Drug effects on melanoma

Koomen, E.R.

Chapter 8

Estrogens, oral contraceptives and hormonal replacement therapy, increase the incidence of cutaneous melanoma: a population-based case control study

8

Els R. Koomen, Arjen Joosse, Ron M.C. Herings, Mariël K. Casparie, Henk-Jan Guchelaar, Tamar Nijsten

Annals of Oncology 2009, 20(2), 358-364

Abstract

Background: Multiple studies showed conflicting results on the association between oral contraceptive use and the development of cutaneous melanoma (CM). We investigated the association between estrogen use and CM incidence.

Patients and methods: Data from PHARMO Pharmacy database and PALGA, the pathology database in the Netherlands, were linked. Women, ≥ 18 years, with a pathology report of a primary CM between January 1st 1991 and December 14th 2004 and ≥ 3 years of follow-up prior to CM diagnosis were eligible cases. Controls were matched for age and geographic region. Multivariate logistic regression was used to calculate adjusted odds ratio (OR) and 95% confidence interval (CI) for the association between CM incidence and estrogen use, oral contraceptives (OC) and hormonal replacement therapy (HRT), separately.

Results: In total, 778 cases and 4072 controls were included. CM risk was significantly associated with estrogen use (≥ 0.5 year; adjusted OR=1.42, 95% CI: 1.19-1.69). This effect was cumulative dose-dependent (p-trend < 0.001). CM risk was also significantly associated with the use of HRT (≥ 0.5 year: OR=2.08; 95% CI: 1.37-3.14) and OC (≥ 0.5 year: OR=1.28; 95% CI: 1.06-1.54).

Conclusion: Our study suggests a cumulative dose-dependent increased risk of CM with the use of estrogens.

Introduction

The influence of estrogens on the incidence of cutaneous melanoma (CM) in women has been supported by a number of observations. Firstly, indicating the effect of estrogens on melanocyte proliferation, hyperpigmentation is a side-effect of oral contraceptive (OC) use and may also occur during pregnancy (chloasma) or with the use of hormonal replacement therapy (HRT). [1] Secondly, until the age of ~45 years, CM incidence rates exceed those in men, after which the incidence rates in men rise markedly, but level off in women. Since the incidence rates of CM in women mimic those of breast cancer, female sex steroids have been hypothesized to be involved in the development of CM in women. [2] Additionally, recent studies have demonstrated improved survival among women compared to men with CM after adjusting for demographic and tumor characteristics. [3, 4] One of the possible explanations may be influence of estrogens because it has been suggested that estrogens are associated with melanomas with a relative good prognosis such as superficial spreading melanomas. [4] In a previous study we observed that ever use of estrogens was associated with an increased incidence of CM. [5] Moreover, women with a history of breast cancer have been reported to be at higher risk of CM and vice versa. [6] Also, estrogen-binding receptors have been detected in melanomas and benign *nevi* [7].

On the basis of these observations, several epidemiological studies have investigated the association between OC use and CM development. These studies show, however, inconsistent results. About 25-30 years ago, a higher CM incidence was suggested among women using OC compared to women who never used OC in three cohort studies. [8-10] Nevertheless, subsequent (case control) studies failed to confirm a significant effect of OC on the incidence of CM. [11-15] However, a few large studies with long-term follow-up and a relatively high proportion of women having used OC for a long period of time did show a significant two- to four-fold increased likelihood to develop CM. [16-18] In a pooled analysis of 10 case-control studies Karagas *et al.* [2] observed no excess CM risk associated with OC use for 1 year or longer compared to non-users. However, these studies are limited in sample size and included selected study populations. Prior estrogen use was assessed by interviews or questionnaires, which may result in a recall bias. Moreover, no differentiation was made between OC and HRT. In this case control study, we linked a population-based pharmacy database with the national pathology database to assess the association between the incidence of CM and estrogen, OC and HRT use, separately.

Patients and methods

Setting

Data were used from the PHARMO database, containing drug-dispensing records of a defined population of more than 2 million Dutch residents representing >12% of the Dutch population. Residents are included regardless of type of health insurance or other relevant factors. [19]

The core file of the PHARMO system is a patient-roster file which includes of all patients an entry- and exitdate. To this roster file, the drug dispensing records of all pharmacies, and pathology records are linked on a patient-centric level. Since most individuals designate a single pharmacy in The Netherlands, dispensing histories are virtually complete. [20] The computerized drug-dispensing histories contain all dispensed prescriptions and include type, quantity, dosage form, strength, dispensing date and prescribed daily dose of the dispensed drug. PHARMO was linked to PALGA, the Dutch nationwide registry of histo- and cytopathology, using a variation of a reliable probabilistic algorithm. [21] PALGA contains abstracts of all pathology reports with encrypted patient identification and diagnostic terms being in scope with SNOMED classification. Since 1990 the registration reached 100% participation and in 2004 over 9 million patients were archived. [22] Therefore, PALGA represents all Dutch patients and is the basis for the Netherlands Cancer Registry.

We reported our study according to the STROBE guidelines. [23]

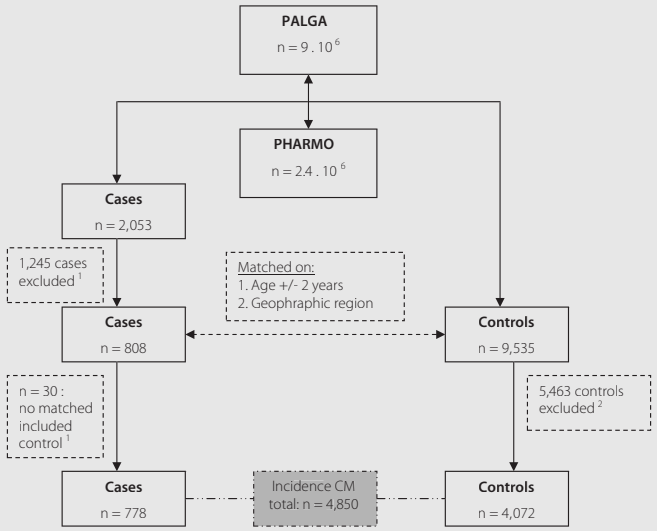
Study population

Cases had a primary CM diagnosis in PALGA from 1 January 1991 to 14 December 2004 and were also followed by PHARMO at any point in this period. End of follow-up was defined as the date of CM diagnosis (index date).

For each case all records in PALGA were read by one of two investigators (AJ, ERK). From these records, ERK and AJ extracted and recorded final diagnosis, date, anatomical location and CM subtype according to WHO classification [24] of the primary CM. To assess interobserver variation, 300 cases were randomly selected and scored by both researchers.

Potential cases were excluded if, in PHARMO, a date of entry was unknown, gender was unknown, follow-up in the 3 years before CM diagnosis was incomplete, or, in PALGA, the date of CM diagnosis was before the age of 18 or before January 1 1991, the CM was not pathologically confirmed, the primary CM was not on the skin (e.g. in the eye) or if the CM was *in situ* (Fig. 1).

Figure 1 Flow chart study population †



[1] Cases excluded	Total	n = 1,275	100 %
1 = Entrydate missing		n=34	2.7 %
2 = Age at diagnosis < 18 yrs		n=15	1.2 %
3 = Follow up 3 yr before CM incomplete		n=810	64 %
4 = CM not pathologically confirmed		n=195	15 %
5 = Primary CM before 1991		n=59	4.6 %
6 = no cutaneous MM (i.e. eye)		n=102	8.0 %
8 = in situ CM		n=30	2.4 %
9 = no matched controls included		n=30	2.4 %

[2] Controls excluded	Total	n= 5,463	100 %
1 = Entrydate missing		n= 373	6.8 %
2 = Age at diagnosis < 18 yrs		n= 9	0.2 %
3 = Follow up 3 yr before CM incomplete		n= 5,079	93 %
4 = possible CM patient (ICD code 'CM')		n= 2	0.04 %

† All numbers presented represent the number of patients (cases or controls) involved.

For every eligible case, an average of five controls was sampled from the population available in PHARMO, matched for date of birth (± 2 years) and geographic region (individual matching). Potential cases could not be selected as controls. To calculate follow-up, controls were assigned the index date of the matched case.

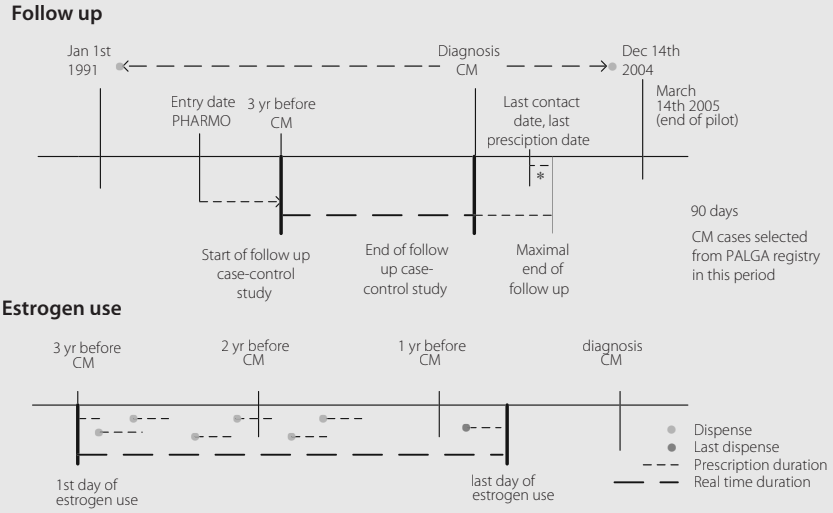
Potential controls were excluded if, in PHARMO, a date of entry was unknown, they were younger than 18 years at the index date, follow-up in the 3 years before index date was incomplete, or if they were diagnosed in PHARMO with previous CM according to the International Classification of Disease (Fig. 1).

Drug Exposure

Estrogen exposure was expressed in defined daily doses (DDDs) according to the WHO definitions. It was defined as the use of one or more estrogens containing formulations for at least 6 months of cumulative prescription duration in the 3 years before CM because a minimal exposure was assumed to be required for the hypothesized biologic mechanism (Fig. 2). All estrogens commercially available and approved in The Netherlands in the study period were included. Estrogen use was differentiated between OC (ATC codes: G03AAXX and G03ABXX) and HRT (ATC code: G03CAXX). For OC, daily use for 21 days and subsequently a 7 days period of non-use was assumed and therefore we divided the prescribed daily dose by 0.75 (= 21 of 28). Some OC formulations are used for 22 days with a 6-day drug-free period. However, this applied to only 6.2% of the prescriptions and the error is < 5%. Therefore, we did not correct for this.

For locally applied HRT, i.e. vaginal therapy, only dispenses with a prescribed dose corresponding with a minimal systemic exposure of 0.25 DDD/day orally were included. To further detail estrogen use, the cumulative dispensed dose and the cumulative prescribed duration were calculated (Fig 2).

Figure 2



Potential confounders

Ever use of drugs possibly related to progression and development of CM such as non-steroidal anti-inflammatory drugs (NSAIDs including COX-2-inhibitors) and statins was assessed [25]. Use of fibrates and lipid-lowering drugs other than fibrates or statins [25] was recorded, but the number of cases and controls using these drugs were too small (<1.0 %) to be used in further analysis.

To estimate health care consumption, which may affect the likelihood of CM diagnosis, the total number of unique prescriptions (ie, number of different ATC codes excluding estrogens) recorded in PHARMO in the 3 years before CM was included.

Statistical analysis

To test for statistical differences, χ^2 and Student's *t*-tests were used for categorical and continuous variables respectively. All statistical tests were performed two-sided with rejection of the null hypothesis at a *p*-value <0.05.

A multivariate logistic regression model was used to calculate adjusted odds ratio (OR) and 95% confidence interval (CI) for the association between CM incidence and estrogen use. In the multivariate model we included confounders with a *p*-value <0.10 in univariate analysis. The different estrogen variables were categorized based on tertiles among all users. We categorized HRT variables across the median because of the relative small numbers.

All statistical analyses were performed using SPSS 14.0 (.2) (SPSS Inc., Chicago, IL).

Results

Study population

In total, 2053 female subjects who were registered in PHARMO had a SNOMED code 'CM' in PALGA, of which 778 (37.9%) met the inclusion criteria (Fig. 1). Most of the potential cases were excluded because the time periods they were registered in PALGA and PHARMO did not match, or the follow-up in PHARMO in the 3 years before CM diagnosis was incomplete. The accordance in extracting the relevant information from the pathology records between the two authors was high (Kappa values >0.85). Of the 9535 controls matched on age (± 2 years) and geographical region, 4072 (42.7%) were eligible to enter the study.

Mean age of cases and controls was 53.6 and 54.6 years (p >0.05; Table 1). The number of unique prescriptions excluding estrogens was borderline significant with cases having more prescriptions than controls (8.25 versus 7.74, $p = 0.047$).

Table 1 Prior estrogen use (hormonal replacement and contraceptives) and characteristics of the study population

		Cases (778)		Controls (4072)		p-value	Adjusted OR [a]	95% CI
		n	%	n	%			
Age at diagnosis [b]	yrs	53.6 ±	16.5	54.6 ±	16.1	0.13	-	-
Total unique diagnoses [b]	number	0.62 ±	1.33	0.59 ±	1.50	0.55	-	-
Total unique prescriptions [c]	number	8.25 ±	7.37	7.74 ±	6.48	0.05	-	-
NSAIDs [d]	yes	388	49.9	1817	44.6		-	-
	no	390	50.1	2255	55.4	<0.01	-	-
Statins [d]	yes	40	5.1	265	6.5		-	-
	no	738	94.9	3807	93.5	0.15	-	-
Estrogen use [e]	non-exposed	577	74.2	3270	80.3		1.00	referent
	exposure >0.5 yr	201	25.8	802	19.7	<0.001	1.42	1.19 - 1.70
Cumulative prescription duration [d,f]	non-exposed	577	74.2	3270	80.3	[g]	1.00	referent
	1-700 days	75	9.6	279	6.9	<0.01	1.51 [h]	1.15 – 1.98
	701-1100 days	63	8.1	301	7.4	0.24	1.19 [h]	0.89 - 1.58
	>1100 days	63	8.1	222	5.5	0.001	1.61 [h]	1.20 - 2.16
Cumulative dose	0 DDD	577	74.2	3270	80.3	[g]	1.00	referent
	1-650 DDD	63	8.1	271	6.7	0.06	1.31 [h]	0.98 - 1.75
	651-1000 DDD	68	8.7	270	6.6	0.01	1.44 [h]	1.08 - 1.90
	> 1000 DDD	70	9.0	261	6.4	<0.01	1.51 [h]	1.14 – 1.99

[a] Adjusted for the total number of unique prescriptions dispensed (excluding estrogens) and the use of NSAIDs.
[b] Mean value presented ± standard deviation, tested for statistical difference with t-test, equal variances assumed.
[c] Mean value presented ± standard deviation, tested for statistical difference with t-test, equal variances not assumed.
[d] Number of cases and controls presented, tested for statistical difference with χ^2-test.
[e] Estrogen use: hormonal replacement therapy (HRT) and oral contraceptives (OC).
[f] Time interval between estimated last day of use (based on last dispense and amount dispensed) and date of diagnosis of CM. The cumulative prescription duration may exceed maximum number of days of follow up (3 years) due to overlapping dispenses.
[g] p-value for trend analysis: <0.001
[h] p-values calculated for each category of estrogen-users compared with non-users.

NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; CI, confidence interval; DDD, defined daily dose.

Estrogen use

Estrogens were used for more than half a year in the study period by 25.8% of the cases and 19.7% of the controls (p <0.001; Table 1). Of estrogens used, 83.4% was ethinyloestradiol, 9.1% was oestradiol, 4.8% were conjugated estrogen, 2.0% was oestriol and 0.6% was mestranol.

Cases and controls did not differ in the average day dose of estrogens. Cases were prescribed an average estrogen day dose of 0.73 DDD/day [standard deviation (SD) 0.19] and controls were prescribed 0.75 DDD/day [SD 0.16].

In univariate analysis, estrogen use (≥ 0.5 year), ever NSAID use, a cumulative prescription duration (1-700 days or >1100 days) or a cumulative dose of estrogens (651-1000 DDD or >1000 DDD) were significantly associated with the incidence of CM (p < 0.05).

After adjusting for confounding factors in a multivariate model, estrogen use (≥ 0.5 year) remained significantly associated with a higher risk of developing CM (adjusted OR = 1.42, 95% CI = 1.19-1.70).

Compared to controls, CM patients were significantly more likely to have used higher cumulative doses. Compared to female non estrogen users, women who used estrogens >1100 days were ~60% more likely to have developed a CM (adjusted OR = 1.61, 95% CI = 1.20-2.16). For the cumulative prescription duration as well as the cumulative dose, a statistically significant trend was detected (p <0.001).

Oral Contraceptives

OC was used in the study period for more than half a year by 21.5% of the cases and 17.7% of the controls. Of the oral contraceptives used, 99.3% was ethinyloestradiol and 0.7% was mestranol. In univariate analysis, OC use (≥ 0.5 year) was significantly associated with the incidence of CM (p < 0.05; Table 2). After adjusting for confounding factors in a multivariate model, OC use remained significantly associated with development of CM (adjusted OR = 1.28, 95% CI = 1.06-1.54). Compared to female non estrogen users, women who used estrogens longer than 1100 days were more likely to have developed CM (adjusted OR = 1.56, 95% CI = 1.16-2.10). Female CM patients were significantly more likely to be included in the highest category of cumulative dose than those without CM (>1000, adjusted OR = 1.44, 95% CI = 1.08-1.94, compared to 0 DDD). For the cumulative prescription duration (p-trend analysis <0.01) as well as the cumulative dose (p = 0.01), a statistically significant trend was found.

Hormonal Replacement Therapy

HRT was used for more than half a year in the study period by 4.2% of the cases and 2.0% of the controls (p = 0.001; Table 2). Of the HRT used, 56.9% was estradiol, 29.9% were

Table 2 Prior use of oral contraceptives (OC) or hormonal replacement therapy (HRT) in the study population

		Cases (778)		Controls (4072)			Adjusted OR [a]	95% CI
ORAL CONTRACEPTIVES		**n**	**%**	**n**	**%**	**p-value**		
Prior OC use [b]	non-exposed	611	78.5	3351	82.3		1.00	referent
	exposure >0.5 yr	167	21.5	721	17.7	*0.01*	1.28	1.06 – 1.54
Cumulative prescription duration [b,c]	0 days	611	78.5	3351	82.3	[d]	1.00	referent
	1-700 days	56	7.2	235	5.8	*0.08*	1.31 [e]	0.96 – 1.77
	701-1100 days	50	6.4	271	6.7	*0.94*	1.02 [e]	0.75 – 1.40
	>1100 days	61	7.8	215	5.3	*<0.01*	1.56 [e]	1.16 – 2.10
Cumulative dose [b]	0 DDD	611	78.5	3351	82.3	[d]	1.00	referent
	1-700 DDD	55	7.1	256	6.3	*0.29*	1.18 [e]	0.87 – 1.60
	701-1000 DDD	50	6.4	230	5.6	*0.28*	1.21 [e]	0.88 – 1.67
	>1000 DDD	62	8.0	235	5.8	*0.01*	1.44 [e]	1.08 – 1.94
HORMONAL REPLACEMENT THERAPY								
Prior HRT use [b]	non-exposed	745	95.8	3990	98.0		1.00	referent
	exposure >0.5 yr	33	4.2	82	2.0	0.001	2.08	1.37 – 3.14
Cumulative prescription duration [b,c]	0 days	745	95.8	3990	98.0	[d]	1.00	referent
	1-671 days	18	2.3	43	1.1	<0.01	2.16	1.24 – 3.78
	>671 days	15	1.9	39	1.0	0.02	1.98	1.08 – 3.62
Cumulative dose [b]	0 DDD	745	95.8	3990	98.0	[d]	1.00	referent
	1-671 DDD	18	2.3	44	1.1	<0.01	2.13	1.22 – 3.71
	>671 DDD	15	1.9	38	0.9	0.01	2.02	1.10 – 3.70

[a] Adjusted for the total number of unique prescriptions dispensed (excluding estrogens) and the use of NSAIDs.
[b] Number of cases and controls presented, tested for statistical difference with χ^2-test.
[c] Time interval between estimated last day of use (based on last dispense and amount dispensed) and date of diagnosis of CM. The cumulative prescription duration may exceed maximum number of days of follow up (3 years) due to overlapping dispenses.
[d] *p*-values calculated for each category of estrogen-users compared with non-users.
[e] *p*-value for trend analysis: ≤0.01

OC, oral contraceptive; HRT, hormonal replacement therapy; OR, odds ratio; CI, confidence interval; DDD, defined daily dose.

conjugated estrogens, 12.5% was estriol and 0.6% was ethinyloestradiol. In univariate analysis HRT use (≥ 0.5 year) and the highest categories of cumulative prescription duration and dose of HRT were significantly associated with the incidence of CM ($p < 0.05$; Table 2). After adjusting for confounding factors in a logistic multivariate model, HRT use (≥ 0.5 year) was still significantly associated with development of CM (adjusted OR = 2.08, 95% CI = 1.37-3.14). In the multivariate model, female CM patients were two folds more likely to have used HRT for a longer duration and higher cumulative doses than those without CM.

Subgroup analysis

Restricting the multivariate analysis to the effect of estrogen use (≥ 0.5 year) on the incidence of superficial spreading CM showed comparable results (adjusted OR = 1.46; 95% CI = 1.18-1.81). If the analysis was restricted to nodular CM, lentigo maligna and others, the risk estimate is slightly lower (adjusted OR = 1.12, 95% CI = 0.74-1.69).

Discussion

Estrogens – in general

Estrogen use, both OC and HRT therapy, was associated with an increased incidence of CM. Although we can only speculate about the causality based on observational studies, the significant dose-effect relationships we detected do support our hypothesis. Previous studies are not in accordance with our findings. This may be due to lower cumulative doses of estrogens being used or limited sample sizes. Early case control studies [8-10], which also supported an increased risk of CM with estrogen use, are likely to have included higher doses (doses of estrogens used have declined since the 1970's). On the based of an overall CM incidence among women in The Netherlands in 2000 of 16 per 100.000 person-years [26], a female population of 8,02 million [27] of which 20% uses estrogens and an estimated relative risk of 1.42, the crude estimate for the incidence of CM among non-users of estrogens would be 15 per 100.000 person-years and would increase to 21 per 100.000 person-years with estrogen use.

Oral Contraceptives versus Hormonal Replacement Therapy

Although the adjusted OR of the association between OC and HRT and CM are not statistically different, the difference is striking (OC: OR = 1.28, 95% CI = 1.06-1.54, HRT: OR = 2.08, 95% CI = 1.37-3.14). Theoretically, there are several important differences between OC and HRT. The age distribution differs (ie, HRT is used in post and OC in pre menopausal

women). However, both age at diagnosis as well as an multiplicative interaction term of estrogen use and age were not statistically significant in multivariate analysis. In this study, it is impossible to differentiate between the effects of the HRT and more variable or lower endogenous estrogens on development of CM. Also, HRT consist of estrogen monotherapy, whereas OC usually is a combination of an estrogen with a progestagen. No effects of progestagens on the incidence of CM have been published. However, as Dobos pointed out, very limited data are available on the progestagen effects on the biological behavior of CM. [28] In contrast to OC, which nearly always contains ethinyloestradiol, HRT mostly contains oestradiol, conjugated estrogens or oestriol. The regimen in which OC and HRT differs because HRT can be used intermittently or continuously and OC is normally used once daily for a period of 21 days/month.

Estrogens used as HRT are often applied locally. To affect CM incidence a systemic exposure is warranted, therefore, we only included HRT dispenses that were likely to result in a minimal systemic exposure of estrogens (≥0.25 DDD/day orally). Obviously, our results also do not apply for estrogens applied locally in relatively low doses (for instance vaginal therapy twice weekly).

Strengths and limitations

This is the largest case-control study exploring the effects of estrogen use on CM incidence including >750 female cases. Both cases and controls in our study were sampled from PALGA and PHARMO. These databases are general population-based and reflect the Dutch population well. [21-22] Moreover, pharmacy data are gathered prospectively avoiding recall bias. Since we had detailed drug dispensing information, we were able to study dose-effect responses, differentiate between OC and HRT, and exclude low-dosed topically applied estrogens. Confounding by indication seems unlikely because risk factors of CM do not affect the prescription of OC. It seems highly unlikely that menopausal complaints (e.g., flushing and vaginal atrophy) or causes of menopause (e.g., hysterectomy) are associated with the incidence of CM, unless estrogens are prescribed for osteoporosis. Because osteoporosis is associated with low endogenous vitamin D levels and low sun exposure, which affect CM incidence in opposite directions [29], the association between HRT use and CM development may be affected. Unfortunately, vitamin D levels and measures of life-style factors such as sun exposure were not available in the PHARMO database. To our knowledge, only one study has studied the association between estrogen use and sun exposure and demonstrated that HRT users did not differ in sun exposure compared non-users, but users were more likely to use sunbeds. [30] For OC, the association with sun (bed) exposure use has not

been documented. To minimize the ascertainment bias, the analyses were adjusted for a proxy of health care consumption (i.e. the number of unique ATC codes). We limited the study to the effects of estrogen use in the 3 years prior to CM diagnosis to not exclude too many patients. For some subgroup analyses, especially for the use of HRT, the sample sizes may be too small. Most cases were excluded because they were registered in PHARMO in a different time period.
Unfortunately, the variation in average day dose of estrogens, expressed in DDD, among users was minimal and therefore any possible associations between average day dose and CM incidence would not have been detectable in our population. Therefore we did not include average day dose in our analysis.

Conclusion

This large observational study suggests a cumulative dose-dependent increased risk of CM with the use of estrogens. In our study, women who used estrogens for more than half a year were about 40% more likely to have developed a CM than women who did not use estrogens or less than half a year (adjusted OR = 1.42, 95% CI = 1.19-1.70). A validation of our findings is warranted, preferably in a (prospective randomized) study with detailed propectively gathered information on both drug use as well as sun (bed) exposure. Moreover, more experimental research is warranted to elucidate the effects of estrogens, progestagens and gender on CM development and progresison.

Acknowledgement
We thank Professor Jan Vandenbroucke for critical discussion of the design of the study and Dr Mark Tinga for data selection in PHARMO.

Reference List

(1) Østerlind A, Tucker MA, Stone BJ, Jensen OM. The Danish case-control study of cutaneous malignant melanoma. III. Hormonal and reproductive factors in women. Int J Cancer 1988, 42, 821-824.

(2) Karagas MR, Stukel TA, Dykes J, Miglionico J, Greene MA, Carey M *et al.* A pooled analysis of 10 case-control studies of melanoma and oral contraceptive use. Br J Cancer 2002, 86, 1085-1092.

(3) Vries E de, Nijsten TEC, Visser O, Bastiaannet A, Hattem S van, Janssen-Heijnen MLG *et al.* Superior survival of females among 10 538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol 2008, 19 (3), 583-589.

(4) Vries E de, Houterman S, Janssen-Heijnen MLG, Nijsten T, Schans SAM van de, Eggermont AMM *et al.* Up-to-date survival estimates and historical trends of cutaneous malignant melanoma in the south-east of The Netherlands. Ann Oncol 2007, 18, 1110-1116.

(5) Koomen ER, Joosse A, Herings RMC, Casparie MK, Bergman W, Nijsten T, Guchelaar HJ. Is statin use associated with a reduced incidence, reduced Breslow thickness or delayed metastasis of melanoma of the skin? Eur J Cancer 2007, 43, 2580-2589.

(6) Schoenberg BS, Christine BW. Malignant melanoma associated with breast cancer. South Med J 1980, 73 (11), 1493-1497.

(7) Walker MJ, Beattie CW, Patel MK, Ronan SM, Das Gupta TK. Estrogen receptor in malignant melanoma. J Clin Oncol 1987, 5 (8), 1256-1261.

(8) Beral S, Ramcharan S, Faris R. Malignant melanoma and oral contraceptive use aming women in California. Br J Cancer 1977, 36, 804-809.

(9) Kay CR. Malignant melanoma and oral contraceptives. Br J Cancer 1981, 44, 479.

(10) Ramcharan S, Pellegrin FA, Ray R, Hsu JP. The Walnut Creek Contraceptive Drug Study: a prospective study of the side effects of oral contraceptives III. NIH Publication No. 81-564.

(11) Adam SA, Sheaves JK, Wright NH, Mosser G, Harris RW, Vessey MP. A case-control study of the possible association between oral contraceptives and malignant melanoma. Br J Cancer 1981, 44, 45-50.

(12) Zanetti R, Francheschi S, Rossa S, Bidoli E, Colonna S. Cutaneous Malignant melanoma in females: The role of hormonal and reproductive factors. Int J Epidemiol 1990, 19 (3), 522-526.

(13) Hannaford PC, Villard-Mackintosh L, Vessey MP, Kay CR. Oral contraceptives and malignant melanoma. Br J Cancer 1991, 63, 430-433.

(14) Holly EA, Cress RD, Ahn DK. Cutaneous melanoma in women. III. Reproductive factors and oral contraceptive use. Am J Epidemiol 1995, 141 (10), 943-950.

(15) Westerdahl J, Olsson H, Måsbäck, C Ingvar, Jonsson N. Risk of malignant melanoma in relation to drug intake, alcohol, smoking and hormonal factors. Br J Cancer 1996, 73, 1126-1131.

(16) Feskanich D, Hunter DJ, Willett WC, Spiegelman D, Stamfer MJ, Speizer FE *et al.* Oral contraceptive use and risk of v in premenopausal women. Br J Cancer 1999, 81 (5), 918-923.

(17) Palmer JR, Rosenberg L, Strom BL, Harlap S, Zauber AG, Warshauer ME *et al.* Oral contraceptive use and risk of cutaneous malignant melanoma. Cancer Causes and Control 1992, 3, 547-554.

(18) Lê MG, Cabanes PA, Desvignes V, Chanteau MF, Mlika N, Avril MF. Oral contraceptive use and risk of cutaneous malignant melanoma in case-control study of French women. Cancer Causes and Control 1992, 3, 199-205.

(19) http://www.pharmo.nl/, PHARMO pharmacy database, visited Nov 23rd 2007.

(20) Lau HS, Boer A de, Beuning KS *et al.* Validation of pharmacy records in drug exposure assessment. J Clin Epidemiol 1997, 50, 619-625.

(21) Herings R. Pharmo, A record linkage system for postmarketing surveillance of prescription drugs in the Netherlands [thesis in pharmaco-epidemiology and pharmacotherapy]. The Netherlands, Utrecht University, 1993: 17-32.

(22) Casparie MK, Tiebosch T, Burger G *et al.* Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cellular Oncology 2007, 29 (1), 19-24.

(23) Vandenbroucke JP, Von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ *et al.* Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. Ann Intern Med 2007, 147, 163-194.

(24) LeBoit PE, Burg G, Weedon D, Sarasin A (editors). WHO Classification of tumours Volume 6, IARC WHO Classification of Tumours No 6, Pathology and genetics of tumours of the skin, 2005.

(25) Freeman SR, Drake AL, Heilig LF, Graber M, McNealy K, Schilling LM, Dellavalle RP. Statins, fibrates, and melanoma risk: a systematic review and meta-analysis. J Natl Cancer Inst, 2006, 98 (21), 1538-1546.

(26) Vries E de, Poll-Franse LV van de, Louwan WJ, Gruijl FR de, Coebergh JWW. Predictions of skin cancer incidence in the Netherlands up to 2015. Br J Dermatol 2005, 152, 481-488.

(27) http://www.statline.cbs.nl/, Centraal Bureau voor de Statistiek, visited February 18th 2007.

(28) Dobos J, Timar J, Bocsi J, Burian Z, Nagy K, Barna G *et al.* In vitro and in vivo antitumor effect of 2-methoxyestradiol on human melanoma. Int J Cancer 2004, 112, 771-776.

(29) Egan KM, Sosman JA, Blott WJ. Sunlight and reduced risk of cancer: is the real story vitamin D? J Natl Cancer Inst 2005, 97 (3), 161-163.

(30) Jensen LB, Hilden J. Sociological and behavioral characteristics of perimenopausal women with an express attitude to hormone substitution therapy. Maturitas 1996, 23, 73-83.