Low-dose aspirin use and the risk of ovarian cancer in Denmark

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Received 10 September 2014; revised 22 October 2014 and 4 December 2014; accepted 9 December 2014

Background: A comprehensive body of evidence has shown that aspirin has cancer-preventive effects, particularly against gastrointestinal cancer, but its effects on the risk of ovarian cancer are less well established. This nationwide case–control study examined the association between low-dose aspirin and the risk of ovarian cancer.

Patients and methods: We identified all patients in the Danish Cancer Registry aged 30–84 years old with a histologically verified first diagnosis of epithelial ovarian cancer during 2000–2011. Each patient was sex- and age-matched to 15 population controls using risk-set sampling. Prescription use, comorbidity, reproductive history, and demographic characteristics data were obtained from nationwide registries. The use of low-dose (75–150 mg) aspirin was defined according to the dose as well as the duration and consistency of use. Conditional logistic regression was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for the association between low-dose aspirin use and the risk of epithelial ovarian cancer, both overall and for specific histological types.

Results: For 4103 ovarian cancer cases and 58,706 population controls, the adjusted OR for epithelial ovarian cancer associated with ever use (≥2 prescriptions) of low-dose aspirin was 0.94 (95% CI 0.85–1.05). ORs for epithelial ovarian cancer were lower with the use of 150 mg aspirin tablets (OR = 0.82; 95% CI 0.68–0.99) and with long-term use (≥5 years) of low-dose aspirin (OR = 0.77; 95% CI 0.55–1.08). Continuous long-term use of low-dose aspirin, defined as close consecutive prescriptions, was associated with a further reduction in OR (0.56; 95% CI 0.32–0.97). For histological types of epithelial ovarian cancer, the strongest inverse associations with low-dose aspirin use were seen for mucinous and endometrioid tumours.

Conclusion: This nationwide case–control study indicates that low-dose aspirin use may be associated with a reduced risk of epithelial ovarian cancer.

Key words: ovarian cancer, aspirin, chemoprevention

introduction

There is convincing evidence that aspirin protects against the development of gastrointestinal cancer [1–4]. Aspirin use may also protect against the development of several non-gastrointestinal cancers, including ovarian cancer [1–5], but the results are less conclusive. Two recent meta-analyses found a slightly reduced risk ratio for the association between aspirin use and ovarian cancer risk without any clear dose–duration relationship [4, 5]. Subsequently, two large observational studies reported that aspirin use was associated with substantial risk reductions for ovarian cancer [6, 7]. The majority of previous studies were based on self-reported drug use information, and only a few evaluated the associations between aspirin use and histological types of epithelial ovarian cancer.

Identifying preventive measures for ovarian cancer is important, since ovarian cancer continues to be the deadliest gynaecologic malignancy in the world [8]. The suggestive evidence of aspirin’s chemopreventive effect and the lack of detailed exposure and outcome information in previous studies prompted us to conduct this nationwide study of the association between low-dose aspirin use and epithelial ovarian cancer risk with detailed information about aspirin exposure and histological types of epithelial ovarian cancer.

methods

setting and data sources

Our study was designed as a nested case–control study based on information from nationwide Danish registries holding information about cancer,
prescription use, medical history, reproductive data, and education [9–13]. In Denmark, unambiguous linkage of individual-level data is secured by the use of a unique civil registry number assigned to all Danish citizens by the Civil Registration System [14]. A detailed description of the included registries with codes for drug exposure and covariates are provided in supplementary Material and Table S1, available at Annals of Oncology online.

All patients aged 30–84 years with a first diagnosis of ovarian cancer during 2000–2011 with no previous history of cancer excluding non-melanoma skin cancer [11] were eligible for inclusion. The ovarian tumours had to be histologically verified epithelial cancers of a well-defined type, i.e. serous, mucinous, endometrioid, or clear cell (supplementary Figure S1, available at Annals of Oncology online). Patients had to be residents of Denmark at the start of the Danish Prescription Registry [10] (January 1995) and at the date of diagnosis (index date).

For each case, we selected 15 female controls matched for date of birth (±1 month) from the Civil Registration System [14] using risk-set sampling [15]. Specifically, controls had to be alive and having no previous cancer (excluding non-melanoma skin cancer) at the time the corresponding case was diagnosed. Thus, the exposure window was the same for cases and controls. The controls fulfilled the same criteria as cases; in addition, we excluded controls with a previous bilateral oophorectomy before the index date.

We identified all prescriptions of low-dose aspirin (75, 100, or 150 mg tablets) redeemed by cases and controls from 1995 to 1 year before the index date. Study subjects were classified as ever users (≥2 prescriptions on separate dates) or non-users (<2 prescriptions) of low-dose aspirin. Ever users were further divided into recent users (≥2 prescriptions within 1–3 years before the index date) and former users (≤1 prescription within 1–3 years before the index date).

Since low-dose aspirin is taken almost exclusively as one tablet daily, we defined the cumulative duration of use based on the low-dose aspirin prescription dates and the number of days covered by the individual prescriptions. The coverage was defined as the number of tablets dispensed plus a 30-day grace period, allowing for some degree of non-compliance. A treatment period continued as long as consecutive prescriptions were redeemed and the cumulative treatment period was classified as short term (<5 years) or long term (≥5 years).

We defined the daily dose of low-dose aspirin as the tablet dose prescribed during the exposure period: 75–100, 150 mg, or mixed strength. We defined continuous low-dose aspirin use as one continuous treatment period, from the start of treatment until 1 year before the index date (i.e. no addition of separate treatment periods).

statistical analyses

Following a predefined statistical analysis plan, we used conditional logistic regression to calculate age-matched and multivariable adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for epithelial ovarian cancer associated with low-dose aspirin. Potential confounders were selected based on current evidence of risk factors for ovarian cancer [16, 17]. The final model adjusted for age (by design), education, parity, infertility, endometriosis, diabetes mellitus, chronic obstructive pulmonary disease or asthma, hysterectomy, tubal sterilization, oral contraceptives, hormonal replacement therapy (HRT), statins, and paracetamol. First, we examined the associations between ever, recent, and former use of low-dose aspirin and ovarian cancer risk. Second, we carried out stratified analyses according to the duration and dose of the low-dose aspirin tablets (75–100 or 150 mg) and the type of ovarian cancer (overall, four histological types). Third, we computed ORs for overall epithelial ovarian cancer according to estimated dose, duration, and continuity of low-dose aspirin use. The reference group in all analyses was non-use of low-dose aspirin.

Finally, we carried out three sensitivity analyses. First, we used the total number of low-dose aspirin tablets redeemed to define exposure to low-dose aspirin. Specifically, we computed ORs for epithelial ovarian cancer within categories of ≤1825 and >1825 low-dose aspirin tablets, assuming that the number of tablets represented the number of days that low-dose aspirin was taken. Second, to evaluate the impact of potential misclassification due to left truncation of aspirin exposure prior to 1995, we applied a new-user design by excluding all cases, with their corresponding controls, and all controls who redeemed a prescription of low-dose aspirin in 1995 (wash-out period). Third, we stratified the analyses according to parity (0, 1, 2, and ≥3 births) and evaluated potential statistical interaction (on a multiplicative scale) between parity and the effect of low-dose aspirin use.

All analyses were carried out using R version 3.0.2 [18], with a significance level of 5%.

results

Our study population consisted of 4103 epithelial ovarian cancer cases and 58,706 population controls. Table 1 summarizes the characteristics of cases and age-matched controls. Compared with controls, cases had higher prevalence of nulliparity, infertility, and HRT use and lower prevalence of tubal sterilization and use of oral contraceptives or paracetamol.

Overall, 12.0% of cases and 12.8% of controls were ever users of low-dose aspirin, yielding an adjusted OR of 0.94 (95% CI 0.85–1.05) for epithelial ovarian cancer (Table 2). No major variation in ORs was observed according to the recency of low-dose aspirin use or to the overall duration of cumulative use (≥5 years: OR = 0.92; 95% CI 0.77–1.10). Exclusive use of 150 mg aspirin tablets was associated with a reduced OR for epithelial ovarian cancer (0.82; 95% CI 0.68–0.99). A similar, albeit not statistically significant, OR was observed in a combined analysis of tablet dose and duration of use (150 mg tablets; ≥5 years: OR = 0.77; 95% CI 0.55–1.08; Table 3).

When restricting low-dose aspirin exposure to continuous use (i.e. overlapping treatment periods defined by the number of tablets and 30-day grace periods), ≥5 years of low-dose aspirin use were associated with an OR for epithelial ovarian cancer of 0.56 (95% CI 0.32–0.97).

In analyses stratified according to the histological type of ovarian cancer, the risk estimates for serous ovarian cancer were similar to those for epithelial ovarian cancer overall (Table 2). Non-statistically significant inverse associations were found between low-dose aspirin use and the risk of endometrioid and mucinous ovarian cancer. For endometrioid cancer, the ORs decreased with an increasing tablet dose (150 mg: OR = 0.68; 95% CI 0.41–1.15) and with an increasing duration of low-dose aspirin use (≥5 years: 0.69; 95% CI 0.42–1.11). For mucinous cancer, there was no clear pattern with tablet dose or duration. For clear cell ovarian cancer, low-dose aspirin use was associated with non-statistically significantly increased ORs without any apparent pattern according to the tablet dose or duration of use (Table 2).

In the sensitivity analysis defining exposure to low-dose aspirin according to the number of low-dose aspirin tablets dispensed, the OR for epithelial ovarian cancer was 0.94 (95% CI
0.79–1.11) associated with >1825 tablets as a proxy for use of low-dose aspirin for ≥5 years and thus similar to the result for ≥5 years of cumulative use of low-dose aspirin defined according to prescription coverage periods. In the new-user analysis, ever use of low-dose aspirin was associated with a neutral OR for epithelial ovarian cancer (0.99; 95% CI 0.89–1.11), whereas ≥5 years of continuous low-dose aspirin was associated with a reduced OR (0.72; 95% CI 0.38–1.37), albeit based on small numbers. Finally, we observed no material variation in risk estimates according to parity (P = 0.32).

**Discussion**

In this nationwide case–control study, we observed a reduced risk of epithelial ovarian cancer associated with the use of low-dose aspirin at an estimated daily tablet dose of 150 mg and with the long-term continuous use of low-dose aspirin as defined by overlapping prescription coverage periods. Although analyses of each of the histological types of epithelial ovarian cancer were based on a limited number of cases, we found substantial type-specific risk variation. The risk estimates for serous ovarian cancer were similar to those of epithelial ovarian cancer overall. In contrast, we observed inverse associations between low-dose aspirin use and the risk of endometrioid or mucinous ovarian cancer, and increased risk estimates for clear cell ovarian cancer.

Our results are in agreement with those of a recent pooled case–control study by Trabert et al. [6], which reported a 36% reduced risk (OR = 0.64; 95% CI 0.50–0.81) for epithelial ovarian cancer associated with daily use of low-dose aspirin. Similarly, another population-based case–control study of 902 epithelial ovarian cancer cases by Lo-Ciganic et al. [19] showed a reduced risk of ovarian cancer associated with continuous (OR = 0.71; 95% CI 0.54–0.94) and low-dose (OR = 0.72; 95% CI 0.53–0.97) aspirin. In the Women’s Health Initiative cohort, Brasky et al. [7] observed a large reduction in ovarian cancer risk among women with consistent aspirin use for 5 or more years [hazard ratio (HR) = 0.37; 95% CI 0.16–0.84]; however, this result was based on just eight exposed cases. Furthermore, in the Iowa Women’s Health Study, Prizment et al. [20] observed a decreasing risk of ovarian cancer with increasing frequency of aspirin use (≥6 times per week: HR = 0.61; 95% CI 0.37–0.99). Finally, frequent use of aspirin for 3 or more years was associated with a 50% reduced risk of ovarian cancer (OR = 0.50; 95% CI 0.30–0.84) in a population-based case–control study by Schildkraut et al. [21]. The majority of the remaining observational studies reported weaker inverse associations between aspirin use and ovarian cancer [22–31]. Some studies reported null associations [32–37], whereas only two studies reported an increased risk of ovarian cancer associated with aspirin use [38, 39].

Our finding of a reduced ovarian cancer risk, particularly among women with the highest compliance with low-dose aspirin use, should be interpreted cautiously. These women comprised only 2–3% of low-dose aspirin users in the study population; thus, the statistical precision of the risk estimates was low for this subgroup. Some of these women might have taken more than one low-dose aspirin tablet per day, although 75–150 mg aspirin is the recommended dose for cardiovascular protection in Denmark [37]. It is also conceivable that these women had a risk profile for ovarian cancer that differed from that of the general female population, although we adjusted for most established ovarian cancer risk factors. Nonetheless, our finding is interesting due to increasing evidence that long-term consistent use of aspirin is necessary to achieve a cancer-preventive effect [2, 40]. Cook et al. [41, 42] found no association between aspirin and ovarian cancer in the Women’s Health Study involving 39 876 women assigned to 100 mg of aspirin or placebo every other day during the intervention period of 10 years [41] or after extended post-trial follow-up [42]. One reason why Cook et al. may not have seen an association is that the aspirin dose, an average of 50 mg daily, was too low. Our results may indicate that a minimum daily dose of 150 mg is needed for aspirin to have a chemopreventive effect against ovarian cancer. However, the optimal dose of aspirin for chemoprevention of ovarian or other cancers remains unclear [2, 43].
## Table 2. Risk of epithelial ovarian cancer and histological types by dose and duration of low-dose aspirin use

<table>
<thead>
<tr>
<th></th>
<th>Epithelial OC</th>
<th>Serous OC</th>
<th>Endometrioid OC</th>
<th>Mucinous OC</th>
<th>Clear cell OC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (N)</td>
<td>Adjusted OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cases (N)</td>
<td>Adjusted OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cases (N)</td>
</tr>
<tr>
<td>Non-use</td>
<td>3609</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2372</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>579</td>
</tr>
<tr>
<td>Ever use&lt;sup&gt;c&lt;/sup&gt;</td>
<td>494</td>
<td>0.94 (0.85–1.05)</td>
<td>359</td>
<td>0.96 (0.85–1.09)</td>
<td>71</td>
</tr>
<tr>
<td>Recent use&lt;sup&gt;d&lt;/sup&gt;</td>
<td>419</td>
<td>0.95 (0.85–1.06)</td>
<td>302</td>
<td>0.96 (0.84–1.10)</td>
<td>64</td>
</tr>
<tr>
<td>Former use&lt;sup&gt;e&lt;/sup&gt;</td>
<td>75</td>
<td>0.92 (0.73–1.17)</td>
<td>57</td>
<td>0.97 (0.73–1.27)</td>
<td>7</td>
</tr>
<tr>
<td>Estimated daily dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>75–100 mg</td>
<td>251</td>
<td>1.01 (0.88–1.16)</td>
<td>182</td>
<td>1.03 (0.87–1.21)</td>
<td>42</td>
</tr>
<tr>
<td>150 mg</td>
<td>125</td>
<td>0.82 (0.68–0.99)</td>
<td>89</td>
<td>0.82 (0.65–1.02)</td>
<td>16</td>
</tr>
<tr>
<td>Mixed dose</td>
<td>118</td>
<td>0.95 (0.78–1.16)</td>
<td>88</td>
<td>1.01 (0.80–1.27)</td>
<td>13</td>
</tr>
<tr>
<td>Duration of use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>341</td>
<td>0.95 (0.84–1.07)</td>
<td>246</td>
<td>0.97 (0.84–1.12)</td>
<td>51</td>
</tr>
<tr>
<td>≥5 years</td>
<td>153</td>
<td>0.92 (0.77–1.10)</td>
<td>113</td>
<td>0.94 (0.77–1.16)</td>
<td>20</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for age, education, parity, infertility, endometriosis, diabetes mellitus, chronic obstructive pulmonary disease or asthma, hysterectomy, tubal sterilization, oral contraceptives, hormonal replacement therapy, statins and paracetamol.

<sup>b</sup>Reference category.

<sup>c</sup>‘Ever use’ was defined as ≥2 prescriptions on separate days.

<sup>d</sup>‘Recent use’ was defined as ≥2 prescriptions in the 1–3 years before the index date.

<sup>e</sup>‘Former use’ was defined as ≤1 prescriptions in the 1–3 years before the index date.

CI, confidence interval; OC, ovarian cancer; OR, odds ratio.
Although the epidemiological literature rather consistently supports an inverse association between aspirin use and ovarian cancer risk, the potential underlying mechanisms are unclear [43]. Low-dose aspirin inhibits the constitutively active cyclooxygenase-1 (COX)-1 enzyme, whereas higher doses also inhibit the inducible COX-2 enzyme [44]. Moreover, aspirin has targets other than COX, so its anti-cancer effects may not be solely attributable to COX inhibition [43–46]. The drug concentrations achieved by low-dose aspirin may be too low for these COX-independent mechanisms [43, 44]; thus, it has been suggested that the antithrombotic effect of low-dose aspirin may play a key role in its chemopreventive activity [44].

The main strengths of our study were the nationwide approach and the reliable, detailed exposure and outcome data that we retrieved from high-quality prescription and cancer registries. The use of the Prescription Registry eliminated recall bias, minimized misclassification, and allowed us to evaluate the effects of the timing, intensity, and duration of low-dose aspirin use. The large study size permitted detailed analyses according to aspirin exposure and the histological type of ovarian cancer. Cases were identified in the Cancer Registry, which is comprehensive and has high completeness and accuracy of diagnoses [11]. By restricting our sample to histologically verified well-defined epithelial ovarian cancer cases, we further enhanced case validity. Moreover, the register-based information on reproductive data and educational level, and the continuously updated data on prescriptions and medical conditions, enabled us to adjust the analyses for the majority of established risk factors for ovarian cancer.

The study had some limitations. We had no information on over-the-counter drug use. However, in Denmark, more than 90% of the total sales of low-dose aspirin are through prescriptions [47]. Moreover, since low-dose aspirin is predominantly used as part of a daily regimen for cardioprotection, it seems appropriate to assume that over-the-counter purchase of low-dose aspirin was mainly by women who also redeemed low-dose aspirin by prescription; thus, misclassification of low-dose aspirin use was most likely minimal. In contrast, high-dose (500 mg) aspirin is primarily sold over-the-counter in Denmark [47]. High-dose aspirin is used mainly for short-term treatment of transient pain; when taken for conditions associated with chronic pain, high-dose aspirin, non-aspirin NSAIDs, and paracetamol are typically redeemed by prescription due to reimbursement of the cost and the need for medical surveillance [17]. Therefore, over-the-counter use of aspirin likely resulted in only minor misclassification of long-term aspirin use. Even so, we cannot rule out that the lack of information on over-the-counter use of high-dose aspirin, non-aspirin NSAIDs, or paracetamol may have introduced some residual confounding.

Finally, left truncation of prescription data may have led to exposure misclassification of women who initiated low-dose aspirin treatment before 1995. Although therapy with low-dose aspirin for cardioprotection was uncommon in Denmark before the start of the Prescription Registry [48], we evaluated the influence of left truncation by applying a new-user design, which yielded results similar to those of the main analyses.

In conclusion, this study indicates that low-dose aspirin use may be associated with a reduced risk of epithelial ovarian cancer. The most pronounced effect was observed for long-term use of 150 mg aspirin and for continuous use of low-dose aspirin. The finding of risk variation according to ovarian cancer histological type warrants additional studies.

funding
Funding was obtained from the Unit of Lifestyle, Virus and Genes, Danish Cancer Society Research Centre (no grant number).

disclosure
The authors have declared no conflicts of interest.

references
3. Rothwell PM, Price JF, Fowkes FG et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of

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Table 3. Risk of epithelial ovarian cancer by dose and by consistency of low-dose aspirin use as stratified according to the duration of use

<table>
<thead>
<tr>
<th>Dose by duration of use</th>
<th>Cases (N)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5 years</td>
<td>195</td>
<td>1.02 (0.87–1.19)</td>
</tr>
<tr>
<td>≥5 years</td>
<td>56</td>
<td>1.00 (0.75–1.32)</td>
</tr>
<tr>
<td>150 mg</td>
<td>89</td>
<td>0.85 (0.68–1.06)</td>
</tr>
<tr>
<td>≥5 years</td>
<td>36</td>
<td>0.77 (0.55–1.08)</td>
</tr>
<tr>
<td>Mixed dose</td>
<td>57</td>
<td>0.93 (0.71–1.23)</td>
</tr>
<tr>
<td>≥5 years</td>
<td>61</td>
<td>0.97 (0.74–1.27)</td>
</tr>
</tbody>
</table>

*Adjusted for age, education, parity, infertility, endometriosis, diabetes mellitus, chronic obstructive pulmonary disease or asthma, hysterectomy, tubal sterilization, oral contraceptives, hormonal replacement therapy, statins and paracetamol.

bReference category.

cContinuous low-dose aspirin use was computed as treatment periods with consecutive prescriptions redeemed within the time window defined by the coverage (number of tablets + grace period) of the preceding prescription. The analysis was restricted to continuous low-dose aspirin use for <5 or ≥5 years according to the timing of the most recent continuous treatment period with coverage up until 1 year before the index date.

CI, confidence interval; OR, odds ratio.