Non-Steroidal Anti-Inflammatory Drugs and Melanoma Risk: Large Dutch Population-Based Case–Control Study

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This case–control study investigates the potential chemoprophylactic properties of non-steroidal anti-inflammatory drugs (NSAIDs) on the incidence of cutaneous melanoma (CM). Data were extracted from the Dutch PHARMO pharmacy database and the PALGA pathology database. Cases had a primary CM between 1991 and 2004, were $\geqslant$18 years, and were observed for 3 years in PHARMO before diagnosis. Controls were matched for date of birth, gender, and geographical region. NSAIDs and acetylsalicylic acids (ASAs) were analyzed separately. Adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated using multivariable logistic regression, and the results were stratified across gender. A total of 1,318 CM cases and 6,786 controls were eligible to enter the study. CM incidence was not significantly associated with ever ASA use (adjusted OR: 0.92, 95% CI: 0.76–1.12) or ever non-ASA NSAID use (adjusted OR: 1.10, 95% CI: 0.97–1.24). However, continuous use of low-dose ASAs was associated with a significant reduction of CM risk in women (adjusted OR: 0.54, 95% CI: 0.30–0.99) but not in men (OR: 1.01, 95% CI: 0.69–1.47). A significant trend ($P=0.04$) from no use, non-continuous use to continuous use was observed in women. Continuous use of low-dose ASAs may be associated with a reduced incidence of CM in women, but not in men.

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INTRODUCTION

Cutaneous melanoma (CM) is a growing health problem, as CM incidence rates are steadily rising in both Europe (de Vries *et al.*, 2003) and the United States (Cancer Facts and Figures 2007, accessed on 5 January 2009, on http://www.cancer.org). However, mortality rates seem to have leveled off, probably because of increased awareness resulting in early detection of CM (de Vries and Coebergh, 2004). Although local CM is generally successfully treated with surgery, for metastatic disease therapeutic results remain disappointing (de Vries *et al.*, 2003; Eigentler *et al.*, 2003). Consequently, focus of the melanoma research has shifted from therapy to prevention and early detection.

Chemoprevention may complement current preventive measures and is defined as the use of natural or synthetic agents to prevent, reverse, suppress, or delay pre-malignant lesions from progressing into invasive cancer (Demierre, 2006). Non-steroidal anti-inflammatory drugs (NSAIDs) have shown promising results in various solid cancers (Harris *et al.*, 2005) and may have chemopreventive potential in CM (Francis *et al.*, 2006). *In vitro* studies in melanoma cell lines have shown that NSAIDs can induce apoptosis (Vogt *et al.*, 2001; Bundscherer *et al.*, 2008) and inhibit tumor growth and invasion (Denkert *et al.*, 2001; Chiu *et al.*, 2005; Bundscherer *et al.*, 2008).

The proposed anticancer mechanism of NSAIDs is inhibition of cyclooxygenase-2 (COX-2). This enzyme is inducible by inflammatory stimuli, is overexpressed in different neoplasms, and is probably linked to carcinogenesis through various mechanisms, for example, angiogenesis, apoptosis, inflammation, and immune function (Xu, 2002; Harris *et al.*, 2005). However, NSAIDs may inhibit cancer through various COX-independent pathways as well (Marx, 2001; Elwood *et al.*, 2009). This could be of particular importance in CM, as NSAIDs inhibit the growth of CM cell lines independent of COX-2 (Vogt *et al.*, 2001; Xu, 2002; Chiu *et al.*, 2005; Bundscherer *et al.*, 2008; Lee *et al.*, 2008), and COX-2 is not consistently expressed in CM (Denkert *et al.*, 2001; Vogt *et al.*, 2001; Goulet *et al.*, 2003; Nettelbeck *et al.*, 2003; Kuzbicki *et al.*, 2006; Lee *et al.*, 2008).

Thus far, most of the epidemiological studies assessing the chemoprophylactic effects of NSAIDs on CM incidence focus

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Abbreviations: ASA, acetylsalicylic acid; ATC, Anatomical Therapeutical Chemical Classification System; CI, confidence interval; CM, cutaneous melanoma; COX-2, cyclooxygenase-2; ICD, International Classification of Disease; NSAIDS, non-steroidal anti-inflammatory drugs; OR, odds ratio; RCT, randomized controlled trial

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on acetylsalicylic acids (ASAs). A randomized controlled trial (RCT) and a large cohort study did not find an association between low- or high-dose aspirin use and CM incidence (Cook *et al.*, 2005; Jacobs *et al.*, 2007). Studies investigating a possible association of CM and non-ASA NSAIDs are limited. Recently, a large cohort study did not observe an association with either ASA or non-ASA NSAIDs on CM incidence (Asgari *et al.*, 2008). However, two smaller epidemiological studies suggested a reduced risk on CM incidence and progression in NSAID users (Harris *et al.*, 2001; Ramirez *et al.*, 2005). Therefore, the potential chemoprophylactic properties of NSAIDs remain unclear due to heterogeneity in study design and conflicting results.

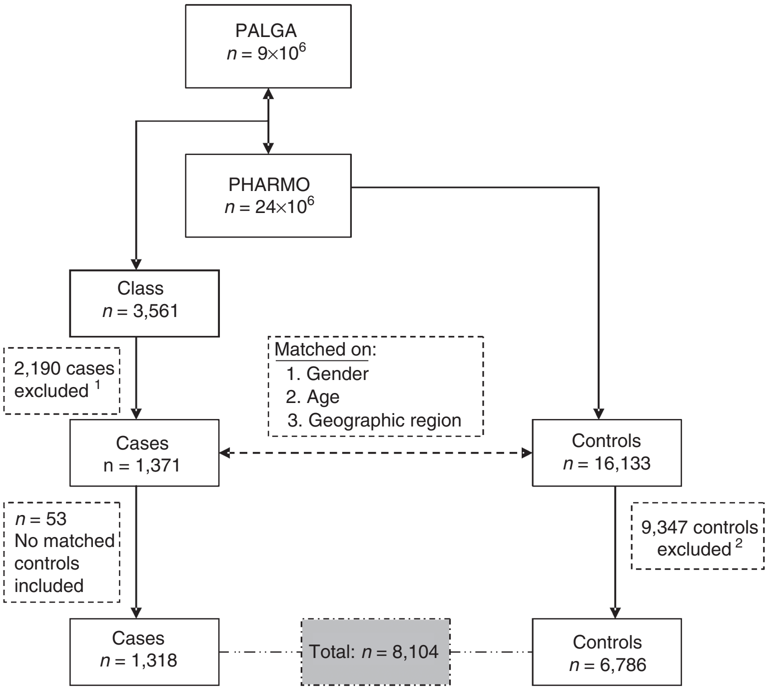
The objective of this study was to investigate a possible protective effect of ASA and non-ASA NSAIDs on CM incidence in a large population-based sample by linking the Dutch pathology registry with a pharmacy database.

RESULTS

Study population

The ascertainment of the cases and controls has been described previously (Koomen *et al.*, 2007). Briefly, of the 3,561 subjects who were registered in PHARMO (Institute for Drug Outcome Research) and had a systemized nomenclature of medicine (SNOMED) code 'melanoma' in PALGA (The Nationwide Network and Registry of Histo- and Cytopathology in The Netherlands), 1,318 (37.0%) cases met the eligibility criteria (Figure 1). Patients were mostly excluded because the registration periods in PHARMO and PALGA did not match, leading to incomplete pharmacy records in PHARMO in the 3-year observation period before CM diagnosis. Of the 16,133 controls matched for gender, age, and geographical region, 6,786 (42.1%) met the inclusion criteria.

About 60% of the study population was female, with a mean age of 55 years (Table 2). Compared with the controls, cases had a significantly higher number of unique non-melanoma International Classification of Disease (ICD) diagnoses (0.71 vs 0.61, $P = 0.04$), which was confirmed in men, but not in women. Also, cases had a higher number of unique medications prescribed (7.53 unique Anatomical Therapeutical Chemical Classification System (ATC) codes vs 6.93, $P < 0.01$), which was confirmed in both men and women. As reported earlier, women with melanoma used more estrogens compared with the control population (31.6 vs 24.8%, $P = < 0.001$) (Koomen *et al.*, 2009).



[1] Cases excluded	Total $n = 2{,}243$	100 (%)
1 = Entry date missing	$n = 78$	3.5
2 = Age at diagnosis <18 years	$n = 19$	0.8
3 = OP 3-year before CM incomplete	$n = 1408$	63
4 = CM not pathologically confirmed	$n = 343$	15
5 = Primary CM before 1991	$n = 93$	4.1
6 = No Cutaneous MM (e.g., eye)	$n = 205$	9.1
7 = *in situ* CM	$n = 44$	2.0
8 = No matched controls included	$n = 53$	2.4

[2] Controls excluded	Total $n = 9347$	100 (%)
1 = Entry date missing	$n = 640$	6.8
2 = Age at diagnosis <18 years	$n = 30$	0.3
3 = OP 3 years before index date incomplete	$n = 8673$	93
4 = possible CM patient (ICD code 'CM')	$n = 4$	0.04

Figure 1. Flow chart of study population. CM, cutaneous melanoma; OP, observation period; MM, malignant melanoma; ICD, International Classification of Disease.

ASA use and CM incidence

More than 40% of the total NSAID use consisted of ASA use (Table 1). The proportion of CM patients who used ASA was comparable with the controls, except for high-dose ASAs (Table 2). Female cases were significantly less likely to be a continuous user of low-dose ASAs than their matched controls (1.7 vs 3.1%, $P=0.03$). In men, no significant difference in the distribution of ASA exposure was observed. After adjusting for age, gender, year of diagnosis, prior use of statins and estrogens, and unique number of ICD and ATC codes in a multivariable model, none of the ASA exposure variables were significantly associated with CM incidence in the total study population and in men (Table 3). However, in women, continuous use of low-dose ASA for 3 years was associated with a reduced risk of developing a CM of almost 50% (adjusted OR: 0.54, 95% CI: 0.30–0.99). In addition, in women, there was a significant dose–response trend for no use, non-continuous use, and continuous use (*P*-value for trend = 0.04).

Table 1. ATC codes and corresponding NSAID[1]

ASAs	ATC code	% of total[2]
Acetylsalicylic acid	B01AC06/N02BA01	22.5
Carbasalate calcium	B01AC08/N02BA15	19.1
ASA use		41.6
Non-ASA NSAIDs	**ATC code**	**% of total[2]**
Diclofenac	M01AB05	20.5
Ibuprofen	M01AE01	14.5
Naproxen	M01AE02	10.0
Rofecoxib[3]	M01AH02	3.0
Diclofenac, combinations	M01AB55	2.5
Indometacin	M01AB01	2.3
Meloxicam	M01AC06	1.6
Piroxicam	M01AC01	1.2
Nabumetone	M01AX01	1.0
Ketoprofen	M01AE03	0.4
Celecoxib	M01AH01	0.3
Sulindac	M01AB02	0.3
Tiaprofenic acid	M01AE11	0.2
Aceclofenac	M01AB16	0.1
Etoricoxib	M01AH05	0.1
Flurbiprofen	M01AE09	0.1
Tenoxicam	M01AC02	0.1
Dexibuprofen	M01AE14	<0.1
Dexketoprofen	M01AE17	<0.1
Diflunisal	N02BA11	<0.1
Tolfenamic acid	M01AG02	<0.1
Metamizole sodium	N02BB02	<0.1
Non-ASA NSAID use		58.4

ASAs, acetylsalicylic acids; ATC, anatomical therapeutic chemical classification system; NSAIDs, non-steroidal anti-inflammatory drugs.

[1] All available NSAID ATC codes were included in the study. Presented are ATC codes corresponding with 1 or more prescription among cases and controls.

[2] Percentage (%) of the total 22,279 prescriptions among cases and controls.

[3] Withdrawn from the Dutch market in 2004.

Non-ASA NSAID use and CM incidence

The most commonly dispensed non-ASA NSAIDs were diclofenac (20.5%), ibuprofen (14.5%) and naproxen (10.0%) (Table 1). Female and, to a lesser extent, male CM patients were more likely to have ever used non-ASA NSAIDs compared with controls (Table 2). Of the non-ASA NSAID users, the overwhelming majority used <600 pills during 3 years and only 2.3 and 2.9% of cases and controls, respectively, used more than 600 pills. In the distribution of the categories of the cumulative number of pills, the only significant difference was observed in the lowest category of 1–600 pills for the total study population and for women.

In the multivariable models that adjusted for multiple confounders, no significant associations were found, although relatively low non-ASA NSAID exposure (1–600 pills) was borderline significantly associated with a modest increase in CM risk (OR: 1.12, 95% CI: 0.98–1.23, Table 3). In further subgroup analysis, the use of 1–4 prescriptions of non-ASA NSAIDs in 3 years was significantly associated with a marginally increased risk of CM (OR: 1.15, 95% CI: 1.01–1.30, data not shown). Higher levels of exposure appeared to be protective for all subgroups, but none of these associations were significant (Table 3).

DISCUSSION

NSAID use and risk of CM

Continuous use of low-dose ASAs during 3 years was associated with a reduced likelihood of developing CM in women but not in men.

In contrast, none of the non-ASA NSAID variables were significantly associated with the risk of having a CM in the multivariable model (Table 3). However, infrequent use of pills (1–600 pills in 3 years) was significantly associated with the incidence of CM in an univariate analysis (Table 2), but this was not significant in the multivariable model after adjusting for health-care consumption (Table 3), suggesting that this and the possible other confounders affected the univiarate model. Interestingly, a similar association has been reported in a case–control study in prostate cancer (Perron *et al.*, 2003). This illustrates that use of health care may be an important confounder in pharmaco-epidemiological studies.

The use of larger quantities of non-ASA NSAIDs (>600 pills in 3 years) seemed to be protective for CMs but did not reach significance, which could be explained, in part, by a relatively short time of observation (3 years), limited sample size in this subgroup (<225 patients), and/or that non-ASA NSAIDs were administered as analgetics (the prescribed frequency of the use by physicians was most often 'when

Table 2. Study group characteristics

	Total group			Males			Females		
	Cases (*n*=1,318)	**Controls (*n*=6,786)**	***P*-value**	**Cases (*n*=540)**	**Controls (*n*=2,714)**	***P*-value**	**Cases (*n*=778)**	**Controls (*n*=4,072)**	***P*-value**
Gender[1]									
Male	540 (41.0%)	2,714 (40.0%)							
Female	778 (59.0%)	4,072 (60.0%)	0.51						
Age at index date[2]									
Years	55.3 (±15.9)	55.9 (±15.5)	0.18	57.7 (±14.6)	58.0 (±14.2)	0.72	53.6 (±16.5)	54.6 (±16.1)	0.13
Total unique diagnoses[2]									
N	0.71 (±1.52)	0.61 (±1.55)	0.04	0.84 (±1.8)	0.66 (±1.6)	0.02[3]	0.62 (±1.3)	0.59 (±1.5)	0.55
Total unique medications prescribed[2]									
N	7.53 (±6.49)	6.93 (±6.78)	<0.01	6.95 (±6.9)	6.24 (±6.3)	0.03[3]	7.93 (±6.2)	7.39 (±7.0)	0.03[3]
Estrogen use[1]									
Ever use	246 (18.7%)	1,009 (14.9%)		—	—		246 (31.6%)	1,009 (24.8%)	
Never use	1,072 (81.3%)	5,777 (85.1%)	<0.01	—	—		532 (68.4%)	3,063 (75.2%)	<0.01
Statin use[1]									
Ever use	115 (8.7%)	574 (8.5%)		75 (13.9%)	309 (11.4%)		40 (5.1%)	265 (6.5%)	
Never use	1,203 (91.3%)	6,212 (91.5%)	0.75	465 (86.1%)	2,405 (88.6%)	0.10	738 (94.9%)	3,807 (93.5%)	0.15
ASA use									
Never use	1,137 (86.3%)	5,853 (86.3%)		435 (80.6%)	2,219 (81.8%)		702 (90.2%)	3,634 (89.2%)	
Ever use	181 (13.7%)	933 (13.7%)	0.99	105 (19.4%)	495 (18.2%)	0.51	76 (9.8%)	438 (10.8%)	0.41
Type of ASA use									
Never use	1,137 (86.3%)	5,853 (86.3%)		435 (80.6%)	2,219 (81.8%)		702 (90.2%)	3,634 (89.2%)	
Low-dose, non-continuous use	76 (5.8%)	455 (6.7%)	0.24	42 (7.8%)	239 (8.8%)	0.53	34 (4.4%)	216 (5.3%)	0.28
Low-dose, continuous use	61 (4.6%)	329 (4.8%)	0.75	48 (8.9%)	204 (7.5%)	0.28	13 (1.7%)	125 (3.1%)	0.03
High-dose use	44 (3.3%)	149 (2.2%)	0.02	15 (2.8%)	52 (1.9%)	0.19	29 (3.7%)	97 (2.4%)	0.04
Non-ASA NSAIDs use									
Never use	700 (53.1%)	3,862 (56.9%)		304 (56.3%)	1,598 (58.9%)	0.27	396 (50.9%)	2,264 (55.6%)	
Ever use	618 (46.9%)	2,924 (43.1%)	0.01	236 (43.7%)	1,116 (41.1%)		382 (49.1%)	1,808 (44.4%)	0.02
Cumulative no. of pills									
No use	700 (53.1%)	3,862 (56.9%)		304 (56.3%)	1,598 (58.9%)		396 (50.9%)	2,264 (55.6%)	
1–600	588 (44.6%)	2,728 (40.2%)	<0.01	226 (41.9%)	1,051 (38.7%)	0.20	362 (46.5%)	1,677 (41.2%)	<0.01
601–1,000	12 (0.9%)	92 (1.4%)	0.29	3 (0.6%)	31 (1.1%)	0.26	9 (1.2%)	61 (1.5%)	0.63
>1,000	18 (1.4%)	104 (1.5%)	0.86	7 (1.4%)	34 (1.3%)	0.85	11 (1.4%)	70 (1.7%)	0.74

ASA, acetylsalicylic acids; NSAIDs, non-steroidal anti-inflammatory drugs.

[1] Number of cases and controls presented, ±SD? tested for statistical difference with χ^2-test.

[2] Mean value presented, tested for statistical difference with *t*-test.

[3] Equal variances not assumed according to Levene's test for equality of variances.

Table 3. ASA/NSAID use and cutaneous melanoma

	Total			Males			Females		
Variable	***n***	**Adjusted OR[1]**	**95% CI**	***n***	**Adjusted OR[1]**	**95% CI**	***n***	**Adjusted OR[1]**	**95% CI**
ASA use									
Overall exposure									
Never ASA use	6,990	1.00	Referent	2,654	1.00	Referent	4,336	1.00	Referent
ASA use	1,114	0.92	0.76–1.12	600	0.92	0.69–1.21	514	0.90	0.68–1.19
Use of ASA									
Never Use	6,990	1.00	Referent	2,654	1.00	Referent	4,336	1.00	Referent
Low dose <3 years[2]	531	0.77	0.58–1.01	281	0.72	0.49–1.06	250	0.82	0.55–1.22
Low dose 3 years[2]	390	0.87	0.64–1.18	252	1.01	0.69–1.47	138	**0.54**	**0.30–0.99**
High dose (ever)[3]	193	1.35	0.96–1.92	67	1.34	0.74–2.43	126	1.37	0.89–2.11
Non-ASA NSAIDs									
Overall exposure									
Never NSAID use	4,562	1.00	Referent	1,902	1.00	Referent	2,660	1.00	Referent
NSAID use	3,542	1.10	0.97–1.24	1,352	1.04	0.86–1.26	2,190	1.13	0.96–1.34
Cumulative pills									
0	4,562	1.00	Referent	1,902	1.00	Referent	2,660	1.00	Referent
1–600	3,316	1.12	0.98–1.23	1,277	1.06	0.87–1.27	2,039	1.15	0.98–1.36
601–1,000	104	0.67	0.36–1.23	34	0.46	0.14–1.51	70	0.82	0.40–1.69
>1,000	122	0.89	0.53–1.43	41	0.96	0.42–2.21	81	0.88	0.46–1.69

ASA, acetylsalicylic acids; CI, confidence interval; NSAIDs, non-steroidal anti-inflammatory drugs; OR, odd ratio.

[1] Adjusted for age, sex (only in total group), year of diagnosis, the use of statins resp. estrogens (only in females), the total of different medical diagnoses, total of different medications prescribed, and the interaction term between the latter two.

[2] Use of 30–100 mg acetylsalicylic acid per unit (⩾990 pills is considered 3 years–continuous use).

[3] Use of >100 mg acetylsalicylic acid per unit.

Bold values are statistically significant.

needed'); thus implying non-continuous exposure. On account of small numbers, separate analyses for selective COX-2 inhibitors could not be carried out.

The observed difference in chemoprophylactic effects between non-ASA NSAIDs and ASAs may be dependent on the patterns of use or on a different mechanism of action. First, low-dose ASAs are most commonly prescribed as daily cardiovascular preventive drugs, whereas non-ASA NSAIDs and high-dose ASAs are commonly used irregularly as analgetics. Second, ASAs may have additional anti-cancer effects in comparison with non-ASAs, such as inhibition of thrombocyte aggregation (Rickles and Falanga, 2001), or effects of cancer-related systems such as apoptosis, NF-êB, DNA repair systems, oxidative stress, or mitochondrial calcium uptake (Elwood *et al.*, 2009).

We did not find a reduced CM incidence among overall non-ASA NSAID or ASA users, which is in accordance with three large observational studies. A large cohort study of regular and high-dose ASA (>325 mg) exposure observed no protective effect on CM (Jacobs *et al.*, 2007). A second cohort confirmed the absence of an association between ASA or non-ASA NSAID use and CM incidence (Asgari *et al.*, 2008). This study, however, has several limitations, that is, low-dose aspirin exposure was excluded in subgroup analyses, ∼40% of cases were CM *in situ*, and stratification across gender was not carried out. Our results, showing an association of low-dose ASA use in women with CM is in contrast with an RCT among females for whom low-dose aspirin use (100 mg every other day) for an average of 10 years did not affect CM incidence (68 vs 70 incident cases, $P=0.87$) (Cook *et al.*, 2005). This study however was limited by a small number of CM cases, non-continuous exposure, and was not population-based.

In other malignancies, multiple studies investigating the chemopreventive properties of ASA and non-ASA NSAIDs have been published. A review showed that in colorectal, breast, and lung cancer, the risk reductions by non-ASA NSAIDs and ASAs were comparable (Harris *et al.*, 2005), which contradicts our results that suggest a different effect. Results of a case–control study on prostate cancer, however, were comparable: prolonged use of ASAs showed a protective effect, whereas use of non-ASA NSAIDs did not (Perron *et al.*, 2003). In lung (Harris *et al.*, 2002), breast (Harris *et al.*, 2006), and prostate (Perron *et al.*, 2003) cancer, exposure to regular or high-dose use of ASAs did, but exposure to low-dose ASA did not, decrease the incidence of these cancers, which is not in line with our findings in CM patients.

However, comparing the results of studies assessing the chemoprophylactic effect of NSAIDs is challenging because studies differ in several important ways such as ascertainment of drug exposure (for example, self-reported or pharmacy database), definition of exposure, type of NSAID (ASA or non-ASA), dose, duration, patterns of use (for example, sporadic, intermittent, chronic), drug adherence, study population (for example, general population, cohorts from tertiary centers), melanoma (for example, invasive or *in situ* CM), sample size, and subgroup analyses (that is, stratification across gender). A pivotal unresolved problem is the definition of the dosage of NSAID, which could have chemoprophylactic effects.

Gender differences

Stratification across gender showed a gender difference in favor of women, especially for continuous use of low-dose ASAs. This apparent discrepancy between men and women is not well understood and may be explained by pharmacological and melanoma differences. Pharmacodynamics and pharmacokinetics of ASA differ between men and women: the effect on platelets that differs across gender and women achieve higher concentrations with equal doses being administrated (Levin, 2005). As ASA may influence oxidative stress, the gender difference in antioxidant enzymes may have a role (May, 2007). Remarkably, a recent RCT investigating antioxidant supplementation showed an increase in the incidence of CM in women, but not in men (Hercberg *et al*., 2007). Another explanation may be that biology of melanoma itself may not be comparable in men and women, as CM survival differs significantly across gender when adjusted for other prognostic factors (de Vries *et al*., 2008; Lasithiotakis *et al*., 2008). Differences in adherence to cardiovascular drugs, however, are not likely to explain the observed gender differences (Kulkarni *et al*., 2006).

Interestingly, we previously reported a gender difference in the effects of statins with regard to CM incidence and progression using the same study population (Koomen *et al*., 2007). Future (epidemiological) studies are warranted to explore CM gender differences**.**

Strengths and weaknesses

This is the largest population-based study that investigates the effect of NSAID use on CM incidence in more than 1,350 cases. The CM cases were confirmed by a pathology report, and drug exposure was prospectively assessed by a highly reliable pharmacy database. (Herings R (1993). PHARMO: a record linkage system for postmarketing surveillance of prescription drugs in the Netherlands. Thesis in pharmacoepidemiology and pharmacotherapy. Utrecht University). In PHARMO, detailed information on drug use was available, such as the number of dispenses, the number of dispensed pills, and dosage. As the dosages (in World Health Organization (WHO)'s Defined Daily Doses) of NSAIDs vary largely between the indications for which they are prescribed, we were not able to include this information. Furthermore, as a large proportion of the NSAIDs are used as analgetics 'on demand,' no data were available regarding the duration of use for these types of NSAIDs. Therefore, duration of use could not be included in the analyses, except based on the number of pills prescribed. As several NSAIDs are available over the counter without a prescription, the actual use of NSAIDs is underestimated. Therefore, if this would influence our results, it is most likely that this would produce bias toward the null. However, this misclassification is likely to be equal among cases and controls; hence, bias is likely to be minimal. In this study, NSAID use was ascertained in the 3 years before CM diagnosis, which may have been too short to detect the effect of NSAID exposure (Harris *et al*., 2005). However, increasing the observation period to 5 years decreased the sample size substantially (from 1,318 to 931 CM cases). Although a proxy for health-care consumption was included in the multivariable model, surveillance bias may still have affected our results. Information on lifestyle factors such as sun exposure was not available, but the confounding effect of sun exposure on NSAID use seems to be limited.

In conclusion, long-term use of (low-dose) ASA was associated with a reduced risk of CM in women, but not in men. Future observational and ultimately interventional clinical studies are needed to confirm these findings.

MATERIALS AND METHODS

Setting

This study was designed as a case–control study, using population-based data from two Dutch databases. PHARMO is a network of linked databases including a pharmacy database containing more than two million Dutch residents, representing 12% of the total Dutch population. The residents were included regardless of the type of insurance (http://www.pharmo.nl/, PHARMO RLS Network, accessed on 5 January 2009). An individual enters the PHARMO database when obtaining the first prescription in a PHARMO pharmacy, and is observed until the last prescription. As most patients in The Netherlands visit a single pharmacy, drug dispensing records are virtually complete (Lau *et al*., 1997). The prospectively gathered computerized drug dispensing records contain the date of dispense, type, quantity, dosage form, strength, and daily dose of the prescribed drug.

PHARMO was linked to PALGA, the Dutch registry of histopathology and cytopathology, using a variation of a reliable probabilistic algorithm. (Herings R (1993). PHARMO: a record linkage system for postmarketing surveillance of prescription drugs in the Netherlands. Thesis in pharmaco-epidemiology and pharmacotherapy. Utrecht University) PALGA contains abstracts of all Dutch pathology reports encrypted with patient identification and diagnostic terms in scope with the SNOMED classification, and reached 100% participation from 1990 onward, and therefore is the basis of the Dutch Cancer Registry (Casparie *et al*., 2007).

The protocol of this study was approved by the scientific and privacy committees of both PALGA and PHARMO, and was granted exempt status by the ethics board of the Leiden University Medical Centre.

Study population

Cases were defined as individuals with a CM diagnosis in PALGA between 1 January 1991 and 14 December 2004 and who were also registered in PHARMO in the same period. The end point of the observation period was defined as the date of CM diagnosis (index date). For each case, two investigators (AJ, ERK) extracted final diagnosis, date, and Breslow's depth from the PALGA pathology reports with high accordance ($\kappa > 0.85$) (Koomen *et al.*, 2007). Cases were excluded if, in PALGA, the date of primary CM diagnosis was before the age of 18 years or before 1 January 1991, the primary melanoma was not pathologically confirmed, was *in situ*, or was non-cutaneous, or in PHARMO, the date of entry was unknown, gender was unknown, or time of observation before CM diagnosis was <3 years (Figure 1).

For every eligible case, an average of five controls matched for gender, date of birth (±2 years), and geographic region (∼100 regions based on clusters of local pharmacies) were sampled from PHARMO. To calculate the time of observation for the controls, they were assigned the index date of the matched case to be able to determine the 3-year observation period. Controls were excluded if, in PHARMO, the date of entry was unknown, they were younger than 18 years at the index date, the time of observation before index date was <3 years, or a diagnosis of melanoma was recorded according to the ICD9-CM in the hospital linkage database of PHARMO (Figure 1).

Drug exposure

For all cases and controls, systemic NSAID use, restricted to the 3-year observation period before the index date, was extracted from the PHARMO database using the ATC codes of the WHO. All NSAIDs, including ASAs, available in The Netherlands were included (Table 1). Drug dispenses containing <7 pills were excluded (for example, after a dental extraction), but weekly prescribed NSAIDs were included (for example, weekly pharmacy deliveries to nursery homes).

ASAs were investigated separately from non-ASA NSAIDs because, next to COX-2 inhibition, they inhibit thrombocyte aggregation, which has been linked to carcinogenesis (Rickles and Falanga, 2001). Furthermore, ASAs are almost exclusively prescribed for long-term continuous use and not for intermittent use as an analgetic, in contrast with non-ASA NSAIDs.

ASA use

Among all users, ASA use was categorized by prescribed dosage. Individuals who used low-dose ASA (30–100 mg daily) were categorized into continuous (that is, use of ⩾990 U of ASA during the observation period of 3 years or 1,095 days) and non-continuous users. Higher doses of ASA (⩾100 mg) were dispensed far less frequently and were mostly prescribed for on-demand use, suggesting temporary use as an analgetic. It was not possible to extract continuous users from this group of high-dose ASA users because of the low cumulative quantities of pills used during the observation period. Therefore, all users of high-dose ASA were analyzed separately.

Non-ASA NSAID use

None-ASA NSAIDs such as, ibuprofen and diclofenac, were prescribed irregularly, with a wide variation of daily prescribed doses, and to be used on demand. Therefore, assumptions for continuous or non-continuous use could not be made, and categorization was limited to the number of pills prescribed. For the categories of cumulative number of pills, the cutoff values were chosen to reflect levels of exposure: non-users, individuals who were likely to be exposed for <2/3 years of the observation period of 3 years (1–600 pills during 1,095 days), individuals using on average more than one pill daily in 3 years (>1,000 pills) and an intermediate group.

Potential confounders

Ever use of drugs related to progression and the development of CM, such as statins (Koomen *et al.*, 2007) and estrogens (Koomen *et al.*, 2009), were considered possible confounders. The use of heparins, fibrates, and other lipid-lowering drugs was also recorded. However, the number of individuals using these drugs was too small (<1.0%) to be used in further analysis. To adjust for a possible surveillance bias (that is, patients who seek medical care are more likely to be diagnosed with other disease including CM), a proxy of health-care and pharmacy-seeking behavior was created by calculating the total number of unique ATC codes (excluding all NSAIDs) and the total number of unique ICD9-CM codes (that were primary discharge diagnosis after hospitalization), which were both recorded in the database in the 3 years before the index date. The ICD code for melanoma found for each case was not included in the total number of unique ICD codes to avoid overmatching. Both confounders proved to be significant in all multivariable analyses carried out and also showed a significant interaction with each other. This interaction term was added in the multivariable analysis ($P < 0.01$).

Statistical analysis

A χ^2-test was used to test for statistical differences between categorical variables, for continuous variables a Student's *t*-test or a Mann–Whitney *U*-test was used as appropriate. A multivariable logistic regression model was used to calculate adjusted ORs and 95% CIs to analyze the association between dependent CM incidence and NSAID use and its defined categorizations of exposure.

As CM development, progression, and survival, as the effect of potential chemoprophylactic drugs, may differ across gender (Levin, 2005; Koomen *et al.*, 2007; de Vries *et al.*, 2008; Lasithiotakis *et al.*, 2008), a pre-specified separate analysis for men, women, and for the total group was carried out.

All statistical tests were two-sided, with a rejection of the null hypothesis at $P < 0.05$. All statistical analyses were carried out using SPSS 14.0 (0.2) (SPSS, Chicago, IL).

CONFLICT OF INTEREST

RMCH is a director of PHARMO, a research institute that performs financially supported studies for several pharmaceutical companies. However, for this study, no research funding from pharmaceutical companies was received. The remaining authors state no conflict of interest.

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