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Use of Aspirin and Statins in Relation to Inflammation in Benign Prostate Tissue in the Placebo Arm of the Prostate Cancer Prevention Trial

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Abstract

Aspirin and statin use may lower risk of advanced/fatal prostate cancer, possibly by reducing intraprostatic inflammation. To test this hypothesis, we investigated the association of aspirin and statin use with the presence and extent of intraprostatic inflammation, and the abundance of specific immune cell types, in benign prostate tissue from a subset of men from the placebo arm of

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the Prostate Cancer Prevention Trial. Men were classified as aspirin or statin users if they reported use at baseline or during the seven-year trial. Presence and extent of inflammation were assessed, and markers of specific immune cell types (CD4, CD8, FoxP3, CD68, c-KIT) were scored, in slides from end-of-study prostate biopsies taken irrespective of clinical indication, per trial protocol. Logistic regression was used to estimate associations between medication use and inflammation measures, adjusted for potential confounders. Of 357 men included, 61% reported aspirin use and 32% reported statin use. Prevalence and extent of inflammation were not associated with medication use. However, aspirin users were more likely to have low FoxP3, a T regulatory cell marker (OR: 5.60, 95% CI: 1.16–27.07), and statin users were more likely to have low CD68, a macrophage marker (OR: 1.63, 95% CI: 0.81–3.27). If confirmed, these results suggest that these medications may alter the immune milieu of the prostate, which could potentially mediate effects of these medications on advanced/fatal prostate cancer risk.

Keywords

prostate; inflammation; aspirin; statin; male

Introduction

A growing body of evidence indicates that chronic inflammation contributes to prostate carcinogenesis (1). Intraprostatic inflammation is highly prevalent in older men with elevated prostate-specific antigen (PSA), abnormal digital-rectal examination (DRE), and benign prostatic hyperplasia (2–4), as well as in older men without clinical indication for prostate tissue removal (5–7). Chronic inflammation in the prostate could arise through exposure to infectious agents, environmental toxins, dietary factors, hormones, or possibly aging-associated factors, and could contribute to carcinogenesis via release of mutagenic reactive oxygen species or pro-proliferative and angiogenic cytokines (1).

However, despite biological plausibility, establishing a direct link between chronic intraprostatic inflammation and prostate cancer risk has been challenging. Inflammation can be assessed in tissue collected for indication (i.e. elevated PSA), but intraprostatic inflammation may also contribute to rising PSA levels (8). As a result, tissue collected for indication is enriched for inflammation regardless of prostate cancer status. Our team previously conducted the only two studies that have examined inflammation in men without indication for biopsy in relation to prostate cancer risk: a case-control study in the Prostate Cancer Prevention Trial (PCPT) that found a positive association between inflammation in at least one biopsy core and overall and high-grade prostate cancer (6), and a prospective study of men who participated in both PCPT and the Selenium and Vitamin E Prostate Cancer Prevention Trial that found a positive trend between increasing mean percent of tissue with inflammation and odds of subsequent prostate cancer diagnosis (7).

If intraprostatic inflammation is causally associated with prostate cancer, then strategies to combat inflammation could plausibly reduce prostate cancer risk. Aspirin and statins are medications commonly used by older adults that are known to have anti-inflammatory properties (9,10). With respect to prostate cancer prevention, meta-analyses of observational

studies support that regular use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDS) (11,12) and statins (13) are modestly inversely associated with prostate cancer risk, and more strongly inversely associated with risk of advanced or fatal disease. Evidence of a relationship between anti-inflammatory medication use and intraprostatic inflammation would enhance the biological plausibility of these findings; however, to our knowledge, this association has not yet been examined in prostate tissue collected without clinical indication.

The purpose of this study was to investigate the association between aspirin, non-aspirin NSAID, and statin use and the prevalence and extent of inflammation, as well as the abundance of specific immune cell types, in benign prostate tissue collected irrespective of indication from men in the placebo arm of the Prostate Cancer Prevention Trial (PCPT). We hypothesized that use of these medications would be associated with a decreased prevalence and extent of intraprostatic inflammation and a differing abundance of specific immune cells.

Methods

Study Sample

This study included a subset of men from the Prostate Cancer Prevention Trial (PCPT), a phase III, randomized, double-blinded, placebo-controlled trial designed to evaluate finasteride for the primary prevention of prostate cancer (14). Between 1993 and 1997, the trial recruited 18,882 men ages 55 years and older with no evidence of prostate cancer at enrollment (normal DRE, PSA ≤3 ng/mL, and International Prostate Symptom Score (IPSS) <20) from 221 study sites across the U.S. Participants underwent annual prostate cancer screening for up to seven years and were recommended for biopsy if their PSA was elevated or their DRE was abnormal. At the end of seven years, all participants not diagnosed with prostate cancer were asked to undergo an end-of-study biopsy, irrespective of indication.

The current study included men from the placebo arm of the PCPT who underwent an end-of-study biopsy and were selected for a nested case-control study of lower urinary tract symptoms (LUTS) incidence and progression (5). Case-control sets for LUTS incidence and progression were selected from men who had International Prostate Symptom Scores (IPSS) <15 at baseline, were not taking LUTS medications, and had no history of benign prostatic hyperplasia (BPH) surgery or physician-diagnosed BPH/LUTS, and were developed based on the International Prostate Symptom Score at baseline and seven years. Participants who had prostate cancer detected at the end-of-study biopsy were not excluded, to minimize potential for selection bias. For the current study, LUTS cases and controls were combined, as LUTS case-control status was only weakly associated with intraprostatic inflammation in biopsies from the periphery of the prostate in the prior study (5). These LUTS cases and controls were used for the current study because data on inflammation and immune cell types were already generated.

This study was conducted in accordance with the U.S. Common Rule. The PCPT was approved by institutional review boards (IRBs) at all participating study sites; the current study was approved by the Johns Hopkins Bloomberg School of Public Health IRB and the

Colorado Multiple IRBs. All participants provided written informed consent to participate in the PCPT.

Measurement of Medication Use and Other Covariates

At enrollment, baseline demographics, medical, and lifestyle factors were collected via questionnaire. Current medication use was assessed with both closed (e.g. “Do you use aspirin?”) and open-ended questions. Participants were also asked to report any new use of medications at each in-person or telephone follow-up, occurring every three months post-baseline. Participants were categorized as users of each medication if they reported use at baseline or any point during the seven-year follow-up.

Baseline weight and height were measured using standardized protocols, and weight was re-measured annually. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Men were asked to complete a food frequency questionnaire at the first annual follow-up. Serum PSA was measured in samples collected from baseline and at annual follow-up visits at a central laboratory.

Measurement of Intraprostatic Inflammation and Immune Cell Markers

We used published (5) and unpublished data previously collected in the LUTS nested case-control study. Briefly, to assess presence and extent of inflammation, an average of 2 (range: 1–6) randomly selected H&E stained slides containing one or more prostate biopsy cores were digitized and reviewed using Aperio ImageScope Viewer Software. In biopsy cores with both tumor and benign tissue, only benign tissue was reviewed. The study pathologists recorded the presence of any inflammatory cells in each biopsy core and the approximate percentage of total biopsy core area with inflammatory cell involvement.

To assess the abundance of specific immune cell types, an average of 2 (range: 1–3) randomly selected unstained slides containing one or more biopsy cores were immunohistochemically (IHC) stained for 1) CD4 (CD4+ T cells), 2) CD8 (CD8+ T cells), 3) FoxP3 (Tregs), 4) CD68+ cells (macrophages), and 5) c-KIT (mast cells). These immune cell types were chosen as cell types that were expected to be observed within the prostate based on prior studies from tissue collected for indication (15). One of two study pathologists visually reviewed and scored each slide on a scale of 0–4, with 0 indicating no cells identified and 4 indicating an extensive number of cells.

Statistical Analysis

Characteristics of users and non-users of each medication were described using medians for continuous variables and proportions for categorical variables. In univariable analyses, proportions, chi-square tests, and Cochran-Armitage trend tests were used to compare each outcome of interest by aspirin, non-aspirin NSAID, and statin use. These outcomes included 1) the presence of intraprostatic inflammation, defined as having at least one biopsy core with inflammation (yes, no), 2) the extent of intraprostatic inflammation, defined as the percentage of biopsy cores with inflammation (categorized as 0%, >0% but <100%, and 100%) and the mean percentage of tissue area with inflammation (categorized as 0%, <3%, ≥3%, because 3% was the median of the non-zero values), and 3) the abundance of markers

of each immune cell type. Because multiple slides per person were reviewed, and because each slide had varying numbers of biopsy cores, a weighted average score for each immune cell marker was calculated based on the number of cores per scored slide. Using this weighted score, the abundance of each immune cell marker was categorized as low (less than the median, i.e. <1), medium (1), or high (>1).

Multivariable regression models were used to examine the association between aspirin and statin use and inflammation/immune cells after adjusting for potential confounders. Multivariable models were not run for non-aspirin NSAID use given the null univariable results. The presence of inflammation was modeled using logistic regression, and the percentage of biopsy cores with inflammation (none [reference], some, high), the mean percentage of tissue area with inflammation (0% [reference], <3%, ≥3%), and abundance of immune cell markers (low, medium [reference], high) were modeled using polytomous logistic regression. Ordinal logistic regression was also attempted, but the proportional odds assumption did not hold. Multivariable models simultaneously included both aspirin and statin use and were adjusted for other potential confounders, including age at biopsy (continuous), race (white, non-white), and baseline BMI (continuous), cigarette smoking status (current, former, never), physical activity (sedentary, light, moderate, active), education (college, no college), and diabetes (yes, no).

As a sensitivity analysis, univariable analyses were repeated after restricting to the LUTS controls (N=86); these were men with IPSS<8 at baseline and at year 7, and men with IPSS<8 at baseline and baseline to year 7 slope <25th percentile. Analyses were also repeated after restricting to men who were not diagnosed with prostate cancer on the end-of-study biopsy (N=295). While in the primary analysis men with a PSA ≥4 ng/mL could have been included if they had a prior negative biopsy during trial follow-up, and were consequently not clinically indicated for biopsy at the end of the trial despite elevated PSA, in a sensitivity analysis we restricted to men with a PSA <4 ng/mL immediately prior to the end-of-study biopsy (N=317). These sensitivity analyses were conducted to ensure that the case-control sampling procedure for LUTS, the inclusion of men with prostate cancer, and the inclusion of who may have been clinically indicated for biopsy under conventional protocols did not meaningfully alter the results. All statistical tests were two-sided, and p-values <0.05 were considered statistically significant. Analyses were conducted in SAS version 9.4.

Results

There were 357 men from the placebo arm of PCPT included in this analysis. The median age at the end-of-study biopsy was 70 years old, and the median PSA was 1.50 ng/mL. Of these men, 218 (61%) reported aspirin use and 115 (32%) reported statin use during the trial period. Eighty-six men (24%) reported use of both aspirin and statins. Other unadjusted characteristics of the study sample are displayed in Table 1. Both aspirin and statin users were less likely than non-users to have a college education and were more likely to be former smokers. PSA concentration at the end-of-study biopsy were slightly lower among men who used aspirin or statins. At the end-of-study biopsy, 19% of aspirin users (vs. 15%

of non-aspirin users) and 21% of statin users (vs. 16% of non-statin users) were diagnosed with prostate cancer.

A median of 4 biopsy cores per man were assessed for inflammation (range: 1–11). The prevalence of having at least one biopsy core with inflammation was similar among aspirin users and non-users (68% vs. 64%) and statin users and non-users (68% vs. 66%, Table 2). The extent of inflammation also did not differ by medication use, both when assessed as the percentage of biopsy cores with inflammation and the mean percentage tissue area with inflammation (Table 2). Consistent with these univariable results, aspirin and statin use were not associated with either the presence or extent of inflammation after multivariable adjustment (Table 3). The presence and extent of inflammation also did not differ for men who reported use of both aspirin and statins (Supplemental Table 1).

There were 321, 326, 315, 325, and 297 men with data on abundance of CD4, CD8, FoxP3, CD68, and c-Kit positive cells, respectively. A median of 4 biopsy cores per person (range: 1–14) were stained for CD4, CD8, FoxP3, and CD68 cells, and a median of 2.5 cores per person (range: 1–10) were stained for c-Kit cells. For all markers, the median and mode weighted scores were 1. In univariable analyses, aspirin users appeared to have lower abundance (i.e. scores <1) of CD4, CD8, and FoxP3 cells compared to non-aspirin users (Table 2). The difference between aspirin users and non-users in FoxP3 cells was statistically significant after multivariable adjustment (OR: 5.60, 95% CI: 1.16–27.07 for low vs. medium staining for FoxP3, Table 4). Statin use appeared to be associated with lower abundance of CD8 and CD68 cells. Associations with CD68 were statistically significant in both univariable and multivariable models (OR: 1.92, 95% CI:1.00–3.71 for low vs. medium staining for CD68 in the model adjusted for age and race), though further adjustment for lifestyle factors attenuated this result (Table 4). Use of both aspirin and statins was also associated with low CD68 (Supplemental Table 1).

Similar patterns were observed for all outcomes in sensitivity analyses restricted to LUTS controls (Supplemental Table 2), men without prostate cancer (Supplemental Table 3), and men with a PSA <4 ng/mL at the end of the trial (Supplemental Table 4).

Thirty-three percent of the study sample reported use of non-aspirin NSAIDs. Non-aspirin NSAID use was not associated with any of the outcomes examined (Supplemental Table 5).

Discussion

This study examined associations between aspirin, non-aspirin NSAID, and statin use and the overall presence and extent of inflammation, as well as markers of specific immune cells, in benign prostate tissue to inform a mechanistic link between use of the medications and prostate cancer prevention. We found that the presence and extent of intraprostatic inflammation was similar among users and non-users of these medications. However, slight differences were observed in the abundance of specific immune cell markers. Specifically, FoxP3, a marker of Tregs, was less abundant in benign prostate tissue of aspirin users compared to non-users, while CD68, a marker of macrophages, was less abundant among statin users.

To our knowledge, this is the first study to examine the relationship between anti-inflammatory medication use and intraprostatic inflammation in men without biopsy indication or other clinical reason for prostate tissue removal. Prior studies have examined statin use in relation to inflammation in negative prostate biopsies that were clinically indicated (16) and in prostatectomy specimens from men with prostate cancer (17), but these tissue specimens may have been enriched for inflammation due to the clinical indication and presence of prostate cancer, respectively. Other studies have examined aspirin (18–28) and statin (29) use in relation to circulating markers of inflammation, but circulating markers are not necessarily indicative of inflammation within the prostate, which is most etiologically relevant for prostate cancer. There is evidence that these drugs have general immune modulatory effects (30,31), but effects on specific immune cells in the prostate have not been examined to date.

Our study observed a slightly lower abundance of Tregs in benign prostate tissue in aspirin users as compared to non-users. This finding is plausible given that aspirin, via inhibition of the cyclooxygenase enzymes, inhibits synthesis of prostaglandin E_2 (PGE_2), which has been shown to promote development of Tregs (32). Inhibition of COX-2/PGE_2 has also been shown to reduce Treg cell activity in murine lung cancer models (33). Tregs downregulate the immune system and may block T cells from mounting an effective anti-tumor response (34–36). In accordance with this proposed pro-tumorigenic role, studies have found Tregs to be more prevalent in tumor versus benign prostate tissue from the same patients, and in peripheral blood of prostate cancer vs. non-prostate cancer donors (37). Greater numbers of epithelial Tregs have also been positively associated with Gleason sum and pathologic stage (38). On the other hand, Tregs may also inhibit cancer development by restraining cancer-promoting inflammation (39). Thus, while our study suggests that aspirin use may lower the number of Tregs in the prostate, additional studies of Tregs and prostate cancer incidence and progression are needed to better understand the implications of this finding.

We also observed a lower abundance of macrophages in benign prostate tissue of men who reported using a statin. Macrophages are one of the most abundant immune cells in the tumor microenvironment and can promote tumor growth and progression via promotion of inflammation, immunosuppression, angiogenesis, invasion, and metastasis (40). M2 macrophages in particular are thought to suppress the anti-tumor immune response and have been associated with poorer prostate cancer prognosis (41–43). Statins may influence macrophage function via the mevalonate biosynthetic pathway and the associated protein farnesylation and small G protein signaling activity (44,45). There is biological evidence for such a link, as statins have been shown to regulate gene expression in human macrophages treated with oxidized low-density lipoprotein (44) and animal studies have found statins to reduce the proliferation and activation of macrophages within atherosclerotic plaques (46). Further research is needed to understand why macrophages but not other immune cells appeared influenced by statins use, to quantify the abundance of specific macrophage phenotypes (i.e. M1 vs. M2), and to determine whether the difference in macrophage abundance observed in this study is clinically meaningful.

In this study, a higher proportion of aspirin users (19%) than non-users (15%) and statin users (21%) than non-users (16%) were diagnosed with prostate cancer at the end-of-study

biopsy (n=62 end-of-study prostate cancers diagnosed total), though neither of these differences was statistically significant (p=0.37 for aspirin, p=0.23 for statins). These results are consistent with studies of the full placebo arm of PCPT, which reported no protective associations between aspirin or statin use and total prostate cancer in this cohort (47,48). While these findings may seem surprising given that aspirin and statins are purported to have anti-carcinogenic effects, they are not in direct conflict with the existing literature, which has shown weaker associations for total prostate cancer risk, but much more consistent and robust inverse associations between aspirin and statin use and advanced, lethal, or fatal prostate cancer (11–13). PCPT participants were also screened annually for prostate cancer, and the lack of inverse associations observed in PCPT could indicate that these medications do not lower prostate cancer risk in highly screened populations, where risk, and particularly risk of advanced disease, may be mitigated more strongly by screening and early detection. Nevertheless, our results were consistent when we excluded the men with prostate cancer detected on the end-of-study biopsy, and our biological findings should apply equally to screened and unscreened populations.

For each immune cell marker, data were not available for 9–17% of the men due to unavailability of slides, insufficient tissue on slides, or problems with IHC staining. Rates of missingness for the immune cell markers were lower among aspirin users, but missingness was not associated with any other demographic or clinical variables and reasons for missingness did not differ by aspirin use, suggesting that lower rates of missing data among aspirin users occurred by chance.

Limitations of this study include the small sample size and cross-sectional study design. Because we tested multiple hypotheses, we also cannot rule out the possibility that our findings related to Tregs and macrophages were false positives, particularly given that null associations were observed for other immune cell types regulated by similar biochemical pathways. Conversely, the null findings for the other immune cell types and for inflammation overall may have been false negatives due to nondifferential misclassification of our exposure or outcome. Misclassification of medication use may have occurred due to our lack of data on the duration of medication use, and specifically on whether men stopped taking aspirin or statins before the end-of-study biopsy. Misclassification of inflammation and immune cell measurements may have occurred as each outcome was visually assessed by pathologists as opposed to quantitatively measured. Study pathologists included multiple genitourinary pathologists trained for the review of inflammation and immune cell markers, but we cannot rule out non-differential misclassification due to differences in pathologists' scoring. As technology is rapidly advancing, future studies will be able to utilize more precise methods for quantifying the extent of inflammation and profiling immune cells in prostate tissue; such studies will be key for confirming both our positive and null results.

This study also has several notable strengths. The study included the use of multiple measures, including both the presence and extent of inflammation, and the abundance of markers of innate and adaptive immune cells. Such detailed assessment allowed us to not only assess the extent of inflammation within prostate tissue, but to understand the specific immune cells that might be modulating the inflammatory response. IHC staining was performed by a single laboratory with trained pathologists using validated, standardized

protocols, thereby minimizing opportunities for error. Importantly, for the majority of men, inflammation and immune cell markers were measured in prostate tissue collected without indication for biopsy, thus avoiding the selection bias that arises when only men with suspected or diagnosed prostate cancer due to elevated serum PSA are included.

This study provides preliminary population-based evidence that aspirin and statin use may influence certain immune cells within the prostate of men without indication for biopsy. Additional research utilizing increasingly precise methodologies is needed to confirm these observational findings and further interrogate the hypothesis that aspirin and statin use may influence advanced/fatal prostate cancer risk via immune modulation. However, given our observed lack of association for aspirin and statins use and the overall presence and extent of intraprostatic inflammation, other potential mechanisms linking aspirin and statins use to prostate cancer should also be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Characteristics of a subset[a] of men from the placebo arm of PCPT, by aspirin use[b] and statins use[b]

	Aspirin Use		Statins Use	
	No	Yes	No	Yes
N	139	218	242	115
Age at end-of-study biopsy, median	69	70	69	71
Race[c], %				
White	96	96	95	97
Non-white	4	4	5	3
College education[c], %	60	48	54	50
Diabetes[c], %	6	6	4	9
BMI[c] (kg/m^2), median	26.7	27.4	26.9	27.3
Smoking status[c], %				
Current	8	8	10	3
Former	48	66	56	64
Never	44	27	34	32
Pack-years of smoking[c], median	21.5	23.1	21.5	23.1
Physical activity[c], %				
Sedentary	19	24	21	23
Light	12	17	14	16
Moderate	58	46	53	45
Very active	11	13	11	16
Missing	1	0	0	0
Daily intake[c], median				
Energy (kcal)	1930	2094	2115.1	1860.5
Vegetables (servings/day)	1.9	2.0	1.8	2.1
Total fat (g)	67.6	74.5	77.9	62.3
Polyunsaturated fatty acids (g)	14.0	15.4	15.4	13.0
Total protein (g)	78.6	87.9	88.0	78.0
Red meat (servings/week)	3.5	3.9	4.0	3.4
Alcoholic beverages (drinks/day)	0.3	0.2	0.4	0.1
PSA at biopsy (ng/mL), median	1.7	1.4	1.6	1.3
Prostate cancer diagnosis at end-of-study biopsy, %	15	19	16	21

[a] From a case-control study of LUTS nested in the placebo arm of PCPT (5). The men did not have a clinical indication for biopsy.

[b] Reported at trial entry or during the 7 years of the trial

[c] Reported at trial entry

PCPT, Prostate Cancer Prevention Trial; BMI, body mass index; PSA, prostate-specific antigen

Table 2.

Presence and extent of intraprostatic inflammation and abundance of immune cell markers[a] by aspirin use and statins use, in a subset[b] of men from the placebo arm of PCPT

	Aspirin Use			Statins Use		
	No, %	Yes, %	*p*-value[c]	No, %	Yes, %	*p*-value[c]
≥1 core with inflammation	64	68	0.5	66	68	0.7
Percent of cores with inflammation						
None	37	32	0.9	35	33	0.9
Some	53	62		57	61	
All	10	6		8	6	
Mean percent tissue area with inflammation						
None	36	32	0.7	34	32	0.8
<3%	30	34		31	37	
≥3%	34	33		35	30	
CD4						
Low	15	20	0.3	19	18	0.8
Medium	43	41		41	44	
High	42	39		41	38	
CD8						
Low	8	12	0.2	9	14	0.1
Medium	69	69		69	70	
High	23	19		22	17	
FoxP3						
Low	2	8	0.1	5	5	0.8
Medium	70	68		68	70	
High	29	25		27	25	
CD68						
Low	13	14	0.4	11	20	**0.02**
Medium	75	76		77	72	
High	13	10		12	8	
c-KIT						
Low	11	8	0.4	9	10	0.2
Medium	73	74		72	77	
High	16	18		20	13	

[a] Abundance was scored on a scale of 0–4. When multiple slides per individual were scored, a weighted average was calculated using the number of cores per slide. Abundance was categorized based on the median value of 1 (low: <1, medium: 1, high: >1)

[b] From a case-control study of LUTS nested in the placebo arm of PCPT (5). The men did not have a clinical indication for biopsy.

[c] p-value from the chi-square test (for dichotomous variables) or Cochran-Armitage trend test (for ordinal variables). Bolded values are statistically significant.

PCPT, Prostate Cancer Prevention Trial

Table 3.

Associations between aspirin use and statins use and the presence and extent of intraprostatic inflammation in a subset[a] of men from the placebo arm of PCPT

	Aspirin Use			Statins Use		
	At least one core with inflammation			At least one core with inflammation		
	No	Yes		No	Yes	
# users / non-users	70 / 50	148 / 89		37 / 83	78 / 159	
Model 1 OR (95% CI)	Ref	1.19 (0.76–1.86)		Ref	1.10 (0.69–1.77)	
Model 2 OR (95% CI)	Ref	1.11 (0.70–1.75)		Ref	1.02 (0.63–1.65)	
Model 3 OR (95% CI)	Ref	1.16 (0.71–1.89)		Ref	1.04 (0.62–1.73)	
	Percent of cores with inflammation			Percent of cores with inflammation		
	None	Some	All	None	Some	All
# users / non-users	70 / 52	135 / 73	13 / 14	35 / 73	63 / 124	6 / 16
Model 1 OR (95% CI)	Ref	1.14 (0.87–2.17)	0.69 (0.30–1.59)	Ref	1.12 (0.69–1.81)	0.77 (0.30–1.99)
Model 2 OR (95% CI)	Ref	1.29 (0.81–2.06)	0.61 (0.26–1.43)	Ref	1.04 (0.63–1.70)	0.67 (0.26–1.75)
Model 3 OR (95% CI)	Ref	1.33 (0.81–2.19)	0.67 (0.26–1.70)	Ref	1.01 (0.60–1.70)	0.92 (0.33–2.56)
	Mean percent tissue area with inflammation			Mean percent tissue area with inflammation		
	None	<3%	≥3%	None	<3%	≥3%
# users / non-users	70 / 50	75 / 42	73 / 47	37 / 83	43 / 74	35 / 85
Model 1 OR (95% CI)	Ref	1.28 (0.76–2.15)	1.11 (0.66–1.86)	Ref	1.30 (0.76–2.24)	0.92 (0.53–1.61)
Model 2 OR (95% CI)	Ref	1.21 (0.70–2.06)	1.02 (0.61–1.73)	Ref	1.23 (0.71–2.15)	0.84 (0.48–1.47)
Model 3 OR (95% CI)	Ref	1.21 (0.68–2.13)	1.12 (0.64–1.95)	Ref	1.23 (0.67–2.20)	0.87 (0.48–1.58)

[a]From a case-control study of LUTS nested in the placebo arm of PCPT (5). The men did not have a clinical indication for biopsy.

Model 1: unadjusted

Model 2: adjusted for age and race

Model 3: adjusted for age (continuous), race (white, non-white), BMI (continuous), smoking status (current, former, never), physical activity (sedentary, light, moderate, active), education (college, no college), diabetes (yes, no), and statins use (for aspirin model) or aspirin use (for statins model) (yes, no)

PCPT, Prostate Cancer Prevention Trial; OR, odds ratio; CI, confidence interval

Table 4.

Associations between aspirin use and statins use and the abundance[a] of immune cell markers in a subset[b] of men from the placebo arm of PCPT

	Aspirin use			Statins Use		
	CD4			CD4		
	Low	Medium	High	Low	Medium	High
# users / non-users	42 / 17	84 / 50	79 / 49	18 / 41	44 / 90	38 / 90
Model 1 OR (95% CI)	1.47 (0.76–2.85)	Ref	0.96 (0.58–1.58)	0.90 (0.46–1.74)	Ref	0.86 (0.51–1.46)
Model 2 OR (95% CI)	1.50 (0.77–2.91)	Ref	0.94 (0.57–1.55)	0.94 (0.48–1.84)	Ref	0.82 (0.48–1.40)
Model 3 OR (95% CI)	1.66 (0.81–3.40)	Ref	1.06 (0.61–1.82)	0.91 (0.45–1.85)	Ref	0.85 (0.49–1.50)
	CD8			CD8		
	Low	Medium	High	Low	Medium	High
# users / non-users	25 / 9	144 / 82	39 / 27	14 / 20	71 / 155	17 / 49
Model 1 OR (95% CI)	1.58 (0.71–3.55)	Ref	0.82 (0.47–1.44)	1.53 (0.73–3.20)	Ref	0.76 (0.41–1.41)
Model 2 OR (95% CI)	1.60 (0.71–3.60)	Ref	0.80 (0.45–1.40)	1.58 (0.75–3.36)	Ref	0.71 (0.38–1.33)
Model 3 OR (95% CI)	1.33 (0.56–3.13)	Ref	0.76 (0.41–1.40)	1.60 (0.72–3.57)	Ref	0.77 (0.40–1.50)
	FoxP3			FoxP3		
	Low	Medium	High	Low	Medium	High
# users / non-users	15 / 2	135 / 80	50 / 33	5 / 12	67 / 148	24 / 59
Model 1 OR (95% CI)	4.44 (0.99–19.94)	Ref	0.90 (0.53–1.51)	0.92 (0.31–2.72)	Ref	0.90 (0.52–1.57)
Model 2 OR (95% CI)	**4.98 (1.10–22.58)**	Ref	0.86 (0.51–1.45)	1.15 (0.38–3.48)	Ref	0.84 (0.48–1.48)
Model 3 OR (95% CI)	**5.60 (1.16–27.07)**	Ref	0.94 (0.53–1.67)	1.01 (0.31–3.23)	Ref	0.97 (0.54–1.77)
	CD68			CD68		
	Low	Medium	High	Low	Medium	High
# users / non-users	30 / 15	157 / 88	20 / 15	21 / 24	74 / 171	8 / 27
Model 1 OR (95% CI)	1.12 (0.57–2.20)	Ref	0.75 (0.36–1.53)	**2.02 (1.06–3.86)**	Ref	0.69 (0.30–1.58)
Model 2 OR (95% CI)	1.09 (0.55–2.14)	Ref	0.73 (0.36–1.51)	**1.92 (1.00–3.71)**	Ref	0.65 (0.28–1.51)
Model 3 OR (95% CI)	0.83 (0.40–1.73)	Ref	0.73 (0.33–1.60)	1.63 (0.81–3.27)	Ref	0.72 (0.30–1.75)

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	c-Kit			c-Kit		
	Low	Medium	High	Low	Medium	High
# users / non-users	15 / 12	137 / 81	34 / 18	9 / 18	72 / 146	12 / 40
Model 1 OR (95% CI)	0.74 (0.33–1.66)	Ref	1.12 (0.59–2.11)	1.01 (0.43–2.37)	Ref	0.61 (0.30–1.23)
Model 2 OR (95% CI)	0.71 (0.32–1.60)	Ref	1.10 (0.58–2.09)	0.95 (0.40–2.25)	Ref	0.58 (0.28–1.19)
Model 3 OR (95% CI)	0.48 (0.19–1.22)	Ref	1.18 (0.60–2.32)	1.23 (0.48–3.19)	Ref	0.62 (0.30–1.31)

[a]Abundance was scored on a scale of 0–4. When multiple slides per individual were scored, a weighted average was calculated using the number of cores per slide. Abundance was categorized based on the median value of 1 (low: <1, medium: 1, high: >1)

[b]From a case-control study of LUTS nested in the placebo arm of the PCPT (5). The men did not have a clinical indication for biopsy. Sample size for analyses of each marker was as follows: CD4, n=321; CD8, n=326; FoxP3, n=315; CD68, n=325; c-Kit, n=297

Model 1: unadjusted

Model 2: adjusted for age and race

Model 3: adjusted for age (continuous), race (white, black), BMI (continuous), smoking status (current, former, never), physical activity (sedentary, light, moderate, active), education (college, no college), diabetes (yes, no), and statins use (for aspirin model) or aspirin use (for statins model) (yes, no)

Bolded values are statistically significant

PCPT, Prostate Cancer Prevention Trial; OR, odds ratio; CI, confidence interval