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Anti-inflammatory drug use and ovarian cancer risk by COX1/COX2 expression and infiltration of tumor-associated macrophages

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Abstract

Background: NSAID use may affect ovarian cancer risk via prostaglandin synthesis and tumor-associated macrophage (TAM) infiltration. We evaluated if associations between aspirin or non-aspirin NSAID use and ovarian cancer risk differed by tumor expression of prostaglandin-related (COX1, COX2) and TAM-related (CD68, CD163) markers.

Methods: We evaluated cases and matched controls from the Nurses' Health Study (NHS), NHSII, and New England Case Control Study (NECC). Cases with immunohistochemistry data on COX1 and COX2 (n=532) or CD68 and CD163 (n=530) were included. We used polytomous logistic regression, adjusted for ovarian cancer risk factors, to estimate odds ratios (OR) for NSAID use and ovarian cancer risk by marker level.

Results: Recent aspirin use had a non-significant inverse association and recent non-aspirin NSAID use had no association with ovarian cancer risk. NSAID use was not differentially associated with ovarian cancer by COX1 or COX2 expression. However, recent aspirin use was

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associated with lower ovarian cancer risk for high (OR=0.54, 95%CI=0.37-0.78), but not low (OR=1.50, 95%CI=0.97-2.31), CD163 density (p-heterogeneity<0.001). Similar results were observed for aspirin duration and tablets and for recent non-aspirin NSAID use. Results were not clearly different by macrophage density defined by the less specific macrophage marker, CD68.

Conclusion: NSAID use was inversely associated with risk of ovarian cancer with high density CD163, a marker for M2-type, immunosuppressive macrophages. However, the relationship did not differ by prostaglandin synthesis markers.

Impact: Future research should explore prostaglandin-independent mechanisms for the association between NSAID use and ovarian cancer risk, including immune mechanisms.

INTRODUCTION

There is growing evidence of an association between anti-inflammatory drug use and ovarian cancer risk (3–8). Recent studies reported a lower ovarian cancer risk among regular aspirin users that was strongest for frequent or low-dose aspirin use (4–8). A key mechanism of action for aspirin and other NSAIDs is down-regulation of prostaglandin synthesis via inhibition of the cyclooxygenase (COX) enzymes, COX1 and COX2 (9–12). Prior work showed a strong inverse association between aspirin use and colorectal cancer risk that was only evident for COX2+ tumors (13). In contrast, the association between aspirin use and breast cancer did not differ by COX2 status, suggesting different mechanistic pathways across cancer sites (14).

Prostaglandins can also modulate immune function, in part by inducing activation and polarization of macrophages (15–23). Tumor-associated macrophages (TAMs) frequently activate and polarize to the M2 phenotype in response to inflammatory signaling (15, 16, 18, 24, 25). Once activated, they alter the inflammatory response, inhibit Type I T-helper (Th1) adaptive immunity, contribute to matrix remodeling, and promote cell proliferation and angiogenesis (20, 26–29). M2-type TAM infiltration has been associated with worse prognosis in breast cancer, while results have been mixed for ovarian cancer (30–32). Most ovarian cancer studies used CD68 as a total macrophage marker and CD163 as an M2-type marker (30–36).

Here, we evaluate if the associations between NSAID use and ovarian cancer risk differ by COX1 or COX2 expression or by infiltration with TAMs. We hypothesized that the inverse association between anti-inflammatory drug use and ovarian cancer would be strongest for tumors with higher levels of COX1 and COX2, a greater number of M2-type macrophages (high CD163), or a greater ratio of M2-type to total macrophages (CD163/CD68).

MATERIALS AND METHODS

We conducted a case-control study, including 450 cases from the Nurses' Health Studies and 157 cases from the New England Case Control Study.

Study population

The Nurses' Health Study (NHS) is a prospective cohort study that enrolled 121,700 female registered nurses aged 30-55 in 1976. The NHSII enrolled 116,429 female registered nurses aged 25-42 in 1989. Women completed a baseline questionnaire on lifestyle and reproductive factors, medication use, and disease outcomes. Updated questionnaires were administered biennially thereafter. Incident epithelial ovarian cancer cases were identified from questionnaires, reports from family, or linkage to the National Death Index. Cases were confirmed by medical record review or cancer registry linkage. To facilitate pooling with the New England Case Control (NECC) study, we matched four controls per case on year of birth and questionnaire completion at the time of case diagnosis. Women were ineligible for selection as controls if they experienced any of the following prior to the case index date: bilateral oophorectomy, pelvic irradiation, history of cancer except non-melanoma skin cancer. Return of self-administered questionnaires was accepted as informed consent. The Institutional Review Board at Brigham and Women's Hospital approved the NHS/NHSII study protocols.

The NECC is a population-based case control study (detailed elsewhere (37, 38)). Briefly, 1,513 cases of epithelial ovarian cancer were identified from statewide cancer registries and tumor boards in Eastern Massachusetts and New Hampshire. Cases were interviewed a median of 8.5 months after diagnosis. Controls were identified via drivers' license registries and town resident lists, and frequency matched to cases by age and state of residence. Of 4,366 potential controls, 1,426 did not meet eligibility criteria, 1,362 declined to participate, and 1,578 were enrolled. Women were ineligible if they were younger than age 18, did not have a phone, did not speak English, moved, died, had a prior bilateral oophorectomy, or their physician declined permission to contact (cases). Each participant provided written informed consent. The Institutional Review Boards at Brigham and Women's Hospital and Dartmouth Medical School approved the study protocols.

We included cases diagnosed 1976-2012 in NHS/NHSII and 1998-2008 in NECC. An expert gynecologic pathologist (JLH) who was blinded to exposure status reviewed case medical records, confirming the diagnosis and recording tumor morphology (invasive, borderline), histology (serous, mucinous, endometrioid, clear cell, other), grade (I, II, III), and stage (I, II, III, IV).

Assessment of anti-inflammatory drug use and covariates

NHS/NHSII assessed aspirin and non-aspirin NSAID use via self-report on biennial questionnaires (8). Women in NHS reported recent regular use (2+ times per week) of aspirin on all biennial questionnaires except 1986. Data on the number of aspirin tablets per week was collected in 1980, 1982 and biennially beginning in 1994. Recent regular use of non-aspirin NSAIDs was queried biennially starting in 1990, and the number of non-aspirin NSAID tablets used per week was collected biennially beginning in 1998. In NHSII, recent regular use of aspirin and non-aspirin NSAIDs was queried in 1989, 1993, and biennially thereafter. Questions on number of tablets per week were added in 1999 and repeated biennially. Data on the majority of ovarian cancer risk factors, including menopausal status, parity, oral contraceptive (OC) use, postmenopausal hormone therapy (HT) use, tubal

ligation, hysterectomy, family history of breast or ovarian cancer, and weight (to calculate BMI) were self-reported on questionnaires every 2-4 years.

The NECC assessed anti-inflammatory drug use by in-person interview. The interviewer asked women to recall the time period from childhood up to one year before diagnosis for cases or up to one year before the interview date for controls, and report any regular analgesic use (i.e., continuous use for six months or longer). For each drug type, women were asked to report age at first use, duration of use, and usual dose for every non-consecutive period of use lasting at least six months. Women were also asked about menopausal status, parity, OC use, HT use, tubal ligation, hysterectomy, family history of breast or ovarian cancer, height and weight.

Tumor block collection and tissue microarrays creation

NHS/NHSII requested paraffin-embedded tissue blocks containing representative tumor samples from cases with a pathology report. Tumors (n=450) were collected. Primary reasons tumor blocks were not collected were: the tissue had been destroyed, the patient was deceased, or the hospital was unable to send a sample (39). The NECC accessed tumor blocks from cases (n=157), most of whom were diagnosed at Brigham and Women's Hospital (n=119). In NECC, funding was available to obtain tissue blocks for only a subset of cases, oversampling high-grade serous tumors. Tissue blocks were reviewed to verify histology and grade and make tissue microarrays (TMAs). TMAs were arrayed at the Dana-Farber/Harvard Cancer Center (DF/HCC) Specialized Histopathology Core by taking three core biopsies with a 1.0mm (NECC) or 0.6mm (NECC/NHS/NHSII) diameter from ovarian cancer tissue blocks and re-embedding the cores into a single block (40, 41).

Immunohistochemistry

Slides were cut from TMA blocks and, within two weeks, stained for a single marker and counterstained for hematoxylin at the DF/HCC Specialized Histopathology Core. Staining was performed on the Leica Bond III staining platform using the Bond Polymer Refine Detection Kit (Leica Biosystems). Primary antibodies, dilutions, and antigen retrieval are in Supplemental Table 1.

Consistent with prior studies (13, 14, 30, 31), staining was evaluated in a quantitative or semi-quantitative manner by one of two gynecologic pathologists (JLH, MG). COX1 was evaluated in four categories: no staining, weak intensity staining in any cell, moderate intensity staining in ≥10% of cells, and high intensity staining in ≥10% of cells. COX2 was evaluated in five categories based on percent staining positive: 0, >0-5, >5-25, >25-75, >75. CD68 and CD163 density were scored separately for tumor stroma and epithelium as: none (1), low (2; <10% of cells, scattered), moderate (3; <10% of cells, with aggregation - at least three aggregates of three macrophages), high (4; >10% of cells macrophages or an area of confluent macrophages). Stromal and epithelial scores were summed to reflect total TAM infiltration. The intraclass correlation coefficients (ICCs) across the three cores were: COX1, 0.81; COX2, 0.72; CD163, 0.71; and CD68, 0.67.

We dichotomized scores at the median to maximize power. Specifically, positive staining was defined as follows: COX1+, moderate to high intensity staining of ≥10% of cells, and

COX2+, >5% of cells stained; otherwise tumors were coded as stain negative. CD68 staining was used to estimate total macrophage density and CD163 to quantify M2-type macrophages (22, 30, 31, 42). Tumors were classified as high density (i.e., CD68 high or CD163 high) when the sum of the epithelium and stromal scores was greater than 4 out of 8. CD163/CD68 was calculated using the summed scores.

Statistical analysis

We created two analytic datasets: one examining COX1 and COX2, and one examining CD163 and CD68. For each dataset, we excluded cases with missing data on either of the relevant markers. We had 532 cases for analyses of COX1 and COX2 and 530 cases for analyses of CD163 and CD68. We then excluded participants with missing data on the exposure of interest (n=80 aspirin and n=109 non-aspirin NSAIDs for COX analyses, and n=79 aspirin and n=106 non-aspirin NSAIDs for TAM analyses).

Aspirin and non-aspirin NSAID use were harmonized and evaluated using three metrics: recent use (recent, past, non-use), duration of use (none to <1, 1 to <5, 5 to <10, and ≥10 years of regular use), and tablets used per week (none to <1, 1 to <6, and ≥6 tablets per week). We captured on-study use (minimum age of 25) for NHS/NHSII and use after age 25 for NECC. Recent use was defined as use during the questionnaire cycle prior to case diagnosis (average lag-time of 3 years) for NHS/NHSII and 1-year prior to the case index date in NECC. Duration of use was assessed in years. Tablets per week reflected cumulative average tablets per week in NHS/NHSII and tablets per week for the longest continuous period of anti-inflammatory drug use for NECC.

We evaluated the correlation among tumor markers using Spearman correlations. We fit logistic regression models to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for NSAID use and ovarian cancer risk in the full study population, and used polytomous logistic regression to estimate ORs and 95% CIs for ovarian cancer defined by COX1(+/−), COX2(+/−), CD163(high/low) and CD68(high/low), and by the ratio CD163/CD68 (<1/≥1). We adjusted for matching factors and ovarian cancer risk factors in multivariate models. Covariates not associated with the tumor markers were constrained to have the same estimate for all tumor subtypes. These included cohort (NHS, NHSII, NECC); age (continuous in years); menopausal status (premenopausal/unknown, postmenopausal) parity (nulliparous, 1, 2, 3, >3 children); estrogen, estrogen plus progestin, and other HT use separately (ever/never); tubal ligation (yes/no); family history of breast or ovarian cancer (yes/no); and BMI (<20, 20-25, 25-30, 30+ kg/m^2). Hysterectomy (yes/no) and OC use (<1, 1-5, 5-10, 10+ years) were differentially associated by tumor marker status for multiple markers, so they were modeled as unconstrained variables with different estimates for each tumor type. There was no evidence of heterogeneity across studies (Supplemental Table 2), so data were pooled.

We conducted planned sensitivity analyses restricting to (1) invasive and (2) high grade serous ovarian cancer. Another sensitivity analysis assessed low-dose aspirin use, though this analysis was restricted by data availability to NHS/NHSII participants with a case index date between 2001 and 2012. We conducted a third sensitivity analysis with a common reference group (no regular use of any NSAID), and a fourth sensitivity analysis to evaluate

the association of analgesic use and risk of COX1+ and COX1- tumors accounting for COX2 status and vice versa, CD68-low and CD68-high tumors accounting for CD163 levels and vice versa, and CD163-low and CD163-high tumors accounting for any positivity of COX1 or COX2 (43). All analyses were conducted using SAS statistical software version 9.4 (SAS Institute, Cary, NC, USA) or Stata statistical software version 12.1 (StataCorp, College Station, TX, USA). Statistical tests were two-sided with p-values <0.05 considered statistically significant.

RESULTS

Cases on TMAs were more likely to be postmenopausal, slightly less likely to use OCs, and had greater parity than observed in the full population of cases from NHS/NHSII and NECC (Supplemental Table 3). In this study population, we did not observe a significant association between aspirin use and risk of ovarian cancer, although there was a suggestion of an inverse association (e.g., OR, recent vs. non-use=0.78, 95%CI=0.55-1.09; Supplemental Table 2). The association between non-aspirin NSAID use and risk of ovarian cancer was generally null, though women with ≥10 years of non-aspirin NSAID use had a 1.8-fold increased risk of ovarian cancer (95%CI=1.17-2.77) relative to those with <1 year of non-aspirin NSAID use.

COX1 and COX2

COX1+ ovarian cancers were more likely to be serous, high grade, and high stage than COX1- cancers, while the distribution of histopathologic features was similar for COX2+ versus COX2- ovarian cancers (Table 1). Further, women with COX1+ cancer were more likely to be postmenopausal, had shorter durations of OC use and reported greater estrogen plus progestin HT use than women with COX1- cancer (Supplemental Table 4). Ovarian cancer cases that were COX2+ versus COX2- had a higher prevalence of tubal ligation as well as longer duration of OC and estrogen plus progestin HT use. The spearman correlation between COX1 and COX2 levels was 0.07 (Table 2).

We observed no evidence of heterogeneity for associations between aspirin or non-aspirin NSAID use and ovarian cancer risk by COX1 or COX2 receptor status, for regular use, duration, and tablets/week (p-heterogeneity≥0.22; Table 3). For example, the OR for recent versus never use of aspirin and ovarian cancer was 0.71 (95%CI=0.50-1.01) for COX1- and 0.87 (95%CI=0.54-1.38) for COX1+ tumors (p-heterogeneity=0.72). Similarly, regular non-aspirin NSAID use was not associated with risk of ovarian cancer for COX1- (OR=0.96, 95%CI=0.68-1.34), COX1+ (OR=1.05, 95%CI=0.66-1.68), COX2- (OR=1.00, 95%CI=0.70-1.41) and COX2+ (OR=1.00, 95%CI=0.65-1.53) cases.

When we cross-classified tumors by COX1 and COX2, there was no significant association between recent aspirin use and any of the four tumor types. For example, the OR for recent aspirin use and risk of COX1-/COX2- ovarian cancer was 0.75 (95%CI=0.50-1.12); while the OR for recent aspirin use and risk of COX1+/COX2+ ovarian cancer was 0.74 (95%CI=0.38-1.44). The ORs for recent non-aspirin NSAID use and risk of COX1-/COX2- and COX1+/COX2+ cancers were similar (OR=0.90, 95%CI=0.60-1.34; OR=0.77, 95%CI=0.37-1.60, respectively).

TAM markers: CD163 and CD68

CD163 and CD68 were strongly correlated with each other (ρ=0.81), and weakly correlated with COX1 and COX2 (ρ=0.09-0.19; Table 2). Tumors with high density CD163 or CD68 were more likely to be serous, high grade and stage III (Table 1). When we considered the distribution of ovarian cancer risk factors by density of CD163 and CD68, OC use, tubal ligation, and greater parity were more common for cases with high density of CD163 or CD68 (Supplemental Table 4).

We observed evidence of a differential association for aspirin use and ovarian cancer risk by CD163 density (Table 4). Recent aspirin use (vs. non-use) was suggestively associated with a higher risk of CD163-low ovarian cancer (OR=1.50, 95%CI=0.97-2.31) and a lower risk of ovarian cancer with high CD163 density (OR=0.54, 95%CI=0.37-0.78; p-heterogeneity<0.001). We observed similar differences for duration of aspirin use (p-heterogeneity=0.012), and tablets per week (p-heterogeneity<0.001). The comparable associations by CD68 density were not significantly different (e.g., recent use vs. non-use $OR_{CD68low}$=0.99, 95%CI=0.63-1.57; $OR_{CD68high}$=0.71, 95%CI=0.49-1.01; p-heterogeneity=0.17). No heterogeneity was observed by the ratio CD163/CD68 (p>0.05).

A subset of associations between non-aspirin NSAIDs and ovarian cancer risk also differed by CD163 and CD68 density. For example when we compared recent vs. non-use of non-aspirin NSAIDs, we observed a 2.00-fold higher risk (95%CI=1.32-3.05) of ovarian cancer with low CD163, and a 0.65 times lower risk (95%CI=0.45-0.93) of ovarian cancer with high CD163 (p-heterogeneity<0.001). When we evaluated tablets per week, associations were similar with a positive association for CD163 low cancer and a possible inverse association for CD163 high cancer, but they were not significantly different (p-heterogeneity=0.35). There was no evidence of an association for tablets per week and ovarian cancer risk by CD68 level (p-heterogeneity=0.73). For non-aspirin NSAID duration, we observed a significant difference for risk of ovarian cancer with low CD163 density versus high CD163 density (p-heterogeneity=0.05), but no difference was evident by CD68 (p-heterogeneity=0.62).

We also considered the cross-classification of CD68 and CD163. Recent aspirin use was most strongly associated with lower risk of ovarian cancer with high levels of CD68 and CD163 (OR=0.59, 95% CI=0.40, 0.86), though the association between recent aspirin use and lower risk of CD68 low/CD163 high tumors was also significant (OR=0.18, 95% CI=0.05-0.62). Associations for the CD68 low/CD163 low (OR=1.38, 95%CI=0.83-2.28) and CD68 high/CD163low tumors (OR=1.87, 95%CI=0.92-3.77) were in the opposite direction, but non-significant. Recent non-aspirin NSAID use was positively associated with risk of ovarian cancer with low levels of CD68 and CD163 (OR=2.10, 95%CI=1.27-3.49), but inversely associated with risk of ovarian cancer with high levels of CD68 and CD163 (OR=0.68, 95%CI=0.47-0.99).

Sensitivity analyses

Results were similar when we restricted our analyses to invasive epithelial ovarian cancer (Supplemental Tables 5 and 6). Notably, the differential association for anti-inflammatory

drug use and ovarian cancer risk by CD163 density remained statistically significant for all measures of aspirin use, and for recent non-aspirin NSAID use. When we evaluated ever versus never low-dose aspirin use, we observed no evidence of heterogeneity (e.g. ever baby aspirin use vs. non-use $OR_{CD163low}$=1.22, 95%CI=0.64-2.31; $OR_{CD163high}$=1.81, 95%CI=1.03-3.18; p-heterogeneity>0.30 for all comparisons), though power was limited (n=161 cases). Results were similar when we restricted to high-grade serous ovarian cancer (Supplemental Tables 7 and 8) and when we considered a common reference group (e.g., recent aspirin use vs. non-use of any NSAID $OR_{CD163low}$=1.69, 95%CI=1.01-2.85; $OR_{CD163high}$=0.50, 95%CI=0.33-0.76). Results were also similar when we evaluated COX1+ and COX1- tumors accounting for COX2 status and vice versa (Supplemental Table 9), and when we evaluated CD163 accounting for COX1 and COX2 (Supplemental Table 10). When we evaluated CD163 accounting for CD68 status (Supplemental Table 9), associations for aspirin and non-aspirin NSAID use with risk of CD163 low ovarian cancer remained positive (e.g. OR=1.68, 95%CI=1.00-2.82 for recent versus no aspirin use), while associations with risk of CD163 high ovarian cancer became more inverse (e.g. comparable OR=0.39, 95%CI=0.23-0.66). Accounting for CD163 status, we observed an inverse association for recent aspirin and risk of CD68 low ovarian cancer, but no association for CD68 high ovarian cancer. The association for recent non-aspirin NSAID use and risk of CD68 low ovarian cancer changed from significantly positive to inverse, while the association for recent non-aspirin NSAID use and risk of CD68 high ovarian cancer did not change substantially.

DISCUSSION

We examined the association between anti-inflammatory drug use and ovarian cancer risk by markers of increased prostaglandin synthesis (COX1 and COX2) and macrophage infiltration (CD68 and CD163). The associations of NSAID use and ovarian cancer risk by COX1 and COX2 expression suggested no evidence of heterogeneity. We observed significant heterogeneity for the association between aspirin use and ovarian cancer risk by density of M2-type macrophage infiltration (CD163), though we did not observe a difference by total macrophage levels (CD68). When we evaluated differences by total macrophage density, accounting for M2-type macrophage density, the results were in the opposing direction of the M2-type results, suggesting that the associations between anti-inflammatory drug use and risk of ovarian cancer by level of other macrophage types (e.g., M1-type) may be in the opposite direction of the associations by level of M2-type macrophages. This is consistent with a possible pro-tumorigenic role of M2-type macrophages and an opposing role of M1-type macrophages (27).

The primary mechanism of action for aspirin or non-aspirin NSAIDs is down-regulation of the prostaglandin synthesis pathway by inhibition of COX1 and COX2 (9–12). Our study observed a non-significant inverse association for aspirin use and a positive association for long durations of non-aspirin NSAID use, a finding consistent with our prior study of the full NHS/NHSII cohorts (8) and with a larger study in the Ovarian Cancer Cohort Consortium (44). As reported in breast cancer (14), we observed no evidence of heterogeneity by COX1 or COX2 expression. This lack of heterogeneity could, in part, be

due to limited case numbers to examine low-dose aspirin, which has previously been more strongly related to ovarian cancer risk (6, 8).

These results do not support prostaglandin synthesis as the primary mechanism by which NSAIDs influence ovarian cancer risk, so we considered other mechanisms. We observed significant heterogeneity by M2-type macrophage density for both aspirin and non-aspirin NSAIDs, suggesting that aspirin and other NSAIDs may work by limiting differentiation of macrophages to the immunosuppressive M2 type. While prostaglandins promote macrophage differentiation (15–17), other molecules and pathways are also involved in the activation, differentiation, and tumor-promoting activity of this immune cell population. For example, monocyte chemoattractant protein 1 (MCP-1/CCL2) is lower in breast and pancreatic cancer cells treated with aspirin, and also affects macrophage infiltration in ovarian cancer (45–47). Further research should consider the effects of aspirin on MCP-1 and other factors that may regulate immune cell recruitment and differentiation in ovarian cancer (48). If associations between NSAID use and ovarian cancer risk are not fully explained by immune mechanisms, they may also reflect NSAID-driven modifications in gene expression. For example, a study of PC3 human prostate cancer cells reported that expression of genes involved in DNA repair, cell growth, and cell proliferation was altered in cells treated with high, but clinically relevant concentrations of multiple NSAIDs (49). Dysregulation of many of these same genes has been reported in ovarian cancer (50), and may be an intermediate step by which NSAIDs influence ovarian cancer risk.

Strengths of our study included the large study population drawn from two prospective studies, and a population-based case-control study. All studies collected detailed exposure and confounder data, including multiple metrics of anti-inflammatory drug use for both aspirin and non-aspirin NSAIDs. Additionally, measures of the tissue markers were reproducible across cores and TMAs were cut and stained by the same laboratories across all three studies, reducing assay variability.

We also acknowledge important limitations of this research. It is possible that bias arose when identifying cases for inclusion (i.e., cases with available tissue blocks). However, cases for whom we had tumor tissue blocks had similar distributions of NSAID use to the full case population (Supplemental Table 3). Further the results of this analysis were similar to those reported in a prospective cohort analysis of the NHS/NHSII (8). Cases in the NECC were enrolled a median of 8.5 months after diagnosis which may have led to survivor bias, and we recognize that information from NECC, a retrospective study with a 1-year exposure lag, may have been affected by recall bias or reverse causation. Notably, results from NECC were not substantially different from NHS/NHSII. Finally, we gained substantial power by pooling NHS/NHSII/NECC; however, even after combining the studies, we did not have adequate power for a detailed analysis of low-dose or daily aspirin use, the subset of aspirin use that has consistently been associated with lower ovarian cancer risk (4–6, 8, 44). Additionally, while the benefits of pooling outweighed the drawbacks, the exposure metrics evaluated in NHS/NHSII and NECC were not directly comparable, so data harmonization resulted in the loss of some metrics of medication use (i.e., frequency), limited the number of categories we could consider for the exposure and covariates, and precluded a detailed

evaluation of dose-response relationships for duration and tablets of anti-inflammatory drug use.

In summary, we observed that the associations between aspirin or non-aspirin NSAID use and risk of ovarian cancer did not differ by levels of COX1 or COX2 expression, suggesting that associations between anti-inflammatory drug use and ovarian cancer risk may act through a prostaglandin-independent biologic pathway. Given that we saw strong differences in the association of NSAID use and ovarian cancer risk by density of M2-type macrophages, which have an immunosuppressive effect on the tumor, alteration of immune pathways may be a mechanism by which these drugs, particularly aspirin, influence ovarian carcinogenesis. Recent large studies have consistently shown a modest inverse association of daily or low-dose aspirin with ovarian cancer risk (4–6, 8, 44); thus, elucidating potential mechanisms by which NSAIDs can alter the tumor microenvironment, particularly with respect to cellular factors such as immunity, is crucial to determining whether aspirin use may prevent ovarian cancer. Further research should leverage both larger population-based studies as well as experimental models, and consider the complex distribution of cell types found in epithelial ovarian cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Distribution of ovarian cancer histopathology by tumor marker in the Nurses' Health Studies and the New England Case Control Study

	COX1*		COX2†		CD163‡		CD68‡	
	−	+	−	+	low	high	low	high
Total, n	378	154	353	179	212	318	183	347
Histology, (%)								
Serous	65.1	83.8	70.0	71.5	53.8	82.1	60.1	76.4
Mucinous	5.3	0.0	1.7	7.8	9.0	0.9	8.7	1.7
Endometrioid	17.5	7.1	15.6	12.3	20.8	9.1	16.4	12.4
Clear cell	7.1	6.5	8.5	3.9	11.3	4.1	9.8	5.5
Other	5.0	2.6	4.2	4.5	5.2	3.8	4.9	4.0
Grade, (%)								
Borderline	12.4	7.1	9.9	12.8	23.1	3.5	23.0	5.2
1	9.5	3.9	7.9	7.8	13.2	4.7	13.1	5.5
2	6.1	2.6	4.8	5.6	8.0	2.8	6.0	4.3
3	70.1	85.1	76.2	70.9	53.8	86.8	57.4	82.1
Unknown	1.9	1.3	1.1	2.8	1.9	2.2	0.5	2.9
Stage, (%)								
1	29.1	19.5	24.4	30.2	40.1	17.0	36.6	20.7
2	9.8	4.5	9.6	5.6	9.9	6.9	7.7	8.4
3	51.1	63.6	55.5	53.1	40.6	64.8	45.4	60.2
4	4.0	3.9	4.0	3.9	2.4	5.0	2.7	4.6
Unknown	6.1	8.4	6.5	7.3	7.1	6.3	7.7	6.1

*Tumors were classified as COX1- when there was no evidence of staining or only weak intensity staining, and COX1+ when there was moderate to high intensity staining in ≥10% of cells.

†Tumors were classified as COX2- when <5% of cells stained, and COX2+ when there was staining in ≥5% of cells.

‡CD68 and CD163 were scored as low when <10% of cells stained and staining was scattered. CD68 and CD163 were scored as high when <10% of cells stained, with aggregation, or when ≥10% of cells stained.

Table 2.

Correlations* among tumor markers in the Nurses' Health Studies and the New England Case Control Study

	COX1	COX2	CD68	CD163	CD68/CD163
COX1	1.00	0.07	0.13	0.19	0.10
COX2		1.00	0.09	0.13	0.08
CD68			1.00	0.81	−0.13
CD163				1.00	0.45
CD68/CD163					1.00

*Spearman correlations were calculated among the 513 cases with data on all 4 tumor markers (COX1, COX2, CD68 and CD163). Correlations with an absolute value ≥0.146 are statistically significant, assuming a two-sided test with α=0.05.

Table 3.

Associations between aspirin and non-aspirin NSAID use and risk of ovarian cancer by COX1/COX2 level in the Nurses' Health Studies and the New England Case Control Study

			OR (95% CI)			OR (95% CI)		
	Controls (n)	**Cases (n)**	**COX1−**	**COX1+**	**P-het**	**COX2−**	**COX2+**	**P-het**
Aspirin								
Regular use								
No regular use	1552	197	(ref)	(ref)		(ref)	(ref)	
Past use	280	92	0.74 (0.49, 1.11)	0.87 (0.50, 1.49)		0.79 (0.52, 1.19)	0.78 (0.47, 1.28)	
Recent use	605	163	0.71 (0.50, 1.01)	0.87 (0.54, 1.38)		0.82 (0.57, 1.17)	0.68 (0.44, 1.06)	
					0.72			0.73
Duration								
<1 year	1616	212	(ref)	(ref)		(ref)	(ref)	
1 to <5 years	206	39	0.68 (0.43, 1.09)	0.72 (0.36, 1.44)		0.67 (0.41, 1.09)	0.75 (0.40, 1.38)	
5 to <10 years	197	62	0.95 (0.62, 1.45)	0.96 (0.53, 1.77)		1.14 (0.75, 1.73)	0.67 (0.36, 1.24)	
10+ years	347	109	0.76 (0.51, 1.13)	0.95 (0.57, 1.59)		0.92 (0.62, 1.37)	0.70 (0.42, 1.17)	
p-trend			0.34	0.92		0.91	0.22	
					0.46			0.22
Tablets								
<1 tablet/week	1728	260	(ref)	(ref)		(ref)	(ref)	
1 to <6 tablets/week	395	131	0.91 (0.66, 1.25)	0.94 (0.61, 1.46)		0.90 (0.65, 1.24)	1.01 (0.67, 1.53)	
6+ tablets per week	292	62	0.76 (0.52, 1.13)	0.87 (0.51, 1.49)		0.82 (0.56, 1.22)	0.79 (0.46, 1.34)	
p-trend			0.17	0.62		0.31	0.40	
					0.69			0.95
Non-aspirin NSAIDs								
Regular use								
No regular use	1600	208	(ref)	(ref)		(ref)	(ref)	
Past use	247	105	1.54 (1.08, 2.20)	1.50 (0.91, 2.47)		1.71 (1.19, 2.44)	1.27 (0.79, 2.04)	
Recent use	460	110	0.96 (0.68, 1.34)	1.05 (0.66, 1.68)		1.00 (0.70, 1.41)	1.00 (0.65, 1.53)	
					0.92			0.55
Duration								

			OR (95% CI)			OR (95% CI)		
	Controls (n)	**Cases (n)**	**COX1−**	**COX1+**	**P-het**	**COX2−**	**COX2+**	**P-het**
<1 year	1680	226	(ref)	(ref)		(ref)	(ref)	
1 to <5 years	347	84	0.92 (0.64, 1.31)	1.03 (0.63, 1.68)		1.12 (0.79, 1.60)	0.67 (0.40, 1.11)	
5 to <10 years	206	72	1.27 (0.86, 1.88)	1.21 (0.70, 2.11)		1.38 (0.93, 2.06)	1.09 (0.65, 1.83)	
10+ years	111	45	1.75 (1.08, 2.82)	1.92 (0.99, 3.72)		1.59 (0.95, 2.68)	2.24 (1.28, 3.93)	
p-trend			0.010	0.05		0.02	0.006	
					0.93			0.51
Tablets								
<1 tablet/week	1478	184	(ref)	(ref)		(ref)	(ref)	
1 to <6 tablets/week	164	50	0.96 (0.59, 1.54)	1.45 (0.78, 2.70)		0.87 (0.51, 1.48)	1.49 (0.87, 2.55)	
6+ tablets per week	225	48	1.03 (0.65, 1.61)	1.07 (0.55, 2.09)		1.01 (0.63, 1.64)	1.08 (0.60, 1.92)	
p-trend			0.97	0.77		0.95	0.71	
					0.82			0.74

Models are adjusted for: cohort, age, menopausal status (pre/post), parity (nulliparous, 1, 2, 3, >3), oral contraceptive use (<1, 1-5, 5-10, 10+ years), estrogen, estrogen+progestin and other HT use (ever/never), tubal ligation (yes/no), hysterectomy (yes/no), family history of breast or ovarian cancer (yes/no), and BMI (<20, 20-25, 25-30, 30+)

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Table 4.

Associations between aspirin and non-aspirin NSAID use and risk of ovarian cancer by levels of CD163, CD68 and their ratio in the Nurses' Health Studies and the New England Case Control Study

			OR (95% CI)			OR (95% CI)			OR (95% CI)		
	Controls (n)	Cases (n)	CD163 low	CD163 high	P-het	CD68 low	CD68 high	P-het	CD163/CD68<1	CD163/CD68≥1	P-het
Aspirin											
Regular use											
No regular use	1552	197	(ref)	(ref)		(ref)	(ref)		(ref)	(ref)	
Past use	280	91	1.43 (0.88, 2.35)	0.56 (0.37, 0.87)		1.12 (0.67, 1.87)	0.67 (0.44, 1.02)		0.95 (0.54, 1.67)	0.74 (0.50, 1.11)	
Recent use	605	163	1.50 (0.97, 2.31)	0.54 (0.37, 0.78)		0.99 (0.63, 1.57)	0.71 (0.49, 1.01)		1.20 (0.75, 1.91)	0.67 (0.47, 0.95)	
					<0.001			0.17			0.07
Duration											
<1 year	1616	213	(ref)	(ref)		(ref)	(ref)		(ref)	(ref)	
1 to <5 years	206	39	1.07 (0.60, 1.92)	0.54 (0.33, 0.90)		0.86 (0.46, 1.60)	0.63 (0.39, 1.02)		0.75 (0.36, 1.58)	0.69 (0.43, 1.08)	
5 to <10 years	197	62	1.70 (1.02, 2.83)	0.67 (0.42, 1.07)		1.06 (0.60, 1.88)	0.91 (0.59, 1.40)		1.73 (1.00, 2.99)	0.74 (0.47, 1.16)	
10+ years	347	107	1.25 (0.78, 2.02)	0.64 (0.42, 0.96)		1.03 (0.63, 1.69)	0.72 (0.48, 1.08)		0.92 (0.52, 1.60)	0.80 (0.54, 1.18)	
p-trend			0.21	0.07		0.71	0.25		0.81	0.37	
					0.012			0.24			0.42
Tablets											
<1 tablet/week	1728	262	(ref)	(ref)		(ref)	(ref)		(ref)	(ref)	
1 to <6 tablets/week	395	127	1.62 (1.09, 2.40)	0.63 (0.44, 0.89)		1.17 (0.77, 1.79)	0.79 (0.57, 1.10)		1.20 (0.77, 1.87)	0.81 (0.59, 1.12)	
6+ tablets per week	292	64	1.58 (1.00, 2.48)	0.53 (0.34, 0.81)		1.15 (0.70, 1.89)	0.69 (0.47, 1.03)		1.17 (0.68, 1.99)	0.71 (0.48, 1.04)	
p-trend			0.04	0.001		0.54	0.06		0.52	0.07	
					<0.001			0.09			0.10
Non-aspirin NSAIDs											
Regular use											
No regular use	1600	208	(ref)	(ref)		(ref)	(ref)		(ref)	(ref)	
Past use	247	105	2.65 (1.69, 4.16)	1.16 (0.80, 1.69)		2.35 (1.45, 3.81)	1.31 (0.91, 1.88)		1.97 (1.21, 3.19)	1.40 (0.98, 2.00)	
Recent use	460	111	2.00 (1.32, 3.05)	0.65 (0.45, 0.93)		1.63 (1.03, 2.58)	0.81 (0.58, 1.14)		1.33 (0.84, 2.11)	0.88 (0.63, 1.24)	
					<0.001			0.02			0.26
Duration											

			OR (95% CI)			OR (95% CI)			OR (95% CI)		
	Controls (n)	**Cases (n)**	**CD163 low**	**CD163 high**	**P-het**	**CD68 low**	**CD68 high**	**P-het**	**CD163/CD68<1**	**CD163/CD68≥1**	**P-het**
<1 year	1680	225	(ref)	(ref)		(ref)	(ref)		(ref)	(ref)	
1 to <5 years	347	81	1.66 (1.07, 2.58)	0.64 (0.43, 0.96)		1.53 (0.96, 2.45)	0.73 (0.50, 1.06)		1.16 (0.69, 1.94)	0.85 (0.60, 1.22)	
5 to <10 years	206	71	2.14 (1.33, 3.46)	0.92 (0.60, 1.41)		1.99 (1.19, 3.32)	1.03 (0.68, 1.54)		1.82 (1.08, 3.08)	1.10 (0.73, 1.64)	
10+ years	111	48	2.79 (1.55, 5.02)	1.60 (0.98, 2.62)		1.86 (0.91, 3.80)	1.96 (1.24, 3.08)		2.96 (1.61, 5.45)	1.59 (0.98, 2.58)	
p-trend			<0.001	0.10		0.01	0.006		<0.001	0.06	
					0.05			0.62			0.06
Tablets											
<1 tablet/week	1478	185	(ref)	(ref)		(ref)	(ref)		(ref)	(ref)	
1 to <6 tablets/week	164	51	2.03 (1.13, 3.64)	0.84 (0.51, 1.39)		1.48 (0.76, 2.85)	1.04 (0.66, 1.66)		1.70 (0.92, 3.12)	0.95 (0.59, 1.54)	
6+ tablets per week	225	48	1.27 (0.67, 2.42)	0.96 (0.61, 1.51)		1.12 (0.56, 2.26)	1.00 (0.64, 1.56)		0.80 (0.38, 1.72)	1.11 (0.72, 1.72)	
p-trend			0.39	0.72		0.71	0.97		0.73	0.73	
					0.35			0.73			0.62

Models are adjusted for: cohort, age, menopausal status (pre/post), parity (nulliparous, 1, 2, 3, >3), oral contraceptive use (<1, 1-5, 5-10, 10+ years), estrogen, estrogen+progestin and other HT use (ever/never), tubal ligation (yes/no), hysterectomy (yes/no), family history of breast or ovarian cancer (yes/no), and BMI (<20, 20-25, 25-30, 30+).