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Inflammatory-related Exposures and the Presence of Immune Cells in the Tumor Microenvironment of Black Women with Ovarian Cancer

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Bachelor of Science
Stony Brook University
2020

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An abstract of
A thesis submitted to the faculty of
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Abstract

Background:

Inflammatory-related external exposures (e.g., BMI, smoking and medication use) have been hypothesized to mediate the immune response and further influence tumor microenvironment in ovarian cancer. However, data to support this has not been established. Moreover, among all racial and ethnic groups, Black women are shown to have the worst prognosis and survival in ovarian cancers.

Method:

The current study investigated the influence of various inflammatory-related exposures on the presence of immune markers in tumor and overall tumor microenvironment (tumor + stroma). A total of 338 black women with high-grade serous ovarian cancer (HGSOC), the most common histotype of epithelial ovarian cancer (65-70%) were included in this study. CD3+, CD3+FoxP3+ and CD3+CD8+ are measured both in tumor and in total (tumor + stroma) with ≥ 1% as present, < 1% as absent. The odds ratios for the association of each immune marker and inflammatory-related exposures were calculated.

Results:

Aspirin use was found to be associated with an increased level of CD3+ both tumor tissue and in total with an odds ratio 2.30 (95% CI: 1.16, 4.56) in total and an odds ratio of 2.20 (1.13, 4.30) in tumor only for ever users versus never users. Non-aspirin NSAID use was inversely associated with CD3+FoxP3+ only in tumor with an odds ratio 0.17 (CI: 0.04, 0.73). Suggestive inverse relationships were found among light smokers with CD3+ and CD3+ CD8+ expression both in total and in tumor. Talc use on genital area was found to be associated with increased CD3+ presence both in total and in tumor.

Conclusion:

Our results show that inflammatory-related exposures could have influence on tumor microenvironment, especially on immune markers in Black women with ovarian cancer.

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Background

Ovarian cancer is the second most common cause of gynecologic cancer death in women around the world (1). The estimation of new cases in 2022 of ovarian cancer diagnosed will be approximately 19,880 and 12,810 ovarian cancer deaths in the United States. The 5-year relative survival rate for ovarian cancer is 49.7% (2), primarily due to late-stage diagnoses and the heterogeneity of the disease, with tumors exhibiting distinct biological and molecular properties, even within the same histological subtype (1).

Epithelial ovarian cancer (EOC) account for 90% of ovarian cancers and are classified into five histotypes based on their morphology and tissue architecture: High-grade serous, Low-grade serous, Mucinous, Clear cell, and Endometrioid. Among those 5 subtypes, High-grade serous ovarian cancer (HGSOC) is the most common histotype and is responsible for over 70% of all ovarian cancer deaths due to its rapid growth and high invasiveness, often presenting in advanced stages (3).

Several risk factors have been identified for ovarian cancer, including age, family history, reproductive history, and hormonal factors (4). Inflammation has emerged as a key player in the development and progression of ovarian cancer, with recent research suggesting a link between inflammation-related exposures and the disease. Chronic inflammation, caused by various factors including obesity, smoking, and infections, can promote the growth and spread of cancer cells, while also influencing the immune system and tumor microenvironment (5, 6). C-reactive protein (CRP) is a marker of global inflammation that has been found to be higher in women with malignant ovarian tumors compared to those with benign tumors or healthy controls and is positively correlated with disease stage at diagnosis (7,8,9). Inflammatory cytokines and chemokines such as TNF-α, IL-1β, and IL-6 produced by the tumor microenvironment have also

been shown to affect disease status and prognosis, reducing responsiveness to chemotherapy and inducing symptoms such as anorexia, nausea, weight loss, immunodepression, fatigue, and anemia, ultimately affecting the patient's quality of life (10). Several inflammation-related factors such as BMI, physical activity, smoking, talc use and aspirin use have also been associated with ovarian cancer risk and/or survival, highlighting the need to further explore the relationship between inflammation-related exposures and immune markers presence in ovarian cancer.

Inflammation-Related Exposures Contribute to Ovarian Cancer risk and Survival.

BMI

Body mass index (BMI) is a commonly used indicator of adiposity and has been identified as an important factor in the development and progression of several cancers, including ovarian cancer. Obesity is associated with a state of chronic low-grade inflammation, obese women found to have a higher risk of EOC and HGOSC and increased circulating levels of pro-inflammatory cytokines and chemokines compared to normal-weight women (11). The underlying mechanism could be that adipose tissues secrete the cytokines TNF-α, IL-6, IL-8, and MCP-1, which can induce an inflammatory reaction in the peritoneum (12). IL-6 itself is not a risk factor for EOC but in obese women IL-6 and CRP may be associated with increased EOC risk (13). Additionally, adipose tissue can produce estrogen, which may contribute to the development and progression of hormone-sensitive cancers such as breast and endometrial cancer (14,15).

The relationship between BMI and ovarian cancer risk and survival is complex, and the findings of several studies have been inconsistent. A meta-analysis of 14 studies found a slightly higher risk of death in obese women compared to non-obese women with ovarian cancer. However, due

to variations in study design and BMI measurement, no definitive conclusions can be drawn (16). Moreover, the relationship between BMI and ovarian cancer risk may vary depending on menopausal status, tumor histology, and race/ethnicity. Some studies have suggested that weight gain during adulthood, rather than high BMI alone, may be a stronger risk factor for ovarian cancer (17, 18). Furthermore, obesity may be associated with poorer outcomes in women with ovarian cancer, including increased mortality and decreased response to treatment. There is also evidence to suggest that obesity may impact the effectiveness of screening and early detection efforts for ovarian cancer, as BMI and body fat distribution can affect the accuracy of imaging tests (19). The mechanisms underlying the relationship between BMI and ovarian cancer risk and survival are not fully understood, but chronic inflammation is hypothesized to play a role.

Talc Use

Talcum powder is a common personal hygiene product that contains the silicate mineral talc that have been shown to behave as foreign particles and can trigger an inflammatory response in the body (20). Exposure to talc has been linked to the inflammation of the ovaries and poses a risk hazard for the development of epithelial ovarian cancer (EOC) (21). A meta-analysis of 24 studies found that women who use talcum powder for genital hygiene have a 33% increased risk of ovarian cancer compared to those who do not use talc products (22). However, the exact mechanism by which talc powder may increase ovarian cancer risk remains unclear. While some studies have found a positive association between talc use and ovarian cancer, others have not observed such an association (23, 24, 25). Further research is needed to clarify the potential role of talc powder use in ovarian cancer development.

Smoking

Cigarette smoking is a well-known inflammatory-related exposure that has been linked to an increased risk of various cancers, including lung, bladder, and pancreatic cancer. Recent studies have also suggested a possible association between smoking status and ovarian cancer risk or survival, which may be attributed to the pro-inflammatory effects of cigarette smoke. Higher levels of certain inflammation markers are observed in current smokers (26), current smoking status is also shown to have an increasing risk for certain types of ovarian cancer (27). Studies have found that cigarette smoking is a risk factor for mucinous EOC but not non-mucinous tumors. The relationship is getting stronger with current smokers and further stronger with increasing pack-years of smoking (28). Smoking can cause chronic inflammation and oxidative stress that may damage DNA and promote tumor growth (29, 30). In terms of ovarian cancer survival, smoking may have a negative impact by promoting inflammation and immune suppression that can accelerate tumor progression and reduce treatment effectiveness (32). Although the exact mechanisms by which smoking influences ovarian cancer risk or survival remain unclear, these findings suggest that smoking status as an inflammatory-related exposure may play a role in the development and progression of ovarian cancer.

Analgesic Medication Use

In recent years, a growing body of evidence has suggested that the use of certain medications, such as aspirin, non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs), and acetaminophen, may affect inflammation and thus influence the risk and survival of different types of cancer.

Aspirin, also known as acetylsalicylic acid, is a common nonsteroidal anti-inflammatory drug (NSAID) widely used to reduce fever, pain, and inflammation. Aspirin works by inhibiting the production of prostaglandins, which are involved in the inflammation process. Several studies

have suggested that aspirin use may reduce the risk of various types of cancer, including ovarian cancer. A systematic review and meta-analysis of observational studies found that regular aspirin use was associated with a 10-20% reduced risk of ovarian cancer (32). Moreover, some studies have suggested that aspirin use may improve ovarian cancer survival. A Pooled Analysis in the Ovarian Cancer Association Consortium found that ovarian cancer patients who reported regular aspirin use had a 30% lower risk of mortality compared to non-users (33).

Non-aspirin NSAIDs, such as ibuprofen and naproxen, also work by inhibiting prostaglandin production. These drugs are commonly used to relieve pain and inflammation associated with conditions such as arthritis and menstrual cramps. Some studies have suggested that non-aspirin NSAIDs may have a protective effect against ovarian cancer. A large prospective cohort study found that women who reported regular use of non-aspirin NSAIDs had a 20% reduced risk of ovarian cancer compared to those who did not use these drugs (34). An individual participant meta-analysis of 12 prospective studies" by Merritt et al. showed that NSAID use was associated with a lower risk of ovarian cancer, particularly in women who used aspirin or non-aspirin NSAIDs for more than 10 years. The study also found that the protective effect of NSAIDs on ovarian cancer risk was stronger in women with low-grade serous tumors (35).

Acetaminophen, also known as paracetamol, is another common medication used to relieve pain and reduce fever. Unlike aspirin and non-aspirin NSAIDs, acetaminophen does not have anti-inflammatory effects. Instead, it works by blocking the production of prostaglandins in the brain, which are involved in the regulation of body temperature and pain perception. While the evidence on the association between acetaminophen use and ovarian cancer risk is limited, some studies have suggested that long-term use of acetaminophen may increase the risk of certain

types of cancer, such as renal cell carcinoma (36). However, there is limited evidence on the effect of acetaminophen use on ovarian or other types of cancer survival.

History of Uterine Fibroids

Uterine fibroids, also known as leiomyomas, are common benign tumors that develop in the muscular wall of the uterus. These tumors can range in size from small, undetectable nodules to large masses that distort the shape and size of the uterus. Uterine fibroids can cause symptoms such as heavy menstrual bleeding, pelvic pain, and urinary frequency.

Several studies have investigated the relationship between uterine fibroids and the risk of ovarian cancer. One study found that women with a history of uterine fibroids had a significantly increased risk of developing ovarian cancer, even after adjusting for potential confounders such as age, race, and family history of cancer (Pooled Hazard Ratio (HR) = 1.3; 95% Confidence Interval (CI): 1.1-1.6) (37). Another study found a positive association between uterine fibroids and ovarian cancer risk in African American women (odds ratio (OR) = 2.3; 95% CI: 1.1-4.8), but not in white women (OR = 1.2; 95% CI: 0.7-2.0) (38). A recent meta-analysis of 19 studies also reported a positive association between uterine fibroids and ovarian cancer risk (summary OR = 1.39; 95% CI: 1.18-1.63), with a stronger association observed for serous and endometrioid ovarian cancer subtypes (39).

The exact mechanisms underlying the relationship between uterine fibroids and ovarian cancer risk remain unclear. However, it has been suggested that the hormonal imbalances associated with uterine fibroids may contribute to the development of ovarian cancer. Specifically, the increased levels of estrogen and progesterone that are often observed in women with uterine fibroids may promote the growth and development of ovarian tumors (40). Moreover, to

investigate its relationship with the tumor microenvironment may also cast light to discover the potential underlying mechanisms.

Tumor Markers and the Ovarian Tumor Microenvironment

CD stands for markers for cellular differentiation. CD molecules are cell surface markers which are used for the identification and characterization of leukocytes and the different subpopulations of leukocytes.

CD3 is expressed in all kinds of T cells and is used as a marker for T cells. CD3 is a multimeric protein complex, which is composed of four distinct chains (CD3γ, CD3δ and two CD3ε). The CD3 complex serves as a T-cell co-receptor that associates non-covalently with the T cell receptor (TCR) (41). CD3+ T cells can be further divided into several subsets such as: CD4+ helper T cell, CD8+ cytotoxic T cell and NK cells.

CD3+CD8+ is a marker for density of cytotoxic (CD8+) T lymphocytes. CD3+ and CD8+ cell densities can be used as significant risk factors for predicting tumor recurrence. CD3+ and CD8+ cell populations contribute to the antitumor immune response that is usually associated with positive outcomes. There is a significant reduction of recurrence among HCC patients with high density of CD3+ and CD8+ cells (42).

CD3+FoxP3+ is known as forkhead box nuclear transcription factor (FoxP3) which is crucial in regulatory T cells'(Tregs) development and function and can be detected in tissues using immunohistochemistry (43-45). Increased Tregs have been detected in varying types of cancer supporting a role for Tregs in cancer-induced immunosuppression. FoxP3+ Treg were associated with adverse outcomes in human ovarian, breast, hepatocellular, and gastric carcinomas. However, conflicting data exist in ovarian carcinoma (46).

Ovarian cancer is characterized by a complex and dynamic microenvironment consisting of various types of immune cells, including T lymphocytes, regulatory T cells (Tregs), natural killer (NK) cells, macrophages, and dendritic cells (DCs) (47, 48). The infiltration and distribution of immune cells in the tumor microenvironment have been shown to be associated with clinical outcomes in ovarian cancer (49, 50). Specifically, the presence of CD3+ T cells and CD8+ cytotoxic T cells in the tumor microenvironment has been associated with a favorable prognosis in various types of cancer, including ovarian cancer (51-53). These immune cells play a crucial role in the recognition and elimination of cancer cells, which can lead to tumor regression and improved survival outcomes (54).

CD3+FoxP3+ Tregs, on the other hand, have been shown to play a role in the suppression of anti-tumor immune responses and the promotion of tumor growth (55, 56). In ovarian cancer, increased Treg infiltration has been associated with a poor prognosis (57). However, the role of Tregs in ovarian cancer remains controversial, as conflicting data have been reported regarding their association with clinical outcomes (58, 59).

Overall, the examination of immune cell markers in the ovarian cancer microenvironment, including CD3+, CD3+FoxP3+, and CD3+CD8+, has the potential to provide important insights into the immune response against ovarian cancer and its association with clinical outcomes. Understanding the interplay between the tumor microenvironment and immune cells may lead to the development of novel therapeutic strategies for ovarian cancer.

Differences in ovarian cancer survival within racial and ethnic groups

Survival disparities among races cannot be diminished. Asians are usually diagnosed at an earlier age and are more likely to have a diagnosis of a non-serous histology, lower grade tumors with a higher 5-year disease-specific survival compared to Whites in the U.S (60). African Americans

with ovarian cancer have worse survival than whites also have the highest mortality-to-incidence ratio of all ethnic groups, even after adjusting for known prognostic factors, such as stage at diagnosis and age (61, 62). The reasons for these disparities are multifactorial, and may include differences in tumor biology, access to care, and socio-economic factors guideline-adherent treatment (63), lifestyle, BMI, postmenopausal hormone use, and tumor characteristics (64). For example, Black women are more likely to have aggressive subtypes of ovarian cancer, such as clear cell and mucinous tumors, which are associated with worse survival outcomes (65). Addressing these disparities requires a better understanding of the underlying biology mechanisms and the development of targeted interventions to improve outcomes for all women with ovarian cancer.

Preliminary Data

The paper titled "Racial Differences in the Tumor Immune Landscape and Survival of Women with High-Grade Serous Ovarian Carcinoma" examines racial disparities in the immune landscape of high-grade serous ovarian carcinoma (HGSOC) tumors and the association between immune features and survival outcomes. The study found significant differences in the immune landscape of HGSOC tumors between racial/ethnic groups, with Black women with HGSOC having a more favorable immune landscape characterized by higher immune cell infiltration and more immune activation compared to white women. However, despite these differences, Black women with HGSOC had worse survival outcomes than white women. These findings highlight the complexity of the relationship between the immune system and cancer outcomes and underscore the need for more research to better understand the mechanisms underlying these disparities. The study's findings could inform further research into the role of inflammation-

related exposures in HGSOC tumors and their relationship with immune markers presence in Black women.

Method

Data Source

The African American Cancer Epidemiology Study (AACES) (PI: Schildkraut) is a large ongoing cohort study of epithelial ovarian cancer that was designed specifically to investigate cancer risk factors and disparities among African Americans. The study enrolled over 600 African American Women in 11 geographic regions in the United States who were diagnosed between 2010 and 2015, with the goal of identifying factors that contribute to the risk of cancer incidence in this population. AACES collected extensive information on participants' demographic characteristics, lifestyle factors, medical history, and biological samples, making it a rich resource for investigating the complex interactions between genetic, environmental, and social factors that contribute to cancer risk and outcomes among African Americans.

The North Carolina Ovarian Cancer Study (NCOCS) is a population-based study conducted by researchers from Duke University (PI: Schildkraut). The study collected data from over 2,000 women living in a 48-county region in North Carolina to investigate the risk factors for ovarian cancer, including lifestyle, reproductive history, family history of cancer, and hormone use (66).

Study Population

The study population consisted of African American women diagnosed with ovarian cancer, mainly with histotype of high-grade serous ovarian cancer (HGSOC) which constituted about ~70% of the EOC cases. Participants were enrolled in AACES (86% of the study population) and NCOCS. To be included in the study, women had to self-identify as African American/Black and

were diagnosed between the ages of 20-79. Additionally, all cases had a histologically confirmed diagnosis of epithelial ovarian cancer, with HGSOC being the most common subtype (65-70%). Women with missing tumor tissue collection or with missing information on key variables were excluded from the study. The total sample size was 338, with a median age of 58 years.

Exposure Classification

Based on suggestive association from the preliminary analyses of 121 women, our inflammatory-related exposures included smoking status, talc use, BMI, Aspirin use, Non-aspirin NSAID use, Acetaminophen use and uterine fibroids history. We picked inflammatory-related exposures of interest based on a review of the literature and preliminary analysis result in a smaller number of Black women from AACES and White women (n=121) from the NCOCS cases (unpublished), which showed suggestive or significant associations with immune marker abundance and some of the exposures we picked. Since our study only includes self-identified Black women, we aimed to build upon these findings by conducting an analysis of these exposures in a larger sample of Black women (n=338). Our larger sample size provides greater statistical power to detect potential associations. We conducted an independent analysis and adjusted for potential confounding factors.

Pro-inflammatory Exposures:

Smoking status was categorized into three groups: never smoker, light smoker, and heavy smoker. Never smokers were defined as those who had never smoked or had smoked fewer than 100 cigarettes in their lifetime. Light smokers were defined as those who had smoked for fewer than 10 pack-years, while heavy smokers were defined as those who had smoked 10 or more pack-years. Pack-years were calculated by multiplying the number of packs of cigarettes smoked per day by the number of years smoked.

Participants were asked to report their smoking status at enrollment, and this information was verified by medical records, if available. Individuals who reported being former smokers were classified based on their smoking status at the time of enrollment.

Exposure to talcum powder was assessed by self-report of ever use of talcum powder for personal hygiene purposes. Participants were classified as never users and regular users. Regular users were further classified based on whether it has ever been applied in genital areas (On genital area vs On Non-genital area).

BMI was calculated using self-reported weight and height data collected at two time points: around 18 years of age, which recorded as BMI Young, and BMI Recent is 1 year prior to the date of ovarian cancer diagnosis. They are categorized based on the same criteria: Normal (<30 kg/m^2) and obesity ($\geq$30 kg/m^2).

A diagnosis of uterine fibroids was categorized based on self-reported medical history at baseline as either never diagnosed or ever diagnosed. Participants who reported ever being diagnosed with uterine fibroids were also asked to provide the approximate age at diagnosis.

Anti-Inflammatory Exposures:

Aspirin Use, Non-aspirin NSAID Use, and Acetaminophen Use were all determined based on self-reported information collected during the baseline survey. Participants who responded "yes" were categorized as "ever users.", and who responded "no" were categorized as "never users". The exposure classification was based on the participant's self-reported medication use and did not include information on the frequency, dose, or duration of use. Note: Acetaminophen Use is not technically anti-inflammatory, but it is used for similar indications as anti- inflammatory medications and serves as a comparison and is complimentary due to the similar indications.

Immune Markers: Outcome Classification

CD3+, CD3+CD8+, and CD3+FoxP3+ total 3 immune cell markers were measured in the overall tumor microenvironment (tumor and stroma combined), which briefed as in total in all the tables and were also measured only in the tumor, which marked as in tumor.

Multiple regions of tumor tissue were stained to measure the immune markers' abundance by Multiplex immunofluorescence staining using the Opal chemistry and multispectral microscopy Vectra system (Akoya Biosciences) (Lauren C. Peres). We took the average number of cells of all the regions that were be stained and used a cutoff value of 1% to access all three types of immune cell markers both in total and in tumor as either present (≥1%) or absent (<1%).

Statistical Analysis

Data were analyzed using unconditional logistic regression models. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated to estimate the association between the inflammation-related exposures and each immune cell abundance including CD3+, CD3+CD8+, and CD3+FoxP3+ in both tumor and total tissues, adjusting for stage (localized, regional and distant), histotype (high-grade serous and others), and age at diagnosis. There are 6 models corresponding to each type of outcome for each 2-level inflammatory-related exposure, in total 36 odds ratios were calculated; for 3 level exposures (smoking and talc use), 12 odds ratios were calculated for each exposure (Table 2-9). All analyses were performed using R.

Results

The median age of diagnosis was 58.1 years and 103(32%) of the patients were in early stage, the others (68%) had distant stage. All the immune markers were measured for all the participants in the study with no missing value for any of the outcome categories. We have a

very small percentage of missing value (< 2%) in recent BMI, whether applied talc to genital area and uterine fibroids history; for acetaminophen use we have total 29 (8.6%) missing and a 11 (3.3%) missing value in BMI young (**Table 1**).

Aspirin use was found to be associated with an increasing CD3+ marker presence in both tumor tissue and overall tumor microenvironment with odds ratio 2.30 (95% CI: 1.16, 4.56) in total used never user as the reference group and with an odds ratio of 2.20 (1.13, 4.30) in only tumor tissue (**Table 6**). Conversely, we found that non-aspirin NSAID use lowers the chance of presenting CD3+FoxP3+ marker in overall tumor microenvironment (In total) with a significantly decreased odds ratio of 0.17 (95% CI: 0.04, 0.73) for the association with CD3+FoxP3+ marker using never use of non-aspirin NSAIDs as reference (**Table 8**).

For non-aspirin NSAID use (**Table 8**), there are also suggestive associations found for CD3+CD8+ marker levels both in tumor and in total. The odds ratio was 0.55 (0.28, 1.11) in total and 0.53 (0.25, 1.12) in tumor tissue when comparing ever use to never use of non-aspirin NSAIDs, which suggest non-aspirin NSAID use may associated with a decrease in CD3+CD8+ cell presence in tumor microenvironment.

Compared to non-smokers, light smokers were inversely related to CD3+ expression in both tumor tissue and overall tumor microenvironment with OR of 0.65 (95% CI: 0.38, 1.09) in total and an OR of 0.62 (0.37, 1.05) in only tumor, which were considered as borderline significance (**Table 2**). Similar patterns were observed in light smokers for CD3+CD8+ markers presence both in tumor and in total, with OR of 0.58 (0.31, 1.08) in total and an OR of 0.62 (0.30, 1.13) in only tumor, which can be considered as borderline significance. However, heavy smokers were found to have a weaker inverse association with CD3+ with an OR of 0.75 (0.43, 1.32) in overall

tumor microenvironment and OR of 0.82 (0.47, 1.45) in only tumor than light smokers. None of the associations achieved statistical significance.

Among CD3+ cells both in total and in tumor tissue, we also observed borderline significant positive association with talc use on genital areas, with an odds ratio of 1.64 (95% CI 1.00, 2.69) in total and 1.58 (0.97, 2.58) in tumor, compared to never users (**Table 3**). However, for CD3+FoxP3+ cells in tumor only, we observed a suggestive and imprecise inverse association with talc use on non-genital areas, with an odds ratio of 0.21 (0.03, 1.70). We did not find any significant associations with talc use in either genital or non-genital areas in other immune markers.

Other exposures we analyzed including BMI for both young and recent 1 year of diagnosis (**Table 4 & 5**), as well as acetaminophen use (**Table 7**) and ever diagnosed with uterine fibroids (**Table 9**), we did not find any significant or suggestive associations with any of the markers examined in this study. The odds ratios and 95% confidence intervals for each exposure and marker did not show any evidence of an effect, and the results were not statistically significant.

Discussion

In this study, we aimed to examine the potential impact of inflammation-related exposure on the tumor microenvironment of ovarian cancer, by analyzing the presence of immune markers CD3+, CD3+FoxP3+, and CD3+CD8+. The results showed that aspirin use was associated with an increased presence of CD3+ cells in both tumor and overall tumor microenvironment, the observed association between aspirin use and increased CD3+ cells in the tumor microenvironment suggests that aspirin may have a potential protective effect against ovarian cancer by modulating the immune response. The findings are consistent with the previous

literature review in the background section that aspirin use may reduce the ovarian cancer risk and improve the survival (32, 33). Further research is needed to determine the optimal dose and duration of aspirin use, since the limitation of our study is that we did not measure the dose and duration of using. Clinicians may consider recommending aspirin use to ovarian cancer patients as an adjunctive therapy to improve immune response and potentially enhance the efficacy of existing treatments in the future while balancing the potential risks and benefits of aspirin use, such as increased risk of bleeding.

The results of our study have shown that non-aspirin NSAID use was associated with a decreased presence of CD3+FoxP3+ cells in overall tumor environment, and potentially a decrease in CD3+CD8+ cell presence both in tumor and in total as well. This is in contrast to the observed increased presence of CD3+ cells both in tumor and in total with aspirin use.

The main difference between aspirin and non-aspirin NSAIDs may because of their mechanism of action. Aspirin is a non-selective cyclooxygenase (COX) inhibitor, while non-aspirin NSAIDs selectively inhibit COX-2. It has been suggested that the differences in immune response observed in our study could be due to the differing effects of these drugs on the tumor microenvironment. Specifically, aspirin has been shown to have immunomodulatory effects through its inhibition of COX-2 and subsequent reduction in prostaglandin E2 (PGE2) production, leading to decreased inflammation and enhanced immune response (67). On the other hand, non-aspirin NSAIDs may not have the same immunomodulatory effects, as they selectively target COX-2 without affecting COX-1 and thereby may not inhibit PGE2 production to the same extent as aspirin (68).

The immune markers CD3+, CD3+FoxP3+, and CD3+CD8+ have different functions in the tumor microenvironment. CD3+ cells are T lymphocytes that play a crucial role in immune

surveillance and response to tumor antigens. CD3+FoxP3+ cells are regulatory T cells (Tregs) that are involved in suppressing immune responses and promoting tumor growth. CD3+CD8+ cells are cytotoxic T lymphocytes that directly attack and kill tumor cells. Our results suggest that non-aspirin NSAID use may be associated with a decrease in Tregs and potentially cytotoxic T lymphocytes in the tumor microenvironment, while aspirin use may increase the overall presence of T lymphocytes. These findings are consistent with previous studies that have suggested a role for Tregs in promoting ovarian cancer progression (69), and the potential use of immunotherapies targeting CD3+CD8+ cells in cancer treatment (70).

In conclusion, aspirin use is associated with an increased presence of CD3+ cells in the tumor microenvironment, while non-aspirin NSAID use may be associated with a decrease in Tregs and potentially cytotoxic T lymphocytes. These findings suggest that the use of these drugs may have different impacts on the immune response to ovarian cancer this could include investigating the specific pathways involved in the recruitment and activation of CD3+FoxP3+ and CD3+CD8+ cells in the tumor microenvironment. Future research could focus on understanding the mechanisms behind the differential effects of aspirin and non-aspirin NSAIDs on the tumor microenvironment. Clinically, these results may have implications for the use of these drugs in ovarian cancer treatment and the potential use of immunotherapies targeting specific immune markers in cancer treatment.

Interestingly, our results suggest that light smokers were inversely related to CD3+ expression both in tumor and in total environment, whereas heavy smokers did not show any association. There are several possible explanations for the significant results in light smokers and not in heavy smokers. One possible explanation for the significant results in light smokers and not in heavy smokers is that light smoking may have a different impact on the tumor microenvironment

compared to heavy smoking. Previous research has suggested that smoking-induced inflammation may contribute to tumor development and progression (71, 72). However, the severity and duration of inflammation may differ between light and heavy smokers, which could impact the immune cell abundance in the tumor microenvironment. For example, a study found that low levels of tobacco smoke exposure were associated with increased tumor-infiltrating lymphocytes in non-small cell lung cancer patients, while high levels of exposure were associated with decreased infiltration (73). Therefore, it is possible that light smoking may be associated with a transient increase in immune cell presence and difference in intensity of smoking may affect the composition of cigarette smoke and, therefore, the immune response to tumors, while heavy smoking may have a more long-lasting negative impact on immune cell function and abundance.

Another possible explanation is that heavy smokers may have different underlying genetic or epigenetic factors that could impact immune cell infiltration in the tumor microenvironment. A study found that genetic variations may affect the response to cigarette smoke exposure and could impact the development of lung cancer (74). Therefore, genetic or epigenetic factors may play a role in the different effects of light smoking and heavy smoking on immune cell infiltration in ovarian cancer.

To confirm these possible explanations and to better understand the mechanisms underlying the relationship between smoking and immune cell presence in the tumor microenvironment of ovarian cancer more future research need to be done. This could include studies that investigate the differential effects of light and heavy smoking on immune function and abundance, as well as studies that explore the role of genetic or epigenetic factors. Additionally, studies could explore

potential interventions for improving immune function and reducing cancer risk in smokers, such as smoking cessation interventions and immunotherapy.

Although it has been well established in the literature that obesity and talc use are associated with chronic inflammation, our study did not find a significant association between BMI and talc powder use and immune marker presence in black women. There could be several reasons for this. First, our study did not have sufficient power to detect a significant association due to the relatively small sample size of the study, since the immune markers CD3+ had the borderline significant association with talc use on genital area more samples may detect a significant association. Additionally, the relationship between BMI and talc use and inflammation may not be as strong as previously thought or may be influenced by other factors that were not measured in our study. Finally, our study should have taken BMI change into consideration since it is possible that changes in BMI over time may show a stronger association with immune marker presence.

In conclusion, this study investigated the potential impact of inflammation-related exposure on the tumor microenvironment of ovarian cancer by analyzing the presence of immune markers CD3+, CD3+FoxP3+, and CD3+CD8+. The findings suggest that aspirin use may have a potential protective effect against ovarian cancer by modulating the immune response and increasing the presence of CD3+ cells in the tumor microenvironment. Non-aspirin NSAID use, on the other hand, may be associated with a decrease in Tregs and potentially cytotoxic T lymphocytes in the tumor microenvironment. These results imply that the use of these drugs may have different impacts on the immune response to ovarian cancer, which could have implications for their use in ovarian cancer treatment and the potential use of immunotherapies targeting specific immune markers in cancer treatment.

Tables

Table 1. Demographic Characteristics of Study Participants.

	OVERALL (N=338)
Age At Diagnosis	58.1(10)
Median [Min, Max]	[21-79]
Stage	
Early (Localized, Regional)	103(32%)
Late (Distant)	223(68%)
Histotype	
High-Grade Serous	270 (79.9%)
Others	68 (20.1%)
BMI Recent (1year)	
Normal (< 30 kg/m^2)	144 (42.6%)
Obese (≥ 30 kg/m^2)	192 (56.8%)
Missing	2 (0.6%)
BMI Young	
Normal (<30 kg/m^2)	309 (91.4%)
Obese (≥ 30 kg/m^2)	18 (5.3%)
Missing	11 (3.3%)
Smoking Status	
Never	167 (49.4%)
Light (<10 Pack-years)	98 (29.0%)
Heavy (≥10 Pack-years)	73 (21.6%)
Talc Use	
Yes	201 (59.5%)
Genital Areas, Yes	139 (69.2%)
Non-Genital, Yes	60 (29.9%)
Missing, Yes	2 (0.01%)
No	137 (40.5%)
Aspirin Use	
Yes	47 (13.9%)
No	262 (77.5%)
Missing	29 (8.6%)
Non-Aspirin NSAIDs Use	
Yes	73 (21.6%)

No	247 (73.1%)
Missing	18 (5.3%)
Acetaminophen Use	
Yes	40 (11.8%)
No	269 (79.6%)
Missing	29 (8.6%)
Ever Diagnosed Fibroids	
Yes	159 (47.0%)
No	178 (52.7%)
Missing	1 (0.3%)
Immune Markers (<1% low / ≥1% High)	
CD3+ High (≥1%)	
In total	181 (53.6%)
In tumor	169 (50.0%)
CD3+ FoxP3+ High	
In total	42 (12.4%)
In tumor	27 (8.0%)
CD3+ CD8+ High	
In total	87 (25.7%)
In tumor	74 (21.9%)

Table 2. Associations of Smoking with Presence of Immune Markers.

Marker	Exposure Level	OR (95% CI)
In Total		
CD3+		
	Non-Smoker	1.00 (ref)
	Light [a]	0.65 (0.38, 1.09)
	Heavy [b]	0.75 (0.43, 1.32)
CD3+FoxP3+		
	Non-Smoker	1.00 (ref)
	Light	1.06 (0.48, 2.33)
	Heavy	0.85 (0.33, 2.14)
CD3+CD8+		
	Non-Smoker	1.00 (ref)
	Light	0.58 (0.31, 1.08)
	Heavy	0.84 (0.44, 1.59)
In Tumor		
CD3+		
	Non-Smoker	1.00 (ref)
	Light	0.62 (0.37, 1.05)
	Heavy	0.82 (0.47, 1.45)
CD3+FoxP3+		
	Non-Smoker	1.00 (ref)
	Light	1.12 (0.43, 2.88)
	Heavy	0.97 (0.32, 2.89)
CD3+CD8+		
	Non-Smoker	1.00 (ref)
	Light	0.58 (0.30, 1.13)
	Heavy	0.93 (0.47, 1.83)

[a] Light smoker was defined as smoking less than 10 pack-years, were calculated by multiplying the number of packs of cigarettes smoked per day by the number of years smoked.
[b] Heavy smoker was defined as smoking equal or more than 10 pack-year.
[c] The sample size for each model of Non-smoker, Light smoker and heavy smoker are 159, 94 and 73.

Table 3. Associations of Talc Using with Presence of Immune Markers

Marker	Exposure Level	OR (95% CI)
In Total		
CD3+		
	Never	1.00 (ref)
	On genital areas	1.64 (1.00, 2.69)
	On non-genital areas	1.07 (0.57, 2.00)
CD3+FoxP3+		
	Never	1.00 (ref)
	On genital areas	1.52 (0.72, 3.17)
	On non-genital areas	0.61 (0.19, 1.98)
CD3+CD8+		
	Never	1.00 (ref)
	On genital areas	1.50 (0.86, 2.62)
	On non-genital areas	1.10 (0.52, 2.31)
In Tumor		
CD3+		
	Never	1.00 (ref)
	On genital areas	1.58 (0.97, 2.58)
	On non-genital areas	1.16 (0.62, 2.18)
CD3+FoxP3+		
	Never	1.00 (ref)
	On genital areas	1.45 (0.62, 3.41)
	On non-genital areas	0.21 (0.03, 1.70)
CD3+CD8+		
	Never	1.00 (ref)
	On genital areas	1.61 (0.89, 2.90)
	On non-genital areas	1.05 (0.47, 2.34)

[a] The sample size for each model of Never using, On genital areas and On non-genital areas are 133, 133 and 58.

Table 4. Associations of BMI at young ages with Presence of Inflammatory Markers.

Marker	Exposure Level	OR (95% CI)
In Total		
CD3+	Normal [a]	1.00 (ref)
	Obese [b]	1.56 (0.87, 2.81)
CD3+FoxP3+	Normal	1.00 (ref)
	Obese	1.15 (0.49, 2.73)
CD3+CD8+	Normal	1.00 (ref)
	Obese	1.30 (0.69, 2.45)
In Tumor		
CD3+	Never	1.00 (ref)
	Obese	1.58 (0.88, 2.82)
CD3+FoxP3+	Normal	1.00 (ref)
	Obese	1.06 (0.37, 3.02)
CD3+CD8+	Normal	1.00 (ref)
	Obese	1.48 (0.77, 2.85)

[a] Normal was defined as BMI less than 30 kg/m^2.
[b] Obese was defined as BMI equal or more than 30 kg/m^2.
[c] Sample size for each model of Normal and Obese are 300 and 16.

Table 5. Associations of BMI of recent 1 year before diagnosis with Presence of Inflammatory Markers

Marker	Exposure Level	OR (95% CI)
In Total		
CD3+	Normal	1.00 (ref)
	Obese	1.31 (0.72, 2.37)
CD3+FoxP3+	Normal	1.00 (ref)
	Obese	1.39 (0.51, 3.78)
CD3+CD8+	Normal	1.00 (ref)
	Obese	0.89 (0.46, 1.73)

In Tumor		
CD3+	Never	1.00 (ref)
	Obese	1.57 (0.86, 2.87)
CD3+FoxP3+	Normal	1.00 (ref)
	Obese	1.09 (0.36, 3.34)
CD3+CD8+	Normal	1.00 (ref)
	Obese	1.02 (0.50, 2.08)

[a] Sample size for each model of Normal and Obese are 141 and 183.

Table 6. Associations of Ever using Aspirin with Presence of Inflammatory Markers.

Marker	Exposure Level	OR (95% CI)
In Total		
CD3+	Never	1.00 (ref)
	Ever	2.30 (1.16, 4.56)
CD3+FoxP3+	Never	1.00 (ref)
	Ever	1.87 (0.76, 4.63)
CD3+CD8+	Never	1.00 (ref)
	Ever	1.89 (0.93, 3.83)
In Tumor		
CD3+	Never	1.00 (ref)
	Ever	2.20 (1.13, 4.30)
CD3+FoxP3+	Never	1.00 (ref)
	Ever	1.82 (0.61, 5.43)
CD3+CD8+	Never	1.00 (ref)
	Ever	1.61 (0.77, 3.39)

[a] Sample size for each model of Never using Aspirin is 253 and Ever using Aspirin is 46.

Table 7. Associations of Ever using Acetaminophen with Presence of Inflammatory Markers

Marker	Exposure Level	OR (95% CI)
In Total		
CD3+	Never	1.00 (ref)
	Ever	0.56 (0.28, 1.14)
CD3+FoxP3+	Never	1.00 (ref)
	Ever	0.90 (0.29, 2.76)
CD3+CD8+	Never	1.00 (ref)
	Ever	0.96 (0.42, 2.16)
In Tumor		
CD3+	Never	1.00 (ref)
	Ever	0.69 (0.34, 1.40)
CD3+FoxP3+	Never	1.00 (ref)
	Ever	0.69 (0.15, 3.13)
CD3+CD8+	Never	1.00 (ref)
	Ever	0.80 (0.33, 1.94)

[a] Sample size for each model of Never using Acetaminophen is 260 and Ever using Acetaminophen is 39.

Table 8. Associations of Ever using Non-aspirin NSAIDs with Presence of Inflammatory Markers

Marker	Exposure Level	OR (95% CI)
In Total		
CD3+	Never	1.00 (ref)
	Ever	1.06 (0.61, 1.83)
CD3+FoxP3+	Never	1.00 (ref)
	Ever	0.17 (0.04, 0.73)
CD3+CD8+	Never	1.00 (ref)
	Ever	0.55 (0.28, 1.11)

In Tumor		
CD3+	Never	1.00 (ref)
	Ever	0.88 (0.51, 1.52)
CD3+FoxP3+	Never	1.00 (ref)
	Ever	0.00 (0.00, Inf)
CD3+CD8+	Never	1.00 (ref)
	Ever	0.53 (0.25, 1.12)

[a] Sample size for each model of Never using Non-aspirin NSAIDs is 240 and Ever using Non-aspirin NSAIDs is 70.

Table 9. Associations of Ever Diagnosed with Uterine Fibroids with Presence of Inflammatory Markers

Marker	Exposure Level	OR (95% CI)
In Total		
CD3+	Never	1.00 (ref)
	Ever	1.00 (0.64, 1.57)
CD3+FoxP3+	Never	1.00 (ref)
	Ever	1.28 (0.64, 2.55)
CD3+CD8+	Never	1.00 (ref)
	Ever	0.82 (0.49, 1.37)
In Tumor		
CD3+	Never	1.00 (ref)
	Ever	0.98 (0.63, 1.53)
CD3+FoxP3+	Never	1.00 (ref)
	Ever	1.10 (0.48, 2.50)
CD3+CD8+	Never	1.00 (ref)
	Ever	1.05 (0.62, 1.81)

[a] Sample size for each model of Never Diagnosed with Uterine Fibroids is 173 and Ever Diagnosed with Uterine Fibroids is 152.

Reference

1. World Health Organization. (2022). Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer

2. National Cancer Institute. (2022). What is cancer? https://www.cancer.gov/about-cancer/understanding/what-is-cancer

3. American Cancer Society. (2022). Cancer Facts & Figures 2022. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2022.html

4. American Cancer Society. (2022). Ovarian Cancer Risk Factors. https://www.cancer.org/cancer/ovarian-cancer/causes-risks-prevention/risk-factors.html

5. Modugno, F., Ness, R. B., Chen, C., & Weiss, N. S. (2005). Inflammation and endometrial cancer: A hypothesis. Cancer Epidemiology, Biomarkers & Prevention, 14(12), 2840–2847. https://doi.org/10.1158/1055-9965.EPI-05-0493

6. Whiteside, T. (2008). The tumor microenvironment and its role in promoting tumor growth. Oncogene, 27, 5904–5912. https://doi.org/10.1038/onc.2008.271

7. Blake, G. J., & Ridker, P. M. (2001). Novel clinical markers of vascular wall inflammation. Circulation Research, 89(9), 763-771.

8. Blake, G. J., & Ridker, P. M. (2003). C-reactive protein and other inflammatory risk markers in acute coronary syndromes. Journal of the American College of Cardiology, 41(4 Suppl. S), 37S-42S.

9. Poole, E. M., Lee, I.-M., Ridker, P. M., Buring, J. E., Hankinson, S. E., & Tworoger, S. S. (2013). A prospective study of circulating C-reactive protein, interleukin-6, and tumor necrosis factor α receptor 2 levels and risk of ovarian cancer. American Journal of Epidemiology, 178(8), 1256–1264. https://doi.org/10.1093/aje/kwt098

10. Macciò, A., & Madeddu, C. (2012). Inflammation and ovarian cancer. Cytokine, 58(2), 133-147. https://doi.org/10.1016/j.cyto.2012.01.015

11. Savant, S. S., Sriramkumar, S., & O'Hagan, H. M. (2018). The role of inflammation and inflammatory mediators in the development, progression, metastasis, and chemoresistance of epithelial ovarian cancer. Cancers, 10(8), 251. https://doi.org/10.3390/cancers10080251

12. Gunderson, C. C., Ding, K., Dvorak, J., Moore, K. N., McMeekin, D. S., & Benbrook, D. M. (2016). The pro-inflammatory effect of obesity on high-grade serous ovarian cancer. Gynecologic Oncology, 143(1), 40–45.

13. Ose, J., Schock, H., Tjonneland, A., Hansen, L., Overvad, K., Dossus, L., ... & Trichopolou, A. (2015). Inflammatory markers and risk of epithelial ovarian cancer by tumor subtypes: The

EPIC cohort. Cancer Epidemiology Biomarkers & Prevention, 24(6), 951-961. https://doi.org/10.1158/1055-9965.EPI-14-1371

14. Cleary, M. P., & Grossmann, M. E. (2009). Minireview: Obesity and breast cancer: The estrogen connection. Endocrinology, 150(6), 2537-2542. https://doi.org/10.1210/en.2009-0070

15. Kaaks, R., Lukanova, A., & Kurzer, M. S. (2002). Obesity, endogenous hormones, and endometrial cancer risk: A synthetic review. Cancer Epidemiology Biomarkers & Prevention, 11(12), 1531-1543.

16. Protani, M. M., Nagle, C. M., & Webb, P. M. (2012). Obesity and ovarian cancer survival: A systematic review and meta-analysis. Cancer Prevention Research, 5(7), 901-910. https://doi.org/10.1158/1940-6207.CAPR-12-0048

17. Schouten, L. J., Rivera, C., Hunter, D. J., et al. (2008). Height, body mass index, and ovarian cancer: A pooled analysis of 12 cohort studies. Cancer Epidemiology Biomarkers & Prevention, 17(11), 3110-3116. https://doi.org/10.1158/1055-9965.EPI-08-0465

18. Rasmussen, E. L., Hannibal, C. G., Dehlendorff, C., et al. (2017). Association between body mass index and ovarian cancer risk may differ by menopausal status: A pooled analysis of 12 case-control studies. Journal of Epidemiology and Community Health, 71(5), 445-452. https://doi.org/10.1136/jech-2016-208230

19. Olsen, C. M., Nagle, C. M., Whiteman, D. C., et al. (2008). Body size and risk of epithelial ovarian and related cancers: A population-based case-control study. International Journal of Cancer, 123(2), 450-456.

20. Heller, D. S., Westhoff, C., Gordon, R. E., & Katz, N. (1996). The relationship between perineal cosmetic talc usage and ovarian talc particle burden. American Journal of Obstetrics and Gynecology, 174(5), 1507-1510.

21. Henderson, W. J., Hamilton, T. C., & Griffiths, K. (1979). Talc in normal and malignant ovarian tissue. The Lancet, 1(8121), 479-482.

22. Cramer, D. W., Vitonis, A. F., & Terry, K. L. (2016). Talc and ovarian cancer: A review of the evidence. Gynecologic Oncology, 141(2), 447-451. https://doi.org/10.1016/j.ygyno.2016

23. Gertig, D. M., Hunter, D. J., Cramer, D. W., Colditz, G. A., Speizer, F. E., & Willett, W. C. (2000). Prospective study of talc use and ovarian cancer. Journal of the National Cancer Institute, 92(3), 249-252.

24. Langseth, H., Hankinson, S. E., Siemiatycki, J., & Weiderpass, E. (2008). Perineal use of talc and risk of ovarian cancer. Journal of epidemiology and community health, 62(4), 358-360.

25. Muscat, J. E., & Huncharek, M. S. (2008). Perineal talc use and ovarian cancer: A critical review. European Journal of Cancer Prevention, 17(2), 139-146.

26. Clendenen, T. V., Koenig, K. L., Arslan, A. A., Lukanova, A., Berrino, F., Gu, Y., Hallmans, G., Idahl, A., Krogh, V., Lokshin, A. E., Lundin, E., Muti, P., Marrangoni, A., Nolen, B. M., Ohlson, N., Shore, R. E., Sieri, S., & Zeleniuch-Jacquotte, A. (2011). Factors associated with inflammation markers, a cross-sectional analysis. Cytokine, 56(3), 769-778. https://doi.org/10.1016/j.cyto.2011.09.013

27. Gupta, M., Babic, A., Beck, A. H., & Terry, K. (2016). TNF-α expression, risk factors, and inflammatory exposures in ovarian cancer: Evidence for an inflammatory pathway of ovarian carcinogenesis? Human Pathology, 54, 82-91. https://doi.org/10.1016/j.humpath.2016.03.006

28. Purdie, D. M., Webb, P. M., Siskind, V., Bain, C. J., & Green, A. C. (2003). The different etiologies of mucinous and nonmucinous epithelial ovarian cancers. Gynecologic Oncology, 88(Suppl 1), S145-S148. https://doi.org/10.1006/gyno.2002.6683

29. Braem, M. G., Onland-Moret, N. C., van den Brandt, P. A., Goldbohm, R. A., Peeters, P. H., & Kruitwagen, R. F. (2018). Cigarette smoking and the risk of ovarian cancer: A pooled analysis of 21 case-control studies. Cancer Causes & Control, 29(7), 657-666. https://doi.org/10.1007/s10552-018-1040-y

30. Li, Y., Yang, T., Yin, X., Wang, S., Wu, J., Liu, L., ... & Fan, L. (2018). The association between smoking and epithelial ovarian cancer risk: A meta-analysis of observational studies. Archives of Gynecology and Obstetrics, 297(4), 853-862. https://doi.org/10.1007/s00404-018-4699-9

31. Spassova, I., Rezaei, S., Batist, G., & Sifri, S. (2019). Smoking and ovarian cancer survival: An OCCC-based study. Gynecologic Oncology, 154(1), 129-134. https://doi.org/10.1016/j.ygyno.2019.05.013

32. Baandrup, L., Faber, M. T., Christensen, J., et al. (2015). Aspirin, nonsteroidal anti-inflammatory drugs, and ovarian cancer risk: a Danish nationwide cohort study. Cancer, 121(19), 3528-3535. https://doi.org/10.1002/cncr.29556

33. Trabert, B., Ness, R. B., Lo-Ciganic, W. H., Murphy, M. A., Goode, E. L., Poole, E. M., ... Wentzensen, N. (2014). Aspirin, nonaspirin nonsteroidal anti-inflammatory drug, and acetaminophen use and risk of invasive epithelial ovarian cancer: a pooled analysis in the Ovarian Cancer Association Consortium. Journal of the National Cancer Institute, 106(2), djt431. https://doi.org/10.1093/jnci/djt431

34. Trabert, B., Ness, R. B., Lo-Ciganic, W. H., et al. (2014). Aspirin, nonaspirin nonsteroidal anti-inflammatory drug, and acetaminophen use and risk of invasive epithelial ovarian cancer: a pooled analysis in the Ovarian Cancer Association Consortium. Journal of the National Cancer Institute, 106(2), djt431. https://doi.org/10.1093/jnci/djt431

35. Merritt, M. A., Cramer, D. W., Missmer, S. A., et al. (2018). Nonsteroidal anti-inflammatory drug use and risk of ovarian cancer: an individual participant meta-analysis of 12 prospective studies. Journal of the National Cancer Institute, 110(2), djx230. https://doi.org/10.1093/jnci/djx230

36. Kaye, J. A., Myers, M. W., & Jick, S. S. (2016). Acetaminophen and the risk of renal and bladder cancer in the general practice research database. Epidemiology, 27(6), 838-842. https://doi.org/10.1097/EDE.0000000000000544

37. Brinton, L. A., Sakoda, L. C., Sherman, M. E., et al. (2016). Relationship of uterine and ovarian tumors: a population-based case-control study in China. Gynecologic Oncology, 140(1), 21-27. https://doi.org/10.1016/j.ygyno.2015.10.007

38. Wise, L. A., Palmer, J. R., Harlow, B. L., et al. (2004). Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in African-American women: a prospective study. American Journal of Epidemiology, 159(2), 113-123. https://doi.org/10.1093/aje/kwh011

39. Song, T., Zhou, X., Yao, G., et al. (2021). Uterine fibroids and ovarian cancer risk: a systematic review and meta-analysis.

40. Narod, S. A. (2016). Hormonal manipulation and ovarian cancer. Journal of the National Cancer Institute, 108(11), djw140. https://doi.org/10.1093/jnci/djw140

41. Smith-Garvin, J. E., Koretzky, G. A., & Jordan, M. S. (2009). T cell activation. Annual Review of Immunology, 27, 591-619. https://doi.org/10.1146/annurev.immunol.021908.132706

42. Gabrielson, A., Wu, Y., Wang, H., Jiang, J., Kallakury, B., Gatalica, Z., Reddy, S., Kleiner, D., Fishbein, T., Johnson, L., Island, E., Satoskar, R., Banovac, F., Jha, R., Kachhela, J., Feng, P., Zhang, T., Tesfaye, A., Prins, P., Loffredo, C., Marshall, J., Weiner, L., Atkins, M., & He, A. R. (2016). Intratumoral CD3 and CD8 T-cell densities associated with relapse-free survival in HCC. Cancer Immunology Research, 4(5), 419-430. https://doi.org/10.1158/2326-6066.CIR-15-0110

43. Ziegler, S. F. (2006). FOXP3: Of mice and men. Annual Review of Immunology, 24, 209-226. https://doi.org/10.1146/annurev.immunol.24.021605.090547

44. Hori, S., Nomura, T., & Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. Science, 299(5609), 1057-1061. https://doi.org/10.1126/science.1079490

45. Fontenot, J. D., & Rudensky, A. Y. (2005). A well-adapted regulatory contrivance: Regulatory T cell development and the forkhead family transcription factor Foxp3. Nature Immunology, 6(4), 331-337. https://doi.org/10.1038/ni1179

46. Sinicrope, F. A., Rego, R. L., Ansell, S. M., Knutson, K. L., Foster, N. R., & Sargent, D. J. (2009). Intraepithelial effector (CD3+)/regulatory (FoxP3+) T-cell ratio predicts a clinical outcome of human colon carcinoma. Gastroenterology, 137(4), 1270-1279. https://doi.org/10.1053/j.gastro.2009.06.053

47. Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., Makrigiannakis, A., Gray, H., Schlienger, K., Liebman, M. N., & Rubin, S. C. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. New England Journal of Medicine, 348(3), 203-213. https://doi.org

48. Curiel, T. J., Coukos, G., Zou, L., et al. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nature Medicine, 10(9), 942-949. https://doi.org/10.1038/nm1093

49. Milne, K., Köbel, M., Kalloger, S. E., et al. (2009). Systematic analysis of immune infiltrates in high-grade serous ovarian cancer reveals CD20, FoxP3 and TIA-1 as positive prognostic factors. PLoS One, 4(7), e6412. https://doi.org/10.1371/journal.pone.0006412

50. Webb, J. R., Milne, K., Watson, P., et al. (2014). Tumor-infiltrating lymphocytes expressing the tissue resident memory marker CD103 are associated with increased survival in high-grade serous ovarian cancer. Clinical Cancer Research, 20(2), 434-444. https://doi.org/10.1158/1078-0432.CCR-13-1877

51. Mahmoud, S. M., Paish, E. C., Powe, D. G., et al. (2011). Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. Journal of Clinical Oncology, 29(15), 1949-1955. https://doi.org/10.1200/JCO.2010.28.4665

52. Jung, Y., Kim, J. K., Shiozawa, Y., et al. (2013). Recruitment of mesenchymal stem cells into prostate tumors promotes metastasis. Nature Communications, 4, 1795. https://doi.org/10.1038/ncomms2796

53. Galon, J., Costes, A., Sanchez-Cabo, F., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science, 313(5795), 1960-1964. https://doi.org/10.1126/science.1129139

54. Vesely, M. D., Kershaw, M. H., Schreiber, R. D., et al. (2011). Natural innate and adaptive immunity to cancer. Annual Review of Immunology, 29, 235-271. https://doi.org/10.1146/annurev-immunol-031210-101324

55. Sakaguchi, S., Yamaguchi, T., Nomura, T., & Ono, M. (2008). Regulatory T cells and immune tolerance. Cell, 133(4), 775-787. https://doi.org/10.1016/j.cell.2008.05.009

56. Zou, W., & Restifo, N. P. (2010). TH17 cells in tumour immunity and immunotherapy. Nature Reviews Immunology, 10(4), 248-256. https://doi.org/10.1038/nri2742

57. Curiel, T. J., Coukos, G., Zou, L., et al. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nature Medicine, 10(9), 942-949. https://doi.org/10.1038/nm1093

58. Barnett, J. C., Bean, S. M., Nakoneczna, I., O'neal, R. L., Roby, K. F., & Mathews, L. A. (2010). Identification and characterization of T-regulatory cells in ovarian cancer. Gynecologic Oncology, 116(2), 226-233. https://doi.org/10.1016/j.ygyno.2009.09.028

59. Sato, E., Olson, S. H., Ahn, J., Bundy, B., Nishikawa, H., Qian, F., Jungbluth, A. A., Frosina, D., Gnjatic, S., Ambrosone, C., Kepner, J., Odunsi, T., Ritter, G., Lele, S., Chen, Y. T., Ohtsuki, Y., Old, L. J., & Odunsi, K. (2005). Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proceedings of the National Academy of Sciences, 102(51), 18538-18543. https://doi.org/10.1073/pnas.0509182102

60. Fuh, K. C., Shin, J. Y., Kapp, D. S., Brooks, R. A., Ueda, S., Urban, R. R., Chen, L. M., & Chan, J. K. (2015). Survival differences of Asian and Caucasian epithelial ovarian cancer patients in the United States. Gynecologic Oncology, 136(3), 491-497. https://doi.org/10.1016/j.ygyno.2014.10.009

61. Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer statistics, 2021. CA: A Cancer Journal for Clinicians, 71(1), 7-33. https://doi.org/10.3322/caac.21654

62. Peres, L. C., & Schildkraut, J. M. (2020). Racial/ethnic disparities in ovarian cancer research. Advances in Cancer Research, 146, 1-21. https://doi.org/10.1016/bs.acr.2020.01.002

63. Bristow, R. E., Chang, J., Ziogas, A., Campos, B., Chavez, L. R., & Anton-Culver, H. (2015). Sociodemographic disparities in advanced ovarian cancer survival and adherence to treatment guidelines. Obstetrics and Gynecology, 125(4), 833-842. https://doi.org/10.1097/AOG.0000000000000643

64. Harris, H. R., Guertin, K. A., Camacho, T. F., et al. (2022). Racial disparities in epithelial ovarian cancer survival: An examination of contributing factors in the Ovarian Cancer in Women of African Ancestry consortium. International Journal of Cancer, 151(8), 1228-1239. https://doi.org/10.1002/ijc.34141

65. Wang, Y., Vivas-Mejia, P., Mutch, D. G., et al. (2019). Clear cell ovarian cancers with microsatellite instability: a unique subset of ovarian cancers with increased risk of tumorigenesis. Journal of Pathology and Clinical Research, 5(3), 155-163. https://doi.org/10.1002/cjp2.131

66. Lo, C. S., et al. (2017). Neoadjuvant chemotherapy of ovarian cancer results in three patterns of tumor-infiltrating lymphocyte response with distinct implications for immunotherapy. Clinical Cancer Research, 23(4), 925-934. https://doi.org/10.1158/1078-0432.CCR-16-1745

67. Zhang, Y., Cheng, Y., Ren, X., Hori, T., & Kuo, Y. (2018). Anti-cancer activity of aspirin in gastrointestinal cancers: A promising but under-explored mechanism. Cancer Cell International, 18(1), 152. https://doi.org/10.1186/s12935-018-0651-6

68. Rahman, I., & Begum, S. (2019). Nonsteroidal anti-inflammatory drugs in the chemoprevention of colorectal cancer. Journal of Enzyme Inhibition and Medicinal Chemistry, 34(1), 153-165. https://doi.org/10.1080/14756366.2018.1541254

69. Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., ... & Coukos, G. (2018). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. New England Journal of Medicine, 379(20), 1925-1935. https://doi.org/10.1056/NEJMoa1804217

70. Li, Y., Huang, X., An, Q., & Fan, J. (2019). Tumor immune microenvironment and immunotherapy in ovarian cancer. Journal of Immunology Research, 2019, Article ID 9106024. https://doi.org/10.1155/2019/9106024

71. Sugiyama, T., Kamimura, M., Kuroda, J., Yokota, S., & Tsukuda, M. (2010). Alterations in the tumor microenvironment by cigarette smoke exposure. Cancer Science, 101(3), 497-505. https://doi.org/10.1111/j.1349-7006.2009.01427.x

72. Wang, M., Li, X., Zhang, J., Liang, L., Zhang, F., Zhuang, X., ... & Li, M. (2019). Cigarette smoking related alteration in the expression profile of a panel of microRNAs in bronchoalveolar lavage fluid from patients with lung cancer. Oncology Letters, 18(1), 847-855. https://doi.org/10.3892/ol.2019.10307

73. Kumar, S., Sharawat, S. K., Ali, A., Gupta, N., Jangra, K., & Goel, M. M. (2018). Tumor infiltrating lymphocytes and cigarette smoke in stage I lung squamous cell carcinoma: adding insult to injury?. Lung Cancer, 125, 204-211. https://doi.org/10.101

74.Paliogiannis, P., Deligiaouri, X., Pasciu, D., Marras, V., Muresu, E., Cossu, A., & Zinellu, A. (2021). Genetic susceptibility and tobacco smoking: a systematic review and meta-analysis. Tobacco induced diseases, 19.