 diabetes mellitus (T2DM), is thought to play an important pathophysiological role in cancer cell proliferation.[1,2] Due to function-altering mutations in receptor-initiated signalling pathways, cancer cells overcome the normal growth factor-dependent uptake and metabolism of nutrients, particularly of glucose.[3] Previous studies have shown that cancer cells mainly depend on glycolysis for energy.[3,4] This results in a high requirement for glucose, as ATP generation by glycolysis requires more glucose than oxidative phosphorylation (Warburg effect).[1] Furthermore, glucose uptake may exceed cellular demand for survival and proliferation.[3] The possibility that inadequate glycaemic control may facilitate cancer cell proliferation therefore is interesting to explore.[1]

Observational studies have suggested an association between inadequate glycaemic control and the risk of cancer, such as gastrointestinal (GI) cancers.[5,6] However, the evidence for an association between various measures of hyperglycaemia and cancer risk has been inconsistent. In one meta-analysis of epidemiological studies, an increased risk of colorectal and pancreatic cancer was found in groups with the highest compared to those with the lowest ranges of markers of glycaemia (e.g. insulin, glycated haemoglobin [HbA1c], fasting blood glucose).[7] Another meta-analysis of the evidence regarding HbA1c levels and the risk of cancer pointed to an increased risk of GI cancers with HbA1c levels in the diabetic range.[8] However, in a meta-analysis of clinical trials the cancer risk did not seem to be affected by the level of glycaemic control (i.e. randomisation to either standard or intensive glycaemic control).[9]

HbA1c is a proxy-indicator for chronic persistent hyperglycaemia, as one single measurement is considered to reflect the blood glucose concentration over the life span of an erythrocyte, which is approximately three months.[2,10-12] In 2004, Brown et al. suggested a novel HbA1c-based measure of hyperglycaemia called 'glycaemic burden' (GB). This measure is a function of the extent and duration that a patient's HbA1c exceeds a predefined threshold.[13] Such a measure would theoretically capture the cumulative effects of long-term hyperglycaemia that may be missed by individual or mean HbA1c measurements.[14]

Glycaemic burden is considered a proxy indicator for at least two of the proposed biological pathways through which type 2 diabetes mellitus may increase cancer risk, i.e. hyperglycaemia and hyperinsulinaemia. We hypothesize that the risk of GI cancer is increased in patients with higher glycaemic burden. Up to now, this association has not been studied. Therefore, our aim was to investigate the influence of GB on the population-based risk of GI cancer in patients with T2DM.

Methods

Data source

Data for this population-based cohort study were obtained from the PHARMO Database Network and linked at the individual patient level to the Eindhoven area of the Netherlands Cancer Registry (E-NCR; formerly described as Eindhoven Cancer Registry (ECR)). The construct and validity of the data linkage have been described elsewhere.[15]
Data from the Eindhoven area of the NCR, maintained by the Netherlands Comprehensive Cancer Organization, covers a demographic region with approximately 2.4 million inhabitants (~15% of the Dutch population) and no academic hospitals. Trained registration clerks actively collect data on newly diagnosed cancers, patient characteristics, staging and initial treatment from hospital medical records. Vital status is obtained by linkage to the Dutch municipal personal records database.
The PHARMO Database Network is a large, patient-centric data network including patient-linked observational databases designed for drug safety and outcomes research. For this study the Out-patient (community) Pharmacy Database was used, which contains longitudinal drug dispensing records, and included information on dispensing date, dose descriptions, and amount dispensed. All drugs are coded according to their Anatomical Therapeutic Chemical/Defined Daily Dose Classification (ATC/DDD) code.[16]
Both the E-NCR and the PHARMO Database Network are recognized as high quality sources for (pharmaco-)epidemiological research that collect information in overlapping regions in the Netherlands for a period of at least 10 years.[15]

Study design and population

We conducted a cohort study of all patients with incident T2DM, aged 30 years or older, who initiated pharmacologic therapy for diabetes with any type of non-insulin anti-diabetic drug (NIAD; ATC code 'A10B') between January 1, 1998 and December 31, 2011 based on their ATC coded dispensing data. The first NIAD dispensing defined their index date (Figure 8.1). To ensure that patients were incident patients, they had to have at least 1 year recorded data between start of enrolment in the PHARMO Database Network and the index date.
Potential patients with T2DM for whom the first recorded anti-diabetic drug was insulin (ATC code 'A10A') were excluded to prevent misclassification by including patients with type 1 diabetes. Patients who eventually required insulin during their follow-up were retained in the cohort to prevent selection bias by restricting the cohort to patients with less advanced T2DM. Furthermore, patients were excluded if they had a history of GI cancer. In addition, the cohort was restricted to incident T2DM patients who had at least

one recorded HbA1c value during the year before the index date, and at least one or more recorded HbA1c value after the index date to ensure calculation of glycaemic burden.

All patients were followed from the index date until a first ever diagnosis of a GI cancer (C15-C26, death from any cause, end of registration within the PHARMO catchment area, or end of data collection (December 31, 2011), whichever came first.

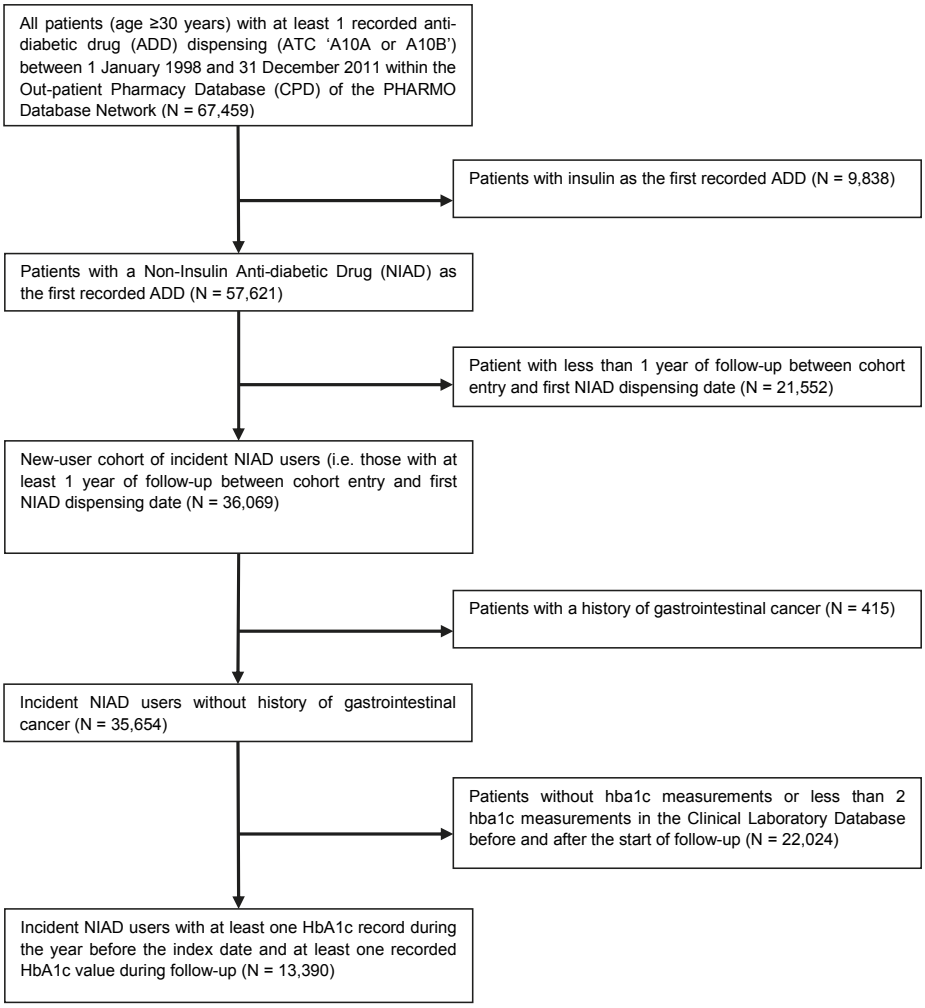

Figure 8.1 Flow-chart of study population. Abbreviations: ADD, anti-diabetic drug; ATC, Anatomical Therapeutic Classification code; CPD, Community Pharmacy Database; Hba1c, glycated hemoglobin; NIAD, Non-Insulin Anti-diabetic Drug.

Outcome

During follow-up, the occurrence of the first GI cancer was obtained from the E-NCR. GI cancers were classified according to the International Classification of Diseases of oncology (ICD-O).[17] These included oesophageal cancer (C15), gastric cancer (C16), small intestinal cancer (C17), colorectal cancer (C18-C20), hepatic cancer (C22), biliary tract cancer (C23: gallbladder, and C24: extrahepatic bile duct cancer), pancreatic cancer (C25), and unspecified GI cancer (C26). GI cancer subsites were defined as 'upper GI cancer' (C15-C17), 'lower GI cancer' (C18-C20), and 'hepato-pancreatico-biliary (HPB-) cancer' (C22-C25).

Glycaemic burden

Follow-up for all patients was divided into time-intervals based on subsequent HbA1c records after the index date. Glycaemic burden was defined as the cumulative amount by which HbA1c exceeded a specified threshold (Figure 8.2). It was calculated as the sum of the differences between a subject's HbA1c value and the threshold. We applied a threshold of 7% (53 mmol/mol) as HbA1c levels of ≥7% serve as marker for inadequate glycaemic control in for clinicians upon which anti-diabetic drug therapy should be changed to reduce the risk of complications of long-term hyperglycaemia over time.[18] All HbA1c values recorded in the Clinical Laboratory Database (CLD) of the PHARMO Database Network between the index date and the end of follow-up were used to calculate cumulative glycaemic burden. We assumed the change between each pair of HbA1c measurements represented a linear function so that the resulting total glycaemic burden approximated the area under the curve measurement. During the last time period the level of HbA1c was assumed to remain constant, if there were no new HbA1c values after the last HbA1c measurement in the CLD and the first ever diagnosis of a GI cancer, death from any cause, end of registration within the PHARMO catchment area, or end of data collection (whichever came first). HbA1c measurements below the 7% threshold did not decrease the cumulative burden. For the statistical analyses, and to account for differences in follow-up, we calculated a yearly estimate of glycaemic burden (glycaemic burden years; GBY), by dividing the cumulative glycaemic burden by the number of years of follow-up at each time interval. The variable GBY was categorized into three categories (0 GBY, >0-1.0 GBY, >1.0 GBY).

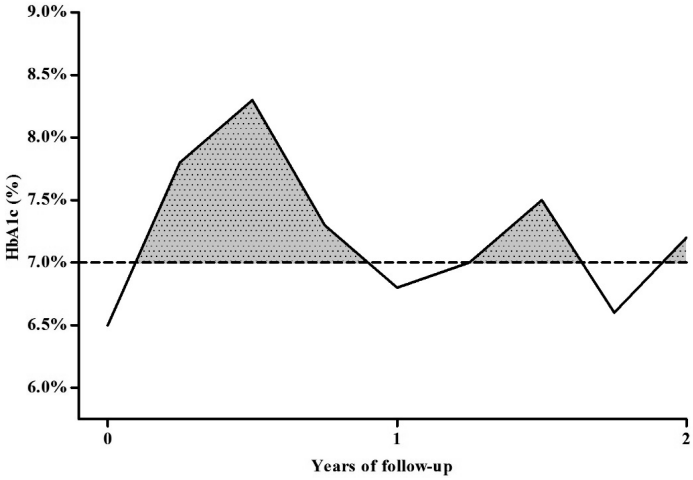

Figure 8.2 Example of glycaemic burden calculation, with glycaemic burden defined as the sum of the differences between a subject's HbA1c values (black line) and a predefined threshold of 7% (53 mmol/mol; dotted black line). The shaded area represents the total glycaemic burden experienced by a hypothetical subject during a follow-up period of two years. Glycaemic burden years (GBY) is calculated by dividing the total glycaemic burden by the amount of follow-up in years.

Covariables

Time-fixed and time-dependent covariables were chosen a-priori based on the current literature and their availability in the E-NCR-PHARMO database. Sex and history of hospitalisation were included as time-fixed covariables. Time-dependent covariables were assessed at each separate time-interval, and included age, duration of T2DM (time since first ever NIAD prescription), exposure to specific antidiabetic drugs suggested to impact cancer risk (metformin, sulfonylureas, insulin), and use of other pharmacologic agents known to impact cancer risk, including non-steroidal anti-inflammatory drugs (NSAIDs, excluding aspirin), aspirin, statins, proton pump inhibitors (PPIs), and anti-hypertensives during the 90 days prior to the start of each time-interval. In addition, the use of anti-anaemic preparations during the 90 days prior to the start of each time-interval was considered as confounding variable as a proxy indicator of anaemia as changes in the lifespan of erythrocytes can affect HbA1c levels.[19,20]

Statistical analyses

Incidence rates of GI cancer per category of GBY were calculated by dividing the number of events by the total person-years of follow-up in each category. Using a time-

dependent Cox proportional hazards model, we calculated hazard ratios (HRs) and 95% confidence intervals (CI) of GI cancer per GBY category. Patients in the >0-1.0 GBY category were set as reference in the main analyses as this category contained the largest number of events and because in clinical practice many patients with T2DM have an HbA1c level above 7% at least at one point in time. After performing age and sex adjusted analyses (Model 1), additional covariables were added to the fully adjusted model (Model 2; Adj. HR) if they changed the β coefficient of the primary exposure variable (GBY) by ≥5% in a univariate analysis.

Sensitivity analyses

As noted in the section on 'glycaemic burden' calculation, the level of HbA1c was assumed to remain constant during the last time period. This is the time between the last recorded Hba1c measurement in the CLD of PHARMO Database Network and the first ever diagnosis of a GI cancer or other right-censoring event (death from any cause, end of registration within the PHARMO catchment area or end of data collection). To test the robustness of this assumption and its effects on GI cancer risk, the following sensitivity analysis was performed. For most patients (90%) the duration that the level of HbA1c was assumed to remain constant during the last time period was 1 year. Therefore, the in the sensitivity analysis the end of follow-up was set 1 year after the last HbA1c measurement, a first ever diagnosis of a GI cancer, death from any cause, end of registration within the PHARMO catchment are or end of data collection, whichever came first.
Additionally, sex-stratified analyses were performed to explore differences in GI cancer risk by sex. All data management and analyses were performed with SAS software version 9.4 (SAS Institute, Cary, NC).

Results

Baseline characteristics

13,990 patients with incident T2DM were included in this study (Table 8.1). The median age was 65 years (interquartile range (IQR) 56-73 years), and 54.3% were male. At the start of follow-up the median HbA1c value was 7.2 (IQR 6.7-8.1). In most patients anti-diabetic drug treatment was initiated with either metformin (79.2%) and/or sulfonylureas (22.4%), although use of various other anti-diabetic drugs also occurred. In addition, two-thirds of the population were using drugs for the treatment of hypertension, and almost 50% were using statins.

Table 8.1 Baseline characteristics of incident type 2 diabetic patients.

Characteristic	N=13,990
Age (median, IQR)	65 (56-73)
Sex (n, %)	7,594 (54.3)
Hba1c (median, IQR)	7.2 (6.7-8.1)
Use of anti-diabetic drugs (n, %)[a]	
Metformin	11,086 (79.2)
Sulfonylureas	3,139 (22.4)
Thiazolidinediones	179 (1.3)
Meglitinides	12 (0.1)
Incretins	20 (0.1)
Insulin	0 (0.0)
Use of other drugs (n, %)[a]	
Anti-anaemic preparations	405 (2.9)
Anti-hypertensives	8,910 (63.7)
Aspirin	2,706 (19.3)
Non-steroidal anti-inflammatory drugs[b]	1,997 (14.3)
Proton pump inhibitors	2,712 (19.4)
Statins	6,523 (46.6)

[a] During the 90 days prior to the start of follow-up;. [b] Excluding aspirin. IQR, interquartile range.

Risk of gastrointestinal cancer

Table 8.2 shows the risk of GI cancer overall and stratified by subsite, per category of glycaemic burden years. During more than 60,000 person-years of follow-up (mean 3.9 [SD 2.9] years per person), 285 GI cancer events were observed. Overall, a statistically significant decreased risk of GI cancer was observed in patients without GBY, compared to patients with >0-1 GBY (fully adjusted HR 0.71, 95% CI 0.53-0.96). No further increased risk of GI cancer was found for patients with >1 GBY compared with >0-1 GBY (fully adjusted HR 1.15, 95% CI 0.84-1.57). When stratified by GI cancer subsite, no statistically significant differences in upper and lower GI cancer risk were observed between categories of GBY. For HPB cancer, a statistically significant decreased risk in patients with 0 GBY, and an increased risk in patients with >1 GBY were observed, compared with >0-1 GBY.

Sensitivity analyses

Table 8.3 shows the results of the sensitivity analysis that explored the effect and robustness of the assumption that the HbA1c level remained constant after the very last recorded value. No considerable change in HRs was observed as compared to the main analysis.

Stratifying the analyses by sex (Supplementary Table S8.1) did not significantly change the results compared with the main analyses for men, with a statistically significant reduced risk of GI cancer overall in patients with 0 GBY compared with >0-1 GBY (fully

adjusted HR 0.65, 95% CI 0.44-0.97) and no further increased risk in patients with >1 GBY (fully adjusted HR 1.16, 95% CI 0.80-1.70). For women, no statistically reduced risk of GI cancer overall was seen in patients with 0 GBY. Women with 0 GBY, in contrast to men, had a statistically significant reduced risk of HPB cancer compared with women with >0-1 GBY in the age-adjusted analysis (HR 0.17, 95% CI 0.04-0.74).

Table 8.2 Risk of gastrointestinal cancer in incident type 2 diabetic patients by glycaemic burden years, overall and by subsites.

			Model 1		Model 2	
Site	Events (*N*=285)	Incidence rate[a]	Hazard ratio	95% CI	Hazard ratio	95% CI
Any GI cancer						
0 GBY	65	334	0.70[b]	0.52-0.94	0.71[b]	0.53-0.96
>0-1.0 GBY	145	500	*Ref.*		*Ref.*	
>1.0 GBY	75	566	1.18	0.89-1.55	1.15	0.84-1.57
Upper GI cancer						
0 GBY	11	57	0.85	0.41-1.76	[c]	[c]
>0-1.0 GBY	21	72	*Ref.*			
>1.0 GBY	7	53	0.75	0.32-1.76	[c]	[c]
Lower GI cancer						
0 GBY	46	236	0.79	0.56-1.13	0.82	0.57-1.17
>0-1.0 GBY	90	310	*Ref.*		*Ref.*	
>1.0 GBY	39	294	0.99	0.68-1.44	0.96	0.64-1.46
HPB cancer						
0 GBY	8	41	0.38[b]	0.18-0.83	0.33[b, d]	0.15-0.73
>0-1.0 GBY	33	114	*Ref.*		*Ref.*	
>1.0 GBY	29	219	1.99[b]	1.21-3.27	2.48[b, d]	1.40-4.40

[a] per 100,000 person-years of follow-up; [b] statistically significant with p>0.05; [c] insufficient number of events for further covariable adjustment; [d] adjusted for age, sex, use of sulfonylurea derivatives, insulins, and duration of type 2 diabetes mellitus. CI, confidence interval; GBY, glycaemic burden years; GI, gastrointestinal. Model 1: age-sex adjusted; Model 2: fully adjusted for age, sex, use of sulfonylurea derivatives, insulins, aspirin, anti-anaemic preparations, history of hospitalisation, duration of type 2 diabetes mellitus.

Table 8.3 Sensitivity analyses for the risk of gastrointestinal cancer in incident type 2 diabetic patients by glycaemic burden years.

				Model 1		Model 2	
Site	Events	Person-years	Incidence rate[a]	Hazard ratio	95% CI	Hazard ratio	95% CI
Any GI cancer							
0 GBY	65	18,794	346	0.71[b]	0.53-0.96	0.72[b]	0.53-0.97
>0-1.0 GBY	142	27,884	509	*Ref.*		*Ref.*	
>1.0 GBY	74	12,893	574	1.17	0.88-1.55	1.14	0.83-1.57

[a] per 100,000 person-years of follow-up; [b] statistically significant with p>0.05. CI, confidence interval; GBY, glycaemic burden years; GI, gastrointestinal. Model 1: age-sex adjusted; Model 2: fully adjusted for age, sex, use of sulfonylurea derivatives, insulins, aspirin, anti-anaemic preparations, history of hospitalisations, duration of type 2 diabetes mellitus.

Discussion

In this population-based cohort study of incident T2DM patients, we observed a decreased risk for GI cancer in patients without GBY, but no increased risk for patients with >1 GBY, compared to patients with up to 1 GBY. When stratifying the analysis by subsite of GI cancer only the risk of HPB cancer was associated with increased numbers of GBY.

To date, there are no observational studies that have used multiple HbA1c records during follow-up to compare levels of glycaemic burden and risk of GI cancer. Previous observational studies on the relationship between single HbA1c values and GI cancer risk have pointed to an increased risk of various GI cancers when comparing the highest levels of HbA1c to the lowest levels of HbA1c[8,12,21,22], however, data were not statistically significant for all types of GI cancer (8). Using data from the large European Prospective Investigation into Cancer and Nutrition (EPIC), Grote et al. found an increased risk of pancreatic cancer with increasing pre-diagnostic HbA1c levels (Odds ratio [OR] 2.42, 95% CI 1.33-4.39 for the highest [≥6.5%] vs. the lowest [≤5.4%] HbA1c category).[21] Furthermore, Rinaldi et al. observed a mild increased risk of colorectal cancer (OR 1.10, 95% 1.01-1.19) with every 10% increase in HbA1c, also using data from the EPIC study.[22] Although these types of observational studies may offer valuable insight into the possible mechanism by which T2DM may increase the risk of GI cancer, stratification of subjects based on the HbA1c level at cohort entry may not grasp the cumulative effects of hyperglycaemia over time.

In this study, higher numbers of GBY were associated with an increased risk of HPB cancer. Several factors may underlie this association. First, an increased risk in the higher GBY categories may reflect molecular pathways via which hyperglycaemia and subsequent hyperinsulinaemia increases the risk of these cancer types in vivo. Chronic hyperglycaemia may support carcinogenesis and cancer growth as many cancer cells rely on glucose-dependent energy metabolism because of the Warburg-effect.[23] In turn, insulin has been shown to possess mitogenic effects on cancer cells in laboratory experiments.[24] By binding to insulin and insulin-like growth factor 1 (IGF-1) receptor, insulin may increase cell proliferation, and prevent cell death via intracellular growth pathways, such as the mTOR signalling pathway (mammalian target of rapamycin signaling pathway).[2,25] Secondly, the increased risk of HPB cancer could be the result of protopathic bias, i.e. where an increase in GBY could be the result of an emerging HPB cancer, especially pancreatic cancer. These cancer types have been shown to induce disturbances in glucose homeostasis and T2DM. Protopathic bias may be avoided by applying a lag-period between cancer diagnosis and glycaemic burden calculation. We

were, however, unable to investigate the possible effects of protopathic bias in this study, as we did not have sufficient cancer events.

In theory, glycaemic burden may well serve as a proxy indicator of total glycaemic control from the moment of diabetes diagnosis. With the increased usage of electronic health care systems, it may become possible to implement glycaemic burden in clinical practice when ultimately proven useful. An increase in glycaemic burden could show long-term non-compliance to drug therapy, or indicate progression of diabetes or other underlying medical problems. In another study using E-NCR-PHARMO database, it was shown that among T2DM patients with colorectal cancer using anti-diabetic drugs, mean HbA1c levels decreased by 0.1-0.2% (1-2 mmol/mol) from two years before the cancer diagnosis till the moment the cancer was diagnosed, and returned back to pre-existent levels during the two years afterwards.[20] The authors observed that these changes were more profound in patients who used anti-anaemic preparations, and may therefore reflect the effects of anaemia on HbA1c values, rather than actual changes in glucose metabolism.[20] For glycaemic burden, this may either result in an attenuated build-up of glycaemic burden or a steady state whenever a patient does not reach HbA1c levels above 7% (53 mmol/mol). Although this may look favourable for the clinician as a sign of improved glycaemic control, it could also be an omen of a looming illness causing anaemia. Additional studies are needed to evaluate whether glycaemic burden could be a useful clinical and research parameter in the future.

Several limitations of this study need to be considered. First, the Clinical Laboratory Database of PHARMO Database Network, wherein values HbA1c values are recorded, does not completely cover the whole Eindhoven region of the linked NCR-PHARMO database. Therefore, a considerable amount of potential study subjects had to be excluded from the study population. This could have caused a selection bias by inclusion of either healthier diabetic patients – those who have regular check-ups at their general practitioner (GP) or diabetes specialist due to their personal interest in their health status – or less healthy diabetic patients for whom the GP or diabetes specialist want more frequent check-ups. Secondly, a sensitivity analysis was performed to test the robustness of the assumption that the level HbA1c remained constant after the last recorded value. This assumption may lead to misclassification of more T2DM patients into a higher GBY category. The analyses showed results with comparable direction, however not all with statistical significance. As of yet it is unclear which of the analyses provides the least amount of misclassification. Therefore, we advise the readers to interpret the results with caution. Moreover, this is the first study looking into the association between glycaemic burden and GI cancer risk. Replication and conformation of our data in separate populations is needed. Third, it is possible that our results are not without any residual confounding, as we were unable to correct for potential

confounders that may influence the level of hyperglycaemia and risk of cancer (e.g. obesity, high caloric diet, physical inactivity).[24] Fourth, the use of non-insulin anti-diabetic drugs was used as a proxy indicator of the onset of T2DM. It is known, however, that T2DM may go undiagnosed for several years until complications have already emerged.[26] In our study, this latent period of T2DM may result in several years of missing exposure time to hyperglycaemia, which may have subsequently resulted in an underestimation of the level of the total glycaemic burden. Also, a longer duration of follow-up increased the likelihood of reaching a higher category of glycaemic burden over time. We corrected for the time-effect by dividing the cumulative glycaemic burden by the number of years of follow-up to create the exposure variable 'glycaemic burden years', and by correcting for duration of T2DM in the analyses. Lastly, site-specific analyses of GI cancer risk could not be performed because the number of specific GI cancer events was too small. By stratifying the outcome of overall GI cancer into broad subgroups, we aimed to explore whether increased glycaemic burden had any impact on specific GI cancer subsites.

The major strength of this study was the availability of longitudinal HbA1c records from the Clinical Laboratory Database of the PHARMO Database Network, which allowed us to model glycaemic control over time for a large group of patients using the HbA1c-based measure 'glycaemic burden'. Furthermore, the Out-patient Pharmacy Database of PHARMO Database Network holds complete and longitudinal drug dispensing data, via which we could time-dependently account for drug exposure during follow-up. In addition, cancer ascertainment could be an important concern in studies using claims databases or data from primary care facilities when they are not linked to a cancer registry, as the possibility of false-positive cancer cases could confound the results. Fortunately, cancer data from the Eindhoven region of the NCR are known to contain high quality data over a wide range of cancers, which guarantees a high level of cancer ascertainment.

In conclusion, using a population-based cohort study we are the first to report on the association between glycaemic burden and risk of GI cancer. We observed that T2DM patients with a higher level of GBY were at an increased risk of GI cancer when compared to patients without glycaemic burden years. These results underline the potential importance of adequate treatment of hyperglycaemia in order to minimize cancer risk in individuals with T2DM. However, due to possible residual confounding and chance of bias caution is advised when interpreting the results. Future studies are needed to replicate our findings and to assess the clinical value of glycaemic burden to guide treatment strategies.

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Supplementary table

Table S8.1 Sex-specific risk of gastrointestinal cancer in incident type 2 diabetic patients by category of glycaemic burden years.

			Model 1		Model 2	
Site	Events (*N*=184)	Incidence rate[a]	Hazard ratio	95% CI	Hazard ratio	95% CI
Men						
Any GI cancer						
0 GBY	36	359	0.63[b]	0.43-0.92	0.65[b]	0.44-0.97
>0-1.0 GBY	94	591	*Ref.*		*Ref.*	
>1.0 GBY	54	716	1.28	0.92-1.79	1.16	0.80-1.70
Upper GI cancer						
0 GBY	9	90	0.83	0.37-1.85	[c]	[c]
>0-1.0 GBY	18	113	*Ref.*			
>1.0 GBY	5	66	0.62	0.23-1.67	[c]	[c]
Lower GI cancer						
0 GBY	21	209	0.58[b]	0.35-0.95	0.61	0.37-1.02
>0-1.0 GBY	59	371	*Ref.*		*Ref.*	
>1.0 GBY	27	358	1.02	0.65-1.61	0.93	0.56-1.54
HPB cancer						
0 GBY	6	60	0.62	0.24-1.57	0.47	0.18-1.21
>0-1.0 GBY	16	101	*Ref.*		*Ref.*	
>1.0 GBY	22	292	3.05[b]	1.60-5.82	5.48[b]	2.71-11.08
Women						
Any GI cancer						
0 GBY	29	307	0.83	0.53-1.31	0.80	0.50-1.28
>0-1.0 GBY	51	390	*Ref.*		*Ref.*	
>1.0 GBY	21	368	0.97	0.59-1.62	1.09	0.62-1.92
Upper GI cancer						
0 GBY	<5	21	0.96	0.16-5.78	[c]	[c]
>0-1.0 GBY	<5	23	*Ref.*			
>1.0 GBY	<5	35	1.54	0.26-9.23	[c]	[c]
Lower GI cancer						
0 GBY	25	265	1.18	0.70-2.00	1.15	0.67-1.98
>0-1.0 GBY	31	237	*Ref.*		*Ref.*	
>1.0 GBY	12	210	0.92	0.47-1.79	0.99	0.47-2.07
HPB cancer						
0 GBY	<5	21	0.17[b]	0.04-0.74	[c]	[c]
>0-1.0 GBY	17	130	*Ref.*			
>1.0 GBY	7	123	0.96	0.40-2.32	[c]	[c]

[a] per 100,000 person-years of follow-up; bstatistically significant with p>0.05; cinsufficient number of events for further covariable adjustement. CI, confidence interval; GBY, glycaemic burden years; GI, gastrointestinal; HPB, hepato-pancreatico-biliary. Model 1: age-sex adjusted; Model 2: fully adjusted for age, sex, use of sulfonylurea derivatives, insulins, aspirin, and anti-anaemic preparations, history of hospitalisations, duration of type 2 diabetes mellitus.

Chapter 9

General discussion

General discussion

In this thesis, new and original data on the risk of gastrointestinal (GI) cancer in patients with type 2 diabetes mellitus (T2DM) have been presented in order to create a better insight into the complexity of the association. We evaluated the association between T2DM and all types of GI cancer in both the Dutch population and population of the United Kingdom (UK), and whether the association is partially explained by detection bias or protopathic bias. Furthermore, the association between the use of metformin and the risk of GI cancers, and between the use of incretin-based anti-diabetic drugs (incretins) and the risk of pancreatitis, was evaluated using a time-dependent definition of drug-exposure. In addition, the association between the level of hyperglycaemia over time and GI cancer risk was evaluated using a novel marker of hyperglycaemia called *glycaemic burden*. Below, the key findings of our studies in relation to the current available literature and the methodological limitations are discussed. Lastly, the general discussion ends with future directions and perspectives for researchers investigating the association between T2DM and GI cancer and the overall conclusions.

The association between type 2 diabetes mellitus and gastrointestinal cancer

The first aim of this thesis was to evaluate the association between T2DM and the risk of GI cancer, and whether the association is partially explained by detection bias or protopathic bias. To date, T2DM has been associated with several GI cancers.[1-3] The highest risk estimates have been reported for liver and pancreatic cancers.[4,5] For colorectal cancer a modest 15-30% increased risk has been found in the literature.[6,7] For other types of GI cancers mixed results have been reported.

In **Chapter 2** we created an overview of the incidence rates (IRs) of all GI cancers in individuals with and without T2DM using the UK CPRD database. Higher IRs of any GI, liver, pancreatic, and colon cancer, a lower incidence of oesophageal cancer, and no significant differences in gastric, small intestinal, biliary, and rectal cancer were found in patients with T2DM compared to non-diabetic controls. These results are in line with recent studies performed in the Netherlands and the UK. Schrijnders et al. found increased standardized incidence ratios (SIR) of obesity-related cancers (including oesophageal adenocarcinoma, liver, pancreas, gallbladder and pancreatic cancer) in both men and women (SIR 1.80, 95% CI 1.59-2.01 overall, SIR 2.21, 95% CI 1.88-2.54 in men, and SIR 1.38, 95% CI 1.11-1.64 in women) in the first year after the diagnosis of T2DM.[8] Also using the UK CPRD database, Peeters et. al. found comparable incidence

rates of colorectal cancer in individuals with or without T2DM.[9] In contrast, using a large cohort of individuals with insulin-treated diabetes, Swerdlow et al. reported no statistically significant differences in GI cancer incidence rates between insulin-treated diabetes patients in the UK compared with the general population.[10] However, the number of cancer events in this study was very low and misclassification of the exposure group (by inclusion of patients with type 1 diabetes mellitus) may have influenced the results.

The study in **Chapter 2** was a descriptive study in which we reported unadjusted incidence rates of GI cancer. The study was not designed to assess a causal relationship between T2DM and GI cancer. Differences in the incidence rates of GI cancers could have been explained by a different distribution of risk factors for GI cancer such as diet, physical inactivity, and obesity among patients with T2DM and non-diabetic controls.[11] Furthermore, the main results may have been distorted by not accounting for detection bias or reverse causality. The possible influence of these biases on the results are discussed in further detail in the section on bias.

In **Chapter 3**, using the E-NCR-PHARMO database, we observed a 50% increased risk of GI cancer in patients with T2DM compared with non-diabetic controls. This overall increased risk of GI cancer in T2DM patients was explained by a four-fold increased risk of Hepato-Pancreatico-Biliary tract (HPB) cancers, which was driven by pancreatic cancer (five-fold increase) and biliary tract cancer (four-fold increase). No difference in risk of other GI cancer types between patients with T2DM and non-diabetic controls was observed.

In a recent summary meta-analysis of other meta-analytical studies (that included observational studies), statistically significant increased risks of all GI cancers but gastric cancer were found in patients with T2DM compared to non-diabetic individuals.[3] However, consistent evidence was only found for intrahepatic cholangiocarcinoma (summary random effects estimate (SRE) 1.97, 95% CI 1.57-2.46) and colorectal cancer (SRE 1.27, 95% CI 1.21-1.34), which may suggest uncertainty about the results of previous meta-analyses for other GI cancer sites.[3] In particular, the differences between observational studies that were included by the original meta-analyses and presence of unaccounted bias or residual confounding could have caused false positive results.[3] While meta-analyses increase statistical power and stronger evidence, a major drawback is that they can also further inflate biased results. These methodological issues, that impact observational studies, are difficult to overcome. Moreover, most studies that are included in meta-analyses did not account for possible detection bias by attributing cancer events to the T2DM population directly after the diagnosis of T2DM, which could have inflated the risk estimates.

The association between T2DM and the risk of HPB type cancers may be more complex as compared with the association between T2DM and the risk of other GI cancer sites. A stronger association was observed in data from the United Kingdom (**Chapter 2**) and The Netherlands (**Chapter 3**). We found larger differences in the IRs of liver and pancreatic cancer between individuals with and without T2DM as compared with other GI cancer sites (**Chapter 2**). In **Chapter 3**, the highest increased risks of GI cancer were observed for liver, biliary tract and pancreatic cancer in patients with T2DM compared with non-diabetic controls. These findings are generally in line with results from previous meta-analyses of observational studies, showing an approximate two-fold increased risk of these cancer types.[3-5] Both the liver and the pancreas are exposed to higher levels of endogenous insulin compared to other organs via the portal venous system. As insulin may be one of the major hormonal contributors to the diabetes-cancer link because of its mitogenic properties, this can explain why higher risk estimates are observed for HPB type cancers.[1]

The association between T2DM and pancreatic cancer is further complicated as pancreatic cancer may manifest itself as diabetes mellitus through various mechanisms.[12-14] These mechanisms include a paraneoplastic syndrome related to diabetogenic substances excreted by pancreatic cancer cells[15], parenchymal atrophy by loss of islet cell mass[16], and direct beta-cell dysfunction.[17] In contrast, patients with T2DM may lose the ability to produce sufficient endogenous insulin over time, requiring exogenous insulin therapy to regulate their blood glucose levels. The loss of the insulin-rich pancreatic milieu may result in the loss of mitogenic stimulus to promote pancreatic hypertrophy and growth. This may explain why studies have shown higher risks of pancreatic cancer in T2DM earlier in the course of follow-up.[12]

Instead of finding a modestly elevated risk of colorectal cancer as was hypothesized based on the current literature, we found no difference in the risk of colorectal cancer between patients with and without T2DM. These findings are in line with previous studies performed in the Netherlands which showed no statistically significant increased risk of colorectal cancer between patients with and without T2DM, while correcting for multiple confounders (e.g. age, sex, body mass index (BMI), smoking status and alcohol use).[18,19] In contrast, de Kort et al. found a statistically significant increased risk of colorectal cancer in patients with T2DM compared to non-diabetic controls also using the E-NCR-PHARMO database.[20] These differences in the results may be explained by methodological variation, such as study design or statistical analyses (e.g. inclusion of specific confounders).

Pathophysiological processes that may promote the proliferation of cancer cells through hyperinsulinaemia, hyperglycaemia, insulin resistance and inflammation may form the basis for the link between T2DM and GI cancer. However, T2DM and GI cancers also

share various risk factors that could (in part) explain the association. Besides non-modifiable risk factors such as older age and race/ethnicity, a range of modifiable risk factors have been identified, including obesity, physical inactivity, smoking, alcohol abuse, and poor dietary habits.[1] Most of these modifiable risk factors have increased following the industrial revolution in Europe and the United States. In observational studies, residual confounding is likely present when not all of these factors are adequately adjusted for.

Unfortunately, in the studies presented in **Chapter 2** and **Chapter 3**, we were unable to correct for several important general confounding factors, including obesity, smoking, alcohol use, and physical inactivity. Especially not having corrected for the presence of obesity may have confounded the results. In a large umbrella review of meta-analyses (N=204), Kyrgiou et al. have analysed the strength and validity of the evidence of the association between adiposity and the risk of cancer.[11] They found that the associations between adiposity and oesophageal adenocarcinoma, cancer of the gastric cardia, colon, rectum, biliary tract, and pancreas were supported by strong evidence.[11] Moreover, previous research has shown that obesity is a known risk factor for the development of T2DM as well.[21]

Temporal relationship between T2DM and GI cancer

Detection bias and protopathic bias

When investigating the link between T2DM and GI cancer it is important to take into account the temporal relationship between both entities. A diagnosis of T2DM often increases the level of medical consultation in comparison to individuals without this disease, which can result in an apparent increase in cancer risk among the T2DM population, representing a form of detection bias.[22] Also, GI cancer – specifically pancreatic cancer – may cause disturbances in glucose metabolism by interfering with endogenous insulin production by the pancreas resulting in T2DM and leading to protopathic bias in epidemiologic studies.[23,24] Therefore, if an individual with T2DM is diagnosed with pancreatic cancer shortly after the diagnosis of T2DM, it is unlikely and methodically incorrect to designate T2DM as a causative factor in the development of the pancreatic cancer. Both types of biases can be minimized by applying a lag period between de diagnosis of T2DM and GI cancer.[24,25]

In both **Chapter 2** and **Chapter 3** sensitivity analyses were performed using a one-year lag period after the diagnosis of T2DM to minimize detection bias and protopathic bias. No clear differences in the incidence rates of GI cancers were seen compared to the main analysis in **Chapter 2**. In **Chapter 3** of this thesis, an attenuation of the risk estimates for

GI cancer and pancreatic cancer was observed, although the risk of these cancers in patients with T2DM were still statistically significantly increased compared to non-diabetic controls. Previous studies investigating the possible effects of detection bias found significantly increased risks of colorectal and pancreatic cancers in the first three to six months following a diagnosis of T2DM.[13,18,20] However, only in the study by Johnson et al. the risk of these cancers and of liver cancer remained elevated – though less prominently – after excluding the first 3 months of follow-up.[13] The results of our study and the studies mentioned above indicate that detection bias and protopathic bias may have inflated the risk estimates of GI cancer in T2DM patients when a lag period after the diagnosis of T2DM is not accounted for. However, they also suggest that the association between T2DM and GI cancer is probably not completely explained by these biases, or that a one-year lag period is insufficient to fully exclude detection bias.

Latency time of cancer

An important aspect of the temporal relationship between T2DM and GI cancer that merits discussion is the latency time of GI cancer – i.e. the time it takes for a GI malignancy to develop from its conception up until diagnosis. When studying an exposure-disease relationship in an observational setting, assumptions need to be made about the role T2DM plays in the development of cancer, such as the time for T2DM to have a biological effect on carcinogenesis (initiation or promotion). In reality, various etiologic factors that contribute to a disease process will probably have complex interrelations.[26]

In 1981, Kenneth J. Rothman proposed that induction and latent periods of disease are distinguishable time windows referring to the period between the causal action of a risk factor and disease initiation, and the period between disease initiation and detection, respectively.[26] The sum of these two intervals is defined as the empirical induction period (**Figure 9.1**). Inappropriate assumptions about the length of the empirical induction period may result in non-differential misclassification bias of risk estimates towards the null, thereby underestimating the effect of a risk factor or even obscuring a true effect.[26,27] Unfortunately, the latent time period of cancer is difficult to estimate. Rough estimates from studies on growth rates of solid cancers show that the latent time period might be approximately 5-7.5 years for colorectal cancer, assuming a linear growth rate.[28] To approach an adequate assumption of the latent time period, analyses with varying latent time periods can be used, as the risk estimate should increase when the assumption becomes more adequate.[27] In **Chapter 3**, the mean follow-up time was 4 years. Therefore, apart from accounting for possible detection bias by applying a one-year lag period in the analyses, sensitivity analyses involving the latent time period of GI

cancers were not possible. To explore the possible effect of non-differential misclassification bias, future observational studies should correct for the latency time of cancer if an adequate follow-up period is available.

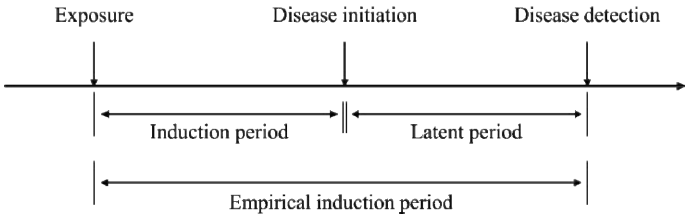

Figure 9.1 Time windows between the exposure to a (causal) risk factor, disease initiation, and disease detection as proposed by Rothman 1981.

Database representativeness

In order to assess to what extent our findings in a representative sample from the total UK population (Chapter 2 and 6) were indirectly applicable to the Netherlands, we compared the age- and sex distribution of the UK CPRD with the total Dutch population for the year 2011 in Chapter 4. The age distribution of men and women in the CPRD population was comparable to that in the Dutch male and female population in the same calendar year. Differences in the distribution of age of more than 10% only occurred in older age categories (75+ in men and 80+ in women). This study, however, was only a first step in evaluating the applicability of CPRD's results to the Dutch population. Obviously, more variables that influence the applicability of a database's results for another population (e.g. prevalence of disease risk factors such as smoking, obesity, comorbidities and drug use) need to be compared, in order to draw firmer conclusions. Currently, pharmaco-epidemiological research, that has been conducted in CPRD, is already being used for regulatory decision making in the Netherlands. For instance through changes in drug use recommendations by the European Medicines Agency.[29] We hope that with our initial study grounds have been laid for future investigators to perform more extensive comparison studies between databases and other populations in order to investigate the applicability of a database's results to a country's population.

The use of anti-diabetic drugs and risk of gastrointestinal cancer

The second aim of this thesis was to evaluate the risk of GI cancer in users of metformin and to evaluate the risk of pancreatitis in users of incretins. Several drugs used in the treatment of T2DM have been reported to be associated with cancer risk.[30] Metformin was chosen as drug of interest in this thesis because of the ongoing debate regarding its possible effect on reducing cancer risk, while many previous studies were affected by time-related biases.[31] In addition, we decided to investigate the effects of incretins on the risk of pancreatitis because of the increasing reports of this adverse event in the literature and its possible consequences for pancreatic cancer risk.

Metformin

In **Chapter 5**, a time-varying approach to determine metformin exposure in T2DM patients was applied in order to evaluate GI cancer risk. During more than 280,000 person-years of follow-up with 1,076 GI cancer events, no reduced risk of GI cancer (or its specific subsites) was found when comparing current use of metformin with current use of other non-insulin anti-diabetic drugs (NIADs). Also, no reduced risks of GI cancers were found when performing stratified analyses by treatment stage or cumulative dose.

Evans et al. were the first to report an association between the use of metformin and cancer risk in 2005.[32] Since then, numerous observational studies have reported on a reduced cancer risk with use of metformin.[33-42] However, not all studies have confirmed this association.[41-44] In fact, an increased risk of colorectal cancer has also been reported.[45]

There is a plausible biological mechanism by which metformin could reduce cancer risk.[46] Metformin has been shown to interact with intracellular energy metabolism and growth pathways in cancer cells, thereby reducing cancer growth.[46] Besides, metformin reduces overall insulin resistance, hyperinsulinaemia and hyperglycaemia, which have been associated with an increased risk of cancer.

Many observational studies on the risk of GI cancer and the use of metformin have been criticized for the presence of time-related biases.[30,31,47] As mentioned in the introduction of this thesis, these time-related biases revolve around two axes.[30] Incorrectly classifying observation time to different exposure categories (immortal time bias), and comparing the use of metformin with the use of anti-diabetic drugs (ADDs) used in a later stage of the disease without accounting for disease duration (time-lag bias or confounding by indication).[30] Studies that have classified observation time correctly have so far not found a decreased risk of cancer with the use of metformin.[48,49] In our study, we tried to

minimize time-related biases by classifying exposure to ADDs time-dependently using 90-day time-intervals. As a result, exposure time could be classified appropriately to use and non-use of metformin. Furthermore, confounding by indication was minimized by comparing the use of metformin with the use of other non-insulin ADDs while adjusting for the use of other ADDs that have been associated with cancer risk and duration of T2DM in the statistical analyses. In addition, stratified analyses for each metformin-user subgroup were performed to reveal any discrepancies. Using this approach, no reduced risks of GI cancers were found in users of metformin compared to users of other NIADs, which is in line with recent results of other well designed observational studies.

Many clinical trials on the possible cancer preventive effect of metformin are still ongoing. One notable example is a placebo-controlled phase III trial of metformin for the secondary prevention of recurrent colorectal polyps and adenomas in individuals who had undergone an index colonoscopy with polypectomy.[50] A statistically significant decrease in the incidence of recurrent polyps (RR 0.67, P=0.034) and adenomas (RR 0.60, P=0.016) was found at 1 year in non-diabetic individuals who were treated with low-dose oral metformin (250 mg daily). However, the absolute number of recurrent polyps and adenomas during follow-up endoscopy was clinically negligible (median number of polyps 0 [interquartile range (IQR) 0–1] in the metformin group versus 1 [IQR 0–1] in the placebo group (p=0.041)).[50] Subsequently, this raises question about the clinical impact of the reported results.

Our study on the use of metformin and GI cancer risk was not without limitations. First of all, we were not able to correct various confounding factors, such as lifestyle factors (e.g., obesity, alcohol use, smoking status, and physical activity), dietary habits, and the presence of unmeasured (disease specific) comorbidities (e.g., gastro-esophageal reflux disease, chronic liver disease, or chronic pancreatitis). Second, a lack of statistical power existed for some cancer sites, such as liver cancer and biliary tract cancer, especially in the sensitivity analyses wherein a new-user cohort was used. This resulted in a limited ability to statistically adjust for confounders in the multivariate analyses. An effective approach to control for all potential confounding factors would be to use propensity score adjustment, especially when the number of outcome events is low.[47] A propensity score incorporates all of the available covariates into one score and represents the probability of receiving one drug over the other.[47] Similarly to a randomized trial, this approach aims to achieve balance between the study groups with regard to measured confounders. Although we acknowledge that propensity score adjustment would be an effective strategy to further reduce residual confounding and limit the number of covariates in the multivariate model, it cannot overcome the unmeasured confounding in the data source and therefore this strategy was not applied.[51,52]

Based on the current evidence as mentioned above and the results found in our study on the risk of GI cancers with the use of metformin, the use of metformin is most likely not associated with a clinically relevant reduced risk of GI cancer. Expensive clinical trials should therefore be discouraged.

Incretin-based anti-diabetic drugs

In **Chapter 6**, a population-based cohort study was conducted using data from the UK CPRD to evaluated the risk of any, acute, and chronic pancreatitis in users of incretins compared to users of other NIADs. Adjusting for lifestyle, disease and drug history, a 1.5-fold statistically significant increased risk of any type of pancreatitis with current use of incretins versus other NIAD use was found. The risk of acute pancreatitis was 1.4-fold greater in current incretin users versus other NIAD users, but this did not reach statistical significance (HR 1.42, 95% CI 0.98-2.06). Furthermore, an association between chronic pancreatitis and incretin use was not detected, but the number of events in this subgroup was small (N=160). Stratified by type of incretin (DPP-4 inhibitors and GLP-1 receptor agonists), the risk of acute pancreatitis was statistically significantly increased in current users of DPP-4 inhibitors only.

Both acute and chronic pancreatitis have been associated with an increased risk of pancreatic cancer, with a 2-fold and 8-fold increased risk of pancreatic cancer more than 5 years after a diagnosis of acute and chronic pancreatitis respectively.[53,54] Concerns about pancreatic cancer risk with use of incretins first arose after an increased risk of pancreatitis was reported in adverse-event databases.[55,56] However, large population-based cohort studies thereafter have shown mixed results related to pancreatic cancer risk.[57-62]

Regarding the risk of pancreatitis, data from the SAFEGUARD consortium – investigating the cardio/cerebrovascular and pancreatic safety of ADDs in T2DM patients – have shown that GLP-1 receptor agonists (exenatide and liraglutide) and some DPP-4 inhibitors (alogliptin, sitagliptin, saxagliptin) show a high risk for acute pancreatitis.[63] This was concluded after evaluating the existing literature at that time (up to 2015), and performing studies using adverse-event databases (e.g. Eudravigilance). However, no increased risk of acute pancreatitis was found when analysing acute pancreatitis risk with the use of incretins in T2DM patients using a combination of multiple large pharmaco-epidemiological databases (e.g. PHARMO Database Network). Furthermore, some of the more recent epidemiological studies have shown no statistically significant association between the use of incretins and the risk of pancreatitis.[64,65] In a meta-analysis of 55 randomised controlled trials (N=33,350), Li et al. found no evidence of an increased risk of pancreatitis with incretins versus controls (OR 1.11, 95% CI 0.57 to

2.17). Analysis by type of incretin showed similar results (OR 1.05, 95% CI 0.37 to 2.94 for GLP-1 agonists versus controls, and OR 1.06, 95% CI 0.46 to 2.45 for DPP-4 inhibitors versus controls).[64] Pooled results from five observational studies (three retrospective cohort studies, and two case-control studies; N=320,289) also showed no statistically significant increased risk of pancreatitis in users of incretins. Also, a recent large nested case-control analysis of over 1.5 million patients, has shown no increased risk of pancreatitis in users of incretins compared to users of at least two NIADs (pooled adjusted HR 1.03, 95% CI 0.87 to 1.22). In addition, clinical trial data have not demonstrated a relationship between the use of incretins and development of pancreatic cancer.[66]

Based on the current evidence in the literature, incretins probably do not convey a clinically relevant increased risk of pancreatitis or pancreatic cancer in patients with T2DM. However, we were the first to separately study the risk of both acute and chronic pancreatitis with the use of incretins, and our results are yet to be replicated. Moreover, the complex interactions between both T2DM, pancreatitis, and pancreatic cancer make it difficult to analyse the potential impact of incretins on the risk of both pancreatitis and pancreatic cancer. Therefore, we think that consequent monitoring of potential side effects by means of periodic safety update reports remains warranted.

Other methodological considerations

Prevalent user bias

Prevalent user bias arises when all users of ADDs from a given calendar date, regardless of the time they had used the drug for, are included in the study population.[30,67] These individuals might be at a lower risk of cancer, as the use of a certain ADD for a long period of time may reflect good glycaemic control. In a cohort of prevalent ADD users correcting for the previous duration of use is not possible, which may introduce bias. To explore any effects of this type of bias we performed sensitivity analyses using a new-user design in **Chapter 5** and **Chapter 6**, where follow-up for included patients started at the date of the first-ever ADD prescription after a period of time wherein no ADD prescriptions were recorded.[67] In the study on the risk of GI cancer with use of metformin (**Chapter 5**) this approach did not result in an alteration of the results. In **Chapter 6** the risks of any and acute pancreatitis in current users of incretins were more pronounced compared to the main analysis.

Hyperglycaemia and gastrointestinal cancer risk

Hyperglycaemia has been associated with both micro- and macrovascular complications in individuals with T2DM.[68,69] Possibly, hyperglycaemia is also associated with an increased risk of cancer.[1] Previous studies investigating the association between hyperglycaemia and (GI) cancer risk have only used individual or mean measures of glycaemia (e.g. HbA1c at start of follow-up) and evidence on the cumulative effects of hyperglycaemia on GI cancer risk are unknown. Therefore, as a means to study the possible biological relationship between T2DM and GI cancer in an observational setting, the final aim of this thesis was to evaluate the association between the level of hyperglycaemia over time and GI cancer risk in patients with T2DM, using a novel marker of hyperglycaemia called glycaemic burden (**Chapter 8**).

Glycaemic burden was defined as the sum of the differences between a subject's HbA1c value and the threshold of 7% (53 mmol/mol). To account for differences in duration of follow-up, a yearly estimate of glycaemic burden was calculated for the analyses (glycaemic burden years; GBY), by dividing the cumulative glycaemic burden by the number of years of follow-up. Comparing to T2DM patients with up to one year of GBY, a decreased risk of GI cancer was found in patients with zero glycaemic burden (HbA1c never above 7% [53 mmol/mol] during follow-up), but no further increased risk for patients with more than one year of GBY. When stratifying the analysis by subsite of GI cancer only the risk of hepato-pancreatico-biliary tract cancers was associated with increased years of GBY.

As noted in **Chapter 8**, no observational studies that have used multiple HbA1c records during follow-up to compare levels of glycaemic burden and risk of GI cancer have been conducted as of yet. Previous observational studies on the relationship between single HbA1c values and GI cancer risk have pointed to an increased risk of various GI cancers when comparing the highest levels of HbA1c to the lowest levels of HbA1c.[70-73] Although these studies may offer valuable insight into the possible mechanism by which T2DM may increase the risk of GI cancer, stratification of subjects based on the HbA1c level at cohort entry may not grasp the cumulative effects of hyperglycaemia over time.

Glucose is a major source of energy for cancer cells, as these cells mainly depend on glycolysis for energy production.[74] Furthermore, function-altering mutations in receptor-initiated signalling pathways overcome growth-factor dependent uptake and metabolism of glucose.[74] This high requirement for glucose and increased glucose uptake in cancer cells may be an explanation for the association between hyperglycaemia and cancer risk in previous studies.[1]

The study in **Chapter 8** had several limitations. There was the possibility of selection bias as a considerable amount of potential study subjects had to be excluded due to a large

number of missing HbA1c values. The included individuals could either represent healthier T2DM patients – those who have regular check-ups at their general practitioner or diabetes specialist due to their personal interest in their health status – or less healthy T2DM patients for whom the GP or diabetes specialist orders more frequent laboratory tests. Furthermore, there is the possibility of residual confounding due to unmeasured confounding factors that may influence the level of hyperglycaemia and risk of cancer (e.g. obesity, high caloric diet, physical inactivity).[1] In addition, we used the start of NIADs as proxy-indicator of the onset of T2DM. This could have caused an underestimation of the cumulative glycaemic burden, as T2DM may go undiagnosed for several years until complications have already emerged. Moreover, as the latent time period for T2DM per individual is unknown, misclassification of GBY categories could have occurred.

The study in **Chapter 8** is the first observational study to evaluate the association between T2DM and glycaemic burden. Therefore, future studies are needed to replicate our findings while taking into account the limitations of our study regarding selection bias and limited ability to correct for confounding factors. Nonetheless, our results do suggest that an association is present between hyperglycaemia over time and the risk of GI cancer in patients with T2DM.

Data source limitations

For the studies presented in this thesis data from the E-NCR-PHARMO database and data from the UK CPRD were used. Although large electronic healthcare databases contain very valuable and detailed information, data were not collected for a specific research question but for routine clinical care/evaluation and/or reimbursement purposes, and the use of such databases may have several limitations.

First, although the E-NCR-PHARMO database contains information on body mass index (BMI), this was available for a limited number of individuals only. This made statistical adjustment for BMI not possible in the studies using the this database. The importance of adjusting for BMI or obesity as confounding variable has previously been mentioned.

Second, a diagnosis of T2DM was based on the use of oral ADDs, using the first-ever recorded drug prescription as the date of onset of T2DM. Hence, individuals who are managed without any medication – i.e. advising dietary and lifestyle changes – were not included within the T2DM population of the studies in this thesis. It is possible that some of these patients were included in the non-diabetic reference population. However, given the limited long-term success of lifestyle modification programs to maintain glycaemic goals in patients with type 2 diabetes, the majority of patients will require

ADD therapy over the course of their diabetes.[69] On the one hand, if the presence of T2DM really contributes to GI cancer development, this could have led to an increased risk of GI cancer in the reference population and caused a bias of the risk estimates towards the null. On the other hand, the presence of this bias also strengthens the significance of the results when finding statistically significant differences between the two populations. Third, non-differential misclassification of exposure could have occurred in the studies on ADD use and GI cancer risk. The longitudinal drug dispensing database of PHARMO Database Network does not provide certainty that dispensed drugs are actually ingested by individuals. Therefore, true exposure to a certain drug – an ADD or a confounder drug – may be overestimated. This misclassification of exposure could have biased the risk estimates towards the null. Ideally, one would want data confirming actual drug use in patients, but this is practically impossible to achieve.
The CRPD GOLD database uses READ codes in order to identify GI cancer events. READ codes are a coded thesaurus of clinical terms used by clinicians to record patient findings and procedures. As READ codes are dependent on the adequate administration by general practitioners in the UK, there is a chance that some GI cancer events already diagnosed yet not registered may be missed, and that patients with a registration of a specific cancer may not have that cancer at all or a different type of cancer. This could lead to a form of information bias. On the one hand, CPRD tries to minimize this registration bias by using their Quality and Outcomes Framework that incentivises general practitioners to optimize their registries. On the other hand, it is possible to link CPRD data to the UK population-based National Cancer Registry. Dregan et al. have previously investigated the validity of cancer diagnoses in CPRD compared with cancer registry data.[75] In a cohort comprised of 42,556 participants, registered with English general practices in the General Practice Research Database (GPRD; currently known as CPRD), they showed the predictive value of a GPRD cancer diagnosis was 96% for gastro-oesophageal cancer and 98% for colorectal cancer.[75] Therefore, a high level of ascertainment is reached based on CPRD cancer records alone. For studies using the UK CPRD database in this thesis linkage to the National Cancer Registry was not performed. We assumed the information bias as mentioned above to be non-differential between exposure groups and of minimal impact on the study results.

Future directions

In this thesis the associations between overall and site-specific GI cancers on the one hand, and T2DM, the use of ADDs, and glycaemic control on the other hand were evaluated, while taking several important methodological challenges into account. The

results presented evoke several questions for which recommendations for future studies can be made. First, because of a limited follow-up time, detection bias may not have been completely accounted for and we could not perform additional analyses to explore the effect of the latent time period of cancer on the risk estimates. Future observational studies on the association between T2DM and GI cancer should therefore focus on minimizing distortion of the results through bias and confounding, and consider evaluating the possible influence of latent time periods of GI cancer. To do so, larger databases with a longer duration of follow-up and higher number of cancer events would be necessary in order to provide enough statistical power for adequate statistical analyses and confounder adjustment. Secondly, given the current evidence, future pharmaco-epidemiological studies on the association between the use of metformin and GI cancer risk will probably not yield new results that would further impact the conclusions that can be drawn from the current literature. Therefore, new studies on this topic are not recommended, unless new methodological issues are detected that can be evaluated with novel statistical methods. Lastly, a novel marker of hyperglycaemia over time – glycaemic burden – was used to evaluate the risk of GI cancer with increasing levels of glycaemic burden for the first time. For future research it would be interesting to investigate if such a marker would have any clinical value in the management of T2DM or to compare glycaemic burden to other types of (HbA1c-based) measures of hyperglycaemia in its ability to predict the risk of complications of T2DM, also including the risk of cancer.

Conclusions

In conclusion, the main objectives of this thesis can be addressed as follows:
First, a diagnosis of T2DM has been associated with a higher risk of GI cancer, driven by an increased risk of pancreatic and liver cancer, but not other GI cancer sites. When (partially) accounting for detection bias the risk estimates of these cancers attenuated, though remained statistically significantly elevated. This indicates that previously found risk estimates in the literature may also have been overestimated due to detection bias. Of note, residual confounding due to unmeasured risk factors may have influenced the results of our studies.
Second, the use of metformin is not associated with a decreased risk of GI cancer or its subsites. Therefore, expensive clinical trials investigating the use of metformin as chemopreventive agent are not recommended. In contrast, we found an increased risk of acute pancreatitis, but not chronic pancreatitis, in users of incretins, which was not consistent with the current evidence in the literature. In light of the mixed results and

the fact that stratified results for acute and chronic pancreatitis have been reported for the first time in this thesis, pharmacovigilance is warranted regarding the use of incretins.

Third, the degree of hyperglycaemia over time, expressed as glycaemic burden, may be associated with a higher GI cancer risk. However, future studies are needed to replicate and build forward on our initial findings. In general, Dutch clinicians should be aware of the association between T2DM and liver and pancreatic cancer, in particular in new or inadequately controlled T2DM patients.

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Summary

Summary

Type 2 diabetes mellitus (T2DM) and gastrointestinal (GI) cancer both are increasing global health problems of which the incidence and prevalence are still rising. Over the past decades a growing body of evidence has been collected to support an association between T2DM and GI cancer risk. However, the association between these two entities is complex and is influenced by a wide range of factors. These include pathophysiological mechanisms (e.g. hyperglycaemia and hyperinsulinaemia), confounding due to common, shared risk factors (e.g. obesity, physical inactivity), drug use (e.g. the use of various anti-diabetic drugs; ADDs), and different study methodologies which may introduce bias.

In this thesis, multiple aspects of the complex association between T2DM and GI cancer were investigated. First, we focussed on the impact of detection bias on the incidence and risk of all types of GI cancer (**Chapter 2** and **Chapter 3**). In this part the thesis, we also paid attention to the comparability of the population in the UK Clinical Practice Research Datalink (CPRD) database for the Dutch population as supportive evidence for the generalizability of study results using the CPRD database (**Chapter 4**). Second, the risk of GI cancer with use of metformin (**Chapter 5**) and the risk of (acute and chronic) pancreatitis with use of incretins (**Chapter 6**) were analysed. Furthermore, the possibility of selection bias in a pharmaco-epidemiological study was highlighted (**Chapter 7**). Third, we evaluated the association between GI cancer risk and glycaemic control by evaluating the degree of hyperglycaemia over time expressed as glycaemic burden (**Chapter 8**).

The general introduction of this thesis gives background information on T2DM and GI cancer (**Chapter 1**). Evidence from previous research on the association between both entities is presented and we explain the motivation for our work. Based on existing data and literature, the association between T2DM and GI cancer is complex, not consistently reported, and subject to various methodological issues and unmeasured distortions, such as confounding and detection bias. Studies on the association between T2DM and cancer often focus on cancer in general, or on highly prevalent cancers, such as colorectal cancer, or cancers for which strong associations have previously been described in the literature, such as liver and pancreatic cancer. Evidence for an association between other types of GI cancer (oesophageal cancer, gastric cancer) is less pronounced.

In **Chapter 2**, incidence rates (IRs; per 100,000 person-years of follow-up) of GI cancers were determined in a British cohort of 333,438 anti-diabetic drug users and 333,438 matched non-diabetic individuals obtained from the CPRD database between 1988 and 2012. This study showed that T2DM patients had higher crude IRs of liver (IR 26, 95% confidence interval [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)

compared to the non-diabetic cohort, whereas the IR of oesophageal cancer was lower (IR 41, 95% CI 39–44 vs. 47, 95% CI 44–51). Similar results were found in a sensitivity analysis that minimized detection bias by excluding 1 year of follow-up after the index date. A possible sign of detection bias was observed for pancreatic cancer, for which the IR declined from 65 to 48 in the diabetic cohort. However, the difference in IRs for pancreatic cancer between the T2DM and non-diabetic cohort remained statistically significantly elevated.

In Chapter 3, the risks of GI cancer and its subsites were analysed in new users of ADDs compared to matched non-diabetic controls. Data were obtained from the linked database of the Eindhoven region of the Netherlands Cancer Registry and PHARMO institute (E-NCR-PHARMO database) from 1998 through 2011. In order to explore the effects of detection bias on the association between T2DM and GI cancer, Cox regression analyses were performed with and without a 1-year lag-period to estimate hazard ratios (HRs) for GI cancer. In the overall analysis, a 50% increased risk of GI cancer was found in T2DM patients compared with controls (HR 1.5, 95% CI 1.3-1.7), which was attenuated to a 40% increased risk following adjustment for potential detection bias in the 1-year lagged analysis (HR 1.4, 95% CI 1.2-1.7). Stratified by cancer subsite, statistically significant increased risks of pancreatic (HR 4.7, 95% CI 3.1-7.2), extrahepatic bile duct (HR 4.2, 95% CI 1.5-11.8) and distal colon cancer (HR 1.5, 95% CI 1.1-2.1) were found, but not for other GI cancer subsites. The results show that T2DM is mainly associated with hepato-pancreatico-biliary (HPB) type cancers and suggest that detection bias may play in important role in the strength of the association. Moreover, future observational studies should include sensitivity analyses in which detection bias is kept to a minimum by including one or more years of lag-time.

In Chapter 4 we compared the age and sex distribution of the UK CPRD with that of the total Dutch population, in order to judge database representativeness. For this study the age and sex distribution of the UK CPRD were visually and numerically compared with Dutch census data from the StatLine database of the Dutch National Bureau of Statistics in 2011. The age distribution of men and women in CPRD was comparable to the Dutch male and female population. Differences of more than 10% only occurred in older age categories (75+ in men and 80+ in women). This unique study was a first step in showing that results from observational studies using CPRD data are applicable to the Dutch population, and thus provide us with a useful resource for decision making in the Netherlands. Nevertheless, in pharmaco-epidemiological studies the generalizability may still be decreased because of differences in drug exposure likelihood between countries, as these differences could cause variations in the actual population studied when selecting individuals based on medication use. We hope that the results of this study

may encourage scientists from other countries with similar healthcare systems to perform studies of CPRD representativeness.

In Chapter 5 a cohort study was presented evaluating the risk of GI cancer in users of metformin by employing the E-NCR-PHARMO database. Previous studies have shown large protective effects of metformin use on GI cancer risk. However, time-related biases and other methodological shortcomings have limited the validity of reported risk reductions by metformin thus far. Moreover, recent studies that have used a time-varying approach of metformin exposure could not confirm lower risks of several types of cancer with use of metformin. In our study, patients who had used ≥1 non-insulin antidiabetic drug (NIAD) from 1998 through 2011 were included (N=57,621). Drug exposure was modelled time-varyingly using 90-day time intervals, with exposure to metformin or other NIADs classified as 'current use' or 'past use' based on the drug prescriptions prior to the start of each 90-day time interval. Time-dependent Cox regression analyses were used to estimate HRs of GI cancers in current metformin users versus current users of other NIADs, adjusted for various confounding variables. Furthermore, sensitivity analyses were performed using a new-user cohort of incident NIAD users only. We showed that current use of metformin was not associated with a decreased risk of GI cancer [HR, 0.97; 95% CI 0.82–1.15] or specific GI cancer sites. The sensitivity analyses yielded comparable results. Also, no dose-response trends were observed with increasing cumulative dose of metformin. In line with the recent evidence, we concluded that no decreased risk of GI cancer was present with current use of metformin compared with current use of other NIADs.

In Chapter 6 we determined the risk of any, acute, and chronic pancreatitis with the use of incretin agents (dipeptidyl peptidase-4 [DPP-4] inhibitors and glucagon-like peptide-1 receptor agonists [GLP-1RA]). A population-based cohort study was conducted using data from the UK CPRD during 2007 to 2012. A total of 182,428 patients with ≥1 NIAD prescription were included and matched to non-diabetic control subjects. Using Cox regression analysis, adjusted HRs of pancreatitis were estimated in incretin-users (N=28,370) compared with non-diabetic controls and with other NIAD users. Focusing on the comparison between incretin-users and users of other NIADs, we found that current incretin users had a 1.5-fold increased risk of any pancreatitis compared with other NIAD users (adjusted HR 1.47, 95% CI 1.06–2.04), but not of acute or chronic pancreatitis (adjusted HR 1.42, 95% CI 0.98–2.06 and adjusted HR 0.87, 95% CI 0.45–1.69 respectively). In incident current incretin users the risk of any and acute pancreatitis was doubled as compared with other NIAD users (adjusted HR 2.12, 95% CI 1.31–3.43 and adjusted HR 1.96, 95% CI 1.13–3.41), whereas there was no increased risk found for chronic pancreatitis (adjusted HR 1.24, 95% CI 0.44–3.50). Interestingly, there was an increased risk of acute pancreatitis in current users of DDP-4 inhibitors only (adjusted HR

1.59, 95% CI 1.05–2.40), suggesting that differences in the pharmacodynamics properties of different types of incretins may be important for the incretin–pancreatitis link. We showed in this study that the use of incretins was associated with an increased risk of pancreatitis. An association with chronic pancreatitis was not observed, possibly due to a low number of events. However, the evidence regarding the association between incretins and pancreatitis remains conflicting in the literature. Therefore, we believe that consequent monitoring of potential side effects by means of periodic safety update reports remains warranted.

In **Chapter 7**, by means of a letter to the editor, we commented on a pharmaco-epidemiological study by Chin-Hsiao Tseng published in the European Journal of Cancer in 2016.[1] The author investigated the risk of kidney cancer with use of metformin using a Taiwanese National Health Insurance reimbursement database. We noted that a significant selection bias may have occurred during the allocation process of individuals to the treatment group of never users of metformin. Patients using other antidiabetic drugs before they start with metformin were excluded from the study population (N=200,785). Therefore, possible follow-up time designated for the group of never users of metformin was wrongfully excluded from the analysis. We suggested Dr. C.-H. Tseng to reanalyse the risk of kidney cancer in users of metformin compared to non-users of metformin without the potential selection bias. Furthermore, in this comment it is also underlined that costly drug trials should be discouraged based on methodologically inaccurate studies.

In **Chapter 8**, we explored whether multiple high levels of glycosylated haemoglobin (HbA1c) over time impacts GI cancer risk in T2DM patients using a novel measure of hyperglycaemia called glycaemic burden (GB). In previous studies it has been shown that high levels of HbA1c are associated with an increased GI cancer risk in T2DM patients. However, these studies mainly use single, time-fixed measures of HbA1c as determinant in regression models, and therefore do not account for fluctuations in the level of hyperglycaemia over time. A cohort study was performed using the E-NCR-PHARMO database. All incident patients aged ≥30 years using ≥1 NIAD were included. All HbA1c measurements recorded between the first NIAD prescription and the end of follow-up were used to calculate GB. GB was based on the extent and duration that HbA1c values exceeded a threshold of 7% (53 mmol/mol) and was expressed as glycaemic burden years (GBY). The association between GBY and GI cancer was analysed using Cox regression analysis, with GBY entered in the regression model as a time-dependent categorical variable (no burden [0 GBY], >0-1.0 GBY, and >1.0 GBY). During >60,000 person-years of follow-up, 285 GI cancers were observed. Compared to patients with >0-1.0 GBY, patients in the 0 GBY category (no burden) had a decreased risk of GI cancer (HR 0.71, 95% CI 0.53-0.96), but patients with >1.0 GBY did not have a further increased

risk of GI cancer (HR 1.15, 95% CI 0.84-1.57). For hepato-pancreatico-biliary tract (HPB) cancer, however, increased levels of GBY were associated with an increased risk of cancer. This was the first study investigating the association between GB and GI cancer risk. Therefore, future studies are warranted to further investigate the clinical value of GB as determinant of cancer risk. Of note, residual confounding and selection bias may have influenced the results.

In **Chapter 9** the general discussion of this thesis is presented. Here the results of our studies are reflected upon in light of the currently available evidence. First, the general association between T2DM and GI cancer and its subsites is discussed in detail, while also looking at pathophysiological mechanisms and important confounders. We concluded that a diagnosis of T2DM is associated with a higher risk of GI cancer, mainly driven by an increased risk of HPB type cancers, but not by other GI cancer sites. However, residual confounding due to unmeasured or unaccounted risk factors may have influenced the results of our studies and those in the literature. Furthermore, detection bias is an important type of bias that may have inflated previously reported risk estimates of GI cancer risk in patients with T2DM, especially for pancreatic cancer. Also, it is still unclear how the latency time of cancer affects the association between T2DM and GI cancer. Second, focussing on the association between the use of anti-diabetic drugs and GI cancer, we discussed that the use of metformin was not associated with a decreased risk of GI cancer or its subsites. Therefore, clinical trials investigating the use of metformin as chemopreventive agent are not recommended. In contrast to more recent evidence, we found an increased risk of acute pancreatitis, but not of chronic pancreatitis, in users of incretins. In light of the mixed results and the fact that stratified results for acute and chronic pancreatitis have been reported for the first time in this thesis, additional studies on this association are warranted. Third, after examining the evidence on the association between hyperglycaemia and GI cancer risk, we concluded that glycaemic burden (the degree of hyperglycaemia over time) may be associated with a higher GI cancer risk. However, future studies are needed to replicate and build upon our initial findings. Overall, Dutch clinicians should be aware of the association between T2DM and GI cancer, especially liver, biliary tract, and pancreatic cancer, in particular in patients newly diagnosed with T2DM or who are inadequately controlled.

References

1. Tseng CH. Use of metformin and risk of kidney cancer in patients with type 2 diabetes. Eur J Cancer 2016;52:19-25.

Nederlandse samenvatting

Nederlandse samenvatting

Diabetes mellitus type 2 (DM2) en gastrointestinale (GI) tumoren vormen wereldwijd een steeds groter probleem. Zowel de incidentie als prevalentie van beide ziekten lopen op. In de afgelopen decennia is meer bewijs ontstaan voor een associatie tussen DM2 en het risico op de ontwikkeling van GI tumoren. Ook is gebleken dat het een zeer complexe associatie is, die wordt beïnvloed door diverse factoren. Deze factoren betreffen: pathofysiologische mechanismen (bijv. hyperglycaemie en hyperinsulinemie), vertekening van studieresultaten door de aanwezigheid van gezamenlijke risicofactoren (bijv. obesitas, onvoldoende lichaamsbeweging), medicatiegebruik (bijv. het gebruik van verschillende anti-diabetica) en variaties in onderzoeksmethodes, waardoor vertekening van resultaten kan optreden.

In dit proefschrift zijn meerdere aspecten van de complexe associatie tussen DM2 en GI tumoren onderzocht. Allereerst hebben we ons gericht op de invloed van detectiebias op de incidentie van, en het risico op GI tumoren (Hoofdstuk 2 en Hoofdstuk 3). Ook schonken we in dit deel van het proefschrift aandacht aan de vergelijkbaarheid van de populatie in de *Clinical Practice Research Datalink* (CPRD) uit het Verenigd Koninkrijk (VK) met de algehele Nederlandse bevolking (Hoofdstuk 4). Daarmee hebben we bewijs willen vinden voor de generaliseerbaarheid van resultaten uit CPRD-studies voor de Nederlandse bevolking. Ten tweede zijn de effecten van het gebruik van twee anti-diabetica onderzocht, respectievelijk in een studie naar het risico op GI tumoren bij gebruikers van metformine (Hoofdstuk 5) en in een onderzoek naar het risico op (acute en chronische) pancreatitis bij gebruik van incretines (Hoofdstuk 6). Daarnaast wierpen we een kritische blik op het optreden van selectiebias in farmaco-epidemiologisch onderzoek (Hoofdstuk 7). Ten derde is de associatie bekeken tussen het risico op GI tumoren en de mate van hyperglycaemie in de tijd. Hierbij hebben we gebruik gemaakt van een nieuwe variabele genaamd 'glycaemische last' (*Glycaemic burden;* Hoofdstuk 8).

Hoofdstuk 1 bevat de algemene introductie. Hierin staat de achtergrondinformatie over DM2 en GI tumoren beschreven, evenals de bewijslast uit voorgaand onderzoek betreffende hun onderlinge associatie, en de onderbouwing voor de studies in dit proefschrift. Tot op heden is gebleken dat de associatie tussen DM2 en GI tumoren complex is. Studieresultaten zijn inconsistent en worden beïnvloed door verschillende methodologische problemen en vertekening, waaronder *confounding* en detectiebias. In het verleden richtten observationele studies zich vooral op kanker in het algemeen, frequent voorkomende tumoren, of tumoren waarvan een sterke relatie al was beschreven in de literatuur. Voor meer zeldzame typen GI tumoren, zoals slokdarm- en maagkanker, is minder bewijslast te vinden in de literatuur. Derhalve zijn in dit proefschrift meerdere aspecten van de complexe associatie tussen DM2 en GI tumoren

onder de loep genomen, met speciale aandacht voor de rol van anti-diabetica, glucoseregulatie, en methodologische aspecten.

In Hoofdstuk 2 werd de incidentie (*incidence rate per 100.000 persoonsjaren; IR*) van GI tumoren berekend in een Brits cohort dat bestond uit 333.438 gebruikers van anti-diabetica (DM2 populatie) en eenzelfde aantal personen zonder DM2. De data werden verkregen van de CPRD database tussen 1988 en 2012. Patiënten met DM2 bleken hogere IR's te hebben voor leverkanker (IR 26, 95% betrouwbaarheidsinterval [BI] 24-28 vs. 8,9, 95% BI 7,7-10), alvleesklierkanker (IR 65, 95% BI 62-69 vs. 31, 95% BI 28-34) en dikkedarmkanker (IR 119, 95% BI 114-124 vs. 109, 95% BI 104-114) vergeleken met individuen zonder DM2. Daarentegen zagen we een lagere IR voor slokdarmkanker in de diabetespopulatie (IR 41, 95% BI 39-44 vs. 47, 95% BI 44-51). Vergelijkbare resultaten werden gezien in de sensitiviteitsanalyse waarbij het eerste jaar na start van follow-up werd geëxcludeerd uit de analyse. Door exclusie van het eerste follow-upjaar in deze sensitiviteitsanalyse werd de invloed van detectiebias op de IR verminderd. In geval van alvleesklierkanker leek detectiebias een rol te spelen. Bij exclusie van het eerste follow-upjaar verminderde de IR voor alvleesklierkanker in de diabetespopulatie namelijk van 65 naar 48. Echter, de IRs voor alvleesklierkanker bij patiënten met DM2 bleef statistisch significant hoger dan bij mensen zonder DM2.

In Hoofdstuk 3 werd het risico op de ontwikkeling van de verschillende GI tumoren geanalyseerd in nieuwe gebruikers van anti-diabetica (patiënten met DM2) ten opzichte van gematchte controlepatiënten zonder anti-diabetica (mensen zonder DM2). Hierbij werd gebruik gemaakt van de koppeling van de kankerregistratie van het IKNL Regio Zuid (voorheen bekend als Integraal Kankercentrum Zuid [IKZ]) aan het datanetwerk van het PHARMO Instituut, oftewel het IKZ-PHARMO cohort, tussen 1998 en 2011. Om de effecten van detectiebias op de associatie tussen DM2 en GI tumoren te analyseren werden Cox regressie analyses verricht met en zonder latente periode van 1 jaar (bijv. tumoren die gediagnosticeerd werden <1 jaar na de start van follow-up werden geëxcludeerd voor de berekening van het kankerrisico). In de analyse zonder latente periode hadden patiënten met DM2 een 50% verhoogd risico op de ontwikkeling van GI tumoren (*hazard ratio* [HR] 1,5, 95% BI 1,3-1,7) vergeleken met individuen zonder DM2. Dit risico verminderde naar 40% na toevoeging van een latente periode van 1 jaar in de analyse, waardoor mogelijke detectiebias afnam (HR 1,4, 95% BI 1,2-1,7). In de subanalyse gestratificeerd naar tumorlocatie vonden we statistisch significant verhoogde risico's voor alvleesklierkanker (HR 4,7, 95% BI 3,1-7,2), extrahepatische galwegkanker (HR 4,2, 95% BI 1,5-11,8) en distale dikkedarmkanker (HR 1,5, 95% BI 1,1-2,1) bij patiënten met DM2 vergeleken met mensen zonder DM2. Voor andere GI tumorsubtypen werden geen verhoogde risico's gevonden. Uit deze resultaten blijkt dat DM2 voornamelijk geassocieerd is met tumoren van alvleesklier- en galwegstelsel.

Bovendien konden we concluderen dat detectiebias een rol speelt bij de sterkte van de associatie ofwel de hoogte van het berekende risico of GI tumoren. Voor toekomstig onderzoek is het daarom van belang om altijd sensitiviteitsanalyses te verrichten waarin detectiebias zoveel mogelijk wordt beperkt.

In **Hoofdstuk 4** werden de leeftijds- en geslachtsverdeling van de CPRD database vergeleken met die van de Nederlandse bevolking. Het doel van deze studie was om een eerste stap te zetten om de toepasbaarheid van de CPRD database te beoordelen voor de Nederlandse populatie. Hiervoor werden voor het jaar 2011 de leeftijds- en geslachtsverdeling van de CPRD vergeleken met Nederlandse censusdata van de Statline database van het Centraal Bureau voor de Statistiek (CBS) en verschillen visueel en numeriek weergegeven. Voor zowel mannen als vrouwen bleek de leeftijdsverdeling tussen de twee databases vergelijkbaar. Verschillen in aantallen personen per leeftijdscategorie van 10% of meer kwamen enkel voor in hogere leeftijdsgroepen (75+ bij mannen en 80+ bij vrouwen). Met deze studie is een eerste stap gezet om te beoordelen of resultaten uit observationeel onderzoek met de CPRD database toepasbaar zijn op de Nederlandse bevolking. Hiermee zou CPRD een zeer behulpzame bron kunnen zijn voor het maken van beleidsplannen in Nederland. Echter, in geval van farmaco-epidemiologisch onderzoek blijft het van belang om verschillen in blootstelling aan medicijnen of voorschrijfgedrag van artsen mee te nemen. Dit kan namelijk een belangrijk effect hebben op de generaliseerbaarheid van studieresultaten. Desalniettemin hopen wij dat deze eerste studie onderzoekers uit andere landen met vergelijkbare zorgsystemen inspireert om dergelijke studies naar toepasbaarheid van databases te verrichten.

In **Hoofdstuk 5** werd een cohortstudie gepresenteerd, waarin het risico op GI tumoren bij gebruikers van metformine werd geëvalueerd. Voor deze studie werd opnieuw gebruik gemaakt van het IKZ-PHARMO cohort. Voorgaande onderzoeken hebben grote beschermende effecten van metformine laten zien op het GI tumorrisico. Echter, het is gebleken dat meerdere van deze studies te maken hadden met methodologische problemen zoals tijd-gerelateerde bias, waarbij blootstelling aan metformine inadequaat werd geclassificeerd. Deze vorm van bias beperkt de validiteit van de gevonden lagere tumorrisico's bij gebruikers van metformine. Recentere studies, waarin blootstelling aan metformine adequaat was geclassificeerd, hebben geen verlaagd tumorrisico kunnen vaststellen bij gebruikers van metformine. Voor onze studie hebben we patiënten geïncludeerd die ≥1 anti-diabeticum hadden gebruikt (exclusief insuline; *non-insulin antidiabetic drug* [NIAD]) tussen 1998 en 2011 (N=57.621). Blootstelling aan medicatie werd tijdsafhankelijk gemodelleerd door gebruik te maken van tijdsintervallen van 90 dagen. Blootstelling aan metformine of andere NIAD's werd geclassificeerd als 'huidig' of 'voorheen' door te kijken naar medicatierecepten voorafgaand aan elk 90-dagen

tijdsinterval. Tijd-afhankelijke Cox regressieanalyses werden toegepast om het risico op GI tumoren te berekenen bij huidige gebruikers van metformine vergeleken met huidige gebruikers van andere NIAD's, gecorrigeerd voor *confounders*. Daarnaast werden sensitiviteitsanalyses verricht waarbij gebruik werd gemaakt van een cohort van incidente (nieuwe) gebruikers van NIAD's (*new-user cohort*). Via deze aanpak konden we laten zien dat huidige gebruik van metformine niet geassocieerd was met een verlaagd risico op GI tumoren [HR 0,97; 95% BI 0,82-1,15]. De sensitiviteitsanalyses met het incidente NIAD cohort toonden vergelijkbare resultaten. Ook werd er geen relatie gevonden tussen het GI tumorrisico en de cumulatieve dosering van metformine. Met deze resultaten konden we concluderen dat gebruik van metformine niet geassocieerd was met een verlaagd GI tumorrisico, zoals ook andere recente studies lieten zien.

In **Hoofdstuk 6** werd het risico bepaald op het optreden van pancreatitis (alvleesklierontsteking) bij gebruik van incretines (dipeptidylpeptidase-4 [DPP-4] inhibitoren en *glucagon-like* peptide-1 receptor agonisten [GLP-1RAn]). Als uitkomstmaat werd zowel gekeken naar pancreatitis in het algemeen als acute en chronische pancreatitis. Er werd een cohortstudie uitgevoerd met data van de CPRD database van 2007 tot 2012. Alle patiënten met ≥1 voorschrift voor een NIAD werden geïncludeerd (N=182.428) en gematcht aan controlepatiënten zonder DM2. Via Cox regressieanalyse werden gecorrigeerde HR's berekend voor pancreatitis bij gebruikers van incretines (N=28.370) vergeleken met gebruikers van andere NIAD's. Huidige gebruikers van incretines hadden een verhoogd risico op pancreatitis in het algemeen (HR 1,47, 95% BI 1,06-2,04), maar geen verhoogd risico op acute of chronische pancreatitis (HR 1,42, 95% BI 0,98-2,06 en HR 0,87, 95% BI 0,45-1,69). Bij incidente, huidige gebruikers van incretines was het risico op pancreatitis in het algemeen en op acute pancreatitis tweemaal zo hoog in vergelijking met gebruikers van andere NIAD's (HR 2,12, 95% BI 1,31-3,43 en HR 1,96, 95% BI 1,13-3,41). Overigens was er een verhoogd risico op acute pancreatitis bij huidige gebruikers van enkel DPP-4 inhibitoren (HR 1,59, 95% BI 1,05-2,40) en niet bij GLP-1RAn, wat suggereert dat verschillen in farmacologische eigenschappen mogelijk een rol spelen bij de relatie tussen incretines en pancreatitis. Concluderend hebben we in deze studie laten zien dat gebruik van incretines geassocieerd is met het risico op pancreatitis. Een associatie met chronische pancreatitis werd niet gezien, mogelijk door een zeer laag aantal gevallen van chronische pancreatitis in de populatie. Echter, de literatuur over de associatie tussen incretines en pancreatitis is vooralsnog zeer verdeeld. Hierdoor adviseren wij dat het monitoren van mogelijke bijwerkingen van incretines via periodieke veiligheidsrapporten van belang blijft.

In **Hoofdstuk 7** gaven we via een ingezonden brief kritiek op een farmaco-epidemiologische studie van Chin-Hsiao Tseng die in 2016 werd gepubliceerd in het

European Journal of Cancer. Deze onderzoeker analyseerde het risico op nierkanker bij gebruikers van metformine met behulp van een Taiwanese nationale zorgverzekeringsdatabase. In het artikel viel ons op dat er mogelijk selectiebias was opgetreden tijdens het toewijzen van individuen aan specifieke behandelgroepen, namelijk de groep van personen die nooit metformine hebben gebruikt (*never users*). Personen met DM2 die andere anti-diabetica gebruikten vóórdat zij startten met het middel metformine werden geëxcludeerd van de studiepopulatie (N=200.785). Derhalve ging er mogelijk *follow-up* tijd verloren die was bestemd voor de groep *never users* van metformine. We hebben Dr. C.-H. Tseng daarom de suggestie gedaan om het risico op nierkanker bij gebruikers van metformine opnieuw te analyseren zonder deze bron van selectiebias. Verder sluit deze reactie op de studie van Dr. C.-H. Tseng aan op het debat over het feit dat dure medicijnproeven moeten worden afgeraden als het onderliggende bewijs methodologisch inaccuraat is.

In **Hoofdstuk 8** werd onderzocht of de mate van hyperglycaemie over de tijd geassocieerd was met een verhoogd risico op GI tumoren. Hoewel een hoog HbA1c-gehalte als maat voor langdurige hyperglycaemie in het verleden geassocieerd is met een verhoogd risico op GI tumoren bij DM2 patiënten, betrof dit meestal studies waarin enkelvoudige of statische variabelen met HbA1c werden gebruikt in de regressiemodellen. Daardoor hielden deze studies geen rekening met fluctuaties van het HbA1c-gehalte in de tijd. Om dit te kunnen analyseren werd een cohortstudie verricht met behulp van het IKZ-PHARMO cohort. Alle incidente gebruikers van NIAD's van 30 jaar of ouder werden geïncludeerd. De mate van hyperglycaemie in de tijd werd berekend tussen de start van de eerste NIAD en het einde van follow-up. Hiervoor werd een nieuwe variabele gebruikt, genaamd 'glycaemische last' (*glycaemic burden; GB*). De GB was gedefinieerd als de mate en de duur dat de HbA1c-waarde van een patiënt boven een drempelwaarde van 7% (53 mmol/mol) uitkwam. Om rekening te houden met verschillen in follow-upduur werd de GB gedeeld door het aantal jaren follow-up, resulterende in de variabele *glycaemic burden years* (GBY) die werd gebruikt in de regressiemodellen. De associatie tussen GBY en GI tumoren werd vervolgens geanalyseerd door middel van Cox regressieanalyse, met GBY als tijd-afhankelijke categorische variabele (geen glycaemische last [0 GBY], >0-1,0 GBY en >1,0 GBY). Tijdens meer dan 60.000 persoonsjaren aan follow-up werden 285 GI tumoren vastgesteld. Vergeleken met patiënten met >0-1,0 GBY hadden patiënten in de categorie 0 GBY (geen glycaemische last) een verlaagd risico op GI tumoren (HR 0,71, 95% BI 0,53-0,96). Daarentegen hadden patiënten in de categorie >1,0 GBY geen verhoogd risico op GI tumoren (HR 1,15, 95% BI 0,84-1,57). Voor HPB-type tumoren vonden we echter wel dat een hogere GBY categorie geassocieerd was met een hoger risico. Met deze studie waren wij de eersten die de associatie tussen GB en de ontwikkeling van GI tumoren

onderzochten. Wel konden onze resultaten onderhevig zijn aan selectiebias en *confounding*. Toekomstig onderzoek zal daarom verder moeten uitwijzen of GB een waardevolle toevoegde waarde heeft voor de klinische praktijk als maat voor GI tumorrisico.

In Hoofdstuk 9 werden de resultaten van onze studies geïnterpreteerd en gereflecteerd aan de hand van de huidige beschikbare literatuur. Allereerst werd de algemene associatie tussen T2DM en GI tumoren in detail besproken. Hierbij werd ook gekeken naar mogelijke pathofysiologische mechanismen en belangrijke *confounders.* We concludeerden dat een diagnose van DM2 geassocieerd is met een hoger risico op GI tumoren en dat dit verhoogde risico voornamelijk lijkt te worden gegenereerd door een hoger risico op HPB-type tumoren. Echter, studieresultaten kunnen onderhevig zijn aan enige vertekening indien voor belangrijke *confounding* factoren niet wordt gecorrigeerd in de analyses. Daarnaast is detectiebias een belangrijke vorm van bias die de hoogte van de gevonden risico's in voorgaande gepubliceerde studies mogelijk heeft verhoogd; dit geldt met name voor alvleesklierkanker. Verder is het op dit moment niet duidelijk welke invloed de latente periode van kanker precies heeft op de associatie tussen DM2 en GI tumoren. Ten tweede werd de associatie tussen anti-diabetica en het risico op GI tumoren besproken. We concludeerden dat metformine niet geassocieerd is met een lager GI tumorrisico, zoals in eerder onderzoek werd gesuggereerd. Om deze reden raden wij het opzetten van medicijnproeven met metformine als anti-tumormedicijn af. In tegenstelling tot recente literatuur, vonden wij bij gebruik van incretines een verhoogd risico op acute pancreatitis, maar niet op chronische pancreatitis. Gezien de tegenstrijdige resultaten in de literatuur, en het feit dat in onze studie voor het eerst gerapporteerd werd over chronische pancreatitis als uitkomstmaat, is verder onderzoek naar de associatie tussen pancreatitis en incretines van belang. Ten derde werd het bewijs voor een associatie tussen hyperglycaemie en GI tumorrisico opgesomd. Uit onze studie bleek dat de mate van hyperglycaemie in de tijd, uitgedrukt in GBY, mogelijk geassocieerd is met een hoger risico op GI tumoren. Echter, verder onderzoek is nodig om onze resultaten te repliceren en om voort te bouwen op onze eerste bevindingen. In het algemeen kunnen we vaststellen dat Nederlandse artsen zich bewust moeten zijn van de associatie tussen DM2 en GI tumoren. Opvallend zijn vooral de associaties tussen DM2 en HPB-type tumoren, met nieuw gediagnosticeerde DM2 en met inadequate glucoseregulatie.

Referenties

1. Tseng CH. Use of metformin and risk of kidney cancer in patients with type 2 diabetes. Eur J Cancer 2016;52:19-25.

Valorisation

Valorisation

Value for society

Over the past decades a rise in the incidence and prevalence of both type 2 diabetes mellitus (T2DM) and gastrointestinal (GI) cancer has been observed. Determining whether T2DM is an important risk factor for the development of GI cancer - and to what degree - helps governmental and medical institutions decide if T2DM is a possible target for cancer prevention or if patients with T2DM form a population that needs targeted (GI) cancer screening. In this thesis, we investigated the complex association between T2DM and GI cancer, focussing on the influence of a variety of factors on the association. In **Chapter 2** we found higher incidence rates of liver, pancreatic and colon cancer in patients with T2DM compared with individuals without T2DM. In **Chapter 3**, a 40% increased risk of GI cancer was found, which seemed mainly driven by increased risks of hepato-pancreatico-biliary type cancers. In both chapters, evidence of detection bias influencing the strength of the association was seen. The results of these studies help to determine the strength of the association between T2DM and GI cancer, which can be used for future scientists and policymakers to decide whether targeted cancer screening of individuals with T2DM is needed.

In the past, multiple studies have pointed to lower risks of (GI) cancers in users of metformin compared to users of other anti-diabetic drugs. The results of these studies formed the basis for the launch of numerous drug trials to investigate the possibility of repurposing metformin as globally used first-line anti-diabetic drug to a more chemotherapeutic agent. There are currently over 100 clinical drug trials being conducted investigating the effect of metformin as therapeutic agent in the treatment of various cancers (www.clinicaltrials.gov; accessed on April 11, 2019). However, evidence has come to light that many previous studies on the risk of cancer with use of metformin have been afflicted by time-related biases, thereby inflating the protective effects of metformin on cancer development. More recent studies and our study presented in **Chapter 5**, using a time-dependent definition of drug exposure in order to minimize time-related bias, showed no differences in risk of GI cancers in users of metformin compared to users of other non-insulin anti-diabetic drugs. Therefore, our results add to the debate whether performing costly drug trials is justified when purely based on (biased) information from observational studies. We recommend to replicate well-designed pharmaco-epidemiological studies with minimal time-related bias to gain more robust evidence that indeed no association is present between the use of metformin and (GI) cancer risk.

Value for professionals

Investigating complications of type 2 diabetes mellitus, including the risk for cancer development, is important for clinical practice. Generally, clinicians managing patients with T2DM are aware of the development of diabetic retinopathy, nephropathy, and neuropathy. If future research shows more concrete evidence of a causal biological link between T2DM and development of cancer, then clinicians can be more aware of this additional serious complication. Based on the evidence presented in this thesis, an association between T2DM and GI cancer is present, with a 40% increased risk of GI cancer in the T2DM population versus the non-diabetic population (**Chapter 2**). However, a causal link could not be proven with the studies in this thesis, and detection bias (**Chapters 2** and **3**) and residual confounding due to unmeasured confounding variables may be present. Furthermore, in **Chapter 8**, a lower risk of GI cancer was found in patients with T2DM without glycaemic burden compared to patients with up to one year of glycaemic burden. Although future studies are needed to confirm these findings, our results indicate that more stringent glycaemic control in patients with T2DM may be beneficial by reducing the risk of GI cancer.

Value for future research

Most of the research in this thesis is part of an endeavour to learn more about the complex association between T2DM and GI cancer. First, the evidence presented in Chapters 2 through 9 will help to develop future research on the link between T2DM and GI cancer. For example, it is important to perform sensitivity analyses that include one or more years of lag period between the (assumed) onset of T2DM and the diagnosis of a (GI) cancer in order to minimize detection bias (**Chapter 2**). Furthermore, when investigating the association between (anti-diabetic) drugs and (GI) cancer, it is important to use a time-dependent covariate of drug exposure in order to capture variations in drug exposure over time (**Chapters 3** and **6**).

Second, in **Chapter 4** we showed that the age- and sex distribution of the Clinical Practice Research Datalink (CPRD) is comparable to that of the total Dutch population. Investigating the generalizability of populations contained in large population-based databases will help translating study results to other populations. The results of our study can encourage scientists from other countries with similar healthcare systems to perform studies of CPRD representativeness.

Third, we were the first to investigate the association between a novel marker of hyperglycaemia of time, called 'glycaemic burden', and the risk of GI cancer (**Chapter 8**). As this is the first study more research is needed to confirm our findings and build on evidence that is currently presented.

Finally, based on our data no causal link between T2DM and GI cancer can be established. As the underlying pathophysiological mechanisms between T2DM and GI cancer remain to be elucidated, more basic research focusing on mechanistic aspects is needed.

In summary, while an association between T2DM and GI cancer has been clearly established, much is to be learned about their causal relationship and biological mechanisms that lead up to it. The results of the studies in this thesis will help develop future (observational) research on the link between T2DM and GI cancer and encourage other scientist to study the complex association.

List of publications

List of publications

Roy G.P.J. de Jong, Johannes T.H. Nielen, Ad A.M. Masclee, Maryska L.G. Janssen-Heijnen, Frank de Vries. Comments on "Use of metformin and risk of kidney cancer in patients with type 2 diabetes", Chin-Hsiao Tseng, Eur J Cancer 2016;52:19-25. European Journal of Cancer 2016;61:157-8

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Lotte M. Knapen, **Roy G.P.J. de Jong**, Johanna H.M. Driessen, Yolande C. Keulemans, Nielka P. van Erp, Marie L. De Bruin, Hubert G.M. Leufkens, Sander Croes, Frank de Vries. Use of incretin agents and risk of acute and chronic pancreatitis: A population-based cohort study. Diabetes Obesity and Metabolism 2017;19:401-411

Roy G.P.J. de Jong, Andrea M. Burden, Sander de Kort, Myrthe P.P. van Herk-Sukel, Pauline A.J. Vissers, Paddy K.C. Janssen, Harm R. Haak, Ad A. Masclee, Frank de Vries, Maryska L.G. Janssen-Heijnen. No decreased risk of gastrointestinal cancers in users of Metformin in the Netherlands; a time-varying analysis of metformin exposure. Cancer Prevention Research 2017;10:290-297

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Book chapters

R.G.P.J. de Jong en Y.C.A. Keulemans. Buikpijn. In A.J.R. Balthasar, J. Bergsma, M.A. Siemonsma, red. Probleemgeöriënteerd denken in de pijngeneeskunde. Utrecht: De Tijdstroom, 2017. p 33-41

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Dankwoord

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Roy
Roermond, April 2019

Curriculum vitae

Curriculum vitae

Ronaldus Gerardus Petrus Jacobus (Roy) de Jong was born on the 11th of January 1990 in Roosendaal, The Netherlands. He grew up in the village of Kruisland, where he finished primary school. After graduation from the Jan Tinbergen College in Roosendaal in 2008, he started medical school at the Maastricht University, Faculty of Health, Medicine, and Life Sciences. During medical school he attended clinical and scientific traineeships at the division of Gastroenterology and Hepatology, department of Internal Medicine, Maastricht University Medical Centre+ (MUMC+). For his scientific traineeship, Roy studied aspects of pain in patients with chronic pancreatitis, and contributed a chapter on pain in chronic pancreatitis to the Dutch textbook 'Problem-based thinking in the specialty of pain medicine' (*Probleemgeöriënteerd denken in de pijngeneeskunde*), under supervision of Dr. Y.C.A. Keulemans. After obtaining his medical degree in 2014, he started his PhD affiliated to the department of Internal Medicine, VieCuri Medical Centre, to the division of Gastroenterology and Hepatology, MUMC+, and to GROW, School for Oncology and Developmental Biology, Maastricht University, under the supervision of Prof. dr. A.A.M. Masclee, Prof. dr. M.L.G. Janssen-Heijnen, and Dr. F. de Vries. In October 2016, Roy started his residency in gastroenterology and hepatology at the department of Internal Medicine of VieCuri Medical Centre. He is currently working as a resident in gastroenterology in Zuyderland Medical Centre under supervision of Dr. Y.C.A. Keulemans. Roy is married to Anne and they live together in Roermond with their daughter Sophie.