Pharmaco-epidemiology as a Tool in Pharmacovigilance: Studying cancer as adverse drug reaction

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The work described in this thesis was conducted at the Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands and the Drug Safety Unit of the Dutch Health Care Inspectorate, the Hague, the Netherlands.

The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NOW), the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, The European Commission (DG XII), the Netherlands Genomics Initiative (NGI) and the Municipality of Rotterdam.

The contribution of the inhabitants, general practitioners and pharmacists of the Ommoord district to the Rotterdam Study are gratefully acknowledged.

The publication of this thesis was financially supported by the Department of Epidemiology, Erasmus Medical Center and the Health Care Inspectorate. Daarnaast wordt de bijdrage van het Nederlands Bijwerkingen Fonds zeer gewaardeerd.

Lay-out and Printed by Optima Grafische Communicatie, Rotterdam (www.ogc.nl)
Cover photograph by Elmira Torabi

ISBN 978-94-6169-162-0

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Farmaco-epidemiologie als instrument in de farmacovigilantie:
het bestuderen van kanker als bijwerking van een geneesmiddel

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam

op gezag van de
rector magnificus

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 1 februari 2012 om 15.30 uur

door

Tanneke Rikje Ruiter
geboren te Wageningen

Promotiecommissie

Promotores:	Prof.dr. B.H.Ch. Stricker
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Chapter 1

General introduction: introduction, outline and aim of the thesis

INTRODUCTION

Pharmacovigilance is defined by the World Health Organization (WHO) as the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problem. [1] There has been concern about the safety of medicines since the discovery of congenital abnormalities in babies delivered by women who had taken thalidomide during pregnancy in 1961. [2] An adverse drug reaction is defined as a response to a medicinal product which is noxious and unintended and which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease or for the restoration or modification of physiological function. Two types of adverse drug reactions are distinguished: type A reactions which are predictable from the known pharmacology of the medical substance and which are dose-dependent, and type B reactions which are idiosyncratic and unpredictable. [3] It is clear that this is a very crude distinction which is, however, useful from the point of view of discovering unknown adverse reactions as early as possible.

During drug development, the efficacy and safety of the active substance is investigated in clinical trials in a relatively small selected homogenous patient population, during a limited period of time. As around 80% of the adverse drug reactions is estimated to be of type A, a large number of the potential adverse drug reactions is documented during the clinical phase. [4] After regulatory review and approval, during which all available information is reviewed, the marketing phase starts. Through marketing, the product is available for the entire population which is obviously far more heterogeneous than the study population. In contrast to the limited timeframe available during the clinical drug development phase, the post-marketing phase continues until the drug is withdrawn from the market. As a consequence, previously unknown adverse drug reactions might come to light, especially those of type B. Therefore, the obligation for the marketing authorization holder, as well as for regulatory authorities, for the continuous evaluation of safety and efficacy during the post marketing phase of a drug, have been legally laid down. [5-7]

Spontaneous reporting of an adverse drug reaction by a health care professional or a consumer is one of the most important sources of information. The marketing authorization holder has the responsibility to collect, evaluate and collate these reports. Serious adverse events (i.e. those which result in death, are life-threatening, require a hospitalization or cause a prolongation of an existing hospitalization, result in persistent significant disability or incapacity, which are congenital anomalies/birth defects, or are otherwise medically significant) need to be forwarded to the competent authorities within 15 calendar days. Although the health care professional has a legal obligation to report potential serious adverse drug reactions, a vast amount of under-reporting exists. [8-9] Under-reporting tends to be selective, as the mild and better-known adverse effects

are less well reported than the serious ones. [10] However, before an adverse drug reaction can be reported, it needs to be recognized. Recognition of an adverse drug reaction can be difficult when the association between the drug and the adverse drug reaction is less well-known, when the background incidence is high, when the attributable proportion is low, or when, for example, the timeframe between first exposure to the drug and the occurrence of the adverse drug reaction is long. Especially the recognition of cancer as a potential adverse drug reaction, if the association is not known, might be underestimated by health care professionals. In addition, since the timeframe between the onset of cancer and its diagnosis (the latent period) might already be several years, the timeframe between start of drug exposure and the diagnosis of cancer (induction period + latent period) might be even longer. As a consequence, evaluating potential safety signals as cancer, based solely on the reporting of adverse events is insufficient and additional measures to evaluate the risk of cancer in drug safety are required.

Post-authorization safety studies, either non-interventional (pharmaco-epidemiological) or interventional (clinical trials), are conducted with the aim of identifying or quantifying a safety hazard related to an authorized medicinal product. A post-authorization clinical trial may be set up to obtain more information on use of a drug in a specific patient population. However, the majority of the post-authorization safety studies is observational and employs pharmaco-epidemiological designs such as case-control or cohort studies. In these pharmaco-epidemiological studies, the determinant of interest is the use of a specified drug. Epidemiology has been of great value in assessing unexpected and unpredictable adverse effects such as smoking and lung cancer, as well as asbestos and mesothelioma. As adverse drug reactions are generally unintended and unpredictable, it has been reasoned that, when taking into account several prerequisites, observational studies can be as credible as randomized controlled trials. [11] To Strengthen the Reporting of Observational Studies in Epidemiology, these fundamentals have been laid down in the STROBE statement. [12]

Since population-based cohort studies often include a large number of participants who are followed over a significant period of time, the opportunity to assess the association between specified drugs and cancer as adverse drug reaction is present. However, as the effect of the drug may vary over time, may be dose-dependent and may be influenced by numerous other factors, such as, for example, genotype, the assessment of the association between drugs and cancer as potential outcome remains a challenge.

In this thesis, several pharmaco-epidemiological cohort studies are presented, describing the association between drug exposure and the occurrence of cancer as adverse drug reaction.

Outline and aim

The aim of this thesis was to gain more insight into the occurrence of cancer as potential adverse drug reaction for the effective assessment of the risk-benefit profile of medicines by performing pharmaco-epidemiological studies and to verify whether these pharmaco-epidemiological studies are indeed helpful in assessing cancer as adverse drug reaction. To this end, we studied cancer as potential adverse reaction to drugs that are frequently used in certain patient groups.

The overall extent to which hospitalizations in the Netherlands are related to adverse events, and their nature, is described in **chapter 2**. In this study, a Dutch nationwide registry of hospital discharges was used. [13] The information on hospitalizations related to an adverse drug reaction was combined with data on dispensed medicinal products in the Netherlands which were obtained from the Dutch Foundation for Pharmaceutical Statistics (SFK). [14]

In **chapter 3,** we describe cancer as adverse drug reaction in patients with diabetes mellitus using insulin glargine and metformin, respectively. These drugs were chosen as both drugs were associated with cancer previously. Insulin glargine has been an issue of debate since 2009 when several reports suggested an increased risk of cancer in participants who used insulin glargine. [15-18] With regard to metformin, the opposite was hypothesized when metformin was suggested to be associated with a decreased risk of cancer. [16, 19-22] In the two studies we performed, associations were analyzed using data from the PHARMO Record Linkage System (RLS). This source includes drug dispensing records from community pharmacies linked on patient level to hospital discharge records from the Dutch National Medical Register [13] concerning approximately 2.5 million individuals in the Netherlands since 1986. [23]

The Rotterdam Study was used to study the effect of drugs, genotype and their interaction in breast cancer patients, as well as the risk of basal cell carcinoma in patients using high-ceiling diuretics. The objectives and design were extensively described earlier. [24-27] In short, the Rotterdam Study, a large prospective population-based follow-up study, was started in 1990. Coverage of prescription-only drugs from pharmacies has been established, as well as the collection of data with regard to morbidity and mortality. In **chapter 4.1**, we describe the potentially modifying effect of the *cyclooxygenase* (*COX)* genotype on the use of non-steroidal anti-inflammatory drugs (NSAIDs) and the risk of breast cancer in a female postmenopausal population. COX-2 enzyme over-expression has been observed in breast cancer tissue and NSAIDs are known to inhibit the synthesis of COX. [28] The association between use of NSAIDs and the risk of breast cancer, as well as the association between *COX*-genotype and the risk of breast cancer have been described extensively. [29-30] However, little is known about the potential interaction between NSAIDs and *COX*-genotype and the risk of breast cancer. [31] In **chapter 4.2** we describe the potentially modifying effect of *CYP2C19*2* and **3* genotype on breast cancer survival

in patients using tamoxifen. Tamoxifen, a drug used for the treatment of breast cancer, is a pro-drug, which is metabolized to its active metabolites by enzymes in cytochrome P450, among which enzymes encoded by the genes *CYP2D6* and *CYP2C19*. [32-33]

In **chapter 5** the results of an analysis of the association between basal cell carcinoma (BCC) and the use of photosensitizing diuretic agents are presented. Despite the photosensitizing abilities of diuretic agents, little is known about a possible association between these frequently used drugs and the risk of BCC. [34-35]

A reflection on the main results from the studies presented in this thesis, as well as a critical appraisal of several methodological issues (e.g., quantifying drug exposure) and future implications can be found in **chapter 6**. Furthermore, we discuss whether pharmaco-epidemiological studies are indeed helpful in assessing the potential of cancer as adverse drug reaction.

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Chapter 2

Trends in adverse drug reaction related hospitalizations in persons aged 55 years and over: a population-based study in the Netherlands

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ABSTRACT

Introduction: elderly appear to be particularly at risk of developing adverse drug reactions (ADRs). The objective of this study was to describe the trends in the incidence of ADR-related hospitalizations, and their nature, over the period 2000 – 2005 in persons aged 55 years and over in the Netherlands and to correlate these ADR-related hospitalizations to the dispensed medicines over the same period.

Methods: data on hospital admissions were obtained from the Dutch nationwide registry of hospital discharges. Data on dispensed medicinal products were obtained from the Dutch Foundation for Pharmaceutical Statistics. Analyses were performed using binary logistic regression and by calculating relative risks.

Results: overall, 26,852 (1.3%) of the 2,127,133 acute, non-planned hospital admissions were attributable to an ADR. When taking into account the number of dispensings, elderly above 75 years were at a statistically significantly increased risk of being hospitalized compared to those 55 – 75 years old with regard to an ADR due to anticoagulants (RR 2.20, 95% CI 2.12 – 2.28), antidiabetic agents (RR 3.53, 95% CI 3.39 – 3.66), salicylates (RR 1.70, 95% CI 1.54 – 1.86) and antirheumatics (RR 2.19, 95% CI 2.06 – 2.33).

Conclusion: in our study, we showed that the elderly above 75 years were at increased risk of being hospitalized for an ADR. Given that it has been estimated that the number of those aged 65 years and over will continue to grow, it is of pivotal importance to further endorse the drug safety in this vulnerable patient group.

INTRODUCTION

An adverse drug reaction (ADR) is a response to a medicinal product which is noxious and unintended and which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease or for the restoration or modification of physiological function. [1] Elderly appear to be particularly at risk of developing ADRs. [2-5] Polypharmacy is more common among elderly and it has been shown that the risk of an ADR is related to the number of drugs prescribed. [6] In addition, renal and/or hepatic function impairment increases with age and thus the potential metabolism and elimination of drugs decreases; as a consequence, drug dose often needs to be adapted in elderly. In addition to age-related pharmacokinetic changes, pharmacodynamic variations may occur as well in elderly patients, increasing or decreasing the sensitivity to a drug. [7] Furthermore, elderly suffer more frequently from substantial co-morbidity, which can influence the pharmacokinetics and pharmacodynamics as well. [7]

Information on adverse drug reactions among elderly is limited as the homogenous population in randomized controlled clinical trials in which new medicines are tested does not represent the heterogeneous population in which the medicine is used after marketing. [8] Especially the elderly population, generally not included in clinical trials, differs from the homogeneous population in which efficacy and safety of active substances is tested initially. In addition, clinical trials are not suitable to assess the drug safety profile completely, due to the small sample size and the limited amount of follow-up time. [8]

In the Netherlands, in 2008, the life expectancy at birth was 76.7 years for men and 82.0 for women; in 2040 it has been estimated to further increase to respectively 82.7 and 85.7 years. [9-10] Likewise, the number of those aged 65 years and over will grow between 2010 and 2040 from 2.4 to 4.6 million. [10] As the contribution of the elderly to the total population increases, the number of ADR-related hospitalizations is expected to increase as well. In line with this consideration, a population-based Dutch study reported that the number of ADR-related hospitalizations in older persons in the Netherlands has increased rapidly since 1981, but temporized during the years 1997 – 2007. [11] However, the authors of this study did not take into account the number of medicines used, which may differ across age groups. [11]

Our objective was to describe the trends in incidence, and the nature of ADR-related hospitalizations over the period 2000 – 2005 in persons aged 55 years and over in the Netherlands and to correlate this number to the amount of dispensed medicines over the same period.

METHODS

Setting

Data on hospital admissions were obtained from the Dutch nationwide registry of hospital discharges. [12] The hospital record database contains detailed information concerning dates of admission and discharge, primary and secondary discharge diagnoses, urgency of admission, as well as special codes indicating drug-related hospitalizations. All diagnoses are coded according to the International Classification of Disease, ninth edition (ICD-9). [13] Characteristics of hospital admissions are coded by professional code clerks on the basis of hospital discharge letters and are coded independently of reimbursement. For every admission, the main discharge diagnosis is mandatory and up to nine additional diagnoses are optional.

Data on dispensed medicinal products in this population-based study were obtained from the Dutch Foundation for Pharmaceutical Statistics (SFK). [14] Since 1990, the SFK collects dispensing data from more than 90% of the 1,900 community pharmacies in the Netherlands. The total number of dispensings per year was available on pharmacological subgroup level of the Anatomic Therapeutical Chemical code (ATC). [15] Data were available in four fixed predefined age categories (55-64, 65-69, 70-74 and over 75 years of age).

Outcome

For this study, all patients older than 55 years with an acute, non-planned admission to a Dutch hospital in the period between 2000 and 2005 were included. An ADR-related hospitalization was defined as an acute, non-planned hospital admission with an E-code between 930 and 949 as secondary diagnosis. [13] E-codes are supplementary to the main discharge diagnosis, and the numbers E930 – E949, as first auxiliary code next to the main diagnosis, are indicating an ADR as the main diagnosis during hospitalization. The E-code is indicative of the drug group involved in the ADR. E-codes referring to intended overdoses, errors in administration and therapeutic failure were not included in the analysis. In addition to this, there were eleven main diagnoses with an ICD code specifically indicating an ADR; these were included as well in the outcome definition: 244.3 (other iatrogenic hypothyroidism), 251.0 (hypoglycemic coma), 323.5 (encephalitis, myelitis, and encephalomyelitis following immunization procedures), 336.8 (other myelopathy, drug induced or radiation induced myelopathy), 357.6 (polyneuropathy due to drugs), 422.9 (other and unspecified acute myocarditis, toxic myocarditis), 573.3 (hepatitis unspecified; toxic (non-infectious) hepatitis), 692.3 (contact dermatitis and other eczema due to drugs and medicines in contact with skin), 693.0 (dermatitis due to substances taken internally; due to drugs and medicaments), 995.2 (Other and unspecified adverse

effect of drug, medicinal and biological substance (due) to correct medicinal substance properly administered) and 995.4 (shock due to anesthesia). [13]

Statistical analysis

Hospitalizations concerning adverse drug reactions were identified overall and for separate age categories. Age-categories were based on the fixed classification of the available prescription data (55-64, 65-69, 70-74 and more than 75 years of age). Binary logistic regression analyses were performed to assess the effect of age, sex and year of hospitalization on the risk of an ADR-related hospitalization.

The ten drug groups most frequently involved in ADR-related hospitalizations were further analyzed. In detail: the E-codes referring to hospitalizations concerning an ADR and indicating specific drug groups were matched to ATC codes on ATC-3 level referring to specific active substances. Within each drug group, relative risks were calculated for each age category taking into account the total number of prescriptions dispensed, with the lowest age category as the reference. Analyses were performed using SPSS software (version 17.0, IBM, US) and Microsoft Office Excel 2003. *P*-values were considered statistically significant if < 0.05.

RESULTS

In the period between 2000 and 2005, a total of 2,127,133 acute, non-planned admissions of persons older than 55 years of age occurred in the Netherlands. The available baseline characteristics are presented in **table 1**. The majority of all hospital admissions occurred in the group aged 75 years and over (45.7%). The number of hospital admissions increased over the study period, from 323,887 admissions in 2000 to 400,243 in 2005. Overall, 26,852 (1.3%) hospitalizations were attributed to an ADR (25,775 hospitalizations were identified through an E-code, and 1,077 through a main discharge code indicating a drug-induced reaction). The percentage hospitalizations attributed to an ADR was stable (≈1.3% per year) during the study period.

Age was found to be a risk factor for an ADR-related hospitalization (unadjusted odds ratio (OR) 1.07, 95% CI 1.05 – 1.08). In addition, female sex was associated with an increased risk of an ADR-related hospitalization (OR 1.29, 95% CI 1.26 – 1.32) in comparison with men. The effect of age on the risk of an ADR-related hospitalization was modified by sex (*p*-value for interaction <0.001). Women aged over 75 years had a 58% higher risk of an ADR-related hospitalization than men aged 55 – 64 years **(table 2)**.

The effect of age on the risk of an ADR-related hospitalization was modified by the calendar year of hospitalization as well (*p*-value for interaction <0.001). The age dependent risk of an ADR-related hospitalization decreased statistically significantly during

Table 1: Baseline characteristics of the acute, non planned hospital admissions in the Netherlands of persons aged 55 years and over during the period 2000 – 2005

Baseline characteristic		*n* (%)
Total number of admissions		2,127,133
Mean age in years (SD)		72.9 (9.9)
Female sex		1,055,608 (49.6)
ADR-related admissions		26,852 (1.3)
Number of admissions per age category	55 – 64	503,569 (23.7)
	65 – 69	297,443 (14.0)
	70 – 74	354,214 (16.7)
	> 75	971,907 (45.7)
Calendar year of admission	2000	323,887 (15.2)
	2001	315,813 (14.8)
	2002	339,735 (16.0)
	2003	363,960 (17.1)
	2004	383,495 (18.0)
	2005	400,243 (18.8)

Abbreviations: *n*: number.

Table 2: Univariate analyses of age and sex as risk factors for an ADR-related hospitalization

		OR	95% CI
Age (per year)		1.07	1.05 – 1.08
Female sex (reference: male)		1.29	1.26 – 1.32
Risk of an ADR stratified for age and sex	Men, 55 – 64	Reference	
	Men, 65 – 69	1.21	1.14 – 1.28
	Men, 70 – 74	1.27	1.20 – 1.35
	Men, > 75	1.28	1.22 – 1.34
	Women, 55 – 64	1.47	1.39 – 1.55
	Women, 65 – 69	1.47	1.38 – 1.56
	Women, 70 – 74	1.54	1.46 – 1.63
	Women, > 75	1.58	1.51 – 1.65

Abbreviations: OR: odds ratio, CI: confidence interval.

the study period, with those with older age having an increased risk of hospitalization of 1.12 (95% CI 1.09 – 1.15) in 2000 decreasing to a statistically non-significant risk of 1.01 in 2005 (95% CI 0.99 – 1.04). The results for different age categories, stratified for sex and calendar year of admission, are presented in **table 3**.

Although the risk of an ADR-related hospitalization generally increased with age, the risk of an ADR-related hospitalization when aged over 75 years did not differ statistically significantly from the risk when aged 55 – 64 years old. For 25,775 hospitalizations, an

Table 3: Age specific risk of an ADR-related hospitalization stratified for sex and calendar year of admission with reference to those aged 55 – 64 years

		65 – 69		70 – 74		≥ 75	
		OR	95% CI	OR	95% CI	OR	95% CI
Men	2000	1.25	1.07 – 1.46	1.33	1.14 – 1.54	1.46	1.29 – 1.66
	2001	1.33	1.14 – 1.55	1.39	1.20 – 1.61	1.45	1.28 – 1.65
	2002	1.14	0.99 – 1.32	1.29	1.13 – 1.48	1.30	1.16 – 1.46
	2003	1.19	1.03 – 1.37	1.24	1.08 – 1.42	1.25	1.11 – 1.40
	2004	1.24	1.09 – 1.41	1.24	1.09 – 1.41	1.19	1.07 – 1.33
	2005	1.15	1.00 – 1.32	1.22	1.07 – 1.39	1.16	1.04 – 1.29
Women	2000	1.16	0.99 – 1.36	1.28	1.11 – 1.49	1.30	1.15 – 1.47
	2001	1.03	0.88 – 1.21	1.02	0.88 – 1.18	1.18	1.05 – 1.32
	2002	1.07	0.92 – 1.25	1.09	0.94 – 1.25	1.24	1.11 – 1.38
	2003	0.88	0.76 – 1.02	0.99	0.87 – 1.13	1.05	0.95 – 1.17
	2004	0.95	0.83 – 1.09	1.06	0.94 – 1.20	0.96	0.87 – 1.06
	2005	0.99	0.87 – 1.14	0.97	0.86 – 1.10	0.89	0.81 – 0.98

Abbreviations: OR: odds ratio, CI: confidence interval.

E-code was supplemented and the age-related risk of a hospitalization concerning an ADR could be calculated relative to the number of prescriptions dispensed in the respective drug category. As can be seen from **table 4,** elderly above 75 years of age were at a statistically significantly increased risk of being hospitalized compared to those younger than 75 years with regard to an ADR concerning anticoagulants (RR 2.20, 95% CI 2.12 – 2.28), insulins and antidiabetic agents (RR 3.53, 95% CI 3.39 – 3.66), salicylates (RR 1.70, 95% CI 1.54 – 1.86) and antirheumatics (RR 2.19, 95% CI 2.06 – 2.33). In contrast, those aged above 75 years of age were at a statistically significantly decreased risk of an ADR-related hospitalization concerning antineoplastic and immunosuppressive drugs (RR 0.34, 95% CI 0.26 – 0.42).

With regard to insulin and antidiabetic agents, the most frequently occurring presentation of an ADR-related hospitalization was an unspecified hypoglycemia (81%) or a hypoglycemic coma (8%). For antineoplastic and immunosuppressive drugs, the most frequently occurring ADR-related hospitalization was a hospitalization concerning fever (27%) or neutropenia (17%). Constipation (29%) and unspecified intestinal obstruction (13%) were the most frequently occurring ADR-related hospitalizations for opiates and related narcotics; volume depletion (31%) and hyposmolality and/or hyponatremia (31%) were the most common ADR-related hospitalizations for high-ceiling diuretics. With regard to cardiotonic glycosides and drugs of similar action, the most frequently occurring presentation of an ADR-related hospitalization was poisoning (42%) followed by unspecified adverse effects of a drug (29%). For other antihypertensive agents, angioneurotic edema (21%) and 'other specified cardiac dysrhythmias' (15%) were most

Table 4: Relative risks for the ten most frequent hospitalizations concerning an ADR relative to the total number of prescriptions dispensed

ICD code explanation (corresponding E-code)	ATC code	n	Age category										
			55 – 64 years		65 – 69 years			70 – 74 years			> 75 years		
			IR	Ref-erence	IR	RR	95% CI	IR	RR	95% CI	IR	RR	95% CI
Anticoagulants (9342)	B01A	6133	13	1	19	1.49	1.39 – 1.59	21	1.63	1.54 – 1.73	28	2.20	2.12 – 2.28
Antineoplastic and immunosuppressive drugs (9331)	L	4760	253	1	273	1.08	1.00 – 1.15	258	1.02	0.95 – 1.10	86	0.34	0.26 – 0.42
Insulins and antidiabetic agents (9323)	A10	1832	5	1	6	1.37	1.18 – 1.56	10	2.18	2.02 – 2.35	16	3.53	3.39 – 3.66
High ceiling diuretics (9444)	C03C	1518	12	1	12	1.05	0.79 – 1.30	16	1.37	1.16 – 1.59	15	1.24	1.06 – 1.42
Salicylates [a] (9353)	B01A, N02B	1286	2	1	3	1.23	1.01 – 1.44	4	1.52	1.33 – 1.71	4	1.70	1.54 – 1.86
Antirheumatics (9356)	M01, M02	1227	4	1	5	1.24	1.05 – 1.43	7	1.66	1.49 – 1.84	10	2.19	2.06 – 2.33
Cardiotonic glycosides and drugs of similar action [b] (9421)	C01A	787	14	1	22	1.56	1.16 – 1.96	25	1.84	1.48 – 2.20	20	1.45	1.13 – 1.78
Other opiates and related narcotics [c] (9352)	N02A	671	7	1	10	1.47	1.21 – 1.73	14	1.89	1.65 – 2.14	13	1.88	1.68 – 2.08
Other antihypertensives [d] (9426)	C02A, C02C	538	44	1	54	1.22	0.94 – 1.50	63	1.44	1.18 – 1.69	58	1.32	1.10 – 1.54
Adrenal cortical steroids [e] (9320)	H02A, H02B	507	9	1	10	1.09	0.79 – 1.39	12	1.37	1.10 – 1.64	11	1.20	0.97 – 1.42

Abbreviations: IR: incidence of hospital admissions concerning an ADR per 100,000 dispensings, RR: relative risk

[a] Acetylsalicylic acid and amino derivatives, salicylic acid salts. [b] digitalis glycosides, digoxin, strophanthins. [c] codeine, morphine, opium (alkaloids), meperidine. [d] clonidine, guanethidine, rauwolfia alkaloids, reserpine. [e] cortisone derivatives, desoxycorticosterone, derivatives, fluorinated corticosteroids.

frequently occurring. An unknown complication of diabetes (27%) and other disorders of pancreatic internal secretion (9%) were the most commonly presented ADR-related hospitalizations for adrenal corticosteroids. An unspecified hemorrhage of the gastrointestinal tract was a frequent ADR-related hospitalization for respectively salicylates (17%) and antirheumatics (9%). Another common ADR-related hospitalization for these drugs was chronic or unspecified gastric ulcer with hemorrhage (salicylates 14% and antirheumatics 11%). Unspecified hemorrhage (22%) and unspecified hemorrhage of the gastrointestinal tract (12%) were also the most common ADR-related hospitalizations for anticoagulants.

DISCUSSION

Our study showed that the proportion of ADR-related hospitalizations was 1.3% of all hospitalizations. This percentage is lower than the percentages found in other studies. [2, 5, 16-17] In our opinion, underestimation of the total number of ADR-related hospitalization is likely, but will probably not have flawed our comparison between sex- and age-groups. In an earlier study, it was described that under-reporting of ADRs causing hospital admissions is considerable. [18] In addition, misclassification of the outcome is likely as not all ADRs will be recognized or mentioned in the discharge letters and coded accordingly. Although the ICD-9 codes are given independently of exposure and independently of reimbursement (yielding non-differential misclassification), it might be that some types of ADRs are more likely to be identified than others because they are easily recognized, severe, or specific (potentially yielding differential misclassification). The proportion of ADR-related hospitalizations in our study was stable during the study period while others found an increase in ADR-related hospitalizations over the period 1981 – 2007 which did, however, temporize since 1997. [11] In our opinion, this difference can be explained by the denominator used. We used the total number of hospitalizations, while Hartholt *et. al.* used the total population of a certain age category within the Netherlands. [11] Although the population growth in the Netherlands decreases, the total population still increases mainly attributable to those older than 65 years. [10]

In our study, higher age was associated with an increased risk of an ADR-related hospitalization but this effect was modified by the calendar year of admission and by sex. Furthermore, as earlier described, we found that female sex was associated with an increased risk of an ADR in comparison with male sex. [19]

At first sight, the risk of an ADR-related hospitalization when aged over 75 years did not differ statistically significantly from the risk of an ADR-related hospitalization when aged 55-64 years old. However, when taking into account the number of dispensings to the different age categories, elderly above 75 years of age were at a significantly

increased risk of an ADR-related hospitalization attributable to anticoagulants, insulins and antidiabetic agents, salicylates or antirheumatics. In contrast, a decreased risk of ADR-related hospitalizations was found for the use of antineoplastic and immunosuppressive drugs in elderly above 75 years of age. This decreased risk may be explained by the burden of co-morbidities in elderly diagnosed with cancer of which the coding may prevail over the coding of ADRs.

The ten drugs which were most frequently associated with drug related hospitalizations (anticoagulants, antineoplastic and immunosuppressive drugs, insulins and antidiabetic agents, high-ceiling diuretics, salicylates, antirheumatics, cardiotonic glycosides and drugs of similar action, other opiates and related narcotics, other antihypertensives and adrenal cortical steroids) were similar to the drugs most frequently incriminated in drug related hospitalizations in other studies. [18, 20-22] Also, the proportion of ADR-related hospitalizations attributable to these drugs (≈75%) is similar to other studies, as well as the presentation of these ADRs. [21-22]

One of the strengths of this study is that we used all admissions to Dutch hospitals between 2000 and 2005. However, as a consequence of the ecological study design we were not able to verify whether, in case of an ADR-related hospitalization, the patient actually used the specified drug and whether this drug indeed caused the ADR. In addition, since no further information was available on factors like polypharmacy and co-morbidities, confounding might play a role as well. Polypharmacy is common in the elderly and it might be that a drug interaction caused the ADR or that another drug than the suspected drug caused the ADR. [6-7]

In our study, we showed that the elderly above 75 years of age are at increased risk of being hospitalized for an ADR. Given that it has been estimated that the number of those aged 65 years and over will grow between 2010 and 2040 from 2.4 to 4.6 million it is of pivotal importance to further endorse the drug safety in this vulnerable patient group. [10] Special attention should be given to anticoagulants, salicylates and antirheumatics (hemorrhage), insulins and antidiabetic agents (hypoglycemia), opiates (constipation), cardiotonic glycosides (intoxication), certain antihypertensives (angioneurotic edema and cardiac dysrhythmias) and diuretics (volume depletion, hyposmolality).

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Chapter 3

Cancer as adverse drug reaction in diabetic patients

Chapter 3.1

Risk of cancer in patients on insulin glargine and other insulin analogues in comparison with those on human insulin: results from a large population-based follow-up study

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Diabetologia 2011 Sep 29. [Epub ahead of print]

ABSTRACT

Introduction: several publications suggest an association between certain types of insulin and cancer, but with conflicting results. We investigated whether insulin glargine is associated with an increased risk of cancer in a large population-based cohort study.
Methods: data for this study were obtained from dispensing records from community pharmacies individually linked to hospital discharge records from 2.5 million individuals in the Netherlands. In a cohort of incident users of insulin, the association between insulin glargine and other insulin analogues, respectively, and cancer was analyzed in comparison with human insulin, using Cox proportional hazard models with cumulative duration of drug use as a time-varying determinant. The first hospital admission with a primary diagnosis of cancer was considered as the main outcome; secondary analyses were performed with specific cancers as outcomes.
Results: of the 19,337 incident insulin users enrolled, 878 developed cancer. Use of insulin glargine was associated with a lower risk of cancer in general in comparison with human insulin (HR 0.75, 95% CI 0.71 – 0.80). In contrast, an increased risk was found for breast cancer (HR 1.58, 95% CI 1.22 – 2.05). Dose-response relationships could not be identified.
Conclusion: users of insulin glargine and users of other insulin analogues had a lower risk of cancer in general than those using human insulin. Both associations might be a consequence of residual confounding, lack of adherence or competing risk. However, as in previous studies, we demonstrated an increased risk of breast cancer in users of insulin glargine in comparison with users of human insulin.

INTRODUCTION

Diabetes mellitus is an important risk factor for cardiovascular disease. [1-2] In addition, diabetes has been associated with an increased risk of colorectal cancer [3-4], breast cancer [4-5], endometrial cancer [4, 6], hepatocellular carcinoma [4, 7], pancreatic cancer [4, 8] and bladder cancer. [4, 9] In contrast, patients with diabetes have a decreased risk of developing prostate cancer. [4, 10] Furthermore, diabetes has been reported as an independent predictor of mortality from cancer. [4, 11-12] However, due to factors such as duration of diabetes, different drugs used to attain metabolic control and presence of other diseases, the assessment of cancer risk in diabetes patients remains difficult. [13-14]

In 2004, a publication with data from the General Practice Research Database in the UK reported that in patients with type 2 diabetes, chronic insulin therapy was associated with a significantly higher risk of colorectal cancer compared with patients with diabetes who did not use insulin. [15] By the end of 2009, articles were published using data from population registries to analyze a possible relationship between the use of hypoglycemic agents and the risk of cancer. [16-19] Of these, three showed an increased risk of cancer with use of insulin glargine (A21Gly,B31Arg,B32Arg human insulin, Lantus®) compared with other types of insulin analogues or human insulin. [16, 18-19] Currie *et al.* did show an increased risk of cancer while using insulin compared with patients using metformin but did not show an increased risk of cancer for those using insulin analogues compared with those using human insulin. [17] More recently, it has been reported that the use of insulin glargine did not increase the risk of overall cancer compared with the use of human insulin. [20]

In addition to these observational studies, reports regarding randomized controlled trials have been published. [21-23] None of these described dissimilarity in cancer incidence between participants treated with insulin glargine and those treated with human insulin or other types of insulin. [21-23] With regard to dose, a dose-dependent relationship has been described for insulin glargine and risk of cancer, but not for other insulin analogues or human insulin. [18, 24] Consequently, whether different types of insulin may be a cause of cancer is an issue of ongoing debate. [25-32]

Therefore, the objective of this study was to analyze the hypothesis that use of insulin glargine is associated with an increased risk of cancer in comparison with use of human insulin.

METHODS

Setting

Data for this study were obtained from the PHARMO Record Linkage System (RLS) which includes drug-dispensing records from community pharmacies linked on a patient level to hospital discharge records from the Dutch National Medical Register for approximately 2.5 million individuals in the Netherlands since 1986. [33-34]

The drug-dispensing database contains the following information per prescription as of 1998: anatomical therapeutic chemical (ATC) classification of the drug, dispensing date, regimen, quantity dispensed and estimated duration of use. [35] The hospital record database contains detailed information concerning primary and secondary discharge diagnoses and dates of admission and discharge. All diagnoses are coded according to the International Classification of Disease, ninth edition (ICD-9). [36]

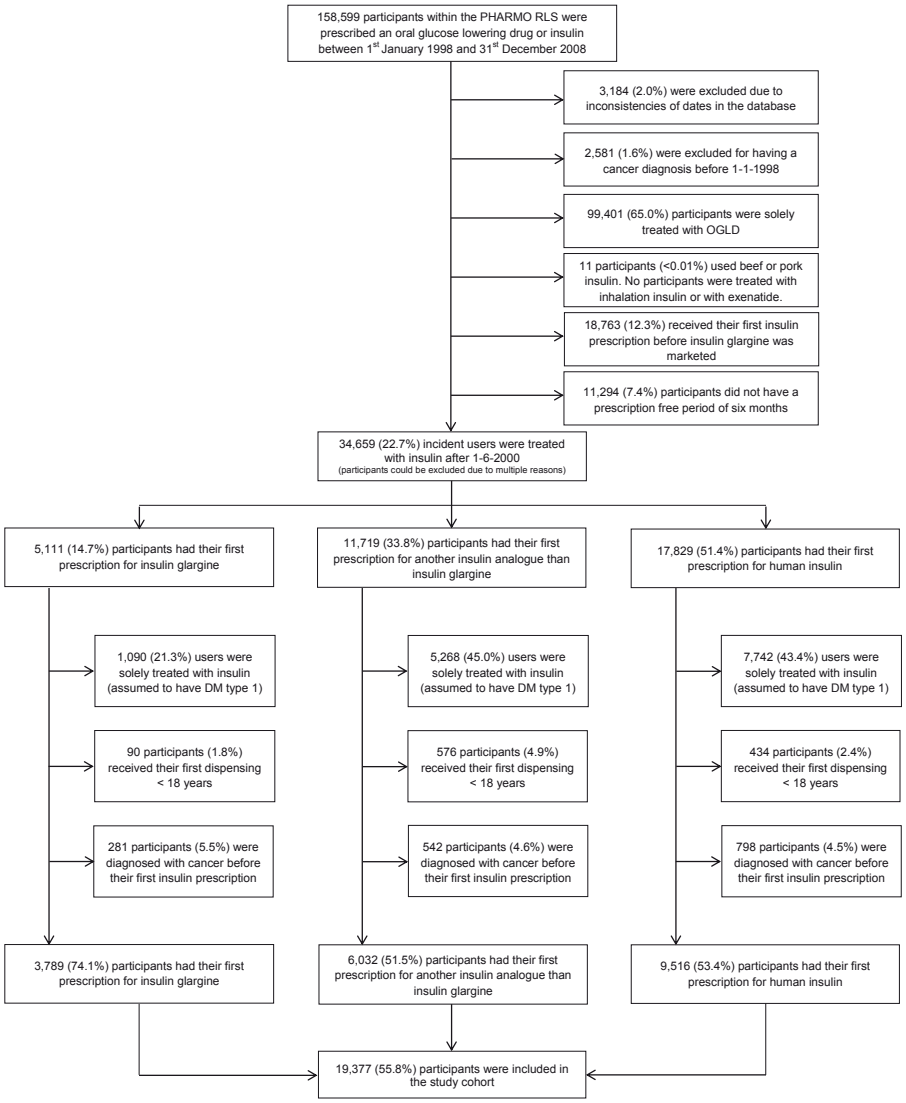
Study population

All participants with a prescription for any hypoglycemic agent, including an oral glucose-lowering drug (OGLD) or insulin, between 1 January 1998 and 31 December 2008 were included in the study cohort. The patient flow is presented visually in a flow diagram (**figure 1**). As insulin glargine has been marketed in the Netherlands since June 2000, participants with a prescription of any insulin before 1 June 2000 were excluded from the cohort. [37] Furthermore, to ensure the study cohort included only incident insulin users, users needed to have had a 6 month period without prescription of insulin (any type) before inclusion. To mimic a study cohort of participants with type 2 diabetes, those using only insulin were assumed to have type 1 diabetes and were excluded from the analysis. In addition, participants with a primary cancer diagnosis before 1 June 2000, a primary cancer diagnosis before prescription of insulin, or who were aged under 18 years at first prescription were excluded. As a consequence, the remaining cohort only included insulin users with prior use of OGLD who were followed over time starting from the first prescription for insulin.

Exposure

The different types of insulin prescribed for diabetes were classified into three mutually exclusive categories according to ATC code: insulin glargine; other insulin analogues; and human insulin (**supplementary material (SM) table 1**). For each participant, the number of cumulative days of insulin use was calculated. The cumulative exposure to each insulin category at any point in time during follow-up was calculated for each participant in days since start of the respective insulin type. Cumulative days of insulin exposure were taken from this time point until death of the participant, end of study,

Figure 1: Flow chart visualizing the flow of participants into the study cohort



first diagnosis of cancer, relocation out of the PHARMO RLS catchment area, or the last day of use of a dispensed agent in the same insulin category.

To visualize participants' drug adherence with different types of insulin, the percentage of participants adherent to therapy was calculated. For every cohort member, the follow-up time was calculated for insulin glargine, other insulin analogues and human insulin, respectively. For every month of follow-up, the number of users was divided by

the total number of those who started minus those who died, those diagnosed with cancer and those who moved out of the PHARMO RLS catchment area.

Outcome

The first hospital admission with a primary diagnosis of any type of cancer, ICD-9 codes 140 – 172, 174 – 209 and 235 – 239, was considered the primary outcome. [36] The secondary outcome measure was diagnosis of one of the following solid cancers: colon cancer (ICD-9 153 or 154), pancreatic cancer (ICD-9 157), breast cancer (ICD-9 174 or 175), prostate cancer (ICD-9 185), endometrial cancer (ICD-9 179 or 182), respiratory tract cancer (ICD-9 160 – 165) and bladder cancer (ICD-9 188). These cancers were selected because they have been associated with diabetes, either with an increased or with a decreased risk. [3, 5-6, 8-10]

Covariables

Age at first insulin prescription, sex, number of unique other drugs used in the year before start of insulin (excluding those prescribed for diabetes), number of hospitalizations in the year before start of insulin and calendar time were considered potential confounders or effect modifiers. The number of days of use of OGLD in the year before start of insulin therapy was calculated, as well as the number of days of OGLD use as of 1 January 1998, to adjust for duration of diabetes. Furthermore, the average dose was calculated per insulin category as average defined daily dose (DDD) over the previously dispensed prescriptions to adjust for severity of glucose intolerance. For all types of insulin, one DDD is equivalent to 40 U insulin. [35]

Statistical analysis

Individuals were followed from their first insulin prescription until the first of one of the following events: cancer as defined above, death, end of data collection in the PHARMO RLS (i.e. the patient moves out of the PHARMO RLS area) or end of the study period at 31 December 2008. The association between insulin and cancer was analyzed using Cox proportional hazard models with duration of cumulative drug use as a time-varying determinant, as described by Stricker and Stijnen. [38] In this model, cumulative exposure in participants with cancer at the date of diagnosis is compared with cumulative exposure in all individuals without cancer with the same duration of insulin exposure in days. Time since start of insulin is used as the underlying timescale in the Cox proportional hazards model. We assumed that cancer risk, after a certain cumulative exposure, does not return to zero after stopping (i.e. in case of switching to another type of insulin). However, time since cessation was taken into account in one of the sub-analyses. In the analysis performed, the actual exposure during follow-up was used. This analysis defines the exposure accurately but may suffer from reverse causation bias. To address this is-

sue, analyses were performed taking into account a latent period before the diagnosis of cancer in which we assumed that cancer was already present 1 year before it was actually diagnosed (for instance, cumulative exposure to 21 June 2007 instead of 21 June 2008). To further deal with the issue of reverse causation, a fixed-cohort analysis was performed in which the first exposure to insulin determined the drug category in which the participant was categorized. To further address potential residual confounding, a propensity-score analysis was performed. The methods and results for the fixed and propensity-score analyses are presented in, respectively, **SM methods and results**.

The ways in which use of OGLD and insulin dose were addressed in the analyses are described in **SM methods**, as are the general statistical methods used.

RESULTS

Setting and characteristics

Within the PHARMO RLS, 158,599 participants were prescribed an OGLD or insulin between 1 January 1998 and 31 December 2008. After applying exclusion criteria, 19,337 (12.2%) participants were included in the study cohort (**figure 1**). As can be seen from **table 1**, there were significant differences at baseline and during follow-up between participants starting on insulin glargine or other insulin analogues and those starting on human insulin.

Users of insulin analogues were significantly younger than those starting on insulin glargine; in contrast, those starting on insulin glargine were more frequently male than those starting on other insulin analogues. The mean number of unique other drugs used and number of hospitalizations in the year before start of insulin did not differ significantly. The first dose prescribed, as well as the average dose calculated over all prescriptions differed significantly for those using other insulin analogues in comparison with those using insulin glargine. The duration of OGLD use prior to start of insulin was significantly shorter for those using other insulin analogues than for those using insulin glargine or human insulin. However, when stratifying for the year in which insulin therapy was started, no clear differences could be seen (**SM table 2**). Last, the duration of days of follow-up since the start of insulin was considerably lower for users of insulin glargine than for those using other insulin analogues. An adherence curve is presented in **figure 2**, in which the percentages of participants adherent to the three different categories of insulin are visualized.

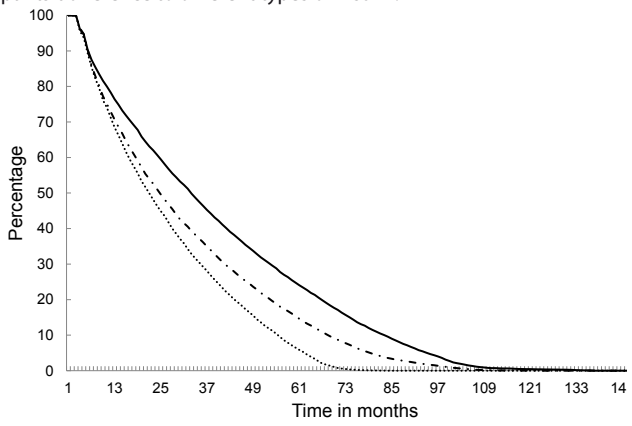
Those dispensed insulin glargine were statistically significantly less adherent to therapy than those dispensed other insulin analogues or human insulin. In **SM figure 1** (insulin glargine), **SM figure 2** (other insulin analogues) and **SM figure 3** (human insulin)

Table 1: Characteristics of participants using insulin glargine, other insulin analogues or human insulin

Variable	Insulin glargine (*n*=3,789)	Other insulin analogues (*n*=6,032)	Human insulin (*n*=9,516)
Age at first prescription of insulin in years (Mean±SD) [a]	63.1±13.7	61.8±13.9	65.0±13.5
Sex, *n* (%)[b]			
Male	1,901 (50.2%)	2,931 (48.6%)	4,423 (46.5%)
Female	1,888 (49.8%)	3,101 (51.4%)	5,093 (53.5%)
Total number of unique other drugs used in the year before first prescription of insulin[c]			
Mean±SD	8.8±5.6	8.9±6.0	9.0±6.2
Median (IQR)	8 (5 – 8)	8 (5 – 8)	8 (5 – 8)
Total number of hospitalizations in the year before first prescription of insulin[c]			
Mean±SD	0.5 (2.1)	0.6 (1.1)	0.6 (1.1)
Median (IQR)	0 (0 – 1)	0 (0 – 1)	0 (0 – 1)
Number of days of OGLD use in the year before first prescription of insulin, median (IQR)[a]	324 (280 – 350)	320 (235 – 348)	324 (276 – 349)
Number of days of OGLD use before first prescription of insulin as of January 1998, median (IQR)[a]	1,567 (548 – 2,474)	1,190 (275 – 2,134)	1,154 (383 – 1,090)
Duration of follow-up since first insulin prescription in days[a]			
Mean±SD	803 (587)	1,186 (823)	1,381 (924)
Median (IQR)	659 (307 – 1,176)	813 (344 – 1,489)	1,629 (755 – 2,350)
Average daily dose of the first insulin prescription (U)[a]			
Mean±SD	21.7 (13.5)	28.1 (46.6)	26.0 (19.8)
Median (IQR)	16.7 (16.7 – 16.7)	16.7 (16.7 – 33.3)	16.7 (16.7 – 33.3)
Average daily dose over all insulin prescriptions in U since first prescription[a]			
Mean±SD	44.1±63.4	15.4±44.5	55.6±112.0
Median (IQR)	34.0 (22.0 – 50.0)	15.0 (14.0 – 18.0)	46.0 (26.0 – 64.0)
Types of insulin (first prescription, *n* (%))			
Fast-acting	-	1,899 (31.5)	1,346 (14.1)
Intermediate fast-acting	-	11 (0.2)	4,479 (47.1)
Intermediate and fast-acting	-	3,065 (50.8)	3,691 (38.8)
Long-acting	3,789 (100.0)	1,056 (17.5)	-

Abbreviations: *n:* number, IQR: interquartile range, OGLD: oral glucose lowering drugs, U: units.
[a] *p*-value following linear regression <0.0001. [b] *p*-value following χ^2 test <0.0001. [c] *p*-value following linear regression not significant.

Figure 2: Participants' adherence to different types of insulin.



Legends: Dotted line: insulin glargine, dotted/dashed line: other insulin analogues, solid line: human insulin.

adherence is presented separately for those who died, those who got diagnosed with cancer and those who were censored at the end of study.

As-treated analyses

Of the 878 participants hospitalized for cancer, 158 were treated with insulin glargine, 423 with other insulin analogues and 592 participants were treated with human insulin. The corresponding incidence rates were, respectively, 11.29, 13.78 and 12.81 cancers per 1,000 patient years. As can be seen from **table 2**, use of insulin glargine was associated with a lower risk of cancer in comparison with use of human insulin (HR 0.71, 95% CI 0.67 – 0.75). In the full model, adjustments did not change the HR (HR 0.75, 95% CI 0.71 – 0.80). Stratifying for prior OGLD use for less or longer than 1 year did not change this point estimate, nor did adjustment for prior days of OGLD used change the point estimates by more than 10%. Adjustments were made by adding dose as an additional time-varying covariable to the model (HR 0.75, 95% CI 0.71 – 0.80) but, as follow-up information was used when applying this method, results from analyses stratified for baseline dose are also presented in **table 2**. As the majority of the cohort members had a median first dose of 16.7 U per day (**table 1**) these analyses were stratified in three strata: more than, less than or equal to the median dose per day. When replacing cumulative exposure at the end of follow-up with attained cumulative exposure 1 year prior to the diagnosis of cancer (in order to minimize the chance of reverse causation) the point estimates remained statistically significantly protective. Proportionality of the full model was tested; *p*-values for insulin glargine and other insulin analogues were, respectively, 0.14 and 0.32.

Table 2: Risk of cancer in patients using insulin glargine or other insulin analogues in comparison with those using human insulin (as-treated analysis)

Covariables [a] included in the model	Insulin glargine		Other insulin analogues	
	HR	95% CI	HR	95% CI
None	0.71	0.67 – 0.75	0.79	0.76 – 0.81
Stratified for dose of first insulin prescription				
< Median	0.71	0.56 – 0.89	0.87	0.76 – 0.98
Median	0.71	0.66 – 0.77	0.75	0.72 – 0.79
> Median	0.68	0.61 – 0.76	0.86	0.82 – 0.91
Age, sex	0.72	0.68 – 0.76	0.80	0.77 – 0.82
Age, sex, calendar time, hospitalizations, unique drugs	0.75	0.71 – 0.79	0.84	0.81 – 0.87
Full model: age, sex, calendar time, hospitalizations, unique drugs, use of other insulin	0.75	0.71 – 0.80	0.85	0.82 – 0.89
Full model adjusted for time since cessation [b]	0.72	0.67 – 0.76	0.82	0.79 – 0.86
Full model adjusted for days of prior OGLD use				
< 1 year OGLD use	0.77	0.65 – 0.90	0.81	0.74 – 0.89
≥ 1 year OGLD use	0.79	0.74 – 0.84	0.93	0.88 – 0.98
Full model, adjusted for use of OGLD (time-dependent)				
Biguanide	0.75	0.71 – 0.80	0.85	0.82 – 0.89
SU	0.76	0.71 – 0.80	0.85	0.81 – 0.88
Other OGLD	0.75	0.71 – 0.80	0.85	0.82 – 0.89
Full model adjusted for average DDD	0.75	0.71 – 0.80	0.85	0.82 – 0.89
Full model, stratified for dose of first insulin prescription				
< Median	0.70	0.54 – 0.89	0.89	0.74 – 1.08
Median	0.79	0.73 – 0.85	0.81	0.76 – 0.85
> Median	0.72	0.64 – 0.80	0.92	0.86 – 0.98
Full model including a latency time of 1 year [c]	0.76	0.71 – 0.81	0.88	0.84 – 0.93

Abbreviations: HR: hazard ratio, CI: confidence interval, OGLD: oral glucose lowering drugs, SU: sulfonylurea derivatives, DDD: defined daily dose.

[a] Covariables: age, age at first insulin prescription; calendar time, time since inclusion of participant in PHARMO RLS; hospitalizations, number of hospitalizations in the year prior to start of insulin; unique drugs, number of unique drugs dispensed in the year prior to start of insulin; days of prior OGLD use, number of days of OGLD use as of January 1998; use of other insulin, in the analysis of insulin glargine, adjustments were made for use of other types of insulin as a time-dependent variable and in the analysis of other insulin analogues, adjustments were made for use of insulin glargine as a time-dependent variable; average DDD, dose calculated over all previous insulin prescriptions. [b] Time since cessation of insulin glargine, other insulin analogues and/or human insulin in days. [c] Model included a 1 year latency period: exposure was cumulated up till one year prior to the date of cancer diagnosis; incidence rate (no. of cancer diagnoses/1,000 patient years) for insulin glargine 6.30; for other insulin analogues 7.90 and for human insulin 9.03.

When specific cancers were used as endpoints (**table 3**) applying the full model, insulin glargine was associated with a significantly lower risk of colon cancer but not of other cancers.

In contrast, use of insulin glargine was associated with an increased risk of breast cancer (HR 1.58, 95% CI 1.22 – 2.05) and prostate cancer (HR 2.76, 95% CI 1.32 – 5.80) in comparison with use of human insulin. The complete analyses for endometrial cancer and pancreatic cancer were not possible because of the low number of cancer diagnoses. Furthermore, with regard to the stratified model for first prescribed dose, analyses were not possible for some of the lowest strata because of the low number of cases (≈ 70% of the participants received a first dose of 16.7 U per day, **table 1**). No clear dose effect could be seen over the different strata of dose. For other insulin analogues, no increased risk of breast cancer or prostate cancer was seen; in addition, no decreased risk of colon cancer was found. However, a decreased risk of bladder cancer, as well as respiratory tract cancer was seen (**table 3**).

In users of insulin glargine the dose was not related to the diagnosis of cancer (crude HR comparing those with an average DDD higher than the median with those having an average DDD lower than the median 1.02, 95% CI 0.77 – 1.34, HR applying full model 0.98, 95% CI 0.74 – 1.29) nor could this be demonstrated for insulin analogues other than insulin glargine (crude HR 1.02, 95% CI 0.99 – 1.04; HR applying full model 0.95, 95% CI 0.76 – 1.18) or for human insulin (HR 0.95, 95% CI 0.82 – 1.09, HR applying a similar full model 0.96, 95% 0.82 – 1.12).

Fixed-cohort analyses and propensity-score analyses

For cancer in general, similar estimates were found in the fixed analyses (**SM table 3**). Similar estimates were gained as well from the propensity-score analyses; these results are presented in the **SM results**. In the analyses with specific cancers as endpoints, the results differed slightly. With regard to insulin glargine, the decreased risk of colon cancer and the increased risk of breast cancer were nearly similar; however, for prostate cancer, no risk deviations could be found. The results for lung cancer were similar, but an increased risk was found for bladder cancer. With regard to other insulin analogues, the results were similar: no increased risk of breast cancer or prostate cancer was seen and no decreased risk of colon cancer was found. However, a decreased risk of bladder cancer as well as respiratory tract cancer was seen (**SM table 4**). As in the as-treated analyses, no dose-response relationships could be determined.

Table 3: Risk of specific cancer in patients using insulin glargine or other insulin analogues in comparison with those using human insulin (as-treated analysis)

Model	Insulin glargine				Other insulin analogues				Human insulin
	IR	*n*	HR	95% CI	IR	*n*	HR	95% CI	IR
Colon cancer									
Full model [a]	1.29	18	0.55	0.39 – 0.76	2.02	62	1.07	0.93 – 1.25	1.90
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			0.72	0.63 – 0.85			1.06	0.91 – 1.23	
SU			0.72	0.62 – 0.84			1.08	0.93 – 1.26	
Other OGLD			0.73	0.63 – 0.85			1.07	0.91 – 1.26	
Full model + average DDD			0.55	0.40 – 0.76			0.97	0.81 – 1.15	
Full model, stratified for dose of first insulin prescription									
< Median		0	–	–		0	–	–	
Median		16	0.30	0.17 – 0.54		44	0.89	0.70 – 1.14	
> Median		2	–	–		18	0.77	0.59 – 1.00	
Full model including a latency time of 1 year [b]	0.86	12	0.61	0.40 – 0.91	1.14	35	1.20	0.97 – 1.48	1.58
Bladder cancer									
Full model [a]	0.79	11	1.89	0.69 – 3.21	0.91	28	0.48	0.34 – 0.69	1.06
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			0.83	0.68 – 1.01			0.80	0.65 – 0.98	
SU			0.84	0.69 – 1.02			0.80	0.66 – 0.98	
Other OGLD			0.83	0.68 – 1.01			0.80	0.66 – 0.98	
Full model + average DDD			1.38	0.70 – 2.70			0.48	0.32 – 0.70	
Full model, stratified for dose of first insulin prescription									
< Median		0	–	–		1	–	–	
Median		8	2.27	1.24 – 4.15		16	0.56	0.39 – 0.79	
>Median		3	–	–		11	0.79	0.60 – 1.04	
Full model including a latency time of 1 year [b]	0.50	7	1.09	0.37 – 3.24	0.61	19	0.66	0.44 – 0.99	0.80

Table 3: Risk of specific cancer in patients using insulin glargine or other insulin analogues in comparison with those using human insulin (as-treated analysis)(cont'd)

Model	Insulin glargine				Other insulin analogues				Human insulin
	IR	*n*	HR	95% CI	IR	*n*	HR	95% CI	IR
Respiratory tract cancer									
Full model [a]	1.64	23	1.03	0.84 – 1.24	1.89	58	0.64	0.54 – 0.77	1.90
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			1.01	0.82 – 1.24			0.87	0.78 – 0.96	
SU			0.97	0.79 – 1.20			0.87	0.78 – 0.96	
Other OGLD			1.02	0.82 – 1.25			0.86	0.78 – 0.95	
Full model + average DDD			0.76	0.61 – 0.96			0.64	0.54 – 0.77	
Full model, stratified for dose of first insulin prescription									
< Median		0	–	–		1	–	–	
Median		15	1.09	0.57 – 2.07		34	0.46	0.30 – 0.71	
> Median		8	1.14	0.79 – 1.63		23	0.93	0.80 – 1.08	
Full model including a latency time of 1 year [b]	1.07	15	1.23	0.94 – 1.62	1.17	36	0.91	0.73 – 1.12	1.32
Prostate cancer									
Full model [a]	0.99	7	2.76	1.32 – 5.80	1.26	19	0.83	0.70 – 1.03	1.55
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			2.74	1.29 – 5.80			0.84	0.70 – 1.03	
SU			3.12	1.35 – 7.19			0.83	0.70 – 1.03	
Other OGLD			2.72	1.28 – 5.79			0.85	0.73 – 1.09	
Full model + average DDD			1.01	0.62 – 1.73			0.99	0.95 – 1.04	
Full model, stratified for dose of first insulin prescription									
< Median		0	–	–		0	–	–	
Median		5	2.21	0.92 – 5.34		11	0.83	0.53 – 1.39	
> Median		2	–	–		8	0.87	0.67 – 1.16	
Full model including a latency time of 1 year [b]	0.71	5	2.19	1.03 – 4.66	0.79	12	0.84	0.68 – 1.43	1.15

Table 3: Risk of specific cancer in patients using insulin glargine or other insulin analogues in comparison with those using human insulin (as-treated analysis) (continued)

Model	Insulin glargine				Other insulin analogues				Human insulin
	IR	*n*	HR	95% CI	IR	*n*	HR	95% CI	IR
Breast cancer									
Full model [a]	4.06	28	1.58	1.22 – 2.05	3.08	48	0.95	0.83 – 1.08	2.81
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			1.58	1.22 – 2.04			0.94	0.81 – 1.08	
SU			1.67	1.28 – 2.19			0.94	0.82 – 1.08	
Other OGLD			1.52	1.16 – 1.98			1.03	0.89 – 1.20	
Full model + average DDD			1.62	1.24 – 2.12			0.90	0.80 – 1.02	
Full model, stratified for dose of first insulin prescription									
< Median		2	–	–		3	–	–	
Median		15	1.22	0.91 – 1.64		29	0.90	0.70 – 1.15	
> Median		11	2.81	1.23 – 6.44		16	0.10	0.02 – 0.47	
Full model including a latency time of 1 year [b]	1.60	11	1.65	1.10 – 2.47	1.99	31	0.99	0.81 – 1.20	2.28

Abbreviations: IR: incidence rate, *n:* number, HR: hazard ratio, CI: confidence interval, OGLD: oral glucose lowering drugs, SU: sulfonylurea derivatives.

[a] In the full model, adjustments were made for age at first insulin prescription, sex, calendar time, number of unique drugs used in the year before start of insulin and number of hospitalizations in the year before start of insulin. Days of prior OGLD use, number of days of OGLD use as of January 1998. Furthermore, when the insulin of interest was insulin glargine, adjustments were made for the use of other insulin analogues as time-dependent variables; when the insulin of interest were other insulin analogues, adjustments were made for the use of insulin glargine. [b] Model included a 1 year latency period: exposure was cumulated up till one year prior to the date of cancer diagnosis.

DISCUSSION

In this study, we found that cumulative use of insulin glargine was associated with a significantly lower risk of cancer in general, and of colon cancer specifically, in comparison with use of human insulin. Similar results were found for the risk of cancer in general and use of other insulin analogues in comparison with human insulin. In contrast, as in other studies, we found an increased risk of breast cancer for insulin glargine in comparison with human insulin. [16,19] However, this has not been consistently presented by others. [17, 22-24, 39-40] For insulin analogues other than insulin glargine, no increased risk of breast cancer was found. With regard to breast cancer, insulin glargine has shown a significantly higher proliferative effect on breast cancer cells compared with human insulin or other insulin analogues. [39] Recently, it was estimated that the serum of type 1 diabetic patients containing insulin glargine was 11% more mitogenic than human insulin containing serum. [40] Our results for other specific cancers were not consistent, with the exception of a decreased risk of colon cancer for the use of insulin glargine and a decreased risk of bladder cancer and respiratory tract cancer for the use of other insulin analogues.

It might be hypothesized that the protective effect of insulin glargine on cancer in general is a result of the lower dose of the first prescription, dispensed to these participants in comparison with the dose prescribed to participants using other insulin analogues or human insulin. Adjustment for dose was performed by adding dose as a time-dependent covariable in the model. Using this method, follow-up information is used, which is prone to reverse causality bias. Therefore, analyses were stratified for the baseline dose. However, in these stratified analyses as well as separate dose analyses, no dose-dependent relations could be demonstrated.

Our results are partly at variance with the earlier published population-based studies that caused alarm. [16-20] The first of these papers concluded that risk of cancer in participants using insulin glargine was higher than in those using human insulin. [18] As a possible explanation, the mitogenic properties of insulin glargine in diabetic patients, as published earlier, were suggested. [41] Another study reported that insulin analogues were not associated with a higher incidence of cancer compared with human insulin. [17] The third one, a Swedish study, did not show an increased risk of any malignancy, but similar to our study, they showed that women using insulin glargine had an increased incidence rate of breast cancer compared with women using other types of insulin analogues or human insulin. [19] The Scottish Diabetes Research Network found that those receiving insulin glargine had the same incidence rate for all cancers as those not receiving insulin glargine. [16] However, a subset of patients using insulin glargine alone had a significantly higher incidence of all cancers, and breast cancer specifically, than those using other types of insulin. [16] Nevertheless, the authors concluded that insulin glargine use was most likely not associated with an increased risk of cancer and that the

finding above should be considered to be biased because of differences in allocation of patients to different types of insulin. [16] More recently, a cohort study of new users of OGLDs showed that the number of insulin doses dispensed (any insulin type) was associated with a higher risk of cancer compared with participants not using insulin.[42] In contrast, it was reported that, in a Taiwanese cohort study [20], use of insulin glargine was not associated with an increased risk of overall cancer while in Chinese individuals with type 2 diabetes, insulin usage (any type) was associated with a reduced risk of cancer compared with non-usage. [43] However, the latter study was severely criticized for the exclusion of follow-up time prior to insulin use. [25]

Limitations of the earlier publications were brought forward, among which short follow-up, failure to correct for body mass index, the impossibility of breaking down the risk of cancer in general to a tumor-specific risk, the inability to consider prior use of insulin before start of study, low numbers of patients using a specified insulin and the absence of dose analyses. [32] In addition, clinical decisions determining each patient's treatment are not random and confounding by severity of glucose intolerance could play an important role in observational studies. [28, 30] Another issue is reverse causality and assessment of etiologically relevant timing of exposure: cancer has a long latency period during which the disease itself may cause changes in treatment. [28, 30] Last, the severity of disease may also be related to the frequency of clinical contact, which may reduce the time between onset and diagnosis of cancer. [28]

As described above, reverse causality may play a role in observational studies, as cancer often has a long latency period between the biological onset of the disease and the clinical diagnosis. During this latency period, symptoms related to still undetected cancer may cause treatment changes. By cumulating exposure to 1 year prior to the diagnosis of cancer, we attempted to minimize reverse causality by taking into account a latent period (i.e. when the disease is already present but not yet diagnosed). To further address reverse causation, we performed a fixed analysis; none of these analyses changed the risk of cancer in general by more than 10%. To address the issue of potential residual confounding a propensity-score analysis was performed from which similar estimates were found. Also, although we assumed that cancer risk does not return to the background rate after a certain cumulative exposure, we performed a sensitivity analysis in which we adjusted for time since cessation. This adjustment was done to investigate whether the risk declined after discontinuation. However, these analyses did not substantially change the risk estimates.

Our study was performed in incident users of insulin: those who had a prescription-free period of 6 months before study entry. By excluding those with prevalent use of insulin, we attempted to make participants more similar with regard to duration and severity of insulin resistance. However, the participants being prescribed insulin glargine differed considerably from those being prescribed other insulin analogues or human insulin.

Insulin glargine is reserved for those suffering from nightly hypoglycemic attacks, partly because of its higher cost in comparison with human insulin. [37] Patients with type 1 diabetes are particularly prone to these attacks as, in contrast to patients with type 2 diabetes, they do not have any remaining insulin production. [1] However, it is possible that under everyday circumstances in the Netherlands, insulin glargine is prescribed more generally to those having difficulties attaining euglycemia. Unfortunately, we were not able to fully differentiate between those receiving insulin for type 1 or for type 2 diabetes; these groups might differ regarding their cancer risk. However, in an attempt to restrict the analysis to those with type 2 diabetes, we included only participants with prior OGLD use. We were able to adjust for the number of unique other drugs used prior to the first prescription of insulin and the number of hospitalizations to adjust for co-morbidity. Nevertheless, it is likely that our findings are confounded as those receiving insulin glargine or other insulin analogues might die earlier because of comorbidity; consequently they would not live long enough to develop cancer or, in other words, they would die of 'competing risks'. [44] Another explanation for our findings might be the significantly lower adherence to insulin glargine in comparison with use of other insulin analogues or human insulin.

In contrast to some former studies, we were not able to adjust for smoking status or body mass index, which might be considerable confounding factors. However, although obesity is associated with an increased risk of developing insulin resistance and type 2 diabetes [45] caution must be made when assessing the relationship with cancer. Furthermore, in previous studies, smoking and body mass index did not change the point estimate by more than 10%. [16, 19]

Last, in our study we used cancer hospitalization as an outcome measure, which is different from pathology data for cancer diagnoses. Some cancers might be diagnosed more frequently in a non-clinical setting. Within each specific cancer, this would, however, lead to non-differential misclassification of the outcome and consequently to dilution of the estimated effect towards the null hypothesis.

In conclusion, in our study of insulin users, users of insulin glargine had a lower risk of specific cancers and of cancer in general in comparison with those on human insulin. Similar results were found for use of other insulin analogues in comparison with human insulin. However, in our opinion, both associations might be a consequence of residual confounding, lack of adherence or competing risk. The fact that we were not able to demonstrate a dose-effect association would also be an argument against a causal relationship. Furthermore, as in previous studies, we demonstrated an increased risk for breast cancer and use of insulin glargine. [16, 19] In our opinion, reasons for concern with regard to the safety of insulin glargine remain and the possible association with cancer, and breast cancer specifically, requires further attention.

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SUPPLEMENTARY MATERIAL

SM Table 1: Categorization of different types of insulin

Category		ATC code	Product Name	Duration of acting after administration
Insulin analogues	Insulin glargine	A10AE04	Insulin Glargine	Long
	Other insulin analogues	A10AB04	Insulin Lispro	Fast
		A10AB05	Insulin Aspart	
		A10AB06	Insulin Glulisine	
		A10AC04	Insulin Lispro	Intermediate
		A10AD04	Insulin Lispro	Combination fast and intermediate
		A10AD05	Insulin Aspart	
		A10AE05	Insulin Detemir	Long
Human Insulin	Human insulin	A10AB01	Human Insulin	Fast
		A10AC01	Human Insulin	Intermediate
		A10AD01	Human Insulin	Combination fast and intermediate
		A10AE01	Human Insulin	Long

SM Table 2: Number of days of Oral Glucose Lowering Drug (OGLD) use prior to start of insulin; stratified per year in which insulin therapy was started

Number of days of OGLD use in the year prior to start of insulin (median, inter-quartile range)				
Year of start with insulin	*n*	Users of insulin glargine	Users of other insulin analogues	Users of human insulin
2000	1,009	318 (226 – 340)	315 (233 – 345)	326 (283 – 351)
2001	1,827	330 (298 – 354)	321 (275 – 350)	326 (282 – 350)
2002	1,757	326 (276 – 349)	323 (287 – 351)	329 (287 – 349)
2003	2,095	331 (300 – 352)	326 (288 – 349)	326 (278 – 350)
2004	2,391	328 (292 – 350)	324 (281 – 347)	327 (281 – 352)
2005	2,341	329 (294 – 351)	323 (280 – 350)	323 (258 – 349)
2006	2,413	324 (273 – 350)	318 (190 – 349)	322 (278 – 348)
2007	2,736	317 (252 – 349)	314 (104 – 344)	309 (196 – 344)
2008	2,768	322 (276 – 349)	313 (120 – 345)	314 (164 – 347)
Number of days of OGLD use prior to start of insulin as of January first 1998 (median, inter-quartile range)				
Year of start with insulin	*n*	Users of insulin glargine	Users of other insulin analogues	Users of human insulin
2000	1,009	816 (245 – 937)	693 (272 – 919)	823 (236 – 940)
2001	1,827	1,050 (393 – 1,205)	841 (400 – 1,163)	1,064 (392 – 1,211)
2002	1,757	1,278 (539 – 1,539)	1,110 (482 – 1,558)	1,281 (554 – 1,538)
2003	2,095	1,195 (705 – 2,046)	1,393 (607 – 1,860)	1,327 (454 – 1,869)
2004	2,391	1,456 (621 – 2,212)	1,416 (489 – 2,168)	1,389 (455 – 2,199)
2005	2,341	1,632 (776 – 2,495)	1,486 (485 – 2,505)	1,495 (429 – 2,448)
2006	2,413	1,760 (502 – 2,676)	1,219 (233 – 2,470)	1,635 (481 – 2,542)
2007	2,736	1,566 (311 – 2,672)	1,113 (83 – 2,561)	1,171 (63 – 2,481)
2008	2,768	1,489 (483 – 2,849)	961 (105 – 2,396)	1,375 (134 – 2,697)

Abbreviations: *n:* number.

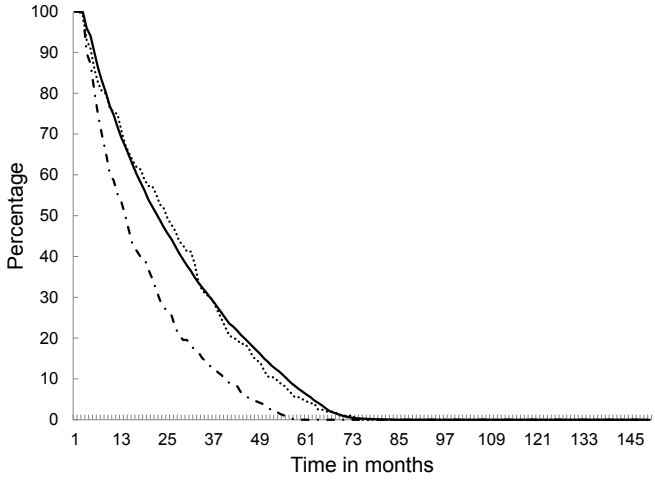
SM Table 3: Risk of cancer in patients using insulin glargine or other insulin analogues in comparison with those using human insulin (fixed analysis)

Covariables [a] included in the model	Insulin glargine		Other insulin analogues	
	HR	95% CI	HR	95% CI
None	0.73	0.69 – 0.77	0.80	0.77 – 0.82
Stratified for dose of first insulin prescription				
< Median	0.67	0.52 – 0.88	0.87	0.76 – 0.99
Median	0.73	0.67 – 0.78	0.76	0.73 – 0.80
> Median	0.63	0.56 – 0.71	0.87	0.83 – 0.92
Age, sex	0.74	0.70 – 0.78	0.80	0.78 – 0.83
Full model: age, sex, calendar time, hospitalizations, unique drugs	0.77	0.73 – 0.82	0.85	0.82 – 0.88
Full model adjusted for time since cessation [b]	0.73	0.69 – 0.78	0.83	0.80 – 0.86
Full model adjusted for days of prior OGLD use				
< 1 year OGLD use	0.75	0.64 – 0.89	0.81	0.75 – 0.88
≥ 1 year OGLD use	0.80	0.75 – 0.86	0.88	0.86 – 0.92
Full model, adjusted for use of OGLD (time-dependent)				
Biguanide	0.77	0.73 – 0.82	0.85	0.82 – 0.88
SU	0.77	0.73 – 0.82	0.85	0.82 – 0.88
Other OGLD	0.77	0.73 – 0.82	0.85	0.82 – 0.88
Full model adjusted for average DDD	0.77	0.72 – 0.82	0.85	0.82 – 0.88
Full model, stratified for dose of first insulin prescription				
< Median	0.65	0.49 – 0.86	0.88	0.76 – 1.02
Median	0.78	0.72 – 0.84	0.81	0.77 – 0.85
> Median	0.66	0.58 – 0.75	0.93	0.88 – 0.99
Full model including a latency time of 1 year [c]	0.75	0.70 – 0.81	0.87	0.84 – 0.91

Abbreviations: HR: hazard ratio, CI: confidence interval, OGLD: oral glucose lowering drugs, SU: sulfonylurea derivatives.

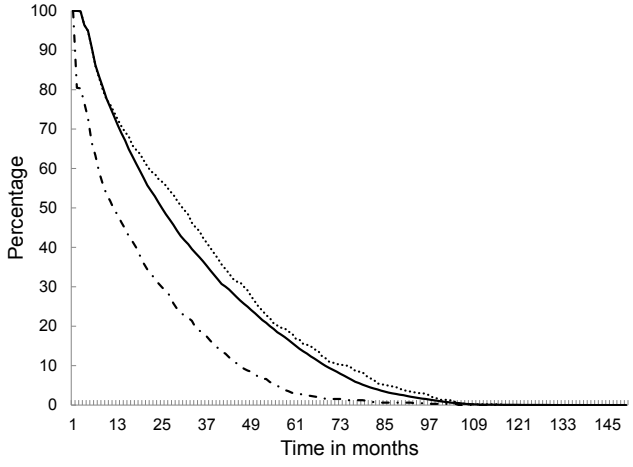
[a] Covariables: age, age at first insulin prescription; calendar time, time since inclusion of participant in PHARMO RLS; hospitalizations, number of hospitalizations in the year prior to start of insulin; unique drugs, number of unique drugs dispensed in the year prior to start of insulin; days of prior OGLD use, number of days of OGLD use as of January 1998; average DDD, dose calculated over all previous insulin prescriptions. [b] Time since cessation of insulin glargine, other insulin analogues and/or human insulin in days. [c] Model included a 1 year latency period: exposure was cumulated up till one year prior to the date of cancer diagnosis; incidence rate (no. of cancer diagnoses/1,000 patient years) for insulin glargine 7.20; for other insulin analogues 7.90 and for human insulin 9.23.

SM Figure 1: Participants' adherence to insulin glargine presented separately for those who died, those who got diagnosed with cancer and those who got censored at the end of study.



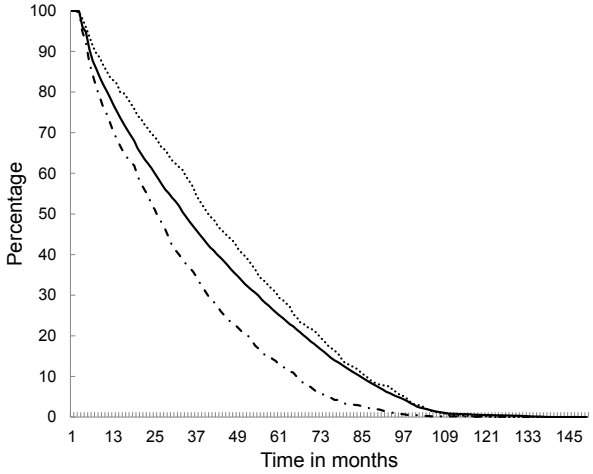
Legends: Dotted line: diagnosed with cancer, dotted/dashed line: died, solid line: censored at the end of study.

SM Figure 2: Participants' adherence to other insulin analogues presented separately for those who died, those who got diagnosed with cancer and those who got censored at the end of study.



Legends: Dotted line: diagnosed with cancer, dotted/dashed line: died, solid line: censored at the end of study.

SM Figure 3: Participants' adherence to human insulin presented separately for those who died, those who got diagnosed with cancer and those who got censored at the end of study.



Legends: Dotted line: diagnosed with cancer, dotted/dashed line: died, solid line: censored at the end of study.

SM METHODS

Fixed cohort analysis

An additional analysis performed was the fixed cohort analysis in which the first exposure to insulin determined in which drug category the participant was categorized. If individuals received additional different types of insulin, switched or discontinued use during follow-up this did not change the exposure status. This was chosen to simulate an intention-to-treat analysis and consequently further avoid reverse causation.

A theoretical example: a participant used six months of insulin glargine, discontinues and starts with another insulin analogue for six months until the end of the study. In the fixed analysis, only the exposure to insulin glargine will be taken into account. In the as treated analysis, the exposure to insulin glargine, as well as the exposure to the other insulin analogue will be taken into account. Follow-up for both situations is from the date of starting the first insulin until the end of study. As a consequence of the example described above, the number of exposed participants to a certain insulin will be similar or larger in the as treated analysis in comparison with the fixed analysis. Consequently, the number of participants with a cancer diagnosis will be similar or larger in the as treated analysis.

Propensity Score Analysis

To further adjust for residual confounding, an analysis using propensity scores was performed. The propensity of treatment with either insulin glargine or other insulin analogues at baseline was calculated, based on adjusted estimates from a binary logistic regression model (treatment of interest yes/no) with the following characteristics: sex, age at first insulin prescription, year of first prescription of insulin, number of unique other drugs used in the year before start of insulin (excluding those prescribed for diabetes), number of hospitalizations in the year before start of insulin, the number of days of use of an oral glucose lowering drug in the year before start of insulin therapy and the number of days of OGLD use as of 1 January 1998. [1-2] The association between insulin glargine and other insulin analogues, respectively, and cancer in comparison with human insulin was analyzed using Cox proportional hazard models with cumulative duration of drug use as a time-varying determinant while adjusting for the respective propensities. Modeling was performed for the fixed analysis, as well as for the as treated analysis. In the as treated analysis, adjustments were also made for the use of other types of insulin than the reference group (human insulin) or the insulin of interest (in the analysis for insulin glargine, adjustments were made for the use of other insulin analogues than insulin glargine and vice versa).

Use of OGLD

Use of OGLD was taken into account in two different ways. Firstly, the full model was stratified for those using less or more than 1 year OGLD prior to start of insulin. These models were adjusted for the number of days of use of OGLD as a proxy for duration of diabetes mellitus. Furthermore, the full model was analyzed while additionally adjusting, in a time varying manner, for cumulative use of biguanides (A10BA), sulfonylurea derivatives (A10BB) and use of other OGLD (A10B minus those mentioned above).

Dose

The average dose per insulin category was used as a time-dependent covariable in the full model. However, since follow-up information is used performing these analyses, which is methodologically less elegant, a second analysis was performed in which the crude model, as well as the full model, were analyzed stratified for the dose of the first dispensed insulin prescription. The latter being less elegant from a clinical point of view, since most participants get initiated on a general dose before being titrated to a more personal dose. Third, a dose analysis was performed within each insulin category, in which the average DDD during follow-up in those with cancer was compared with the average DDD in all individuals without cancer, with the same duration of insulin exposure in days. In these analyses, those with an average DDD higher than the median were compared with those with an average DDD lower than the median.

General statistical methods

Covariables that changed the hazard ratio (HR) of cancer risk by more than 10%, or were considered clinically relevant, were taken into account as confounders. [3] To test for effect modification by covariables, interaction terms were introduced in the model and stratified analyses were performed if the interaction term was significant. Non-parametric tests (Kruskal-Wallis) and linear regression were applied to verify differences between the treatment groups for continuous variables. These were preferred over ANOVA, since there was no equality of variance among the different treatment groups. Differences in categorical variables between the groups were tested with a chi-square test. Analyses were performed using SPSS software (version 16.0, IBM, US) and SAS software (version 9.1, SAS institute, Cary, US). Proportionality of the full model was tested by adding an interaction term of the determinant and time. *P*-values are two-sided and were considered statistically significant if $p < 0.05$.

SM RESULTS

Fixed cohort analysis

878 participants were hospitalized for cancer, 101 of these started insulin therapy on insulin glargine, 251 started on other insulin analogues and 526 participants started on human insulin. The corresponding incidence rates were respectively 12.12, 12.81 and 14.61 cancers per 1000 patient years. As can be seen from **supplementary material (SM) table 3**, use of insulin glargine was associated with a lower risk of cancer in comparison with users of human insulin (HR 0.73, 95% CI 0.69 – 0.77).

In the full model, adjustments were made for age at first insulin prescription, sex, calendar time, number of unique drugs used and number of hospitalizations in the year before start of insulin (HR 0.73, 95% CI 0.73 – 0.82). Stratifying for prior OGLD use, for less or longer than 1 year, did not change this point estimate, nor did adjustment for prior days of OGLD used, change the point estimate more than 10%. Adjustments were made by adding dose as an additional time-varying covariable to the model (HR 0.79, 95% CI 0.72 – 0.82) but since follow-up information is used applying this method, stratified analyses for baseline dose are presented in **SM table 3**. Since the vast majority of the cohort members had a median first dose of 16.7 U per day **(table 1)**, these analyses were stratified in three strata: more than, less than or equal to the median dose per day. When replacing cumulative exposure at end of follow up with attained cumulative exposure one year prior to end of follow-up (in order to minimize the chance of reverse causation), the point estimates remained statistically significantly protective. Proportionality of the full model was tested; the assumption of proportional hazards was complied with (*p*-values respectively 0.14 and 0.67).

When specific cancers were used as endpoints (**SM table 4**) applying the full model, insulin glargine was associated with a significantly lower risk of colon cancer but not of other cancers. In contrast, use of insulin glargine was associated with an increased risk of breast cancer in comparison with human insulin (HR 1.39, 95% CI 1.08 – 1.79). The complete analyses for endometrial cancer and pancreatic cancer were not possible due to a low number of cancer diagnoses (respectively *n*=2 and *n*=7). Furthermore, with regard to the stratified model for first prescribed dose, analyses were not possible for some of the lowest quartiles due to a low number of cases. The low number was a consequence of the issue that ≈70% of the participants received a first dose of 16.6 U per day resulting in an unequal distribution (**table 1**). No clear dose effect could be seen over the different strata of dose. For other insulin analogues, no increased risk of breast cancer was seen (HR 1.00, 95% CI 0.93 – 1.09), however, a decreased risk of colon cancer, bladder cancer, respiratory tract cancer and prostate cancer was found.

Dose-response relations could not be identified for users of insulin glargine (crude HR comparing those with an average DDD higher than the median with those having an average DDD lower than the median: 1.14, 95% CI 0.77 – 1.69, HR applying full model 1.06, 95% CI 0.71 – 1.29) , nor could this be demonstrated for other insulin analogues than insulin glargine (crude HR 1.06, 95% CI 0.79 – 1.42, HR applying full model 1.04, 95% CI 0.78 – 1.39) or for human insulin (crude HR 1.01, 95% CI 0.86 – 1.20), adjusted HR applying a similar full model HR 0.94, 95% CI 0.79 – 1.12).

Propensity Score Analysis

In the fixed analysis, the use of insulin glargine was associated with a lower risk of cancer in comparison with users of human insulin (HR 0.73, 95% CI 0.69 – 0.77). The use of other insulin analogues was associated with a lower risk of cancer in comparison with users of human insulin as well (HR 0.80, 95% CI 0.78 – 0.83). Similar estimates were found for the as treated analysis. The use of insulin glargine was associated with a lower risk of cancer in comparison with users of human insulin (HR 0.72, 95% CI 0.68 – 0.76), as was the use of other insulin analogues in comparison with use of human insulin (HR 0.82, 95% CI 0.79 – 0.86).

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SM Table 4: Risk of specific cancer in patients using insulin glargine or other insulin analogues in comparison with those using human insulin (fixed analysis)

Model	Insulin glargine				Other insulin analogues				Human insulin
	IR	*n*	HR	95% CI	IR	*n*	HR	95% CI	IR
Colon cancer									
Full model [a]	1.65	13	0.61	0.43 – 0.88	2.36	38	0.87	0.78 – 0.97	2.83
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			0.74	0.64 – 0.87			0.92	0.85 – 0.99	
SU			0.74	0.64 – 0.86			0.91	0.84 – 0.99	
Other OGLD			0.74	0.64 – 0.87			0.92	0.85 – 0.99	
Full model + average DDD			0.64	0.45 – 0.90			0.73	0.64 – 0.84	
Full model, stratified for dose of first insulin prescription									
< Median		0	–	–		0	–	–	
Median		10	0.29	0.16 – 0.53		29	0.70	0.60 – 0.82	
> Median		3	–	–		9	0.76	0.58 – 1.00	
Full model including a latency time of 1 year [b]	1.03	8	0.83	0.54 – 1.28	0.94	15	0.88	0.77 – 1.00	1.71
Bladder cancer									
Full model [a]	1.02	8	1.77	1.04 – 3.00	0.93	15	0.69	0.57 – 0.84	1.00
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			0.82	0.67 – 1.01			0.84	0.75 – 0.95	
SU			0.83	0.68 – 1.01			0.84	0.75 – 0.95	
Other OGLD			0.82	0.67 – 1.01			0.84	0.74 – 0.95	
Full model + average DDD			1.26	0.63 – 2.49			0.61	0.47 – 0.78	
Full model, stratified for dose of first insulin prescription									
< Median		0	–	–		0	–	–	
Median		7	2.26	1.17 – 4.39		11	0.49	0.36 – 0.66	
> Median		1	–	–		4	0.77	0.57 – 1.02	
Full model including a latency time of 1 year [b]	0.64	5	2.06	0.83 – 5.12	0.69	11	0.84	0.69 – 1.02	0.74

SM Table 4: Risk of specific cancer in patients using insulin glargine or other insulin analogues in comparison with those using human insulin (fixed analysis) (cont'd)

Model	Insulin glargine				Other insulin analogues				Human insulin
	IR	*n*	HR	95% CI	IR	*n*	HR	95% CI	IR
Respiratory tract cancer									
Full model [a]	1.53	12	0.92	0.74 – 1.16	2.11	34	0.70	0.62 – 0.78	1.74
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			0.90	0.71 – 1.13			0.86	0.78 – 0.94	
SU			0.81	0.61 – 1.06			0.85	0.78 – 0.93	
Other OGLD			0.94	0.75 – 1.19			0.85	0.78 – 0.93	
Full model + average DDD			0.57	0.42 – 0.79			0.70	0.62 – 0.78	
Full model, stratified for dose of first insulin prescription									
< Median		0	–	–		1	–	–	
Median		8	0.72	0.44 – 1.18		21	0.69	0.58 – 0.82	
> Median		4	0.88	0.62 – 1.24		12	0.83	0.73 – 0.95	
Full model including a latency time of 1 year [b]	1.16	9	1.06	0.81 – 1.39	1.25	20	0.77	0.68 – 0.88	1.29
Prostate cancer									
Full model [a]	1.06	4	1.17	0.84 – 1.62	1.26	10	0.82	0.69 – 0.97	1.74
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			1.24	0.81 – 1.57			0.82	0.69 – 0.97	
SU			1.18	0.85 – 1.63			0.76	0.63 – 0.93	
Other OGLD			1.21	0.85 – 1.73			0.51	0.37 – 0.69	
Full model + average DDD			1.01	0.61 – 1.67			0.98	0.81 – 1.18	
Full model, stratified for dose of first insulin prescription									
< Median		0	–	–		0	–	–	
Median		3	0.96	0.57 – 1.64		6	0.78	0.46 – 1.31	
> Median		1	–	–		4	0.87	0.65 – 1.16	
Full model including a latency time of 1 year [b]	0.76	3	2.19	1.03 – 4.66	0.64	5	0.84	0.68 – 1.03	1.28

SM Table 4: Risk of specific cancer in patients using insulin glargine or other insulin analogues in comparison with those using human insulin (fixed analysis) (cont'd)

Model	Insulin glargine				Other insulin analogues				Human insulin
	IR	*n*	HR	95% CI	IR	*n*	HR	95% CI	IR
Breast cancer									
Full model [a]	5.40	21	1.39	1.08 – 1.79	2.81	23	1.00	0.93 – 1.09	2.88
Full model, adjusted for use of OGLD (time-dependent)									
Biguanide			1.40	1.09 – 1.79			1.00	0.92 – 1.09	
SU			1.46	1.12 – 1.90			1.01	0.93 – 1.09	
Other OGLD			1.59	1.21 – 2.08			1.03	0.94 – 1.11	
Full model + average DDD			1.43	1.08 – 1.88			0.94	0.86 – 1.03	
Full model, stratified for dose of first insulin prescription									
< Median		1	–	–		2	–	–	
Median		13	1.49	1.12 – 1.98		15	1.10	0.96 – 1.26	
> Median		7	1.01	0.62 – 1.63		6	0.28	0.11 – 0.71	
Full model including a latency time of 1 year [b]	2.35	9	2.19	1.44 – 3.33	1.48	12	1.01	0.92 – 1.11	2.36

Abbreviations: IR: incidence rate, *n:* number of events, HR: hazard ratio, CI: confidence interval, OGLD: oral glucose lowering drugs, SU: sulfonylurea derivatives.
[a] In the full model, adjustments were made for age at first insulin prescription, sex, calendar time, number of unique drugs used in the year before start of insulin and number of hospitalizations in the year before start of insulin. Days of prior OGLD use, number of days of OGLD use as of January 1998. [b] Model included a 1 year latency period: exposure was cumulated up till one year prior to the date of cancer diagnosis.

Chapter 3.2

Lower risk of cancer in patients on metformin in comparison with those on sulfonylurea derivatives: results from a large population-based follow-up study

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Diabetes Care 2011. [Epub ahead of print]

ABSTRACT

Introduction: numerous studies suggested a decreased risk of cancer in diabetics on metformin. Since different comparison groups were used, the effect magnitude is difficult to estimate. Therefore, the objective of this study was to further analyze whether, and to which extent, use of metformin is associated with a decreased risk of cancer in a cohort of incident users of metformin in comparison with users of sulfonylurea derivatives.
Methods: data for this study were obtained from dispensing records from community pharmacies, individually linked to hospital discharge records from 2.5 million individuals in the Netherlands. The association between use of metformin and cancer, in comparison with use of sulfonylurea derivatives, was analyzed using Cox proportional hazard models, with cumulative duration of drug use as a time-varying determinant.
Results: use of metformin was associated with a lower risk of cancer in general (HR 0.90, 95% CI 0.88 – 0.91) in comparison with use of sulfonylurea derivatives. When specific cancers were used as endpoints, similar estimates were found. Dose-response relations were identified for users of metformin, but not for users of sulfonylurea derivatives.
Conclusion: in our study, cumulative exposure to metformin was associated with a lower risk of specific cancers and cancer in general, in comparison with cumulative exposure to sulfonylurea derivatives. However, whether this should indeed be seen as a decreased risk of cancer for the use of metformin, or as an increased risk of cancer for the use of sulfonylurea derivatives, remains to be elucidated.

INTRODUCTION

As drug of first choice in diabetes mellitus type 2, metformin is the most widely prescribed oral glucose lowering drug (OGLD). [1-2] However, the decision to prescribe metformin also depends on patient characteristics. In those with renal failure, cardiac or hepatic failure, use of metformin is contra-indicated. [2]

In 2004, a statistically non-significant relationship between use of metformin and the risk of colon cancer was described. [3] However, one year later, metformin was found to be associated with a decreased risk of cancer in general in a case-control study in a diabetic population. [4] Numerous studies followed; among which studies confirming the association between use of metformin and decreased risk of cancer in general [5-8] or in specific cancers. [5-6, 9-14] However, for breast cancer [5-6] and prostate cancer, [5, 14] the decreased risk was not consistently demonstrated; for other cancers no association with use of metformin was found. [6, 12] Hence, there is heterogeneity among published studies on cancer in diabetics on metformin, [15] partly because different comparison groups were used such as non metformin users, users of other OGLDs or users of insulin. Higher endogenous insulin levels have been linked to an increased risk of certain cancers. [16] Moreover, specifically for insulin glargine, the debate whether this specific insulin increases the risk of cancer is ongoing. [17-21]

Due to factors such as different drugs used to attain metabolic control, the duration of diabetes and the presence of other diseases, the assessment of cancer risk in diabetic patients remains difficult. Therefore, the objective of this study was to analyze whether, and to which extent, use of metformin is associated with a decreased risk of cancer in a cohort of incident users of metformin, in comparison with use of sulfonylurea derivatives.

METHODS

Setting

Data for this study were obtained from the PHARMO Record Linkage System (RLS), which includes drug dispensing records from community pharmacies linked at a patient level to hospital discharge records from the Dutch National Medical Register for approximately 2.5 million individuals in the Netherlands since 1986. The drug dispensing database contains detailed information per prescription as of 1998. The hospital record database contains information on discharge diagnoses and the dates of admission and discharge, coded according to the International Classification of Disease ninth edition (ICD).

Study Population

All individuals with more than one prescription for any hypoglycemic drug, between 1 January 1998 and 31 December 2008 were eligible. To ensure a study cohort of incident OGLD users, participants needed to have a six month period without prescription of any hypoglycemic drug before inclusion. Patients using only insulin were excluded; in addition, those who started on other OGLD than biguanides or sulfonylurea derivatives or were under 18 years of age at first prescription and patients with a primary cancer before first prescription of OGLD were excluded from the analysis.

Exposure

The OGLD were classified into two mutually exclusive categories according to ATC-code: biguanides (A10BA) and sulfonylurea derivatives (A10BB). In the Netherlands, metformin is the only biguanide available. To obtain a valid estimate, use of sulfonylurea derivatives was chosen as comparator since, in our opinion, a comparison should be made to participants with diabetes to reduce the risk of confounding by indication. In addition, a single drug category, for the same indication, and of sufficient size, is the most straightforward comparator. Besides metformin, sulfonylurea derivatives are most frequently used. The cumulative exposure to each OGLD category was calculated for each participant in days since the start of the respective OGLD type until death of the participant, diagnosis of cancer, removal out of the PHARMO RLS catchment area, the last day of use of a dispensing in the same OGLD category, start of insulin or another OGLD than metformin or sulfonylurea derivatives, or end of the study period at 31 December 2008. To visualize drug adherence, the percentage participants adherent to therapy was calculated: for all patients the follow-up time on metformin and sulfonylurea derivatives was calculated. For every month of follow-up, the number of users of each drug was divided by the total number of users of that drug at study start.

Outcome

The primary outcome was first hospital admission with a primary diagnosis of any type of cancer, ICD-9 codes 140 – 172, 174 – 209 and 235 – 239. Sub-analyses were performed for the following specific cancers: esophagus cancer (ICD-9 150), stomach cancer (ICD-9 151), colorectal cancer (ICD-9 153 – 154), primary liver cancer (ICD-9 155), pancreatic cancer (ICD-9 157), respiratory tract cancer (ICD-9 160 – 165), breast cancer (ICD-9 174 – 175) and prostate cancer (ICD-9 185). These cancers were selected because they have been previously studied in association with the use of metformin.

Covariables

Age at first OGLD prescription, sex, number of unique other drugs used in the year before start of OGLD, number of hospitalizations in the year before start of OGLD and

calendar time were considered as potential confounder or effect modifier. For each dispensing, the dose was available. The average dose was calculated for metformin and sulfonylurea derivatives as the average defined daily dose (DDD) over the previously dispensed prescriptions.

Statistical analysis

The association between metformin and cancer was analyzed using Cox proportional hazards models with duration of cumulative drug use as a time-varying determinant, as described earlier. [22] In this model, cumulative exposure to metformin in participants with cancer at the date of diagnosis was compared to cumulative exposure to sulfonylurea derivatives in the remaining cohort members at the same date of follow-up, i.e. with the same duration of OGLD exposure in days. Time since start of OGLD was used as underlying timescale in the Cox proportional hazard model. Participants were censored at time of start of insulin or another OGLD than the drug of interest (metformin) or the reference drugs (sulfonylurea derivatives); in case of multiple cancer diagnoses, additional censoring occurred at the first cancer.

Sub-analyses

Different sub-analyses were performed to assess the robustness of the results. To address possible reverse causation, a latency period was taken into account (**sub-analysis a**); we assumed that cancer was already present one year before it was actually diagnosed (i.e. end of cumulation of exposure on 21 June 2007 when the cancer was diagnosed at 21 June 2008). In order to assess the effects of long term use another sub-analysis was performed in patients using metformin or sulfonylurea derivatives for at least 365 days (**sub-analysis b**). Since metformin users are frequently additionally treated with sulfonylurea derivatives and vice versa, a sub-analysis was performed in which additional censoring of the participants took place at the moment that participants on metformin started on sulfonylurea derivatives and the moment participants on sulfonylurea derivatives started on metformin (**sub-analysis c**). Furthermore, a sub-analysis was performed in those who were solely treated with monotherapy with either metformin or sulfonylurea derivatives (**sub-analysis d**) and a sub-analysis was performed in those who were treated with metformin as well as with sulfonylurea derivatives but not with any other hypoglycemic (**sub-analysis e**) during the study period.

Also, the effect of dose was assessed in additional analyses in which the full model was adjusted for dose in a time dependent manner. However, since follow-up information is used performing this analysis, a second analysis was performed in which the full model was stratified for the dose of the first OGLD. In these analyses, those with a higher than the mean first dose of metformin were compared with those with a higher than the mean first dose of sulfonylurea derivatives. In addition, those with a lower than the mean

first dose of metformin are compared with those with a lower than the mean first dose of sulfonylurea derivatives. Third, a dose analysis was performed within respectively users of metformin and sulfonylurea derivatives in which the average DDD during follow-up in those with cancer was compared with the average DDD in all individuals without cancer.

General statistics

Covariables that changed the hazard ratio (HR) of cancer risk by more than 10%, or which were considered clinically relevant, were included in the model. To test for effect modification, interaction terms were introduced in the model and stratified analyses were performed. Non-parametric tests (Kruskal-Wallis) and linear regression were applied to verify differences between the treatment groups for continuous variables. These were preferred over ANOVA, since there was no equality of variance among the different treatment groups. Differences in categorical variables between the groups were tested with a chi-square test. Analyses were performed using SAS software (version 9.2, SAS institute, Cary, US). *P*-values are two-sided and were considered statistically significant if $p < 0.05$.

RESULTS

Within the PHARMO RLS, 158,599 participants were prescribed an OGLD or insulin between 1 January 1998 and 31 December 2008. 3,184 participants (2.0%) were excluded due to inconsistencies in the database, 6,638 (4.2%) for having a cancer diagnosis before 1 January 1998 or before exposure. Another 14,016 (8.8%) were solely treated with insulin and 47,997 (30.3%) did not have a prescription free period of six months before starting an OGLD. 1,390 (0.9%) participants were exposed before the age of 18 year and 1,866 (2.1%) had their first prescription for another oral glucose lowering drug than metformin or a sulfonylurea derivative. After applying exclusion criteria, 85,289 (53.8%) participants were included in the study cohort (participants could be excluded due to several reasons).

Between participants starting metformin and those starting sulfonylurea-derivatives, significant differences were present at baseline and during follow-up (**table 1**). Although those prescribed metformin were significantly younger, the age distribution was similar between users of metformin and sulfonylurea derivatives. Patient starting with metformin used less other drugs and had fewer hospitalizations in the year before start of OGLD than those starting on sulfonylurea derivatives. The duration of follow-up since first OGLD was significantly shorter for those who started with metformin than for those who started with sulfonylurea derivatives.

Table 1: Characteristics of participants using metformin or sulfonylurea derivatives

Characteristic	Incident users of metformin (*n*=52,698; 61.8%)	Incident users of SU (*n*=32,591; 38.2%)
Age at first prescription of OGLD in years [a]		
Mean±SD	61.8±13.4	65.6±13.8
Median (IQR)	62.1 (52.8 – 71.7)	66.7 (56.2 – 76.0)
Male sex [b] (%)	24,432 (46.4%)	15,699 (48.2%)
Total number of unique other drugs used in the year before first prescription of OGLD [a]		
Mean±SD	6.0±4.8	6.1±5.3
Median (IQR)	5 (2 – 8)	5 (2 – 9)
Total number of unique hospitalizations in the year before first prescription of OGLD [a]		
Mean±SD	0.3±0.8	0.3±0.9
Median (IQR)	0 (0 – 0)	1 (0 – 1)
Duration of follow-up since first OGLD prescription in days [a]		
Mean±SD	1,031±853	1,697±1,071
Median (IQR)	825 (348 – 1526)	1,639 (791 – 2534)
Average daily dose of the first OGLD prescription in DDD		
Mean±SD	0.55±2.17	1.04±0.91
Median (IQR) [a]	0.45 (0.25 – 0.50)	0.67 (0.50 – 1.33)
Average daily dose over all OGLD prescriptions since first prescription in DDD		
Mean±SD	0.69±1.73	1.49±1.13
Median (IQR) [a]	0.50 (0.38 – 0.85)	1.14 (0.65 – 2.00)
Solely treated with metformin or SU (%)	27,129 (51.5%)	13,045 (40.0%)
Additional treatment with either metformin or SU (%)	19,068 (36.2%)	16,950 (52.0%)
Hospitalized for cancer diagnosis (%)	1,590 (3.0%)	1,962 (6.0%)
Censored because of death (%)	6,501 (12.3%)	3,459 (10.6%)
Censored because of start of other OGLD or insulin (%)	11,909 (22.6%)	8,781 (26.9%)

Abbreviations: OGLD: oral glucose lowering drug. SD: standard deviation; IQR inter-quartile range; DDD: defined daily dose; SU: sulfonylurea derivatives.

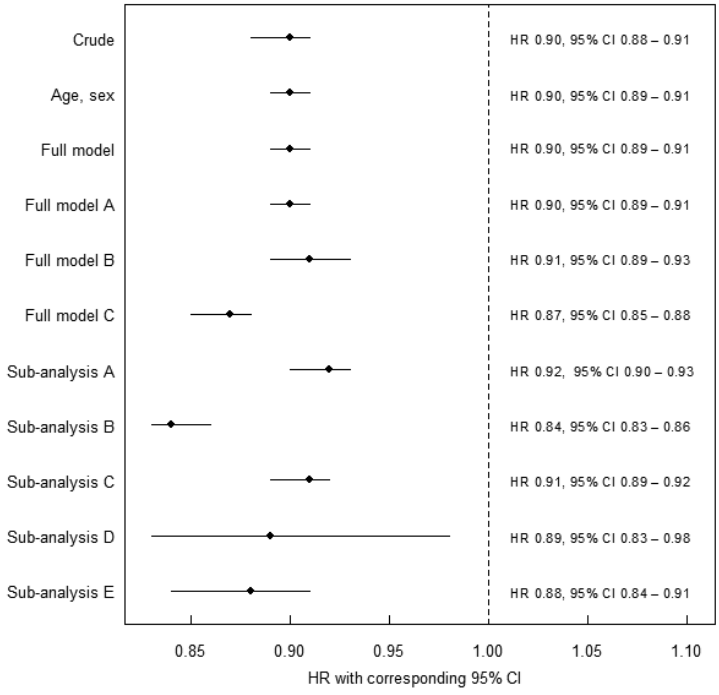
[a] *p*-value following linear regression <0.001. [b] *p*-value following chi-square test <0.001.

An adherence curve is presented in **supplementary figure 1**; the adherence to therapy between those on metformin and those on sulfonylurea derivatives differed statistically significantly (*p*-value < 0.001) with those on metformin being less adherent.

Of the 3,552 participants hospitalized for cancer, 1,590 started with metformin and 1,962 started with sulfonylurea derivatives. The incidence rates were respectively 10.69

and 12.96 cancers per 1,000 patient years. Cumulative exposure to metformin was associated with a lower risk of cancer in comparison with cumulative exposure to sulfonylurea derivatives (HR 0.90, 95% CI 0.88 – 0.91; **figure 1**). In the full model, adjustments were made for age at first OGLD prescription, sex, calendar time, number of unique drugs used and number of hospitalizations in the year before start of OGLD (HR 0.90, 95% CI 0.89 – 0.91). Further adjustments by adding dose as an additional time-varying covariable to the model yielded a similar HR (HR 0.90, 95% CI 0.89 – 0.91); since follow-up information is used applying this method, stratified analyses for baseline dose were also

Figure 1: risk of cancer in patients when comparing cumulative exposure to metformin to cumulative exposure to sulfonylurea derivatives.



Legends: The **full model** included the covariables: Age: age at first OGLD prescription; Sex; Calendar time: calendar year in which the first prescription was dispensed; Hospitalizations: no. of hospitalizations in the year prior to start of OGLD; Unique drugs: no. of unique drugs dispensed in the year prior to start of OGLD. **Full model A** additionally included the average DDD: dose calculated over all previous OGLD prescriptions. **Full model B** was stratified for dose of first OGLD prescription lower than the median dose; **Full model C** was stratified for dose of first OGLD prescription higher than the median dose. **Sub-analysis A** included a 1 year latency period: exposure was cumulated up till one year prior to the date of cancer diagnosis. **Sub-analysis B** included only those with more than 1 year of exposure since start of OGLD. In **sub-analysis C** additional censoring took place at the moment metformin users started SU and at the moment SU users started metformin. **Sub-analysis D** included only those treated with monotherapy metformin or SU during the study period and **sub-analysis E** included only those who were treated with as well metformin as sulfonylurea derivatives during the study period. HR: Hazard Ratio. CI: confidence interval.

performed (**figure 1**). In these analyses, those with a dose higher than the median dose had a lower hazard (HR 0.87, 95% CI 0.85 – 0.88) than those starting on a lower dose than the median dose (HR 0.91, 95% CI 0.89 – 0.93).

The robustness of the results was tested performing different sub-analyses (**figure 1**); in none of these analyses the HR changed more than 10%. Furthermore, the full model was analyzed stratified for those older than the median age and those younger. For those younger than the median age, a lower HR for the risk of cancer (HR 0.86; 95% CI 0.84 – 0.88) was found than for those aged older than the median age (HR 0.93, 95% 0.91 – 0.95). In addition, the full model was analyzed stratifying for those who had been hospitalized prior to the start of OGLD and those who had not been hospitalized. Those hospitalized prior to the first dispensing of OGLD had a lower risk of cancer (HR 0.84, 95% CI 0.81 – 0.87) than those not hospitalized (HR 0.91, 95% CI 0.89 – 0.92).

The full model was applied in all sub-analyses in which specific cancers were used as endpoints as well; these results are presented in **table 2.** As with the analysis on cancer in general, additional adjustment by average DDD did not change the point estimates. Furthermore, for all specific cancers, it was found as well that a baseline dose of more than the median had a slightly higher protective effect than a baseline dose below the median. With regard to the exposure of more than 365 days, this also resulted in lower estimates for all outcomes with exception of stomach cancer, this point estimate did not change.

Table 2: risk of specific cancer in patients when comparing cumulative exposure to metformin to cumulative exposure to sulfonylurea derivatives

	Metformin		SU		HR of metformin with reference to SU	
	n [a]	IR [b]	*n* [a]	IR [b]	HR [c]	95% CI
Esophagus	45	0.30	46	0.30	0.90	0.82 – 0.97
Stomach	47	0.32	70	0.46	0.83	0.76 – 0.90
Colon	228	1.53	299	1.97	0.91	0.88 – 0.94
HCC	16	0.11	15	0.10	0.67	0.53 – 0.86
Pancreas	60	0.40	106	0.70	0.73	0.66 – 0.80
Respiratory	203	1.36	251	1.66	0.87	0.84 – 0.91
Breast	207	2.63	217	2.81	0.95	0.91 – 0.98
Prostate	90	1.28	136	1.83	0.92	0.88 – 0.97

Abbreviations: *n:* number, IR: incidence rate, HR: hazard ratio, CI: confidence interval, OGLD: oral glucose lowering drugs, SU: sulfonylurea derivatives.

[a] *n*: number of events. [b] IR: Incidence rate / 1000 patient years. [c] HR: Hazard Ratio applying the full model in which adjustments were made for age at first OGLD prescription, sex, year in which the first OGLD prescription was dispensed, no. of unique drugs used in the year and no. of hospitalizations in the year before start of OGLD.

Dose-response relations could be identified for use of metformin (crude HR comparing those with an average DDD higher than the median with those having an average DDD lower than the median: 0.80, 95% CI 0.72 – 0.89, HR applying full model 0.89, 95% CI 0.80 – 0.99), but not for sulfonylurea derivatives (crude HR 1.00, 95% CI 0.99 – 1.01, HR applying full model 1.00, 95% CI 0.99 – 1.01).

DISCUSSION

In this study, we found that use of metformin was associated with a significantly lower risk of cancer in general and of specific cancers, in comparison with the use of sulfonylurea derivatives. The HR of 0.90 found in our study (95% CI 0.88 – 0.91) is similar to the odds ratio of 0.86 found by Evans *et. al*. (95% CI 0.73 – 1.02, with reference to no metformin use). [4] However, they presented a subset of patients included in a study published later, in which a lower HR for the use of metformin of 0.63 (95% CI 0.53 – 0.75, adjusted) was described in comparison with no use of metformin. [6] In addition, in an Italian case control study exposure to metformin and gliclazide was associated with a reduced risk of cancer of 0.28 (95% CI 0.13 – 0.57) in comparison with no exposure. [8] Others found that use of metformin monotherapy, in comparison with sulfonylurea derivative monotherapy, was associated with a decreased risk of cancer of 0.74 (95% CI 0.65 – 0.84). [5, 15]

In our opinion, the differences in estimates can largely be explained by differences in the study populations, designs, methods of collecting risk factors and estimation of the exposure to metformin (duration and dose), the comparators used and the start of follow-up.

The association with age in our study can be explained by the increased risk of cancer at higher age; the association with hospitalization prior to start of OGLD might be explained by better screening and earlier diagnosis. Dose-dependent relations could be demonstrated for metformin, but not for sulfonylurea derivatives. With regard to the differences in mean average DDD between those using metformin (0.7) and those using sulfonylurea derivatives (1.5), we hypothesized that this can be explained by a lower tolerability of participants to metformin in comparison with sulfonylurea derivatives.

Since diabetes itself is associated with cancer, our study included only incident users of metformin or sulfonylurea derivatives, which was defined as a prescription free period of six months before study entry. [23] As follow-up started at the date of first prescription of an OGLD, adjustment for duration of diabetes in our study was optimal and as a consequence, all participants had a more or less similar duration of diabetes mellitus. However, we were not able to filter out those who used metformin for other indications (e.g., polycystic ovarian disease). Such diseases occur at a low frequency and these indications are not registered in the Netherlands. Consequently, the number of those using

metformin for other indications than diabetes is most likely too low to bias the risk estimates in our study. In addition, as this study included only those with diabetes who were treated with drugs, no comparison could be made with those who were treated with lifestyle changes. Furthermore, since no information was available on cause of mortality, we were not able to verify whether use of metformin is associated with a decreased risk of cancer death in comparison with sulfonylurea derivatives as published earlier. [24]

We were indirectly able to adjust for co-morbidity because we had information on other drugs used and on the number of hospitalizations prior to the first prescription of OGLD. However, in contrast to some former studies, we were not able to adjust for smoking status or BMI, which might be considerable confounding factors. Similar to others, one of the most important issues which we could not address was the clinical decision making process, determining each patient's treatment.

Reverse causality may play a role in observational studies since cancer often has a long latency period during which the disease is already present but has not yet been diagnosed. During this long latency period, the disease itself may cause changes in treatment and therefore, the assessment of etiologically relevant timing of exposure is of pivotal importance. [18] By taking into account a latent period (i.e. when disease is already present but not yet diagnosis) by cumulating exposure to one year prior to the date of diagnosis, we attempted to minimize reverse causality; this did not change the HR. Other sensitivity analyses to test the robustness of our results were performed as well, none of them changing the HR more than 10%.

As PHARMO RLS is a population-based database, selection bias is negligible as everybody using any prescription at any time is enrolled. Misclassification of exposure is unlikely as all information on dispensed prescriptions is gathered prospectively and automatically. Furthermore, misclassification of the outcome is unlikely as this is collected independently of the exposure of interest in our study. However, we used cancer hospitalization as outcome measure, which is different from pathology data on cancer diagnoses. Some cancers might be diagnosed and treated more frequently on an outpatient basis. However, as the cancers are coded independently of the exposure, within each specific cancer, this would lead to non differential misclassification of the outcome and consequently to dilution of the estimated effect towards the null-hypothesis.

Several possible explanatory biological mechanisms that might explain the protective effect of metformin on the risk of cancer have been described. [25] However, it should be emphasized that these are largely speculative. The decreased risk of cancer in those using metformin in comparison with those using sulfonylurea derivatives could also be explained as an increased risk of cancer of those using sulfonylurea derivatives in comparison with those using metformin. As sulfonylurea derivatives increase the levels of endogenous insulin, this would be a plausible underlying mechanism as well. How-

ever, this option seems less likely, as results in the group treated with a combination of metformin and sulfonylurea derivatives were similar to those on monotherapy with metformin. Despite this, it is premature to draw any conclusions from these two subanalyses.

In conclusion, in our study cumulative exposure to metformin was associated with a lower risk of cancer in general and of specific cancers, in comparison with cumulative exposure to sulfonylurea derivatives. However, whether this should indeed be seen as a decreased risk of cancer for the use of metformin in comparison to the use of sulfonylurea derivatives or as an increased risk of cancer for the use sulfonylurea derivatives in comparison to the use of metformin remains to be elucidated.

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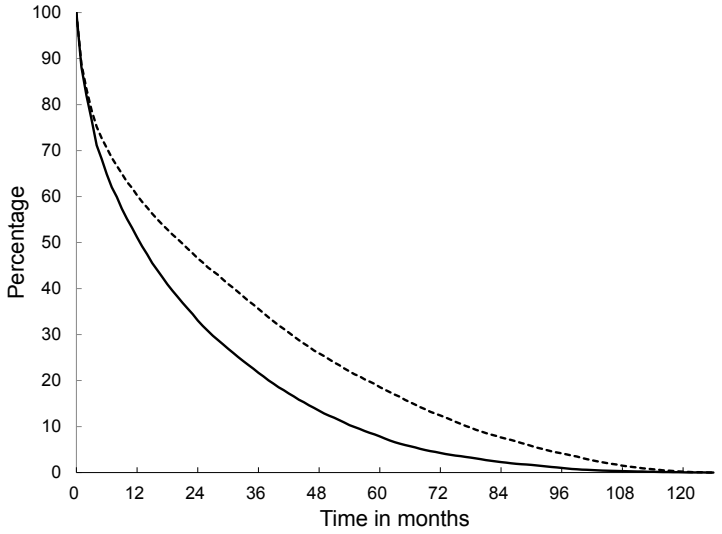
SUPPLEMENTARY MATERIAL

SM Figure 1: Participants' adherence to metformin and sulfonylurea derivatives.



Legends: Dotted line: sulfonylurea derivatives, solid line: metformin.

Chapter 4

Drugs, genotype and their interaction in breast cancer patients

Chapter 4.1

Use of NSAIDs, *COX* genotype and the risk of breast cancer in postmenopausal women

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Submitted

ABSTRACT

Introduction: the associations between *cyclooxygenase* (*COX*) genotype and breast cancer, and non-steroidal anti-inflammatory drugs (NSAIDs) and breast cancer have been frequently studied. Only few studies considered the interaction between use of NSAIDs and *COX* genotype and the risk of breast cancer. In our study, we hypothesized that the use of NSAIDs may decrease the risk of breast cancer and that this effect may be modified by *COX-1* or *COX-2* genotype.

Methods: data were obtained from a large population-based prospective cohort study. Genome wide genotype data on *COX-1* and *COX-2* genes as well as detailed information on drug dispensing and cancer diagnoses were available. Logistic regression analysis was used to assess the association between single nucleotide polymorphisms (SNPs) in the *COX-1* and *COX-2* genes and the risk of breast cancer. Cox proportional hazards models were used to assess the association between the use of NSAIDs and the risk of breast cancer with cumulative drug use as a time-varying determinant. The presence of multiplicative and additive effect modification was assessed by using interaction terms and by calculating the relative excess risk due to interaction respectively.

Results: none of the SNPs in the *COX-1* or *COX-2* gene region was associated with the risk of breast cancer. The use of COX-non-selective NSAIDs was associated with an increased risk of breast cancer of 13% (HR 1.13, 95% CI 1.02 – 1.25). Use for more than two years was associated with a twofold increased risk of breast cancer (HR 2.04, 95% CI 1.14 – 3.67). Neither additive, nor multiplicative effect modification by the SNPs under analysis in the *COX-1* or *COX-2* genes was present.

Conclusion: in our study, use of COX-non-selective NSAIDs was associated with an increased risk of breast cancer. The effect of NSAIDs on the risk of breast cancer was not modified by the SNPs under analysis in the *COX-1* or *COX-2* genotype. In light of the results of our study, additional research might be necessary to further elucidate the association between the use of NSAIDs and the risk of breast cancer.

INTRODUCTION

Nearly 30% of all female cancer diagnoses in Europe concern breast cancer. Although the incidence varies considerably in Europe, the Netherlands is one of the countries with the highest rate (91 per 100,000 person years). [1] However, five-year survival rates have improved, partly due to earlier detection, improved treatment and the decreased use of hormone replacement therapy. [1-3]

Aspirin and the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen and naproxen are among the most frequently used drugs (prescription and over the counter (OTC)) in the United States. [4] In Europe, NSAIDs represent 7.7% of all prescriptions. [5] Most likely, these rates are underestimated because of OTC use. NSAIDs are not innocuous agents since use of these drugs increases the risk of gastrointestinal (GI) complications. [6] NSAIDs inhibit cyclooxygenase (COX), of which there are two types: COX-1 is constitutively expressed and is not inducible, and COX-2, which is expressed in response to growth factors, tumor promoters and cytokines. GI adverse events appear to be caused by COX-1 inhibition; therefore it was hypothesized that COX-2 inhibitors may provide a safer alternative to COX-non-selective NSAIDs. However, COX-2 specific inhibitors are associated with a moderate increase in the risk of cardiovascular events, largely attributable to a twofold increased risk of myocardial infarction. [7] In contrast, beneficial effects have been reported as well: NSAIDs are hypothesized to decrease the risk of colon cancer. [8] Celecoxib, a COX-2 specific inhibitor has been recommended as an oral adjunct treatment for individuals with familial adenomatous polyposis who are prone to develop colon cancer. [9]

The association between use of NSAIDs and the risk of breast cancer, as well as the relation between *COX*-genotype and risk of breast cancer were frequently studied. [10-11] However, only few studies investigated the interaction between *COX-1* or *COX-2* genotype and use of NSAIDs and the risk of breast cancer. [12-16] In these studies, exposure to NSAIDs was based on interview data and information on day-to-day use and the specification of type of NSAIDs was not always available. With regard to genotype, the effect on the risk of breast cancer was assessed for 11 different single nucleotide polymorphisms (SNPs) in the *COX* genotype but not all SNPs were included in every study. In our study using drug dispensing data and genome wide genotype data, we hypothesized that the use of NSAIDs may decrease the risk of breast cancer and that this effect may be modified by *COX* genotype.

METHODS

Setting

Data were obtained from the Rotterdam Study, a large population-based prospective cohort study. The objectives and design were extensively described earlier. [17] In summary, since 1991, inhabitants of the suburb Ommoord, aged 55 years or older were invited to participate. Of all 10,275 invited subjects 7,983 entered the study (78%). In 1999, 3,011 participants (of 4,472 invitees, 67%) who were 55 years of age or older, were added to the second cohort (Rotterdam Study II).

Baseline examinations consisted of a home interview and a clinical workup at the research center. During follow-up, additional interviewing, laboratory assessments, clinical examinations and imaging procedures were carried out every 3-4 years. As all pharmacies which serve the Ommoord district are on one computer network, detailed information on drug dispensing was available for all participants as of 1 January 1991. The vital status of the participants was obtained regularly from the municipal population registry. Morbidity and mortality were assessed by information from the general practitioner, by linkage to a registry of histo- and cytopathology (PALGA), or, in case of hospitalization, by discharge reports from the medical specialists. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center and all participants gave written informed consent.

All women in the Rotterdam Study for whom genome wide genotype data was available and who were not previously diagnosed with breast cancer were included in the study cohort. Participants were followed since inclusion in the Rotterdam Study until the diagnosis of breast cancer, death or end of the study period (31 December 2008), whichever came first.

Exposure

Use of NSAIDs (ATC-code M01A) in the Rotterdam Study was categorized into three different groups: use of COX-1 selective, COX-2 selective and COX-non-selective NSAIDs (**table 1**). [18-19] The use of acetylsalicylic acid and carbasalate calcium as platelet aggregretion inhibitors (ATC codes B01AC06 and B01AC08), as well as the use of salicylates used as analgesics (ATC code N02BA) was assessed as 'salicylates' as well as categorized as COX-1 selective. [19]

The duration of a prescription was calculated as the total number of delivered units divided by the prescribed daily number of units. Participants could contribute cumulative exposure time to all categories. Cumulative exposure was calculated from start of study until the diagnosis of breast cancer, death or the end of the study period whichever came first. The effect of cumulative exposure was assessed continuously per year use as well as categorized: no use, 1-30 days use, 30-365 days, 365-730 days and more than 730

Table 1: classification of NSAIDs and salicylates used by women in the Rotterdam Study according to COX selectivity

Active Substance	ATC-code	COX selectivity
Acetylsalicylic acid	B01AC06, N02BA01, N02BA51	COX-1
Azapropazone	M01AX04	COX-1
Carbasalate calcium	B01AC08, N02BA15	COX-1
Dexibuprofen	M01AE14	COX-1
Dexketoprofen	M01AE17	COX-1
Diflunisal	N02BA11	COX-1
Flurbiprofen	M01AE09	COX-1
Indomethacin,	M01AB01	COX-1
Ketoprofen	M01AE03	COX-1
Piroxicam,	M01AC01	COX-1
Tenoxicam	M01AC02	COX-1
Tolmetin	M01AB03	COX-1
Celecoxib	M01AH01	COX-2
Etoricoxib	M01AH05	COX-2
Meloxicam	M01AC06	COX-2
Rofecoxib	M01AH02	COX-2
Valdecoxib	M01AH03	COX-2
Aceclofenac	M01AB16	Not specific
Benzydamine	M01AX07	Not specific
Diclofenac	M01AB05, M01AB55	Not specific
Glucosamine	M01AX05	Not specific
Ibuprofen	M01AE01	Not specific
Nabumeton	M01AX01	Not specific
Naproxen	M01AE02	Not specific
Phenylbutazone	M01AA01	Not specific
Sulindac	M01AB02	Not specific
Tiaprofenic acid	M01AE11	Not specific
Tolfenamic acid	M01AG02	Not specific

days of use. The average DDD over all previous prescriptions was calculated to analyze the effect of dose on the risk of breast cancer.

At baseline, blood was taken from which DNA was isolated. To obtain a larger genome coverage, imputation was performed using standard procedures. All imputed SNPs (*n*=105) in the *COX-1* region (Chromosome 9, Bp 124,173,050 to 124,197,802 ± 50 kb) and the *COX-2* region (Chromosome 1, Bp 184,907,567 to 184,916,182 ± 50 kb) were extracted. Haploview tagger was used to identify SNPs in high linkage disequilibrium ($r^2 > 0.8$) leading to the exclusion of 46 SNPs leaving 59 SNPs for the analysis. [20]

Outcome

Two research physicians independently assessed the diagnosis of breast cancer on the basis of pathology data and medical records. In case of discrepancy, consensus was sought or a cancer epidemiologist decided. All events were classified according to the International classification of disease (ICD) tenth edition. [21] Only cases confirmed by pathology were considered in the analyses.

Covariables

The following covariables were assessed as potential confounders and/or effect modifiers: age, body mass index (BMI; kg/m2), waist-hip-ratio, smoking status (yes/no/past), age at which the participant became parent (<20 years, 20-24 years, 25-29 years or no birth, ≥ 30 years), age at onset of menarche and menopause, postmenopausal hormone use (ever/never) and oral contraceptive (OC) use (ever/never). Of these, height, weight, hip and waist circumference were assessed at baseline at the research center. All other covariables were assessed via an interview at baseline. Covariables that changed the point estimate by more than 10%, or which were considered to be clinically relevant, were included in the full model.

Statistical analysis

Logistic regression analysis was used to assess the association between the SNPs and the risk of breast cancer. Cox proportions hazards models were used to assess the association between the use of NSAIDs and the risk of breast cancer with cumulative drug use as a time-varying determinant. At the time of diagnosis cumulative exposure in participants with breast cancer was compared to cumulative exposure in participants without breast cancer with the same days of follow-up. A sensitivity analysis was performed in which time since cessation (days) was taken into account. To this end, the number of days from the start of the last prescription up till the end of follow-up was calculated. In another sub-analysis, a latency period was taken into account. This was done to address the issue of potential reverse causation where the disease is already present but not diagnosed yet causes a change in exposure. To address this issue, analyses were performed taking into account a latent period before the diagnosis of cancer in which we assumed that cancer was already present one year before it was actually diagnosed (for instance, exposure cumulated until 21 June 2007 instead of 21 June 2008). To test for multiplicative effect modification by covariables mentioned above, interaction terms were introduced in the statistical model and stratified analyses were performed. The presence of additive interaction was assessed by calculating the relative excess risk due to interaction (RERI). Multiple imputations (ten times) using linear regression were used to assess the effect of missing values. All genotypes were tested for Hardy–Weinberg equilibrium using a χ^2 test. Analyses were performed using SPSS software (version 17.0, IBM, US) and SAS (version

9.2, SAS institute, Cary, US). All *p*-values are two-sided and were considered significant if $p < 0.05$. For the analysis assessing the association between the SNPs and the risk of breast cancer, as well as for the assessment of presence of effect modification by SNPs a Bonferroni correction was applied, *p*-values were considered significant if $< 0.05/59 = 8.5 * 10^{-4}$.

RESULTS

Genotype data was available for 4,720 women, of whom 212 (4.5%) developed breast cancer. The baseline characteristics are presented in **table 2**. All genotypes were found to be in Hardy Weinberg Equilibrium. There were no statistically significant differences between women with and without breast cancer with regard to smoking status, prior use of postmenopausal hormones or OC, age at first parenthood, age at menarche, age at menopause, BMI and waist-hip-ratio. However, women diagnosed with breast cancer were statistically significantly younger (66.7 years) than those not diagnosed with breast cancer (69.1 years). Although missing values were present, their presence was neither related to the outcome, nor to the exposure under analysis. Analyses performed using multiple imputations with linear regression for missing values yielded similar results. To avoid a reduction in power, the analyses presented are those using imputed data for missing values unless stated otherwise.

Table 2: baseline characteristics of the 4,720 women for whom genotype data was available

Characteristic		*n*=4,720
Cohort – RS I		3,547 (75%)
Age at start of study (years, SD)		69.0 (9.6)
BMI (kg/m2, SD)		26.9 (4.2)
Waist-hip-ratio		0.9 (0.09)
Age at first parenthood	< 20 years	275 (5.8%)
	20 – 24 years	1,337 (28.3%)
	25 – 30 years or no birth	2,500 (53.0%)
	≥ 30 years	608 (12.9%)
Age at first menarche (years, SD)		13.0 (1.6)
Age at menopause (years, SD)		50.0 (3.9)
Smoking	Current	860 (18.2%)
	Former	1,419 (30.1%)
	Never	2,317 (49.1%)
Ever use of postmenopausal hormones		863 (19.4%)
Ever use of oral contraceptive use		1,402 (29.7%)

Abbreviations: SD: standard deviation. Missing values: BMI: 179 (3.8%), Waist-hip-ratio: 464 (9.8%), Age at first menarche: 426 (9.0%), Age at menopause 1,859 (39.4%), smoking status: 122 (2.6%).

None of the 59 SNPs within the *COX-1* or *COX-2* genes was statistically significantly associated with the risk of breast cancer. Use of any NSAIDs or salicylates was not associated with breast cancer (HR 1.03, 95% CI 0.99 – 1.08). Although the use of salicylates was not associated with breast cancer (HR 1.02, 95% CI 0.96 – 1.19), the use of NSAIDs was (HR 1.09, 95% CI 1.01 – 1.19). The use of NSAIDs or salicylates was further analyzed with regard to COX-selectivity. Use of COX-1 selective NSAIDs, including salicylates, was not associated with breast cancer (HR 1.02, 95% CI 0.96 – 1.08), neither was the use of COX-2 selective NSAIDs (HR 0.96, 95% CI 0.54 – 1.69). In contrast, the use of COX-non-selective NSAIDs was associated with an increased risk of breast cancer of 12% (HR 1.12, 95% CI 1.01 – 1.24). Although none of the covariables changed the HR by more than 10%, the model was adjusted for all available covariables. In the full model, use of COX-non-selective NSAIDs was associated with an increased risk of breast cancer of 13% (95% CI 1.02 – 1.25, **table 3**).

Table 3: Risk of breast cancer for different categories of NSAIDs and salicylates drug use

Exposure [a]	*n*	Crude model		Full model [b]	
		HR	95% CI	HR	95% CI
Any NSAID or salicylate	167	1.03	0.99 – 1.08	1.04	0.99 – 1.09
Any NSAID	155	1.09	1.01 – 1.19	1.10	1.00 – 1.20
Any Salicylate	69	1.02	0.96 – 1.08	1.02	0.97 – 1.09
COX-1 selective NSAIDs and salicylates	91	1.02	0.96 – 1.08	1.03	0.97 – 1.08
COX-2 selective NSAIDs	23	0.96	0.54 – 1.69	0.93	0.52 – 1.68
COX-non-selective NSAIDs	150	1.12	1.01 – 1.24	1.13	1.02 – 1.25

Abbreviations: *n:* number, HR: hazard ratio, CI: confidence interval.

[a] exposure as categorized in table 1. [b] adjustments were made for age, body mass index (BMI; kg/m2), waist-hip-ratio, smoking status (yes/no/past), age at which the participant became parent (<20 years, 20-24 years, 25-29 years or no birth, ≥ 30 years), age at onset of menarche and menopause, postmenopausal hormone use (ever/never) and oral contraceptive (OC) use (ever/never).

Sensitivity analyses were performed in which time since cessation or a latency period were taken into account; point estimates remained similar. Categorized, use of any NSAIDs for more than two years was associated with a 85% higher risk compared with no use (HR 1.85, 95% CI 1.07 – 3.18). Short-term use of salicylates was associated with an increased risk of breast cancer of 1.75 (95% CI 1.07 – 2.86) compared with no use. When analyzed according to COX-selectivity, use of COX-non-selective NSAIDs for more than two years was associated with a more than twofold increased risk of breast cancer in comparison with no use (HR 2.04, 95% CI 1.14 – 3.67; **table 4**).

For none of the NSAIDs or salicylates, dose was found to be associated with the risk of breast cancer. Effect modification (multiplicative or additive) of the association between

Table 4: Risk of breast cancer categorized for duration of NSAIDs and salicylates use

		Full model [a]	
		HR	95% CI
Any NSAID or salicylate	No use	Reference	
	1 – 30 days use	0.86	0.55 – 1.34
	30 – 365 days use	1.27	0.86 – 1.87
	365 – 730 days use	1.07	0.58 – 1.98
	> 730 days use	1.39	0.89 – 2.15
Any NSAID	No use	Reference	
	1 – 30 days use	0.90	0.62 – 1.33
	30 – 365 days use	1.22	0.85 – 1.74
	365 – 730 days use	1.41	0.76 – 2.62
	> 730 days use	1.85	1.07 – 3.18
Any salicylate	No use	Reference	
	1 – 30 days use	1.75	1.07 – 2.86
	30 – 365 days use	0.77	0.43 – 1.39
	365 – 730 days use	0.94	0.49 – 1.78
	> 730 days use	1.13	0.76 – 1.69
COX-1 selective NSAIDs and salicylates	No use	Reference	
	1 – 30 days use	1.13	0.73 – 1.75
	30 – 365 days use	1.06	0.68 – 1.66
	365 – 730 days use	0.93	0.45 – 1.89
	> 730 days use	1.18	0.79 – 1.75
COX-2 selective NSAIDs	No use	Reference	
	1 – 30 days use	0.98	0.51 – 1.87
	30 – 365 days use	1.22	0.64 – 2.34
	365 – 730 days use	2.62	0.97 – 7.09
	> 730 days use	-	-
COX-non-selective NSAIDs	No use	Reference	
	1 – 30 days use	0.91	0.62 – 1.33
	30 – 365 days use	1.25	0.88 – 1.78
	365 – 730 days use	1.57	0.81 – 3.01
	> 730 days use	2.04	1.14 – 3.67

Abbreviations: HR: hazard ratio, CI: confidence interval.

[a] adjustments were made for age, body mass index (BMI; kg/m2), waist hip ratio, smoking status (yes/no/past), age at which the participant became parent (<20 years, 20-24 years, 25-29 years or no birth, ≥ 30 years), age at onset of menarche and menopause, postmenopausal hormone use (ever/never) and oral contraceptive (OC) use (ever/never).

use of COX-non-selective NSAIDs and the risk of breast cancer by the 59 SNPs in the *COX-1* or *COX-2* genes was not present.

DISCUSSION

Use of COX-non-selective NSAIDs was associated with an increased risk of breast cancer. Use of COX-2 selective NSAIDs was associated with a decreased risk of breast cancer, but this estimate did not reach statistical significance. Our finding of an increased risk of cancer for the use of COX-non-selective NSAIDs is at variance with results from previous studies. For breast cancer, the potential association with use of NSAIDs has been recently analyzed in a review of 26 studies. [11] This meta-analysis suggested a slightly decrease of the risk of breast cancer with a marginal statistically significant difference. [11] Although it was a large meta-analysis (*n*=528,705), shortcomings were present: there was high heterogeneity between different studies included and comparisons were only assessed as non-use vs. any use, non-regular use vs. regular use and in a sub-analysis use of more than 5 years, compared to less than five years instead of a more accurate assessment of duration. Furthermore, time since cessation, the different types of NSAIDs (beyond aspirin and ibuprofen) and dose were not taken into account. In our study we used drug dispensing data to assess the association between use of NSAIDs and the risk of cancer while all previous studies assessing the interaction between use of NSAIDs and *COX* genotype on breast cancer risk used interview data. [12-16]

As *COX-2* over-expression has been detected in approximately 40% of human breast cancer cases [22], the association between *COX-2* SNPs and breast cancer has been frequently analyzed as well. [10] Recently, a meta-analysis on studies assessing the relationship between the three most frequently studied SNPs in *COX-2* and breast cancer risk concluded that none of these is associated with the risk of breast cancer. [10] The SNPs in these studies were mainly assessed via TaqMan. When analyses concern a small number of SNPs the use of TaqMan assays can be efficient; however, when a more complete overview of the effect of genotypic variations is warranted, the use of other methods such as the Illumina BeadChip with additional imputation is preferred. In contrast to the earlier studies, we were able to assess the effect of 59 SNPs (covering a total of 105) in the *COX-1* and *COX-2* region in relation to breast cancer. Like an earlier study, we did not find a statistically significant relation between rs2745557 and the risk of breast cancer. [13] In contrast, others did describe an increased risk of the risk of breast cancer in those carrying the homozygote or heterozygote variant genotype. [12] However, this effect disappeared after additional adjustments. None of the other SNPs in our analysis reached the Bonferroni-corrected significance level and consequently, none of the SNPs in our study was associated with the risk of breast cancer which is consistent with the absent relationship between rs5275, rs20417 and rs5277 and breast cancer as recently described in a meta-analysis. [10]

The interaction between *COX* genotype and use of NSAIDs and their effect on the risk of breast cancer has been studied earlier. [12-16] However, in contrast to these earlier

studies who used interview data on drug exposure, we used drug dispensing data. As a consequence, duration assessment could be more accurately estimated; furthermore, as genome wide genotype data was available, a wide range of genetic variations in the *COX-1* and *COX-2* genes could be evaluated as potential effect modifier as well. Using this data, we could not confirm the earlier described effect modification of the use of NSAIDs and the risk of breast cancer by rs2745557 and rs2143416 (which is in r^2 with rs20417). [13] In this earlier study, women carrying the homozygous variant and who were non-user were at a significantly higher risk of breast cancer than those carrying the heterozygote or wild type genotype and using NSAIDs (OR for rs2745557 3.9, 95% CI 1.2 – 12.7 and for rs20417 4.9, 95% CI 1.5 – 16.2, respectively, *p*-values for interaction not available). [13] Furthermore, we could not confirm that for rs4648261, users of NSAIDs with the variant heterozygote or variant homozygote genotype had a nearly statistically significant decreased risk of breast cancer compared to non-users with the referent genotype (OR 0.54, 95% CI 0.29 – 1.01, *p*-value for interaction 0.04). [12] However, it is of importance to apply a Bonferroni correction when testing multiple SNPs; in these two studies, five and eight SNPs were tested respectively, but the Bonferroni correction was not applied. Similar to other studies, we did not find effect modification of the association between use of NSAIDs and the risk of breast cancer by 3 other SNPs in the *COX-2* region. [14-16]

One of the strengths of our study is that we assessed the association between use of NSAIDs and *COX-1* and *COX-2* genotype with regard to the risk of breast cancer using drug dispensing data and genome wide genotype data. This way, the assessment of duration of exposure could be evaluated very accurately and time since cessation could be taken into account. In addition, dose-response relationship could be evaluated and the effect of different types of NSAIDs could be assessed as well. Population-based cohort studies may be affected by selection bias, information bias and confounding. Selection bias probably did not occur because all breast cancer patients were ascertained independently of their NSAID exposure status within a large population-based cohort study. Information bias is unlikely as all information was gathered prospectively and without knowledge of the research hypothesis. Although we were able to adjust for several potential confounding factors which did not change the point estimate, residual confounding can always be present. A potential confounding factor could be that obese women – who have a higher chance of breast cancer – are prescribed more regularly NSAIDs (e.g., for artrosis); but as we adjusted for BMI and since this did not change the point estimate, this seems unlikely. Furthermore, although all breast cancer cases were proven by pathology, the hormonal status was not known. As a consequence, analyses could not be stratified for estrogen and progestagen receptor status. This is of importance since as suggested by previous research the etiology of different breast cancer subtypes may be heterogeneous and the potential effect of e.g., aspirin or ibuprofen may vary as well. [23]

Another limitation of our study is the limited number of breast cancer cases. Although these low numbers might explain why we did not find a statistically significant protective effect for the use of COX-2 inhibitors, we were adequately powered to assess a 20% decreased risk (α 0.05; β 0.80) for use of NSAIDs in one of the other exposure categories. The increased risk we found for short-term use of salicylates and the risk of breast cancer in comparison with no use can be explained by confounding by indication. This bias arises when the indication (or contra-indication) of the treatment is a risk factor for the outcome under study. However, the increased risk of cancer for the use of COX-non-selective NSAIDs cannot easily be explained by confounding by indication. Misclassification of exposure is another potential bias. Although there is no reason to expect that the resulting underestimation would be different for those with and without breast cancer, it may be hypothesized though, that women without breast cancer used more OTC NSAIDs than women with breast cancer. The fact that we were not able to demonstrate a dose-effect association would also be an argument against a causal relationship. However, although our finding is counterintuitive, it could also be a true finding with yet unknown etiology.

In conclusion, our results suggest that none of the analyzed SNPs in the *COX-1* and *COX-2* genes is associated with the risk of breast cancer. In contrast to earlier studies, in our study use of COX-non-selective NSAIDs was associated with an increased risk of breast cancer. The effect of NSAIDs on the risk of breast cancer was not modified by the SNPs available in the *COX-1* or *COX-2* genotype. In light of the results of our study, additional research might be necessary to further elucidate the association between the use of NSAIDs and the risk of breast cancer.

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Chapter 4.2

*CYP2C19*2* polymorphism is associated with increased survival in breast cancer patients using tamoxifen

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Pharmacogenomics. 2010 Oct;11(10):1367-75.

ABSTRACT

Introduction: variant alleles of the *CYP2C19* gene were recently associated with survival in breast cancer patients on tamoxifen therapy. CYP2C19 is one of the enzymes involved in the metabolism of tamoxifen into active metabolites. We investigated the hypothesis that *CYP2C19*2* and **3* variants, known for absent enzyme activity, are associated with an increased breast cancer mortality rate in patients using tamoxifen.

Methods: in the prospective population-based Rotterdam Study, the association between *CYP2C19*2* and **3* carriers and breast cancer mortality was studied among 80 incident users of tamoxifen. Survival was analyzed with life tables and Cox regression analysis with drug exposure as a time dependent variable. Adjustments were performed for calendar time, average tamoxifen dose, age, the indication for tamoxifen, *CYP2D6*4* genotype and concomitant use of CYP2C19 inhibitors or inducers.

Results: in patients on tamoxifen, *CYP2C19 *2* carriers were associated with a fourfold longer breast cancer survival than patients with the wild type (hazard ratio 0.26, 95% CI 0.08 – 0.87).

Conclusion: this study suggested that *CYP2C19*2* polymorphism may possibly be a predictive factor for survival in breast cancer patients using tamoxifen.

INTRODUCTION

Although breast cancer is a major public health problem and its incidence is rising, mortality is decreasing in most industrialized countries thanks to earlier detection and surgical treatment, as well as adjuvant therapy. [1] Still, approximately 14% of all female cancer deaths is caused by breast cancer, making it one of the leading causes of cancer mortality in women. [2] Tamoxifen, a selective estrogen receptor modulator, has been successfully used since 1977 for the treatment of estrogen receptor positive breast cancer. [3]

Tamoxifen has a lower affinity for the estrogen receptor and is less potent than its metabolites 4-hydroxy-tamoxifen and endoxifen (4-hydroxy-N-desmethyl-tamoxifen). Jordan et al. described that 4-hydroxy-tamoxifen had a higher affinity and a 50-100 fold potency compared to tamoxifen itself. [4] Endoxifen was identified in the 1980s and was found to have similar affinity and potency as 4-hydroxy-tamoxifen, but due to its higher concentration in plasma, it is considered to be responsible for the in vivo cytostatic activity. [5-7] Tamoxifen is predominantly metabolized by the cytochrome P450 system amongst which CYP3A4, CYP2B6, CYP2C9 and CYP2C19 and CYP2D6 are presumed to be the most important isoenzymes. [8-13] Plasma concentrations of tamoxifen and its metabolites vary widely between patients. [14] This variation is suggested to be due to genetic variability of genes which encode for cytochrome P450 enzymes involved in metabolizing tamoxifen. [9,13,15-16]

*CYP2D6*4* polymorphism, a common non-functional variant allele in Caucasians leading to absent enzyme activity, has been correlated with lower concentrations of endoxifen. [15,17] In addition, it has been associated with a higher risk of breast cancer recurrence. [18-25] Furthermore, co-administration of CYP2D6 inhibitors resulted in lower concentrations of endoxifen. [15,17,22] However, others could not confirm this association [26-30] and whether there is a place for *CYP2D6*-genotype-guided tamoxifen therapy is still an issue of debate. [31-32]

Another enzyme involved in the metabolism of tamoxifen into its active metabolites is CYP2C19. [8,11,13,15-16] In contrast to *CYP2D6*, relatively little is known about *CYP2C19* and tamoxifen efficacy. [30,33-34] Okishiro et al. did not find an association between *CYP2C19*2* and **3*, genetic polymorphisms leading to absent enzyme activity, and recurrence rate of breast cancer in users of tamoxifen [30] neither did others find a correlation between *CYP2C19* and tamoxifen efficacy. [33] However, in a multicenter study, it was found that *CYP2C19*2* predicts a favorable outcome of tamoxifen treatment for advanced breast cancer. [34] *CYP2C19*2* has a minor allele frequency of around 13% in Caucasians. [35] Its functionality has been established, for example, regarding proton pump inhibitors where the *CYP2C19*2* polymorphism leads to significantly higher drug exposure and a better response. [36]

As mentioned earlier, CYP2C19 is involved in the metabolism of tamoxifen to its active metabolites 4-hydroxy tamoxifen and endoxifen and therefore we hypothesized that *CYP2C19*2* and **3* variants, which are known for their absent enzyme activity, are associated with an increased breast cancer mortality rate in breast cancer patients using tamoxifen.

METHODS

Setting

Data were obtained from the Rotterdam Study, a large population-based cohort study. The objectives and design were extensively described earlier. [37-39] In brief, nearly all patients were of Caucasian origin. Of the 10,275 eligible persons, aged 55 years and over and living in the suburb Ommoord, 7,983 (78%) participated and are followed since inclusion. The study was approved by the Medical Ethical Committee of the Erasmus Medical Center and all participants gave written informed consent. All participants were examined in detail at baseline. They were interviewed at home by trained interviewers and during two subsequent visits at the research center they underwent additional interviewing, laboratory assessments, clinical examinations and imaging procedures. Follow-up examinations took place every 3-4 years. The vital status of the participants was obtained regularly from the municipal population registry. Morbidity and mortality were assessed by information from the general practitioner or, in case of hospitalization, by discharge reports from the medical specialists. Data concerning drug utilization was provided by the seven computerized pharmacies in Ommoord. Information on all prescriptions was available as of 1 January 1991 and included the product name, the Anatomical Therapeutical Chemical (ATC) code, the dispensing date, dose and regimen.

Study population, design and outcomes

To ensure that only incident users of tamoxifen were included, the study cohort consisted of all women in the Rotterdam Study who received a first prescription of tamoxifen between 1 April 1991 and 1 July 2005 for breast cancer, who had used tamoxifen for at least 180 days during follow-up, and for whom genotype data was available.

Patients were followed until the outcome of interest, i.e. death due to breast cancer, death due to another cause, or end of the study period, whichever came first. Two research physicians independently assessed the diagnosis of breast cancer and the cause of death on the basis of pathology data and medical records. All events were classified according to the International Classification of Disease (ICD) tenth edition. In case of discrepancy, consensus was sought or a cancer epidemiologist decided.

Covariables

Calendar time, average tamoxifen dose, age at the date of diagnosis, the indication for tamoxifen (adjuvant or palliative), *CYP2D6*4* genotype, and use of concomitant medication that can induce or inhibit the enzyme CYP2C19 were taken into account as potential confounders. As CYP2C19 inhibitors lansoprazole, omeprazole, pantoprazole, rabeprazole, cimetidine, indomethacine, oxcarbazepine and topiramate were considered. As CYP2C19 inducers, carbamazepine, norethindrone, prednisone and rifampicine were considered. Use was defined as filling a prescription within 90 days before the index date.

Genotyping

At the baseline examination of the Rotterdam Study blood was taken from which DNA was isolated. *CYP2C19*2* (681G>A, rs4244285), *CYP2C19*3* (636G>A, rs4986893) and *CYP2D6*4* (1846G>A, rs3892097) genotyping was performed, using TaqMan allelic discriminatory assays (Applied Biosystems) on an ABI Prism 7000 Sequence Detection system (Applied Biosystems). Each assay consisted of two allele-specific minor groove binding (MGB) probes, labeled with the fluorescent dyes VIC and FAM. The thermal profile consisted of an initial denaturation step at 95°C for 15 minutes, followed by 50 cycles of denaturation at 92°C for 15 seconds and annealing and extension at 60°C for 1 minute. Genotypes were scored by measuring allele-specific fluorescence using the SDS 2.2.2 software for allelic discrimination (Applied Biosystems).

Subjects were defined as poor metabolizer (PM) if they were homozygous for the **2* or **3* allele, which is known to encode absence of enzyme activity. In case of heterozygosity participants were defined as intermediate metabolizers (IM). When subjects did not have one of these variant alleles they were defined as extensive metabolizers (EM).

Statistical analysis

The association between *CYP2C19*2* and **3* genotype and breast cancer mortality in tamoxifen users was analyzed using Cox proportional hazard models with drug exposure as a time dependent variable. The date of mortality was taken as the index date for the case. At the index date, all persons still alive were matched to the case based on days of duration of tamoxifen use. Consequently, at the date of mortality, the cumulative exposure duration to tamoxifen in patients who died of breast cancer was compared to a similar duration of tamoxifen exposure in patients who did not die of breast cancer. [40] The relationship between *CYP2C19*2* and **3* genotype and breast cancer mortality was analyzed with a genotypic model (**1/*1*, **1/*2* and **2/*2; *1/*1, *1/*3* and **3/*3*), an allele effect model, a dominant model (**1/*1* versus **1/*2* and **2/*2*; **1/*1* versus **1/*3* and **3/*3*) and a recessive model (**2/*2* versus **1/*2* and **1/*1; *3/*3* versus **1/*3* and **1/*1*).

In addition, three further analyses were performed using Cox proportional hazard models with adjustments for age. Firstly, the role of *CYP2C19*2* and **3* genotype was analyzed in all women with breast cancer not using tamoxifen in the Rotterdam Study. Secondly, the association between overall survival, excluding breast cancer survival, and *CYP2C19*2* and **3* genotype was analyzed in all women with breast cancer in the Rotterdam Study. Finally, the role of *CYP2C19*2* and **3* genotype and risk of diagnosis of breast cancer was analyzed in all women in the Rotterdam Study.

All genotypes were tested for Hardy Weinberg equilibrium using a chi-square test. Differences between the two groups were tested for significance with the unpaired T-test and for categorical variables with a chi-square test. Analyses were performed using SPSS software (version 15.0, IBM, US). All *p*-values are two-sided and were considered significant if $p < 0.05$.

RESULTS

Within the Rotterdam Study, 286 out of 4,878 women were diagnosed with breast cancer, of whom 85 were treated with tamoxifen. For 215 women, of whom 80 had used tamoxifen, *CYP2C19*2* and **3* genotype was known. The baseline characteristics are presented in **table 1**. The mean age at the first prescription of tamoxifen was approximately 75 years. The mean prescribed daily dose was 34 mg with a mean total duration of two years. In 54% of the patients, tamoxifen was prescribed as an adjuvant and 20% had metastases at the moment of prescription. Although all patients definitely had breast cancer, the precise indication (adjuvant or palliation) for tamoxifen was not known in 26%. Twelve percent of breast cancers was estrogen receptor negative and 50% was estrogen receptor positive. Of the 215 women, 90 persons (41.9%) died during the study period of whom 45 died of breast cancer (50%). Of the tamoxifen users, 20% (*n*=16) used CYP2C19 inhibitors and 3% (*n*=3) used CYP2C19 inducers in the 90 days before the index date.

Minor allele frequencies of *CYP2C19*2* and *CYP2C19*3* were 15.8% and <0.01%, respectively. *CYP2C19*3* was in Hardy Weinberg equilibrium (p= 0.79), but due to its low variant allele frequency, not further analyzed. As shown in **table 2**, *CYP2C19* heterozygous **2* carriership was associated with a longer survival among tamoxifen users with a hazard ratio of 0.26 (95% CI 0.08 – 0.87). This analysis was adjusted for calendar time, age, average dose, indication of tamoxifen and *CYP2D6*4* genotype (point estimates with confidence intervals of these covariables from the multivariate model were respectively 1.0 (1.0 – 1.0), 0.99 (0.92 – 1.06), 2.42 (0.66 – 8.94), 6.9 (2.74 – 17.51) and 4.19 (1.83 – 9.66)). A dominant model and an allele effect model yielded similar results. A recessive model could not be fitted due to absence of homozygous cases. The unadjusted effect

Table 1: Characteristics of the 215 women with breast cancer and a known *CYP2C19* genotype in the Rotterdam Study

Number of patients [a]		tamoxifen users (*n*=80)	non users (*n*=135)
Age at diagnosis in years (SD)		72 (8.6)	70 (10.4)
Age at first prescription (SD)		75.5 year (8.8)	-
Mean prescribed daily dose (SD)		33.7 mg (8.7)	-
Mean tamoxifen duration since first prescription (SD)		2.13 year (1.8)	-
Indication tamoxifen (%)	adjuvant	43 (54.8)	
	palliative	16 (20)	-
	unknown	21 (26.2)	
CYP2C19 genotype (%) [b]	*1/*1	48 (60)	94 (69.6)
	*1/*2	30 (37.5)	39 (28.9)
	*2/*2	2 (2.5)	2 (1.5)
CYP2D6 genotype (%) [c]	*1/*1	50 (62.5)	91 (67.4)
	*1/*4	27 (33.7)	33 (24.4)
	*4/*4	3 (3.8)	11 (8.1)
Breast cancer mortality (%)		36 (45)	9 (6.7)
Other cause mortality (%)		13 (16.3)	32 (23.8)

Abbreviations: SD: standard deviation. [a] Unpaired t-tests and chi-square tests did not show any statistical differences between the groups. [b] Genotype in Hardy Weinberg equilibrium (p=0.42). [c] Genotype in Hardy Weinberg equilibrium (p=0.99).

Table 2: Association between *CYP2C19* genotype and risk of mortality in patients using tamoxifen

		Cases	Adjusted Hazard ratio [a]	95% CI	*P*-value
CYP2C19	*1/*1	28	reference		
	*1/*2	8	0.26	0.08 – 0.87	0.03
	*2/*2	0	-		

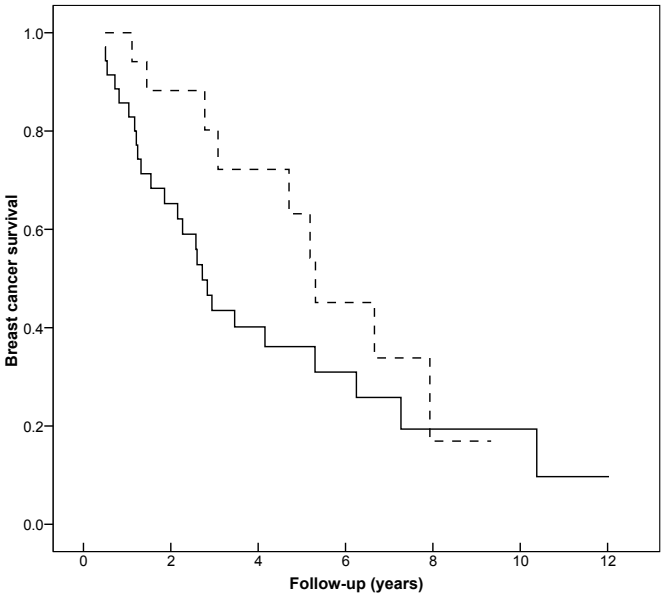
Abbreviations: CI: confidence interval.

[a] Hazard ratio adjusted for calendar time, age, daily dose, indication for tamoxifen and *CYP2D6*4* genotype.

of *CYP2C19*2* genotype on breast cancer survival in tamoxifen users is presented graphically in **figure 1** (n=80). When excluding participants with ER negative tumors, numbers were even lower but *CYP2C19* heterozygous **2* carriership was nevertheless associated with a longer survival with a hazard ratio of 0.006 (95% CI 0.00 – 0.52, p-value 0.02).

There was no significant effect modification by the use of CYP2C19 inhibitors or inducers (p-values for interaction term respectively 0.8 and 0.6). The effect of concomitant use of CYP2C19 inhibitors and inducers was assessed as confounder. Adjusted for use of CYP2C19 inhibitors the risk lowered further to 0.21 (95% CI 0.05 – 0.81) while adjusting for CYP2C19 inducers yielded a higher risk of 0.28 (95% CI 0.08 – 0.98).

Figure 1: Kaplan Meier breast cancer survival curve in tamoxifen users for *CYP2C19*2* genotype.



Legends: Number of events: 36, event distribution: 28 events in participants with *CYP2C19 *1/*1* genotype, 8 events in participants with **1/*2 CYP2C19* genotype, number of participants censored: 16, *p*-value=0.01. Dashed line: participants with **1/*2 CYP2C19* genotype, solid line: participants with **1/*1 CYP2C19* genotype.

In an exploratory analysis, the effect of *CYP2C19*2* polymorphism was stratified according to *CYP2D6*4* genotype. Although there was no significant effect modification (p=0.8), point estimates varied between the different *CYP2D6* strata. Within the group of *CYP2D6 *1/*1, CYP2C19*2* was associated with a risk of death from breast cancer of 0.61 (95% CI 0.2 – 2.4, n=19). Because patients carrying one **4* variant allele are also known as extensive metabolizers, we combined the group *CYP2D6*1/*1* with *CYP2D6 *1/*4* which led to a hazard ratio of 0.33 (95% CI 0.09 – 0.87) of *CYP2C19*2* on breast cancer mortality. [41] Unfortunately, it was not possible to analyze the association with *CYP2C19 *2* variant alleles within participants with *CYP2D6 *4/*4* genotype due to low numbers.

In addition, three further analyses were done to assess the role of *CYP2C19*2* in different patient groups within the Rotterdam Study. These results are shown in **table 3**. Firstly, there was no significant association between *CYP2C19*2* genotype and breast cancer mortality in the reference group, namely women with breast cancer using no tamoxifen in the Rotterdam Study (n=135, HR 1.98, 95% CI 0.56 – 7.43). Secondly, there was no significant association between overall survival, excluding breast cancer survival, and *CYP2C19*2* genotype within all women with breast cancer in the Rotterdam Study (n=215, p=0.86). Finally, *CYP2C19*2* genotype was not associated with the risk of breast cancer diagnosis (p=0.15). However, carriership of one **2* variant allele was associated

Table 3: Associations with *CYP2C19* genotype in different patient groups within the Rotterdam Study

		Cases	Adjusted Hazard ratio [a]	95% CI	*P*-value
CYP2C19 [b] and risk of breast cancer mortality in non-users of tamoxifen.	*1/*1	5	reference		
	*1/*2	4	1.98	0.56 – 7.43	0.33
	*2/*2	0	-		
CYP2C19 [c] and risk of overall mortality in breast cancer patients.	*1/*1	26	reference		
	*1/*2	16	1.15	0.62 – 2.15	0.65
	*2/*2	1	1.46	0.20 – 10.80	0.71
CYP2C19 [d] and risk of breast cancer diagnosis in women.	*1/*1	142	reference		
	*1/*2	69	1.29	0.97 – 1.72	0.08
	*2/*2	4	0.69	0.26 – 1.86	0.46

Abbreviations: CI: confidence interval.

[a] Hazard ratios are adjusted for age. [b] Analysis in 135 women with breast cancer, a known genotype and no tamoxifen use. [c] Analysis in 215 women with a known genotype and breast cancer, excluding breast cancer mortality. [d] Analysis in 3728 women with a known genotype.

with a hazard ratio of breast cancer diagnosis of 1.29 (95% CI 0.97 – 1.72, *n*=3728, *p*=0.08) in comparison with individuals with *CYP2C19* wild type.

DISCUSSION

In this population-based cohort study in breast cancer patients using tamoxifen, we showed that *CYP2C19* heterozygous **2* carriership is not associated with increased breast cancer mortality, as was hypothesized, but with increased survival. To further explore this association, we analyzed the effect of *CYP2C19*2* in three different patient groups within the Rotterdam Study. Firstly, in non-users of tamoxifen there was no significant association between *CYP2C19*2* and breast cancer mortality. In addition, we showed that in women with breast cancer *CYP2C19*2* genotype was not associated with overall mortality. Furthermore, we did not show a significant association between *CYP2C19*2* genotype and the risk of breast cancer diagnosis. Apparently, the *CYP2C19*2* genotype is not an independent risk factor for cancer survival, but modifies the risk when combined with tamoxifen. However, the relation we found in our analysis, i.e. that carriership of one **2* polymorphism was associated with increased survival, seems to be counterintuitive. Consequently, it is either a finding by chance, or a valid finding with unknown etiology.

CYP2C19 is involved in the metabolism of tamoxifen into its active metabolites.[8,11,13,15-16] However, different associations between *CYP2C19* genotype and breast cancer outcome have been described [18-30] making it hard to adequately describe the genotype-phenotype relation. [31-32] It might be speculated that the genotype *CYP2C19 *1/*2* has

a corresponding extensive metabolizer phenotype or even ultra-rapid metabolizer phenotype, but since we are not aware of a biological plausibility, we refute this option.

Justenhoven et. al. suggested that increased metabolism of estrogens by *CYP2C19*17* may lead to decreased estrogen levels and therefore reduces breast cancer risk. [42] Also in another study, *CYP2C19*17*, a variant allele which in contrast to **2,* is leading to increased enzyme activity, was associated with more favorable clinical outcomes in users of tamoxifen. [21] Unfortunately, we were not able to complement our analysis with *CYP2C19*17* polymorphism to see whether our results might be explained by *CYP2C19*17*.

Another theoretical explanation for our finding might be that individuals with *CYP2C19 *1/*1* genotype have more adverse reactions due to higher levels of endoxifen and are consequently less compliant. In our study, the mean dose of tamoxifen was 34 mg. Only in 2005, the Early Breast Cancer Trialists' Collaborative Group reported that the proportional risk reductions produced by tamoxifen in breast cancer patients appear to be about the same in trials of 20 mg/day as in trials of 30 – 40 mg/day. [3] It seems possible that women with the *CYP2C19* wild type, by producing more active metabolites on a higher dose than women with the risk alleles, are at increased risk of hot flashes and other adverse effects, which might influence compliance and consequently the survival benefit. [43] Although we were not able to verify this, we think that this option is not likely considering the strong indication for compliance in this patient group.

The association between *CYP2D6*4* carriership and increased breast cancer mortality which we found has been described before. [18-25] In an exploratory analysis, we analyzed the effect of *CYP2C19*2* polymorphism according to *CYP2D6*4* genotype. The hazard ratio varied between the different *CYP2D6* strata with a higher hazard ratio in *CYP2D6*1/*1* genotype and a lower hazard ratio in carriers of a single **4* variant allele. The numbers in this exploratory analysis are too small to draw conclusions, but might indicate that the contribution of *CYP2C19*2* is relatively high in patients carrying a *CYP2D6*4* variant allele. A proposed mechanism that could be considered as a possible explanation for this finding is that of competitive action between CYP2D6 and CYP2C19. In this hypothesis it can be speculated that the genotype *CYP2C19 *1/*2* has a greater impact on survival in participants with the genotype *CYP2D6 *4/*4* than in participants with the genotype *CYP2D6 *1/*1* or even **1/*4*.

Potential biases of population-based studies are selection bias, information bias and confounding. In this study, selection bias probably did not occur because all breast cancer patients were selected independently of their *CYP2C19*2* genotype within a large cohort study. Furthermore, although availability of *CYP2C19*2* and **3* genotype was an inclusion criterion, it is not likely that this criterion is related to the genotypic status itself, nor to the availability of a blood sample nor to the successfulness of the genotyping. Information bias is unlikely as all information was gathered prospectively and

without knowledge on the research hypothesis and genotype. Although, for example the indication of tamoxifen (adjuvant or palliative) is of great impact on the survival of breast cancer patients, in essence this covariable cannot be assumed to be a confounder, since it most likely is not related to *CYP2C19*2* genotype. Nevertheless, we adjusted for average tamoxifen dose, calendar time, age, the indication of tamoxifen (palliative or adjuvant) and *CYP2D6*4* genotype. Additional adjusting for either CYP2C19 inducers or inhibitors, respectively, gave either a higher or a lower risk compared to the, for use of concomitant drugs, unadjusted analysis. Unfortunately, numbers were too low for stratification and, at this moment, it is too premature to draw any further conclusions with regard to phenotype in these drug categories. In addition, we cannot exclude that our findings occurred by chance. In our study we did not have complete data on breast cancer stage and additional therapies. Therefore, we cannot exclude the possibility that our finding is confounded by a baseline difference in prognosis of the participants under study. Neither did we have complete information on estrogen status but associations between *CYP2C19*2* genotype and tumor size, nodal status, histological grade or estrogen receptor status are unlikely. [21] In the present study, 12% of the breast cancers was estrogen receptor status negative, 50% was estrogen receptor positive. For others it was not known (38%). Under-reporting of estrogen receptor positive status and over-reporting of negative status due to the decreased survival of patients with estrogen receptor negative status is likely. [44] These figures thus reflect the higher rate of estrogen receptor positive status in postmenopausal women.

In conclusion, this study suggests that *CYP2C19*2* genotype may possibly be a predictive factor for survival in breast cancer patients using tamoxifen. As none of the explanations above is satisfactory, only replication in other studies can shed more light on our findings and could verify whether this finding has any clinical relevance in a larger population.

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Chapter 5

Basal cell carcinoma as adverse reaction to use of photosensitizing diuretics: high-ceiling diuretics are associated with an increased risk of basal cell carcinoma in a population-based follow-up study

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Eur J Cancer. 2010 Sep;46(13):2467-72. Epub 2010 Jun 3.

ABSTRACT

Introduction: in Caucasians, basal cell carcinoma (BCC) is among the most frequently diagnosed cancers and its incidence is increasing. Known risk factors for the development of BCC are age, sun exposure, and certain skin characteristics. Despite photosensitizing abilities of diuretic agents, little is known about a possible association with BCC.

Methods: data were obtained from the Rotterdam Study; a large prospective population-based follow-up study with coverage of prescription-only drugs from pharmacies. The diagnoses of BCC were obtained through general practitioners, and by linkage with a registry of histo- and cytopathology. Cumulative use of diuretics at the date of diagnosis was categorized into quartiles for users of high-ceiling diuretics, potassium sparing agents and thiazides. The association between these drugs and BCC was assessed by Cox proportional hazard modeling with adjustment for age, gender and potential confounders. Effect modification was tested with interaction terms.

Results: use of high-ceiling diuretics in the highest quartile (> 3.7 years cumulative exposure) was associated with an increased hazard of BCC of 62 percent compared to no use (HR 1.62; 95% CI 1.09 – 2.42). Patients who used high-ceiling diuretics and had a high tendency to sunburn had a higher risk of diagnosis of a BCC than non-users who do not have this tendency (*p*-value for interaction 0.03). Neither the use of potassium sparing agents, nor the use of thiazides was associated with BCC.

Conclusion: in our study, cumulative use of high-ceiling diuretics was associated with an increased risk of diagnosis of BCC. This effect is stronger in patients with a high tendency to sunburn.

INTRODUCTION

In Caucasians, basal cell carcinoma (BCC) is among the most frequently diagnosed cancers [1] and its incidence is increasing. [2-4] In a large region of the Netherlands, the age-adjusted incidence for men rose from 40 per 100,000 person years in 1973 to 92 per 100,000 person years in 2000. For women, the incidence rate rose from 34 to 79 per 100,000 person years during the same period. [2] However, mortality rates are low since BCC metastasizes rarely. [5-6] Nonetheless, morbidity can be high due to local tissue destruction, and residual scarring after surgery. Cosmetic considerations and the high incidence make BCC among the five most costly cancers to treat. [7]

Known risk factors for the development of BCC are age and phenotypic characteristics, such as hair color, eye color and skin phototype. In addition to genodermatoses (specific inherited genetic skin conditions), genetic risk factors have been elucidated. [8-10] The major environmental risk factor for the development of BCC is excessive exposure to ultraviolet radiation (UV), both chronic and intermittent. [11] UV-B causes specific DNA mutations and UV-A indirectly damages the DNA via reactive oxygen molecules. [12-14] UV induced DNA damage, and therefore the risk of BCC, may be enhanced in patients with increased photosensitivity because they are more likely to get (severe) sunburns due to a lower Minimal Erythema Dose. A wide range of drugs have photosensitizing abilities including sulfonylurea derivatives used in diabetes mellitus, nonsteroidal anti-inflammatory drugs, antipsychotic drugs, antibiotics, antimalarials, amiodarone, diuretics and cardiovascular drugs. [15-17] Of these drugs, amiodarone has been associated with the development of BCC [18] and self-reported use of photosensitizing drugs in general was associated with an increased risk of BCC and squamous cell carcinoma (SCC). [19] Furthermore, an association between the total dispensed amount of photosensitizing diuretics in milligram (i.e., thiazides, potassium sparing agents and furosemide) and risk of SCC and malignant melanoma has been described. [20] However, no clear associations were described between use of diuretics and BCC.

Despite the photosensitizing abilities of diuretic agents, little is known about a possible association between use of these frequently used drugs and the risk of BCC. [19-20] The objective of this study was to test the hypothesis that long-term use of diuretics is associated with an increased risk of BCC.

METHODS

Setting

Data were obtained from the Rotterdam Study, a large population-based follow-up study. The objectives and design were extensively described earlier. [21-23] In the Rotter-

dam Study I, 7,983 of 10,275 eligible persons aged 55 years and over, participated and are followed since inclusion. They are inhabitants of the suburb Ommoord and mainly Caucasians (90%). In 1999, 3,011 participants (out of 4,472 invitees) who had become 55 years of age or older, were added to the cohort (Rotterdam Study II).

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center and all participants gave written informed consent. All participants were examined in detail at baseline. Participants were interviewed at home by trained interviewers and investigations took place during two subsequent visits at the research center. During follow-up, they underwent additional interviewing, laboratory assessments, clinical examinations and imaging procedures every 3-4 years. The vital status of the participants was obtained regularly from the municipal population registry. Morbidity and mortality were assessed by information from the general practitioner or, in case of hospitalization, by discharge reports from medical specialists.

Data concerning filled prescription-only drugs are provided by the seven computerized pharmacies in Ommoord that dispense out-patient prescriptions. Information on prescriptions was available as of 1 January 1991 and included product name, Anatomical Therapeutical Chemical (ATC) code [24], dispensing date, total amount of drug units per prescription, prescribed daily number of units, dose and regimen.

Study population and outcome

To ensure that only incident users of diuretics were included, the study cohort consisted of all patients in the Rotterdam Study who did not receive a prescription of diuretics before 1 April 1991. Complete coverage of pharmacy data started namely only in 1 January 1991 and prescriptions in the Netherlands have a maximum of 90 days. The diagnoses of BCC were obtained through the general practitioners and by linkage with a nationwide registry of histo- and cytopathology in the Netherlands (PALGA) from 1 January 1986 to 31 December 2007. Two research physicians independently assessed the first date and diagnosis of BCC. All events were classified according to the International Classification of Disease (ICD) tenth edition. [25] In case of discrepancy, consensus was sought or a cancer epidemiologist decided. The index date was defined as the date of the first diagnosis of BCC in the pathology data. Patients were followed since inclusion in the Rotterdam Study until the diagnosis of BCC, death, or end of the study period (31 December 2007), whichever came first.

Exposure

Cumulative time of use and average defined daily dose of diuretic dispensings were calculated over the period 1 April 1991 through 31 December 2007. Each participant could contribute cumulative exposure time to one or more of three categories, i.e. high-ceiling diuretics (ATC-code C03C), potassium sparing agents (ATC-code C03D), thiazides

including chlortalidon (ATC-code C03A) and thiazides in combination with other drugs (C03EA). The potential association was assessed continuously per additional year of cumulative use and categorically by dividing cumulative use at the index date into four quartiles for each drug group. Quartiles were preferred over other cut-off points to establish equal power in all groups, and because it guarantees unbiased cut-off points. To analyze the effect of dose on the risk of BCC we categorized the average defined daily dose (DDD, calculated over available prescriptions) [24] of diuretic users into four quartiles for each drug group.

Covariables

The following baseline patient characteristics, all determined by baseline interview or during the visit to the examination center, were individually assessed as potential confounders and/or effect modifier: gender, age, smoking status (current smoker, former smoker or never smoked), self-reported tendency to sunburn (high or low), outdoor work (> 4 hours daily for > 25 years), history of living in a country with a high sun exposure (>1 year), ethnicity, natural hair color during childhood (blond, brown, red or black), natural hair color when adult (black or brown; blond or red), eye color (blue, intermediate or brown) and cohort (Rotterdam Study I or Rotterdam Study II).

Furthermore, concomitant use of other diuretics and/or other photosensitizing drugs was considered as potential confounder and/or effect modifier. The following drugs, known for their photosensitizing abilities were included: amiodarone, quinidine, calcium antagonists, sulfonylurea derivatives used in diabetes mellitus (tolbutamide, glibenclamide, gliclazide, glimepiride), non-steroidal anti-inflammatory drugs (piroxicam, flurbiprofen, ibuprofen, ketoprofen, naproxen, celecoxib and diclofenac), antipsychotics (chlorpromazine, haloperidol, phenothiazines), antibiotics (tetracyclines, fluoroquinolones, sulfonamides) and antimalarial drugs (aminoquinoline and methanolquinolines). Use was assessed in days of cumulative exposure at the index date.

Statistical analysis

The association between diuretics and BCC was analyzed using Cox proportional hazard models with cumulative drug use as a time-varying determinant, while adjusting for age at baseline and gender. [26] At the date of diagnosis cumulative exposure in participants with a BCC was compared to cumulative exposure in all individuals without a BCC with the same follow-up time in days. To encounter the exponential age-related risk of cancer, a sub-analysis was done in which the comparison was further restricted to participants who also had the same age as the persons with BCCs (plus or minus 180 days).

Covariables that changed the hazard ratio of BCC risk by more than 10%, ore were considered clinically relevant, were taken into account as confounders. [27] To test for effect modification by covariables mentioned above, interaction terms were introduced

in the statistical model and separate analyses were performed in different categories. In addition, proportionality of the model was tested by adding an interaction term of the determinant and the follow-up time. Analyses were performed using SPSS software (version 15.0, IBM, US) and SAS software (version 9.1, SAS institute, Cary, US). All *p*-values are two-sided and were considered significant if $p < 0.05$.

RESULTS

We excluded 14 participants from the study population (10,994) who had a diagnosis of BCC and another 288 because they had a prescription for a diuretic before 1 April 1991. The baseline characteristics for the remaining study cohort (10,692) are presented in **table 1**. During the period of 1 April 1991 through 31 December 2007, 522 first diagnoses basal cell cancer were made. Of these, 193 patients had drug dispensing data for a diuretic of whom 137 had one or more prescriptions for thiazides (ATC-codes C03A and C03EA), 110 for high-ceiling diuretics (C03C) and 26 participants with a BCC had one or more prescriptions for potassium sparing agents (C03D).

After adjusting for age and gender, cumulative use of high-ceiling diuretics was statistically significantly associated with an increased hazard ratio of BCC of 1.07 per year (95% CI 1.01 – 1.13). Use of high-ceiling diuretics in the highest quartile (> 3.7 years of cumulative use) was associated with a 62% increased risk of BCC compared to no use (HR 1.62, 95% CI 1.09 – 2.42). Neither the use of potassium sparing agents, nor the use of thiazides was associated with a statistically significantly increased hazard ratio of BCC (**table 2)**. Use of high-ceiling diuretics in the highest dose quartile (> 1.16 average DDD) during the whole period of use was associated with a slightly higher risk of BCC (HR 1.48, 95% CI 0.99 – 2.21, *p*-value for trend 0.03) but these results were not significantly different from those using a dose in other quartiles (lowest quartile (<0.72 average DDD) HR 1.15, 95% CI 0.77 – 1.72, second quartile (average DDD 0.72 – 1.00) HR 1.33, 95% CI 0.82 – 2.16 and third quartile (average DDD 1.00 – 1.16) HR 1.43, 95% CI 0.86 – 2.40).

None of the covariables was found to be a confounder. With regard to concomitant drug use, this was tested performed as well in a cumulative manner (any use of another diuretics and/or other photosensitizing drugs) as on drug specific level (per drug). Tendency to sunburn was an effect modifier (*p*-value for interaction 0.03). Patients who did not use high-ceiling diuretics and who did not have a high tendency to sunburn were used as reference. Patients who did not have a high tendency to sunburn and who use high-ceiling diuretics had a 3% higher risk of a BCC (95% CI 0.77 – 1.39); those who had a high tendency to sunburn and did not use a high-ceiling diuretic had a 17% increased risk of a BCC (95% CI 0.95 – 1.44) while those who had a high tendency to sunburn and

Table 1: Baseline characteristics of the study population (*n*=10,692)

Characteristic [a]		*n*
Gender	Men	4288 (40%)
	Women	6404 (60%)
Age at entry in years (SD)		69 (9.7)
Cohort of entry	Rotterdam Study I	7770 (73%)
	Rotterdam Study II	2922 (27%)
High tendency to sunburn	Yes	3216 (30%)
	No	6607 (62%)
Outdoor work (> 4 hours daily for > 25 years)	Yes	1187 (11%)
	No	6047 (57%)
Living in a sunny country (> 1 year)	Yes	1017 (9%)
	No	8929 (84%)
Hair color when young	Blond	2245 (21%)
	Brown	6402 (60%)
	Red	295 (3%)
	Black	1000 (9%)
Hair color at present time	Blond or red	2540 (24%)
	Black or brown	7402 (69%)
Eye color	Blue	6239 (58%)
	Intermediate	769 (7%)
	Brown	2231 (21%)
Smoking status	Current smoker	2286 (21%)
	Former smoker	4644 (42%)
	Never smoked	3707 (34%)
Ethnicity	Caucasian	9645 (90%)
	Other	212 (2%)

Abbreviations: *n:* number.

[a] If numbers do not add up to 10,692 or 100% this is due to missing values.

used high-ceiling diuretics had an increased risk of 58% for the diagnosis of a BCC (95% CI 1.14 – 2.19).

To further encounter the age-specific risk of cancer, a sub-analysis was done. The comparison was further restricted to participants who had the same age (plus or minus 180 days) at the date of diagnosis. Although slightly lower, the gender-adjusted, hazard ratio for developing a BCC was 1.04 per year (95% CI 1.01 – 1.07) when compared to participants with the same age. Proportionality of the models used, yielded no statistically significant deviations from the null.

Table 2: Age and gender adjusted risk of basal cell carcinoma during use of diuretics

		n	Hazard Ratio	95% confidence interval
Thiazides		137	1.00	0.95-1.05
	No use	385	Reference	
	< 94 days	34	1.02	0.72 – 1.45
	94 – 524 days	35	0.98	0.69 – 1.39
	524 – 1646 days	34	0.86	0.60 – 1.22
	>1646 days	34	1.10	0.77 – 1.58
K^+ sparing agents		26	1.04	0.93 – 1.17
	No use	496	Reference	
	< 152 days	6	0.73	0.32 – 1.63
	152 – 475 days	7	1.23	0.58 – 2.61
	475 – 923 days	7	1.90	0.90 – 4.02
	> 923 days	6	0.92	0.41 – 2.08
High-ceiling diuretics [a]		110	1.07	1.02 – 1.13
	No use	412	Reference	
	< 82 days	27	0.97	0.65 – 1.44
	82 – 400 days	28	1.11	0.75 – 1.65
	400 – 1360 days	28	1.23	0.83 – 1.81
	> 1360 days	27	1.62	*1.09 – 2.42*

Abbreviations: *n:* number. [a] *p*-value for trend: 0.01

DISCUSSION

Although UV exposure is a well-established risk factor for BCC, little is known about the contribution of photosensitizing drugs to BCC development. [18-20, 28] In this study, cumulative exposure time of high-ceiling diuretics was associated with an increased risk of BCC but a significant dose-dependency was not demonstrated. A significantly higher risk of BCC was observed in users of high-ceiling diuretics who have a high tendency to sunburn. An explanation could be that the use of high-ceiling diuretics might lower the Minimal Erythema Dose.

BCC characteristically appear on body areas exposed to the sun, with 80% appearing on the head and neck. [29] After all, sunlight remains one of the major risk factors for non-melanoma skin cancer. [12-14] In addition, it has been postulated that photosensitizing reactions followed by sun exposure may enhance the risk of sunburns and photo damage and subsequently the risk of skin cancer. [30] Our findings are in line with these hypotheses.

In our analysis, we did not find an increased risk of BCC to thiazides despite earlier publications. [31] Furthermore, we did not verify whether the increased risk of BCC dimin-

ishes after discontinuation of diuretic therapy. However, use of diuretic agents is mainly a long-term treatment. In addition, in the well-known association between oral psoralen and ultraviolet-A light (PUVA) therapy for psoriasis and squamous cell carcinoma, a persistent risk of non-melanoma skin cancer was seen after discontinuation of therapy. [32]

The association between high-ceiling diuretics and BCC may be possibly explained through the fact that the phototoxic potential of two frequently prescribed diuretics, furosemide and chlorothiazide may vary in the different UV spectra. These drugs both contain a sulfa-group. Sulfonamides are known for their photosensitizing abilities through phototoxic oxygen dependent reactions, but also act through photo-allergic reactions. [16, 33-34] A phototoxic reaction is the more common of the two and resembles sunburn. Photo-allergy is an acquired immune response through antigen-antibody or cell-mediated mechanisms. Photosensitivity is a broader term for the entities phototoxicity and photo-allergy. [16, 33] A possible explanation for our finding might be that furosemide acts as a photosensitizer through UvA and chlorothiazide acts through UvB. [35] However, as in our study, we did not find an association for the use of thiazides and the risk of BCC we think this option is less likely.

Population-based studies may be affected by selection bias, information bias and confounding. In this study, selection bias probably did not occur because all BCC patients were ascertained independently of their diuretic exposure status within a large population-based cohort study. Information bias is also unlikely as all information was gathered prospectively and without knowledge of the research hypothesis. Although there will probably be an underestimation of the number of pathologically proven BCCs, this most likely resulted in non-differential misclassification.

Acute and intermittent ultraviolet exposure at young age is one of the risk factors for which we could not adjust. However, in our opinion this variable will not be a true confounder, since it is probably not associated with the exposure. Hence, as was described earlier, adjustment of the association with ultraviolet exposure for high-ceiling diuretics did not change the risk. [36]

The long follow-up of almost 20 years is one of the strengths of this study. When analyzing drug exposure and a risk of cancer this is of pivotal importance, since cancer usually has a long induction and latent time. In addition, the complete prospectively collected information on drug dispensing excludes the possibility that our findings can be explained by recall bias or other types of information bias. The latter may explain why our study found this association and others did not. [20] In addition, information on co-factors was extensive in our study.

In conclusion, in our study, cumulative exposure time to high-ceiling diuretics was associated with an increased risk of BCC. This effect is more pronounced in patients who have a high tendency to sunburn. Patients on high-ceiling diuretics should be more carefully advised to undertake measures to protect themselves against sun exposure.

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Chapter 6

General discussion

GENERAL DISCUSSION

The aim of this thesis was to provide reliable, balanced information with regard to the occurrence of cancer as potential adverse drug reaction of medicines by performing pharmaco-epidemiological studies and to verify whether these pharmaco-epidemiological studies are indeed helpful in assessing cancer as potential long-term adverse drug reaction. To this end, we studied cancer as potential adverse reaction to drugs that are frequently used in certain patient groups.

In this chapter, the main findings of the pharmaco-epidemiological studies performed in this thesis will be discussed. A critical appraisal of several methodological issues (e.g., quantifying drug exposure) will be discussed as well to facilitate a proper interpretation. Furthermore, we discuss whether pharmaco-epidemiological studies are indeed helpful in assessing cancer as adverse drug reaction, and discuss future implications of the research presented.

Main findings

Adverse drug reaction related hospitalizations

Pharmacotherapy is the most frequently employed intervention in current medical care. However, although intended effects usually predominate, there are adverse effects as well. In **chapter 2** of this thesis, we estimated the incidence of hospitalizations attributable to adverse drug reactions (ADR) in the Netherlands. Our study showed that 1.3% of all hospitalizations was related to an ADR. This percentage is lower than the percentages found in other studies. [1-4] Moreover, only 4 out of the total of 26,852 hospitalizations related to an adverse drug reaction had a cancer diagnosis as main outcome. As described earlier, under-reporting of ADRs causing hospital admissions is considerable and this might be an issue in this study. [5] Part of this under-reporting can be explained by misclassification of the etiology as not all ADRs will be recognized, mentioned in the discharge letters, or registered by the code clerks. Recognition of cancer as an ADR might especially be underestimated by health care professionals as the time interval between start of drug exposure and the diagnosis of cancer is usually very long. Of course, such an association is more easily recognized after chronic drug use than after short-term incidental use of a certain drug in the distant past. Also, recognition of such a delayed ADR might be easier in large data collections of cumulative healthcare data than on a case-by-case basis under everyday clinical circumstances by general practitioners or medical specialists.

Therefore, using only data concerning drug-related hospitalization admission diagnoses, such as in earlier studies, [6] does not suffice to evaluate cancer as adverse drug reaction and additional measures to evaluate drug safety with regard to the risk of

cancer are required. Thus, we set out to assess the association between the use of drugs and the occurrence of cancer as an adverse drug reaction using different data sources. Because the topic is extensive, only a number of issues were addressed, according to the way they presented themselves during the research period at the Drug Safety Unit of the Inspectorate of Healthcare.

Cancer as adverse drug reaction in diabetic patients

Diabetes mellitus has been associated with an increased risk of colorectal cancer [7,8], breast cancer [7,9], endometrial cancer [7,10], hepatocellular carcinoma [7,11], pancreatic cancer [7,12] and bladder cancer. [7,13] In contrast, patients with diabetes seem to have a decreased risk of developing prostate cancer. [7,14] Furthermore, diabetes has been reported as an independent predictor of mortality from cancer. [7,15,16] However, due to factors such as duration of diabetes, different drugs used to attain metabolic control and presence of other diseases, assessment of cancer risk in diabetes patients remains difficult. [17,18]

Numerous articles have been published using data from population registries to analyze a possible relationship between use of hypoglycemic agents and the risk of cancer. For use of insulin glargine, an increased risk of cancer has been published, although not consistently. [19-23] Consequently, whether different types of insulin may be a cause of cancer is an issue of ongoing debate. [24-31]

In **chapter 3.1**, we analyzed the hypothesis that use of insulin glargine is associated with an increased risk of cancer in comparison with use of human insulin. In our study, users of insulin glargine had a lower risk of cancer in general compared with those on human insulin. However, significant differences between users of insulin glargine and users of human insulin were present. Although there were no differences between the number of different drugs used and the number of hospitalizations in the year prior to start of insulin, those using insulin glargine used oral glucose lowering drugs (OGLD) for a longer period prior to start of insulin than those using human insulin. Partly due to its higher costs in comparison with human insulin, insulin glargine is reserved for those suffering from nightly hypoglycemia attacks. [32] Especially patients with type 1 diabetes are prone to these attacks, since, in contrast to patients with diabetes mellitus type 2, they do not have a remaining insulin production. [33] However, it is possible that under everyday circumstances insulin glargine is prescribed more generally to those having difficulties attaining euglycemia. Although we were able to adjust for the number of other drugs used prior to the first prescription of insulin and the number of hospitalizations to adjust for co morbidity, it is still likely that our findings are confounded, since those receiving insulin glargine or other insulin analogues might die earlier due to co-morbidity. As a consequence, they will not live long enough to develop cancer, or, in other words, die of 'competing risks'. [34] Another explanation for our findings might be the significantly lower adherence to therapy of those using insulin glargine in comparison with those

using human insulin. Therefore, in our opinion, this association might be a consequence of residual confounding, lack of adherence or competing risk.

However, like previous studies, we demonstrated an increased risk of breast cancer in users of insulin glargine in comparison with human insulin users. [20,23] Breast cancer has been associated with higher levels of endogenous insulin and as insulin is a growth factor for a number of epithelial tumors and as hyperinsulinemia also produces a secondary increase in the availability of insulin growth factor-1 this has been hypothesized as a possible explanatory mechanism. [31] With regard to breast cancer, insulin glargine has shown a significantly higher proliferative effect on breast cancer cells than human insulin or other insulin analogues. [35] Recently, it was estimated that the serum of type 1 diabetic patients containing insulin glargine was 11% more mitogenic than human insulin containing serum. [36] However, our finding of an increased risk of breast cancer for users of insulin glargine in comparison with those using human insulin has not been consistently confirmed by others [21,37-41] and as the number of cases in our study was relatively low, these results need to be interpreted with caution.

As drug of first choice in diabetes mellitus type 2, metformin is the most widely prescribed OGLD. [42,43] As additional beneficial effect of metformin, a decreased risk of cancer has been suggested. [44] Several studies have analyzed the association between use of metformin and the risk of cancer, but with conflicting results. [21,45-54] Therefore, we analyzed the association between use of metformin and the risk of cancer hypothesizing that the use of metformin decreases the risk of cancer (**chapter 3.2**). In our study, users of metformin had a lower risk of cancer in general and of specific cancers, in comparison with sulfonylurea derivatives. Those aged younger had a lower risk of cancer than those aged older. This can be explained by the increased risk of cancer at higher age. Those hospitalized prior to the first dispensing of OGLD had a lower risk of cancer than those not hospitalized; this can be explained by better screening and earlier diagnosis of the cancer, or, on the other hand, they could also die earlier. Dose-dependent relations could be demonstrated for metformin, but not for sulfonylurea derivatives.

Several possible explanatory mechanisms that might explain the protective effect of metformin on the risk of cancer have been described. [44] Metformin activates 5' adenosine monophosphate protein kinase (AMPK), an energy sensor in the cell which enables muscles to take up glucose from the blood and inhibits gluconeogenesis in hepatocytes during cellular stress. [55] Insufficient activity of AMPK allows uncontrolled cell growth during cellular stress which occurs, for example, during carcinogenesis. Metformin activates AMPK via the upstream LKB1 kinase, [56] a tumor suppressor gene known to be mutated in the Peutz–Jeghers syndrome. [57,58] As extensively described by Jalving *et. al.* other anti-tumor effects of metformin, in addition to AMPK activation, have been described as well. [44] The suggested mechanisms may explain the decreased risk of cancer in users of metformin but it should be emphasized that they are largely speculative. Moreover,

as sulfonylurea derivatives increase the levels of endogenous insulin, this would be a plausible biological underlying mechanism as well. However, this option seems less likely as results in our study, for those treated with a combination of metformin and sulfonylurea derivatives were similar to those who were treated with metformin monotherapy. Despite this, since numbers are low, it is too premature to draw any conclusions from these two sub-analyses.

In addition, our study had limitations. In contrast to some former studies, we were not able to adjust for smoking status or BMI which might be considerable confounding factors. Since previous studies did not always report the actual effect of BMI and/or smoking status on the point estimate, it is not clear to what extent these factors actually confounded our results. In addition, one of the most important issues which we could not address was the clinical decision making process, determining each patient's treatment. The choice to start with or switch to another OGLD is not a random decision and depends largely on patient characteristics such as renal function and other co morbidities like hepatic or cardiac dysfunction. These treatment decisions might have influenced our results.

In conclusion, in our study cumulative exposure to metformin was associated with a lower risk of cancer in general and of specific cancers, in comparison to cumulative exposure to sulfonylurea derivatives. However, whether this should indeed be seen as a decreased risk of cancer for the use of metformin in comparison to the use of sulfonylurea derivatives or as an increased risk of cancer for the use sulfonylurea derivatives in comparison to the use of metformin remains to be elucidated.

Drugs, genotype and their interaction in breast cancer patients

Breast cancer is a major health problem and its incidence is rising. Currently, nearly 30% of all female cancer diagnoses in Europe concerns breast cancer. [59] However, five-year survival rates have improved, partly due to earlier detection, improved treatment and the decreased use of hormone replacement therapy. [59-61] Nevertheless, approximately 14% of all female cancer deaths are caused by breast cancer, making it one of the leading causes of cancer mortality in women. [62]

As the potential effect of drugs on the outcome under analysis may be modified by certain gene products, we verified in **chapter 4.1**, whether the effect of use of NSAIDs on the risk of cancer may be modified by *cyclooxygenase*-genotype (*COX*). COX is an enzyme of which there are two types: COX-1 is constitutively expressed and is not inducible and COX-2 is expressed in response to growth factors, tumor promoters and cytokines. *COX* has been associated with the risk of breast cancer and COX-2 over-expression has been detected in approximately 40% of human breast cancer cases. [63] As a consequence, the association between *COX* SNPs and breast cancer has been frequently analyzed. [64] Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the formation of COX and

therefore also the use of NSAIDs has been studied frequently in the relation to breast cancer. [65] However, only few studies investigated the interaction between *COX-1* and *COX-2* genotype and use of NSAIDs on the risk of breast cancer. [66-70] In our study, use of COX-non-selective NSAIDs was associated with a 13% increased risk of breast cancer. This increased risk was unexpected and could not easily be explained by confounding by indication. Misclassification of exposure is another potential bias. Although there is no reason to expect that the resulting underestimation would be different for those with and without breast cancer, it may be hypothesized though, that women without breast cancer used more OTC NSAIDs than women with breast cancer. The fact that we were not able to demonstrate a dose-effect association would also be an argument against a causal relationship. However, although our finding is counterintuitive, it could also be a true finding with yet unknown etiology. In contrast to our hypothesis, the effect of NSAIDs on the risk of breast cancer was not modified by the available SNPs in the *COX-1* or *COX-2* gene. Or, in other words, we could not determine differences in the effect of NSAIDs on the risk of breast cancer between those carrying zero, one or two variant alleles of the available SNPs in the *COX-1* or *COX-2* gene region.

In **chapter 4.2** we analyzed the effect of *CYP2C19*2* and **3* genotype on the survival of breast cancer patients using tamoxifen. Tamoxifen is a pro-drug predominantly metabolized into its active metabolites 4-hydroxy-tamoxifen and endoxifen (4-hydroxy-*N*-desmethyl-tamoxifen) by the CYP450 system, amongst which CYP2D6, CYP3A4, CYP2B6, CYP2C9 and CYP2C19 are presumed to be the most important isoenzymes. [71-76] *CYP2D6*4* polymorphism, with absent enzyme activity, has been associated with a decreased disease free survival. [77-84] However, relatively little was known about *CYP2C19* variants with absent enzyme activity and tamoxifen efficacy. [85-87] In our study in breast cancer patients using tamoxifen, *CYP2C19 *2* carriership was not associated with increased breast cancer mortality, as was hypothesized, but with increased survival. To further explore this association we analyzed the effect of *CYP2C19*2* in three different patient groups within the Rotterdam Study. Firstly, in non-users of tamoxifen there was no significant association between *CYP2C19*2* and breast cancer mortality. Secondly, we showed that in women with breast cancer *CYP2C19*2* genotype was not associated with overall mortality. Thirdly, we did not show a significant association between *CYP2C19*2* genotype and the risk of breast cancer diagnosis. Apparently, the *CYP2C19*2* genotype is not an independent risk factor for cancer survival but modifies the risk when combined with tamoxifen. However, others could not confirm the beneficial association between *CYP2C19*2* and tamoxifen efficacy in a Japanese study population. [86] In contrast, more recently, similar results were described in a multicenter study in which it was found that *CYP2C19*2* predicts a favorable outcome of tamoxifen treatment in patients with advanced breast cancer. [88] A theoretical explanation for our finding might be that individuals with *CYP2C19 *1/*1* genotype have more adverse reactions due to higher levels

of endoxifen and are consequently less adherent. It seems possible that women with the *CYP2C19* wild type, by producing more active metabolites than women with the risk alleles, are at increased risk of hot flashes and other adverse effects which might influence compliance and consequently the survival benefit. [89] Although we were not able to verify this, we think that this option is not likely considering the strong indication for compliance in this patient group. Furthermore, in our study we did not have complete data on breast cancer stage and additional therapies. Therefore we cannot exclude the possibility that our finding is confounded by a baseline difference in prognosis of the participants under study. Neither did we have complete information on estrogen status but associations between *CYP2C19*2* genotype and tumor size, nodal status, histological grade or estrogen receptor status are unlikely. [83] Consequently, the relationship we found in our study, namely that carriership of one **2* variant allele was associated with increased survival, seems to be counterintuitive and is either a chance finding or a valid finding with unknown etiology.

Basal cell carcinoma as ADR to use of photosensitizing diuretics

In **chapter 5** we describe the association between use of high-ceiling diuretics, which are known for their photosensitizing abilities, and the risk of basal cell carcinoma (BCC). BCC is among the most frequently diagnosed cancers and its incidence is increasing. [90-92] Known risk factors for the development of BCC are age and phenotypic characteristics such as hair color, eye color and skin phototype. The major environmental risk factor for the development of BCC is excessive exposure to ultraviolet radiation (UV), both chronically and intermittently. [93] A wide range of drugs have photosensitizing abilities, but despite the photosensitizing abilities of high-ceiling diuretic agents, little is known about a possible association between use of these frequently used drugs and the risk of BCC. [94,95] In our study, long-term use of high-ceiling diuretics was associated with an increased hazard of BCC of 62% compared to no use. This effect was modified by skin phototype: patients who used high-ceiling diuretics and had a high tendency to sunburn had a higher risk of BCC than users who do not have this tendency. An explanation for this could be that the use of high-ceiling diuretics might lower the Minimal Erythema Dose. In our analysis, we did not find an increased risk of BCC to use of photosensitizing thiazides despite earlier publications. [96]

Information on co-factors was extensive in our study, yet, acute and intermittent ultraviolet exposure at young age is one of the risk factors for which we could not adjust. Whether this covariable is a true confounder is questionable, since it is probably not associated with the exposure to high-ceiling diuretics. The complete prospectively collected information on drug dispensing excludes the possibility that our findings can be explained by recall bias or other types of information bias. However, it might be that detection bias plays a role in our analysis. High-ceiling diuretics are indicated for those

suffering from heart failure, while thiazides are indicated for those with hypertension. It may be hypothesized that patients with heart failure are more closely followed by their physicians, including physical examination, in contrast to those who receive a thiazide for hypertension. However, as BCC characteristically appear on body areas exposed to the sun, with 80% appearing on the head and neck, it is questionable whether detection bias could play such a major role. [97] Therefore, we concluded that cumulative exposure time to high-ceiling diuretics was associated with an increased risk of BCC and that this effect is more pronounced in patients who have a high tendency to sunburn. Therefore, patients on high-ceiling diuretics might be more carefully advised to undertake measures to protect themselves against sun exposure.

Methodological considerations

Study setting and design

Most studies described in this thesis were embedded in the Rotterdam Study, a large prospective population-based cohort study. The objectives and design were extensively described earlier. [98-101] In summary, since 1991, inhabitants of the suburb Ommoord, aged 55 years or older were invited to participate. In the Rotterdam Study I, 7,983 of 10,275 eligible persons, mainly Caucasians, participated and are followed since inclusion. In 1999, 3,011 participants (of 4,472 invitees) who were 55 years of age or older, were added to the cohort (Rotterdam Study II). The study was approved by the Medical Ethics Committee of the Erasmus Medical Center and all participants gave written informed consent.

All participants were examined in detail at baseline. Participants were interviewed at home by trained interviewers and investigations took place during two subsequent visits at the research center. Blood was taken from which DNA was isolated; to obtain a larger coverage, imputation was performed using standard procedures. During follow-up, they underwent additional interviewing, laboratory assessments, clinical examinations and imaging procedures every 3–4 years. The vital status of the participants was obtained regularly from the municipal population registry. Morbidity and mortality were assessed by routinely collected information from the general practitioner, by linkage to a registry of histo- and cytopathology (PALGA), or, in case of hospitalization, by discharge reports from medical specialists. Two research physicians independently assessed the diagnoses of cancer on the basis of pathology data and medical records. In case of discrepancy, consensus was sought or a cancer epidemiologist decided. All events were classified according to the International Classification of Disease (ICD) tenth edition. [102] Only cases confirmed by pathology were considered in the analyses. As all pharmacies which serve the Ommoord district are on a digital network, detailed information on drug dispensing was available for all participants as of 1 January 1991. Information on prescriptions

included product name, Anatomical Therapeutical Chemical (ATC) code [103], dispensing date, total amount of drug units per prescription, prescribed daily number of units, dose and regimen.

The advantage of the Rotterdam Study is that follow-up duration is relatively long – i.e. now more than 20 years – and that extensive information is available on all participants. However, for some of the studies presented in this thesis, the sample size of the Rotterdam Study was too small to perform an adequate analysis. Therefore, data from the PHARMO Record Linkage Study (RLS) was used as well. PHARMO RLS includes drug dispensing records from community pharmacies linked on a patient level to hospital discharge records from the Dutch National Medical Register, [104] concerning approximately 2.5 million individuals, representative of the whole Dutch population, since 1986. [105] The drug dispensing database contains similar information as in the Rotterdam Study. The hospital record database contains detailed information concerning primary and secondary discharge diagnoses and dates of admission and discharge. Diagnoses are coded according to the International Classification of Disease, ninth edition (ICD). [106] Unfortunately, little information on co-factors is available: co-morbidity can be assessed by calculating the number of hospitalizations or the number of different drugs used over a specified period of time but information on, for example, smoking status or BMI is not available.

Bias and confounding

Similar to other types of observational research, the validity of pharmaco-epidemiological studies may be affected by selection bias, information bias and confounding. As PHARMO RLS is a population based database, selection bias is negligible, as everybody using any prescription at any time is enrolled in the geographical regions where PHARMO RLS obtains its data. For the large prospective population-based Rotterdam Study, selection bias is unlikely as well, because all cancer patients were ascertained independently of their exposure status.

Misclassification of exposure is as well unlikely in both, the PHARMO RLS and the Rotterdam Study, as all information on dispensed prescriptions is gathered prospectively and automatically. Differential misclassification of the outcome is unlikely as the outcomes under analyses are collected independently of the exposure of interest. However, non-differential misclassification might have occurred in two ways in the studies performed in diabetic patients (**chapter 3**). First, we used cancer hospitalization as outcome measure, which is different from pathology data on cancer diagnoses. Some cancers might be diagnosed and treated more frequently on an outpatient basis. However, as the cancers are coded independently of the exposure, within each specific cancer, this would lead to non-differential misclassification of the outcome and consequently to dilution of the estimated effect towards the null-hypothesis. Second, non-differential

misclassification might have occurred through reverse causality. In reverse causality, an association may really exist, but the cause and effect are reversed in a way that the cancer itself may cause a change in treatment. With regard to cancer, the time period between the onset of cancer and its diagnosis (the latent period) might be several years, but the period between start of drug exposure and the diagnosis of cancer (induction period + latent period) might be even longer. As a consequence, to obtain a valid estimate, the timing of the outcome should be adequate. When the timing is inadequate, a participant may be coded as not having cancer, while in reality, the cancer might already be present but not diagnosed yet. Since the actual latent period for cancer is not known and may as well vary for different cancers, a predefined latent period (e.g., 1 year) can be taken into account. When taking into account a latent period, the exposure is cumulated up till 1 year prior to the actual diagnosis. This way, sensitivity analyses can be performed to verify whether reverse causality has an impact or not. Of course, required latent periods of more than 1 year may seem more plausible, but as follow-up time may be limited, this is not always feasible.

Furthermore, confounding can be an issue in pharmaco-epidemiological studies. Confounding by indication or by contra-indication can bias the results and is a common problem. It arises when the indication (or contra-indication) of the treatment is a risk factor for the outcome under study. When assessing the association between use of insulin glargine and the risk of cancer, confounding by indication plays a major role as diabetes itself is associated with the risk of cancer as well. To overcome this issue in our study, participants were followed over time starting from their first prescription for insulin, and as a consequence, all participants had the same indication for insulin. Furthermore, participants were more similar with regard to duration and severity of insulin resistance, which addresses the issue of confounding by severity in this analysis. Last, residual confounding might have played a role as well in the studies presented in this thesis. For example, smoking status is not available within the PHARMO RLS and as a consequence, adjustment for this co-factor was not possible. However, as it is unlikely that, for example in the analysis on insulin glargine, all those using human insulin were heavy smokers while those using insulin glargine were all non-smokers the effect of the absence of this covariable may be ignorable.

Despite the rich source of covariables available in the Rotterdam Study, residual confounding may have been present as well in our studies embedded in this population-based cohort. In our study on the effect of *CYP2C19*2* genotype on breast cancer survival in tamoxifen users, we did not have complete data on breast cancer stage and additional therapies. Therefore, we could not exclude the possibility that our finding was confounded by a baseline difference in prognosis of the participants under study. However, although, for example, the indication of tamoxifen (adjuvant or palliative) is of great impact on the survival of breast cancer patients, essentially this covariable cannot

be assumed to be a confounder since it is most likely not related to *CYP2C19*2* genotype. For our study assessing the association between use of photosensitizing high-ceiling diuretics and the risk of BCC, acute and intermittent ultraviolet exposure at young age is one of the risk factors for which we could not adjust. However, in our opinion this variable will not be a true confounder as well, since it is probably not associated with the exposure.

Genotype

In **chapter 4** we analyzed the potential modifying effect of gene products on the association between a drug and a certain outcome. In **chapter 4.1** analyses were performed for 59 SNPs which were available in the *COX-1* and *COX-2* region. In **chapter 4.2** the analysis was performed for 1 SNP in *CYP2C19*. These genes were chosen as candidate genes to test the a priori hypotheses that the effect of NSAIDs on the risk of breast cancer is modified by genetic variation in the *COX* genes and that the effect of tamoxifen on the survival of breast cancer is modified by *CYP2C19*2* respectively. The 59 SNPs analyzed in the *COX-1* and *COX-2* genes tagged a total of 105 SNPs in these genes and included nine of the eleven SNPs which have been previously analyzed with regard to the risk of breast cancer. For some of the SNPs included in the analysis, the potential ability to influence the gene expression has been described (rs20417, rs689466 and rs5275), [107] while for another SNP the functionality could not be established (rs5273). [108] *CYP2C19*2,* known for its absent enzyme activity, has been previously analyzed with regard to tamoxifen efficacy. The minor allele frequency in Caucasians for *CYP2C19*2* is relatively high with 13%. [109]

However, genetic variation in other genes might be modifying the associations under analysis as well. For common genetic variants, genome wide association studies (GWAs) can be performed to assess the effect of common genetic variation in the human genome. Over 600 GWAs have been published during the period November 2002 - July 2010. [110] However, although GWAs have become the primary approach for identifying common SNPs influencing complex diseases, these SNPs are hypothesized to account for only a small fraction of disease heritability. [111] Following the 'Common Disease, Rare Variant' hypothesis it has been argued that common diseases in the population are influenced by numerous rare or low-frequency variants with large effects on disease risk. [111] As both the *CYP2C19* and the two *COX* genes contain many more (rare) SNPs, which were not covered in our analyses, additional analyses of these SNPs might further elucidate their potential relationship with breast cancer. In addition, since it is possible to sequence the entire human genome, novel potential genetic effect modifiers for these and other associations in other genes might be detected as well. However, as this new technology will identify a large number of rare variants and might have a relatively

high proportion of sequence errors and missing values, analyzing these new data will be challenging. [112]

Pharmaco-epidemiological studies and future directions

Although clinical trials are of great value to assess the efficacy and effectiveness of a drug, these are assessed in a homogeneous population during a limited period of time. To evaluate the occurrence of cancer as adverse drug reaction, a large, heterogeneous patient population should be followed over a considerable amount of time. To assess the occurrence of cancer as adverse drug reaction in a clinical trial setting might therefore be very costly. Hence, spontaneous reports of serious adverse events by health care professionals are gathered and analyzed. However, using spontaneous reports has three significant limitations. First, not all adverse events are recognized and under-reporting is present. Second, whether the drug actually caused the adverse event is unknown; it is possible that the drug did not cause the adverse event. Third, spontaneous reports do not give information on the amount and duration of use which is pivotal for the correct interpretation.

Therefore, we set out to verify whether the use of a pharmaco-epidemiological study design can be of important additive value to assess the incidence of cancer as adverse drug reaction. In this thesis, as hypothesized, we found a lower risk of cancer for use of metformin (**chapter 3.1**). However, in contrast to our hypotheses, a lower risk of cancer for use of insulin glargine (**chapter 3.2**) and a counterintuitive finding for survival in breast cancer patients using tamoxifen and carrying a variant of the *CYP2C19*2* genotype (**chapter 4.2**) was found. We described a relatively unexpected increased risk of breast cancer for use of NSAIDs (**chapter 4.1**), but, we also presented an increased risk of basal cell cancer for the use of photosensitizing diuretics, as was hypothesized (**chapter 5**). So, can we conclude that observational pharmaco-epidemiological studies are indeed of additive value when studying cancer as adverse drug reaction despite these controversial findings? Yes, we can and yes, we have to continue performing such studies. Up till this moment, no other alternatives are available to study the incidence of cancer as adverse drug reaction sufficiently. Therefore, if properly designed, to avoid potential bias and confounding, and if tentatively interpreted, observational pharmaco-epidemiological studies can be of added value. Within an aging population, an increasing number of drugs is used chronically, therefore, the limited knowledge of cancer as potential adverse drug reaction is a deficit. Hence, more efforts should be made to study cancer as potential adverse drug reaction by performing pharmaco-epidemiological studies. In our opinion, the legal responsibility for this kind of research first lies with the pharmaceutical industry. However, as the competent authorities in the Netherlands also have the responsibility to conduct research to verify the condition of public health and its determinants and, where necessary, to identify and promote resources for improvement, they have a role in studying cancer as adverse drug reaction as well. [113,114]

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Chapter 7

Summary / Samenvatting

SUMMARY

Introduction and aim of the thesis

Pharmacovigilance is defined by the World Health Organization (WHO) as the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problem. In this field, post-authorization safety studies, either non-interventional (pharmaco-epidemiological) or interventional (clinical trials), are therefore conducted with the aim of identifying or quantifying a safety hazard relating to an authorized medicinal product. The recognition of cancer as an adverse drug reaction is difficult though, as firstly, the association between the drug and the adverse drug reaction may be unknown. Secondly, the background incidence can be relatively high while the attributable proportion of the drug is rather low. And thirdly, the timeframe between first exposure to the drug and the occurrence of the adverse drug reaction can be relatively long. As the effect of the drug may vary over time, may be dose-dependent and may be influenced by numerous other factors, such as, for example, genotype, the assessment of the association between drugs and cancer as potential outcome remains a challenge. However, the association between specified drugs and cancer as adverse drug reaction can be assessed in population-based cohort studies, which often include a large number of participants who are followed over a significant period of time.

The aim of this thesis was to gain more insight with regard to the occurrence of cancer as potential adverse drug reaction by performing pharmaco-epidemiological studies and to verify whether these pharmaco-epidemiological studies are indeed helpful in assessing cancer as potential adverse drug reaction.

Adverse drug reaction related hospitalizations

In **chapter 2** of this thesis, we estimated that in the Dutch nationwide registry of hospital discharges the incidence of hospitalizations attributable to adverse drug reactions in the Netherlands is 1.3%. This percentage is lower than the percentages found in other studies. Moreover, cancer was not coded as potential adverse event. Therefore, using only data concerning drug-related hospitalization admission diagnoses does not suffice to evaluate cancer as adverse drug reaction and additional measures to evaluate drug safety with regard to the risk of cancer are required. Thus, we set out to assess the association between the use of drugs and the occurrence of cancer as an adverse drug reaction using different data sources.

Cancer as adverse drug reaction in diabetic patients

Diabetes has been associated with an increased risk of several cancer types. Numerous articles have been published, using data from population registries, to analyze a possible

relationship between use of hypoglycemic agents and the risk of cancer. For use of insulin glargine, an increased risk of cancer has been published, although this finding was not consistent. In **chapter 3.1**, we analyzed the hypothesis that use of insulin glargine is associated with an increased risk of cancer in comparison with use of human insulin. In our study, users of insulin glargine had a lower risk of cancer in general compared with those on human insulin. However, like previous studies, we demonstrated an increased risk of breast cancer in users of insulin glargine in comparison with human insulin users. But as this finding could not be consistently confirmed by others, and as the number of cases in our study was relatively low, these results need to be interpreted with caution. Although we were able to adjust for the number of other drugs used prior to the first prescription of insulin and the number of hospitalizations, to adjust for co morbidity, it is still likely that our findings are confounded, since those receiving insulin glargine or other insulin analogues might die earlier due to co-morbidity. As a consequence, they will not live long enough to develop cancer, or, in other words, die of 'competing risks'. Another explanation for our findings might be the lower adherence to therapy of those using insulin glargine in comparison with those using human insulin. Therefore, in our opinion, this association might be a consequence of residual confounding, lack of adherence or competing risk.

As drug of first choice in diabetes mellitus type 2, metformin is the most widely prescribed oral glucose lowering drug. As additional beneficial effect of metformin, a decreased risk of cancer has been suggested. Therefore, we analyzed the association between use of metformin and the risk of cancer, hypothesizing that the use of metformin decreases the risk of cancer (**chapter 3.2**). In our study, users of metformin had a lower risk of cancer in general and of specific cancers, in comparison with sulfonylurea derivatives. Dose-dependent relations could be demonstrated for metformin, but not for sulfonylurea derivatives.

Although several possible explanatory mechanisms explaining the protective effect of metformin on the risk of cancer in comparison with the use of sulfonylurea derivatives have been hypothesized, biological plausible mechanisms explaining a potential increase in cancer risk for the use of sulfonylurea derivatives in comparison with the use of metformin have been suggested as well. Therefore, we concluded that whether the results of our study should indeed be seen as a decreased risk of cancer for the use of metformin or as an increased risk of cancer for the use sulfonylurea derivatives remains to be elucidated.

Drugs, genotype and their interaction in breast cancer patients

As the potential effect of drugs on the outcome under analysis may be modified by certain gene products we verified in **chapter 4.1** whether the effect of use of non-steroidal anti-inflammatory drugs (NSAIDs) on the risk of cancer may be modified by

cyclooxygenase-genotype (*COX*). COX-2 overexpression has been observed in breast cancer tissue and in addition, NSAIDs are known to inhibit the synthesis of cyclooxygenase. Therefore also the use of NSAIDs has been studied frequently in the relation to breast cancer. However, only few studies investigated the interaction between *COX-1* and *COX-2* genotype and use of NSAIDs on the risk of breast cancer. In our study, use of COX-non-selective NSAIDs was associated with a 13% increased risk of breast cancer. This could be a true finding with unknown etiology, however, the fact that we were not able to demonstrate a dose-effect association would be an argument against a causal relationship. In contrast to our hypothesis, the effect of NSAIDs on the risk of breast cancer was not modified by the SNPs analyzed in the *COX-1* or *COX-2* gene.

In **chapter 4.2** we analyzed the effect of *CYP2C19*2* genotype on the survival of breast cancer patients using tamoxifen. Tamoxifen is a pro-drug predominantly metabolized into its active metabolites by the hepatic CYP450 system, amongst which CYP2D6, CYP3A4, CYP2B6, CYP2C9 and CYP2C19 are presumed to be the most important isoenzymes. Relatively little is known about *CYP2C19* variants with, according to the literature, absent enzyme activity and tamoxifen efficacy. In our study in breast cancer patients using tamoxifen, *CYP2C19 *2* carriership was not associated with increased breast cancer mortality, as was hypothesized, but with increased survival. Additional analyses showed that *CYP2C19*2* genotype is not an independent risk factor for breast cancer survival but modifies the risk only when combined with tamoxifen.

In **chapter 5** we describe the association between use of high-ceiling diuretics and the risk of basal cell carcinoma (BCC). BCC is among the most frequently diagnosed cancers and its incidence is increasing. Despite the photosensitizing abilities of high-ceiling diuretic agents, little is known about a possible association between use of these frequently used drugs and the risk of BCC. In our study, long-term use of high-ceiling diuretics was associated with an increased hazard of BCC of 62% compared to no use. This effect was modified by skin phototype: patients who used high-ceiling diuretics and had a high tendency to sunburn had a higher risk of BCC than users who do not have a high tendency to sunburn. Therefore, we concluded that patients on high-ceiling diuretics might be more carefully advised to undertake measures to protect themselves against sun exposure.

Conclusion and future directions

A reflection on the main results from the studies presented in this thesis, as well as a critical appraisal of several methodological issues (e.g., the complexity of quantifying drug exposure) can be found in **chapter 6**. In addition, it was discussed that evaluating cancer as adverse drug reaction by performing clinical trials or by analyzing spontaneous reports has significant limitations. At this moment, besides observational research, no other alternatives are available to study the incidence of cancer as adverse drug reaction

sufficiently. Therefore, if properly designed, to avoid potential bias and confounding, and if tentatively interpreted, observational pharmaco-epidemiological studies can be of important additive value to assess the incidence of cancer as adverse drug reaction. Within an aging population, an increasing number of drugs is used chronically, therefore, the limited knowledge of cancer as potential adverse drug reaction is a deficit. Hence, more efforts should be made to study cancer as potential adverse drug reaction by performing pharmaco-epidemiological studies. In our opinion, the legal responsibility for this kind of research first lies with the pharmaceutical industry. However, as the competent authorities in the Netherlands also have the responsibility to conduct research to verify the condition of public health and its determinants and, where necessary, to identify and promote resources for improvement, they have a role in studying cancer as adverse drug reaction as well.

SAMENVATTING

Inleiding en doel van dit proefschrift

Geneesmiddelenbewaking wordt door de Wereldgezondheidsorganisatie (WHO) gedefinieerd als de wetenschap en de activiteiten die betrekking hebben op de opsporing, beoordeling, kennis en preventie van bijwerkingen of van andere mogelijk aan het gebruik van geneesmiddelen toegeschreven problemen. Hiertoe worden, nadat een middel is toegelaten tot de markt, studies verricht naar de veiligheid van een geneesmiddel. Deze kunnen observationeel (farmaco-epidemiologisch) of interventioneel (experimenteel onderzoek) van aard zijn en worden uitgevoerd met als doel het identificeren of kwantificeren van mogelijke veiligheidsproblemen van een reeds tot de markt toegelaten geneesmiddel. Het herkennen van kanker als mogelijke bijwerking is echter moeilijk. Dit heeft verschillende oorzaken. Ten eerste zal een associatie tussen het geneesmiddel en de bijwerking kanker vaak onbekend zijn. Ten tweede kan de achtergrond incidentie hoog zijn, terwijl het oorzakelijk aandeel van het geneesmiddel (attributief risico) laag is. Ten derde kan de tijdspanne tussen de eerste blootstelling aan het geneesmiddel en het optreden van de bijwerking kanker lang zijn. Daarnaast blijft het een uitdaging om de validiteit van de associatie tussen het gebruik van geneesmiddelen en het risico op kanker als mogelijke bijwerking te bevestigen omdat het effect van het geneesmiddel tijdsafhankelijk en dosis-afhankelijk is en beïnvloed kan worden door een groot aantal andere factoren zoals genotype. De associatie tussen specifieke geneesmiddelen en kanker als bijwerking kan worden onderzocht in zogenaamde op de populatie gebaseerde cohort studies. Deze cohorten includeren vaak een groot aantal deelnemers die voor een aanzienlijke periode worden gevolgd. Het doel van dit proefschrift was om meer inzicht te verkrijgen in het ontstaan van kanker als mogelijke bijwerking van een geneesmiddel door het uitvoeren van farmaco-epidemiologische studies en om na te gaan of deze farmaco-epidemiologische studies nuttig zijn bij de beoordeling van kanker als potentiële bijwerking van een geneesmiddel.

Aan bijwerkingen toegeschreven ziekenhuisopnames

In **hoofdstuk 2** van dit proefschrift beschrijven wij dat in de Landelijke Medische Registratie (LMR), 1.3% van de ziekenhuisopnames in Nederland toegeschreven wordt aan een bijwerking van een geneesmiddel. Dit percentage is lager dan de percentages die gevonden zijn in andere studies. Bovendien wordt kanker niet als een mogelijke bijwerking gecodeerd. Daaruit kan men concluderen dat gegevens over aan bijwerkingen toegeschreven ziekenhuisopnames niet volstaan om kanker als bijwerking van een geneesmiddel te evalueren en dat aanvullende gegevens nodig zijn om de veiligheid van geneesmiddelen met betrekking tot het risico op kanker te onderzoeken. Daarom werd besloten om de associatie tussen het gebruik van geneesmiddelen en het optreden van

kanker als bijwerking te analyseren door gebruik te maken van andere gegevensbronnen.

Kanker als bijwerking bij patiënten met diabetes mellitus

Diabetes mellitus wordt beschouwd als een risicofactor voor de meeste vormen van kanker. Vele artikelen zijn gepubliceerd die, met behulp van gegevens uit bevolkingsregisters, een mogelijke relatie hebben bestudeerd tussen het gebruik van bloedglucose verlagende middelen en het risico op kanker. Gebruik van insuline glargine is in de literatuur in verband gebracht met een verhoogd risico op kanker, alhoewel deze bevinding niet consistent gereproduceerd werd. In **hoofdstuk 3.1**, analyseerden we de hypothese dat het gebruik van insuline glargine geassocieerd is met een verhoogd risico op kanker in vergelijking met het gebruik van humaan insuline. Echter, in onze studie hadden gebruikers van insuline glargine een lager risico op kanker dan gebruikers van humaan insuline. Wel was in onze studie het gebruik van insuline glargine geassocieerd met een verhoogd risico op borstkanker in vergelijking met gebruik van humaan insuline. Omdat deze bevinding niet altijd kon worden bevestigd in ander onderzoek en gezien het relatief lage aantal gevallen van borstkanker in onze studie, moeten deze resultaten echter met de nodige voorzichtigheid worden geïnterpreteerd. Hoewel we in staat waren om te adjusteren voor het gebruik van andere geneesmiddelen en voor het voorafgaand aantal ziekenhuisopnames als maat voor co-morbiditeit, is het waarschijnlijk dat het niet mogelijk was om geheel te corrigeren voor vertekening van de resultaten. Omdat degenen die insuline glargine of andere insuline-analogen gebruiken mogelijkerwijs eerder zouden kunnen overlijden als gevolg van co-morbiditeit, zouden ze onvoldoende lang kunnen leven om kanker te ontwikkelen. Naast dit vroeger overlijden door een zogenoemd 'competing risk', zou een alternatieve verklaring kunnen worden gevormd door de lagere therapietrouw van de gebruikers van insuline glargine in vergelijking met de therapietrouw van gebruikers van humaan insuline. Samenvattend zou de in onze studie beschreven associatie daarom een gevolg kunnen zijn van 'residual confounding', een gebrek aan therapietrouw of de aanwezigheid van 'competing risk'.

Als middel van eerste keus bij diabetes mellitus type 2, is metformine het meest voorgeschreven orale bloedglucose verlagende geneesmiddel. Als bijkomstig gunstig effect van metformine, is een verminderd risico op kanker gesuggereerd. Daarom analyseerden we de associatie tussen het gebruik van metformine en het risico op kanker volgens de hypothese dat het gebruik van metformine het risico op kanker zal verlagen (**hoofdstuk 3.2**). In onze studie hadden gebruikers van metformine in vergelijking met gebruikers van sulfonylurea derivaten een lager risico op kanker in het algemeen en op specifieke vormen van kanker. Daarnaast waren wij in staat om dosis-effect relaties aan te tonen voor het gebruik van metformine, maar niet voor het gebruik van sulfonylurea derivaten. Hoewel er verscheidene mogelijke verklarende biologische mechanismen

zijn beschreven welke een beschermend effect van metformine op het risico van kanker zouden kunnen verklaren, zijn er ook biologisch plausibele mechanismen beschreven welke een verhoogd risico op kanker voor het gebruik van sulfonylurea derivaten zouden kunnen verklaren. Daarom is op dit moment onduidelijk of onze resultaten gezien moeten worden als een verlaagd risico op kanker voor gebruikers van metformine in vergelijking tot gebruikers van sulfonylurea derivaten of als een verhoogd risico op kanker voor gebruikers van sulfonylurea derivaten in vergelijking tot gebruikers van metformine.

Geneesmiddelen, genotype en hun interactie bij borstkanker patiënten

Omdat het potentiële effect van geneesmiddelen gemodificeerd kan worden door bepaalde genetische producten, zijn we in **hoofdstuk 4.2** nagegaan of het effect van het gebruik van prostaglandinesynthetaseremmers (NSAID's) op het risico op kanker gemodificeerd wordt door het *cyclo-oxygenase*-genotype (*COX*). Het is beschreven dat borstkankerweefsel een verhoogde expressie van COX-2 kan tonen, en gezien het feit dat NSAID's de vorming van COX remmen is de associatie tussen het gebruik van NSAID's en het risico op borstkanker ook frequent onderzocht. Er is echter maar een klein aantal studies waarin de interactie tussen *COX* genotype en het gebruik van NSAID's op het risico van borstkanker werd geanalyseerd. In onze studie was het gebruik van COX-niet-selectieve NSAID's geassocieerd met een verhoogd risico op borstkanker van 13 procent. Alhoewel dit een valide bevinding zou kunnen zijn met een vooralsnog onbekende etiologie, pleit de afwezigheid van een dosis-afhankelijke relatie hier tegen. In tegenstelling tot onze hypothese werd in onze studie de associatie tussen NSAID's en borstkanker niet gemodificeerd door de geanalyseerde SNPs in het *COX-1* of *COX-2* genotype.

In **hoofdstuk 4.2** hebben we de invloed bestudeerd van *CYP2C19*2* genotype op de overleving van borstkankerpatiënten, die behandeld werden met tamoxifen. Tamoxifen is een zogenoemde 'pro-drug', die voornamelijk door het enzymsysteem CYP450 in de lever omgezet wordt naar de actieve metabolieten. Van de iso-enzymen CYP2D6, CYP3A4, CYP2B6, CYP2C9 en CYP2C19 wordt verondersteld dat zij hieraan een belangrijke bijdrage leveren. Er is relatief weinig bekend over *CYP2C19* varianten, die volgens de literatuur geen enzymactiviteit zouden vertonen, en de werkzaamheid van tamoxifen. In tegenstelling tot onze hypothese was *CYP2C19*2* dragerschap in patiënten met borstkanker, die behandeld werden met tamoxifen, niet geassocieerd met een verhoogde sterfte door borstkanker maar met een langere overleving. Aanvullende analyses toonden aan dat *CYP2C19* genotype geen onafhankelijke risicofactor voor de overleving van borstkanker was maar dat zij het risico alleen modificeerde in combinatie met de aanwezigheid van behandeling met tamoxifen.

In **hoofdstuk 5** beschrijven we de associatie tussen het gebruik van lisdiuretica en het risico op een basaalcelcarcinoom (BCC). Het BCC is een van de meest gediagnosticeerde vormen van kanker en de incidentie hiervan neemt toe. Ondanks het feit dat lisdiuretica fotosensibiliserend zijn, is er weinig bekend over een mogelijke associatie tussen het gebruik van deze frequent voorgeschreven geneesmiddelen en het risico op BCC. In onze studie was langdurig gebruik van lisdiuretica geassocieerd met een verhoogd risico op BCC van 62 procent ten opzichte geen gebruik. Dit effect werd gemodificeerd door het huidtype: patiënten die lisdiuretica gebruikten en een sterke neiging hebben tot huidverbranding door zonlicht hebben een hoger risico op BCC dan gebruikers die deze neiging niet hebben. Daarom concluderen wij dat patiënten die lisdiuretica gebruiken wellicht ook geadviseerd moeten worden om maatregelen te nemen om zichzelf te beschermen tegen zonnebrand.

Conclusie en toekomst

Een reflectie op de belangrijkste resultaten van de studies welke in dit proefschrift worden beschreven, evenals een kritische evaluatie van verschillende methodologische aspecten (bijvoorbeeld de complexiteit bij het kwantificeren van de blootstelling aan een geneesmiddel) zijn te vinden in **hoofdstuk 6**. Daarnaast wordt hier besproken dat de evaluatie van kanker als bijwerking van een geneesmiddel middels het uitvoeren van klinisch onderzoek of door analyse van spontane meldingen doorgaans onvoldoende is. Op dit moment zijn er geen goede alternatieven voor observationeel onderzoek om de incidentie van kanker als bijwerking voldoende te bestuderen. Mits goed opgezet, om vertekening van de resultaten door potentiële bias en 'confounding' te voorkomen, en behoedzaam geïnterpreteerd, zijn observationele farmaco-epidemiologische studies van belangrijke toegevoegde waarde om de incidentie van kanker als mogelijke bijwerking te bestuderen. Omdat binnen een verouderende bevolking veel geneesmiddelen in toenemende mate chronisch gebruikt worden, is de geringe kennis over kanker als bijwerking een tekortkoming. Daarom zou het uitvoeren van farmaco-epidemiologische studies met betrekking tot dit onderwerp meer aandacht moeten krijgen. De wettelijke verantwoordelijkheid voor het opzetten en uitvoeren van dergelijk onderzoek ligt in eerste instantie bij de farmaceutische industrie. Echter, omdat de autoriteiten ook een eigen verantwoordelijkheid hebben in het verrichten van onderzoek naar de staat van de volksgezondheid en de determinanten daarvan, alsmede, waar nodig, het aangeven en bevorderen van middelen tot verbetering daarvan, hebben zij ook een rol bij het bestuderen van kanker als mogelijke bijwerking van een geneesmiddel.

Chapter 8

Acknowledgement / Dankwoord

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About the Author

PhD Portfolio

ACKNOWLEDGEMENT / DANKWOORD

Op deze plaats wil ik graag iedereen bedanken, die betrokken is geweest bij het tot stand komen van mijn proefschrift.

Allereerst mijn promotores, Prof.dr. B.H.Ch. Stricker, Prof.dr. A.G. Uitterlinden en mijn co-promotor Dr. L.E. Visser. Beste Bruno, ik wil je bedanken voor de afgelopen paar jaren. Ik heb veel geleerd van de methodologische discussies die we hadden en met plezier geluisterd naar je verhalen over vroeger, onze hedendaagse maatschappij én plannen om allerhande associaties te gaan analyseren. Daarnaast wil ik je graag bedanken voor de laagdrempeligheid waarmee je te benaderen bent en je immer snelle en waardevolle, kritische reacties. Beste professor Uitterlinden, bedankt dat u me de gelegenheid hebt gegeven deel te nemen aan de wereld van genetisch onderzoek. De samenwerking binnen het CHARGE-consortium was een waardevolle aanvulling op de studies in dit proefschrift. Beste Loes, jouw Friese nuchterheid en heldere aanpak waardeer ik zeer. Wanneer een overleg dreigde te verzanden in oneindigheid wist jij het altijd weer op de rails te krijgen. Je kritische blik op mijn stukken afgewisseld met mental support als het even tegenzat zal ik niet snel vergeten.

Prof.dr. J.W.W. Coebergh, Prof.dr. A. Hofman en Prof.dr. J.W.A. Smit wil ik bedanken voor hun bereidheid om plaats te nemen in de kleine promotiecommissie en voor de inhoudelijke beoordeling van het manuscript. Professor Coebergh, uw bijdrage aan de kankersurveillance in Nederland is van groot belang, het was een genoegen met u samen te mogen werken. Professor Hofman, voor het grootste deel van de studies waaraan ik heb mogen meewerken, werd gebruik gemaakt van het Erasmus Rotterdam Gezondheid Onderzoek (ERGO). Hartelijk bedankt voor de mogelijkheid om hieraan deel te hebben mogen nemen! Professor Smit, ik kijk er naar uit om onder uw supervisie te starten met de opleiding tot internist. Dr. T.P. van Staa en Dr. S.M.J.M. Straus wil ik bedanken voor hun bereidheid plaats te nemen in de grote promotiecommissie.

Daarnaast wil ik hier ook alle co-auteurs bedanken voor hun bijdrage en waardevolle commentaar op de verschillende manuscripten. Graag zou ik in het bijzonder Dr. R.M.C. Herings en Dr. M.P.P. van Herk-Sukel danken voor de plezierige samenwerking met betrekking tot de manuscripten waarin wij gebruik maakten van PHARMO data.

Op deze plek ook een bedankje voor al mijn collega's van de afdeling Epidemiologie en Medische Informatica. Jullie zijn met teveel om individueel op te noemen, maar zonder jullie was het de afgelopen jaren een stuk minder gezellig geweest! Een speciaal dankwoord voor mijn kamergenoten: Daan, Laura, Mark, Marlies, Monique, Nikkie, Till

en Toke. Mark en Monique, dank voor de eerste opvang! Daan, kletskous, ik ken weinig mensen die zo oprecht behulpzaam zijn als jij, dankjewel hiervoor. En verder, luisteren naar Dolly Parton zal nooit meer hetzelfde zijn… Toke, heel veel succes met alle statines!

Graag wil ik ook gebruik maken van de gelegenheid om de 15,000 mensen die deelnemen aan het ERGO onderzoek te bedanken. Zonder al jullie informatie zou het überhaupt niet mogelijk zijn om in dit verband onderzoek te verrichten. Tevens wil ik de huisartsen en apothekers bedanken voor hun medewerking en de onderzoek medewerkers, zowel op de faculteit als het ERGO-centrum, voor hun inzet. Met name wil ik hier de dames op het ERGO-centrum onder leiding van Anneke noemen. Daarnaast dient het werk van de fuppers genoemd te worden, en natuurlijk de inzet van Jolande en collegae voor het verwerken en managen van al deze gegevens. Frank, dank voor het aanleveren van de databestanden. En Nano, bedankt voor de eerste hulp bij computerproblemen, alles goed? Ook de dames van het secretariaat wil ik hierbij bedanken voor hun ondersteuning.

De helft van mijn tijd bracht ik bij programma 8, geneesmiddelen en medische technologie, van de inspectie voor de gezondheidszorg door. De collega's hier wil ik hartelijk bedanken voor de afgelopen tijd. Met name Daan, Diederike, Eline, Frank, Hanneke, Janny, Jeanet, Jitske, Judith, Karina, Maris en Willem. Janny, dank voor je hulp bij alle formulieren, maar, belangrijker, voor de roddels!

Op deze plek zou ik niet zijn aangekomen zonder de steun van vrienden en familie. B, Juut, eerst belangrijker zaken, ik ben heel benieuwd hoe het jullie zal verlopen. Ferry en Lorens, Harm en Trui, Lenny en Boy, Marjan en Martijn, Roy, dank voor de broodnodige gezellige Bourgondische avonden! Karlijn Vermeulen, hoogste tijd om weer eens bij te praten, dank voor je relativeringsvermogen en humor...

Eline, als twee compleet verschillende mensen zijn we dit traject begonnen. Jij als het zonnetje in huis, altijd in voor een praatje – maar vaker een luisterend oor. Ik heb heel veel van je geleerd en hoop dat onze vriendschap nog vele koppen thee zal duren. Lieve Karlijn, dank voor onze talloze gesprekken en je wijze woorden. Als er één iemand kan verwoorden wat er op spiritueel, emotioneel en fysiek niveau allemaal plaats vindt, en hier ook nog verschillende invalshoeken in kan belichten dan ben jij het. Meiden, ik ben er trots op dat jullie vandaag naast mij willen staan.

Bijna tien jaar geleden kreeg ik er 'opeens' een hele nieuwe familie bij, en wat voor één. Thijs en Truus, wat een geluk stralen jullie uit, ben blij met jullie als schoonouders! Raoul

en Marielle, Aniek en Boy, men zegt wel eens "vrienden kies je, familie heb je", voor jullie geldt beide. Opa en Enny, sjun tse zië wie d'uur van ut leëve jenist!

Mijn lieve grootmoeder in het hoge noorden, dank voor uw betrokkenheid. Lief klein omaatje, wat jammer dat je er niet meer bent, ik mis je.

Aafke, Sanne, lieve zusjes, ik ben trots op jullie. Aaf, succes met je tweede studie, jij komt er wel. Sanne, wat kun je toch grappig uit de hoek komen, wanneer gaan we pizza eten? Josse, lieve broer, dank voor al je support, computer-technisch maar bovenal emotioneel, en dank voor je heerlijke nuchtere kijk op het leven.

Josine en Hans, ik waardeer onze om-en-om-uit-eten-gaan-afspraken zeer en ben blij dat jullie het samen zo gezellig hebben. Maar bovenal, lieve mam, ben ik opgelucht dat ik je terug gevonden heb.

Jaap en Caroline, dank voor alle gezelligheid en de lange avonden vol beschouwende gesprekken. En dank voor de heerlijke zeiltochten, al was het veelal zonder wind. Ik weet nu van wie ik het geduld in ieder geval niet heb.

Allerliefste schatje, Thierry. De alom bekende quotes 'liefde is, samen…' bestaan niet voor niets en zonder jou zou het leven zeker minder kleur hebben. Liefde is samen lachen, samen de wereld verkennen, maar ook aan een woord genoeg hebben en elkaar de vrijheid geven om je ambities na te streven.

'Love is composed of a single soul inhabiting two bodies'.

Bedankt voor alles. Ik houd van je.

Rik.

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Submitted

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Tanneke Rikje Ruiter was born on October 20th, 1982 in Wageningen, the Netherlands. In 2000, she graduated from the "Ulenhof college" (athenaeum-beta) in Doetinchem and subsequently started medical school at the Radboud University in Nijmegen. In 2004 she participated in a research on the management of mental health problems by Slovene general practitioners at the Department of Family Medicine at the University of Ljubljana, Slovenia (supervisors: Dr. D. Rotar Pavlič, Prof.dr. I. Švab and Dr. H. van Rijswijk). In 2005 she received her "doctorandus" degree in Medicine, and after completing her medical training, she received her medical degree in 2007. Afterwards, she worked for one year as a resident in Internal Medicine at the "Medisch Centrum Haaglanden" in The Hague (head: Dr. P.H. Geelhoed-Duijvestijn).

In June 2008, she started the work described in this thesis at the Pharmaco-Epidemiology unit (head: Prof.dr. B.H.Ch. Stricker) of the Department of Epidemiology (head: Prof. dr. A. Hofman) of the Erasmus MC in Rotterdam. During this period, she also worked at the Drug Safety Unit of the Dutch Inspectorate of Health Care.

In 2010, she obtained a Master of Science in Health Science, specialization Clinical Epidemiology, at the Netherlands Institute for Health Sciences (NIHES).

In January 2012, she will start her residency in Internal Medicine at the "Groene Hart Ziekenhuis" in Gouda (head: Dr. J.T.M. van der Heyden) as a part of her specialty training at the Leiden University Medical Center (head: Prof.dr. J.W.A. Smit).

PHD PORTFOLIO

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1. PHD TRAINING

Research Skills

Statistics and Methodology

2008-2010 Master of Science in Health Science, specialization Clinical Epidemiology, Netherlands Institute for Health Sciences, Rotterdam, the Netherlands, 30 ECTS.

Presentations

Oral Presentations

2010 High-ceiling diuretics are associated with an increased risk of basal cell carcinoma in a population-based follow-up study, 26th International conference on Pharmaco-epidemiology & Therapeutic Risk Management, Brighton, UK.

2011 Risk of cancer in patients on insulin glargine in comparison to those on human insulin: results from a large population – based follow-up study, 23e Internistendagen, Maastricht, the Netherlands.

2011 Trends in dispensing patterns of rosiglitazone and pioglitazone in the Netherlands following safety signals during the period 1998 – 2008, 27th International Conference on Pharmaco-epidemiology & Therapeutic Risk Management, Chicago, USA.

2011 Risk of cancer in patients on insulin glargine in comparison to those on human insulin: results from a large population – based follow-up study, Bijeenkomst Diabetes Platform, Rotterdam, the Netherlands.

Poster Presentations

2009 *CYP2C19*2* polymorphism is associated with increased survival in breast cancer patients using tamoxifen, 25th International Conference on Pharmaco-epidemiology & Therapeutic Risk Management, Providence, Rhode Island, USA.

2011 Risk of cancer in patients on insulin glargine in comparison to those on human insulin: results from a large population – based follow-up study. 27th International Conference on Pharmaco-epidemiology & Therapeutic Risk Management, Chicago, Illinois, USA.

International conferences

2010 26th International Conference on Pharmaco-epidemiology & Therapeutic Risk Management in Brighton, UK.

2011 Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (CHARGE) meeting, Boston, Massachusetts, USA.

2011 27th International Conference on Pharmaco-epidemiology & Therapeutic Risk Management, Chicago, USA.

Seminars and workshops

2008-2011 Research seminars, department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands.

2008 Business training Feedback, Training and Consultancy Bureau Vergouwen Overduin, The Hague, the Netherlands.

2009 Business meeting, Dutch Association of Pharmaceutical Medicine, Pharmacovigilance Platform Netherlands, Oss, the Netherlands.

2009 Pharmacovigilance Inspectors Working Group Training, European Medicines Authority, Antwerp, Belgium.

2010 Business training Communication, ICM training Bureau, Utrecht, the Netherlands.

Other

2010-2011 Reviewing articles.

2. TEACHING ACTIVITIES

Lecturing

Supervising practicals

2009-2010 Methods of Clinical and Epidemiological Research, 4th year medical students, Erasmus MC, Rotterdam, the Netherlands.

2009-2011 Pharmaco-epidemiology, 4th year medical students, Erasmus MC, Rotterdam, the Netherlands.

2010-2011 Pharmaco-epidemiology and Drug Safety, Erasmus Winter Programme, NIHES, Rotterdam, the Netherlands.

Supervising Master of Science students

2009-2010 K.E. van den Hondel, "The Effect of Short-Term NSAID Use on Echocardiographic Parameters in the Elderly: a Population Based Cohort Study", Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands.

2010-2011 J.A. Rugeles Mindiola, "Is the risk of breast cancer in postmenopausal females within the Rotterdam Study modified by the use of NSAIDs and/or *COX* genotype?", Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands.

Cancer is the Latin word for crab, which explains the symbolic value of this small animal for the disease cancer. The sand bubbler crabs, of which one is pictured on the front of this thesis, are known for their artworks made out of little sand balls. At low tide, the sand bubbler crabs emerge from their holes beneath the sand to gather food that the tide has brought along. They do this by collecting and sifting the sand, and rolling those parts devoid of anything useful for them into little balls. These sand balls symbolize the different drugs prescribed to patients, while the crab emerging from its hole at low tide represents the potential of cancer as adverse drug event.