Drugs with potential chemopreventive properties in relation to epithelial ovarian cancer – a nationwide case-control study

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1. THE THREE ORIGINAL PAPERS ARE

1. Baandrup L, Friis S, Dehlendorff C, Andersen KK, Olsen JH,
Kjaer SK. Prescription use of paracetamol and risk for ovarian cancer in Denmark. Journal of the National Cancer Institute 2014, 106(6)

2. Baandrup L, Kjaer SK, Olsen JH, Dehlendorff C, Friis S. Use
of low-dose aspirin and risk of ovarian cancer in Denmark. Annals of Oncology 2015, 26: 787–792

3. Baandrup L, Dehlendorff C, Friis S, Olsen JH, Kjær SK. Statin use and risk for ovarian cancer: a Danish nationwide case–control study. British Journal of Cancer 2015, 112: 157–161

2. BACKGROUND

OVARIAN CANCER

Worldwide, ovarian cancer is diagnosed in about 240,000 women each year (1). Northern Europe is a high-incidence area (1), and in Denmark ovarian cancer is diagnosed in about 600 women annually, corresponding to an age-standardized (Nordic population) incidence of 17.9 per 100,000 women (2). As most women have no symptoms of the cancer before it has spread beyond the ovaries, localized disease is diagnosed in only a small proportion of patients (approximately 20% in Danish data) (3). Consequently, ovarian cancer has a poor prognosis and is the most lethal gynecologic malignancy (1). Overall 5-year survival is approximately 40%, ranging from 10% in patients with distant metastases to 80% in patients with localized disease (3). Each year, ovarian cancer causes approximately 150,000 deaths worldwide (1) and nearly 400 deaths in Denmark (2). Although, the mortality rate from ovarian cancer in Denmark has improved over the past few decades (3–5), Denmark continues to have one of the highest mortality rates from ovarian cancer in the world (1).

The primary mode of treatment of ovarian cancer is surgery with radical removal of tumor tissue or maximal reduction of tumor tissue and precise staging. Depending on the stage of ovarian cancer, most patients also receive post-operative chemotherapy containing carboplatin and taxane (6). To improve detection of ovarian cancer, several screening strategies have been investigated, including vaginal ultrasound and the tumor marker CA 125 (7). The sensitivity and specificity of these measures, however, have been found to be inadequate for the purpose of general screening, and screening may be associated with unnecessary surgery, leading to important morbidity (7).

CLASSIFICATION, ORIGIN, AND PATHOGENESIS OF EPITHELIAL OVARIAN CANCER

Ovarian tumors are categorized into three main types, epithelial, sex cord-stromal, and germ cell tumors (8). Epithelial tumors account for approximately two-thirds of all ovarian tumors and 90% of ovarian cancers (8). A subset of epithelial ovarian tumors consists of borderline ovarian tumors, which can spread as extra-ovarian lesions without invasive growth (9). Epithelial ovarian cancer is categorized into five main types according to the cell type that the tumors most closely resemble (10;11). A recent pathology review of ovarian cancer reported that serous tumors constituted approximately 70–75% of epithelial ovarian cancers, the remaining types being endometrioid (~10%), clear cell (~10%), mucinous (~3%), and others (12). A small subset of epithelial ovarian cancers is classified as mixed or undifferentiated tumors (10;11).

Epithelial ovarian cancer has been thought to originate from the ovarian surface epithelium, with subsequent cell differentiation into the specific histologic types (8); however, recent studies have challenged this view and strongly suggest that epithelial ovarian cancer may originate in other pelvic organs and involve the ovary secondarily (13;14); for example, serous tumors arise from the Fallopian tube epithelium and endometrioid and clear cell tumors from endometriosis caused by retrograde menstruation. There has been speculation about the origin of mucinous tumors with a suggestion that they may arise from the transitional-type mesothelium located at the tubal-mesothelial junction, where the fimbriae make contact with the peritoneum (13;14).

A dichotomous model for classification of epithelial ovarian cancer was proposed recently, i.e., type I and II tumors (13). Type I tumors include low-grade serous, low-grade endometrioid, clear cell, and mucinous tumors, while type II tumors show less distinct morphologic differences and are diagnosed as high-grade serous, high-grade endometrioid, and undifferentiated tumors (13). Type II tumors are the commonest (~75%) (13). Type I tumors are considered to evolve from well-established precursor lesions, such as borderline ovarian tumors or endometriosis, and to develop in a slow, stepwise fashion, usually presenting at a localized stage (13–15). In contrast, type II tumors are aggressive and typically present in advanced stages. Because of the rapid development of type II tumors, it has not yet been possible to identify precursor lesions (13). Recent studies, however, have identified a lesion in the Fallopian tube, termed 'serous tubal intraepithelial carcinoma', which may be a precursor of type II tumors of serous origin (16).

DETERMINANTS OF EPITHELIAL OVARIAN CANCER

The etiology of epithelial ovarian cancer is multifactorial, and several factors have been suggested to influence risk of developing the disease. Moreover, epithelial ovarian cancer is a heterogeneous disease, and the risk factor profile appears to differ by histologic type. The best-established determinants of epithelial ovarian cancer and histologic type-specific variations are described below.

Age

Epithelial ovarian cancer is strongly associated with age. In Danish data, the disease is rare in women under 40 years of age; thereafter, the incidence increases steadily until it reaches a plateau among women aged 70 years or older (4).

Reproductive factors, ovulation, and hormonal factors

It is well established that the risk of epithelial ovarian cancer decreases with the number of pregnancies, late menarche, early menopause, breastfeeding, and use of oral contraceptives, and increases with use of hormonal replacement therapy (HRT) (17–25). Some studies have reported a protective effect of high parity and oral contraceptive use mainly for non-mucinous epithelial ovarian cancer (25;26).

Several hypotheses have been proposed of the underlying biologic mechanisms of the associations between reproductive factors and risk of epithelial ovarian cancer. The 'incessant ovulation' hypothesis is based on the consistent finding of a reduced risk of epithelial ovarian cancer associated with factors that reduce the lifetime number of ovulations. In this hypothesis, ovulation increases the risk of epithelial ovarian cancer by causing repeated trauma and subsequent proliferation of the ovarian surface epithelium (27;28). The 'gonadotropin' hypothesis suggests that excessive exposure to gonadotropins (follicle-stimulating and luteinizing hormones) leads to malignant transformation of the ovarian surface epithelium, either directly or by stimulating estrogen production (27;29;30). According to this hypothesis, the protective effect of oral contraceptives is due to suppression of gonadotropin secretion (27;31). The 'hormonal' hypothesis proposes that stimulation of the ovarian surface epithelium by androgens increases the risk of epithelial ovarian cancer, whereas stimulation by progesterone decreases the risk (27;32). In this hypothesis, oral contraceptives protect against epithelial ovarian cancer because they decrease levels of androgens and/or because they contain high levels of progestin (synthetic progesterone) (27;31).

There is firm evidence that infertility is associated with an increased risk of epithelial ovarian cancer (33). Although it is debated whether the association is induced by the underlying causes of infertility or the drugs used in fertility treatment, recent data, including a Cochrane review (34), have found no convincing evidence of an increased risk of epithelial ovarian cancer associated with fertility drug treatment (34;35).

Inflammation

A fourth hypothesis for the etiology of epithelial ovarian cancer is the 'inflammatory' hypothesis (36). This theory is closely related to the three hypotheses described above, as inflammation occurs with ovulation, and pro-inflammatory cells and molecules may be recruited by elevated estrogen levels (36). Involvement of inflammation in the development of epithelial ovarian cancer is further supported by reports of an increased risk associated with external pro-inflammatory agents ascending from the lower female genital tract to the Fallopian tube. These agents include asbestos and talc (23;37), and sexually transmitted infections causing pelvic inflammatory disease (37;38). The association between endometriosis and ovarian cancer risk might also be explained by inflammation, as implantation of endometrial tissue outside the uterus induces a local inflammatory reaction (23;37;39).

Surgical procedures

Tubal ligation and hysterectomy have been shown consistently to protect against epithelial ovarian cancer (40–43). The proposed mechanisms include prevention of the passage of endometrial tissue or carcinogenic agents from the vagina through the Fallopian tube and reduced ovarian blood flow resulting in altered hormone levels (40;41;44). Recent evidence, also compatible with the above described theory that the various histologic types of epithelial ovarian cancer have different origins, is that the effect of tubal ligation may be type-specific, predominately reducing the risks of endometrioid and clear cell ovarian cancer (40;41;43).

Lifestyle and behavioral factors

Smoking moderately increases the risk of epithelial ovarian cancer and the association seems to be confined to mucinous tumors (45;46). Some evidence also indicates that alcohol intake slightly increases the risk of epithelial ovarian cancer; however, the results are not conclusive (47;48). An association between consumption of dairy products and increased epithelial ovarian cancer risk has been proposed, but these results are also equivocal (49;50). Finally, obesity has been associated with an increased risk of epithelial ovarian cancer, primarily of the non-serous types (51;52).

Family history and hereditary ovarian cancer

Hereditary ovarian cancer comprises approximately 10% of all epithelial ovarian cancers (53). BRCA1 and BRCA2 mutations are responsible for most families with multiple cases of epithelial ovarian cancer (53), and a Danish study reported that approximately 6% of women with epithelial ovarian cancer carry these mutations (54). Most of the remaining cases of hereditary epithelial ovarian cancer are attributable to the hereditary non-polyposis colorectal cancer syndrome (53).

POTENTIAL CHEMOPREVENTION OF OVARIAN CANCER

Identification of protective factors against ovarian cancer is of public health interest in view of the difficulty of early detection of the disease and its poor prognosis. It has been suggested that some commonly used drugs may have a protective effect against cancer, including ovarian cancer. These drugs include aspirin and non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, and statins.

Aspirin and non-aspirin non-steroidal anti-inflammatory drugs

High-dose aspirin and non-aspirin NSAIDs are used to treat inflammatory conditions and mild-to-moderate pain, whereas low-dose aspirin, which has antithrombotic properties, is used in the treatment and prevention of occlusive cardiovascular or cerebrovascular disease (55;56). The most severe adverse effect of aspirin use is bleeding, especially gastrointestinal bleeding and peptic ulcer. Hemorrhagic stroke is rarer but potentially fatal (56;57). Non-aspirin NSAIDs also increase the risk of peptic ulcer. Increased risk of ischemic cardiovascular and cerebrovascular disease is an additional serious adverse effect associated with use of non-aspirin NSAIDs, in particular for selective cyclooxygenase (COX)-2 inhibitors, but also for traditional non-aspirin NSAIDs with high COX-2 selectivity (55;56;58;59).

Compelling evidence suggests that use of aspirin and other NSAIDs protect against colorectal cancer (57;60;61). NSAID use may also protect against several non-gastrointestinal cancers, including ovarian cancer; however, the results for non-gastrointestinal cancer are less conclusive. In a meta-analysis of 21 studies (62), we recently found suggestive evidence of an inverse association between aspirin and non-aspirin NSAIDs and ovarian cancer risk; however, we identified substantial across-study heterogeneity in both exposure definition and the reported results. Subsequently, two large studies based on multicenter case-control data (63) and on the US prospective Women's Health Initiative (64) reported inverse associations between aspirin use and ovarian cancer risk that were stronger than our estimates in the meta-analysis.

The mechanism by which aspirin and non-aspirin NSAIDs might inhibit the development of cancer has not been firmly established (65;66). The main mode of action of aspirin and non-aspirin NSAID is inhibition of the COX enzymes, which convert arachidonic acid into prostanoids, including prostaglandins, thromboxane, and prostacyclin (66–68). Prostanoids are potent signaling lipids involved in a wide range of physiological processes (68;69). COX-1 is constitutively expressed in most tissues and plays an important role in platelet aggregation and gastric cytoprotection, whereas COX-2 is induced in many tissues by inflammatory and proliferative reactions, including neoplasia (67;68). Aspirin inactivates the COX enzymes irreversibly. Low-dose aspirin is relatively specific for COX-1, whereas high-dose aspirin and most non-aspirin NSAIDs inhibit both COX-1 and COX-2 (65;66;68;70). One main hypothesis is that the anti-cancer action of aspirin and non-aspirin NSAIDs is mediated through COX-2 inhibition (65;66;71). Several procancerous effects of COX-2 have been described, including stimulation of angiogenesis, resistance to apoptosis, increased invasiveness, and increased DNA mutagenesis (72;73). Furthermore, COX-2 may stimulate expression of aromatase and thereby increase the synthesis of estrogen from androgens (74). These COX-2 mediated mechanisms cannot, however, explain the anti-cancer effect of low-dose aspirin (66). Several COX-independent mechanisms have also been suggested (e.g. inhibition of NF-kB, a transcription factor that activates genes involved in inflammation and apoptosis), but they also require higher concentrations of aspirin than achieved by low-dose aspirin treatment (66;68;75). Another hypothesis providing a rationale for a chemopreventive effect of low-dose aspirin is that the COX-1-mediated antithrombotic effect of low-dose aspirin plays a central role (66;75). Specifically, inhibition of COX-1 in platelets by low-dose aspirin may suppress the induction of COX-2 and subsequent downstream signaling in adjacent cell types (66;75).

Paracetamol

Paracetamol is an antipyretic and mildly analgesic drug (76;77), thus sharing indications with high-dose aspirin and non-aspirin NSAIDs (56;77). In contrast to those drugs, however, paracetamol has only weak anti-inflammatory activity (76;77). Serious adverse effects of paracetamol at short-term use in therapeutic doses are extremely rare, whereas renal and hepatic toxicity may occur with higher doses or prolonged treatment (77).

Although paracetamol does not belong to the pharmaceutical class of NSAIDs (77), this drug has been included in many studies evaluating the potential chemopreventive effect of NSAIDs. A meta-analysis from 2005 suggested that paracetamol may protect against ovarian cancer (78). Subsequent observational studies of this association, however, have yielded inconclusive results (63;79–85).

The pharmacological mechanisms of action of paracetamol are complex and not fully established. Previously, paracetamol was considered to act within the central nervous system; more recently however, effects have also been demonstrated in peripheral tissue (76;77). Suggested mechanisms for the therapeutic effects of paracetamol include inhibition of the COX enzymes and stimulation of the descending serotoninergic pathways involved in inhibition of pain sensation (77). Information about the biologic mechanisms underlying a potential chemopreventive effect of paracetamol against ovarian cancer is also limited. Due to chemical similarities with estradiol and progesterone, it has been suggested that paracetamol has anti-gonadotropic properties (78;86–89). Alternately, glutathione depletion from paracetamol metabolism may decrease the effective concentration of follicle-stimulating hormone, because glutathione is required for both the release of this hormone and its binding to the receptor (78;88). A third hypothesis is that paracetamol inhibits 'macrophage migration inhibitory factor' activity, which is an important regulator of the inflammatory response (78;90).

Statins

Statins are the most commonly used cholesterol-lowering drugs (91–93), and they are considered relatively safe, having few side-effects (56;94).

It has been suggested that statins have chemopreventive properties (95–97); however, only a few observational studies have examined the association between statin use and risk of ovarian cancer (98–101). Three studies reported a statistically insignificantly reduced risk of ovarian cancer associated with statin use (98;99;101), whereas one study found no association (100).

Statins exert their primary pharmacodynamic effect, lowering of cholesterol, in the liver by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme required for conversion of HMG-CoA to the cholesterol precursor, mevalonic acid (92;102). By reducing intracellular hepatic cholesterol, statins induce expression of liver cell low-density lipopro-

tein receptors enabling removal of cholesterol from the circulation (102;103). The proposed anti-neoplastic effects of statins are related to inhibition of the synthesis of mevalonic acid, as it is also a precursor of nonsterol isoprenoid metabolites, including geranylgeranyl and farnesyl pyrophosphates (92;102). These pyrophosphates are lipid attachments that are fundamental for the transport and activation of cell signaling proteins, which control several cell signaling pathways (92;102). Disruption of these processes in cancer cells may explain the potential anti-cancer effect of statins (104).

3. AIMS

The literature on chemoprevention of ovarian cancer is sparse, and the results are inconclusive. Furthermore, most previous studies had substantial methodological limitations, including limited sample size and self-reporting of drug use, which could have introduced recall bias and misclassification. Furthermore, few studies reported risk estimates according to the intensity or duration of drug use or histologic type of ovarian cancer. As Denmark has exceptionally good conditions for pharmaco-epidemiologic research because of its unique national registries, we conducted a register-based case-control study nested in the entire Danish female population with the overall aim of studying drugs with potential chemopreventive properties in relation to epithelial ovarian cancer.

This PhD thesis comprises three papers:

- In Paper 1, we investigated whether use of paracetamol or non-aspirin NSAIDs is associated with a reduced risk of epithelial ovarian cancer. The mutual indications for paracetamol and non-aspirin NSAIDs provided an obvious rationale for a combined analysis.
- In Paper 2, we addressed the association between use of low-dose aspirin and the risk of epithelial ovarian cancer.
- In Paper 3, we investigated the association between statin use and epithelial ovarian cancer risk.

4. MATERIALS AND METHODS

DATA SOURCES

This thesis is based on information from the national registries listed below, which cover the entire Danish population. Unambiguous linkage of information among registries is facilitated by the unique personal identification number encoding gender and age, which has been assigned to all Danish residents since 1968.

The Danish Civil Registration System

The Danish Civil Registration System (105) administers personal identification numbers and contains continuously updated information on dates of birth and death, addresses, and migration to and from Denmark.

The Danish Cancer Registry

The Danish Cancer Registry (106;107) contains detailed data on incident cases of cancer in Denmark since 1943. Since 1987, the reporting of cancer cases has been mandatory; however, the registration was nearly complete before that time. Until 2003, registration was based on written notifications from clinicians and pathologists, supplemented with annual electronic linkage to the Danish National Patient Register (see below) and the Danish Causes of Death Registry. Since 2004, registration has been automatized by linkage between the Patient Register (primary data source), the Danish Pathology Registry, and the Causes of Death Registry. This ensures virtually complete registration, in addition to high quality information on diagnoses. During the study period covered by this thesis, approximately 90% of tumors were verified histologically. Cancer diagnoses are recorded according to the 10th revision of the International Classification of Diseases (ICD-10) and the ICD for Oncology, version 3 (ICD-O-3) for topography and morphology codes.

The Danish Prescription Registry

The Danish Prescription Registry (108) holds information on all prescription drugs dispensed at pharmacies in Denmark. The Registry was initiated on January 1, 1995 when recording was considered to be complete. Prescription data include the date of dispensing, the substance, brand name, and quantity; the dosing schedule and indication(s) are not available. Drugs are categorized according to the Anatomic Therapeutic Chemical (ATC) system (109). The amount of drug dispensed is expressed as the number and strength of the pharmaceutical entity (typically, tablets), and the defined daily dose (DDD). The DDD of a drug is the typical maintenance dose of a drug used by an adult for its main indication; e.g. the DDD for paracetamol is 3 g (109).

The Danish National Patient Register

The Danish National Patient Register (110) comprises information on diagnoses and surgical codes for all hospitalizations for somatic conditions since 1977 and for all outpatient contacts since 1995. Diagnoses were coded according to ICD-8 from 1977 to 1993 and to ICD-10 from 1994 onwards. Surgical procedures were classified according to the Danish Classification of Surgical Procedures and Therapies (111) until the end of 1995 and according to the Nordic Classification of Surgical Procedures (112) thereafter.

Registries in Statistics Denmark

The Fertility Database (113) contains information on parity for all women in Denmark aged 13–49 years in 1980 and thereafter. The oldest women recorded in the Fertility Database were born in 1930, and the Database is considered to have total coverage of the fertility of women born from 1945 onwards. Statistics Denmark also administers several other nationwide demographic registries, holding information on education (114) and income (115).

The codes used to identify cases, exposure, and potential confounding factors are listed in Table 1.

STUDY POPULATION

Eligible cases were all women with a first diagnosis of ovarian cancer identified in the Cancer Registry. In paper 1, the study period included the years 2000–2009. In Papers 2 and 3, we were able to extend the study period with two more years of cancer registration (2000–2011). Apart from this difference, the same method was used for establishment of the study population in all three papers. Cases had to have resided in Denmark on the date of diagnosis and on January 1, 1995 (the date of establishment of the Prescription Registry), be without a previous history of cancer (except non-melanoma skin cancer), and aged between 30–84 years at diagnosis.

Ovarian cancer diagnoses were established on the basis of ICD-10 (DC56) with histological verification by ICD-O-3 diagnoses. We restricted cases to invasive epithelial ovarian tumors of well-defined histology, including serous (ICD-O-3 84413, 84603, 84613, and 90143), endometrioid (ICD-O-3 83803, 83813, 85703, 89333, and 89803), clear cell (ICD-O-3 83103, 83133, and 84903), and

mucinous (ICD-O-3 84703, 84713, 84803, 84813, and 90153) tumors.

During 2000–2011, we identified 5304 ovarian cancer cases. Of these, 5012 (94.5%) were epithelial. Of the epithelial ovarian cancers, 4103 (77% of total) were well defined histologically (serous, clear cell, endometrioid, or mucinous), and these cases constituted the final case population in Papers 2 and 3. In Paper 1, a total of 3471 epithelial ovarian cancer cases were included.

For each case, we randomly selected 15 female population controls matched on date of birth (±1 month) from the Civil Registration System by risk-set sampling; i.e., the controls were alive and at risk of a first cancer (except non-melanoma skin cancer) at the time the corresponding case was diagnosed, i.e. the index date. Women were eligible as controls before they became cases. Thus, the calculated odds ratios (ORs) are unbiased estimates of the incidence rate ratios that would have emerged from a cohort study in the source population (116). The controls fulfilled the same selection criteria as cases. In addition, we required that controls have no history of bilateral oophorectomy or salpingo-oophorectomy before the index date. The final study population included 58,706 controls in Papers 2 and 3 and 50,576 in Paper 1.

Table 1

Codes from the Danish national registries used in the analyses

The Danish Cancer Registry	
ICD-10 codes	
Ovarian cancer	DC56
ICD-O-3 codes	
Epithelial ovarian cancer	
Serous	84413, 84603, 84613, and 90143
Mucinous	84703, 84713, 84803, 84813, and 90153
Endometrioid	83803, 83813, 85703, 89333, and 89803
Clear cell	83103, 83133, and 84903
Other epithelial ovarian cancer	80123, 80223, 80333, 80503, 80703, 80713, 81203, 81303, 81403, 81413, 82303, 82313, 82603, 83233, 84403, 84503, 85103, 85603, 85713, 89343, 89503, 89513, 89903, and 90003
Non-epithelial ovarian cancer	82403, 82433, 82463, 86003, 86203, 86213, 86303, 86313, 86403, 88003, 88103, 88303, 88503, 88903, 88913, 89003, 89353, 90603, 90643, 90703, 90713, 90803, 90813, 90843, 90853, 91003, 91013, 91103, 91203, 91303, 91503, 91803, 95403, and 95803
The Danish Prescription Registry	
ATC codes	
Paracetamol	N02BE01
Low-dose aspirin	B01AC06
Non-aspirin NSAIDs	M01A
HRT	G03C, G03D, G03F, and G03HB01
Oral contraceptives	G03A
Fertility drugs	G03DA04, G03GA, and G03GB02
Statins	C10AA
Antidiabetics	A10A and A10B
The Danish National Patient Register	
Hospital discharge codes	
Infertility	
ICD-8	628
ICD-10	N97
Endometriosis	
ICD-8	625.29–625.39
ICD-10	N80
Diabetes mellitus	
ICD-8	249 and 250
ICD-10	E10–E14
COPD	
ICD-8	491–493
ICD-10	J41–J46
Ischemic cardio- and cerebrovascular disease	
ICD-8	410–414, 431–434, and 440
ICD-10	I20, I21, I23–I25, I61, I63, and I64
Musculoskeletal disorders, headache and migraine	
ICD-8	346, 711–715, 717, 718, 722.09, 722.20–722.99, 724, 725, 728, 731, and 732
ICD-10	G43, G44, M02, M03, M05–M07, M10–M19, M45–M54, M60–M79, M93, and M94
Procedure codes	
Bilateral oophorectomy and salpingo-oophorectomy	
Danish Classification	60120 and 60320
Nordic Classification	LAE20, LAE21, LAF10, and LAF11
Tubal ligation	
Danish Classification	60800, 60810, 60820, 60830, and 60840
Nordic Classification	LGA
Hysterectomy	
Danish Classification	61000, 61020, 61040, and 61100
Nordic Classification	KLCC10, KLCC11, KLCC20, KLCD00, KLCD01, KLCD04, KLCD10, KLCD11, KLCD30, KLCD31, KLCD40, KLCD96, KLCD97, KLCE00, KLCE10, KLCE20, KLCE96, KLEF13, and KMCA33

ASSESSMENT AND DEFINITION OF EXPOSURES

From the Danish Prescription Registry, we identified all prescriptions for paracetamol (ATC N02BE01), non-aspirin NSAIDs (ATC M01A), low-dose aspirin (75, 100, and 150 mg per tablet) (ATC B01AC06), and statins (ATC C10AA) redeemed by cases and controls in the period between 1995 and 1 year before the index date. Prescriptions redeemed within 1 year before the index date were disregarded in order to minimize 'reverse causation' (116).

Ever use of the individual study drug was defined as ≥2 prescriptions redeemed on separate dates; women who had redeemed <2 prescriptions (non-users) served as the reference group. Ever use was divided into recent use, i.e. ≥2 prescriptions 1–3 years before the index date, and former use, i.e. ≥2 prescriptions overall but ≤1 prescription 1–3 years before the index date.

In Paper 1, the duration of paracetamol and non-aspirin NSAID use was defined as the period between the first and last prescription plus 60 days and classified into <5, 5–10 or >10 years. Intensity of use was defined as the cumulative number of DDDs divided by the duration of use in days, and was classified into approximate tertiles of low (lower tertile), medium (middle tertile), and high (upper tertile) intensity. We also defined continuous use of paracetamol or non-aspirin NSAID as ≥2 redeemed prescriptions per year from 1 year before the index date and for ≥5 years.

In Paper 2, duration of low-dose aspirin use was calculated on the assumption that low-dose aspirin is taken as one tablet daily. Thus, the cumulative duration of use depended on the dates of prescription and the number of days covered by each prescription. The coverage of each prescription was defined as the number of tablets dispensed plus a 30-day grace period, allowing some degree of non-compliance. The grace periods contributed exposure time until a new prescription, if any, was redeemed. For users with more than one treatment period, the duration of separate treatment periods was added, and the cumulative treatment period was classified as <5 or ≥5 years. We estimated the daily low-dose aspirin dose as the tablet dose during the exposure period, categorized as 75–100 mg, 150 mg, or mixed strength. Finally, we also evaluated continuous low-dose aspirin use, defined as one consecutive treatment period from the start of treatment until 1 year before the index date, i.e. overlapping treatment periods defined by the number of tablets and 30-day grace periods.

In Paper 3, duration of statin use was defined as the period between the first and last prescription plus 60 days and dichotomized into <5 or ≥5 years. Intensity of statin use was defined as tertiles of low, medium, or high intensity according to the cumulative number of DDDs divided by the duration of use in days. In addition, statins were classified according to their lipid solubility, and categorized into 'exclusive use of lipophilic statins' or 'ever use of hydrophilic statins' (including exclusive use of hydrophilic statins and mixed use of lipophilic and hydrophilic statins). Lipophilic statins comprised simvastatin, lovastatin, fluvastatin, atorvastatin and cerivastatin, while pravastatin and rosuvastatin were hydrophilic.

POTENTIAL CONFOUNDERS

Potential confounding factors were selected á priori from the literature (4;17;21;24;33;39-41;43;45;117;118) and depending on the availability of information in the registries. For all covariates, we disregarded the year before the index date. From the Prescription Registry, we retrieved information on use (≥2 prescriptions on separate dates) of oral contraceptives, HRT, fertility drugs, and anti-diabetic medications. From the Patient Registry, we obtained information on diagnoses of endometriosis, hysterectomy, tubal ligation, and comorbid conditions including diabetes mellitus, chronic obstructive pulmonary disease (COPD) (proxy measure of heavy smoking) and asthma, ischemic cardiovascular and cerebrovascular disease, and musculoskeletal disorders and headache including migraine (proxy measure of over-the-counter analgesic drug use). The exposure window for the comorbid conditions was left-truncated to 23 years before the index date to achieve the same length of hospital history for all women in the study. A history of infertility or diabetes mellitus was defined as a composite measure of hospital diagnoses and/or prescriptions for fertility drugs or anti-diabetic drugs. We retrieved information on parity from the Fertility Database and on highest achieved education and personal income from registries in Statistics Denmark. Finally, by linking of information in the Civil Registration System and the Cancer Registry, we identified mothers and sisters of women born after 1953 and assessed the family history of ovarian and breast cancer in this subpopulation.

STATISTICAL ANALYSIS

The prevalence of each covariate (including subcategories) among cases and controls were calculated. Conditional logistic regression was used to estimate ORs and 95% confidence intervals (CIs) for epithelial ovarian cancer associated with use of paracetamol and non-aspirin NSAIDs (Paper 1), low-dose aspirin (Paper 2), and statins (Paper 3). In all analyses, non-use (<2 prescriptions of the study drug) constituted the reference group.

The multivariate model in all three papers adjusted for the core confounding covariates, consisting of age (by design), parity (0, 1, 2, ≥3), oral contraceptive use (ever/never), HRT use (ever/never), infertility (ever/never), endometriosis (ever/never), hysterectomy (ever/never), tubal ligation (ever/never), diabetes mellitus (ever/never), COPD and asthma (ever/never), and education (basic, higher, vocational, unknown). Additional covariates included use of the individual study drugs (ever/never) (Papers 1–3) and personal income (approximate tertiles of low, medium, high) (Paper 3).

We performed stratified analyses by histologic type of epithelial ovarian cancer and by duration and intensity of study drug use. We also evaluated associations according to exposure categories defined by duration and intensity combined (Papers 1–3) and by continuity of study drug use (Papers 1 and 2). The analyses in Paper 3 on statins were stratified according to type of statin (exclusive use of lipophilic statins and ever use of hydrophilic statins). In Papers 1 and 3, we further tested for a linear trend on the intensity variable (DDD) using likelihood ratio tests.

We also performed a number of sensitivity analyses. In Paper 1, we evaluated potential misclassification due to left truncation of study drug use before 1995 by applying a new-user design (119), excluding all cases, their corresponding controls, and all controls who redeemed a prescription of paracetamol or non-aspirin NSAIDs during 1995–1996. This approach was based on the assumption that use in these 2 years indicated use before establishment of the Prescription Registry. In a post-publication analysis, we repeated the main analyses for paracetamol and non-aspirin NSAID use with additional adjustment for musculoskeletal disorders and headache. In Paper 3, we evaluated potential effect measure modification by a history of ischemic cardio- or cerebrovascular disease by including interaction terms between statin use and a history of these diseases. We also conducted stratified analyses to determine whether the effect of the

study drugs varied by menopausal status (≤50 years as proxy for pre-/perimenopausal; >50 years as proxy for postmenopausal). To explore potential residual confounding by oral contraceptive use, we repeated the main analyses with and without adjustment for oral contraceptive use among women aged ≤50 years, for whom there were more complete records of oral contraceptive use. Lastly, for women born after 1953, we repeated the main analyses with adjustment for a family history of ovarian or breast cancer.

5. SUMMARY OF RESULTS

CHARACTERISTICS OF THE STUDY POPULATION

Table 2 presents the characteristics of cases and age-matched controls in Papers 2 and 3 (2000–2011).

Table 2
Characteristics of the study population in Papers 2 and 3

Characteristics	Cases N (%)		Controls N (%)	
Histology				
Serous	2731	(66.6)	—	—
Endometrioid	650	(15.8)	—	—
Mucinous	459	(11.8)	—	—
Clear cell	263	(6.4)	—	—
Age (years)				
30–39	104	(2.5)	1454	(2.5)
40–49	480	(11.7)	6879	(11.7)
50–59	1041	(25.4)	14,991	(25.5)
60–69	1233	(30.1)	17,632	(30.0)
70–84	1245	(30.3)	17,750	(30.2)
Education				
Basic	82	(2.0)	1522	(2.6)
Higher	853	(20.8)	11,576	(19.7)
Vocational	3026	(73.8)	43,606	(74.3)
Unknown	142	(3.5)	2002	(3.4)
Income				
Low	1427	(34.8)	19,854	(33.8)
Medium	1372	(33.4)	19,507	(33.2)
High	1304	(31.8)	19,345	(33.0)
Parity				
Nulliparous	901	(22.0)	9528	(16.2)
1	797	(19.4)	10,641	(18.1)
2	1521	(37.1)	23,218	(39.5)
≥3	884	(21.5)	15,319	(26.1)
Surgical procedure				
Hysterectomy	369	(9.0)	4772	(8.1)
Tubal ligation	200	(4.9)	3453	(5.9)
Medical history				
Infertility	163	(4.0)	1443	(2.5)
Endometriosis	71	(1.7)	857	(1.5)
Diabetes mellitus	189	(4.6)	2792	(4.8)
COPD/asthma	167	(4.1)	2882	(4.9)
Drug use				
Oral contraceptives	224	(5.5)	5070	(8.6)
HRT	1484	(36.2)	18,850	(32.1)
Paracetamol	587	(14.3)	9513	(16.2)
Non-aspirin NSAIDs	2026	(49.4)	28,561	(48.7)
Low-dose aspirin	494	(12.0)	7536	(12.8)
Statins	434	(10.6)	6445	(11.0)

USE OF PARACETAMOL AND NON-ASPIRIN NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND EPITHELIAL OVARIAN CANCER RISK (PAPER 1)

More controls than cases had ever used paracetamol (15.1% versus 13.2%), whereas use of non-aspirin NSAIDs was similar among cases and controls (45.8% and 46.6%). Long-term (≥5 years) use of paracetamol was observed for 41.4% of cases and 43.0% of controls. The median DDDs of paracetamol in the three categories of intensity of use (low, medium, and high) were 0.09, 0.28, and 0.67 DDDs, respectively. High-intensity use of paracetamol was observed among 28.8% of cases and 33.2% of controls.

Ever use of paracetamol was associated with an 18% reduction in risk of epithelial ovarian cancer (OR, 0.82; 95% CI, 0.74–0.92) compared with non-use (Table 3). The risk reduction was highest for recent use of paracetamol. A similar risk reduction was seen for individual histologic types of epithelial ovarian cancer, albeit with lower statistical precision (data not shown).

In analyses of duration and intensity of paracetamol use combined, we observed a clear, albeit statistically insignificant, inverse trend in ORs for epithelial ovarian cancer (Table 3). Notably, long-term (>10 years), high-intensity use of paracetamol was associated with an OR for epithelial ovarian cancer of 0.45 (95% CI, 0.24–0.86). A similar pattern was found when the analysis was restricted to serous ovarian cancer or recent use of paracetamol (data not shown). Continuous use (≥2 prescriptions per year) of paracetamol for ≥5 years was associated with a 27% decrease in the OR for epithelial ovarian cancer (OR, 0.73; 95% CI, 0.56–0.96) (data not shown).

Ever use of non-aspirin NSAIDs was associated with an OR for epithelial ovarian cancer of 1.07 (95% CI, 0.99–1.15) (Table 4). In combined analyses of duration and intensity of non-aspirin NSAID use, we observed increased ORs for epithelial ovarian cancer

Table 3

Risks of epithelial ovarian cancer associated with paracetamol use, overall and according to timing, duration, and intensity of use

Paracetamol use	Cases/Controls N	Adjusted OR (95% CI)	
Non-use	3012/42,951	1.00	(referent)
Overall use			
Ever use	459/7625	0.82	(0.74–0.92)
Recent use	292/5078	0.78	(0.69–0.89)
Former use	167/2547	0.90	(0.76–1.06)
Use for <5 years			
Low-intensity	96/1372	0.96	(0.78–1.19)
Medium-intensity	94/1547	0.84	(0.68–1.04)
High-intensity	79/1427	0.76	(0.60–0.95)
P trend			*0.11*
Use for 5–10 years			
Low-intensity	59/913	0.87	(0.67–1.15)
Medium-intensity	51/792	0.87	(0.65–1.16)
High-intensity	43/812	0.71	(0.52–0.97)
P trend			*0.33*
Use for >10 years			
Low-intensity	12/215	0.75	(0.42–1.35)
Medium-intensity	15/249	0.80	(0.47–1.37)
High-intensity	10/295	0.45	(0.24–0.86)
P trend			*0.25*

associated with long-term use of non-aspirin NSAIDs (e.g., >10 years, high-intensity: OR, 1.28; 95% CI, 0.98–1.67). Continuous use of non-aspirin NSAID for ≥5 years was associated with an OR of 1.18 (95% CI, 0.91–1.53). No apparent variation in risk was seen by histologic type of epithelial ovarian cancer (data not shown).

Although use of paracetamol and non-aspirin NSAIDs was strongly correlated (p<0.01), similar ORs were found for long-term, high-intensity paracetamol use in strata defined by ever use (OR, 0.46; 95% CI, 0.23–0.91) and non-use (OR, 0.38; 95% CI, 0.05–2.78) of non-aspirin NSAIDs (p interaction=0.85). This analysis was, however, based on small numbers (data not shown).

The three sensitivity analyses, application of a new-user design, additional adjustment for musculoskeletal disorders and headache, and update through 2011 (adding data from 2010–2011) all yielded similar results to those of the main analyses (data not shown).

Table 4

Risks of epithelial ovarian cancer associated with non-aspirin NSAID use, overall and according to timing, duration, and intensity of use

Non-aspirin NSAID use	Cases/Controls N	Adjusted OR (95% CI)	
Non-use	1852/27,398	1.00	(referent)
Overall use			
Ever use	1619/23,178	1.07	(0.99–1.15)
Recent use	728/10,404	1.08	(0.98–1.19)
Former use	891/12,774	1.06	(0.97–1.16)
Use for <5 years			
Low-intensity	223/3397	0.99	(0.86–1.15)
Medium-intensity	286/4347	1.01	(0.88–1.15)
High-intensity	317/4670	1.04	(0.92–1.18)
Use for 5–10 years			
Low-intensity	273/3577	1.18	(1.03–1.35)
Medium-intensity	186/2725	1.07	(0.91–1.25)
High-intensity	142/2088	1.09	(0.90–1.31)
Use for >10 years			
Low-intensity	56/649	1.39	(1.04–1.85)
Medium-intensity	66/809	1.33	(1.02–1.73)
High-intensity	68/889	1.28	(0.98–1.67)

USE OF LOW-DOSE ASPIRIN AND EPITHELIAL OVARIAN CANCER RISK (PAPER 2)

The prevalence of low-dose aspirin use was 12.0% among cases and 12.8% among controls (Table 2), and the prevalence of long-term (≥5 years) use was 31.0% and 31.7%, respectively. Most low-dose aspirin users were recent users (cases, 84.8%; controls, 84.6%). About one half (cases, 50.8%; controls, 47.0%) of the women had used low-dose aspirin exclusively at an estimated daily dose of 75–100 mg.

Overall, we observed no apparent association between ever use of low-dose aspirin and risk of epithelial ovarian cancer (OR, 0.94; 95% CI, 0.85–1.05) (Table 5). The ORs were not markedly influenced by timing of use or by overall cumulative duration of use (≥5 years of use: OR, 0.92; 95% CI, 0.77–1.10) (data not shown). Exclusive use of 150 mg aspirin tablets was associated with a reduced OR of 0.82 (95% CI, 0.68–0.99), which decreased further with long-term (≥5 years) use of 150 mg tablets (OR, 0.77; 95% CI, 0.55–1.08) (Table 5). In contrast, exclusive use of 75–100 mg aspirin tablets or mixed use of 75–100 mg and 150 mg tablets was associated with ORs for epithelial ovarian cancer close to unity. Continuous use of low-dose aspirin (i.e. overlapping treatment periods defined by number of tablets and 30-day grace periods) was associated with a 44% decreased risk of epithelial ovarian cancer (OR, 0.56; 95% CI, 0.32–0.97) (Table 5).

Table 5

Risks of epithelial ovarian cancer associated with low-dose aspirin use, overall and according to estimated daily dose, duration, and consistency of use

Low-dose aspirin use	Cases/Controls N	Adjusted OR (95% CI)	
Non-use	3609/51,170	1.00	(referent)
Ever use	494/7536	0.94	(0.85–1.05)
Dose by duration of use			
75–100mg	251/3539	1.01	(0.88–1.16)
<5 years	195/2748	1.02	(0.87–1.19)
≥5 years	56/791	1.00	(0.75–1.32)
150mg	125/2194	0.82	(0.68–0.99)
<5 years	89/1504	0.85	(0.68–1.06)
≥5 years	36/690	0.77	(0.55–1.08)
Mixed strength	118/1803	0.95	(0.78–1.16)
<5 years	57/896	0.93	(0.71–1.23)
≥5 years	61/907	0.97	(0.74–1.27)
Continuous use by duration of use			
<5 years	384/5754	0.96	(0.86–1.08)
≥5 years	13/332	0.56	(0.32–0.97)

In analyses stratified according to histologic type of epithelial ovarian cancer, the ORs for serous ovarian cancer were similar to those for epithelial ovarian cancer overall (Table 6). Reduced ORs were also observed for endometrioid and mucinous ovarian cancer. For endometrioid ovarian cancer, we observed decreasing ORs with increasing aspirin tablet dose (150 mg: OR, 0.68; 95% CI, 0.41–1.15) and increasing duration of use (≥5 years: OR, 0.69; 95% CI, 0.42–1.11). A similar dose-response association was not seen for mucinous ovarian cancer. The ORs for clear cell ovarian cancer were elevated in all categories of dose and duration of low-dose aspirin use.

USE OF STATINS AND EPITHELIAL OVARIAN CANCER RISK (PAPER 3)

Ever use of statins was similar among cases and controls (10.6% and 11.0%, respectively) (Table 2). Of all statin users, 94.5% were recent users, and 87.6% were users of lipophilic statins exclusively. Statins had been used for ≥5 years by 28.6% of cases and 27.3% of controls. The median DDDs of statin per day in the categories of low-, medium-, and high-intensity use were 0.40, 0.73, and 1.36 DDDs, respectively.

Ever use of statins was not associated with risk of epithelial ovarian cancer overall (OR, 0.98; 95% CI, 0.87–1.10) or of serous ovarian cancer (OR, 1.03; 95% CI, 0.90–1.19) (Table 7). Likewise, the OR did not change materially in analyses of recent statin use, ever use of lipophilic statins exclusively, or ever use of hydrophilic statins (data not shown). In analyses of the non-serous histologic types of epithelial ovarian cancer, ever use of statins was inversely associated with risk of mucinous (OR, 0.63; 95% CI, 0.39–1.00)

Table 6

Risks of histologic types of epithelial ovarian cancer associated with low-dose aspirin use, overall and according to duration and intensity of use

	Serous			Endometrioid			Mucinous			Clear cell		
Low-dose aspirin use	**Cases N**	**Adjusted OR (95% CI)**		**Cases N**	**Adjusted OR (95% CI)**		**Cases N**	**Adjusted OR (95% CI)**		**Cases N**	**Adjusted OR (95% CI)**	
Non-use	2372	1.00	(referent)	579	1.00	(referent)	424	1.00	(referent)	234	1.00	(referent)
Ever use	359	0.96	(0.85–1.09)	71	0.85	(0.65–1.13)	35	0.73	(0.50–1.08)	29	1.36	(0.87–2.11)
Dose												
75–100 mg	182	1.03	(0.87–1.21)	42	1.08	(0.76–1.53)	14	0.63	(0.36–1.11)	13	1.24	(0.67–2.29)
150 mg	89	0.82	(0.65–1.02)	16	0.68	(0.41–1.15)	10	0.70	(0.36–1.36)	10	1.68	(0.84–3.35)
Mixed strength	88	1.01	(0.80–1.27)	13	0.62	(0.35–1.11)	11	0.97	(0.51–1.85)	6	1.21	(0.50–2.94)
Duration of use												
<5 years	246	0.97	(0.84–1.12)	51	0.94	(0.68–1.28)	24	0.70	(0.45–1.10)	20	1.21	(0.73–2.00)
≥5 years	113	0.94	(0.77–1.16)	20	0.69	(0.42–1.11)	11	0.82	(0.43–1.57)	9	1.93	(0.91–4.08)

Table 7

Risks of histologic types of epithelial ovarian cancer associated with statin use, overall and according to duration and intensity of use

	Epithelial			Serous			Endometrioid			Mucinous			Clear cell		
Statin use	**Cases N**	**Adjusted OR (95% CI)**		**Cases N**	**Adjusted OR (95% CI)**		**Cases N**	**Adjusted OR (95% CI)**		**Cases N**	**Adjusted OR (95% CI)**		**Cases N**	**Adjusted OR (95% CI)**	
Non-use	3669	1.00	(referent)	2411	1.00	(referent)	593	1.00	(referent)	434	1.00	(referent)	231	1.00	(referent)
Ever use	434	0.98	(0.87–1.10)	320	1.03	(0.90–1.19)	57	0.80	(0.58–1.10)	25	0.63	(0.39–1.00)	32	1.48	(0.92–2.38)
Duration of use															
<5 years	310	0.96	(0.84–1.10)	227	1.01	(0.87–1.18)	45	0.88	(0.62–1.24)	17	0.57	(0.33–0.96)	21	1.32	(0.77–2.24)
≥5 years	124	1.04	(0.85–1.27)	93	1.11	(0.88–1.40)	12	0.57	(0.31–1.07)	8	0.85	(0.39–1.83)	11	2.05	(0.98–4.29)
Intensity of use															
Low	162	1.11	(0.93–1.32)	117	1.15	(0.94–1.41)	20	0.89	(0.54–1.45)	11	0.83	(0.43–1.58)	14	1.76	(0.93–3.33)
Medium	130	0.86	(0.71–1.04)	88	0.82	(0.65–1.03)	24	0.98	(0.62–1.54)	11	0.81	(0.42–1.56)	7	1.01	(0.44–2.32)
High	142	0.98	(0.81–1.17)	115	1.13	(0.92–1.39)	13	0.54	(0.30–0.96)	3	0.23	(0.07–0.74)	11	1.62	(0.80–3.26)

and endometrioid (OR, 0.80; 95% CI, 0.58–1.10) tumors. In contrast, we observed an elevated OR for clear cell ovarian cancer (OR, 1.48; 95% CI, 0.92–2.38) associated with ever use of statins (Table 7).

Table 7 presents results stratified by duration and intensity of statin use. The risks of epithelial ovarian cancer overall and of serous ovarian cancer did not change with increasing duration or intensity of statin use. Similar results were obtained for exclusive lipophilic statin use (data not shown). For epithelial and serous ovarian cancer, we also analyzed the combined effect of duration and intensity of statin use. We found no trend in risk of epithelial or serous ovarian cancer with increasing intensity of statin use among short-term (epithelial tumors: p trend=0.22; serous tumors: p trend=0.98) or long-term (epithelial tumors: p trend=0.68; serous tumors: p trend=0.78) statin users (data not shown).

With regard to non-serous epithelial ovarian cancer, we observed reduced ORs for mucinous ovarian cancer in relation to short-term (OR, 0.57; 95% CI, 0.33–0.96) and high-intensity (OR, 0.23; 95% CI, 0.07–0.74) statin use (Table 7). For endometrioid tumors, the ORs associated with long-term (OR, 0.57; 95% CI, 0.31–1.07) and high-intensity (OR, 0.54; 95% CI, 0.30–0.96) statin use were reduced, whereas those for clear cell ovarian cancer were elevated in all categories of duration and intensity of use.

Finally, we evaluated the association between statin use and epithelial ovarian cancer risk according to previous ischemic cardio- or cerebrovascular disease. The OR for epithelial ovarian cancer associated with ever statin use was slightly increased (OR, 1.23; 95% CI, 0.98–1.55) among women with history of ischemic cardio- or cerebrovascular disease, whereas a neutral association (OR, 0.96; 95% CI, 0.83–1.10) was found among women with no history of these diseases (p interaction=0.06) (data not shown).

Table 8

Risks of epithelial ovarian cancer associated with use of paracetamol, non-aspirin NSAIDs, low-dose aspirin, and statins according to menopausal status

	Paracetamol			Non-aspirin NSAIDs			Low-dose aspirin			Statins		
	Cases N	Adjusted OR (95% CI)		Cases N	Adjusted OR (95% CI)		Cases N	Adjusted OR (95% CI)		Cases N	Adjusted OR (95% CI)	
≤50 years												
Non-use	493	1.00	(referent)	317	1.00	(referent)	577	1.00	(referent)	670	1.00	(referent)
Ever use	13	0.52	(0.30–0.93)	189	1.00	(0.82–1.22)	7	1.14	(0.52–2.49)	14	1.30	(0.74–2.29)
>50 years												
Non-use	2591	1.00	(referent)	1535	1.00	(referent)	3032	1.00	(referent)	2999	1.00	(referent)
Ever use	446	0.84	(0.75–0.94)	1430	1.06	(0.98–1.14)	487	0.94	(0.84–1.05)	420	0.97	(0.86–1.10)

SENSITIVITY ANALYSES ACROSS PAPERS

Table 8 shows the results of analyses stratified according to menopausal status. For paracetamol use, we observed a larger reduction in risk for epithelial ovarian cancer associated with ever use of paracetamol among pre-/perimenopausal women (≤50 years) (OR, 0.52; 95% CI, 0.30–0.93) than among postmenopausal women (>50 years) (OR, 0.84; 95% CI, 0.75–0.94). We found slightly decreased ORs for epithelial ovarian cancer among postmenopausal women who had used low-dose aspirin or statins and slightly higher ORs among pre-/perimenopausal women, but no change in OR according to estimated menopausal status associated with use of non-aspirin NSAIDs. For all study drugs, the ORs for epithelial ovarian cancer were not influenced by use of oral contraceptives among pre-/perimenopausal women, who had the most complete prescription histories for oral contraceptives. Additional adjustment for a family history of ovarian or breast cancer among women born after 1953 yielded risk estimates similar to those of the main analyses for all study drugs (data not shown).

6. DISCUSSION

MAIN FINDINGS

An interesting finding in this PhD thesis is the inverse association between use of paracetamol and epithelial ovarian cancer risk. Notably, we found that the risk of this cancer decreased with increasing duration and intensity of paracetamol use, reaching a risk reduction larger than 50% for the longest duration (>10 years) and the highest doses of paracetamol. In contrast to the findings for paracetamol, use of non-aspirin NSAIDs was not associated with decreased risk of epithelial ovarian cancer. For both paracetamol and non-aspirin NSAIDs, we observed no apparent difference in risk according to histologic type of epithelial ovarian cancer.

Regarding low-dose aspirin, we observed a reduced risk of epithelial ovarian cancer among women with estimated daily use of 150 mg of aspirin. The largest risk reduction was seen for long-term, continuous use of low-dose aspirin, defined as overlapping prescription coverage periods. The associations between low-dose aspirin use and epithelial ovarian cancer risk differed by histologic type. The risk estimates for serous ovarian cancer were similar to those for epithelial ovarian cancer overall, and inverse (albeit weaker) associations were also observed for endometrioid and mucinous ovarian cancer. In contrast, low-dose aspirin use was associated with an increased risk of clear cell ovarian cancer.

The results of this thesis do not indicate a strong association between statin use and risk of epithelial ovarian cancer, although some variation was found by histologic type. No consistent trend in risk estimates for epithelial ovarian cancer overall was found according to the duration, intensity, or lipophilicity of statin use. In the analyses stratified according to the histologic type, we observed an inverse association between statin use and risk of mucinous ovarian cancer, which was strongest for high-intensity statin use. We also found reduced risk estimates for endometrioid ovarian cancer, whereas the risk estimates for clear cell tumors were increased.

COMPARISONS WITH PREVIOUS STUDIES

Paracetamol and non-aspirin non-steroidal anti-inflammatory drugs

Our findings for paracetamol are compatible with those of a meta-analysis published in 2005 that showed an overall inverse association between paracetamol use and ovarian cancer risk and an indication of a dose-response relation (78). Two of the studies in the meta-analysis had findings similar to ours (88;120). Cramer et al. (88) reported that regular use of over-the-counter paracetamol was associated with a 48% reduction in risk of epithelial ovarian cancer, with 26 exposed (of 563) cases and 46 exposed (of 523) population controls. A similar risk reduction (OR, 0.56; 95% CI, 0.34–0.86) for epithelial ovarian cancer associated with paracetamol use was reported by Moysich et al. (120) in a case-control study of 547 cases and 1094 hospital controls. In line with our findings, the inverse association between paracetamol use and epithelial ovarian cancer risk in these two studies increased with increasing duration and intensity of paracetamol use.

Most of the remaining studies of paracetamol use and ovarian cancer risk have shown weaker inverse associations (80–82;121;122) or neutral risk estimates (63;83;85;123;124), except for three studies that reported increased risk estimates (79;84;125). Among the studies in which a neutral risk estimate was found, the study of Trabert et al. within the Ovarian Cancer Association Consortium (63) is worth mentioning. The authors pooled data from 12 case-controls studies for a total of 7776 epithelial ovarian cancer cases. Paracetamol use was analyzed in relation to epithelial ovarian cancer risk according to the frequency, dose, and duration of use, with largely neutral associations in all analyses. The discrepancy between those findings (63) and ours is not readily explainable. In the study of Trabert et al., however, there might have been recall bias, as drug use was self-

reported, and there were inconsistencies in the definitions of paracetamol use in the studies included. Interestingly, the Danish study in the Ovarian Cancer Association Consortium, the Danish Malignant Ovarian Cancer Study, gave results indicative of an inverse association between paracetamol use and epithelial ovarian cancer risk (63;81).

We observed a somewhat lower OR for epithelial ovarian cancer associated with paracetamol use among pre-/perimenopausal women (≤50 years) than among postmenopausal women. This finding is interesting because hereditary ovarian cancer occurs among young women (53), and women with a family history of ovarian or breast cancer would be potential targets for chemopreventive treatment. The variation in risk according to age at diagnosis should, however, be interpreted cautiously as only 14% of the women in our study were aged ≤50 years, limiting the statistical precision of the results for these women. Further, previous studies in which results were reported according to age at diagnosis showed no major age difference in the risk pattern for ovarian cancer associated with paracetamol use (63;84;85;125).

Our null findings for ever use of non-aspirin NSAIDs are largely compatible with the results of meta-analyses of studies of the association between these drugs and ovarian cancer risk (62;126;127). A number of the previous studies on non-aspirin NSAID use and risk of ovarian cancer also indicated a null association (64;81;82;85;123;125;128). Although several studies reported inverse associations between ever use of non-aspirin NSAIDs and ovarian cancer risk (63;80;83;121;124;129–133), solid overall risk estimates were reported in only a few studies (121;124;132), and only Trabert et al. (63) observed a dose-response. In our study, we found a slightly increased risk of epithelial ovarian cancer associated with long-term non-aspirin NSAID use. Two previous studies have also reported increased risk estimates for the association between non-aspirin NSAID use and epithelial ovarian cancer risk (79;88). Cramer et al. (88) reported increased, albeit statistically insignificant, risk of epithelial ovarian cancer associated with over-the-counter use of ibuprofen (OR, 1.20; 95% CI, 0.74–1.95). A stronger association was reported by Wu et al. (79) in a population-based case-control study of 609 cases and 688 controls. In particular, increased risk of epithelial ovarian cancer was observed among women with >10 years of non-aspirin NSAID use (relative risk, 2.18; 95% CI 1.03–4.63). Although the authors concluded that their results raised concern, they also provided several alternative explanations for their findings (e.g. recall bias, surveillance bias and confounding by indication).

Low-dose aspirin

Our finding of a marginally reduced risk of epithelial ovarian cancer associated with ever use of low-dose aspirin is in line with the results of our (62) and other previous meta-analyses (126;127;134). Also, the larger risk reductions associated with an estimated daily dose of 150 mg and with long-term continuous use are more in line with the results of two large and recently published observational studies (63;64). Trabert et al. (63) found a 36% reduction in risk (95% CI, 0.50–0.81) for epithelial ovarian cancer associated with daily use of low-dose aspirin. However, only three of the 12 pooled case-control studies included data for the effect of frequency and dose of aspirin use on ovarian cancer risk. The second study, based on the Women's Health Initiative cohort, showed a large, albeit statistical imprecise, reduction in ovarian cancer risk among long-term (≥5 years), consistent users of aspirin (OR, 0.37; 95% CI, 0.16–0.84) (64). Previous studies include a population-based case-control study by Lo-Ciganic et al. (85), who reported similar risk reductions for ovarian cancer with continuous (OR, 0.71; 95% CI, 0.54–0.94) and low-dose (OR, 0.72; 95% CI, 0.53–0.97) aspirin use; the Iowa Women's Health Study by Prizment et al. (129), who found a decreasing risk of ovarian cancer with increasing frequency of aspirin use (≥6 times per week; hazard ratio, 0.61; 95% CI, 0.37–0.99); and Schildkraut et al. (80), who observed an OR of 0.50 (95% CI, 0.30–0.84) for ovarian cancer associated with frequent use of aspirin for ≥3 years in a population-based case-control study. The remaining studies of the association between aspirin use and ovarian cancer risk have shown weaker inverse associations (81;82;88;121;123;125;132;133;135;136), null associations (83;120;124;128;130;137), and, in two studies, an increased risk (79;84).

Our finding of a substantial reduction in epithelial ovarian cancer risk among women with the highest compliance with low-dose aspirin use (continuous use for ≥5 years) is interesting. The analysis was based on small numbers, and we cannot rule out the possibility that the risk profile for ovarian cancer among women compliant with drug use is different from that of the general female population. Nevertheless, the observation is in line with accumulating evidence that long-term, consistent use of aspirin is necessary to achieve a chemopreventive effect (60). In the Women's Health Study randomized trial, Cook et al. (138;139) found no association between assignment to 100 mg aspirin on alternate days and ovarian cancer risk during a 10-year intervention period (138) or 8-year extended post-trial follow-up (139). The authors did, however, find a reduction in colorectal cancer risk associated with aspirin use during the extended follow-up period (139). One reason for the null findings reported by Cook et al. might be that the average daily dose of 50 mg aspirin was not sufficiently high for prevention of ovarian cancer. Our results may indicate that a daily dose of 150 mg aspirin is required to obtain a chemopreventive effect against ovarian cancer. However, the optimum dose of aspirin for chemoprevention of colorectal or other cancers is unknown (57;66).

Statins

Few observational studies have been conducted of the association between statin use and ovarian cancer risk. Only two studies of gynecological malignancies (99;101) and three of multiple cancer sites (98;100;140) presented risk estimates for statin use associated with ovarian cancer (98–101) or female genital cancer overall (140). Consistent with our findings, Kaye et al. (100) observed no association between statin use and ovarian cancer risk in a population-based case-control study based on prescription data in the General Practice Research Database. The three other studies were also based on prescription data and found inverse statistically insignificant associations between statin use and ovarian cancer risk (98;99;101).

In two of the previous studies, duration of statin use was examined (98;101). In a study by Friedman et al. (98), a nearly 50% reduction in ovarian cancer risk was seen among women with ≥5 years of statin use (RR, 0.54; 95% CI, 0.27–1.09), although this result was based on only eight cases. Lavie et al. (101) reported a tendency toward decreasing ovarian cancer risk estimates with increasing duration of statin use. The results of these two studies (98;101) were pooled in a recent meta-analysis of long-term statin use (141) together with extended analysis of data for ovarian cancer from a randomized clinical trial of statin use and coronary heart disease (142). The pooled analysis indicated that long-term statin use (>5 years) was associated with a reduced risk of

ovarian cancer (141). In our study, we found no apparent variation in ovarian cancer risk according to duration of statin use, irrespective of intensity of use.

To our knowledge, no previous studies have examined associations between statin use and specific histologic types of epithelial ovarian cancer. Our results should be interpreted cautiously because of limited precision, notably in the analyses by duration and intensity of statin use. Nonetheless, a noteworthy observation is the inverse association between statin use and mucinous ovarian cancer. Previous studies have shown that mucinous tumors differ from non-mucinous types of epithelial ovarian cancer with regard to risk factors (25;26) and tissue of origin (13). Some mucinous ovarian cancers may be metastases from gastrointestinal cancers (143), e.g. colorectal cancer, for which there is some evidence of an inverse association with statin use (144).

Previous observational studies of statin use and ovarian cancer risk (98–101) also did not address potential effect modification according to type of statin. Our null findings for both lipophilic and hydrophilic statins are in line with the results of a meta-analysis of randomized controlled trials (97).

METHODOLOGICAL CONSIDERATIONS

Strengths

One of the main strengths of this PhD thesis is the large study population, comprising more than 4000 cases of epithelial ovarian cancer in the studies of low-dose aspirin and statins (Papers 2 and 3) and nearly 3500 cases in the studies of paracetamol and non-aspirin NSAIDs (Paper 1). The large study size allowed detailed analyses according to duration and intensity of study drug use and histologic type of epithelial ovarian cancer.

The national registries comprise a unique data source in Denmark due to the free access to health services, independent of income, and the personal identification numbers assigned to all citizens. The nationwide coverage and the completeness of the data eliminate recall bias and minimize selection bias, which is often a limitation in epidemiologic studies based on surveys. The data are collected prospectively and independently of any specific research question; thus, any misclassification of exposure or outcome would typically be non-differential.

Cases included in this thesis were identified in the Danish Cancer Registry, which has been shown to have high levels of completeness and accuracy (106;107;145). By restricting our sample to histologically verified, well-defined epithelial ovarian cancer cases, we further enhanced the case validity. Controls were sampled randomly from the general Danish female population by risk-set sampling, ensuring similar exposure periods among cases and controls and risk estimates equivalent to those that would have emerged in a cohort study in the base population (116).

We also consider that the information on exposure to the drugs studied was valid, as the Prescription Registry contains information on all prescription drugs dispensed at pharmacies in Denmark since 1995 (108). By use of the Prescription Registry, we reduced exposure misclassification and avoided recall bias, which are potential biases in many previous pharmaco-epidemiologic studies. Furthermore, the Prescription Registry provided us with detailed information on the specific types of drug, tablet doses, and timing of use. In our exposure definition, we applied a 1-year lag before the index date in order to reduce the risk for 'reverse causation' (116;146). This was particularly important in the study of paracetamol and non-aspirin NSAID use in relation to epithelial ovarian cancer (Paper 1), as prescription of these drugs preceding diagnosis might have been for symptoms of ovarian cancer (e.g. pain).

Register-based studies can be limited by lack of information on potential confounding factors (residual confounding) (147); however, by retrieving information from several Danish registries, we were able to adjust for most of the well-known risk factors for ovarian cancer, including parity, oral contraceptive use, HRT use, infertility, endometriosis, hysterectomy, and tubal ligation. Reassuringly, the associations between these factors and epithelial ovarian cancer were in the expected directions, lending credibility to our findings.

Limitations

In the study in Paper 1, we were unable to capture over-the-counter use of paracetamol and non-aspirin NSAIDs, which might have introduced misclassification bias and residual confounding. During the study period, about 40–45% of purchased paracetamol and 80–85% of non-aspirin NSAIDs were prescribed (55;148). The influence of use of over-the-counter paracetamol and non-aspirin NSAIDs on our results was probably limited for several reasons. First, the prevalence of over-the-counter purchase of these drugs was most likely lower among chronic users, as 50% of the cost of drugs is reimbursed if they are prescribed by a physician. Secondly, over-the-counter use of paracetamol and non-aspirin NSAIDs was most likely similar among cases and controls, thus introducing mainly non-differential exposure misclassification (149). Thirdly, adjustment for history of musculoskeletal disorders or headache, as proxy measure of over-the-counter analgesic drug use, yielded risk estimates similar to those of the main analyses. Fourthly, the observed associations between paracetamol and non-aspirin NSAIDs and risks of epithelial ovarian cancer were compatible with the results of a previous Danish study based on self-reported drug use (81). In our study of low-dose aspirin use and ovarian cancer risk (Paper 2), over-the-counter use had minimal influence on the primary exposure, as approximately 90% of low-dose aspirin in Denmark is dispensed by prescription (55); however, over-the-counter use of high-dose aspirin constituted a potential limitation according to the above considerations. In Paper 3, over-the-counter drug use did not influence the primary exposure, as statins are available only by prescription in Denmark.

A general limitation of register-based pharmaco-epidemiologic studies is lack of information on compliance. In our study, however, use of the study drugs required filling ≥2 prescriptions, and part of the cost of the drugs was paid by the patients, implying high compliance. Furthermore, because of our large study size, we were able to restrict analyses to continuous users only who can be assumed to be highly compliant (Papers 1 and 2). We did not evaluate continuous use of statins, because previously published Danish data have shown that adherence to therapy is high (150), which is consistent with our finding that 94.5% of the statin users were recent users.

We had no information on drug use before establishment of the Prescription Registry in 1995, which might have introduced some misclassification (left truncation), as some non-users might have used a study drug before 1995 and some users might have used a drug for longer or at higher intensity than recorded in the Prescription Registry. Potential left truncation of exposure information was minor in Paper 2 because the prevalence of low-dose aspirin use was low in the first half of the 1990s (151). Similarly, although statins (Paper 3) were marketed in 1989, these drugs were used only rarely in Denmark at the beginning of the 1990s

(152;153). Left truncation was a potential limitation in Paper 1 on use of paracetamol and non-aspirin NSAIDs. We addressed this possibility by applying a new-user sensitivity analysis, which yielded results similar to those of the main analyses. Left truncation also explained the low prevalence of oral contraceptive use in our study population of predominantly middle-aged and elderly women. We addressed this possible misclassification of exposure by examining the effect of oral contraceptive use on epithelial ovarian cancer risk among pre-/perimenopausal women who had the most comprehensive histories of oral contraceptive use. The results of these analyses indicated that left exposure truncation for oral contraceptives had no major influence on our results.

Information on drug exposure from the Prescription Registry spanned from 5 to 17 years. Thus, for women with the earliest index dates (2000), the drug history might have been too short to reveal an association with risk of epithelial ovarian cancer, which is likely to have a long latency (154).

One more possible limitation was the lack of information on indications for the study drugs, which could have introduced confounding (116;155). In Paper 1, we were challenged by the fact that analgesics are used for a broad, often nonspecific range of symptoms (155). The different risk estimates for paracetamol and non-aspirin NSAIDs, which are used for the same pain conditions, suggest, however, that use of analgesics was not systematically biased for cases and controls. With regard to low-dose aspirin use, there is no reason to expect that the effect on risk of cancer would vary by indication (60), since low-aspirin is used almost exclusively for cardioprotection among adults (56;156). In Paper 3, we stratified the analyses of ever use of statins by ischemic cardiovascular or cerebrovascular disease, which are indications for statin use (91;93), and found slightly increased, statistically insignificant risk estimates for epithelial ovarian cancer among women with these diseases. An ideal supplementary analysis would have been comparison with non-statin cholesterol-lowering medications (140); however, use of non-statins was low and decreased during the study period, precluding meaningful comparisons with statins.

Adherence to preventive drug therapy might be associated with health behavior, i.e. the 'healthy-user effect' (157). This potential limitation applied to use of low-dose aspirin (particularly continuous use) and statins. Although we adjusted for socioeconomic factors, which have been shown to correlate with lifestyle factors such as smoking, physical activity, and diet (118;158), we had no direct information on health-seeking behavior. The healthy-user effect might, however, have been offset to some extent by the fact that users of low-dose aspirin and statins are likely to be at increased risk of cardiovascular disease; these include smokers and obese people, who are also at increased risk of cancer (60).

Finally, histologic categorization of epithelial ovarian cancer is difficult, and our case material was not reviewed by a pathologist. We restricted epithelial ovarian cancer cases to histologically verified diagnosis and included only well-defined tumors (serous, endometrioid, clear cell, or mucinous), which resulted in exclusion of 909 epithelial ovarian cancers. The distribution of cell types of epithelial ovarian cancers included in our study, however, was not substantially different from that in centralized pathology reviews of ovarian tumors (12), except for a somewhat higher frequency of mucinous tumors. Although it would have been interesting to further classify the epithelial ovarian tumors by grade, this information is not available in the Danish Cancer Registry.

7. CONCLUSIONS AND PERSPECTIVES

The results of this PhD thesis add important knowledge about potential chemopreventive agents in relation to epithelial ovarian cancer. We found a substantial reduction in risk of epithelial ovarian cancer associated with paracetamol use, which increased with the duration and intensity of use, reaching a reduction of more than 50% for the longest duration and highest doses. Although we are aware of the limitations in our study, any unknown or unmeasured confounder would have had to be an extraordinarily strong risk factor for epithelial ovarian cancer. In addition, it is unlikely that even such strong confounders would readily explain the observed duration- and dose-response relations. Biologic mechanisms have been suggested to explain an inverse association between paracetamol use and epithelial ovarian cancer (78;86–89), although the evidence is inconclusive.

Our study supports the existence of an inverse association between low-dose aspirin use and epithelial ovarian cancer risk, consistent with most previous studies. Our results indicate that continuous, long-term use of low-dose aspirin at a minimum daily dose of 150 mg is necessary to obtain a protective effect against epithelial ovarian cancer. Although the potential chemopreventive properties of low-dose aspirin have been studied more extensively than for paracetamol, the precise mechanisms by which aspirin inhibits the development of cancer remain unresolved (65;66).

The results of this thesis do not support any major chemopreventive effect of statin use on the risk of epithelial ovarian cancer. We did observe an inverse association between statin use and mucinous ovarian cancer. Although this may be a chance finding, the fact that the risk factor profile (25;26) and origin (13) of mucinous ovarian tumors differ from those of the other types of epithelial ovarian cancer warrant additional research. Several experimental studies have demonstrated anti-neoplastic effects of statins (159–162). The discrepancy between these findings and those of epidemiologic studies may be that the serum level of statins achieved in the treatment of hypercholesterolemia is not sufficiently high to impose chemopreventive effects, due to extensive first-pass metabolism of statins in the liver (162). Finally, our results did not support a chemopreventive effect of non-aspirin NSAID use against epithelial ovarian cancer. In fact, we found increased risk estimates for long-term use of non-aspirin NSAIDs. However, in context with the results of most previous studies this result does not provide compelling evidence for an increased risk of epithelial ovarian cancer associated with non-aspirin NSAID use.

When considering a given chemopreventive treatment, safety is of particular concern because a large number of healthy people have to be treated to prevent disease in a small percentage of those treated (67). It is important to take into account the possibility that some individuals will not tolerate the treatment. Chemoprevention should primarily be targeted to individuals at high risk of developing the disease, such as women with a family history of ovarian or breast cancer, including carriers of BRCA1 or BRCA2 mutations, or women with the hereditary non-polyposis colorectal cancer syndrome. Interestingly, Cuzick et al. (57), in a recent review of the benefits and harm of prophylactic use of aspirin in the general population, concluded that there appears to be a net benefit of daily aspirin use for a minimum of 5 years among individuals aged 50–65 years. Reductions in incidence of cancer, myocardial infarction, and stroke accounted for the overall benefit, and the reduced cancer incidence was largely due to reduced risk of colorectal cancer for which the evidence of chemopreventive properties of aspirin is convincing (57;61;65).

Given the poor prognosis of epithelial ovarian cancer and the difficulty of early diagnosis, identification of preventive measures is important. The observed reductions in epithelial ovarian cancer risk associated with paracetamol and low-dose aspirin use in this PhD thesis are promising, and further studies of the potential chemopreventive effect of these agents against ovarian cancer are warranted. Studies should also be conducted to evaluate the influence of these two drugs on ovarian cancer prognosis, as two studies (163;164), including one in Denmark (164), suggest that paracetamol improves survival after ovarian cancer. It has also been suggested that statin use improves survival (101;165) or prevents the progression of epithelial ovarian cancer, possibly by a synergistic effect with chemotherapy (161;166). Epidemiologic studies should therefore focus on these aspects of statin use in regard to epithelial ovarian cancer.

8. SUMMARY

Ovarian cancer has a poor prognosis because the disease in the majority of patients is diagnosed at an advanced stage as a result of nonspecific symptoms and lack of efficient screening methods. Because of the poor prognosis of ovarian cancer and the challenge of early detection of the disease, identification of protective factors is important. It has been suggested that some commonly used drugs may have a protective effect against cancer, including ovarian cancer; however, the literature on chemopreventive measures for ovarian cancer is sparse and the results are inconclusive. Most previous studies have substantial methodological constraints, including limited study size and self-reporting of drug use, which introduces potential recall bias and misclassification.

This PhD thesis includes a nationwide case-control study to evaluate associations between use of drugs with potential chemopreventive properties and risk of epithelial ovarian cancer. The study is nested in the entire Danish female population using data from the following nationwide registries: the Danish Cancer Registry, the Danish Civil Registration System, the Danish Prescription Registry, the Danish National Patient Register, and registries in Statistics Denmark on fertility, education, and income. Information from the included registries is linked by use of the unique personal identification number assigned to all Danish citizens.

The cases were all women in Denmark with epithelial ovarian cancer diagnosed during 2000–2009 (Paper 1) and 2000–2011 (Papers 2 and 3), identified in the Cancer Registry. Age-matched female population controls were randomly selected from the Civil Registration System by risk-set sampling. We required that cases and controls have no history of cancer (except non-melanoma skin cancer) and that controls not previously have undergone bilateral oophorectomy or salpingo-oophorectomy. The total study population comprised 3741 epithelial ovarian cancer cases and 50,576 controls in Paper 1, and 4103 epithelial ovarian cancer cases and 58,706 controls in Papers 2 and 3. We used the Danish Prescription Registry to assess use (≥2 prescriptions on separate dates) of paracetamol, non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs), low-dose aspirin, and statins. Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for epithelial ovarian cancer associated with use of the study drugs, with adjustment for potential confounding factors selected á priori. We performed detailed analyses according to duration, intensity, and continuity of study drug use, and the analyses were stratified according to specific histologic types of epithelial ovarian cancer. In all studies, non-use (<2 prescriptions) of the individual study drugs was defined as the reference group.

A striking result of the PhD thesis was a strong inverse association between prescription use of paracetamol and risk of epithelial ovarian cancer. The risk estimates decreased with increasing duration and intensity of paracetamol use, reaching a more than 50% reduction for the longest duration (>10 years) and the highest doses (OR, 0.45; 95% CI, 0.24–0.86). In contrast, we did not observe an inverse association between use of non-aspirin NSAIDs and risk of epithelial ovarian cancer. Moreover, this thesis provides further evidence that use of low-dose aspirin is associated with a reduced risk of epithelial ovarian cancer. In particular, long-term (≥5 years) continuous use of low-dose aspirin, defined as overlapping prescription coverage periods, was associated with a large reduction in risk (OR, 0.56; 95% CI, 0.32–0.97). Finally, we found no apparent association between statin use and epithelial ovarian cancer risk, although the analysis by histologic type suggested an inverse association with the risk of mucinous tumors.

The results of this PhD thesis add important knowledge to the area of chemoprevention in relation to epithelial ovarian cancer. As for any observational study, we cannot exclude potential confounding and exposure misclassification; however, methodological limitations appear unlikely to fully explain the observed reductions in epithelial ovarian cancer risk associated with paracetamol and low-dose aspirin use. Additional research, ideally from clinical trials, is needed before our observations may lead to recommendations for chemopreventive measures against ovarian cancer. In case consensus points to a true protective effect of paracetamol or low-dose aspirin, comprehensive risk-benefit evaluations will also have to be performed. We hope that our results will encourage researchers to look more deeply into the potential chemopreventive effects of the study drugs against epithelial ovarian cancer risk.

9. ABBREVIATIONS

ATC	Anatomic Therapeutic Chemical Index
BRCA	Breast Cancer Early Onset
CA 125	Cancer antigen 125
CI	Confidence interval
COX	Cyclooxygenase
COPD	Chronic obstructive pulmonary disease
DDD	Defined daily dose
g	Gram
ICD	International Classification of Disease
ICD-O	International Classification of Disease for Oncology
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HRT	Hormonal replacement therapy
mg	Milligram
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio

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