Menopausal hormone therapy and risk of biliary tract cancers

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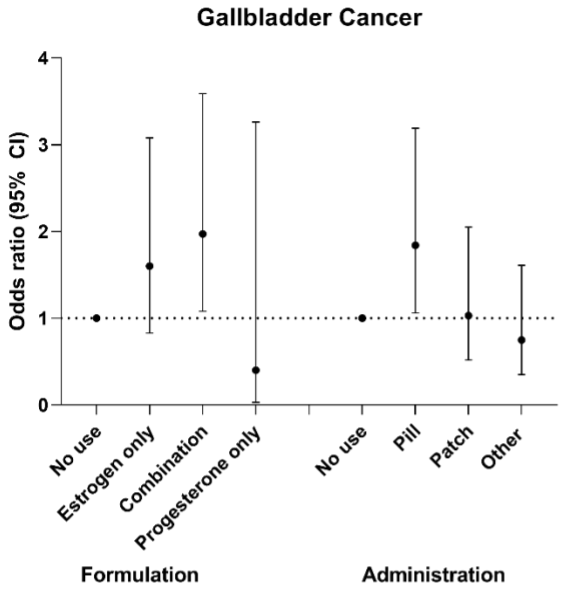
Abstract

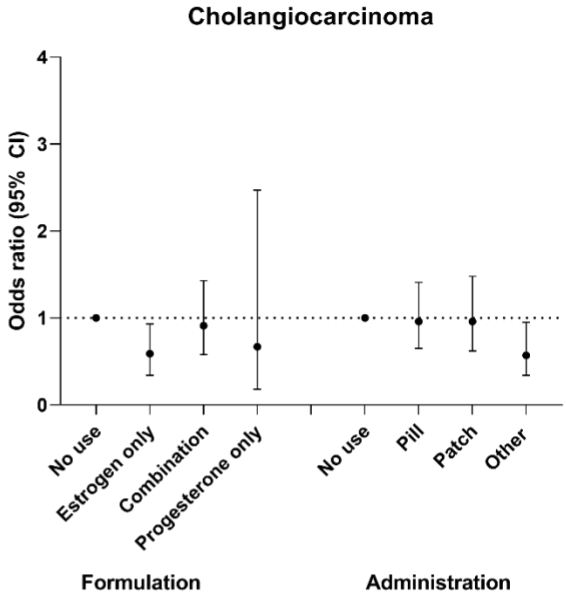
Background: Gallbladder cancer (GBC) has a female predominance, while the other biliary tract cancers (BTC) have a male predominance, suggesting sex hormones may be involved in carcinogenesis. We sought to evaluate the association between menopausal hormone therapy (MHT) and the risk of BTC in women.

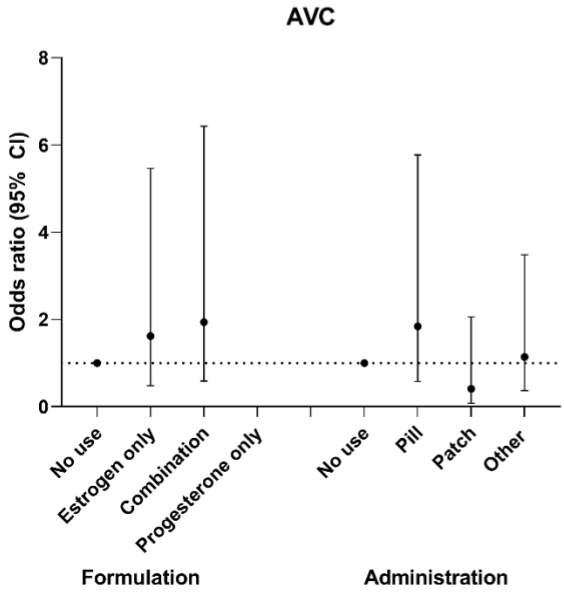
Methods: This nested case-control study was conducted in the UK Clinical Practice Research Datalink. Cases diagnosed between 1990-2017 with incident primary cancers of the gallbladder (GBC), cholangiocarcinoma, ampulla of Vater (AVC), and mixed type were matched to five controls on birth year, diagnosis year, and years in the general practice using incidence density sampling. Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for associations between MHT use and BTC type.

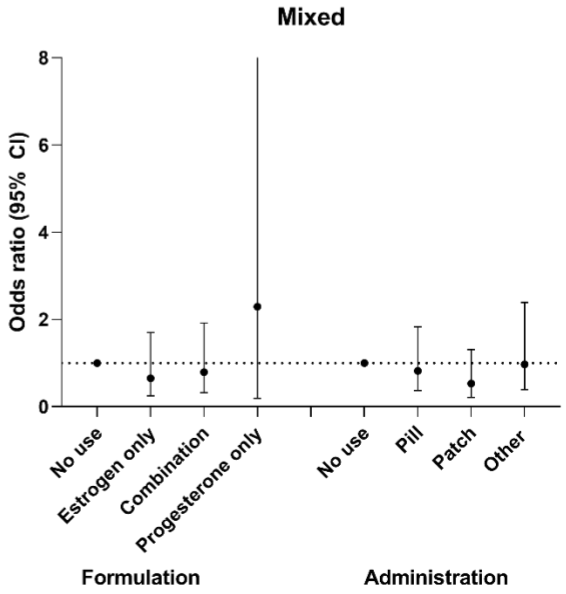
Results: The sample consisted of 1,682 BTC cases (483 GBC, 870 cholangiocarcinoma, 105 AVC, and 224 mixed) and 8,419 matched controls with a mean age of 73 (standard deviation: 11) years. Combined formulations (estrogen-progesterone) were associated with an increased GBC risk (OR: 1.97; 95% CI: 1.08, 3.59). Orally administered MHT was associated with an increased GBC risk (OR: 2.28, 95% CI: 1.24, 4.17). Estrogen-only formulations (OR: 0.59; 95% CI: 0.34, 0.93) and cream or suppository administrations (OR: 0.57; 95% CI: 0.34, 0.95) were associated with decreased cholangiocarcinoma risk. The number of prescriptions, dose, duration of use, and time since last use were not associated with GBC or cholangiocarcinoma risk. MHT use was not associated with risk of AVC or mixed cancer.

Conclusion: Combination MHT formulations and oral administrations are associated with increased GBC risk, while estrogen-only formulations were associated with a lower cholangiocarcinoma risk. MHT formulation and administration should be carefully considered when prescribing.


Gallbladder Cancer
Odds ratio (95% CI)
0
1
2
3
4
No use
Estrogen only
Combination
Progesterone only
No use
Pill
Patch
Other
Formulation
Administration


Cholangiocarcinoma
Odds ratio (95% CI)
0
1
2
3
4
No use
Estrogen only
Combination
Progesterone only
No use
Pill
Patch
Other
Formulation
Administration


AVC
Odds ratio (95% CI)
0
2
4
6
8
No use
Estrogen only
Combination
Progesterone only
No use
Pill
Patch
Other
Formulation
Administration


Mixed
Odds ratio (95% CI)
0
2
4
6
8
No use
Estrogen only
Combination
Progesterone only
No use
Pill
Patch
Other
Formulation
Administration

Background

Biliary tract cancers (BTCs) are rare, but highly fatal and aggressive tumors arising from the biliary epithelium, encompassing cancers of the gallbladder (GBC), intra- and extrahepatic bile ducts (cholangiocarcinoma), and ampulla of Vater (AVC). Overall, the estimated five-year survival rate for GBC is 19% and for cholangiocarcinoma is 17.6 %.[1,2] The etiology of BTCs are not well understood, but they are thought to be separate and epidemiologically distinct cancers given the different embryological origin of the biliary tract epithelia.[3] GBC is one of the only cancers with a female predominance, while the other cancers of the biliary tract have a male predominance.[4] This sex disparity suggests that sex hormones may be involved in carcinogenesis.

The sex steroid hormone estrogen has a lithogenic effect, increasing hepatic output of newly synthesized cholesterol, resulting in increased transfer of cholesterol to bile.[5] Progesterone contributes to biliary stasis by impairing the smooth muscle contractility of the biliary tract resulting in reduced emptying of the gallbladder.[6-8] Together, elevation of these sex steroid hormones can result in the formation of cholesterol gallstones, which greatly increase the risk of GBC.[4,9] Further, estrogen receptors (ERα, ERβ) and progesterone receptors (PR) are present in healthy gallbladder epithelial cells, but their overexpression has been observed in GBC cases compared to those with gallstones only.[10-13] Both ERs are physiologically present in cholangiocytes, and their expression, especially ERβ, increases during proliferation.[14,15]

Several studies have shown that female reproductive factors such as parity and the number of reproductive years are associated with increased risks of GBC and intrahepatic cholangiocarcinoma.[16-22] However research on exogenous hormone use has produced conflicting results. Two randomized clinical trials,[23,24] demonstrated that the use of menopausal hormone therapy (MHT) was associated with an increased risk of symptomatic gallbladder disease and cholecystectomy.[25,26] However, other results from research examining the association between MHT use and BTCs have been mixed.[18,27-30] Further, some of these studies were conducted in small samples[27,28] or were not able to examine associations by cancer site

in the biliary tract.[28,30] Therefore, we sought to examine the relationship between MHT use and cancer along the biliary tract in a large primary care database.

Methods

This analysis used two linked databases, the United Kingdom (UK) Clinical Practice Research Datalink (CPRD) Gold and Hospital Episode Statistics Admitted Patient Care (HES APC). Established in 1987, CPRD Gold contains the primary care data from ~8.5% of the UK population and is representative of the general UK population with regards to age and sex.[31] These data include detailed patient demographics, tests conducted, and medications prescribed. Clinical diagnoses, physical findings, symptoms, and specialist referrals are recorded by general practitioners using Read codes, a standard clinical terminology used in the UK.[31] The validity of recorded health data in CPRD has been verified by several studies, which indicate that recorded clinical diagnoses are complete and accurate, with >90% of primary cancers confirmed.[32] HES APC contains records of all inpatient hospital admissions for patients at NHS hospitals in England. Primary and secondary diagnoses were coded using International Statistical Classification of Diseases, Tenth Revision) and procedures were coded using Classification of Interventions and Procedures from the Office of Population Census and Surveys (OPCS) version 4.4. The HES database linkage was initiated April 1, 1997 and is limited to English practices, representing ~50% of all CPRD practices.[31]

This study is based on data from the November 2017 CPRD Gold database release and the HES database (Linkage Set 20), obtained from the UK Medicines and Healthcare Products Regulatory Agency database, reused with the permission of The Health & Social Care Information Centre. The interpretation and conclusions of this analysis are those of the authors alone. The study was approved by the Independent Scientific Advisory Committee of the CPRD (proposal#17_160.R).

We used a nested case-control study design to sample cases and controls from all women whose most recent active registration at a CPRD Gold practice overlapped with the study period from 1990 (three years after the establishment of the database) through 2017. Eligible cases included women with a

first, primary BTC diagnosis without a prior history of cancer (except non-melanoma skin cancer) (Read codes provided in **Supplemental Table 1**) identified in CPRD Gold. The index date was defined as the date of BTC diagnosis date minus one year. We excluded the 1-year period prior to the index date from the exposure assessment to minimize the possibility of reverse causation (i.e., incipient cancer affecting MHT use). All cases were required to have at least two years of recorded activity in the CPRD prior to the date of cancer diagnosis. We excluded women younger than 45 years and older than 95 years at the index date and cases with unknown cancer site information.

Five female controls per case were randomly selected using incidence density sampling. Controls were individually matched on year of birth (±3 years), index year (±3 years), year of registration in the practice, and in the CPRD prior to the case's index date (one year prior to case's diagnosis date)). All controls were required to be alive, cancer-free (except for non-melanoma skin cancer), and have at least two years of recorded activity in the CPRD prior to their case's diagnosis date, Controls to GBC cases were excluded if they had a history of cholecystectomy in the CPRD record.

MHT exposure was defined as having two or more MHT prescriptions recorded in the patient's medical record at least one year prior to the participant's index date. Women were defined as non-users if they had no or only one recorded prescription in the medical record. A prescription was defined in 28 day increments so that non-pill forms of MHT (e.g. injectables) would be comparable. Duration of use and time since last use was calculated in months. Time since last use was estimated from the year prior to the index date to the last recorded prescription accounting for the duration of the prescription.

Known risk factors for BTC were collected from the patient's medical record, including body mass index (BMI),[33] smoking status and alcohol use (current user, former user, or never user),[34-36] and diagnoses of gallstones,[4,9] diabetes (types 1 and 2),[4,35] liver cirrhosis,[37] hepatitis,[37] and dyslipidemia.[4] We also extracted history of oophorectomy and hysterectomy from the patient's medical records as these procedures are associated with MHT use. Because evidence suggests that the use of aspirin[38] and statins[39] are associated with a reduced risk of BTC, we measured exposure to these medications as having one or more prescriptions for aspirin or statins in the medical record.

Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (95%CIs) to assess the relationship between MHT use and biliary tract cancer by site. Odds ratios were interpreted as relative risks given that controls were selected using incidence density sampling and matched to cases on follow-up time.[40,41] We examined ever MHT use (the main exposure of interest), and in addition the number of prescriptions, duration of use, and time since last use. We also examined MHT formulation (combination estrogen-progesterone, estrogen only, and progesterone only), MHT administration via pill, patch, or other (cream, ring, implant, or suppository), and MHT dose (high and low dosing for pills and patches separately). We further examined the association between age at initiation of MHT and time since last use only among women who used MHT. Possible confounders were chosen based on a review of the literature and retained based on best fit (e.g., lowest AIC) to obtain the most parsimonious model. GBC models were adjusted for age at index date (continuous), index year, BMI (<18.5 kg/m^2, 18.5–<25 kg/m^2, 25–30 kg/m^2, ≥ 30 kg/m^2), history of gallstones, diabetes, hysterectomy, and oophorectomy. These models excluded controls with a history of cholecystectomy. Cholangiocarcinoma models were adjusted for age at index date, index year, BMI, smoking (current, former, never), alcohol use (current, former, never), aspirin use, and statin use, history of diabetes, hysterectomy, and oophorectomy. AVC models were adjusted for age at index date, index year, BMI, smoking, history of diabetes, hysterectomy, and oophorectomy. Mixed type models were adjusted for age at index date, index year, BMI, smoking, alcohol use, history of gallstones, hysterectomy, and oophorectomy.

Multiple imputation with chained equations as implemented in PROC MI (SAS 9.4) was used to impute missing values for BMI, smoking status, and alcohol use. We used age at study entry, year of birth, year of practice entry into CPRD, CPRD practice, MHT exposure, time since menopause, hysterectomy, oophorectomy, diabetes, gallstones, and aspirin and statin use to predict the missing values separately in cases and controls 4. We created 10 imputed sets for cases and controls that we then analyzed. The results from the 10 imputed datasets were then combined using Rubin's formula for the variance as implemented in PROC MIANALYZE.

Premenopausal hysterectomy/oophorectomy is associated with greater risk of mortality and some cancers,[42-44] and women who have had premenopausal hysterectomy/oophorectomy are more likely to take MHT than women who underwent natural menopause.[45] Therefore, we examined associations between MHT and GBC and cholangiocarcinoma, respectively, stratified by history of hysterectomy/oophorectomy. To disentangle the effects of gallstones on GBC risk, we additionally conducted analyses for GBC among those who had no known history of gallbladder disease (cholelithiasis or cholecystitis, **Supplemental Table 2)**. First, we repeated the analysis for GBC only among patients who were eligible for linkage (only patients registered to practices in England) to the HES APC for GBC. We conducted the analysis again restricted to women without evidence of gallbladder disease or cholecystectomy recorded in CPRD Gold or HES APC (**Supplemental Tables 2 – 4**). As a sensitivity analysis, we calculated E-values[46] to estimate the minimum strength of association on the risk ratio scale an unmeasured confounder would have to have with both the outcome and exposure to completely explain away our observed associations between MHT use and GBC and cholangiocarcinoma, respectively.[46]

All statistical tests were 2-sided; for the main exposure (MHT use) we considered $P< 0.05$ statistically significant, for the other variables we adjusted for the fact that for each cancer we tested six MHT associated variables and used $P<0.05/6$ ($P<0.008$) to indicate significance. All analyses were conducted in SAS v9.4 (The SAS Institute, Cary, NC), except for sensitivity analyses estimating E-values which were conducted in R Studio v1.4.1717.

Results

The analysis included 1,682 BTC cases (483 GBC, 870 cholangiocarcinoma, 105 AVC, and 224 mixed) and 8,232 matched cancer-free controls. Cases and controls had an average of 10 years in CPRD prior to the study index date. Cases were more likely than controls to be current smokers; have a history of diabetes, gallstones, liver cirrhosis, hepatitis, bilateral oophorectomy, and use of aspirin (**Table 1**).

Ever use of MHT, defined as any use of ≥2 prescriptions at least 1 year prior to the index date, was associated with increased GBC risk (OR: 1.78; 95% CI: 1.01, 3.12) (**Table 2)**. MHTs formulated with combined estrogen and progesterone were associated with nearly twice the risk of GBC (OR: 1.97; 95% CI: 1.08, 3.59), while estrogen-only formulations were associated with decreased risk of cholangiocarcinoma (OR: 0.59; 95% CI: 0.34, 0.93). However, the *P*-heterogeneity for both outcomes was not significant for MHT formulation overall When based on the *P*-heterogeneity there was no association overall for MHT administration. MHT administration in pill form was associated with an increased GBC risk (OR: 2.28, 95% CI: 1.24, 4.17). Other MHT formulations including creams and suppositories, were associated with a decreased risk of cholangiocarcinoma (OR: 0.57; 95% CI: 0.34, 0.95). Number of prescriptions, dose, duration of use and time since last use were not associated with GBC or cholangiocarcinoma. Among MHT users, age at initiation and time since last use were not associated with these cancers either. MHT use was not associated with AVC or mixed cancer types (**Table 3**).

There were no differences in the associations between MHT use and GBC (**Supplemental Table 5)** or cholangiocarcinoma (**Supplemental Table 6)** among women who had undergone hysterectomy/oophorectomy and those who have no evidence of a history of hysterectomy/oophorectomy . Among women without a recorded history of gallbladder disease (cholelithiasis or cholecystitis) in CPRD, the associations with ever MHT use and MHT formulation and GBC were attenuated and no longer reached statistical significance (**Table 4**). MHT pill use was associated with increased risk of GBC (OR: 1.91; 95% CI: 1.04, 3.51) similar to the main analysis. MHT use was not associated with mixed cancers. The results were not materially different when this analysis was repeated for patients eligible for linkage to HES APC (**Supplemental Table 7**). In the sensitivity analysis, we estimated that a potential unmeasured confounder would have to have a risk ratio of at least 3.0 and 2.9 with both cancer and MHT use to explain away our observed associations for GBC and cholangiocarcinoma, respectively (**Supplemental Table 8**).

Discussion

In this large nested case-control study MHT use was associated with an increased risk of GBC and a reduced risk of cholangiocarcinoma. There was a two-fold increase in risk in GBC with estrogen and progesterone formulations and over twice the risk with orally administered MHT. The association with oral MHT persisted after excluding women with gallbladder disease, though the associations with ever use and formulation were attenuated. Conversely, estrogen-only formulations and other preparations (such as topical creams and suppositories) were associated with a reduced risk of cholangiocarcinoma. The number of prescriptions, dose, duration of use, and time since last use were not associated with GBC or cholangiocarcinoma risk after adjusting for the form of administration.

Sex steroid hormones may increase GBC risk by increasing the risk of gallstones in women. Results from the Heart and Estrogen/progestin Replacement Study Follow-up, a randomized trial of estrogen plus progestin, found a 70% increased risk of cholecystectomy in women allocated to the hormone arm.[47] The Women's Health Initiative also found increased risk of gallbladder disease diagnoses (cholecystitis and cholelithiasis) and cholecystectomy in women allocated to both the estrogen plus progestin and estrogen-only arms.[23] More recently in The Million Women Study, Liu et al. found a substantially lower risk of gallbladder disease with transdermal MHT use compared with pill administrations.[48] Similar to our results, Liu et al also did not find an association between duration of MHT use and gallstone disease. While one explanation for the lack of association might be that the association is not causal, another hypothesis is that form of administration is important.[48] Orally administered MHT are metabolized in the liver before entering systemic circulation, and metabolites are excreted in the bile and urine.[48] Transdermal administration avoids this first-pass metabolism in the liver and is absorbed through the skin directly into systemic circulation.[48] Further, transdermal MHT is prescribed in lower doses than oral hormones to achieve the same physiologic effects.[48,49] These results agree with our observations of increased GBC risk with oral administration of MHT and null associations with other forms of MHT administration.

Two previous hospital-based case-control studies conducted in Italy reported at least 2-fold increased odds of GBC with MHT use.[27,30] Similar to our observations, Gallus et al.[27] found that the

increased odds did not differ by duration of use (<2 years and ≥2years) or by time since last use (<10 years and ≥10 years). However, these studies were based on small sample sizes (n=31 for both) and were not able to examine MHT formulation or administration, or assess cancer risk across the biliary tract.[27,30] Using a matched cohort design in the Swedish registries, Kilander et al.[29] found a significant decreased odd of GBC with any MHT use. Interestingly, while they found a nearly 7-fold increase odds of gallstones with MHT use, the association with GBC was attenuated and no longer significant after adjusting for gallbladder disease. However, this analysis had fewer GBC cases (n=219), relatively short follow-up time (5.6 years), combined extrahepatic cholangiocarcinoma with AVC, and did not examine oral and transdermal administrations separately as we did.[29]

We also observed that oral MHT administration still conferred a nearly two-fold increased risk of GBC in the subset of women who did not have a diagnosis of gallbladder disease. Though many of these women may have had asymptomatic gallstones, the strength of this association suggests sex steroid hormones may have a direct effect on GBC formation. Gabbi et al. showed that estrogen acts independently on gallbladder carcinogenesis. Indeed, mice lacking the Liver X Receptor β, a sensor for oxysterols,[50] do not develop gallstone disease but undergo a process of gallbladder carcinogenesis from inflammation to dysplasia/metaplasia and carcinoma in situ as they age. The elimination of estrogens with ovariectomy early in life halted this progression.[51] Taken together, these findings suggest that estrogen likely has a direct effect, as well as an indirect effect through gallstones, on GBC.

Previous research found a non-significant increased risk of intrahepatic cholangiocarcinoma with use of estrogen-only formulations of MHT (HR: 1.44; 95% CI: 0.91–2.28).[18] Further, Petrick et al. found that increased levels of circulating estradiol were associated with a 40% increased risk of intrahepatic cholangiocarcinoma.[19] Indeed, *in vitro*, 17β-estradiol has been shown to stimulate proliferation and inhibit apoptosis of intrahepatic cholangiocarcinoma cell lines.[14] In contrast, *in vitro,* a lower proliferative response to estradiol was seen in extrahepatic cholangiocarcinoma cell lines, which demonstrated enhanced ER-β expression and no ER-α expression.[14] Indeed ER-α is known to have pro-proliferative action while ER-β antiproliferative effects.[52] In the current study, we were unable to distinguish between

intrahepatic and extrahepatic cholangiocarcinoma However, worldwide approximately 90% of cholangiocarcinoma are extrahepatic.[1,53] In addition, even cases coded as intrahepatic cholangiocarcinoma could actually be extrahepatic cholangiocarcinoma cases as studies in both the UK[54] and the United States [55] have demonstrated extensive misclassification of perihilar extrahepatic cholangiocarcinoma as intrahepatic cholangiocarcinoma, providing further support that the majority of these tumors included in our study are extrahepatic. That the use of estrogen-only MHT formulations was associated with a decreased risk of cholangiocarcinoma in our study suggests that estrogen signaling acts through the ER-β pathway, producing an anti-proliferative response in the extrahepatic bile duct.

The strengths of this study include the relatively large number of rare cancers, the ability to examine associations across BTC sites, accurate prescription records, the establishment of temporality by excluding exposure that occurred in the year prior to diagnosis, and the use of multiple imputation to reduce bias due to missing covariates. However, some limitations should be noted. We could not account for MHT use prior to the patients' enrollment in a CPRD practice, which may contribute to the observed lack of association with duration of use, number of prescriptions, or time since last use and BTC. Power was limited to detect differences between subgroups due to the small number of MHT users exposed to less commonly prescribed administrations or formulations. However, findings similar to ours have also been observed in another similar study, which relied on self-reported MHT use.[48] There is also no reason to believe that this misclassification of MHT use duration would be different for cases and controls as the women in our study were matched on year of birth and time in the practice. Because important covariates such as race/ethnicity were not recorded uniformly in the CPRD, we were unable to control for these risk factors. However, our estimated e-values suggest that only a very strongly associated unmeasured confounder could fully explain away our observed results. Finally, though previous validation studies have reported that cancer diagnoses recorded in CPRD are accurate and complete,[32] we were unable to differentiate between intra- and extrahepatic cholangiocarcinoma as the majority (93%) of these tumors were coded only as cholangiocarcinoma in the patients' primary care records. However, as approximately

8–10% of all cholangiocarcinoma are intrahepatic,[1,53] the tumors in the present study are likely to be majority extrahepatic.

The results of this large observational study suggest that MHT use is associated with increased risk of GBC and a lower risk of extrahepatic cholangiocarcinoma. Further research is needed to confirm ours and others' observations that transdermal administration is associated with a lower risk of GBC than oral administrations. Providers may prefer transdermal formulations for high-risk groups such as those with primary sclerosing cholangitis given that this administration avoids this first-pass metabolism in the liver. These findings support the need for future investigations into sex steroid hormone etiology of BTCs.

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Table 1. Characteristics of biliary tract cancer cases and matched controls enrolled in the United Kingdom's Clinical Practice Research Datalink

Variable	Case N =1,682 (%)	Control N = 8,232 (%)	*P*-value
Cancer			
Gallbladder cancer	483 (28.7)		
Cholangiocarcinoma	870 (51.7)		
Ampulla of Vater cancer	105 (6.2)		
Mixed cancer	224 (13.3)		
Diagnosis/selection year			
1990 – 1999	181 (10.8)	878 (10.7)	
2000 – 2004	291 (17.3)	1,430 (17.4)	
2005 – 2009	436 (25.9)	2,138 (25.9)	
2010 – 2017	774 (46.0)	3,786 (46.0)	
CPRD entry year, mean (SD)	1998 (6.0)	1998 (6.1)	
Years in CPRD, mean (SD)	9.8 (6.0)	9.8 (6.0)	
Age at diagnosis/selection			
45 – 49	43 (2.6)	235 (2.9)	
50 – 59	175 (10.4)	851 (10.3)	
60 – 69	372 (22.1)	1,811 (22.0)	
70 – 79	539 (32.1)	2,637 (32.0)	
80 – 95	553 (32.9)	2,698 (32.8)	
Body mass index			
<18.5 kg/m^2	49 (2.9)	211 (2.6)	
18.5 – 24.9 kg/m^2	538 (32.0)	2,688 (32.6)	
25.0 – 29.9 kg/m^2	520 (30.9)	2,402 (29.2)	0.40
≥30 kg/m^2	373 (22.2)	1,662 (20.2)	
Missing	202 (11.9)	1,269 (15.4)	
Smoking status			
Never	394 (23.4)	2,078 (25.2)	
Former	754 (44.8)	3,732 (45.3)	<0.0001
Current	367 (21.8)	1,345 (16.3)	
Missing	167 (10.0)	1,077 (13.1)	
Alcohol use			
Never	308 (18.3)	1,583 (19.2)	
Former	71 (4.2)	336 (4.1)	0.87
Current	977 (58.1)	4,572 (55.5)	
Missing	308 (18.3)	1,741 (21.2)	
Gallstones	291 (17.3)	353 (4.2)	<0.0001
Chronic disease history			
Diabetes	222 (13.2)	762 (9.3)	<0.0001
Cirrhosis	10 (0.6)	22 (0.3)	0.03
Hepatitis	3 (0.2)	4 (0.1)	0.06
Dyslipidemia	245 (14.6)	1,304 (15.8)	0.19
Bilateral oophorectomy	89 (5.3)	301 (3.7)	0.002
Hysterectomy	322 (19.4)	1,436 (17.4)	0.10
Statin use	496 (28.5)	2,290 (27.8)	0.16
Aspirin use	533 (31.7)	2,352 (28.6)	0.01

Table 2. Odds ratios for the association between menopausal hormone therapy use and odds of gallbladder cancer and cholangiocarcinoma

Gallbladder (excluding controls with history of cholecystectomy)				Cholangiocarcinoma			
Variable	**Cases** **n** = 483 (%)	**Controls** **n** = 2,230 (%)	**OR (95% CI)**	**Variable**	**Cases** **n** = 870 (%)	**Controls** **n** = 4,360 (%)	**OR (95% CI)**
Ever MHT use[a]				**Ever MHT use**[g]			
No use	383 (79)	1,776 (80)	1.00 (Reference)	No use	694 (80)	3,425 (79)	1.00 (Reference)
Ever	100 (21)	454 (20)	**1.78 (1.01, 3.12)**	Ever	176 (20)	935 (21)	0.76 (0.50, 1.17)
P-heterogeneity[b]			0.05	*P*-heterogeneity[b]			0.21
MHT formulation[a]				**MHT formulation**[g]			
No use	383 (79)	1,776 (80)	1.00 (Reference)	Non–use	694 (80)	3,425 (78)	1.00 (Reference)
Estrogen only	32 (7)	142 (6)	1.60 (0.83, 3.08)	Estrogen only	46 (5)	310 (7)	**0.59 (0.34, 0.93)**
Estrogen and progesterone	67 (14)	304 (14)	**1.97 (1.08, 3.59)**	Estrogen and progesterone	127 (15)	606 (14)	0.91 (0.58, 1.43)
Progesterone only	1 (0.2)	8 (0.1)	0.40 (0.03, 6.26)	Progesterone only	3 (0.3)	19 (0.4)	0.67 (0.18, 2.47)
P-heterogeneity[b]			0.06	*P*-heterogeneity[b]			0.14
MHT administration[a,c]				**MHT administration**[c,g]			
No use	383 (79)	1,776 (80)	1.00 (Reference)	None	694 (80)	3,425 (78)	1.00 (Reference)
Pill	86 (18)	370 (16)	**1.84 (1.06, 3.19)**	Pill	147 (17)	750 (17)	0.96 (0.65, 1.41)
Patch	20 (4)	101 (4)	1.03 (0.52, 2.05)	Patch	40 (5)	201 (5)	0.96 (0.62, 1.48)
Other[d]	10 (2)	70 (3)	0.75 (0.35, 1.61)	Other[d]	18 (2)	152 (3)	**0.57 (0.34, 0.95)**
P-heterogeneity[b]			0.04	*P*-heterogeneity[b]			0.06
MHT dose				**MHT dose**			
No use	383 (79)	1,776 (80)	1.00 (Reference)	No use	694 (80)	3,425 (78)	1.00 (Reference)
Pill low dose	66 (14)	262 (12)	1.16 (0.81, 1.64)	Pill low dose	95 (11)	539 (13)	0.79 (0.50, 1.25)
Pill high dose	11 (2)	102 (5)	0.86 (0.49, 1.49)	Pill high dose	38 (4)	195 (5)	0.97 (0.37, 2.55)
Patch low dose	16 (4)	73 (4)	0.94 (0.47, 1.88)	Patch low dose	29 (4)	164 (4)	0.80 (0.61, 1.04)
Patch high dose	2 (1)	16 (1)	1.08 (0.25, 4.61)	Patch high dose	7 (1)	19 (1)	0.82 (0.56, 1.20)
P-heterogeneity[b]			0.94	*P*-heterogeneity[b]			0.95
Number of prescriptions[e]				**Number of prescriptions**[h]			
No use (0–1 prescriptions)	383 (79)	1,776 (80)	1.00 (Reference)	No use (0–1 prescriptions)	694 (80)	3,425(79)	1.00 (Reference)
2–9 prescriptions	59 (12)	229 (10)	1.37 (0.96, 1.94)	2–9 prescriptions	81 (9)	443 (10)	0.84 (0.65, 1.11)
≥10 prescriptions	41 (9)	225 (10)	0.85 (0.55, 1.30)	≥10 prescriptions	95 (11)	492 (11)	0.88 (0.67, 1.15)

P–trend[f]			0.44	*P*–trend[f]			0.24
Duration of use[e]				**Duration of use**[h]			
No use (0–1 month)	383 (79)	1,776 (80)	1.00 (Reference)	No use (0–1 month)	694 (80)	3,425 (79)	1.00 (Reference)
>1–<24 months	49 (10)	180 (8)	1.36 (0.54, 3.42)	>1–<24 months	60 (7)	349 (8)	0.80 (0.59, 1.08)
24–<48 months	17 (4)	81 (3)	1.01 (0.34, 3.01)	24–<48 months	30 (3)	153 (3)	0.90 (0.59, 1.38)
≥48 months	34 (7)	192 (9)	0.77 (0.27, 2.20)	≥48 months	86 (10)	433 (10)	0.91 (0.68, 1.20)
P–trend[f]			0.10	*P*–trend[f]			0.38
Time since last use[e]				**Time since last use**[h]			
No use	383 (79)	1,776 (80)	1.00 (Reference)	No use	694 (80)	3,426 (79)	1.00 (Reference)
>1–<24 months	15 (3)	115 (5)	0.66 (0.22, 1.98)	>1–<24 months	42 (5)	223 (5)	0.86 (0.44, 1.68)
24–<36 months	10 (2)	58 (3)	1.04 (0.32, 3.33)	24–<36 months	23 (3)	144 (3)	0.77 (0.38, 1.57)
36–<84 months	22 (4)	88 (4)	1.41 (0.51, 3.91)	36–<84 months	39 (4)	220 (5)	0.80 (0.41, 1.56)
≥84 months	53 (11)	193 (8)	1.37 (0.53, 3.52)	≥84 months	72 (8)	347 (8)	0.96 (0.51, 1.78)
P–trend[f]			0.05	*P*–trend[f]			0.88
Among MHT users only				**Among MHT users only**			
Age at initiation[e]				**Age at initiation**[g]			
<50 years	19 (19)	111 (24)	1.00 (Reference)	<50 years	49 (28)	253 (27)	1.00 (Reference)
50–55 years	39 (39)	149 (33)	1.74 (0.55, 5.49)	50–55 years	68 (39)	294 (31)	1.16 (0.63, 2.14)
56–60 years	21 (21)	86 (19)	2.13 (0.45, 10.06)	56–60 years	33 (19)	167 (18)	1.34 (0.51, 3.57)
>60 years	21 (21)	108 (24)	1.48 (0.22, 9.75)	>60 years	26 (15)	221 (24)	0.64 (0.17, 2.36)
P–trend[e]			0.60	*P*–trend[f]			0.88
Time since last use[e]				**Time since last use**[h]			
>1–<24 months	15 (3)	115 (5)	1.00 (Reference)	>1–<24 months	42 (24)	223 (24)	1.00 (Reference)
24–<36 months	10 (2)	58 (3)	0.90 (0.30, 2.70)	24–<36 months	23 (13)	144 (15)	0.72 (0.43, 1.20)
36–<84 months	22 (4)	88 (4)	0.87 (0.27, 2.78)	36–<84 months	39 (22)	220 (23)	0.74 (0.49, 1.13)
≥84 months	53 (11)	193 (8)	0.71 (0.23, 2.23)	≥84 months	72 (41)	347 (37)	0.97 (0.70, 1.35)
P–trend[f]			0.56	*P*–trend[f]			0.31

Controls were individually matched to cases on year of birth, index year, and number of years in the general practice and in the CPRD prior to diagnosis/selection date. All models were analyzed with conditional logistic regression.

[a]Model adjusted for age at index date, index year, duration of MHT use, BMI, diabetes, gallstones, hysterectomy, and oophorectomy.

[b]*P*<0.008 indicates statistical significance after adjusting for multiple comparisons (*P*<0.05/6)

[c]Cells do not add up to the total as women could have taken more than one type of MHT.

[d]Other includes cream, nasal spray, implant, ring, or suppository.

[e]Model adjusted for age at index date, index year, MHT administration, BMI, diabetes, gallstones, hysterectomy, and oophorectomy.
[f]The Wald test was used to test for a linear trend across categories of exposure and biliary tract cancer site.
[g]Model adjusted for age at index date, index year, duration of MHT use, BMI, diabetes, smoking status, alcohol use, hysterectomy, oophorectomy, aspirin use, and statin use.
[h]Model adjusted for age at index date, index year, MHT administration, BMI, diabetes, smoking status, alcohol use, hysterectomy, oophorectomy, aspirin use, and statin use.

Table 3. Odds ratios for the association between menopausal hormone therapy use and odds of ampulla of Vater and mixed cancers

Ampulla of Vater				Mixed			
Variable	**Cases** **n** = 105 (%)	**Controls** **n** = 523 (%)	**OR (95% CI)**	**Variable**	**Cases** **n** = 224 (%)	**Controls** **n** = 1,119 (%)	**OR (95% CI)**
Ever MHT use[a]				**Ever MHT use**[g]			
No use	80 (76)	423 (81)	1.00 (Reference)	No use	173 (77)	880 (79)	1.00 (Reference)
Ever	25 (24)	100 (19)	1.71 (0.57, 5.09)	Ever	51 (23)	239 (21)	0.77 (0.33, 1.77)
P-heterogeneity[b]			0.33	*P*-heterogeneity[b]			0.53
MHT formulation[a]				**MHT formulation**[g]			
No use	80 (76)	423 (81)	1.00 (Reference)	Non–use	173 (77)	880 (79)	1.00 (Reference)
Estrogen only	9 (9)	35 (7)	1.62 (0.48, 5.46)	Estrogen only	16 (7)	79 (7)	0.65 (0.25, 1.70)
Estrogen and progesterone	16 (15)	63 (12)	1.94 (0.59, 6.43)	Estrogen and progesterone	34 (15)	158 (14)	0.79 (0.32, 1.92)
Progesterone only	0 (0)	2 (0)	--	Progesterone only	1 (0.5)	2 (0.2)	2.29 (0.19, 27.36)
P-heterogeneity[b]			0.84	*P*-heterogeneity[b]			0.92
MHT administration[a,c]				**MHT administration**[c,g]			
No use	80 (76)	423 (81)	1.00 (Reference)	None	173 (77)	880 (79)	1.00 (Reference)
Pill	21 (20)	78 (15)	1.84 (0.58, 5.77)	Pill	44 (20)	198 (18)	0.82 (0.37, 1.83)
Patch	2 (2)	18 (3)	0.41 (0.08, 2.06)	Patch	9 (4)	54 (5)	0.53 (0.21, 1.31)
Other[d]	5 (5)	23 (4)	1.14 (0.37, 3.48)	Other[d]	7 (3)	32 (3)	0.97 (0.39, 2.39)
P-heterogeneity[b]			0.90	*P*-heterogeneity[b]			0.80
MHT dose				**MHT dose**			
No use	80 (76)	423 (81)	1.00 (Reference)	No use	173 (77)	880 (79)	1.00 (Reference)
Pill low dose	12 (12)	56 (11)	1.02 (0.48, 2.16)	Pill low dose	28 (13)	133 (12)	1.10 (0.66, 1.83)
Pill high dose	4 (4)	18 (3)	1.06 (0.33, 3.42)	Pill high dose	12 (5)	56 (5)	1.07 (0.54, 2.10)
Patch low dose	2 (2)	15 (3)	0.35 (0.07, 1.76)	Patch low dose	6 (3)	41 (4)	0.80 (0.30, 2.13)
Patch high dose	0 (0)	1 (0)	--	Patch high dose	1 (1)	7 (1)	0.56 (0.06, 4.99)
P-heterogeneity[b]			0.84	*P*-heterogeneity[b]			0.81
Number of prescriptions[e]				**Number of prescriptions**[h]			
No use (0–1 prescriptions)	80 (76)	423 (80)	1.00 (Reference)	No use (0–1 prescriptions)	173 (77)	880 (79)	1.00 (Reference)
2–9 prescriptions	15 (14)	50 (10)	1.67 (0.82, 3.40)	2–9 prescriptions	19 (8)	106 (9)	0.89 (0.52, 1.53)
≥10 prescriptions	10 (10)	50 (10)	1.02 (0.46, 2.29)	≥10 prescriptions	32 (14)	133 (12)	1.20 (0.73, 1.98)
P–trend[f]			0.63	*P*–trend[f]			0.57

Duration of use[e]				**Duration of use**[h]			
No use (0–1 month)	80 (76)	423 (81)	1.00 (Reference)	No use (0–1 month)	173 (77)	880 (79)	1.00 (Reference)
>1–<24 months	5 (5)	30 (6)	1.07 (0.15, 7.80)	>1–<24 months	15 (7)	87 (8)	0.88 (0.30, 2.75)
24–<48 months	5 (5)	20 (3)	1.02 (0.10, 10.35)	24–<48 months	9 (4)	38 (3)	1.28 (0.33, 4.99)
≥48 months	8 (7)	37 (7)	0.86 (0.09, 8.57)	≥48 months	27 (12)	114 (10)	1.37 (0.37, 5.12)
P–trend[f]			0.74	*P*–trend[f]			0.31
Time since last use[e]				**Time since last use**[h]			
No use	80 (76)	423 (81)	1.00 (Reference)	No use	173 (77)	880 (79)	1.00 (Reference)
>1–<24 months	10 (10)	13 (2)	3.05 (0.34, 27.01)	>1–<24 months	14 (6)	61 (5)	0.96 (0.25, 3.73)
24–<36 months	0 (0)	14 (3)	--	24–<36 months	6 (3)	23 (3)	1.22 (0.31, 4.72)
36–<84 months	7 (6)	29 (5)	0.78 (0.10, 5.96)	36–<84 months	12 (5)	59 (5)	0.82 (0.24, 2.87)
≥84 months	8 (8)	44 (8)	0.60 (0.07, 5.43)	≥84 months	19 (9)	96 (8)	0.81 (0.24, 2.73)
P–trend[f]			0.06	*P*–trend[f]			0.60
Among MHT users only				**Among MHT users only**			
Age at initiation[e]				**Age at initiation**[g]			
<50 years	5 (20)	15 (15)	1.00 (Reference)	<50 years	14 (6)	61 (5)	1.00 (Reference)
50–55 years	8 (32)	34 (34)	0.99 (0.13, 7.59)	50–55 years	6 (3)	23 (3)	0.72 (0.17, 3.11)
56–60 years	8 (32)	22 (22)	0.78 (0.04, 70.48)	56–60 years	12 (5)	59 (5)	0.77 (0.09, 6.46)
>60 years	7 (28)	29 (29)	0.11 (0.01, 70.75)	>60 years	19 (9)	96 (8)	0.14 (0.01, 1.55)
P–trend[e]			0.83	*P*–trend[f]			0.42
Time since last use[e]				**Time since last use**[h]			
>1–<24 months	10 (10)	13 (2)	1.00 (Reference)	>1–<24 months	12 (23)	56 (23)	1.00 (Reference)
24–<36 months	0 (0)	14 (3)	--	24–<36 months	18 (35)	89 (37)	7.28 (0.74, 71.22)
36–<84 months	7 (6)	29 (5)	0.58 (0.06, 5.65)	36–<84 months	10 (20)	39 (16)	0.67 (0.14, 3.12)
≥84 months	8 (8)	44 (8)	0.20 (0.02, 2.12)	≥84 months	11 (22)	55 (23)	0.78 (0.14, 4.15)
P–trend[f]			0.81	*P*–trend[f]			0.38

Controls were individually matched to cases on year of birth, index year, and number of years in the general practice and in the CPRD prior to diagnosis/selection date. All models were analyzed with conditional logistic regression.

[a]Model adjusted for age at index date, index year, duration of MHT use, BMI, diabetes, smoking status, hysterectomy, and oophorectomy.

[b]$P<0.008$ indicates statistical significance after adjusting for multiple comparisons ($P<0.05/6$)

[c]Cells do not add up to the total as women could have taken more than one type of MHT.

[d]Other includes cream, nasal spray, implant, ring, or suppository.

[e]Model adjusted for age at index date, index year, MHT administration, BMI, diabetes, smoking status, hysterectomy, and oophorectomy.

[f]The Wald test was used to test for a linear trend across categories of exposure and biliary tract cancer site.
[g]Model adjusted for age at index date, index year, duration of MHT use, BMI, diabetes, gallbladder disease, smoking status, alcohol use, hysterectomy, and oophorectomy.
[h]Model adjusted for age at index date, index year, MHT administration, BMI, diabetes, gallbladder disease, smoking status, alcohol use, hysterectomy, and oophorectomy.

Table 4. Odds ratios for the association between menopausal hormone therapy use and odds of gallbladder and mixed cancers among those without diagnosed gallbladder disease

Gallbladder (excluding controls with history of cholecystectomy)				Mixed			
Variable	**Cases** **n** = 349 (%)	**Controls** **n** = 2,160 (%)	**OR (95% CI)**	**Variable**	**Cases** **n** = 195 (%)	**Controls** **n** = 1,062 (%)	**OR (95% CI)**
Ever MHT use[a]				**Ever MHT use**[g]			
No use	274 (79)	1,716 (79)	1.00 (Reference)	No use	148 (76)	840 (79)	1.00 (Reference)
Ever	75 (21)	444 (21)	1.59 (0.85, 2.96)	Ever	47 (24)	222 (21)	0.87 (0.37, 2.08)
P-heterogeneity[b]			0.14	*P*-heterogeneity[b]			0.77
MHT formulation[a]				**MHT formulation**[g]			
No use	274 (79)	1,716 (79)	1.00 (Reference)	Non–use	148 (76)	840 (79)	1.00 (Reference)
Estrogen only	22 (6)	137 (6)	1.45 (0.71, 2.98)	Estrogen only	15 (8)	73 (7)	0.79 (0.30, 2.11)
Estrogen and progesterone	53 (15)	301 (14)	1.84 (0.94, 3.61)	Estrogen and progesterone	31 (16)	147 (14)	0.86 (0.33, 2.21)
Progesterone only	0 (0)	6 (0.3)	--	Progesterone only	1 (0.2)	2 (0.2)	2.33 (0.19, 27.99)
P-heterogeneity[b]			0.72	*P*-heterogeneity[b]			0.63
MHT administration[a,c]				**MHT administration**[c,g]			
No use	274 (79)	1,716 (79)	1.00 (Reference)	None	148 (76)	840 (79)	1.00 (Reference)
Pill	67 (19)	362 (17)	**1.91 (1.04, 3.51)**	Pill	40 (20)	184 (17)	0.89 (0.37, 2.14)
Patch	11 (3)	96 (4)	0.85 (0.39, 1.85)	Patch	9 (5)	46 (4)	0.74 (0.29, 1.87)
Other[d]	7 (2)	67 (3)	0.33 (0.36, 1.90)	Other[d]	7 (4)	31 (3)	1.03 (0.41, 2.59)
P-heterogeneity[b]			0.09	*P*-heterogeneity[b]			0.62
MHT dose				**MHT dose**			
No use	274 (79)	1,716 (79)	1.00 (Reference)	No use	148 (76)	840 (79)	1.00 (Reference)
Pill low dose	51 (15)	255 (12)	1.20 (0.82, 1.76)	Pill low dose	26 (14)	125 (10)	1.35 (0.79, 2.32)
Pill high dose	9 (3)	99 (5)	0.82 (0.45, 1.47)	Pill high dose	10 (5)	53 (5)	1.11 (0.53, 2.32)
Patch low dose	10 (3)	70 (4)	0.83 (0.39, 1.78)	Patch low dose	6 (4)	34 (4)	1.28 (0.47, 3.53)
Patch high dose	1 (0)	15 (1)	1.08 (0.21, 5.50)	Patch high dose	1 (1)	7 (1)	0.67 (0.07, 6.76)
P-heterogeneity[b]			0.14	*P*-heterogeneity[b]			0.63
Number of prescriptions[e]				**Number of prescriptions**[h]			
No use (0–1 prescriptions)	274 (79)	1,716 (79)	1.00 (Reference)	No use (0–1 prescriptions)	148 (76)	840 (79)	1.00 (Reference)
2–9 prescriptions	45 (13)	226 (10)	1.35 (0.92, 1.99)	2–9 prescriptions	18 (9)	101 (10)	1.08 (0.62, 1.91)
≥10 prescriptions	30 (9)	218 (10)	0.84 (0.53, 1.32)	≥10 prescriptions	29 (15)	121 (11)	1.62 (0.94, 2.79)

P–trend[f]			0.18	*P*–trend[f]			0.10
Duration of use[e]				**Duration of use**[h]			
No use (0–1 month)	274 (79)	1,716 (79)	1.00 (Reference)	No use (0–1 month)	148 (76)	840 (79)	1.00 (Reference)
>1–<24 months	36 (10)	177 (8)	0.95 (0.31, 2.92)	>1–<24 months	15 (7)	83 (8)	1.10 (0.34, 3.52)
24–<48 months	12 (3)	81 (4)	0.62 (0.17, 2.30)	24–<48 months	7 (4)	37 (3)	1.30 (0.30, 5.37)
≥48 months	27 (8)	186 (9)	0.59 (0.17, 2.03)	≥48 months	25 (13)	102 (10)	1.76 (0.44, 6.98)
P–trend[f]			0.13	*P*–trend[f]			0.24
Time since last use[e]				**Time since last use**[h]			
No use	274 (79)	1,716 (79)	1.00 (Reference)	No use	148 (76)	840 (79)	1.00 (Reference)
>1–<24 months	12 (3)	111 (5)	0.56 (0.16, 2.00)	>1–<24 months	12 (6)	56 (5)	0.94 (0.23, 3.94)
24–<36 months	9 (3)	56 (3)	0.87 (0.22, 3.44)	24–<36 months	6 (3)	22 (2)	1.37 (0.35, 5.36)
36–<84 months	18 (5)	84 (4)	1.27 (0.37, 4.31)	36–<84 months	10 (5)	55 (5)	0.81 (0.22, 3.02)
≥84 months	36 (10)	193 (9)	0.87 (0.27, 2.76)	≥84 months	19 (10)	89 (8)	1.15 (0.33, 3.97)
P–trend[f]			0.44	*P*–trend[f]			0.81
Among MHT users only				**Among MHT users only**			
Age at initiation[e]				**Age at initiation**[g]			
<50 years	12 (16)	107 (24)	1.00 (Reference)	<50 years	12 (26)	52 (23)	1.00 (Reference)
50–55 years	34 (45)	146 (33)	2.05 (0.60, 7.02)	50–55 years	15 (32)	83 (37)	0.68 (0.14, 3.16)
56–60 years	15 (20)	85 (19)	2.66 (0.49, 14.38)	56–60 years	10 (21)	36 (16)	0.95 (0.10, 9.99)
>60 years	14 (19)	106 (24)	2.10 (0.28, 15.69)	>60 years	10 (21)	51 (23)	0.22 (0.01, 8.64)
P–trend[e]			0.41	*P*–trend[f]			0.52
Time since last use[e]				**Time since last use**[h]			
>1–<24 months	12 (3)	111 (5)	1.00 (Reference)	>1–<24 months	12 (6)	56 (5)	1.00 (Reference)
24–<36 months	9 (3)	56 (3)	1.32 (0.41, 4.31)	24–<36 months	6 (3)	22 (2)	20.07 (0.74, 548.63)
36–<84 months	18 (5)	84 (4)	0.94 (0.28, 3.15)	36–<84 months	10 (5)	55 (5)	0.95 (0.10, 9.45)
≥84 months	36 (10)	193 (9)	0.57 (0.17, 1.93)	≥84 months	19 (10)	89 (8)	0.80 (0.12, 15.76)
P–trend[f]			0.36	*P*–trend[f]			0.94

Controls were individually matched to cases on year of birth, index year, and number of years in the general practice and in the CPRD prior to diagnosis/selection date. All models were analyzed with conditional logistic regression.

[a]Model adjusted for age at index date, index year, duration of MHT use, BMI, diabetes, hysterectomy, and oophorectomy.

[b]*P*<0.008 indicates statistical significance after adjusting for multiple comparisons (*P*<0.05/6)

[c]Cells do not add up to the total as women could have taken more than one type of MHT.

[d]Other includes cream, nasal spray, implant, ring, or suppository.

[e]Model adjusted for age at index date, index year, MHT administration, BMI, diabetes, hysterectomy, and oophorectomy.
[f]The Wald test was used to test for a linear trend across categories of exposure and biliary tract cancer site.
[g]Model adjusted for age at index date, index year, duration of MHT use, BMI, diabetes, smoking status, alcohol use, hysterectomy, and oophorectomy.
[h]Model adjusted for age at index date, index year, MHT administration, BMI, diabetes, smoking status, alcohol use, hysterectomy, and oophorectomy.