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Hormone replacement therapy and the risk of melanoma in post-menopausal women

Running title: Hormone replacement therapy and melanoma

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Keywords: hormone replacement therapy, melanoma, skin cancer, pharmacoepidemiology

Abbreviations: HRT, hormone replacement therapy; UV, Ultraviolet; COPD, Chronic Obstructive Pulmonary Disorder; OR, Odds ratio; CI, confidence interval; DDD, defined daily dose; BMI, body mass index

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ABSTRACT

Study question: Is hormone replacement therapy (HRT) associated with an increased risk of melanoma skin cancer or prognostic outcomes among post-menopausal women?

Summary answer: While we found evidence of an association with melanoma risk, the lack of dose-response, associations observed with recent use, localized disease and intravaginal oestrogens suggests this is a non-causal association.

What is known already: Evidence on HRT and melanoma risk remains inconclusive, with studies providing conflicting results. Furthermore, evidence on melanoma survival is sparse, with only one previous study reporting protective associations with HRT use, likely attributable to immortal time bias.

Study design, size, duration: We conducted a nation-wide population based case-control study and a retrospective cohort study utilizing the Danish healthcare registries. Case control analyses included 8,279 women aged 45 to 85 with a first-ever diagnosis of malignant melanoma between 2000 and 2015, matched by age and calendar time to 165,580 population controls. A cohort of 6,575 patients with a diagnosis of primary malignant melanoma between 2000 and 2013 and followed through 2015 was examined to determine if HRT use had an impact on melanoma survival outcomes.

Participants/materials, setting, methods: Based on prescriptions dispensed since 1995, ever-use of HRT was defined as having filled at least one prescription for HRT prior to the index date. In total 2,629 cases (32.8%) and 47,026 controls (28.4%) used HRT. Conditional logistic regression was used to calculate odds ratios (ORs) for melanoma risk according to HRT use, compared to non-use, adjusting for potential confounders. For cohort analyses Cox proportional hazards models was used to estimate adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for second melanoma incidence and all-cause mortality associated with HRT.

Main results and the role of chance: High use of HRT was associated with an OR of 1.21 (95%CI 1.13 to 1.29) for melanoma risk, with no evidence of a dose-response pattern. Results were most pronounced among recent high users (OR, 1.28 95%CI 1.17-1.41), for localized disease (OR, 1.25 95%CI 1.15 - 1.36) and for intravaginal oestrogen therapy (OR, 1.38 95%CI 1.13- 1.68). Compared with non-use there was no evidence of an association for secondary melanoma for post-diagnostic new-use (fully adjusted HR, 1.56 95%CI 0.64-3.80)

or continuous HRT use (fully adjusted HR, 1.26 95%CI 0.89-1.78). Similar associations were observed for all-cause mortality.

Limitations, reasons for caution: Despite the large sample size and the use of robust population-based registries with almost complete coverage, we lacked information on some important confounders including sun exposure.

Wider implications of the findings: While we cannot rule out an association between HRT use and melanoma risk, the associations observed are also compatible with increased healthcare utilization and thus increased melanoma detection among HRT users. No association between HRT use and melanoma survival outcomes was observed. This should provide some reassurance to patients and clinicians, particularly concerning the use of HRT in patients with a history of melanoma.

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Introduction

A number of risk factors for melanoma have been established including exposure to ultraviolet light (Gandini *et al.*, 2005a), fair skin (Gandini *et al.*, 2005b), and immune suppression (Olsen *et al.*, 2014a; Green and Olsen, 2015). Gender disparities in incidence have also been noted. While men have a higher incidence of melanoma overall, women have the highest incidence among individuals approximately 55 years and younger, with a third of cases in women occurring during childbearing age.(NORDCAN, 2019) Increases in melanoma risk during pregnancy, with oral contraceptive use and other reproductive factors (including parity, age at menarche and menopause) have been noted (Gandini *et al.*, 2011; Kvaskoff *et al.*, 2011). Furthermore, a number of epidemiological studies have identified sex as a prognostic factor among melanoma patients, with survival rates higher among females than males (Bay *et al.*, 2015; Enninga *et al.*, 2017; El Sharouni *et al.*, 2019). Therefore, there has been speculation that hormonal factors may influence melanoma incidence and survival. Indeed, preclinical studies suggest that oestrogen may play a role in melanoma carcinogenesis, with oestrogen receptors ER α and ER β both located on melanocytes (Marzagalli *et al.*, 2016).

However, evidence surrounding HRT use and melanoma risk has been inconclusive. Recent studies reported null associations between HRT use and melanoma risk (Gandini *et al.*, 2011; Tang *et al.*, 2011; Donley *et al.*, 2019). Contrastingly, a recent study observed increases in melanoma risk, with stronger associations among past users (Cervenka *et al.*, 2019). While another study also reported increases in melanoma risk with oestrogen use, reductions in risk were observed in dose-response analysis for progestogen (Botteri *et al.*, 2017).

Studies investigating HRT and melanoma prognosis have been sparse. The only study to date investigated HRT use and melanoma disease free survival observing protective associations, however, these results were likely influenced by immortal time bias (Mackie

and Bray, 2004). Despite the lack of evidence, it has been noted that oncologists will often advise women with a history of cancer against the use of HRT, even for tumours which are not hormonal-dependent (Biglia *et al.*, 2004).

Given the potential role of hormones in melanoma carcinogenesis and the conflicting and limited epidemiological evidence on HRT and melanoma risk and progression we conducted two nationwide studies using the Danish health registries. We aimed to examine whether HRT was associated with increased risk of melanoma in a nested case-control study and with survival and risk of recurrent melanoma in a cohort of patients diagnosed with melanoma.

Materials and Methods

Data Sources

We obtained data from six nationwide registry sources: the Danish Cancer Registry (Gjerstorff, 2011), the Danish Pathology Registry (Bjerregaard and Larsen, 2011), the National Prescription Registry (Wallach Kildemoes *et al.*, 2011), the National Patient Registry (Schmidt *et al.*, 2015a), Registers in Statistics Denmark on educational level (Jensen and Rasmussen, 2011) and the Civil Registration System (Schmidt *et al.*, 2014). Supplementary Material provides a description of these registries (**Supplemental Method 1**) with codes for diagnoses, drug exposure and covariates (**Supplemental Table I**). All linkages were performed by Statistics Denmark.

Virtually all medical care in Denmark is funded by the Danish National Health Service, allowing true population-based register linkage studies covering all residents of Denmark (Thygesen *et al.*, 2011). Data were linked by a unique personal identification number, assigned to all residents. Linkages were performed by Statistics Denmark.

Investigation of Melanoma Risk: Case Control Study

Selection of melanoma cancer cases and population controls

From the Danish Cancer Registry we identified cases as all women with a primary, histologically verified diagnosis of cutaneous melanoma between January 1st 2000 and December 31st 2015. The date of diagnosis corresponded to the index date. We included only patients between the ages of 45-85 years at the index date and excluded patients with any residency outside of Denmark within 10 years prior to the index date. We further excluded

those who had a history of primary ovarian failure, radical hysterectomy, bilateral salpingo-oophorectomy/oophorectomy, those patients with a previous history of cancer (excluding non-melanoma skin cancers) and those with xeroderma pigmentosum. Finally we excluded those with a history of organ transplantation, HIV diagnosis or use of azathioprine, cyclosporine or mycophenolate mofetil as immunosuppression has been associated with an increased risk of skin cancer (Dahlke *et al.*, 2014; Olsen *et al.*, 2014b; Fattouh *et al.*, 2017).

Controls were selected using risk set sampling. For each case, we selected 20 controls among Danish women matched by age and calendar time, applying the same selection criteria as for cases. Controls were assigned the same index date as the case to whom they were matched. Subjects were eligible for sampling as controls before they became cases. Thereby, the calculated ORs provide direct estimates of the incidence rate ratios (IRRs) from a cohort study utilizing the source population (Rothman and Lash, 2008).

Exposure definition: Systemic hormone replacement therapy

Based on prescriptions dispensed since 1995, ever-use of HRT was defined as having filled at least one prescription for HRT prior to the index date. HRT included all systemic agents available in Denmark during the study period including oestrogen only, progestogen only and oestrogen and progestogen combination therapies. Hormonal intrauterine devices were not included. Intravaginal oestrogens were also not included in our HRT exposure definition as the primary indications for intravaginal oestrogens are local complaints including vaginal atrophy. Furthermore, doses administered with intravaginal therapy are markedly lower than systemic oestrogen and have minimal systemic absorption, with previous studies finding use of low-dose intravaginal oestrogens does not result in sustained serum oestrogen levels exceeding normal menopausal range.(Rigg *et al.*, 1978; Simunić *et*

al., 2003; Santen, 2015) However, intravaginal oestrogens were investigated in sensitivity analyses. High levels of HRT use were defined as filled prescriptions equivalent to ≥ 1000 defined daily doses (DDDs) of HRT corresponding to approximately 3 years of cumulative use. This corresponded to a cumulative use of for example 200 mg of estriol or 5000 mg of norethisterone (World Health Organisation, 2019). For all analyses, prescriptions filled in the year prior to the index period were disregarded. This 1-year lag period was introduced to allow for a minimum latency time window and to minimize reverse causality (Rothman and Lash, 2008). The length of the lag period was varied in sensitivity analyses.

Potential Confounders

We defined potential confounders as the following: a) drugs suggested to have photosensitizing properties including oral retinoids, topical retinoids, tetracycline, macrolides, fluroquinolones and aminoquinolines, amiodarone, methoxypsoralene and hydrochlorothiazide (Stern *et al.*, 1984; Kaae *et al.*, 2010; Schmidt *et al.*, 2015b) b) oral contraceptive use c) drugs suggested to potentially modify the risk of cancer including low-dose aspirin, NSAIDs and statins (Jensen *et al.*, 2009; Muranushi *et al.*, 2015, 2016; Lin *et al.*, 2018) d) history of comorbidities (defined by diagnosis codes and related medications) including diabetes, chronic obstructive pulmonary disease, chronic renal insufficiency, diseases associated with heavy alcohol consumption, inflammatory bowel disease, psoriasis, sarcoidosis and stroke (Henderson *et al.*, 2015; Dąbrowski *et al.*, 2016; Tseng *et al.*, 2016; Groothoff *et al.*, 2018) e) Modified Charlson comorbidity Index (CCI) score (0 low; 1-2 medium; ≥ 3 high) based on the prevalence of 19 chronic conditions (Charlson *et al.*, 1987); d) highest achieved education (basic, medium, higher or unknown). Exposure to the drugs outlined above was defined as two or more filled prescriptions prior to the index date and

hospital histories of comorbidities were defined as a primary or secondary discharge or outpatient diagnosis. For all covariates, information within one year prior to the index date was disregarded.

Statistical Analyses

Conditional logistic regression was used to calculate ORs and 95% confidence intervals (CIs) for malignant melanoma associated with the use of HRT compared to never-use, adjusting for all potential confounders outlined previously. We also performed secondary analyses to examine a potential dose-response association, stratifying cumulative HRT use by predefined categories (1-99 DDDs, 100-499 DDDs, 500-999 DDDs, 1000-2000 DDDs and >2000 DDDs). Analyses were carried out by HRT type (including oestrogen, progestogen and oestrogen/progestogen combinations, not restricted to exclusive use) and by route of HRT admission including oral HRT (oestrogen, progestogen and oestrogen/progestogen) and transdermal (oestrogen and oestrogen/progestogen). Analyses were also conducted to investigate associations with recent high use, defined as a cumulative use of $\geq 1,000$ DDDs (including the 1-year lag-time) among users with a filled prescription in the two years prior to the index date. In all analyses, never use of HRT served as the reference category.

Subgroup and Sensitivity Analyses

Pre-specified subgroup and sensitivity analyses were also conducted, including analyses by stage, for intravaginal oestrogens as a control exposure and for new users, as described in detail in **Supplementary Method II**.

Investigation of Melanoma Prognosis: Cohort Analysis

Study Population

We conducted a nationwide cohort study to investigate the risk of second primary melanoma associated with the use of HRT among women aged 45-85 years diagnosed with a previous melanoma. From the cases identified previously for case-control analyses, we identified those with incident melanoma between January 1st 2000 and December 31st 2013 (to ensure sufficient follow-up time). Follow-up time began one year after melanoma diagnosis and continued until a new melanoma diagnosis, death from any cause or end of the study period (31st December 2015), whichever occurred first. The first year of follow-up was excluded for latency purposes, to minimise detection bias due to increased contact with healthcare professionals and to ensure a true second primary melanoma diagnosis.

Exposure Definition

Exposure to HRT was defined into five mutually exclusive groups (**Supplementary Figure I**); new users of HRT were those patients who had filled at least one prescription for HRT in the year post-diagnosis of melanoma but not in the 5 years prior to cohort entry. Continuous users were defined as those who received at least one prescription for HRT in the 2 years prior to cohort entry and in the year post cohort entry, past users were those who filled at least one prescription for HRT between 5 and 2 years pre-diagnosis but not since then, and pre-diagnostic users were defined as those patients who received at least one prescription for HRT in the 2 years prior to cohort entry but not in the year post cohort entry. Non-users were those patients who did not use HRT (excluding intravaginal oestrogens) in the 5 years prior to diagnosis and to one year after diagnosis and were considered the reference category for all analyses. Secondary analyses investigated HRT type and route of

HRT admission. For analyses investigating associations with intravaginal oestrogen, the reference category was non-use of all HRT, including intravaginal oestrogen.

Statistical Analyses

Cox proportional hazard models, using time from diagnosis as the time scale, were used to estimate hazard ratios and 95% CIs of second primary melanoma associated with the use of HRT compared with non-use. Models were adjusted for the confounders listed previously with the addition of melanoma stage (TNM). The proportional hazards assumption was assessed using Schoenfeld residuals. Analyses also investigated the association between HRT and the secondary outcome of all-cause mortality. Sensitivity analyses were conducted restricting the follow-up period to five years.

RESULTS

Investigation of Melanoma Risk: Case Control Study Results

We identified 14,183 cases of melanoma between January 1st 2000 and December 31st 2015. Following exclusions, 8,279 cases were matched to 165,580 cancer-free controls (**Figure I**). Compared to controls, cases had a lower prevalence of alcohol related disorders and COPD, were less likely to have a low comorbidity score and had longer durations of education. Other characteristics were similar between cases and controls (**Table I**).

Overall, 31.8% of cases and 28.4% of controls filled a prescription for HRT (**Table II**) yielding an adjusted OR of 1.18 (95%CI 1.12 to 1.24). A greater proportion of cases exhibited high use of HRT ($\geq 1,000$ DDDs) than controls (15.5% vs. 13.3%) which corresponded to an adjusted OR of 1.21 (95%CI 1.13 to 1.29). ORs remained elevated across all cumulative DDD categories with no evidence of a dose-response (P for trend=0.59). In recent high users, associations were more marked than in distant high users (OR, 1.28 95%CI 1.17 to 1.41; OR, 1.14, 95%CI 1.04 to 1.25, respectively). Both oral and transdermal HRT

were associated with increases in melanoma risk, which was more marked with transdermal HRT use (OR, 1.37, 95%CI 1.17 to 1.61).

Analyses by HRT type and melanoma risk are presented in **Supplementary Table II**. Overall, positive associations were observed with all HRT types. Similar to the primary analyses, for each HRT type, there was no evidence of dose-response relationships, and associations were more pronounced with recent high use. Results were similar among patients with exclusive use of oestrogen and oestrogen/progestogen combination therapy (**Supplementary Table III**).

Sub-group analyses are presented in **Table III**. Overall sub-group analyses revealed similar results. However, null associations were observed for women <50 years, for nodular and acral lentiginous melanoma and for melanoma of unspecified location and of the upper limb. Additionally analysis by stage revealed associations only with localized melanoma (OR, 1.25, 95%CI 1.15 to 1.36). Tests for effect measure modification showed that clinical stage modified the association ($p < 0.001$), while there was less evidence for effect modification by localization ($p = 0.09$), age ($p = 0.45$), or melanoma type (0.37). Additional analyses investigated the risk of melanoma associated with intravaginal oestrogen use, corresponding to an OR of 1.38 (95%CI 1.13 to 1.68) for high use ($\geq 1,000$ DDDs) [**Supplementary Table V**]. There was some evidence of a dose-response relationship and associations were more marked for recent high users. In sensitivity analyses utilizing a new user design (**Supplementary Table VI**) estimates were attenuated, including for high HRT use (OR, 1.13 95%CI 0.99 to 1.28). Similarly, null associations were observed by recency of use. Finally, analyses increasing lag periods (**Supplementary Table VII**) revealed results similar to primary analyses.

Investigation of Melanoma Prognosis: Cohort Study Results

From 8,279 melanoma cases, 6,575 patients with melanoma were included after excluding 1,445 patients diagnosed after 2013 and 259 patients with less than one-year follow-up. Patients in the cohort were followed for a median (IQR) of 5.1 (2.6-8.6) years. **Table IV** presents baseline characteristics by HRT use. Overall, new users of HRT with melanoma were younger, more likely to have a history of oral contraceptive use, had a lower comorbidity score and longer education. Other characteristics remained largely similar between groups.

Results for HRT use associated with secondary melanoma diagnosis are presented in **Table V**. Compared with non-use of HRT, the use of HRT was not associated with an increased risk of secondary melanoma for post-diagnostic new-users (fully adjusted HR, 1.56 95%CI 0.64 to 3.80) or continuous HRT users (fully adjusted HR, 1.26 95%CI 0.89 to 1.78). Similarly, pre-diagnostic HRT use and distant use of HRT were not associated with secondary melanoma risk. Analyses by HRT type revealed null associations for oestrogen and combination therapy. New-use of progestogen post-diagnosis and continuous progestogen use were associated with increases in risk of secondary melanoma however these were based on a small number of events ($n \leq 6$).

While an association was observed for all-cause mortality and post-diagnostic new users (adjusted OR, 0.31 95%CI 0.10 to 0.96) this was based on a small number of events ($n < 5$) (**Table VI**). Associations by other HRT user groups and by HRT type revealed null associations. There was no evidence of an association with HRT use categories and secondary melanoma diagnosis or all-cause mortality in analyses by route of admission (**Supplementary Table VIII & IX**) and restricting the follow-up period to a maximum of 5 years (**Supplementary Table X & XI**).

DISCUSSION

In this nationwide observational study, we found no evidence of an association between HRT use and melanoma prognostic outcomes. While we observed associations for increased melanoma risk, these did not appear to follow a dose-response pattern. Further, analyses by disease stage revealed that associations were only evident for localized disease. Although we cannot rule out a causal association, taken together, these results appear to suggest that the associations observed may be a result of detection bias, likely from more intensive contact with healthcare professionals among HRT users.

Our results for melanoma risk correspond with that observed in a recent meta-analysis, which included a smaller number of cases than our study (n=2,816) and reported a RR of 1.16 (95%CI 0.93 to 1.44) for the association between ever use of HRT and melanoma (Gandini *et al.*, 2011). Contrastingly, subsequent studies utilizing both the Nurse's Health Initiative trial and the NIH-AARP Diet and Health study reported null associations (Tang *et al.*, 2011; Donley *et al.*, 2019). In a recent French prospective study ever HRT use was associated with an increase in melanoma risk (HR=1.35 95%CI 1.07-1.71) (Cervenka *et al.*, 2019). Contrary to our results, the authors report the highest associations among past users (HR, 1.55 95%CI 1.17-2.07). It is unclear why there is a discrepancy in results. Possible explanations include differing exposure definitions, with past-use defined as no HRT use within one year, and based on self-reported use. Furthermore, the authors failed to properly account for latency considerations and included a smaller number of melanoma cases (n=444). There is also a variation in HRT types predominantly used worldwide, which could explain some of the difference in results. In our study, the most commonly used progestogen was medroxyprogesterone and most common combination was norethisterone/oestrogen which differed from Cervenka *et al.*. In an additional study, HRT use was defined as current, non-use or past use (defined as >4 months since last prescription) with a short lag-time period

(3 months), revealing associations with melanoma for current HRT use (RR 1.23 95%CI 1.05 to 1.45) but not past use (RR, 1.00 95%CI 0.80 to 1.25) (Botteri *et al.*, 2017). These results are similar to those observed in analyses of recency of use in this study. The authors also observed associations for current oestrogen use, including for intravaginal oestrogens (RR, 1.45 95%CI 1.12 to 1.88) but not combination therapy. While intravaginal oestrogens may increase oestrogen levels in serum and thus may exert systemic effects (Labrie *et al.*, 2009; Wills *et al.*, 2012; Santen, 2015), the absorbed doses are considerably smaller than the doses delivered with systemic oestrogen therapy. We observed higher estimates for intravaginal oestrogen use than systemic oestrogen therapy, with evidence of dose response ($p < 0.01$). Additionally, associations were more marked for recent users than distant users of intravaginal oestrogens. Given these findings, it is likely associations with intravaginal oestrogens are also subject to detection bias.

To the best of our knowledge, only one study to date had investigated the association between HRT use and melanoma survival. In contrast to our study, MacKie *et al* reported marked increases in disease free survival associated with HRT use in melanoma patients (adjusted HR, 0.17 95%CI 0.05 to 0.62) (Mackie and Bray, 2004). However this was a small study of only 206 melanoma cases, which suffered from a number of methodological shortcomings including failure to use a lag period and potential immortal time bias.

A number of studies have demonstrated that HRT users tend to exhibit healthier behaviour or having more favourable socio-economic characteristics than non-users such as higher education, higher socioeconomic status and lower BMI (Li *et al.*, 2000; Jensen and Hilden, 1996; Lambert *et al.*, 2003). We observed higher estimates with recent use and localized disease, suggestive of increased detection of melanoma in HRT users and healthy user bias. Unfortunately, we did not have information on general practice healthcare visits or proxies for health seeking behaviour e.g. cancer screening to investigate this. However,

previous studies have demonstrated that HRT users are more likely to have increased healthcare utilization, including uptake in cancer screening services such as mammography.(Li *et al.*, n.d.; MacLennan *et al.*, 1998; Cook *et al.*, 2009) Despite this, it is not possible to rule out a potential carcinogenic or tumour promoting effect of HRT on melanoma.

While the association between oestrogen and melanoma is biologically plausible the mechanisms through which sex hormones may exert their effects on melanoma remain unclear. Pre-clinical studies have suggested that oestrogens may be associated with proliferative action while progesterones may exert anti-proliferation and anti-apoptosis effects (Wiedemann *et al.*, 2009; De Giorgi *et al.*, 2011). This is in contrast with the results of our study, which found elevated HRs for both oestrogen and progesterones and melanoma risk. The oestrogen receptors ER α and ER β have both been located on melanocytes,¹⁵ however, pre-clinical evidence suggests that that these receptors may have opposing effects in melanoma with ER α associated with proliferative action and ER β with anticancer effects (Marzagalli *et al.*, 2016). The most commonly used oestrogens within our cohort were estradiol and estriol, both of which bind to both receptors with a similar binding affinity and transactivational activity (Marzagalli *et al.*, 2016). Thus it is unclear if we would expect different formulations of oestrogen to differentially affect melanoma, rather this would likely depend on the ER α /ER β ratio and the specific cell context.

Strengths and Limitations

This study has several strengths, including a large sample size and the use of robust population-based registries with almost complete population coverage. Use of the Danish National Prescription Registry ensured the complete and high-quality assessment of

prescription drug use up to a maximum of 20 years of drug exposure history (Pottegård *et al.*, 2016). Melanoma diagnoses were identified via the Danish Cancer Registry and the Danish Pathology registry and were histologically verified, thus increasing validity.

Despite these strengths, this study also had a number of limitations. Firstly, this study lacked information on a number of important risk factors for melanoma including ethnicity, skin phenotype and UV exposure. However, the majority of the Danish population is of white origin. Additionally, in a previous study conducted in Denmark, sun exposure was found to be similar between HRT users and non-users, although HRT users were more likely to use solariums (Jensen and Hilden, 1996). While this study also lacked information on BMI and smoking status, we adjusted for COPD as a crude proxy for smoking. However, as smoking and obesity have been found to exert differing effects of melanoma risk, it is difficult to predict the direction in which these factors might bias estimates (Song *et al.*, 2012; Sergentanis *et al.*, 2013). We also lacked information on menopausal status and age and menopause, as well as other hormonal factors such as age at menarche and parity.

Conclusion

In this population-based study, we identified a slightly increased risk of melanoma associated with HRT use. While we cannot rule out an aetiological effect of HRT on melanoma incidence the results are compatible with increased healthcare utilization and thus increased melanoma detection among HRT users.

Author Contributions

All authors conceived and designed the study, analysed and interpreted the data and critically revised the manuscript for important intellectual content. AP acquired the data. KK analysed the data. BH wrote the manuscript and all authors participated in the interpretation of the results and critical revision of the manuscript.

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Figure I Flow chart of case selection

† Immunosuppressant drug use includes use of azathioprine, cyclosporine or mycophenolate mofetil.

601 **Figure Legends**

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