# Menstrual factors, reproductive history, hormone use, and Urothelial carcinoma risk: A prospective study in the EPIC cohort

Leila Lujan-Barroso 1,2,3, Edoardo Botteri 4, 5, Saverio Caini 6, Börje Ljungberg 7, Nina Roswall 8, Anne Tjønneland 8,9, Bas Bueno-de-Mesquita 10, 11, 12, 13, Inger T. Gram 14, Rosario Tumino 15, Lambertus A. Kiemeney 16, Fredrik Liedberg 17, Tanja Stocks 18, Marc J. Gunter 19, Neil Murphy 19, Iris Cervenka 20, Agnès Fournier 20, Marina Kvaskoff 20, Christel Häggström 21, 22, Kim Overvad 23, Eiliv Lund 14, Marit Waaseth 24, Renée Turzanski Fortner 25, Tilman Kühn 25, Virginia Menéndez 26, Maria-Jose Sánchez 27,28,29,30 Carmen Santiuste 29,31, Aurora Perez-Cornago 32, Raul Zamora-Ros 1,2, Amanda J. Cross 33, Antonia Trichopoulou 34, Anna Karakatsani 34,35, Eleni Peppa 34, Domenico Palli 6, Vittorio Krogh 36, Veronica Sciannameo 37, Amalia Mattiello 38, Salvatore Panico 38, Carla H. van Gils 39, N. Charlotte Onland-Moret 39, Aurelio Barricarte 29, 40, 41, Pilar Amiano 29, 42, Kay-Tee Khaw 43, Heiner Boeing 44, Elisabete Weiderpass[*] 19, Eric J. Duell[*] 45, 46.

1. Unit of Nutrition and Cancer. Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO), Gran Via de L'Hospitalet 199-203, 08908, L'Hospitalet de Llobregat, Barcelona, Spain.
2. Bellvitge Biomedical Research Institute – IDIBELL, Gran Via de L'Hospitalet 199-203, 08908, L'Hospitalet de Llobregat, Barcelona, Spain.
3. Department of Nursing of Public Health, Mental Health and Maternity and Child Health School of Nursing Universitat de Barcelona, Carrer de la Feixa Llarga s/n, 08907, L'Hospitalet de Llobregat, Barcelona, Spain
4. Cancer Registry of Norway, Oslo University Hospital, Ullernchausseen 64, 0379, Oslo, Norway.
5. Norwegian National Advisory Unit for Women's Health, Women's Clinic, Oslo University Hospital, Sognsvannsveien 20, 0372, Oslo, Norway.
6. Cancer Risk Factors and Lifestyle Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network (ISPRO), Via Cosimo il Vecchio 2, 50139, Florence, Italy
7. Department of surgical and perioperative sciences, urology and andrology, Umeå University, 901 85, Umeå, Sweden.

8. Diet, Genes and Environment, Danish Cancer Society Research Center, Strandboulevarden 49, DK-2100, Copenhagen Copenhagen, Denmark.

9. Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Øster Farimagsgade 5, 1014 Copenhagen.

10. Former senior scientist, Dept. for Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands.

11. Former associate professor, Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The Netherlands.

12. Visiting professor, Dept. of Epidemiology and Biostatistics, The School of Public Health, Imperial College London, St Mary's Campus, Norfolk Place, London, W2 1PG London, United Kingdom.

13. Academic Icon / visiting professor, Dept. of Social & Preventive Medicine, Faculty of Medicine, University of Malaya, Pantai Valley, 50603, Kuala Lumpur, Malaysia.

14. Department of Community Medicine, University of Tromsø , The Arctic University of Norway, N – 9037, Tromsø, Norway.

15. Cancer Registry and histopathology Department, "Civic -M.P. Arezzo" Hospital, ASP Ragusa, 97100, Ragusa, Italy.

16. Radboud university medical center, Radboud Institute for Health Sciences, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

17. Department of Urology Skåne University Hospital and Institution of Translational Medicine, Lund University, Jan Waldenströms gata 5, 205 02, Malmö, Sweden.

18. Department of Clinical Sciences Lund, Lund University, Barngatan 4, 222 42, Lund, Sweden.

19. International Agency for Research on Cancer / World Health Organization, 150 cours Albert Thomas 69372, Lyon CEDEX 08, France.

20. Inserm U1018, Centre for Research in Epidemiology and Population Health (CESP) "Health across Generations" Team, Gustave Roussy 114 rue Edouard Vaillant, F-94805, Villejuif, France.

21. Department of Biobank Research, Umeå University, SE-901 87, Umeå, Sweden.

22. Department of Surgical Sciences, Uppsala University, Akademiska sjukhuset entrence 70, 1 tr SE-751 85, Uppsala, Sweden.

23. Department of Public Health, Section for Epidemiology, Aarhus University, Bartholins Allé 2 DK-8000, Aarhus, Denmark.

24. Department of Pharmacy, University of Tromsø , The Arctic University of Norway, N – 9037, Tromsø, Norway.

25. Division of Cancer Epidemiology, German Cancer Research Center (DFKZ), Im Neuenheimer Feld 280, · 69120, Heidelberg, Germany.

26. Public Health Directorate, C/Ciriaco Miguel Virgil 9, 33006, Oviedo, Asturias, Spain

27. Escuela Andaluza de Salud Pública (EASP), Cuesta del Observatorio 4, 18011 Granada, Spain

28. Instituto de Investigación Biosanitaria ibs.GRANADA, Av. de las Fuerzas Armadas 2, 18014 Granada, Spain

29. Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Av. Monforte de Lemos 3-5, 28029 Madrid, Spain.

30. Universidad de Granada, Av. del Hospicio 1, 18012 Granada, Spain

31. Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Ronda de Levante 11, 30008, Murcia, Spain.

32. Cancer Epidemiology Unit, Nuffield Department of Population Health University of Oxford, OX3 7LF,Oxford, United Kingdom.

33. Faculty of Medicine, Imperial College London, Norfolk Place, London W2 1PG, London, UK.

34. Hellenic Health Foundation, Kaisareias 13 & Alexandroupoleos, GR-115 27, Athens, Greece.

35. 2nd Pulmonary Medicine Department, School of Medicine, National and Kapodistrian University of Athens, "ATTIKON" University Hospital, 12462, Haidari, Greece.

36. Epidemiology and Prevention Unit. Fondazione IRCCS Istituto Nazionale dei Tumouri, Via Venezian 1, 20133 Milano-Italy.

37. Unit of Epidemiology, Regional Health Service ASL TO3, 10095 Grugliasco (Turin), Italy.

38. Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Via Pansini 5, 80131, Naples, Italy.

39. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, P.O. Box 85500, 3508 GA Utrecht, The Netherlands.

40. Navarra Public Health Institute, C/Leyre 15, 31003, Pamplona, Spain.

41. Navarra Institute for Health Research (IdiSNA), C/Irunlarrea 3, 31008, Pamplona, Spain.

42. Ministry of Health of the Basque Government, Public Health Division of Gipuzkoa. Biodonostia Research Institute: Paseo Doctor Begiristain s/N, 20014 Donostia/Gipuzkoa, Gipuzkoa, Spain

43. Department of Public Health and Primary Care, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Hills Rd, Cambridge CB2 0SP, United Kingdom.

44. German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Arthur-Scheunert-Allee 114 – 116, 14558 Nuthetal, Germany.

45. Unit of Biomarkers and Susceptibility, Oncology Data Analytics Program, Catalan Institute of Oncology (ICO), Gran Via de L'Hospitalet 199-203, 08908, L'Hospitalet de Llobregat, Barcelona, Spain.

46. ONCOBELL Program, Bellvitge Biomedical Research Institute (IDIBELL), Gran Via de L'Hospitalet 199-203, 08908, L'Hospitalet de Llobregat, Barcelona, Spain.

*These authors contributed equally to this work

**Running title:** Reproductive factors and Urothelial carcinoma

**Abbreviations list:**

UC: Urothelial carcinoma

EPIC: European Prospective Investigation into Cancer and Nutrition Cohort

FTP: Number of full-term pregnancies

MHT: Menopausal hormone therapy

OC: Oral contraceptives

WHI: Women's Health Initiative

CIS: Carcinoma *in situ*

HR: Hazard ratio

CI: Confidence interval

BMI: Body mass index

AIC: Akaike information criterion

LRT: Likelihood ratio test

PAHs: Polycyclic aromatic hydrocarbons

ER: Oestrogen receptors

PR: Progesterone receptors

**Corresponding author**:

Leila Lujan-Barroso, MSc
Unit of Nutrition and Cancer
Cancer Epidemiology Research Program
Cantalan Institute of Oncology (ICO-IDIBELL)
Avda. Gran Via 199-203
08908 L'Hospitalet de Llobregat, Barcelona, Spain
Tel: +34 93 260 7401
Fax: +34 93 260 7787
email: llujan@iconcologia.net
ORCID: 0000-0001-6224-1764

**Conflict of interest:** The authors declare that they have no conflicts of interest.

## Abstract:

**Background:** Urothelial carcinoma (UC) is the predominant (95%) bladder cancer subtype in industrialised nations. Animal and epidemiological human studies suggest that hormonal factors may influence UC risk**.**

**Methods:** We used an analytic cohort of 333 919 women from the European Prospective Investigation into Cancer and Nutrition Cohort (EPIC). Associations between hormonal factors and incident UC (overall and by tumour grade, tumour aggressiveness, and non-muscle invasive UC) risk were evaluated using Cox proportional hazards models.

**Results:** During a mean of 15 years of follow-up, 529 women developed UC. In a model including number of full-term pregnancies (FTP), menopausal status, and menopausal hormone therapy (MHT), number of FTP was inversely associated with UC risk ($HR_{\geq 5 vs 1}$=0.48, 0.25-0.90; *P*-trend in parous women=0.010) and MHT-use (compared to non-use) was positively associated with UC risk (HR=1.27, 1.03-1.57), but no dose-response by years of MHT-use was observed. No modification of HRs by smoking status was observed. Finally, sensitivity analyses in never-smokers showed similar HR patterns for the number of FTP, while no association between MHT-use and UC risk was observed. Association between MHT-use and UC risk only remained significant in current-smokers. No heterogeneity of the risk estimations in the final model was observed by tumour aggressiveness or by tumour grade. A positive association between the MTH-use and non-muscle invasive UC risk was observed.

**Conclusion:** Our results support that increasing the number of FTP may reduce UC risk.

**Impact:** More detailed studies on parity are needed to understand the possible effects of perinatal hormone changes in urothelial cells.

**Key words:** Bladder cancer; menopausal hormone therapy; menstrual and reproductive factors; parity; urothelial carcinoma.

## Introduction:

Bladder cancer is the 12th most common cancer in the world, accounting for 4.8% and 1.5% of incident cancers in men and women, respectively(1). In 2018, the estimated male:female sex ratio in Europe was 4.7 to 1(1). Although, men are at higher risk than women of developing bladder cancer; women present more advanced stages at diagnosis(2). In Europe, the 5-year relative survival rate is 84% in men and 75% in women(3). The predominant bladder cancer subtype is urothelial carcinoma (UC), accounting for 95% of all cases in industrialised nations(4) and almost 71% of men and 63% of women are diagnosed non-muscle invasive UC(2).

Between 50-64% of UC cases in men and 20-50% in women are attributable to tobacco use; and the risk increases with both intensity and duration of smoking(5). Other established risk factors for UC include occupational exposure to aromatic amines and dyes, ingestion of inorganic arsenic via drinking water, a positive family history, and constitutional variants in at least a dozen genes(4,6).

Sex differences in UC incidence may be explained to a large extent by sex differences in the prevalence and intensity of exposure to known risk factors(4). However, after adjusting for these factors differential risk of bladder cancer persists(2). Thus, several studies support that female hormones may have a beneficial effect on UC risk. An experimental animal study that examined the effect of the hormones on oncogenesis in male rat bladders showed that induced incidence of bladder cancer was higher in the group injected with testosterone supplementation than in the group injected with

oestrogen supplementation(7). Moreover, castration of male mice and pregnancy and/or lactation in female mice can decrease the growth of bladder cancer(8). Previous epidemiological studies have reported a reduced risk of UC in parous women compared to nulliparous women(9–12); and an increased risk in postmenopausal women, particularly those with an earlier age at menopause(11,13,14). In general, no associations between age at menarche, use of oral contraceptives (OC), age at first full-term pregnancy, breastfeeding and UC risk were observed(9–19). A meta-analysis by menopausal hormone therapy (MHT) formulation(11), based on four studies, showed a possible reduction in risk of UC in women who used oestrogen plus progestin MHT compared to never users of MHT. Nevertheless, in the Women's Health Initiative (WHI), which included a clinical trial of MHT component and an observational study of MHT component, no such association was observed(18). To our knowledge, previous studies examining the association of reproductive factors with UC risk did not stratified by tumour characteristics (based on tumour grade and tumour stage).

We used a large number of cases (most of them with detailed UC's characteristics) within a large multi-centric prospective study of European women with a long follow-up (15-years) to assess the associations between menstrual factors, reproductive history, use of exogenous hormones, and the risk of developing UC, overall and by tumour grade, tumour aggressiveness, and non-muscle invasive UC, and accounting for smoking status.

## Methods:

### Study design and population

The European Prospective Investigation into Cancer and Nutrition Cohort (EPIC) is an ongoing multicentre cohort study that recruited participants from 23 centres located in

ten European countries. The EPIC study was performed in accordance with the Declaration of Helsinki. All participants signed an informed consent form, and each centre obtained approval from the local Ethics Committee. At recruitment (baseline), information on diet, lifestyle, and anthropometric measurements was collected. Lifestyle questionnaires included questions on education, occupation, medical history, lifetime history of consumption of tobacco, alcoholic beverages, and physical activity. Questionnaires specific to women were used to collect information on menstrual factors, reproductive history, and use of exogenous hormones. Details on the study design have been described previously(20). A total of 521 324 participants were recruited between 1992 and 2000.

Participants with prevalent cancers, except non-melanoma skin cancer, or participants with missing follow-up information were excluded (n=29 332). Only women were eligible for the present analysis (n=343 985). Women with incomplete information on dietary intake or lifestyle or who had extreme or implausible caloric intake (top or bottom 1% of the ratio of energy intake to estimated energy required(21)) were excluded (n=10 066). After these exclusions, the present analysis included 333 919 women.

**Hormonal and reproductive factors**

Self-reported menstrual factors, and exogenous hormone use included: age at menarche (<12, 12, 13, 14, >14 years), history (yes/no) and duration of OC use (non-user, >0-≤1, >1-5, >5-10 years), menopausal status at baseline (premenopausal: ≥9 cycles over the past 12 months, perimenopausal: <9 cycles, natural menopause in case of no menses, and surgical menopause in case of bilateral oophorectomy), age at natural menopause (surgical menopause were excluded, ≤46, 47-49, 50-52, ≥53 years) , age at any menopause (surgical and natural, ≤46, 47-49, 50-52, ≥53 years) , MHT-use (yes/no) and

duration (non-user, >0-≤1.25, >1.25-4, >4 years), type of MHT (oestrogen alone, progestin alone, or oestrogen plus progestin), oophorectomy (yes/no), hysterectomy (yes/no), and calculated cumulative duration of menstrual cycling. Cumulative duration of menstrual cycling (in years) is an accepted proxy for total endogenous exposure and was calculated as follows(14,22): for postmenopausal women, it was the difference between the age at menopause and the age at menarche minus the total time pregnant (number of full-term pregnancies (FTP) x 9 months, due to the absence of menstrual cycles of 9 months for each pregnancy). For pre- and perimenopausal women, cumulative duration of menstrual cycling was the difference between age at recruitment and age at menarche minus the total time pregnant. Total time taking OCs was subtracted from cumulative duration of menstrual cycling for pre-, peri-, and postmenopausal women. To assess for hormonal changes during pregnancy and exogenous hormones through OC use, those models were additionality adjusted for number of FTP and OC-use.

Self-reported reproductive history included: parity (yes/no), number of FTP (including livebirths and stillbirths; 0, 1, 2, 3, 4, ≥5), age at first FTP (in parous women; ≤20, 21-13, 24-25, 26-30, ≥30 years), number of induced (never pregnant, 0, 1, ≥2) and spontaneous abortions (never pregnant, 0, 1, ≥2), breastfeeding (in parous women; yes/no), and duration of breastfeeding (in parous women who breastfeed; 0>-≤3, >3-12, >12 months).

**Bladder cancer assessments**

Incident bladder cancers were identified through population registries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden, and United Kingdom) and active follow-up, including use of health insurance records, hospital registries, and direct contacts with participants or next-of-kin (France, Germany, and Greece). For these analyses, the

follow-up for UC was completed between December 2011 and December 2013, depending on the centre.

Bladder cancers were defined by ICD-O-3, including first invasive cancer (coded C67 based) and UC (morphology codes 812*–813*)(23). Only incident UC was included in the present analyses; since it represents 95% of all bladder cancers. Definitions of UC subtype classifications are heterogeneous in the literature. In previous EPIC studies, UC was classified by pathology reports as aggressive (pT1 and higher or carcinoma *in situ* (CIS) or World Health Organization (WHO) Grade 3), and non-aggressive (pTa Grade 1 and 2)(23). We also analysed UC by tumour grade (using WHO-defined Grades 2 and 3 as "high-grade" and Grade 1 as "low-grade")(24). Finally, in centres where tumour stage information was available (available in all centres except San Sebastian, United Kingdom, Greece, Malmö, and Norway), we analysed UC restricted to non-muscle invasive subtype (pT1, pTa, or CIS).

**Statistical analysis**

To evaluate associations between hormonal factors and UC risk, Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (95%CI). Ordinal variables were scored and trend tests were calculated on these scores, "unknown" category was excluded for trend test calculation. Estimations of "unknown" categories were provided when more than 10% of the cases were classified as "unknown". Age was used as the time scale, with age at recruitment as the entry time, and age at the date of UC or the end of follow-up (whichever came first) as the exit time. Additional models were performed to describe the risk of UC by tumour aggressiveness, tumour grade (using the Wald test statistic to assess the heterogeneity of the risk between outcomes using the SAS macro *%subtype*(25)), and non-muscle invasive UC. All models were stratified by age at recruitment (1 year-categories) and

study centre. Stratified models by center allowed us to give each center its own baseline hazard, thus the variation in menstrual and reproductive history, hormone use, and cancer patterns across centers were included in the model. Further, stratified by age provided left truncation of the data (the risk of developing the outcomes of interest was only included during the follow-up). Finally, these stratified models assumed proportional hazard between the centers. All models were adjusted for smoking status and intensity at baseline (never-smokers, current smokers ≤15 cigarettes/day, current smokers >15 cigarettes/day, ex-smokers ≤10 years, ex-smokers >10 years, current: pipe/cigar/occasional cigarette smokers, current/former: missing intensity, and unknown), and fruit and vegetable intakes (both entered as continuous variable g/d) (4), which change estimate effect of the hormone variables by more than >10%. Physical activity and body mass index (BMI) were not included as adjustment covariates because they did not change effect estimates >10%. Occupations with potential exposure to bladder carcinogens are potential confounder given the established effect of a number of chemicals and substances (e.g. heavy metal, dyes, and polycyclic aromatic hydrocarbons [PAHs]) on sex hormones levels among healthy women(26–28). Other potential confounders were occupations with potential exposure to bladder carcinogens. To adjust models for occupational exposure a dichotomous score (yes/no) was defined, where it was coded as "yes" if the participant worked in occupations with potential exposure to heavy metals (present in foundries, in metal industries, and in occupations related to welding, turning and electroplating), aromatic amines (present in, e.g. dye production, textile and leather dying, and hairdressers), PAHs (associated with refineries, asphalt work, the transport sector, and car repair stations), and environmental tobacco smoking (particularly elevated for workers in bars and restaurants), detailed information in Büchner *et al* (2009)(29). Nevertheless, occupation was ultimately not

included in the multivariable-adjusted models because <7% of women worked in a job/occupation with potential exposure to bladder carcinogens, and adjusting for occupational exposure did not change any estimated HRs. To evaluate all identified factors in one model, mutually-adjusted models were evaluated. The proportional hazard assumption was checked using Schoenfeld residuals. Also, all the time-dependent variables (interactions of predictors and time) were included in the mutually-adjusted model and evaluated. Restricted cubic splines with 3-5 knots were used to explore linearity in the trend in the risk with number of FTP. Akaike information criterion (AIC) was used to select the best representation of the relation between number of FTP (among parous women) and UC risk (Supplemental Figure 1).

Modification of the HRs by tobacco use at baseline (never, former, and current) was evaluated using a likelihood ratio test (LRT). Joint effect variables (with a common referent group) for tobacco with each variable included in the final model were also evaluated.

Sensitivity analyses were performed in never smokers to reduce the likelihood of residual confounding by smoking at baseline. Finally, to address possible changes in the reproductive history during the follow-up, a sensitivity analysis including only women with completed reproductive history (peri-/postmenopausal women at recruitment) was performed for the final model.

All statistical tests were two-sided and evaluated at α-level 0.05. All analyses were performed using SAS v. 9.4 (Cary, North Carolina, USA).

## Results:

**Descriptive statistics**

After a median follow-up time of 15 years, 529 UC cases were identified including 146 non-aggressive tumours, 230 aggressive tumours, and 153 with unknown tumour aggressiveness; and among the 529 cases, there were 80 low-grade tumours, 233 high-grade tumours, and 216 with unknown tumour grade. The median age at recruitment was 51 years (y) ($25^{th}$ and $75^{th}$ percentile (p25-p75): 45-58-y) for the whole cohort and 58-y (p25-p75: 52-63-y) for UC cases. The median age at diagnosis was 68-y (p25-p75: 62-74-y). Baseline characteristics of participants by country are presented in Table 1.

**Menstrual factors, and exogenous hormone use**

Age at menarche, cumulative duration of menstrual cycling, history and duration of OC use, age at natural menopause, oophorectomy, and hysterectomy showed no association with UC risk (Table 2, Table 3). Elevated and statistically significant HRs for UC were observed for postmenopausal status (natural or surgical) compared to premenopausal status ($HR_{postnaturalvspre}$: 1.88; 95%CI, 1.09-3.25; $HR_{postsurgicalvspre}$: 2.15; 95%CI, 1.10-4.20) (Table 1). MHT use in peri-/postmenopausal women (natural or surgical) was positively associated with overall UC independently of the duration of MHT use (Table 3). For the 67% (n=52,892, 82 cases) of women with information on formulation of MHT available, 25% (n=13,123, 32 cases) took oestrogen alone (HR: 1.43; 95%CI: 0.97-2.10). No association was observed for use of oestrogen plus progestin MHT formulations (HR: 1.08; 95%CI, 0.77- 1.51) (Table 3).

**Reproductive factors**

There was a statistically significant inverse association for number of FTP and UC risk ($HR_{3vs1FTP}$: 0.70; 95%CI, 0.52-0.94; $HR_{\geq 5vs1FTP}$: 0.46; 95%CI, 0.25-0.88; *P*-trend in parous women only = 0.008). No statistically significant associations were observed for the other variables in Table 4.

**Mutually-adjusted Cox proportional hazards regression for UC**

Models included number of FTP and menopausal status, where peri-/postmenopausal women were further classified by MHT history. Statistically significant inverse associations between number of FTP and UC risk were observed ($HR_{3vs1FTP}$: 0.70; 95%CI, 0.52-0.94; $HR_{\geq 5vs1FTP}$: 0.48; 95%CI, 0.25-0.90; *P*-trend in parous women only 0.010) (Table 5). Further, the HR for peri-/postmenopausal MHT-users compared to peri-/postmenopausal women never-users was 1.27 (95%CI, 1.03-1.57) (Table 5).

**Study of the heterogeneity of the risk between non-aggressive tumours and aggressive tumours**

MHT-use was positively associated with risk of non-aggressive UC ($HR_{yesvsno}$: 1.93; 95%CI, 1.29- 2.87). Parity was inversely associated with non-aggressive UC risk ($HR_{yesvsno}$: 0.59; 95%CI, 0.39- 0.90). Natural and surgical menopause were statistically significantly associated with risk of aggressive UC ($HR_{naturalvspre}$: 2.47; 95%CI, 1.01-6.03; $HR_{surgicalvspre}$: 3.25; 95%CI, 1.18-8.97) (Supplemental Table 1). Despite these statistically significant individual associations, statistically significant heterogeneity of the risk for menstrual factors and exogenous hormone use by tumour aggressiveness was not observed for each individual model, and for the mutually-adjusted model (all $P_{het}$-value > 0.05).

**Study of the heterogeneity of the risk between low-grade tumours and high-grade tumours**

MHT-use was positively associated with low-grade tumours (HR: 2.37; 95%CI, 1.37-4.12), while the number of spontaneous abortions (comparisons based on 17 women in the referent group) was statistically significant and inversely associated with the risk of low-grade tumours. Parity was inversely associated with low-grade tumours ($HR_{yesvsno}$:

0.44; 95%CI, 0.26- 0.75; comparisons based on 18 women in the referent group). No associations were observed between hormonal factors and high-grade UC risk (Supplemental Table 1).

Statistically significant heterogeneity in the risk estimates by tumour grade was observed in relation to the number of spontaneous abortions ($P_{het}$-value=0.026) and parity ($P_{het}$-value=0.011). Finally, once the identified variables were included in one model, estimations of the risk were similar by tumour grade ($P_{het}$-value=0.079).

**Risk estimation between hormonal and reproductive factors and non-muscle invasive UC**

Positive association was observed between MHT-users and non-muscle invasive UC risk (HR: 1.38; 95%CI, 1.01-1.90), especially in women which treatment's formulation was oestrogen alone (HR: 1.90; 95%CI, 1.15-3.13) (Supplemental Table 1).

**Modification of the HRs by tobacco**

No evidence for modification of HRs for each factor and UC by cigarette smoking status was found (all likelihood ratio statistics *P*-value>0.05) with the exception of induced abortions (*P*-value=0.028). Different estimations of the HR of the number of induced abortions were observed by smoking status. While no association between number of induced abortions and the risk of UC was observed; HR for never smoking women with at least 2 induced abortions compare to 0 abortions was 2.52 (95%CI: 1.33- 4.78, *P*-trend = 0.012) (Supplemental Table 2).

No modification of HRs by cigarette smoking status in the mutually-adjusted model was observed. Nonetheless, the higher risk of MHT-use was only observed in peri-/postmenopausal women (natural or surgical) who were smokers at baseline (HR: 1.56;

95%CI: 1.10, 2.21) (Supplemental Table 3). No statistically significant associations were observed when joint-effect variables for tobacco and FTP, and tobacco and menopausal status were evaluated.

**Sensitivity analyses**

In general, patterns of HRs did not change substantially when we restricted analyses to the subgroup of never smokers (Supplemental Table 2 and Table 5), or in the subgroup of participants who were peri-/postmenopausal at recruitment (Table 5). In never smokers, no association between MHT-use and UC risk was observed in the final mutually adjusted model (Table 5).

## Discussion:

The present analyses based on 529 women, showed evidence that women who had experienced more than one birth are at lower risk of developing UC compared to uniparous women; further, we observed evidence of an inverse trend between UC risk and number of births. No associations were observed for the remaining menstrual factors, reproductive history variables, or exogenous hormone use variables. We observed no evidences of differences in the estimations of UC risk by the number of full-term pregnancies or other menstrual factors, reproductive history factor, or exogenous hormone use according to tumour characteristics (based on tumour grade and tumour stage).

Previous studies(11,12,18) and two meta-analyses(10,17) observed a reduced risk of UC in parous women, independent of the number of births(10,11,13,14,16–18). Nearly all these studies used “nulliparous” as the referent category(11,13,14,16,17). Nulliparous women likely represent a heterogeneous group that includes women with and women

without fertility problems. In our study, “one birth” was used as a referent category, and we found a linear trend of decreasing UC risk with increasing number of FTP. This reduction in risk with increasing FTP was also observed in never-smokers. The observed trend in our study was similar to the trend reported by Weibull et al. (HR for ≥3 vs. 1 FTP: 0.76; 95%CI: 0.68-0.86)(12).

Women experience several hormonal changes during pregnancy, including an increase in oestrogen and progesterone levels(30). An animal study observed that these increased levels, particularly progesterone levels, may be related with changes in the bladder structure related to greater bladder capacity and compliance(31). Further, it has been shown that oestrogen receptors (ER) and progesterone receptors (PR), that mediate oestrogen and progesterone levels, are expressed in both normal and cancerous urothelial cells(32,33). ERs have different roles in cancer biology, in general ER-α has been related with cell growth, while ER-β has been suggested to act as a suppressor of tumour growth, thus ER-α and ER-β may have opposing effects on cellular processes(34). It has been observed that ER-β is the dominant receptor expressed in urothelial carcinoma cells(8,32). Few studies have been done in relation to ERs and progesterone in urothelial carcinoma cells, but it has been suggested that progesterone suppresses ER expression during pregnancy(35). Consequently, it can be hypothesized that these increased levels of oestrogen and progesterone may reduce UC risk in parous women(9–12,17,36).

Two previous studies have examined the association between induced abortions and the risk of UC (15,37). These two case-control studies did not observe that the number of induced abortions was associated with UC risk. Our results on never-smokers were based on a small number of cases, and in view of the large number of associations tested, the association in never-smokers between induced abortion and UC risk may be

due to chance.

It has been hypothesized that earlier age at menopause increases UC risk due to lower levels of oestrogen after menopause(14). Earlier age at menopause (natural or surgical) was associated with an increased risk of UC in a meta-analysis(17), that included 4 case-control studies and 3 cohort studies. We observed no association between earlier age at menopause and UC, in agreement with other recent prospective cohort studies(10,11,18).

The higher UC risk we observed in peri-/postmenopausal MHT users, when compared to peri-/postmenopausal non-users, is inconsistent with previous studies which found no relation(10,17,18). Our results and previous studies showed no dose-response by years of MHT-use(10,11,13,16,18). The WHI found no influence of the formulation of MHT on the risk of UC (results for oestrogen: n=136 cases; HR: 0.93; 95%CI: 0.74-1.17; results for oestrogen plus progestin: n=103 cases; HR: 1.05; 95%CI: 0.81-1.36)(18). A meta-analysis (based on 4 cohort studies) of MHT by formulation (oestrogen or oestrogen plus progestin) showed a 39% decreased UC risk in users of oestrogen plus progestin (n=84 cases; RR: 0.61; 95%CI: 0.47-0.78), and no effect for users of oestrogen alone (n=217 cases; RR: 1.03; 95%CI: 0.87-1.24)(11). Our results, based on smaller sample sizes (52 UC for oestrogen, and 30 UC for oestrogen plus progestin), were in agreement with those from the WHI, however we observed a positively statistically significant estimation in current-smokers who used oestrogen alone or reported unknown type of MHT. Since we observed no association in never-smokers, and the MHT effect (overall and by formulation) only remained significant in current-smokers, residual confounding from tobacco smoking and possible chance are a likely explanation for our MHT results.

Our study strengths include its prospective cohort design and a relatively large number of incident cases from 10 European countries, which allowed us to investigate associations by strata of smoking status. To our knowledge, this is the first study on menstrual factors, reproductive history, hormone use, and UC risk that includes information on tumour classification. However, non-muscle invasive UC classification was not available in San Sebastian, Oxford, Cambridge, Malmö, and Norway centres.

One potential weakness of our analysis is that information on reproductive history and hormone use was available only at cohort enrolment; however, we noted that 78.7% of the cases were postmenopausal at recruitment, so reproductive history was essentially complete for most participants. We performed sensitivity analyses restricted to postmenopausal women, whose reproductive exposures were unlikely to change. We observed similar results for the final mutually-adjusted model in the analysis restricted to postmenopausal women as we observed for all study participants, suggesting our results were unlikely to be affected by any changes in reproductive history after enrolment. Another potential weakness of our study was the large number of missing values in the MHT variables (duration and formulation). Also, information on MHT was not periodically updated, and therefore, we could not evaluate risk in women who started using MHT or who modified their use after enrolment. Further, tumour grade and tumour aggressiveness had a large number of missing values which could bias HR estimates. We would also like to highlight that information on smoking habits, and fruit and vegetables intakes were not periodically updated, so could not evaluate changes after baseline for any variables. Results from the sensitivity analyses in never smoking women showed that, except for MHT, our results were not affected by residual confounding by smoking status. Finally, we could not consider occupational exposure in our analysis, as not all EPIC-centres collected such information. Further, occupational

exposure was available for 32% (n=169) of UC cases; of which 10% (n=17) reported jobs considered at risk. Despite this, a sensitivity analysis was performed including occupational exposures in the final UC model and similar HR estimates for menopausal status, MHT-use, and number of full-term pregnancies were observed.

## Conclusion:

Our results confirm the increasing benefit of each birth after the first on UC risk. More studies on number of FTP are needed to elucidate the putative protective effects of parity. Further investigations of the role of perinatal hormonal changes and how these changes may affect ER and PR levels and urothelial cells in the bladder are needed.

## Additional Information:

**Disclaimer:** Where authors are identified as personnel of the International Agency for Research on Cancer / World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization.

**Funding:** None

**Author's contribution**

LLB, EB, SC, EW, and EJD analyzed and interpreted the data. LLB and EJD wrote the manuscript. BL, NR, AT, BBdM, ITG, RT, LAK, FL, TS, MG, NM, IC, AF,MK, CH, KO, EL, MW, RTF, TK, VM, MJS, CS, APC, RZR, AJC, AT, AK, EP, DP, VK, VS, AM, SP, CHvG, NCOM, AB, PA, KTK, HB, and EW collected the data and provided critical comments on the manuscript.

## Acknowledgments:

We thank CERCA Program / Generalitat de Catalunya for institutional support. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by: Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Compagnia di SanPaolo (Naples, Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Comprehensive Cancer Center The Netherlands (IKNL), Zorg Onderzoek Nederland Medische Wetenschappen (ZONMW), World Cancer Research Fund (WCRF), Dutch Cancer Society (KWF), Statistics Netherlands (The Netherlands), Health Research Fund (FIS) - Instituto de Salud Carlos III (ISCIII), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, and the Catalan Institute of Oncology - ICO (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk, MR/M012190/1 to EPIC-Oxford) (UK). Raul Zamora-Ros would like to thank the "Miguel Servet" program (CP15/00100) from the Institute of Health Carlos III and European Social Fund (ESF). For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php.

## References:


1. Global Cancer Observatory [Internet]. [cited 2018 Oct 23]. Available from: http://gco.iarc.fr/

2. Shariat SF, Sfakianos JP, Droller MJ, Karakiewicz PI, Meryn S, Bochner BH. The effect of age and gender on bladder cancer: a critical review of the literature. BJU Int. 2010;105:300–8.

3. European Cancer Information System [Internet]. [cited 2019 Apr 24]. Available from: https://ecis.jrc.ec.europa.eu/explorer.php?$0-2

4. Malats N, Real FX. Epidemiology of bladder cancer. Hematol Oncol Clin North Am. 2015;29:177–89, vii.

5. Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC. Association between smoking and risk of bladder cancer among men and women. JAMA. 2011;306:737–45.

6. Bladder cancer statistics | World Cancer Research Fund International [Internet]. [cited 2017 Apr 11]. Available from: http://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/bladder-cancer-statistics

7. Tanahashi NK, Suzawa N, Azuma C. Effects of sex hormones on oncogenesis in rat urinary bladder by N-butyl-N-(4-hydroxybutyl)-nitrosamine. Int J Clin Pharmacol Biopharm. 1977;15:101–5.

8. Johnson AM, O'Connell MJ, Messing EM, Reeder JE. Decreased bladder cancer growth in parous mice. Urology. 2008;72:470–3.

9. Huang A-T, Kogevinas M, Silverman DT, Malats N, Rothman N, Tardon A, et al. Bladder cancer and reproductive factors among women in Spain. Cancer Causes Control. 2009;20:1907–13.

10. Davis-Dao CA, Henderson KD, Sullivan-Halley J, Ma H, West D, Xiang Y-B, et al. Lower risk in parous women suggests that hormonal factors are important in bladder cancer etiology. Cancer Epidemiol Biomarkers Prev. 2011;20:1156–70.

11. Daugherty SE, Lacey JV, Pfeiffer RM, Park Y, Hoover RN, Silverman DT. Reproductive factors and menopausal hormone therapy and bladder cancer risk in the NIH-AARP Diet and Health Study. Int J Cancer. 2013;133:462–72.

12. Weibull CE, Eloranta S, Altman D, Johansson ALV, Lambe M. Childbearing and the risk of bladder cancer: a nationwide population-based cohort study. Eur Urol. 2013;63:733–8.

13. McGrath M, Michaud DS, De Vivo I. Hormonal and reproductive factors and the risk of bladder cancer in women. Am J Epidemiol. 2006;163:236–44.

14. Prizment AE, Anderson KE, Harlow BL, Folsom AR. Reproductive risk factors for incident bladder cancer: Iowa Women's Health Study. Int J Cancer. 2007;120:1093–8.

15. Pelucchi C, La Vecchia C, Negri E, Dal Maso L, Franceschi S. Smoking and other risk factors for bladder cancer in women. Prev Med. 2002;35:114–20.

16. Cantwell MM, Lacey JV, Schairer C, Schatzkin A, Michaud DS. Reproductive factors, exogenous hormone use and bladder cancer risk in a prospective study. Int J Cancer. 2006;119:2398–401.

17. Dietrich K, Demidenko E, Schned A, Zens MS, Heaney J, Karagas MR. Parity, early menopause and the incidence of bladder cancer in women: a case-control study and meta-analysis. Eur J Cancer. 2011;47:592–9.

18. Kabat GC, Kim MY, Luo J, Hou L, Cetnar J, Wactawski-Wende J, et al. Menstrual and reproductive factors and exogenous hormone use and risk of transitional cell bladder cancer in postmenopausal women. Eur J Cancer Prev. 2013;22:409–16.

19. Fernandez E, Gallus S, Bosetti C, Franceschi S, Negri E, La Vecchia C. Hormone replacement therapy and cancer risk: a systematic analysis from a network of case-control studies. Int J Cancer. 2003;105:408–12.

20. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002;5:1113–24.

21. Ferrari P, Slimani N, Ciampi A, Trichopoulou A, Naska A, Lauria C, et al. Evaluation of under- and overreporting of energy intake in the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr. 2002;5:1329–45.

22. al DE et. Menstrual and reproductive factors, exogenous hormone use, and gastric cancer risk in a cohort of women from the European Prospective Investigation... - PubMed - NCBI [Internet]. [cited 2018 Jan 9]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/?term=duell+gastric+cancer+hormones

23. Roswall N, Freisling H, Bueno-de-Mesquita HB, Ros M, Christensen J, Overvad K, et al. Anthropometric measures and bladder cancer risk: a prospective study in the EPIC cohort. Int J Cancer. 2014;135:2918–29.

24. Compérat EM, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, et al. Grading of Urothelial Carcinoma and The New "World Health Organisation Classification of Tumours of the Urinary System and Male Genital Organs 2016." Eur Urol Focus. 2018;5:457–66.

25. Wang M, Spiegelman D, Kuchiba A, Lochhead P, Kim S, Chan AT, et al. Statistical Methods for Studying Disease Subtype Heterogeneity. Stat Med. 2016;35:782–800.

26. Nagata C, Wada K, Tsuji M, Hayashi M, Takeda N, Yasuda K. Association of hair dye use with circulating levels of sex hormones in premenopausal Japanese women. Eur J Public Health. 2015;25:895–9.

27. Yin S, Tang M, Chen F, Li T, Liu W. Environmental exposure to polycyclic aromatic hydrocarbons (PAHs): The correlation with and impact on reproductive hormones in umbilical cord serum. Environ Pollut. 2017;220:1429–37.

28. Pollack AZ, Schisterman EF, Goldman LR, Mumford SL, Albert PS, Jones RL, et al. Cadmium, Lead, and Mercury in Relation to Reproductive Hormones and Anovulation in Premenopausal Women. Environ Health Perspect. 2011;119:1156–61.

29. Büchner FL, Bueno-de-Mesquita HB, Ros MM, Kampman E, Egevad L, Overvad K, et al. Consumption of vegetables and fruit and the risk of bladder cancer in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2009;125:2643–51.

30. Modugno F, Laskey R, Smith AL, Andersen CL, Haluska P, Oesterreich S. Hormone response in ovarian cancer: time to reconsider as a clinical target? Endocr Relat Cancer. 2012;19:R255–79.

31. Rodrìguez LV, Wang B, Shortliffe LMD. Structural changes in the bladder walls of pregnant and hormone-treated rats: correlation with bladder dynamics. BJU Int. 2004;94:1366–72.

32. Shen SS, Smith CL, Hsieh J-T, Yu J, Kim IY, Jian W, et al. Expression of estrogen receptors-alpha and -beta in bladder cancer cell lines and human bladder tumor tissue. Cancer. 2006;106:2610–6.

33. Blakeman PJ, Hilton P, Bulmer JN. Oestrogen and progesterone receptor expression in the female lower urinary tract, with reference to oestrogen status. BJU Int. 2000;86:32–8.

34. Thomas C, Gustafsson J-Å. The different roles of ER subtypes in cancer biology and therapy. Nat Rev Cancer. 2011;11:597.

35. Batra SC, Iosif CS. Progesterone receptors in the female lower urinary tract. J Urol. 1987;138:1301–4.

36. Bai Y, Wang X, Yang Y, Tang Y, Wang J, Han P. Parity and bladder cancer risk: a dose-response meta-analysis. BMC Cancer [Internet]. 2017 [cited 2017 May 31];17. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5219774/

37. La Vecchia C, Negri E, Franceschi S, Parazzini F. Long-term impact of reproductive factors on cancer risk. Int J Cancer. 1993;53:215–9.

Table 1: Baseline characteristics of women in the EPIC cohort by country

| | Cohort (n= 333 919) | France (n= 67 403) | Italy (n= 30 513) | Spain (n= 24 850) | United Kingdom (n= 52 566) | The Netherlands (n= 26 912) | Greece (n= 15 233) | Germany (n= 27 379) | Sweden (n= 26 368) | Denmark (n= 28 720) | Norway (n= 33 975) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Urothelial Carcinoma cases** | 529 | 40 | 72 | 32 | 68 | 80 | 7 | 25 | 105 | 80 | 20 |
| **Age at recruitment(years)[a]** | 51 (45- 58) | 51 (47- 57) | 51 (44- 57) | 48 (41- 55) | 48 (36- 58) | 53 (46- 59) | 54 (43- 64) | 48 (41- 57) | 51 (47- 60) | 56 (53- 60) | 48 (44- 52) |
| **Age at diagnosis(years)[a]** | 68 (62- 74) | 65 (60- 71) | 65 (59- 71) | 64 (57- 71) | 63 (52- 73) | 67 (59- 73) | 65 (54- 75) | 59 (52- 67) | 69 (60- 78) | 72 (68- 76) | 61 (58- 65) |
| **Body mass index(kg/m$^2$)[a]** | 24.1 (21.9- 27.2) | 22.5 (20.8- 24.7) | 25.0 (22.6- 27.9) | 27.5 (24.7- 30.9) | 23.4 (21.4- 26.1) | 24.5 (22.3- 27.3) | 28.2 (24. 8- 31.6) | 24.7 (22.3- 28.0) | 24.1 (21. 9- 27.0) | 24.8 (22.5- 27.8) | 23.8 (21.8- 26.2) |
| **Physical activity [b]** | | | | | | | | | | | |
| **Inactive** | 73 114 (21.9) | 12 623 (18.7) | 11 201 (36.7) | 12 071 (48.6) | 12 581 (23.9) | 1 897 (7.1) | 8 157 (53.6) | 4 756 (17.4) | 5 532 (21.0) | 3 050 (10.6) | 1 246 (3.7) |
| **Moderately inactive** | 113 292 (33.9) | 26 969 (40.0) | 11 940 (39.1) | 8 745 (35.2) | 18 867 (35.9) | 6 410 (23.8) | 3 997 (26.2) | 10 378 (37.9) | 9 480 (36.0) | 9 235 (32.2) | 7 271 (21.4) |
| **Moderately active** | 90 980 (27.3) | 21 813 (32.4) | 4 557 (14.9) | 2 983 (12.0) | 12 075 (23.0) | 6 480 (24.1) | 2 460 (16.2) | 7 110 (26.0) | 6 912 (26.2) | 7 148 (24.9) | 19 442 (57.2) |
| **Active** | 50 782 (15.2) | 5 998 (8.9) | 2 815 (9.2) | 1 051 (4.2) | 8 056 (15.3) | 9 399 (34.9) | 619 (4.1) | 5 129 (18.7) | 4 400 (16.7) | 9 265 (32.3) | 4 050 (11.9) |
| **Smoking status and intensity[b]** | | | | | | | | | | | |
| **Never** | 161 061 (48.2) | 25 164 (37.3) | 12 657 (41.5) | 17 740 (71.4) | 31 544 (60.0) | 10 938 (40.6) | 1 1101 (72.9) | 15 333 (56.0) | 12 436 (47.2) | 12 563 (43.7) | 11 585 (34.1) |
| **Current ≤15 cigarettes/day** | 40 802 (12.2) | 2 971 (4.4) | 4 611 (15.1) | 2 950 (11.9) | 3 675 (7.0) | 4 435 (16.5) | 1 425 (9.4) | 3 491 (12.8) | 4 482 (17.0) | 5 978 (20.8) | 6 784 (20.0) |
| **Current >15 cigarettes/day** | 21 318 (6.4) | 1 924 (2.9) | 3 360 (11.0) | 1 660 (6.7) | 1 409 (2.7) | 2 540 (9.4) | 1 162 (7.6) | 1 467 (5.4) | 1 512 (5.7) | 2 954 (10.3) | 3 330 (9.8) |
| **Former quit ≤ 10 years** | 27 394 (8.2) | 3 628 (5.4) | 2 959 (9.7) | 1 473 (5.9) | 4 887 (9.3) | 3 011 (11.2) | 478 (3.1) | 2 363 (8.6) | 2 349 (8.9) | 2 322 (8.1) | 3 924 (11.6) |
| **Former quit >10 years** | 44 918 (13.5) | 8 581 (12.7) | 3 188 (10.5) | 936 (3.8) | 8 977 (17.1) | 5 215 (19.4) | 298 (2.0) | 4 361 (15.9) | 3 482 (13.2) | 4 268 (14.9) | 5 612(16.5) |
| **Current, pipe/cigar/ occasional cigarette smokers** | 27 610 (8.3) | 21 818 (32.4) | 3 719 (12.2) | 13 (0.1) | 145 (0.3) | 46 (0.2) | 44 (0.3) | 21 (0.1) | 1 672 (6.3) | 68 (0.2) | 64 (0.2) |
| **Current/Former, missing** | 4 854 (1.5) | 1 312 (2.0) | 18 (0.1) | 66 (0.3) | 907 (1.7) | 633 (2.4) | 46 (0.3) | 294 (1.1) | 310 (1.2) | 505 (1.8) | 763 (2.3) |
| **Vegetables intake(g/day)[a]** | 186 (118-286) | 264 (189-356) | 162 (109-232) | 216 (138-315) | 256 (186-347) | 127 (98-162) | 412 (317-527) | 117 (89-156) | 119 (70-184) | 172 (112-244) | 126 (87-179) |
| **Fruit intake(g/day)[a]** | 216 (125-332) | 242 (153-339) | 320 (221-443) | 286 (176-436) | 229 (143-345) | 195 (123-288) | 344 (244-457) | 126 (92-204) | 179 (114-269) | 172 (100-276) | 138 (79-219) |
| **Job exposure [b, c, d], yes** | 6 920 (6.4) | | | 1 177 (4.7) | 599 (5.2) | | 465 (3.1) | 2 479 (9.1) | | 2 200 (7.7) | 6 920 (6.4) |
| **Diabetes [b], yes** | 7 422 (2.4) | 1 379 (2.1) | 633 (2.1) | 1 124 (4.5) | 633 (1.7) | 581 (2.2) | 1 016 (6.7) | 775 (2.8) | 445 (1.8) | 430 (1.5) | 406 (1.5) |

Numbers may not sum to totals due to missing values

[a] Median (percentile 25$^{th}$ and percentile 75$^{th}$) // [b] n (%) // [c] Available in Spain, Cambridge, Greece, Germany, Denmark, and Norway // [d] Job exposure was coded as "yes" if the participant worked in jobs with potential exposure to heavy metals, aromatic amines, polycyclic aromatic hydrocarbons, and environmental tobacco smoke.

Table 2: Multivariable-adjusted models for each individual menstrual factor in relation to UC risk in EPIC Women.

| | Person-years | Cases (%) n=529 | HR (95%CI) [a] | *P*-trend |
|---|---|---|---|---|
| **Age at menarche, years** | | | | |
| **<12** | 678 236 | 64 (12.1) | 1.00 (referent) | 0.845 |
| **12** | 955 271 | 103 (19.5) | 1.10 (0.80- 1.51) | |
| **13** | 1 166 665 | 128 (24.2) | 1.05 (0.78- 1.43) | |
| **14** | 976 383 | 108 (20.4) | 0.92 (0.67- 1.26) | |
| **>14** | 718 342 | 113 (21.4) | 1.07 (0.78- 1.48) | |
| **Cumulative duration of menstrual cycling, accounting for OC use, years [b]** | | | | |
| | | | | |
| **<23** | 960 018 | 72 (13.6) | 1.00 (referent) | 0.924 |
| **23- <30** | 693 105 | 96 (18.2) | 1.01 (0.73- 1.39) | |
| **30- <35** | 920 740 | 108 (20.4) | 0.87 (0.63- 1.21) | |
| **≥35** | 805 979 | 142 (26.8) | 1.00 (0.71- 1.40) | |
| **Unknown** | 1 011 360 | 111 (21.0) | 1.05 (0.74- 1.48) | |
| **Menopausal status** | | | | |
| **Premenopausal** | 1 654 703 | 49 (9.3) | 1.00 (referent) | |
| **Perimenopausal** | 896 065 | 64 (12.1) | 1.32 (0.77- 2.8) | |
| **Natural postmenopausal** | 1 992 700 | 394 (74.5) | 1.88 (1.09- 3.25) | |
| **Surgical postmenopuasal** | 117 733 | 22 (4.2) | 2.15 (1.10- 4.20) | |
| **Age at natural menopause, years [c]** | | | | |
| **≤46** | 385 834 | 85 (21.6) | 1.17 (0.87- 1.58) | 0.527 |
| **47- 49** | 337 177 | 68 (17.3) | 1.08 (0.79- 1.48) | |
| **50 - 52** | 509 460 | 97 (24.6) | 1.00 (referent) | |
| **≥53** | 305 850 | 79 (20.1) | 1.33 (0.99- 1.80) | |
| **Unknown** | 454 379 | 65 (16.5) | 1.21 (0.86- 1.70) | |
| **Age at any menopause, years** | | | | |
| **≤46** | 450 220 | 100 (24.0) | 1.21 (0.91- 1.60) | 0.853 |
| **47- 49** | 360 268 | 70 (16.8) | 1.04 (0.76- 1.42) | |
| **50 - 52** | 527 478 | 101 (24.3) | 1.00 (referent) | |
| **≥53** | 315 160 | 80 (19.6) | 1.31 (0.97- 1.77) | |
| **Unknown** | 457 307 | 65 (15.6) | 1.20 (0.86- 1.68) | |
| **Oophorectomy [d]** | | | | |
| **No** | 3 407 081 | 344 (76.1) | 1.00 (referent) | |
| **Unilateral** | 145 533 | 28 (6.2) | 1.32 (0.90- 1.95) | |
| **Bilateral** | 131 175 | 23 (5.1) | 1.12 (0.73- 1.72) | |
| **Unknown** | 965 580 | 55 (12.2) | 0.91 (0.47- 1.78) | |
| **Hysterectomy [d]** | | | | |
| **No** | 3 640 275 | 344 (76.1) | 1.00 (referent) | |
| **Yes** | 472 260 | 76 (16.8) | 1.09 (0.84- 1.40) | |

UC: Urothelial Carcinoma // OC: oral contraceptive // Numbers may not sum to totals due to missing values

Estimation of "Unknown" category is provided when more than 10% of the cases are classified as "Unknown".

[a] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake.

[b] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake, OC use, and full-term pregnancies

[c] Women who had surgical menopause were excluded.

[d] Available in all centres except Malmö.

Table 3: Multivariable-adjusted models for each individual exogenous hormone use in relation to UC risk in EPIC Women.

| | Person-years | Cases (%) n=529 | HR (95%CI) [a] | *P*-trend |
|---|---|---|---|---|
| **Use of OC** | | | | |
| **No** | 1 859 302 | 278 (52.6) | 1.00 (referent) | |
| **Yes** | 2 668 828 | 239 (45.2) | 0.93 (0.77- 1.14) | |
| **Unknown** | 133 072 | 12 (2.3) | | |
| **Duration OC use, years** | | | | |
| **No** | 1 859 302 | 278 (52.6) | 1.00 (referent) | 0.259 |
| **>0- ≤1** | 495 753 | 34 (6.4) | 0.70 (0.49- 1.01) | |
| **>1- 5** | 780 263 | 63 (11.9) | 0.94 (0.71- 1.26) | |
| **>5- 10** | 594 859 | 69 (13.0) | 1.22 (0.92- 1.63) | |
| **>10** | 546 567 | 51 (9.6) | 0.82 (0.59- 1.13) | |
| **Unknown duration** | 251 386 | 22 (4.2) | | |
| **Missing use of OC** | 133 072 | 12 (2.3) | | |
| **Use of MHT [b]** | | | | |
| **No** | 1 740 862 | 247 (51.5) | 1.00 (referent) | |
| **Yes** | 1 072 357 | 172 (35.8) | 1.28 (1.04- 1.58) | |
| **Unknown** | 193 278 | 61 (12.7) | 1.32 (0.90- 1.95) | |
| **Duration MHT use, years [b]** | | | | |
| **No** | 1 740 862 | 247 (51.5) | 1.00 (referent) | 0.152 |
| **>0- ≤1.25** | 321 348 | 51 (10.6) | 1.33 (0.98- 1.81) | |
| **>1.25-4** | 336 578 | 47 (9.8) | 1.37 (0.99- 1.90) | |
| **>4** | 310 366 | 56 (11.7) | 1.27 (0.93- 1.73) | |
| **Unknown duration** | 104 065 | 18 (3.8) | | |
| **Unknown use of MHT** | 193 278 | 61 (12.7) | 1.03 (0.74- 1.43) | |
| **Type of MHT [b, c]** | | | | |
| **Non-users of MHT** | 1 527 202 | 215 (58.0) | 1.00 (referent) | |
| **Oestrogen alone** | 178 339 | 32 (8.6) | 1.43 (0.97- 2.10) | |
| **Oestrogen + Progestin** | 527 153 | 50 (13.5) | 1.08 (0.77- 1.51) | |
| **Unknown type of MHT** | 329 620 | 74 (20.0) | 1.37 (1.04- 1.81) | |

UC: Urothelial Carcinoma // OC: oral contraceptive // MHT: menopause hormone therapy

Estimation of "Unknown" category is provided when more than 10% of the cases are classified as "Unknown".

a Cox proportional hazards model stratified by centre and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake.

[b] In peri- and postmenopausal (natural or surgical).

[c] Available in France, Italy, Spain, United Kingdom, The Netherlands, Germany, Denmark, and Norway.

Table 4: Multivariable-adjusted models for each individual reproductive factor in relation to UC risk in EPIC Women.

| | Person-years | Cases (%) n=529 | HR (95%CI)[a] | *P*-trend |
|---|---|---|---|---|
| **Parity** | | | | |
| **No** | 686 624 | 73 (13.8) | 1.00 (referent) | |
| **Yes** | 3 774 138 | 440 (83.2) | 0.87 (0.68- 1.12) | |
| **Number of full-term pregnancies**[b] | | | | |
| **0**[c] | 686 624 | 69 (13.5) | 0.92 (0.67- 1.25) | 0.008[d] |
| **1** | 663 853 | 99 (19.4) | 1.00 (referent) | |
| **2** | 1 787 539 | 192 (37.6) | 0.80 (0.62- 1.02) | |
| **3** | 845 995 | 89 (17.4) | 0.70 (0.52- 0.94) | |
| **4** | 253 868 | 35 (6.9) | 0.79 (0.53- 1.18) | |
| **≥5** | 110 467 | 11 (2.2) | 0.47 (0.25- 0.88) | |
| **Age at first full-term pregnancy, years**[d] | | | | |
| **≤20** | 546 150 | 68 (15.5) | 1.00 (referent) | 0.688 |
| **21- 23** | 1 001 554 | 119 (27.1) | 1.03 (0.76- 1.40) | |
| **24- 25** | 742 124 | 73 (16.6) | 0.86 (0.61- 1.20) | |
| **26- 30** | 1 086 162 | 139 (31.6) | 1.03 (0.76- 1.39) | |
| **≥30** | 382 435 | 40 (9.1) | 0.89 (0.59- 1.32) | |
| **Breastfeeding**[d, e] | | | | |
| **No** | 523 624 | 57 (14.1) | 1.00 (referent) | |
| **Yes** | 2 984 829 | 341 (83.8) | 0.85 (0.64- 1.14) | |
| **Duration of breastfeeding, all pregnancies, months**[e, f] | | | | |
| **>0-≤3** | 854 602 | 115 (33.7) | 1.00 (referent) | 0.092 |
| **>3- 12** | 1 327 975 | 142 (41.6) | 0.73 (0.56- 0.95) | |
| **>12** | 771 517 | 79 (23.2) | 0.78 (0.55- 1.09) | |
| **Induced abortions**[g] | | | | |
| **Never pregnant** | 483 030 | 48 (12.4) | 1.19 (0.91- 1.56) | 0.759 |
| **0** | 2 466 069 | 269 (69.7) | 1.00 (referent) | |
| **1** | 404 767 | 45 (11.7) | 1.12 (0.81- 1.56) | |
| **≥2** | 176 646 | 19 (4.9) | 1.01 (0.62- 1.64) | |
| ***P*-trend** | | | | |
| **Spontaneous abortions**[h] | | | | |
| **Never pregnant** | 508 626 | 56 (12.1) | 1.14 (0.85- 1.52) | 0.497 |
| **0** | 2 469 123 | 295 (63.7) | 1.00 (referent) | |
| **1** | 587 558 | 78 (16.9) | 1.10 (0.86- 1.42) | |
| **≥2** | 200 186 | 27 (5.8) | 1.05 (0.71- 1.56) | |
| **Infertility problems**[i] | | | | |
| **No** | 2 872 888 | 255 (83.3) | 1.00 (referent) | |
| **Yes** | 142 531 | 16 (5.2) | 1.61 (0.97- 2.69) | |
| **Unknown** | 151 702 | 35 (11.4) | 1.72 (0.24- 12.51) | |

UC: Urothelial Carcinoma // Numbers may not sum to totals due to missing values

Estimation of "Unknown" category is provided when more than 10% of the cases are classified as "Unknown".

a Cox proportional hazards model stratified by centre and age at recruitment and adjusted by smoking status and intensity, fruits and vegetables intake.

[b] Available in all centres except Bilthoven.

[c] Including nulliparous women and women without full-term pregnancies.

[d] In parous women.

[e] Available in all centres except Bilthoven and Umeå.

[f] In parous women who has ever breastfed.

[g] Available in all centres except Bilthoven, Malmö, Umeå, and Norway.

[h] Available in all centres except Bilthoven, Umeå, and Norway.

[i] Available in France, Italy, Spain, United Kingdom, Utrecht, Greece, and Germany.

Table 5: Mutually-adjusted models for menopause status, MHT, and parity in relation to UC risk in EPIC women.

| | Overall | | | Never smokers | | | Postmenopausal | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cases (%) n=529** | **HR (95%CI)[a]** | ***P*-trend** | **Cases (%) n=195** | **HR (95%CI)[b]** | ***P*-trend** | **Cases (%) n=195** | **HR (95%CI)[b]** | ***P*-trend** |
| **Menopausal status & use of MHT** | | | | | | | | | |
| **Premenopausal** | 49 (9.26) | 0.73 (0.43- 1.22) | | 18 (9.23) | 1.23 (0.52- 2.43) | | | | |
| **Peri-/Postmenopausal & non-users of MHT** | 247 (46.7) | 1.00 (referent) | | 105 (53.9) | 1.00 (referent) | | 247 (51.5) | 1.00 (referent) | |
| **Peri-/Postmenopausal & users of MHT** | 172(32.5) | 1.27 (1.03- 1.57) | | 52 (26.7) | 1.02 (0.71- 1.47) | | 172 (35.8) | 1.28 (1.04- 1.59) | |
| **Peri-/Postmenopausal & unknown MHT-use** | 61 (11.5) | 1.35 (0.88- 2.07) | | 20 (10.26) | 1.12 (0.53- 2.39) | | 61 (12.7) | 1.34 (0.89- 2.02) | |
| **Number of full-term pregnancies [c]** | | | | | | | | | |
| **0 [d]** | 69 (13.5) | 0.92 (0.67- 1.25) | 0.010[e] | 19 (9.7) | 0.72 (0.40- 1.29) | 0.069[e] | 66 (14.1) | 1.03 (0.73- 1.39) | 0.008 [e] |
| **1** | 99 (19.4) | 1.00 (referent) | | 32 (16.4) | 1.00 (referent) | | 88 (18.8) | 1.00 (referent) | |
| **2** | 192 (37.6) | 0.80 (0.62- 1.02) | | 83 (42.6) | 0.95 (0.63- 1.45) | | 171 (36.5) | 0.79 (0.61- 1.03) | |
| **3** | 89 (17.4) | 0.70 (0.52- 0.94) | | 39 (20.0) | 0.85 (0.52- 1.37) | | 82 (17.5) | 0.71 (0.52- 0.97) | |
| **4** | 35 (6.9) | 0.80 (0.54- 1.19) | | 9 (4.6) | 0.57 (0.27- 1.21) | | 35 (7.5) | 0.85 (0.57- 1.27) | |
| **≥5** | 11 (2.2) | 0.48 (0.25- 0.90) | | 5 (2.6) | 0.49 (0.18- 1.29) | | 11 (2.4) | 0.51 (0.27- 0.97) | |

UC: Urothelial Carcinoma // MHT: menopausal hormone therapy // Numbers may not sum to totals due to missing values
Estimation of "Unknown" category is provided when more than 10% of the cases are classified as "Unknown".

[a] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by menopausal status and MHT, number of full-term pregnancies, smoking status and intensity, fruits and vegetables intake.

[b] Cox proportional hazards model stratified by centre and age at recruitment and adjusted by menopausal status and MHT, number of full-term pregnancies, fruits and vegetables intake.

[c] Available in all centres have information except Bilthoven.

[d] Including nulliparous women and women without full-term pregnancies.

[e] In parous women