NSAIDS FOR THE PREVENTION AND CONTROL OF LETHAL PROSTATE CANCER

by

Lauren M. Hurwitz

A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

April 2018

Abstract

Background: Modifiable risk factors for prostate cancer, and specifically lethal prostate cancer, are needed. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) may protect against prostate cancer development and progression, but the current evidence base is limited. To elucidate the potential role of NSAIDs in the primary and tertiary prevention of lethal prostate cancer, this dissertation investigated associations between NSAID use and (1) prostate cancer incidence, including incidence of lethal disease, and mortality, (2) prostate cancer outcomes among men diagnosed with prostate cancer, and (3) inflammation and markers of specific immune cells in benign prostate tissue.

Methods: Associations between NSAID use and prostate cancer incidence and mortality were estimated for men in the Atherosclerosis Risk in Communities (ARIC) study, which enrolled participants from four communities in1987-1989. Associations between NSAID use and case-fatality were studied among men diagnosed with prostate cancer during ARIC follow-up (1987-2012), and associations between NSAID use and prostate cancer recurrence were studied among men treated surgically for localized prostate cancer at the Johns Hopkins Hospital (JHH) between 1993-2006. Associations between aspirin use and the presence and extent of inflammation, as well as markers of specific immune cells, were examined in benign prostate tissue collected without indication from a subset of men from the placebo arm of the Prostate Cancer Prevention Trial (PCPT), who were enrolled in 1993-1997.

Results: In the ARIC study, aspirin but not non-aspirin NSAID use was inversely associated with lethal and fatal prostate cancer. Aspirin use prior to diagnosis was also associated with prostate cancer case-fatality. In the JHH study, neither aspirin nor non-aspirin NSAID use pre- or post-surgery were inversely associated with prostate cancer recurrence. For aspirin, there was suggestive evidence of a positive association. In the PCPT study, aspirin use at trial entry was

inversely associated with the extent of inflammation and the abundance of FoxP3, a marker of T regulatory cells, in benign prostate tissue collected seven years later.

Conclusions: This dissertation provides support for the role of aspirin in the primary prevention of lethal prostate cancer, and suggests that aspirin may act by reducing the extent of inflammation within the prostate.

Thesis Committee Advisors and Readers

Elizabeth Platz, ScD, MPH
Advisor, Department of Epidemiology

Corinne Joshu, PhD, MPH, MA
Thesis Committee and Reader, Department of Epidemiology

Miranda Jones, PhD, MHS
Thesis Committee and Alternate Reader, Department of Epidemiology

Misop Han, MD
Thesis Committee, School of Medicine

Paul Strickland, PhD, MSc
Thesis Reader, Department of Environmental Health and Engineering

Christopher Heaphy, PhD
Thesis Reader, School of Medicine

Jay Bream, PhD
Alternate Thesis Reader, Department of Molecular Microbiology and Immunology

Acknowledgments

This dissertation would not have been possible without the support of so many individuals. First and foremost, thank you to my advisor, Dr. Elizabeth Platz, for all of your advice and mentorship over the years. Thank you for having a thoughtful response to every question and for always guiding me in the right direction. I am continually inspired by your dedication, creativity, and passion for science, and have learned so much from working with you.

Thank you to my thesis committee members, Drs. Corinne Joshu, Miranda Jones, and Misop Han. We have had such helpful and productive committee meetings, and I am so grateful for your support throughout this process.

Thank you to the many professors that I have gotten to interact with and learn from during my time at Hopkins, and to the other students, who have also taught me so much. Thanks especially to the students in my cohort, who have been such a pillar of support, and to the cancer epidemiology students, who have discussed methodological issues with me at length and made our office so lively.

Thank you to friends from near and far, who have encouraged me, explored Baltimore with me, and made the last few years so enjoyable. Special thanks to Rae for answering my endless grammar questions, to Angela for proofreading parts of the dissertation, and to Nandita for helping so much with Echo.

Finally, thank you to my family – to Mom, Dad, and Jacob, and to my entire extended family. You have always instilled in me the importance of education, and have supported me through every step of this process. Thank you for the encouragement, the scientific articles, the groceries, the dog-sitting, and everything else you've done to help me along. I really could not have done this without you.

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Chapter 1. Introduction

Background

Prostate cancer is one of the leading causes of cancer incidence and mortality in the U.S. and worldwide. In the U.S., approximately 164,690 new cases and 29,430 deaths from prostate cancer are expected to occur in 2018, representing 19% and 9% of all new cases and deaths among men, respectively (1). Globally, prostate cancer accounts for approximately 15% of incident cancers and 7% of cancer deaths among men (2). Prostate cancer deaths result in hundreds of thousands of years of potential life lost (YPLL) in the U.S. per year; there were an estimated 378,069 YPLL due to prostate cancer in 2010, and this number is projected to rise to 951,753 by 2050 (3).

The mortality rate from prostate cancer has declined over the past 20 years (1), likely due to the advent of prostate-specific antigen (PSA) screening, improved early detection and early treatment, and improved treatment of advanced prostate cancer (4). Early detection and treatment cannot curb all deaths from prostate cancer, however, as some prostate cancers progress despite early intervention. Moreover, PSA screening has led to vast overdiagnosis and overtreatment of prostate cancers unlikely to cause harm, with 20-50% of screen-detected prostate cancers currently thought to be overdiagnosed (5). Overdiagnosis and overtreatment of prostate cancer result in unnecessary healthcare costs and subject patients to undue physical and psychological harms. To circumvent problems related to overdiagnosis and overtreatment, and to further reduce morbidity and mortality due to prostate cancer, increased focus on prostate cancer prevention is needed.

Current challenges to prostate cancer prevention

Unfortunately, prevention of prostate cancer has proven difficult. This is in part due to the fact that the strongest risk factors for prostate cancer incidence and mortality – increasing age, African-American ancestry, and family history of prostate cancer – are all non-modifiable

(1). There is general consensus that cigarette smoking is associated with prostate cancer mortality and case-fatality (6) and obesity is associated with advanced and fatal prostate cancer (7), but there are otherwise no modifiable risk factors definitively linked to prostate cancer.

The complex etiology and heterogeneity of prostate cancer has also likely hindered efforts to identify risk factors to target for prevention. Prostate cancer outcomes vary widely; most prostate cancers follow an indolent disease course and are unlikely to cause harm during a man's lifetime, while others progress rapidly and become lethal (8). Indolent and lethal prostate tumors appear to have distinct etiologies with differing sets of risk factors (9, 10), and ideally would be studied as distinct prostate cancer subtypes. However, there is currently no ideal method for distinguishing between indolent and lethal tumors at the time of prostate cancer diagnosis. Stage, grade, and diagnostic PSA value are widely used as prognostic indicators (11), but these variables are imperfect predictors of lethal potential (10), particularly in the presence of PSA screening and the resultant lead-time bias. Due to the difficulty of distinguishing indolent from lethal prostate cancers, as well as the low incidence of lethal prostate cancer, most epidemiologic studies have historically examined these cancers in combination, despite the fact that this approach may obscure true associations with lethal disease.

The high prevalence of indolent prostate cancer has also rendered studies subject to detection bias, or bias arising from the association between potential risk factors and the likelihood of undergoing diagnostic procedures (12). The vast majority of prostate cancers are asymptomatic and detected incidentally via screening (13). As a result, it is difficult to discern whether certain factors are associated with risk of developing prostate cancer, or with the propensity to undergo prostate cancer screening and/or biopsy and have prostate cancer detected. Several cancer risk factors have been associated with the likelihood of undergoing prostate biopsy, including age, positive family history, body mass index, smoking status, and use of common medications (14). Detection bias in studies of prostate cancer risk factors is thus

likely, and failure to account for differential opportunities for detection may lead to inferences that are quantitatively or qualitatively biased (15). Because lethal prostate cancer is rare and more likely to become symptomatic and detected regardless of screening, studies using lethal prostate cancer as an endpoint may be less influenced by detection bias. Prostate cancer prevention research should arguably focus on lethal prostate cancer moving forward, both to limit the impact of detection bias, and to identify risk factors for the subset of prostate cancers that cause the greatest morbidity and mortality.

NSAIDs as promising cancer preventive agents

One potential modifiable risk factor for lethal prostate cancer is regular use of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are a class of drugs widely used to treat pain, inflammation, and fever. Aspirin is also indicated for primary and secondary prevention of cardiovascular disease (CVD). While the cardioprotective benefits of aspirin are well-established, potential benefits of aspirin and non-aspirin NSAIDs (NA-NSAIDs) for other chronic diseases such as cancer are not yet fully understood.

Mechanisms of action of NSAIDs

NSAIDs primarily act by inhibiting the cyclooxygenase enzymes (COX-1, COX-2), thereby inhibiting the conversion of arachidonic acid into prostanoids (prostaglandins, prostacyclin, thromboxane A_2, Figure 1-1). COX-1 is constitutively expressed in most cells and synthesizes prostanoids for housekeeping functions, such as gastrointestinal mucosal defense, platelet aggregation, renal homeostasis, and parturition (16). COX-2, on the other hand, is induced in the presence of inflammation and synthesizes prostanoids that serve as inflammatory mediators (16, 17).

Most NSAIDs, including aspirin, are non-selective COX inhibitors, meaning that they inhibit both COX-1 and COX-2 enzymes. Inhibition of COX-2 leads to the anti-inflammatory property of NSAIDs, but simultaneous inhibition of COX-1 can disrupt homeostatic processes, leading to adverse events such as bleeding and ulcers (16). In an attempt to preserve the anti-inflammatory effects of NSAIDs resulting from COX-2 inhibition while avoiding the risk of gastrointestinal toxicities resulting from COX-1 inhibition, selective COX-2 inhibitors (coxibs) were developed and marketed. However, these drugs were found to significantly increase risk of adverse cardiovascular events, and all but one were withdrawn from the U.S. market in the early 2000s.

Aspirin is unique from other NSAIDs in that it acetylates and *irreversibly* inhibits the COX-1 and COX-2 enzymes. Unlike other NSAIDs, aspirin also preferentially inhibits COX-1 over COX-2 when taken at low doses (18). For this reason, aspirin is more effective than non-aspirin NSAIDs (NA-NSAIDs) as an antiplatelet agent. Aspirin's inactivation of COX-1 in platelets leads to decreased production of thromboxane A_2, a promoter of platelet aggregation and vasoconstriction, and because platelets lack nuclei and cannot regenerate COX-1, the effects of aspirin persist for the duration of the platelet lifespan. Low doses of aspirin (75-100 mg per day) are sufficient to bring about these antithrombotic effects, while higher doses of aspirin (≥300 mg per day) are needed for aspirin to inhibit COX-2 and reduce inflammation (18).

Mechanisms of action against cancer

Aspirin and NA-NSAIDs are hypothesized to protect against cancer via both COX-dependent and COX-independent mechanisms. COX-dependent mechanisms include inhibition of COX-2, which is overexpressed in prostate cancer tissue (19), as well as in tissue from several other cancer types (20-24). Increased expression of COX-2 in diagnostic tumor tissue is also positively associated with risk of biochemical recurrence and metastases in men treated for

prostate cancer (25). Decreased COX-2 expression may hinder cancer development and progression by blocking synthesis of the pro-inflammatory prostaglandin E_2 (PGE_2), which inhibits apoptosis, stimulates cell proliferation, migration, and invasion, and suppresses immune responses (26, 27). In general, chronic inflammation is a well-accepted enabling characteristic of cancer (28) and has been implicated in prostate cancer specifically (29); it thus seems plausible that the anti-inflammatory properties of NSAIDs could lead to antineoplastic effects as well.

NSAIDs may also reduce risk of cancer metastases via inhibition of COX-1 in platelets. Activated platelets are thought to facilitate cancer metastases through the bloodstream by binding to tumor cells in circulation and shielding them from immune detection (30, 31). Platelets also release mediators and growth factors that may promote tumor cell extravasation, proliferation, and angiogenesis at new, distant sites (30, 31). By impeding platelet function, NSAIDs and aspirin in particular may disrupt these tumor cell-platelet interactions and encumber cancer spread.

COX-independent mechanisms linking NSAIDs to cancer incidence and progression are not well understood, but may involve AMPK and mTOR signaling, NF-kB signaling, ERK signaling, the Wnt/beta-Catein pathway, inhibition of AP-1, and/or acetylation of non-COX proteins (16, 32).

Review of the current epidemiologic literature

Randomized controlled trials of aspirin and cancer

Aspirin has shown promise as an effective anti-cancer agent according to secondary analyses of randomized controlled trials (RCTs) of aspirin and cardiovascular outcomes. A meta-analysis of six RCTs of daily low-dose aspirin for primary prevention of vascular disease found that daily aspirin reduced overall cancer risk (hazard ratio (HR): 0.88, 95% confidence

interval (CI): 0.80-0.98), with the benefit increasing with increasing duration follow-up (33). In a meta-analysis of 34 RCTs of daily low- or high-dose aspirin for primary or secondary CVD prevention, allocation to daily aspirin was also observed to reduce total cancer deaths, particularly after five years or more of follow-up (after 5 years, odds ratio (OR): 0.63, 95% CI: 0.49-0.82) (33). Furthermore, in a meta-analysis of five of these RCTs from the United Kingdom, where cancer registration and death certification procedures were well-validated and reliable, daily aspirin was found to reduce risk of cancer with distant metastasis (HR: 0.64, 95% CI: 0.48-0.84), including metastasis at diagnosis and at subsequent follow-up (34). However, these meta-analyses excluded results from two U.S. trials examining alternate-day, low-dose aspirin for CVD and cancer prevention, both of which reported null findings for total cancer mortality (35, 36).

From these studies, it is also difficult to discern whether results are driven by reductions in colorectal cancer incidence and mortality, or whether incidence and mortality from prostate and other cancer types are also reduced. Prostate cancer mortality was examined in a pooled analysis of six primary and secondary CVD prevention trials for which there were individual patient data available; in this analysis, allocation to daily aspirin was associated with a non-significant reduction in prostate cancer mortality during the trial treatment period (after ≥5 years of follow-up, HR: 0.52, 95% CI: 0.20-1.34) (37). However, only 37 prostate cancer deaths were observed during the treatment period of these trials. In three of these trials with extended follow-up of participants and 210 prostate cancer deaths, allocation to daily aspirin for at least five years was possibly associated with prostate cancer mortality (across 20 years of follow-up, HR: 0.81, 95% CI: 0.61-1.06); this result was not statistically significant, but a moderate effect of aspirin on prostate cancer mortality could not be ruled out (37). After consideration of all available RCTs, the U.S. Preventive Services Task Force (USPSTF) concluded in 2016 that there was enough evidence to recommend aspirin for the joint primary prevention of colorectal

cancer and CVD, but that the evidence supporting an overall cancer mortality benefit and benefit for other cancer types, including prostate cancer, was inconclusive (38, 39).

Observational studies of NSAIDs and prostate cancer incidence

Several observational studies have examined regular aspirin use in relation to prostate cancer incidence and mortality. Algra et al. found that observational studies of aspirin and cancer can produce estimates similar to those from RCTs, so long as there is "an adequate definition of aspirin exposure, updated assessment of exposure during the follow-up period, and appropriate adjustment for imbalances in baseline characteristics" (40).

Observational studies that meet these criteria have found regular aspirin use to be associated with an approximately 10-20% reduced risk of total incident prostate cancer (41, 42). Aspirin use has also been associated with an approximately 20% reduced risk of advanced prostate cancer, with advanced disease most often defined by cancer stage and/or grade at diagnosis (41, 42). The pooled estimates for total prostate cancer have shown evidence of heterogeneity (*p-heterogeneity*=0.05) and appear to vary by study characteristics such as geographic location, source of medications data, and methods for accounting for detection bias; in contrast, pooled estimates for advanced prostate cancer have been relatively consistent (*p-heterogeneity*=0.66) (42). NA-NSAID use has been associated with a 0-10% reduced risk of total incident prostate cancer and does not appear associated with advanced prostate cancer, though there has been significant heterogeneity across study (41, 42). Few studies have investigated the relationship between aspirin use and lethal prostate cancer specifically (Table 1-1). In the Health Professionals Follow-up Study (HPFS), current use of aspirin at least two times per week was non-significantly associated with a reduced risk of lethal prostate cancer, defined as prostate cancer that was metastatic at diagnosis or that metastasized or caused death during follow-up (HR: 0.84, 95% CI: 0.69-1.02) (43). Former aspirin use was also possibly

inversely associated with lethal prostate cancer (HR: 0.86, 95% CI: 0.67-1.11). A significant dose-response was observed for increasing number of tablets per week (p-trend=0.04), but not for increasing days per week of use or cumulative duration of use. Similarly, in the Physicians' Health Study (PHS), in which aspirin use was initially randomized (1981/2-1988) and then offered to study participants after trial completion, aspirin use at least three days per week for one year or longer was significantly associated with lethal prostate cancer, both for current use (HR: 0.68, 95% CI: 0.52-0.89) and former use (HR: 0.54, 95% CI: 0.40-0.74) (44). The inverse association with current aspirin use was consistent regardless of duration of use, and the association with former use was consistent regardless of time since stopping. Interestingly, associations for current and former use were only observed for cases diagnosed before the PSA era; associations in the PSA era were null. A more recent analysis in HPFS reported no association between aspirin use at least twice per week and prostate cancer that was metastatic or regionally advanced at diagnosis (T3b, N1, or M1) or that metastasized or caused death during follow-up (HR: 0.97, 95% CI: 0.85-1.10) (45). This discordance across HPFS analyses could be due to the inclusion of T3b cancers in the definition of lethal prostate cancer (in the second analysis), or to the differing study periods (1988-2006 for the first analysis, 1986-2012 for the second analysis, which overlaps more with the PSA era). In both of these studies of aspirin and lethal prostate cancer, very few non-white study participants were included (Table 1-1). To our knowledge, the association between NA-NSAID use and lethal prostate cancer has not yet been studied.

Observational studies of NSAIDs and prostate cancer survival

Several studies have also examined aspirin and NA-NSAID use and prostate cancer outcomes following diagnosis. Specifically, studies have examined both pre-diagnostic aspirin use (46-50) and post-diagnostic aspirin use (46, 48-53) in relation to prostate cancer case-fatality, but results have been largely inconsistent, with results ranging from protective to null to

harmful (Table 1-2). Results for NA-NSAID use have been similarly conflicting (49, 50, 53). These inconsistencies could be due to differences in the way NSAID use was measured and operationalized; studies assessed NSAID use at varying time intervals before and after diagnosis, defined “regular” use in different ways, and used various sources of medications data, including self-report and prescription medication databases. Differences between the studies could also be driven by differences in study populations, in outcome ascertainment methods, or in the prevalence or ability to adjust for confounders. As with the studies of aspirin use and lethal prostate cancer, most studies of NSAID use and prostate cancer outcomes have been conducted in primarily white study populations, with the exception of two small studies (Table 1-2).

NSAID use, inflammation, and immune cell profiling in the prostate

Despite the multitude of observational studies on NSAID use and prostate cancer incidence and survival, little is known about the biological effects of NSAIDs on prostate tissue. It is hypothesized that NSAIDs reduce chronic inflammation in the prostate, and that chronic inflammation could therefore mediate relationships between NSAID use and prostate cancer risk and progression, but the association between NSAID use and intraprostatic inflammation has not been studied directly. Associations between aspirin use and serum C-reactive protein, serum pro-inflammatory cytokines, or other circulating markers of inflammation have been examined in randomized (54-59) and non-randomized studies (60-64), with mostly null results. However, it is unknown if serum markers of inflammation are correlated with inflammation in prostate tissue.

One of the major barriers to this work arises from the challenge of assessing inflammation in normal prostate tissue. Inflammation can be studied in tissue removed for prostate biopsy, but biopsies are typically only done for suspicion of prostate cancer, i.e.

elevated PSA or abnormal digital-rectal examination. Prostate tissue may also be removed for treatment of benign prostatic hyperplasia and prostate cancer. However, tissue removed for these indications is inherently different from tissue of men without prostate conditions. Higher serum PSA has also been positively associated with inflammation in men without indication for biopsy (65). Thus, tissue removed for the indication of elevated PSA is more likely to harbor inflammation, and studying only tissue removed for indication can lead to biased associations between intraprostatic inflammation and prostate cancer risk factors or outcomes.

If able to circumvent this challenge, epidemiologic studies could investigate associations between NSAID use and the presence of any inflammation, the extent of inflammation, or the type of inflammation (acute vs. chronic) in prostate tissue. Studies could also examine whether NSAID use modifies the immune cell profile within the prostate. Via COX-dependent and COX-independent mechanisms (66), NSAID use might alter the infiltration of specific innate and adaptive immune cells present in the prostate (67), including:

- **Macrophages**, phagocytic cells that digest microbes and cellular debris, secrete pro-inflammatory cytokines, and present antigens to helper T cells (68).
- **Mast cells**, granulocytes that release lipid mediators (such as prostaglandins) and cytokines (such as TNF-α) that recruit macrophages to sites of inflammation, and may contribute to T cell-mediated chronic inflammation (68, 69).
- **Helper T cells** ($CD4^+$ T cells), which help B lymphocytes produce antibodies, macrophages destroy ingested microbes, and cytotoxic T cells kill infected cells (68).
- **Cytotoxic T cells** ($CD8^+$ T cells), which recognize and kill infected host cells and tumor cells (68).
- **Regulatory T cells (FOXP3+ T cells; Tregs)**, which downregulate effector T cells and mediate peripheral tolerance to self-antigens (68, 70).

The effects of NSAID use on the presence, abundance, or function of these immune cell types in prostate tissue collected without clinical indication is currently unknown, but could help to elucidate the biological mechanisms linking NSAID use to prostate cancer pathogenesis.

Current knowledge gaps

As discussed above, most studies conducted to date on NSAIDs and prostate cancer incidence and progression have included mostly white study participants. This is problematic given the documented racial disparities in prostate cancer incidence and mortality. In particular, black men are more likely than men of other races to develop and die from prostate cancer (71). Moreover, compared to white men, black men tend to have molecularly distinct prostate cancers (72) with differing relationships to risk factors (73-75). It is thus unclear if the current studies can be generalized to these men. Other limitations of the current literature include the limited research focusing on lethal prostate cancer specifically, the discordant results for NSAID use and prostate cancer case-fatality, and the lack of established biological mechanisms linking NSAID use to prostate cancer development and progression. As a result of these uncertainties, aspirin and NA-NSAID use are not currently recommended for primary or tertiary prevention of prostate cancer, despite accumulating evidence that they may be beneficial.

Summary of the dissertation aims and potential impact

This dissertation aims to clarify the potential role of aspirin and NA-NSAIDs in the prevention and control of prostate cancer, with a focus on prostate cancers that are potentially lethal (Figure 1-2). The first study investigates the relationship between aspirin and NA-NSAID use and prostate cancer incidence (total and lethal) and mortality in the Atherosclerosis Risk in Communities (ARIC) study. This aim augments the existing literature by examining risk of *lethal*

prostate cancer in particular, and by exploring this question in a community-based cohort that includes both white and black men.

This dissertation also aims to determine whether aspirin and NA-NSAID use may be beneficial in improving cancer outcomes among men already diagnosed with prostate cancer. Specifically, this dissertation examines the relationship between aspirin and NA-NSAID use and prostate cancer case-fatality in the ARIC study, and prostate cancer recurrence after surgery among a cohort of patients treated at the Johns Hopkins Hospital. Use of these complementary study populations is intended to improve the generalizability of the findings, and to help shed light on why prior studies of NSAID use and prostate cancer outcomes have reported conflicting results.

The third study examines aspirin use and the presence and extent of inflammation within the prostate, a possible mediator of the relationship between NSAID use and prostate cancer incidence and progression. This study also investigates the relationship between aspirin use and the abundance of specific immune cell types in benign prostate tissue. These associations are examined among men from the Prostate Cancer Prevention Trial, a study population that provides the unique opportunity to examine prostate tissue collected without clinical indication. To our knowledge, this study is the first to investigate whether aspirin use may actually alter the extent of inflammation and the immune cell profile in the prostate of men without clinical indication for prostate biopsy, and aims to elucidate the biological plausibility of aspirin influencing prostate cancer endpoints via reductions in intraprostatic inflammation.

This research has the potential to impact public health practice by informing relevant guidelines, such as those released by the USPSTF. There are known risks to taking NSAIDs regularly; as a result, benefits must clearly outweigh potential harms for aspirin or NA-NSAID use to be advisable. Currently, the benefits of regular aspirin use are thought to outweigh the harms only for certain individuals at high risk of cardiovascular disease (i.e. individuals with a 10% or greater 10-year risk) or colorectal cancer (i.e. individuals with Lynch syndrome) (39, 76).

Benefits related to prostate cancer are considered too uncertain to be factored into risk-benefit calculations (38, 77). However, if regular aspirin or NA-NSAID use is consistently shown to protect against lethal prostate cancer, either among men not yet diagnosed or among men already diagnosed and treated, then the benefit-to-harm ratio may be bolstered for additional subgroups. This could lead to the expansion of current USPSTF guidelines, to new guidelines for aspirin or NA-NSAID use among men already diagnosed with prostate cancer, and ultimately to a reduction in the burden of deaths due to prostate cancer.

Chapter 1 Tables

Table 1-1. Observational studies of NSAID use and lethal prostate cancer

Study	Data Source	Study Design	% AA	Exposure	Definition of Lethal Prostate Cancer	Confounders	Hazard Ratio (95% CI)	Key Limitations
Dhillon et al. 2011 (43)	Health Profession-als Follow-up Study (n=51,529)	Cohort	NS (~90% white)	Aspirin use (≥2x per week)	M1 at diagnosis or development of bony metastases or prostate cancer death	Age, time period, race, family history, height, BMI, smoking status, vitamin D, total kilocalories, intake of tomato sauce, fish, red meat, physical activity, statin use	Current use vs. no use: 0.84 (0.69, 1.02)	Generalizability may be limited due to homogeneity of cohort
Cao et al. 2016 (45)	Health Profession als Follow-up Study (n=47,881)	Cohort	NS (~90% white)	Aspirin use (≥2x per week)	Regionally invasive or metastatic at diagnosis (T3b, N1, or M1 or worse), or development of metastases or prostate cancer death	Age, calendar year, race, height, BMI, family history of cancer, physical exam in the past 2 years, history of cancer screening (colonoscopy, sigmoidoscopy, PSA test), smoking status, physical activity, alcohol intake, vitamin use, total energy intake, meat intake, calcium intake, Alternate Healthy Eating Index 2010	Current regular use: 0.97 (0.85, 1.10)	Generalizability may be limited due to homogeneity of cohort
Downer et al. 2017 (44)	Physicians' Health Study (n=22,037)	RCT (1981/2 -1988), Cohort (Post-1988)	NS (~92% white)	Aspirin use (>3 days per week for ≥1 year)	Metastases or prostate cancer death	Age, race, BMI, height, smoking status, hypertension, type II diabetes	Current use: 0.68 (0.52, 0.89) Former use: 0.54 (0.40, 0.74)	Generalizability may be limited due to homogeneity of cohort

Abbreviations: AA, African American; CI, confidence interval; NS, not stated; BMI, body mass index; PSA, prostate specific antigen; RCT, randomized clinical trial

Table 1-2. Observational studies of pre- and post-diagnostic NSAID use and prostate cancer outcomes following diagnosis

Study	Data Source	Study Design	% AA	Exposure	Primary Outcome	Confounders	Hazard Ratio/Odds Ratio (95% CI)		Key Limitations
							Pre-Dx Use	Post-Dx Use	
Flahavan et al. 2014 (47)	National Cancer Registry of Ireland linked to pharmacy claims data (n=2,936)	Cohort	NS	Prescription aspirin	Case-fatality	Age, smoking status, grade, tumor size, comorbidities (based on prescribed medications), year of dx, statin use, treatment	0.88 (0.67, 1.15)		Limited information on lifestyle factors
Cardwell et al. 2014 (46)	UK Clinical Practice Research Datalink (n=1,184 cases and 3,531 matched controls)	Nested case-control	NS	Low-dose aspirin	Case-fatality	Grade, treatment, smoking status, alcohol use, BMI, comorbidities prior to diagnosis	1.04 (0.89, 1.22)	1.02 (0.78, 1.34)	Relies on prescription data and physician notes (may miss over-the-counter use), no adjustment for stage
Jacobs et al. 2014 (48)	CPS-II Nutrition Cohort (n=8,427)	Cohort	1.4%	Daily aspirin	Case-fatality	Age, race, year of dx, stage, nodal involvement, grade, treatment, CVD, cholesterol-lowering medications, history of PSA testing	0.92 (0.72, 1.17)	0.98 (0.74, 1.29)	Potential confounders omitted (i.e. BMI, smoking status)
Veitonmaki et al. 2015 (49)	Finnish Prostate Cancer Screening Trial (n=6,537)	Cohort	NS ("almost entirely Caucas-ian")	Prescription aspirin and NA-NSAIDs	Case-fatality	Age, use of other medications, screening attendance, stage, grade, PSA	For aspirin: 0.96 (0.54, 1.70); For all NSAIDs: 1.30 (1.07, 1.58)*	For aspirin: 0.78 (0.42, 1.46); For all NSAIDs: 0.42 (0.34, 0.51)**	Only prescription medication use included (does not account for over-the-counter use), no adjustment for lifestyle factors
Zhou et al. 2017 (50)	NIH-AARP Diet and Health Study (n=19,474 cases), PLCO	Meta-analysis of two cohorts	AARP: 6% non-white, PLCO: 11% non-white	Regular aspirin and NA-NSAID use within the past year (none,	Case-fatality	Age (as the time scale), grade, stage, treatment, intervention arm (PLCO), race, marital status, BMI, smoking status,	For daily aspirin: 0.98 (0.83, 1.17); For daily NA-NSAIDs: 0.86 (0.67, 1.10)	For daily aspirin: 0.81 (0.58, 1.14); For daily NA-NSAIDs: 0.99 (0.58, 1.68)	Included men with regional/distant spread at dx, NSAID use was self-reported at limited time points

	(n=7,916 cases)			<1/day, ≥1/day)		history of CVD and diabetes, history of screening (AARP), self-perceived general health status (AARP)			
Choe et al. 2012 (52)	CaPSURE Registry (n=5,955)	Cohort	NS	Aspirin	Case-fatality	PSA, stage, grade, treatment, non-aspirin anticoagulants, statins		0.43 (0.21, 0.87)	No adjustment for age or lifestyle factors
Dhillon et al. 2012 (53)	Health Professionals Follow-up Study (n=3,986)	Cohort	NS	Aspirin and NA-NSAIDs	Mets or prostate cancer death	Age, year, race, family history, height, BMI, smoking status, tomato sauce intake, vitamin D intake, total kilocalories, fish, red mean, physical activity, statin use, grade, stage, treatment, pre-dx aspirin use		Aspirin: 0.94 (0.68, 1.30) NA-NSAIDs:0.81 (0.39, 1.67)	Generalizability may be limited due to homogeneity of cohort
Assayag et al. 2015 (51)	UK National Cancer Data Repository (n=11,779)	Cohort	0.9%	Aspirin	Case-fatality	Age, year of dx, race, obesity, smoking status, SES, CVD comorbidity, use of other medications, PSA, grade, treatment		1.46 (1.29, 1.65)	Aspirin use based on prescription database (does not account for over-the-counter use), no adjustment for stage
Osborn et al. 2016 (78)	African American men treated with radiation at the NY Harbor Dep. of Veteran Affairs	Cohort	100%	Aspirin	BCR, distant mets, prostate cancer death	Age, hormone therapy, radiation technique, other medication use, NCCN risk group		For BCR: 0.56 (0.34, 0.93); For mets: 0.23 (0.06, 0.91)	Small sample size, only one treatment group included, no adjustment for lifestyle factors

	(n=286)								
Downer et al. 2017 (44)	Physicians' Health Study (n=3,462 non-metastatic prostate cancers)	Cohort	NS (92% white)	Regular aspirin use (>3 tablets/week for ≥1 year)	Metastases or prostate cancer death	Age, year of dx, race, CCI, BMI, smoking status, PSA, grade, stage, primary treatment		Current use: 0.68 (0.52, 0.90); Past use: 1.50 (1.10, 2.05)	Generalizability may be limited due to homogeneity of cohort
Smith et al. 2017 (79)	NCI-Maryland Prostate Cancer Case-Control Study (n=218 cases with information on disease-free survival)	Cohort (of cases from the parent case-control study)	~50%	Regular aspirin use one year prior to interview	Recurrence (BCR or mets)	Age, race/ethnicity, grade, stage, smoking status, education		Overall: 0.57 (0.26, 1.23); Among AA men: 0.17 (0.03, 0.84)	Small sample size, no details given on outcome ascertainment, unclear whether aspirin use occurred before or after dx, no adjustment for comorbidities

Abbreviations: AA, African American; CI, confidence interval; NS, not stated; Dx, diagnosis; BCR, biochemical recurrence; BMI, body mass index; CVD, cardiovascular disease; PSA, prostate specific antigen; SES, socioeconomic status; NCCN, National Comprehensive Cancer Network; CCI, Charleston Comorbidity Index

*for analyses of medications used during the year of diagnosis

**for analyses that excluded medications used within the last 3 years of follow-up

Chapter 1 Figures

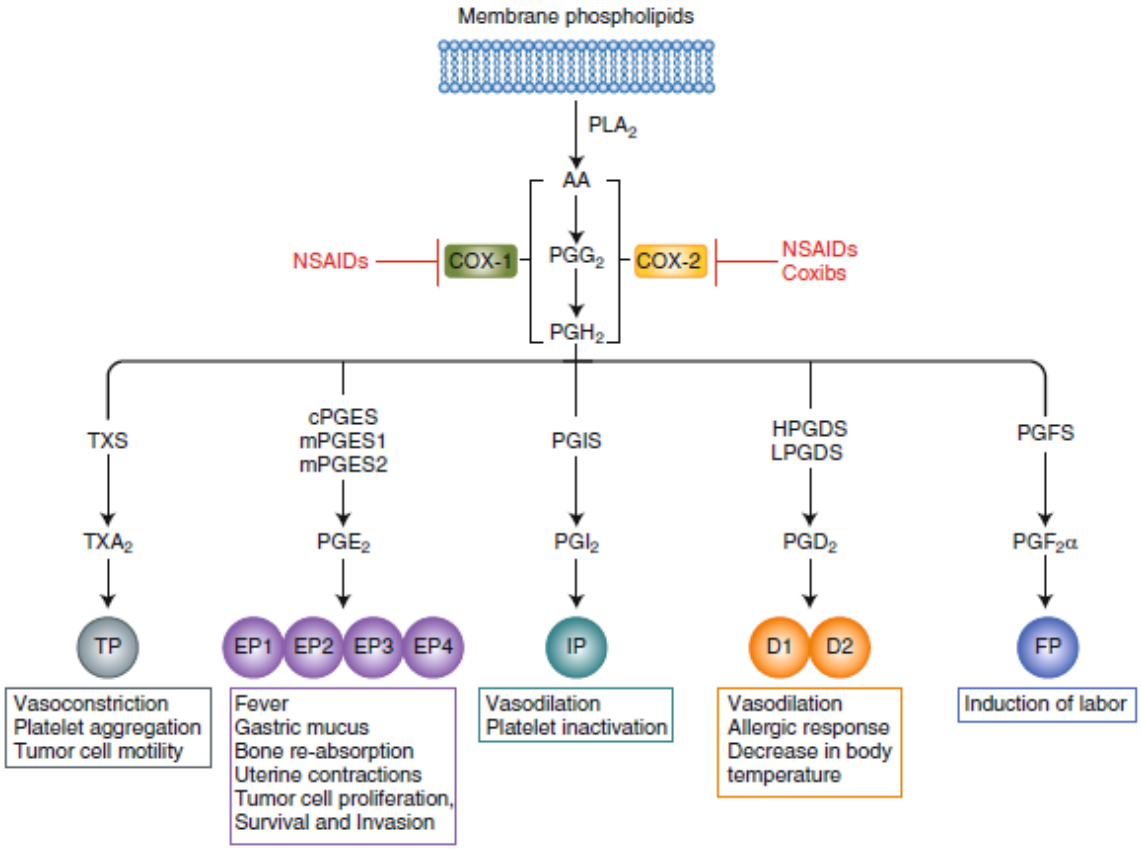


Figure 1-1. NSAIDs and the prostanoid synthesis pathway. Aspirin, other non-selective NSAIDs, and selective COX-2 inhibitors (coxibs) act by inhibiting the COX enzymes, which convert arachidonic acid (AA) into prostaglandin H_2 (PGH_2). PGH_2 is then converted by synthases into thromboxane A_2 (TXA_2), prostaglandin E_2 (PGE_2) prostacyclin (PGI_2), prostaglandin D_2 (PGD_2), and prostaglandin $F_2\alpha$ ($PGF_2\alpha$), which contribute to several biological processes, including but not limited to those listed above. Figure from *NSAIDs and Aspirin: Recent Advances and Implications for Clinical Management*, page 176 (16).

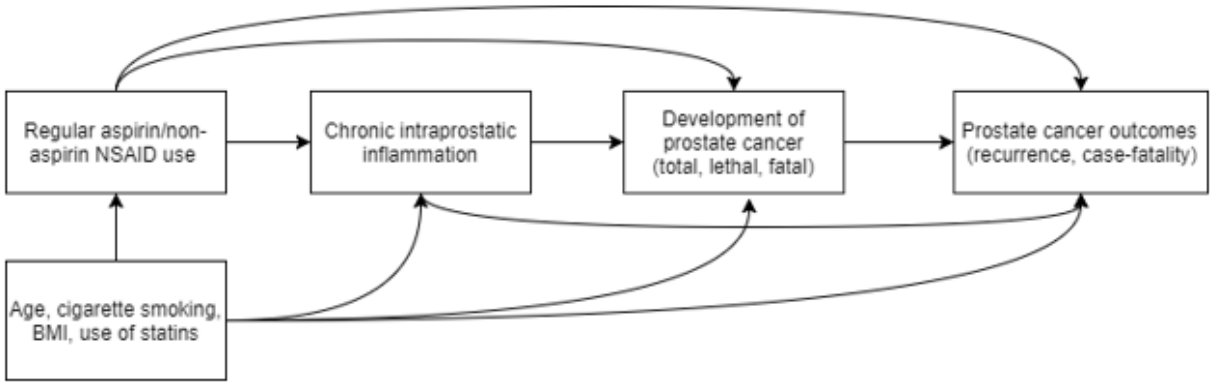


Figure 1-2. Conceptual Framework. We hypothesized that regular use of aspirin and non-aspirin (NA)-NSAIDs reduce risk of prostate cancer, and specifically risk of prostate cancer with lethal potential (Aim 1). We also hypothesized that among men with prostate cancer, regular use of aspirin and NA-NSAIDs reduce risk of disease recurrence and case-fatality (Aim 2). Finally, we hypothesized that these effects are partially mediated by chronic intraprostatic inflammation, and that aspirin and NA-NSAID use would thus be associated with the presence and extent of inflammation in prostate tissue (Aim 3). To examine these relationships, we had to account for confounding by factors such as age, smoking status, obesity, and concurrent use of statin

Chapter 2. Aspirin and Non-Aspirin NSAID Use and Prostate Cancer Incidence, Mortality, and Case-Fatality in the Atherosclerosis Risk in Communities Study

Abstract

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain and inflammation, and aspirin is recommended for some individuals for prevention of cardiovascular disease (CVD) and colorectal cancer. Observational studies suggest that NSAIDs may also lower risk of prostate cancer. However, there is limited evidence on whether NSAIDs protect against lethal prostate cancer in particular, and on whether benefits are consistent in black and white men. This study sought to determine the association between aspirin and non-aspirin NSAID (NA-NSAID) use and risk of total, lethal, and fatal prostate cancer and prostate cancer case-fatality, overall and by race, among men in the Atherosclerosis Risk in Communities (ARIC) study.

Methods: The ARIC study enrolled individuals from four U.S. communities in 1987-89. This analysis was restricted to white and black men from this cohort who had information on medication use and who did not have a cancer history at baseline. Aspirin and NA-NSAID use was assessed at four study visits, with additional detailed information on aspirin use collected at the fourth study visit. Cancer outcomes were ascertained through 2012. Cox proportional hazards regression was used to estimate age- and multivariable-adjusted cause-specific hazard ratios (HRs) and 95% confidence intervals (CIs) for total incident prostate cancer, incident lethal prostate cancer, prostate cancer mortality (i.e. fatal prostate cancer), and case-fatality. Stratified models and likelihood ratio tests were used to test for effect modification by race.

Results: There were 6,594 men (5,060 white, 1,534 black) at risk for prostate cancer. Aspirin and NA-NSAID use were not associated with total prostate cancer. However, aspirin use was inversely associated with lethal (HR: 0.58, 95% CI: 0.35-0.95) and fatal (HR: 0.59, 95% CI: 0.36-0.96) prostate cancer. These associations were consistent among white and black men and most evident among men using aspirin regularly for CVD prevention. Aspirin use was also inversely associated with prostate cancer case-fatality (HR: 0.45, 95% CI: 0.22-0.94). NA-

NSAID use was not associated with lethal or fatal prostate cancer, or with prostate cancer case-fatality.

Conclusions: Aspirin use was inversely associated with lethal and fatal prostate cancer, as well as case-fatality, among white and black men. If confirmed by additional research, benefits of aspirin pertaining to lethal prostate cancer may need to be factored into risk-benefit calculations of men considering starting an aspirin regimen.

Introduction

Unlike other leading causes of cancer, little is known about how to prevent prostate cancer. There is strong evidence that older age, African-American ancestry, and a positive family history increase risk of prostate cancer, but these risk factors are all non-modifiable. Cigarette smoking and obesity both appear associated with advanced and/or lethal prostate cancer (6, 7), but these risk factors can be difficult to modify. There is a need to identify additional modifiable risk factors for prostate cancer, and specifically for lethal prostate cancer, so that preventive strategies can be developed and morbidity and mortality can be reduced.

One potential modifiable factor is regular use of aspirin and non-aspirin (NA) nonsteroidal anti-inflammatory drugs (NSAIDs), which are hypothesized to prevent cancer incidence and progression via anti-inflammatory and anti-platelet mechanisms (80, 81). Secondary analyses of randomized controlled trials (RCTs) of aspirin for cardiovascular disease (CVD) have shown that daily aspirin reduces overall cancer incidence, development of cancer metastases, and cancer mortality, particularly after five or more years of use (33, 34, 37). However, RCTs have not been designed to examine effects of regular aspirin or NA-NSAID use on prostate cancer incidence and mortality specifically.

In observational studies, aspirin and NA-NSAID use have been associated with a moderately reduced risk of total prostate cancer (pooled odds ratio (POR) for aspirin: 0.83, 95% confidence interval (CI): 0.77-0.89; POR for NA-NSAIDs: 0.89, 95% CI: 0.78-1.02) (42). Aspirin but not NA-NSAID use has also been associated with a reduced risk of advanced prostate cancer (POR for aspirin: 0.81, 95% CI: 0.72-0.92) (42). However, most studies have defined advanced prostate cancer based on cancer stage or grade at diagnosis. Diagnostic stage and grade are imperfect indicators of disease lethality, particularly in settings with routine PSA screening for early detection and the resultant lead time (82, 83). Additional studies are thus needed to determine whether aspirin and NA-NSAID use protect against aggressive, potentially

lethal prostate cancer. Furthermore, previous studies have been conducted primarily among white men, and so generalizability to other groups is unknown.

The goal of this study was to investigate associations between aspirin and NA-NSAID use and total, lethal, and fatal prostate cancer, overall and by race, among men who did not have a cancer history at baseline in the Atherosclerosis Risk in Communities (ARIC) study. This study also examined associations between aspirin and NA-NSAID use and prostate cancer case-fatality among men diagnosed with prostate cancer during ARIC follow-up. It was hypothesized that aspirin and NA-NSAID use would be inversely associated with these endpoints, and that the magnitude of the associations would be similar for white and black men.

Methods

Study Population

This study included men from the Atherosclerosis Risk in Communities (ARIC) study, a prospective cohort study that was designed to assess the etiology and natural history of CVD (84). A total of 15,792 men and women ages 45-64 years were recruited from four U.S. communities (Forsyth County, North Carolina; Jackson, Mississippi; the Minneapolis area, Minnesota; Washington County, Maryland) in 1987-89. Participants attended six in-person study visits (Visit 1: 1987-89, Visit 2: 1990-92, Visit 3: 1993-95, Visit 4: 1996-98, Visit 5: 2011-13, Visit 6: 2016-17) and were interviewed by telephone annually. Each study field center received institutional review board approval, and all participants provided informed consent.

For analyses of prostate cancer incidence and mortality, the study population was restricted to men without a history of cancer at their baseline visit in 1987-89 (Figure 2-1). The study population was further restricted to men who self-reported as either white or black, since race is an important determinant of prostate cancer incidence and mortality, and since there were not enough men of other racial groups to examine these groups separately. There were

also very few black men enrolled from the Washington County and Minneapolis field centers; these men were excluded as well, to avoid potential confounding by race/geography. Lastly, men with missing baseline medications data were excluded.

For analyses of prostate cancer case-fatality, the study population included men diagnosed with prostate cancer during follow-up (1987-2012), irrespective of whether prostate cancer was the first or subsequent primary. Exclusion criteria were the same as above, with some additions: cases identified by death certificate only were excluded, as these men had no follow-up time post-diagnosis, and men missing diagnostic stage were also excluded, as stage is a critical covariate in case-fatality analyses.

Assessment of Covariates

At each study visit, participants were asked to bring in the containers for all medications that they had used within the past two weeks. Medication names, concentrations, source (prescribed, over-the-counter, or shared), and use over the past 24 hours were recorded. Current aspirin and NA-NSAID use were ascertained from these forms. See Appendix 2-1 for a detailed description of how the measure of current aspirin use was defined and validated.

Additional information on regular aspirin use was collected at Visit 4. During this visit, participants were asked if they took aspirin on a regular basis, defined as use at least once a week for several months. If they responded yes, they were asked to provide the strength of the aspirin used (<300, 300-499, ≥500 mg), days per week of use, number of pills taken per week, their reason for taking aspirin, and the date that they began taking aspirin regularly. Participants were also asked whether they were currently taking aspirin regularly on annual follow-up interviews starting in 1998.

Other covariates of interest, identified a priori as potential confounders and/or effect modifiers, included body mass index (BMI), cigarette smoking status, use of statin drugs, prevalent coronary heart disease (CHD), self-reported diabetes, use of diabetes medications,

serum glucose concentration (all assessed at each of the four study visits), glycated hemoglobin (ascertained at Visit 2), education level (ascertained at Visit 1), family history of prostate cancer (ascertained at Visit 3), and frequency of routine physical examinations (ascertained at Visit 2). Using these variables, diabetes status for each individual at each visit was classified as either diagnosed diabetes (self-reported diabetes or use of diabetes medications at the current visit, or any previous visit), undiagnosed diabetes (fasting serum glucose ≥126 mg/dL or non-fasting serum glucose ≥200 mg/dL), prediabetes (100 mg/dL ≤ fasting serum glucose <126 mg/dL, or 140 mg/dL ≤ non-fasting serum glucose <200 mg/dL), or no diabetes (fasting serum glucose <100 mg/dL or non-fasting serum glucose <140 mg/dL).

Outcome Ascertainment

Cancer diagnoses and cancer deaths among study participants, as well as tumor characteristics, are identified and adjudicated through linkage to state cancer registries and review of hospital records and death certificates (85). Cancer outcomes are currently ascertained through 2012.

For this study, there were four endpoints of interest: (1) total prostate cancer, defined as diagnosis of a first primary prostate cancer, (2) lethal prostate cancer, defined as diagnosis of a first primary prostate cancer that was advanced stage (T4, N1, or M1) at diagnosis or that caused death during follow-up, (3) fatal prostate cancer, defined as death from prostate cancer, irrespective of whether other cancers were also diagnosed during follow-up, and (4) prostate cancer case-fatality, defined as death from prostate cancer among men diagnosed with prostate cancer, irrespective of whether other cancers were also diagnosed before or after prostate cancer.

Statistical analysis

For each exposure of interest and for each primary outcome, cause-specific hazard ratios (HRs) and 95% CIs comparing NSAID users to non-users were calculated via Cox proportional hazards regression. For analyses of total prostate cancer, men were censored if lost-to-follow-up, if diagnosed with another cancer before prostate cancer, at death, or at end of follow-up in 2012. For analyses of lethal prostate cancer, men were also censored if they were diagnosed with a non-lethal prostate cancer. For analyses of fatal prostate cancer and prostate cancer case-fatality, men were censored at date of death from a cause other than prostate cancer, or administratively in 2012. For all models, the proportional hazards assumption was verified via Schoenfeld residuals.

For analyses of current aspirin and NA-NSAID use and total, lethal, and fatal prostate cancer, age was the time metric and age 45 was the time origin. Aspirin and NA-NSAID use were treated as dichotomous exposures and updated at each study visit (through Visit 4). If missing, the last observed data point was carried forward. Other covariates included in the models were race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (never, quit >10 years ago, quit within 10 years or current smoker), BMI (continuous), statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no), years of education (basic [≤11 years], intermediate [12-16 years], advanced [≥17 years]), and family history of prostate cancer (yes, no). Cigarette smoking status, BMI, statin use, diabetes, and CHD were treated as time-updated covariates; all other covariates were time-fixed. Time-updated covariates were also carried forward from the prior study visit when missing. When time-updated covariates were missing at Visit 1 or when time-fixed covariates were missing (0.2% missing for BMI, 0.2% for education, 2.0% for CHD, 7.4% for family history of prostate cancer, and 0.1% for diabetes), data were imputed using simple mean imputation.

For analyses of regular aspirin use and total, lethal, and fatal prostate cancer, only men who responded to the questions on regular aspirin use and who did not have a cancer history at

Visit 4 were included. Age was the time metric, and the time origin was age 55 (the lower bound of the age range of participants at Visit 4, when information on regular aspirin use was ascertained). HRs were calculated comparing regular aspirin use, dose of aspirin use (<300, 300-499, ≥500 mg), and indications for use (cardiovascular disease prevention, other) to no use. For these analyses, all aspirin variables and other covariates were treated as time-fixed and based on information collected at Visit 4.

For analyses of prostate cancer case-fatality, prostate cancer diagnosis was the time origin and time since diagnosis was the time metric. For these analyses, the exposures of interest were aspirin and NA-NSAID use at the visit prior to prostate cancer diagnosis. All covariates were time-fixed and included age, stage (T1, T2, T3, T4), and grade (low, moderate, high, missing) at diagnosis, race/center, years between the prior ARIC study visit and prostate cancer diagnosis (continuous), birth cohort, education, family history of prostate cancer, and smoking status, BMI, statin use, diabetes, and prevalent CHD from the visit prior to prostate cancer diagnosis.

To assess possible effect measure modification by race (white, black) and by frequency of routine physical examinations (≥ once every 5 years, < once every 5 years), analyses were repeated stratified by these variables. Statistical interaction was tested via the likelihood ratio test. To examine absolute differences in rates of each outcome among current aspirin and NA-NSAID users and non-users, incidence rates were calculated using Poisson regression, with age category (45-54, 55-64, 65-74, 75-85 years), race, and aspirin or NA-NSAID use as covariates and robust variance estimation.

Finally, several sensitivity analyses were conducted to verify the findings for current aspirin use and total, lethal, and fatal prostate cancer. First, because it was hypothesized that the influence of aspirin on cancer endpoints is not immediate, analyses were repeated with values for current aspirin use lagged one year. Aspirin use was only updated every three years and thus already lagged to some extent in the primary analysis, but this sensitivity analysis was

conducted to test whether additional lagging altered the findings. Second, because aspirin is often used concurrently with statins, and because statins have been associated with a reduced risk of total and advanced prostate cancer within ARIC (86) and other studies (87), analyses were repeated among non-statins users only. Too few men reported using both statins and aspirin to examine their joint effects. Third, to test the assumption that carrying forward the last observed value was an adequate approach for handling missing data on time-updated covariates, analyses were repeated with missing data imputed using multiple imputation by chained equations (MICE). Ten imputed datasets were derived, based on ten iterations each, with missing data predicted using all other covariates in this analysis. Finally, to examine the impact of current aspirin use on the cumulative incidence of each outcome, in the presence of competing events, subdistribution hazard ratios were calculated using Fine and Grey regression. Analyses were conducted in SAS Version 9.4 and R Version 3.4.

Results

There were 6,594 men who met study inclusion criteria. The mean age at Visit 1 was 54 years old. Seventy-seven percent of the study population was white; 23% was black. Of these men, 5,976 (91%) attended Visit 2, 5,339 (81%) attended visit 3, and 4,821 (73%) attended Visit 4.

At Visits 1, 2, 3, and 4, 29% 33%, 37%, and 44% of men reported current aspirin use, respectively, while 13%, 16%, 20%, and 23% of men reported current NA-NSAID use. Patterns in current aspirin and NA-NSAID use across study visit are displayed in Figure 2-2. At Visit 4, 37% of men reported regular aspirin use. Among regular aspirin users at Visit 4, 24% used baby aspirin (<300 mg), 68% used regular strength aspirin (300-499 mg) aspirin, and 4% used extra-strength aspirin (≥500 mg). Eighty percent of regular aspirin users reported using aspirin for CVD prevention, while 20% reported using aspirin for other indications.

Through the end of 2012, 817 total incident prostate cancers, including 97 lethal prostate cancers, were diagnosed. There were 90 deaths from prostate cancer.

Current Aspirin Use at Visits 1-4 and Total, Lethal, and Fatal Prostate Cancer

Compared to non-users at Visit 1, current aspirin users were more likely to be white (90% vs. 72%) and have prevalent CHD (17% vs. 4%). Current aspirin users were also slightly more likely to have an advanced degree (45% vs. 37%), prediabetes (44% vs. 38%), and go in for a routine physical exam at least once every five years (66% vs. 59%, Table 2-1).

After adjusting for potential confounders, there was no association between current aspirin use and total prostate cancer (HR: 1.05, 95% CI: 0.91-1.22). However, current aspirin use was inversely associated with both lethal prostate cancer (HR: 0.58, 95% CI: 0.35-0.95) and fatal prostate cancer (HR: 0.59, 95% CI: 0.36-0.96, Table 2-2). Results were consistent when current aspirin use was lagged by one year (Supplemental Table 2-1), when the analysis was restricted to non-users of statins (Supplemental Table 2-2), when missing data were imputed using multiple imputation (Supplemental Table 2-3) and when subdistribution hazard ratios were calculated (Supplemental Table 2-4).

In race-stratified analyses, the association for total prostate cancer remained null for white men, but appeared positive for black men (HR: 1.30, 95% CI: 0.98-1.72, *p*-interaction=0.13). For lethal prostate cancer, results were consistent across race (HR: 0.64, 95% CI: 0.36-1.14 for white men; HR: 0.45, 95% CI: 0.16-1.29 for black men, *p*-interaction=0.46). Similar results were observed for fatal prostate cancer (Table 2-3).

When stratified by frequency of routine physical exams, current aspirin use was associated with a borderline significant increased risk of prostate cancer among men who reported frequent routine physical exams (HR: 1.18, 95% CI: 0.98-1.41). In contrast, there was a non-statistically significant inverse association between current aspirin use and total prostate

cancer among men who reported infrequent routine physical exams (HR: 0.85, 95% CI: 0.65-1.10, *p*-interaction=0.07). Associations with lethal and fatal prostate cancer did not differ by routine physical exam frequency (Table 2-3).

For all age and race categories, the incidence rate for lethal prostate cancer was lower among current aspirin users compared to non-users (

Figure **2-3**). Incidence rate differences (IRDs) between current aspirin users and non-users were greater among black men than white men (among men age 65-74 years: IRD: -51 cases/100,000 person-years, 95% CI: -94- -8 for white men; IRD: -119 cases/100,000 person-years, 95% CI: -213- -24 for black men).

Regular Aspirin Use at Visit 4 and Total, Lethal, and Fatal Prostate Cancer

There were 4,527 men who attended Visit 4, completed the questionnaire items on regular aspirin use, and were still at risk for the outcomes of interest (i.e. not yet diagnosed with any cancer, Table 2-4). Among these men, 506 total incident prostate cancers, 38 lethal prostate cancers, and 36 fatal prostate cancers were observed.

Overall, regular aspirin use at Visit 4 was not statistically significantly associated with total (HR: 1.08, 95% CI: 0.89-1.32), lethal (HR: 0.95, 95% CI: 0.46-1.95), or fatal (HR: 0.81, 95% CI: 0.38-1.70) prostate cancer (Table 2-5). When looked at by indication for use, aspirin used regularly for CVD prevention was not associated with total prostate cancer (HR: 1.04, 95% CI: 0.84-1.29) but was possibly inversely associated with lethal prostate cancer (HR: 0.63, 95% CI: 0.27-1.50) and fatal prostate cancer (HR: 0.57, 95% CI: 0.24-1.37, Table 2-6). Aspirin used regularly for other indications was non-significantly positively associated with all three outcomes. When looked at by dose of aspirin use, regular use of extra-strength aspirin was positively associated with total prostate cancer (HR: 1.84, 95% CI: 1.03-3.30, Table 2-7). For

lethal and fatal prostate cancer, low-dose and regular strength aspirin appeared to be associated with a reduced risk, while extra-strength aspirin appeared harmful.

Current NA-NSAID Use at Visits 1-4 and Total, Lethal, and Fatal Prostate Cancer

Men who reported current NA-NSAID use at Visit 1 were slightly older than non-users (55.0 vs. 54.4 years) and more likely to be white (82% vs. 76%, Table 2-8). Current NA-NSAID users were also slightly more likely to have prevalent CHD (10% vs. 8%) and diagnosed diabetes (9% vs. 7%). Current NA-NSAID users were more likely to go in for a routine physical examination at least once every five years (69% vs. 60%).

In both age-adjusted and multivariable-adjusted models, there was no association between current NA-NSAID use and total, lethal, or fatal prostate cancer (Table 2-9). For total prostate cancer, the multivariable-adjusted HR was 1.16 (95%: CI 0.98-1.37). For lethal prostate cancer, the multivariable-adjusted HR was 0.85 (95% CI: 0.49-1.45), and for fatal prostate cancer, the multivariable-adjusted HR was 1.03 (95% CI: 0.62-1.71). No effect modification by race or frequency of routine physical exams was observed (Table 2-10).

Aspirin and NA-NSAID Use and Prostate Cancer Case-Fatality

There were 676 men included in case-fatality analyses. Of these men, 6% were diagnosed with prostate cancer between Visit 1 and Visit 2, 9% were diagnosed between Visit 2 and Visit 3, 16% were diagnosed between Visit 3 and Visit 4, and 69% were diagnosed after Visit 4. The mean age at diagnosis was 69 years, 82% were diagnosed with early stage disease (≤T2), and 69% were diagnosed with low or moderate grade disease (Gleason sum ≤7). Thirty-nine percent of men reported using aspirin and 22% reported using NA-NSAIDs at the visit prior to diagnosis. Other demographic and clinical characteristics, by aspirin and NA-NSAID use, are

described in Table 2-11. Within this group of men, 65 deaths from prostate cancer were observed.

After multivariable adjustment, current aspirin use at the visit prior to diagnosis was inversely associated with prostate cancer case-fatality (HR: 0.45, 95% CI: 0.22-0.94). NA-NSAID use was possibly inversely associated with case-fatality, but results were non-significant (HR: 0.69, 95% CI: 0.28-1.65).

Discussion

In this study population, current aspirin use was inversely associated with lethal and fatal prostate cancer, but not total prostate cancer. Inverse associations for regular aspirin use were only apparent for aspirin used for CVD prevention. Current aspirin use at the visit prior to diagnosis was also inversely associated with prostate cancer case-fatality. Current NA-NSAID use was not statistically significantly associated with any of the endpoints of interest.

For current aspirin use, the magnitude of the associations with lethal and fatal prostate cancer were fairly large, with current aspirin users exhibiting a 42% reduced risk of lethal prostate cancer and 41% reduced risk of fatal prostate cancer relative to non-users. These inverse associations did not appear to be due to concurrent use of statins, as exclusion of current statin users did not affect the results. Two previous cohort studies also observed inverse associations between aspirin use and lethal prostate cancer: in the Health Professionals Follow-up Study, the HR for lethal prostate cancer comparing current aspirin use to non-use was 0.84 (95% CI: 0.69-1.02) (43), while in the Physicians' Health Study, in which aspirin use was initially randomized and then offered to all participants after trial completion, the HR was 0.68 (95% CI: 0.52-0.89) (44). Both of these studies included primarily white men. Importantly, our study extends these findings to black men, who are more likely than other racial/ethnic groups to develop and die from prostate cancer (1). In this study, we observed similar relative

associations for lethal and fatal prostate cancer across race, but a much greater association on the absolute scale among black men. Given that black men are less likely than white men to use aspirin, both in this study and in others (88, 89), encouraging guideline-concordant use of aspirin among black men may have the potential to help lower lethal prostate cancer rates among black men and attenuate the current racial disparity, if the association between aspirin use and lethal prostate cancer does prove to be causal.

In analyses of regular aspirin use reported at Visit 4, inverse associations with lethal and fatal prostate cancer did not hold. It is unclear why these associations became null; there may have been loss of precision, due to the limited follow-up time after Visit 4 and the small number of events, or unknown sources of bias. However, compared to non-use, regular use of aspirin for CVD prevention and regular use of low- or standard-dose aspirin were possibly inversely associated with lethal and fatal prostate cancer. Though confidence intervals in these analyses were wide, these patterns indicate that the effect of aspirin on lethal prostate cancer may vary by dose or indication, a finding that warrants further investigation.

At first glance, our null results for current aspirin use and total prostate cancer appear inconsistent with the prior literature, which has most often reported slight but significant inverse associations (42). However, our overall null results did not account for detection bias, i.e. bias resulting from the fact that NSAID users may be more health-conscious, or in greater contact with the healthcare system, and thus more likely to be screened for prostate cancer. Prior studies have shown that regular aspirin users are more likely to undergo prostate biopsy (14), and that accounting for detection bias leads to stronger inverse associations between aspirin use and total prostate cancer (42), and so detection bias in our study was likely. While our study was not able to adjust for prostate cancer screening history directly, we were able to stratify by frequency of routine visits to the doctor. We assumed that detection bias would be most evident among men who frequently visited the doctor for routine examinations, and who thus had greater opportunity to undergo prostate cancer screening, and minimal among men who

infrequently visited the doctor for routine examinations. Of note, the association among men who frequently visited the doctor was positive, consistent with the expected effect of detection bias, while the association among men who infrequently visited the doctor for physical examinations was moderately inverse. Though not statistically significant, the inverse association for men who infrequently visited their doctor for routine physical examinations is most consistent with prior studies and suggests that aspirin may protect against development of total prostate cancer, once the influence of detection bias is reduced.

Current aspirin use at the visit prior to prostate cancer diagnosis was also associated with a 57% reduction in case-fatality. The inverse association remained despite adjustment for stage and grade at diagnosis, implying that the association was not simply due to earlier detection and increased lag time among aspirin users. This finding conflicts with the prior literature, which has consistently reported null associations between pre-diagnostic aspirin use and prostate cancer case-fatality (46-50). Our results may differ due to differing time intervals between measurement of aspirin use and prostate cancer diagnosis (median=5.7 years in our study vs. 1-3 years in the previous studies, with the exception of the study by Zhou et al. (50)), or differing study time frames (our study period overlapped both the pre-PSA and PSA era, while all previous studies were conducted solely in the PSA era). It is possible that aspirin is only beneficial earlier in the natural history of prostate cancer, several years before diagnosis, or that the predominance of indolent prostate cancer in the PSA era has obscured associations between pre-diagnostic aspirin use and survival among those with more aggressive disease. There could also be unmeasured confounding in our study by access to or receipt of treatment, a determinant of prostate cancer mortality that could potentially differ by aspirin use. Finally, our case-fatality analysis was limited by a small number of events, and further research is needed to explore whether our results were spurious, confounded, or indicative of a true causal relationship between pre-diagnostic aspirin use and prostate cancer death.

Current NA-NSAID use did not appear to be associated with total, lethal, or fatal prostate cancer or with prostate cancer case-fatality in this study population. The null associations for NA-NSAIDs, in contrast to the inverse associations for aspirin and lethal and fatal prostate cancer, could have several potential explanations. First, there may have been inadequate power to observe associations for NA-NSAID use, as there were fewer NA-NSAID users than aspirin users and thus fewer events among the NA-NSAID exposed. The group of NA-NSAID users was also likely a heterogeneous group, consisting of men using NA-NSAIDs for different indications and for different lengths of time. The mixing of regular users and sporadic users, including men using NA-NSAIDs short-term to alleviate acute pain, muscle pain, aches and pain due to colds, or headaches, could have biased results towards the null, as short-term use is unlikely to influence cancer outcomes. Finally, the discordant findings for aspirin and NA-NSAIDs could indicate that any chemopreventive effects of aspirin against lethal prostate cancer are due to an aspirin-specific biological mechanism. For example, unlike other NSAIDs, aspirin has a prolonged inhibitory effect on platelets, which are thought to facilitate metastases through the bloodstream (30). If platelet inhibition is the primary mechanism of action through which aspirin protects against lethal prostate cancer, then similar effects for NA-NSAIDs would not be expected. Additional studies with greater power and detailed assessment of frequency and indication for NA-NSAID use are needed to rule out the first two explanations and provide support for or against the third.

Aside from small sample size, the primary limitation of this study was the potential for misclassification of aspirin and NA-NSAID use. First, men were asked to self-report medication use by bringing bottles of all current medications to each study visit; failure to bring in all bottles could have resulted in underreporting of use. Second, medication use was assessed only at each study visit, and it was assumed that medication use remained constant during the three-year interval between visits, or indefinitely after Visit 4. Third, though the effect of regular, long-term use of aspirin and NA-NSAIDs was of most interest in this study, our definitions of current

aspirin and NA-NSAID use likely captured both short-term and long-term users. For aspirin, current use at Visit 4 was highly concordant with self-reported regular use at Visit 4, but concordance between current and regular NA-NSAID use was unknown. Fourth, additional information on dose, frequency of use, indication, and lifetime cumulative duration of use would have been informative, but these data were only available for aspirin at Visit 4. Finally, we did not have information on whether participants used aspirin or NA-NSAIDs prior to ARIC enrollment. Other limitations include the small number of lethal and fatal events, which limited power to detect more moderate effects and effects in stratified analyses, and the collinearity of race and US residence in this cohort, which limited our ability to tease apart the influence of race and geography.

Despite this collinearity, the racial and geographic diversity of the cohort was a major study strength that improves generalizability of the results. There was also extremely thorough and time-updated assessment of potential CVD-related confounders. For example, body weight and height were measured at each study visit instead of self-reported, and diabetes status was assessed using a combination of self-report and markers of glycemia. Given that aspirin and NA-NSAIDs are used for specific indications and that these indications may share risk factors with total, lethal, or fatal prostate cancer, careful and complete adjustment for these risk factors is necessary to minimize confounding.

In conclusion, this prospective, community-based study of white and black men provides evidence that aspirin may protect against development and progression of lethal prostate cancer. Additional studies are needed to build support for a causal relationship, and to continue to explore the influence of dose, duration, and timing of aspirin use. If confirmed, benefits of aspirin pertaining to lethal prostate cancer could eventually be incorporated into clinical guidelines or factored into individual risk-benefit calculations of men considering starting an aspirin regimen.

Grant Support and Acknowledgments

The Atherosclerosis Risk in Communities study has been funded in whole or in part with federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, and Department of Health and Human Services, under the following contract numbers: HHSN268201700001I, HHSN268201700003I, HHSN268201700005I, HHSN268201700004I, HHSN268201700002I. Studies on cancer in Atherosclerosis Risk in Communities (ARIC) are also supported by the NCI (U01 CA164975). This research was additionally supported by an NCI Cancer Center Support Grant (P30 CA006973). Lauren Hurwitz was supported by an NCI National Research Service Award (T32 CA09314). The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Cancer incidence data have been provided by the Maryland Cancer Registry, Center for Cancer Surveillance and Control, Maryland Department of Health, 201 W. Preston Street, Room 400, Baltimore, MD 21201. We acknowledge the State of Maryland, the Maryland Cigarette Restitution Fund, and the National Program of Cancer Registries of the Centers for Disease Control and Prevention for the funds that helped support the availability of the cancer registry data.

Thank you to the collaborators from ARIC-Cancer and JHU who contributed to this work. Thank you also to the staff and participants of the ARIC study for their important contributions.

Chapter 2 Tables

Table 2-1. Baseline (1987-1989) demographic and clinical characteristics of 6,594 men in the ARIC study, by current aspirin use

	Current Aspirin Use at Visit 1	
	No	**Yes**
N	4743	1851
Age, mean (sd)	54.3 (5.8)	54.9 (5.6)
BMI, mean (sd)	27.4 (4.2)	27.8 (4.3)
Race, n (%)		
White	3403 (72)	1675 (90)
Black	1340 (28)	194 (10)
Center, n (%)		
Forsyth	1207 (25)	524 (28)
Jackson	1188 (25)	153 (8)
Minneapolis	1136 (24)	666 (36)
Washington County	1212 (26)	508 (27)
Cigarette Smoking Status, n (%)		
Current/Recent (quit <10 years ago)	2084 (44)	810 (44)
Former (quit ≥10 years ago)	1285 (27)	572 (31)
Never	1374 (29)	469 (25)
Education, n (%)		
Basic	1242 (26)	356 (19)
Intermediate	1734 (37)	659 (36)
Advanced	1756 (37)	834 (45)
Missing	11 (0)	2 (0)
Family History of PCa*, n (%)		
Yes	275 (6)	100 (5)
No	4121 (87)	1608 (87)
Missing	347 (7)	143 (8)
Statin Use, n (%)**		
Yes	17 (0)	20 (1)
No	4726 (100)	1831 (99)
Prevalent CHD, n (%)		
Yes	209 (4)	322 (17)
No	4431 (93)	1501 (81)
Missing	103 (2)	28 (2)
Diabetes, n (%)		
Diagnosed diabetes	342 (7)	138 (7)
Undiagnosed diabetes	213 (4)	80 (4)

Prediabetes	1790 (38)	809 (44)
No diabetes	2391 (50)	824 (45)
Missing		
Frequency of Routine Physical Examinations		
≥Once every 5 years	2779 (59)	1230 (66)
<Once every 5 years	1949 (41)	620 (34)
Missing	15 (0)	1 (0)

sd, standard deviation; BMI, body mass index; PCa, prostate cancer; CHD, coronary heart disease

*Reported at Visit 3

**Statin use is low at baseline because the first statin was not FDA approved until 1987. For a better comparison of statin use by aspirin use, see Table 2-4.

Table 2-2. Associations between current aspirin use and total, lethal, and fatal prostate cancer among 6,594 men in the ARIC study, 1987-2012

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No Aspirin	503 / 75897	1.00	--	1.00	--
Aspirin	314 / 43478	0.96	(0.84, 1.11)	1.05	(0.91, 1.22)
Lethal Prostate Cancer					
No Aspirin	74 / 76025	1.00	--	1.00	--
Aspirin	23 / 43572	0.48	(0.30, 0.77)	0.58	(0.35, 0.95)
Fatal Prostate Cancer					
No Aspirin	65 / 84565	1.00	--	1.00	--
Aspirin	25 / 48951	0.52	(0.33, 0.82)	0.59	(0.36, 0.96)

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), current statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

Table 2-3. Associations between current aspirin use and total, lethal, and fatal prostate cancer among 6,594 men in the ARIC study, 1987-2012, stratified by race and frequency of routine physical examinations

	White Men					Black Men					*p*-value*
		Age-Adjusted		Multivariable-Adjusted**			Age-Adjusted		Multivariable-Adjusted**		
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	
Total Prostate Cancer											
No Aspirin	316 / 55777	1.00	--	1.00	--	187 / 20120	1.00	--	1.00	--	
Aspirin	243 / 38271	1.00	(0.85, 1.18)	0.97	(0.82, 1.16)	71 / 5207	1.28	(0.97, 1.68)	1.30	(0.98, 1.72)	0.13
Lethal Prostate Cancer											
No Aspirin	40 / 55898	1.00	--	1.00	--	34 / 20127	1.00	--	1.00	--	
Aspirin	19 / 38365	0.62	(0.36, 1.07)	0.64	(0.36, 1.14)	4 / 5207	0.40	(0.14, 1.12)	0.45	(0.16, 1.29)	0.46
Fatal Prostate Cancer											
No Aspirin	36 / 62036	1.00	--	1.00	--	29 / 22529	1.00	--	1.00	--	
Aspirin	21 / 42823	0.66	(0.39, 1.14)	0.67	(0.38, 1.19)	4 / 6127	0.39	(0.14, 1.10)	0.41	(0.14, 1.20)	0.32

	Frequent Routine Physical Examinations***					Infrequent Routine Physical Examinations***					*p*-value*
		Age-Adjusted		Multivariable-Adjusted**			Age-Adjusted		Multivariable-Adjusted**		
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	
Total Prostate Cancer											
No Aspirin	298 / 43895	1.00	--	1.00	--	205 / 31730	1.00	--	1.00	--	
Aspirin	223 / 27806	1.06	(0.89, 1.26)	1.18	(0.98, 1.41)	91 / 15643	0.79	(0.62, 1.02)	0.85	(0.65, 1.10)	0.07
Lethal Prostate Cancer											
No Aspirin	41 / 43974	1.00	--	1.00	--	33 / 31778	1.00	--	1.00	--	
Aspirin	14 / 27856	0.48	(0.26, 0.88)	0.56	(0.30, 1.06)	9 / 15686	0.50	(0.24, 1.04)	0.64	(0.30, 1.39)	0.95

Fatal Prostate Cancer											
No Aspirin	38 / 49264	1.00	--	1.00	--	27 / 35010	1.00	--	1.00	--	
Aspirin	14 / 31508	0.46	(0.25, 0.86)	0.49	(0.26, 0.94)	11 / 17412	0.62	(0.31, 1.26)	0.78	(0.37, 1.63)	0.49

[*] *p*-value is from the likelihood ratio test comparing the multivariable model with vs. without an interaction term between aspirin use and race/frequency of routine physical exams

[**] Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), current statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

[***] Frequent routine physical examination defined as an examination at least once every 5 years, infrequent routine physical examination defined as an examination less that once every 5 years or no routine physical

Table 2-4. Visit 4 (1996-1998) demographic and clinical characteristics of 4,527 men in the ARIC study, by regular aspirin use

	Current Aspirin Use at Visit 4	
	No	**Yes**
N	2852	1675
Age, mean (sd)	62.4 (5.7)	63.8 (5.6)
BMI, mean (sd)	28.4 (4.6)	28.6 (4.4)
Race, n (%)		
White	2188 (77)	1496 (89)
Black	664 (23)	179 (11)
Center, n (%)		
Forsyth	678 (24)	435 (26)
Jackson	601 (21)	163 (10)
Minneapolis	778 (27)	582 (35)
Washington County	795 (28)	495 (30)
Cigarette Smoking Status, n (%)		
Current/Recent (quit <10 years ago)	838 (29)	454 (27)
Former (quit ≥10 years ago)	1107 (39)	731 (44)
Never	907 (32)	490 (29)
Education, n (%)		
Basic	589 (21)	284 (17)
Intermediate	1073 (38)	611 (36)
Advanced	1183 (41)	777 (46)
Missing	7 (0)	3 (0)
Family History of PCa*, n (%)		
Yes	183 (6)	113 (7)
No	2664 (93)	1556 (93)
Missing	5 (0)	6 (4)
Statin Use, n (%)		
Yes	186 (7)	399 (24)
No	2666 (93)	1276 (76)
Prevalent CHD, n (%)		
Yes	101 (4)	363 (22)
No	2696 (95)	1284 (77)
Missing	55 (2)	28 (2)
Diabetes, n (%)		
Diagnosed diabetes	329 (12)	288 (17)
Undiagnosed diabetes	149 (5)	82 (5)
Prediabetes	1131 (40)	650 (39)
No diabetes	1243 (44)	655 (39)

Frequency of Routine Physical Exams		
≥ Once every 5 years	1656 (58)	1077 (64)
< Once every 5 years	1190 (42)	597 (36)
Missing	6 (0)	1 (0)

sd, standard deviation; BMI, body mass index; PCa, prostate cancer; CHD, coronary heart disease

*Reported at Visit 3

Table 2-5. Associations between regular aspirin use at Visit 4 and total, lethal, and fatal prostate cancer among 4,527 men in the ARIC study, 1996-2012

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No Aspirin	321 / 34363	1.00	--	1.00	--
Aspirin	185 / 19097	1.03	(0.86, 1.23)	1.09	(0.89, 1.32)
Lethal Prostate Cancer					
No Aspirin	24 / 34410	1.00	--	1.00	--
Aspirin	14 / 19119	1.01	(0.52, 1.96)	0.95	(0.46, 1.95)
Fatal Prostate Cancer					
No Aspirin	23 / 38667	1.00	--	1.00	--
Aspirin	13 / 21663	0.86	(0.44, 1.70)	0.81	(0.38, 1.70)

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status at Visit 4 (current/recent, former, never), BMI at Visit 4 (continuous), statin use at Visit 4 (yes, no), diabetes at Visit 4 (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD at Visit 4 (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

Table 2-6. Associations between indication for regular aspirin use at Visit 4 and total, lethal, and fatal prostate cancer among 4,527 men in the ARIC study, 1996-2012

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No Aspirin	321 / 34363	1.00	--	1.00	--
Aspirin for CVD prevention	142 / 15101	1.00	(0.82, 1.21)	1.04	(0.84, 1.29)
Aspirin for other indication	43 / 3925	1.16	(0.84, 1.60)	1.25	(0.91, 1.73)
Lethal Prostate Cancer					
No Aspirin	24 / 34410	1.00	--	1.00	--
Aspirin for CVD prevention	8 / 15111	0.73	(0.33, 1.62)	0.63	(0.27, 1.50)
Aspirin for other indication	6 / 3938	2.11	(0.86, 5.15)	2.31	(0.92, 5.80)
Fatal Prostate Cancer					
No Aspirin	23 / 38667	1.00	--	1.00	--
Aspirin for CVD prevention	8 / 17140	0.65	(0.29, 1.56)	0.57	(0.24, 1.37)
Aspirin for other indication	5 / 4452	1.76	(0.67, 4.63)	1.83	(0.68, 4.89)

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status at Visit 4 (current/recent, former, never), BMI at Visit 4 (continuous), statin use at Visit 4 (yes, no), diabetes at Visit 4 (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD at Visit 4 (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

5 aspirin users of unknown indication were excluded

Table 2-7. Associations between dose of regular aspirin use at Visit 4 and total, lethal, and fatal prostate cancer among 4,527 men in the ARIC study, 1996-2012

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No Aspirin	321 / 34363	1.00	--	1.00	--
Baby Aspirin	47 / 4499	1.10	(0.81, 1.49)	1.15	(0.84, 1.58)
Regular Strength	120 / 13170	0.97	(0.78, 1.19)	1.03	(0.82, 1.29)
Extra Strength Aspirin	12 / 800	1.58	(0.89, 2.82)	1.85	(1.04, 3.32)
Lethal Prostate Cancer					
No Aspirin	24 / 34410	1.00	--	1.00	--
Baby Aspirin	3 / 4501	0.91	(0.28, 3.04)	0.78	(0.22, 2.71)
Regular Strength	8 / 13191	0.83	(0.37, 1.85)	0.79	(0.34, 1.87)
Extra Strength Aspirin	2 / 800	3.50	(0.83, 14.84)	3.86	(0.86, 17.20)
Fatal Prostate Cancer					
No Aspirin	23 / 38667	1.00	--	1.00	--
Baby Aspirin	2 / 5211	0.56	(0.13, 2.37)	0.49	(0.11, 2.17)
Regular Strength	8 / 14834	0.75	(0.34, 1.69)	0.71	(0.30, 1.71)
Extra Strength Aspirin	2 / 922	3.33	(0.78, 14.10)	3.38	(0.75, 15.28)

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status at Visit 4 (current/recent, former, never), BMI at Visit 4 (continuous), statin use at Visit 4 (yes, no), diabetes at Visit 4 (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD at Visit 4 (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

Baby aspirin = <300mg, regular aspirin = 300-499mg, extra strength aspirin = ≥500mg

50 aspirin users of unknown dose were excluded

Table 2-8. Baseline (1987-1989) demographic and clinical characteristics of 6,594 men in the ARIC study, by current NA-NSAID use

	Current NA-NSAID Use at Visit 1	
	No	**Yes**
N	5718	876
Age, mean (sd)	54.4 (5.8)	55.0 (5.8)
BMI, mean (sd)	27.4 (4.2)	28.4 (4.6)
Race, n (%)		
White	4340 (76)	720 (82)
Black	1378 (24)	156 (18)
Center, n (%)		
Forsyth	1477 (26)	254 (29)
Jackson	1459 (26)	261 (30)
Minneapolis	1219 (21)	122 (14)
Washington County	1563 (27)	239 (27)
Cigarette Smoking Status, n (%)		
Current/Recent (quit <10 years ago)	2523 (44)	371 (42)
Former (quit ≥10 years ago)	1570 (27)	287 (33)
Never	1625 (28)	218 (25)
Education, n (%)		
Basic	1371 (24)	277 (26)
Intermediate	2059 (36)	334 (38)
Advanced	2275 (40)	315 (36)
Missing	13 (0)	0 (0)
Family History of PCa*, n (%)		
Yes	319 (6)	56 (6)
No	4977 (87)	752 (86)
Missing	422 (7)	68 (8)
Aspirin Use, n (%)		
Yes	1623 (28)	228 (26)
No	4095 (72)	648 (74)
Statin Use, n (%)**		
Yes	29 (1)	8 (1)
No	5689 (99)	868 (99)
Prevalent CHD, n (%)		
Yes	441 (8)	90 (10)
No	5163 (90)	769 (88)
Missing	114 (2)	17 (2)
Diabetes, n (%)		
Diagnosed diabetes	401 (7)	79 (9)

Undiagnosed diabetes	245 (4)	48 (5)
Prediabetes	2246 (39)	353 (40)
No diabetes	2822 (49)	393 (45)
Missing	4 (0)	3 (0)
Frequency of Routine Physical Exams		
≥ Once every 5 years	3405 (60)	604 (69)
< Once every 5 years	2299 (40)	270 (31)
Missing	14 (0)	2 (0)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug; sd, standard deviation; BMI, body mass index; PCa, prostate cancer; CHD, coronary heart disease

*Reported at Visit 3

**Statin use is low at baseline because the first statin was not FDA approved until 1987

Table 2-9. Associations between current NA-NSAID use and total, lethal, and fatal prostate cancer among 6,594 men in the ARIC study, 1987-2012

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No NA-NSAID	633 / 96626	1.00	--	1.00	--
NA-NSAID	184 / 22749	1.15	(0.98, 1.36)	1.16	(0.98, 1.37)
Lethal Prostate Cancer					
No NA-NSAID	81 / 96799	1.00	--	1.00	--
NA-NSAID	16 / 22797	0.79	(0.46, 1.34)	0.85	(0.49, 1.45)
Fatal Prostate Cancer					
No NA-NSAID	71 / 107724	1.00	--	1.00	--
NA-NSAID	19 / 25792	1.02	(0.62, 1.70)	1.02	(0.62, 1.71)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), current statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

Table 2-10. Associations between current NA-NSAID use and total, lethal, and fatal prostate cancer among 6,594 men in the ARIC study, 1987-2012, stratified by race and frequency of routine physical examinations

	White Men					Black Men					*p*-value*
		Age-Adjusted		Multivariable-Adjusted**			Age-Adjusted		Multivariable-Adjusted**		
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	
Total Prostate Cancer											
No NA-NSAID	428 / 75450	1.00	--	1.00	--	205 / 21176	1.00	--	1.00	--	
NA-NSAID	131 / 18597	1.18	(0.97, 1.44)	1.17	(0.96, 1.43)	53 / 4151	1.15	(0.85, 1.55)	1.13	(0.83, 1.54)	0.81
Lethal Prostate Cancer											
No NA-NSAID	50 / 75617	1.00	--	1.00	--	31 / 21182	1.00	--	1.00	--	
NA-NSAID	9 / 18646	0.70	(0.35, 1.43)	0.74	(0.36, 1.52)	7 / 4151	0.99	(0.44, 2.25)	1.14	(0.50, 2.62)	0.66
Fatal Prostate Cancer											
No NA-NSAID	47 / 83945	1.00	--	1.00	--	24 / 23780	1.00	--	1.00	--	
NA-NSAID	10 / 20915	0.82	(0.41, 1.61)	0.79	(0.40, 1.58)	9 / 4877	1.43	(0.66, 3.09)	1.71	(0.78, 3.76)	0.33

	Frequent Routine Physical Examinations***					Infrequent Routine Physical Examinations***					*p*-value*
		Age-Adjusted		Multivariable-Adjusted**			Age-Adjusted		Multivariable-Adjusted**		
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	
Total Prostate Cancer											
No NA-NSAID	397 / 57433	1.00	--	1.00	--	236 / 39193	1.00	--	1.00	--	
NA-NSAID	124 / 14569	1.17	(0.96, 1.43)	1.16	(0.94, 1.42)	60 / 8179	1.12	(0.84, 1.48)	1.20	(0.90, 1.60)	0.95
Lethal Prostate Cancer											
No NA-NSAID	44 / 57542	1.00	--	1.00	--	37 / 39257	1.00	--	1.00	--	
NA-NSAID	11 / 14590	0.94	(0.48, 1.81)	1.03	(0.53, 2.01)	5 / 8208	0.60	(0.23, 1.52)	0.69	(0.27, 1.76)	0.39

Fatal Prostate Cancer											
No NA-NSAID	41 / 64362	1.00	--	1.00	--	30 / 43362	1.00	--	1.00	--	
NA-NSAID	11 / 16732	0.97	(0.50, 1.89)	0.98	(0.50, 1.92)	8 / 9060	1.13	(0.52, 2.46)	1.16	(0.52, 2.57)	0.90

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

[*] *p*-value is from the likelihood ratio test comparing the multivariable model with vs. without an interaction term between NA-NSAID use and race/frequency of routine physical exams

[**] Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), current statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

[***] Frequent routine physical examination defined as an examination at least once every 5 years, infrequent routine physical examination defined as an examination less that once every 5 years or no routine physical

Table 2-11. Demographic and clinical characteristics of 676 men diagnosed with prostate cancer in the ARIC study, 1987-2012, by current aspirin and NA-NSAID use at the visit prior to diagnosis

	Current Aspirin Use		Current NA-NSAID Use	
	No	Yes	No	Yes
N	411	265	530	146
Age at Diagnosis, mean (sd)	69.0 (6.2)	69.4 (6.5)	69.0 (6.2)	69.6 (6.5)
BMI, mean (sd)	28.0 (4.3)	28.5 (4.1)	28.1 (4.4)	28.4 (3.7)
Race, n (%)				
White	294 (72)	223 (84)	395 (76)	122 (84)
Black	117 (28)	42 (16)	135 (25)	24 (16)
Center, n (%)				
Forsyth	123 (30)	66 (25)	143 (27)	46 (32)
Jackson	97 (24)	39 (15)	115 (22)	21 (14)
Minneapolis	99 (24)	79 (30)	133 (25)	45 (31)
Washington County	92 (22)	81 (31)	139 (26)	34 (23)
Cigarette Smoking Status, n (%)				
Current/Recent (quit <10 years ago)	123 (30)	62 (23)	151 (28)	34 (23)
Former (quit ≥10 years ago)	154 (37)	115 (43)	205 (39)	64 (44)
Never	134 (33)	88 (33)	174 (33)	48 (33)
Education, n (%)				
Basic	88 (21)	43 (16)	104 (20)	27 (18)
Intermediate	159 (39)	94 (35)	202 (38)	51 (35)
Advanced	164 (40)	128 (48)	224 (42)	68 (47)
Family History of PCa, n (%)				
Yes	47 (11)	32 (12)	57 (11)	22 (15)
No	357 (87)	229 (86)	462 (87)	124 (85)
Missing	7 (2)	4 (2)	11 (2)	0 (0)
Statin Use, n (%)				
Yes	17 (4)	47 (18)	48 (9)	16 (11)
No	394 (96)	218 (82)	482 (91)	130 (89)
Prevalent CHD, n (%)				
Yes	11 (3)	51 (19)	55 (10)	7 (5)
No	390 (95)	209 (79)	464 (88)	135 (92)
Missing	10 (2)	5 (2)	11 (2)	4 (3)
Diabetes, n (%)				
No diabetes	178 (43)	114 (43)	232 (44)	60 (41)
Diagnosed diabetes	32 (8)	31 (12)	51 (10)	12 (8)
Undiagnosed diabetes	17 (4)	20 (8)	31 (6)	6 (4)
Prediabetes	184 (45)	100 (38)	216 (41)	68 (47)

Frequency of Routine Physical Exams				
≥ Once every five years	245 (60)	185 (70)	334 (63)	96 (66)
< Once every five years	166 (40)	80 (30)	196 (37)	50 (34)
Cancer Stage (Clinical or Pathologic)				
T1	22 (5)	10 (4)	23 (4)	9 (6)
T2 or SEER Summary Stage 1	314 (76)	208 (78)	405 (76)	117 (80)
T3 or SEER Summary Stage 2	47 (11)	36 (14)	67 (13)	16 (11)
T4 or SEER Summary Stage 3, 4, or 7	28 (7)	11 (4)	35 (7)	4 (3)
Cancer Grade at Diagnosis				
Low	96 (23)	49 (18)	112 (21)	33 (23)
Moderate	187 (46)	135 (51)	245 (46)	77 (53)
High	109 (27)	74 (28)	149 (28)	34 (23)
Missing	19 (5)	7 (3)	24 (5)	2 (1)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug; sd, standard deviation; BMI, body mass index; PCa, prostate cancer; CHD, coronary heart disease; SEER, Surveillance, Epidemiology, and End Results

Table 2-12. Associations between current aspirin and NA-NSAID use at the visit prior to diagnosis and prostate cancer case-fatality among 676 men diagnosed with prostate cancer in the ARIC study, 1987-2012

		Age-Adjusted		Multivariable-Adjusted*		Multivariable-Adjusted**	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Aspirin Use							
No	49 / 3417	1.00	--	1.00	--	1.00	--
Yes	16 / 2275	0.49	(0.28, 0.86)	0.55	(0.29, 1.05)	0.45	(0.22, 0.94)
NA-NSAID Use							
No	57 / 4535	1.00	--	1.00	--	1.00	--
Yes	8 / 1157	0.55	(0.26, 1.16)	0.76	(0.33, 1.72)	0.69	(0.28, 1.65)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for age at diagnosis, stage (T1, T2, T3, T4), grade (low, moderate, high, missing), race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), and years between the prior ARIC study visit and diagnosis

**Adjusted for age at diagnosis, stage (T1, T2, T3, T4), grade (low, moderate, high, missing), race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), years between the prior ARIC study visit and diagnosis, birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), current statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

Chapter 2 Figures

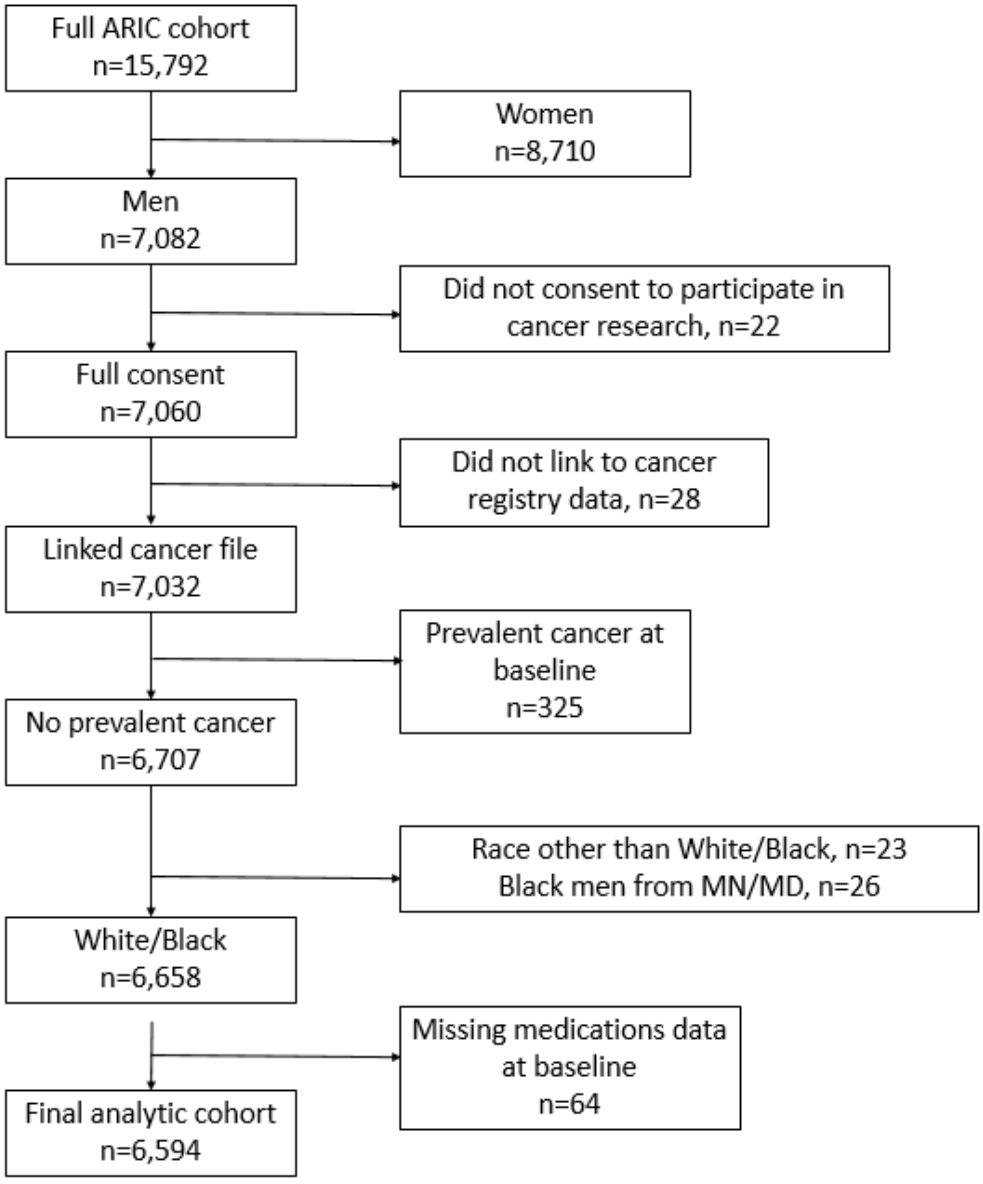


Figure 2-1. Study Inclusion Criteria. The primary analysis was restricted to men from the ARIC study without a prevalent cancer at baseline and with non-missing data on medication use at baseline. Individuals identifying as a race other than white or black, and black men from the MN/MD field centers were also excluded due to the small number of individuals in these groups.

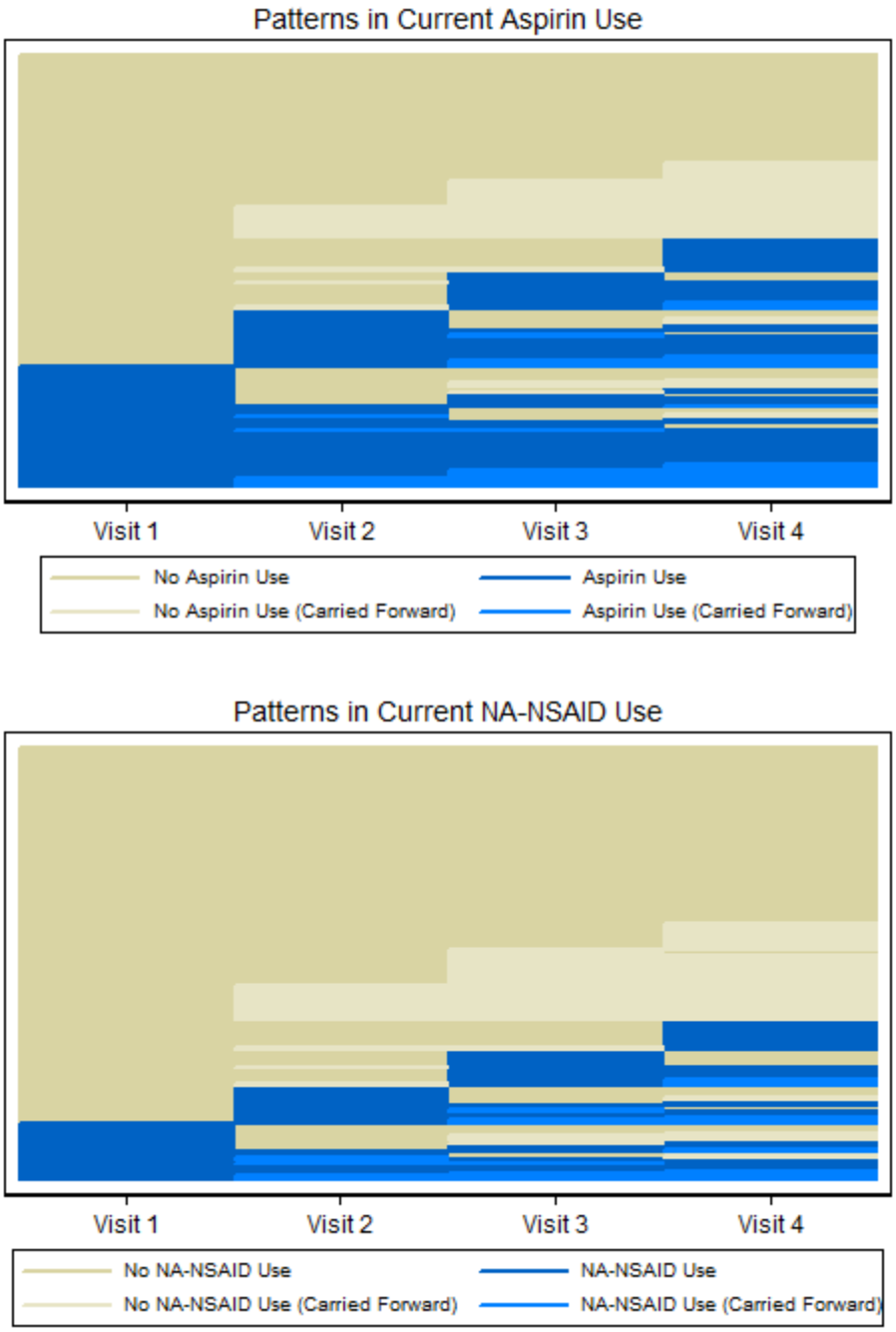


Figure 2-2. Patterns in Current Aspirin and NA-NSAID Use. Patterns in aspirin and NA-NSAID use across the four study visits are shown in the lasagna plots above. When aspirin or NA-NSAID use was unknown, values were carried forward from the previous visit (as indicated by the lighter shading). Overall, there were more current aspirin than NA-NSAID users at each study visit. Once initiating aspirin use, men were more likely to continue using aspirin for the remainder of the study period. NA-NSAID use appeared slightly more irregular.

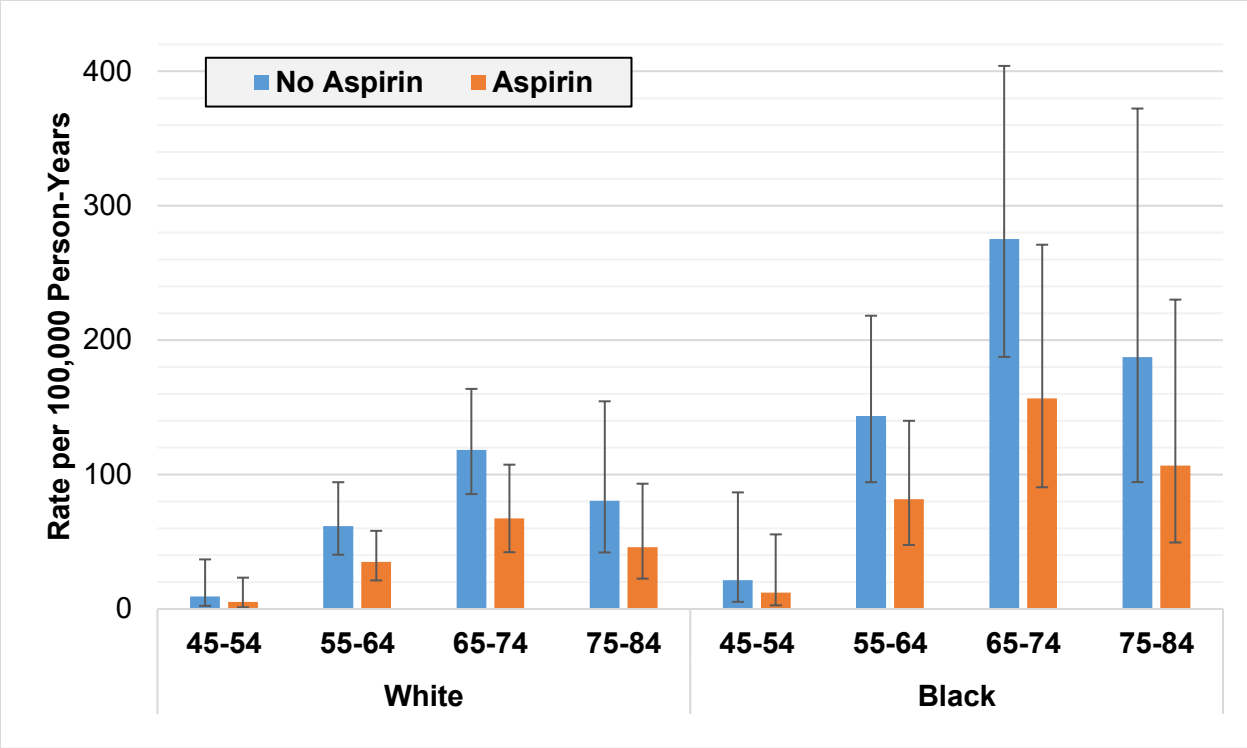


Figure 2-3. Incidence Rates for Lethal Prostate Cancer for Men in the ARIC Study, 1987-2012, by Age, Race, and Current Aspirin Use. For all age and race categories, incidence rates for lethal prostate cancer were lower among current aspirin users as compared to non-users. However, absolute differences in incidence rates between aspirin and non-aspirin users were more pronounced for black men. For example, for white men ages 65-74, the difference in incidence rates of lethal prostate cancer between aspirin users and non-users was -51 cases per 100,000 person-years (95% CI: -94, -8). For black men ages 65-74, the difference was -119 cases per 100,000 person-years (95% CI: -213, -24).

Appendix 2-1. Comparison and Validation of Aspirin Variables in ARIC

There were several variables that we could have used to define current aspirin use at ARIC Visits 1-4. The ARIC datasets included a derived variable on aspirin use during the two weeks prior to each study visit, created using medication codes for the medication bottles that participants brought with them to each study visit. For this variable, participants were considered an aspirin user if they brought in a medication that fit one of the following codes:

"641000" = "SALICYLATES"
"641099" = "SALICYLATE COMBINATIONS"
"642000" = "ANALGESICS OTHER"
"642099" = "ANALGESICS - OTHER COMBINATIONS"
"649900" = "ANALGESIC COMBINATIONS"
"649910" = "ANALGESIC-SEDATIVES"
"649920" = "ANALGESIC-ANTICHOLINERGICS"
"659900" = "NARCOTIC COMBINATIONS"
"659910" = "CODEINE COMBINATIONS"
"659913" = "DIHYDROCODEINONE COMBINATIONS"
"659917" = "HYDROCODONE COMBINATIONS"
"659920" = "PROPOXYPHENE COMBINATIONS"
"659930" = "MEPERIDINE COMBINATIONS"
"659940" = "PENTAZOCINE COMBINATIONS"

However, because this variable likely captured aspirin use as well as use of other non-aspirin analgesics, a second derived variable was created. This variable defined aspirin users as individuals who brought in medications fitting the following two codes only:

"641000" = "SALICYLATES"
"641099" = "SALICYLATE COMBINATIONS"

Finally, we considered use of a third variable based on self-reported use of aspirin, cold medication, or headache powder during the two weeks prior to each study visit.

For the purpose of this study, we were most interested in long-term, regular use of aspirin, and not short-term or sporadic use for treatment of headaches, colds, or acute pain. Thus, to determine which of these variables best captured regular aspirin use, we compared values for these three variables at visit 4 (V4) to our "gold standard" measure: self-reported regular aspirin use at V4. We selected the variable with the highest concordance, and the best balance of sensitivity and specificity, for use in our primary analysis.

Variables:

1. Self-reported regular aspirin use at V4 ("gold standard")
2. Original derived variable for aspirin use within the past two weeks, based on medication codes, for V1-V4
3. Modified derived variable for aspirin use within the past two weeks, based on medication codes (modified to exclude codes for drug combinations that do not necessarily contain aspirin), for V1-V4
4. Self-reported use of aspirin, cold medication, or headache powder within the past two weeks, for V1-V4

Concordance between variables 1 & 2 at V4:

	Variable 1		
Variable 2	**No**	**Yes**	**Total**
No	1882	35	1917
Yes	954	1638	2592
Total	2836	1673	4509

Percent concordance: 78%
Kappa: 0.58
Sensitivity: 98%
Specificity: 66%

Concordance between variables 1 & 3 at V4:

	Variable 1		
Variable 3	**No**	**Yes**	**Total**
No	2422	95	2517
Yes	412	1578	1990
Total	2834	1673	4507

Percent concordance: 89%
Kappa: 0.77
Sensitivity: 94%
Specificity: 85%

Concordance between variables 1 & 4 at V4:

	Variable 1		
Variable 4	**No**	**Yes**	**Total**
No	2097	37	2134
Yes	734	1638	2372
Total	2831	1675	4506

Percent concordance: 83%
Kappa: 0.67
Sensitivity: 98%
Specificity: 74%

Appendix 2-2. Supplemental Tables

Supplemental Table 2-1. Associations between current aspirin use lagged by one year and total, lethal, and fatal prostate cancer among 6,594 men in the ARIC study, 1987-2012

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No Aspirin	508 / 72009	1.00	--	1.00	--
Aspirin	301 / 40821	0.94	(0.81, 1.08)	1.03	(0.88, 1.20)
Lethal Prostate Cancer					
No Aspirin	75 / 72137	1.00	--	1.00	--
Aspirin	20 / 40915	0.42	(0.26, 0.69)	0.52	(0.31, 0.87)
Fatal Prostate Cancer					
No Aspirin	67 / 80662	1.00	--	1.00	--
Aspirin	23 / 46284	0.47	(0.29, 0.75)	0.52	(0.32, 0.86)

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), current statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

All time-updated covariates were lagged one year

Supplemental Table 2-2. Associations between current aspirin use and total, lethal, and fatal prostate cancer among 6,594 men in the ARIC study, 1987-2012, restricted to non-current users of statins

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No Aspirin	479 / 73272	1.00	--	1.00	--
Aspirin	265 / 37629	0.97	(0.83, 1.12)	1.06	(0.90, 1.24)
Lethal Prostate Cancer					
No Aspirin	73 / 73400	1.00	--	1.00	--
Aspirin	19 / 37720	0.46	(0.27, 0.75)	0.54	(0.32, 0.91)
Fatal Prostate Cancer					
No Aspirin	63 / 81493	1.00	--	1.00	--
Aspirin	20 / 42074	0.49	(0.30, 0.81)	0.56	(0.33, 0.95)

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

Supplemental Table 2-3. Associations between current aspirin use and total, lethal, and fatal prostate cancer among 6,594 men in the ARIC study, 1987-2012, with missing data imputed via multiple imputation

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No Aspirin	504 / 75881	1.00	--	1.00	--
Aspirin	313 / 43494	0.97	(0.84, 1.11)	1.06	(0.91, 1.23)
Lethal Prostate Cancer					
No Aspirin	74 / 76008	1.00	--	1.00	--
Aspirin	23 / 43588	0.48	(0.30, 0.76)	0.58	(0.35, 0.94)
Fatal Prostate Cancer					
No Aspirin	65 / 84546	1.00	--	1.00	--
Aspirin	25 / 48970	0.52	(0.33, 0.82)	0.59	(0.36, 0.96)

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), current statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

Supplemental Table 2-4. Associations between current aspirin use and cumulative incidence of total, lethal, and fatal prostate cancer, accounting for non-prostate cancer death as a competing event, among 6,594 men in the ARIC study, 1987-2012

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Total Prostate Cancer					
No Aspirin	503 / 75897	1.00	--	1.00	--
Aspirin	314 / 43478	0.89	(0.77, 1.02)	0.99	(0.86, 1.15)
Lethal Prostate Cancer					
No Aspirin	74 / 76025	1.00	--	1.00	--
Aspirin	23 / 43572	0.51	(0.34, 0.77)	0.63	(0.42, 0.94)
Fatal Prostate Cancer					
No Aspirin	65 / 84565	1.00	--	1.00	--
Aspirin	25 / 48951	0.54	(0.35, 0.82)	0.62	(0.41, 0.95)

*Adjusted for race/center (White/Forsyth, Black/Forsyth, Black/Jackson, White/Minneapolis, White/Washington County), birth cohort (in 5 year categories), smoking status (current/recent, former, never), BMI (continuous), current statin use (yes, no), diabetes (diagnosed diabetes, undiagnosed diabetes, prediabetes, no diabetes), prevalent CHD (yes, no, missing), education (basic, intermediate, advanced, missing), family history of prostate cancer (yes, no, missing)

Chapter 3. Aspirin and Non-Aspirin NSAID Use and Prostate Cancer Recurrence after Radical Prostatectomy

Abstract

Background: Use of aspirin and non-aspirin nonsteroidal anti-inflammatory drugs (NA-NSAIDs) has been associated with a reduced risk of developing certain cancers, including prostate cancer. However, associations with prostate cancer outcomes following diagnosis are unclear. This study examined associations between aspirin and NA-NSAID use and prostate cancer recurrence in a clinical cohort of prostate cancer patients treated surgically.

Methods: The study population included patients with clinically localized prostate cancer who underwent surgery between 1993 and 2006 by a single surgeon at the Johns Hopkins Hospital. Medication use was systematically solicited during the preoperative consultation and later abstracted from patient medical records. Medications used ≥2 times per week pre- and post-surgery were also ascertained via a survey mailed to surviving men in 2007. Patients were followed through 2014 for prostate cancer recurrence, defined as biochemical recurrence, local recurrence, development of metastatic disease, or prostate cancer death. Cox proportional hazards regression was used to estimate associations between aspirin and NA-NSAID use (at the preoperative consult, pre-surgery, and post-surgery) and recurrence, adjusted for age, pathologic stage, grade, and potential confounders. Effect modification by stage/grade and year of surgery was assessed using stratified models.

Results: There were 2,364 men in the full cohort, and 1,508 men who completed the 2007 survey. Among men in the full cohort, 9.69% used aspirin and 5.67% used an NA-NSAID at the preoperative consultation. After multivariable adjustment, aspirin use (HR: 1.16, 95% CI: 0.79-1.69) and NA-NSAID use (HR: 1.06, 95% CI: 0.64-1.75) at the preoperative consultation were not associated with recurrence. Among men who completed the survey, aspirin use pre-surgery (HR 1.14, 95% CI 0.75-1.72) and post-surgery (HR 1.25, 95% CI 0.83-1.89), and NA-NSAID use pre-surgery (HR 0.84, 95% CI 0.50-1.41) and post-surgery (1.29, 95% CI 0.83-2.00) were

also not associated with recurrence. Results were overall consistent when stratified by stage/grade and year of surgery.

Conclusions: Within this study population, aspirin and NA-NSAID use at various time intervals were not inversely associated with recurrence in men treated with radical prostatectomy for clinically localized prostate cancer.

Introduction

There are currently more than three million prostate cancer survivors living in the U.S. (90). Prostate cancer often has a favorable prognosis, and the vast majority of men with prostate cancer live for years, or even decades, after their initial diagnosis. However, some may experience disease recurrence after treatment and/or progression to metastatic disease. Identification of modifiable risk factors for prostate cancer recurrence and progression may provide a means for lowering the risk of these outcomes, in conjunction with conventional treatments and management strategies.

One potential modifiable risk factor for prostate cancer recurrence is regular use of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs). These drugs are hypothesized to reduce risk of cancer progression via anti-inflammatory and antiplatelet mechanisms. Specifically, inhibition of COX-2 blocks production of pro-inflammatory prostaglandins that promote cell proliferation, angiogenesis, and invasion, while inhibition of COX-1 impedes platelets, which are thought to facilitate metastases through the bloodstream (30, 80, 91). In support of these hypotheses, secondary analyses of randomized controlled trials have found that allocation to daily aspirin reduces overall cancer mortality (37) and risk of cancer with distant metastases, both at initial presentation and at follow-up (34).

For prostate cancer, the literature on aspirin and non-aspirin NSAID (NA-NSAID) use and cancer outcomes following diagnosis has been mixed. Observational studies of aspirin use pre-diagnosis and prostate cancer case-fatality have largely been null (46-50), though an inverse association was observed in the Atherosclerosis Risk in Communities study (see Chapter 2 of this dissertation). Studies of aspirin use post-diagnosis and case-fatality have reported results ranging from null (46, 48-50, 53, 79), to protective (44, 52, 78), to harmful (51). Few studies have reported specifically on NA-NSAID use and prostate cancer outcomes (49, 50, 53). Moreover, very few studies have been able to examine prostate cancer recurrence as

an outcome. Prostate cancer recurrence, typically marked by biochemical recurrence (BCR), i.e. rising PSA after PSA nadir post-radical prostatectomy, often precedes death from prostate cancer by several years, and may thus be an early indicator of poor disease outcome less subject to bias due to differential access to salvage treatments or competing risks.

The purpose of this study was to investigate whether aspirin and NA-NSAID use were associated with risk of prostate cancer recurrence among a cohort of men surgically treated for prostate cancer at the Johns Hopkins Hospital (JHH). We hypothesized that aspirin and NA-NSAID use reported at the time of the preoperative consultation, pre-surgery, and-post-surgery would be associated with a reduced risk of prostate cancer recurrence in this study population.

Methods

Study Population

This secondary data analysis included men treated with radical prostatectomy by a single surgeon at JHH. All men were diagnosed with clinically localized disease, and all underwent radical prostatectomy between January 1, 1993 and March 31, 2006. This study period was chosen to restrict to men both diagnosed in the post-PSA era and eligible for the 2007 survey. Men who underwent hormone or radiation therapy before radical prostatectomy were excluded. The study was approved by institutional review boards at the Johns Hopkins University School of Medicine and Johns Hopkins Bloomberg School of Public Health.

Ascertainment of NSAID Use and Other Covariates

Age, race/ethnicity, first-degree family history of prostate cancer, preoperative PSA, year of surgery, positive surgical margins, pathologic stage, and Gleason sum are routinely abstracted for patients undergoing radical prostatectomy at JHH. To obtain information on medication use and comorbidities at the time of the preoperative consultation, electronic and

paper medical records pertaining to this visit were also reviewed by a single abstractor blinded to outcome. During the preoperative consultation, the surgeon routinely asked about and recorded current use of aspirin and NA-NSAIDs in patient medical records, as use of these drugs conferred an increased risk of bleeding during the surgery.

Men alive as of November 2007 and residing in the United States were also mailed a survey on lifestyle and medical factors. The survey ascertained additional information about over-the-counter or prescription medications used regularly, defined as use more than two times per week. Specifically, men were asked to self-report whether they regularly used specific medications (aspirin, ibuprofen, other anti-inflammatory analgesics, acetaminophen, statins, and other cholesterol-lowering drugs) pre- and/or post-surgery (yes, no), and if yes, the duration of use (<1 year, 1-4 years, 5-9 years, or 10+ years) pre- and post-surgery. Men also self-reported their height, weight (five years before surgery, one-year post-surgery, and current), and cigarette smoking history (including number of cigarettes smoked daily at several age intervals).

Outcome Ascertainment

The primary outcome for this analysis was prostate cancer recurrence after radical prostatectomy, defined as a composite outcome of BCR, local recurrence, metastasis, or death from prostate cancer (whichever outcome occurred first). As a secondary outcome, time to metastatic prostate cancer was also examined. To monitor for disease recurrence, men were evaluated by their primary care physicians with PSA tests and digital-rectal examinations every 3 months for the first year after surgery, semiannually during the second year, and annually thereafter. BCR was defined as a confirmed PSA value ≥0.2 ng/mL after PSA nadir. Prostate cancer deaths were determined through linkage to the National Death Index. For this analysis, outcomes ascertained through 2014 were used.

Statistical analysis

Age-adjusted baseline characteristics of the cohort were compared by aspirin and NA-NSAID use at the preoperative consultation, and by pre-surgical use reported via the survey. Cox proportional hazards regression was then used to calculate cause-specific hazard ratios (HRs) and 95% confidence intervals (CIs) for recurrence comparing users versus non-users of aspirin and NA-NSAIDs. Three sets of analyses were conducted:

1) Using data from the medical record abstraction, aspirin and NA-NSAID users at the time of the preoperative consultation were compared to non-users of aspirin and NA-NSAIDs, respectively. In these analyses, year of surgery was the time origin and years since surgery was the time metric. All men in the cohort were included. Covariates were obtained from the medical record abstraction and included and categorized as age (continuous), year of surgery (continuous), race (white, nonwhite), pathologic stage (<T3, ≥T3), pathologic grade (Gleason sum <4+3, ≥4+3), body mass index (BMI) (<25, 25-30, ≥30 kg/m^2, missing), use of a statin at the time of the preoperative consultation (yes, no), history of cardiovascular disease (CVD) (yes, no), and history of arthritis (yes, no).

2) Using data from the 2007 survey, associations for ever use of aspirin pre-surgery, ever use of aspirin post-surgery, ever use of NA-NSAIDs pre-surgery, and ever use of NA-NSAIDs post-surgery were examined in separate models. For pre-surgical use of aspirin and NA-NSAIDs, duration of use (categorized as <1 year, 1-4 years, 5-9 years, 10+ years) was also considered. Joint categories of pre- and post-surgical use (never use, use pre-surgery only, use post-surgery only, and use pre- and post-surgery) were examined to assess for potential interaction. For all of these models, the time metric was years since surgery and the time origin was one-year post-surgery (since certain covariates, such as BMI and smoking status, were assessed

one-year post-surgery, and because it was assumed that events occurring within one year were not truly recurrent events but evidence of more advanced disease at the time of diagnosis). Men who completed the 2007 survey, who were at risk for the event one-year post-surgery (i.e. did not recur or were not lost to follow-up at one year), and who had non-missing medications data were included. Models were adjusted for age, year of surgery, race, pathologic stage, pathologic grade, history of CVD, or arthritis (from the medical record abstraction, treated as above), and BMI one-year post-surgery (continuous), weight change from five-years pre-surgery to one-year post-surgery (>2.2, ≤2.2 kg, missing), cigarette smoking status one-year post-surgery (current, former, never, missing), and use of statins pre-surgery (yes, no, missing) from the 2007 survey.

3) The analyses from 2) were repeated with 2007 (the year of administration of the survey) as the time origin, and years since 2007 as the time metric. This analysis was conducted to remove potential effects of recall bias, as all events included in this analysis were ascertained prospectively in relation to the survey. All men who completed the 2007 survey, had non-missing medications data, and were still at risk for recurrence in 2007 (i.e. had not recurred prior to 2007) were included. Models were adjusted for the same set of covariates as above.

Covariates for these models were chosen a priori, based on prior studies from within this cohort and other cohorts. Covariates included age, established risk factors for prostate cancer recurrence (race) and factors that had previously been associated with recurrence in this cohort (smoking status, BMI, weight change from five years before surgery to one year after surgery, use of statins) (92-94). Models were also adjusted for indications for aspirin and NA-NSAID use, including history of CVD and history of arthritis, since these conditions are associated with aspirin and NA-NSAID use and may share risk factors with prostate cancer recurrence. Year of surgery was included in the models to account for possible secular trends and, in the latter two analyses, for the differing amounts of elapsed time between surgery and survey administration.

Finally, models were adjusted for pathologic stage and grade, since the goal of this analysis was to determine whether aspirin and NA-NSAID use influence risk of prostate cancer outcomes independent of disease severity at diagnosis and surgery.

For all analyses, men were censored at the date of death or last known follow-up. The proportional hazards assumption was tested through Schoenfeld residuals and plots of the log cumulative hazard function. Stratified analyses were used to test for possible effect measure modification by stage and grade at surgery (stage ≥T3 or Gleason sum ≥4+3 vs. stage <T3 and Gleason sum <4+3) and by year of surgery (pre-2000 vs. post-2000). To examine our secondary outcome of prostate cancer metastases, models were repeated with time to metastasis as the outcome. All analyses were conducted using SAS version 9.4.

Results

Aspirin and NA-NSAID use at the time of the preoperative consultation and prostate cancer recurrence

There were 2,364 men in the full cohort. The mean age at surgery was 56 years. The majority of men were white and had non-advanced disease at surgery. 9.69% of the cohort used aspirin at the time of the pre-surgical consultation and 5.67% used NA-NSAIDs. After adjusting for age, most baseline characteristics were similar among aspirin users and non-users at the time of the preoperative consultation, with the exception of statin use and history of CVD and arthritis, which were all more prevalent among aspirin users (Table 3-1). Compared to non-users, NA-NSAID users were more likely to have high-grade disease at surgery and a history of arthritis, after adjusting for age (Table 3-1).

During 25,622 person-years at risk, 300 recurrence events were observed. These recurrences included 185 men who experienced biochemical recurrence only, 25 with local recurrences, and 84 who developed metastatic prostate cancer. There were 48 deaths from prostate cancer in this cohort, and 28 deaths from other causes but with prostate cancer

progression. The median time to recurrence after surgery was 4 years, while the median time to metastatic prostate cancer after surgery was 7 years.

Overall, aspirin and NA-NSAID use at the time of the preoperative consultation were not associated with prostate cancer recurrence (for aspirin: HR: 1.16, 95% CI: 0.79-1.69; for NA-NSAIDs: HR: 1.06, 95% CI: 0.64-1.75, Table 3-2). Results remained null when stratified by stage and grade at diagnosis and by year of diagnosis (Table 3-3). Aspirin and NA-NSAID use at the time of the preoperative consultation were also not associated with prostate cancer metastasis (for aspirin: HR: 1.00, 95% CI: 0.48-2.07; for NA-NSAIDs: HR: 1.16, 95% CI: 0.48-2.79, Table 3-4).

Aspirin and NA-NSAID use pre- and post-surgery and prostate cancer recurrence, with time at risk beginning one-year post-surgery

There were 1505 men (64% of the full cohort) who returned the survey in 2007. A comparison of men who did vs. did not complete the survey is presented in Table 3-5. Compared to the men who did not respond or were ineligible to receive the survey (i.e. died before 2007 or no longer resided in the U.S.), the survey respondents were older, had more favorable clinical characteristics at diagnosis (less advanced stage and lower PSA values), were more likely to be white, and less likely to have a history of CVD. Of the 1505 survey respondents, 20 were not at risk for recurrence one-year post-surgery (including 19 who recurred and one who was lost to follow-up within one year of surgery) and were consequently excluded from further analyses.

Of the 1485 men, 41.8% reported regular aspirin use pre-surgery and 55.0% reported regular aspirin use post-surgery. 24.6% used NA-NSAIDs pre-surgery, and 28.7% used NA-NSAIDs post-surgery. Age-adjusted characteristics by aspirin and NA-NSAID use pre-surgery are displayed in Table 3-6. There were 150 recurrences among these men, including 41 metastases.

After adjusting for stage and grade at surgery and potential confounders, regular aspirin use pre-surgery was not associated with prostate cancer recurrence (HR: 1.29, 95% CI: 0.91-1.82, Table 3-7). Associations did not vary by duration of pre-surgical use. Regular use of aspirin post-surgery was associated with a borderline-significant increased risk of recurrence (HR: 1.37, 95% CI: 0.96-1.96). When joint categories of aspirin use pre- and post-surgery were examined, the elevated risk of recurrence was most apparent in men who reported using aspirin both pre- and post-surgery, compared to the reference group of never users (HR: 1.47, 95% CI: 0.99-2.19). For both pre- and post-surgical aspirin use, associations were similar when stratified by stage/grade at surgery, but more positive among men undergoing surgery after 2000 (for pre-surgical aspirin: HR: 2.39, 95% CI: 1.20-4.77; for post-surgical aspirin: HR: 2.02, 95% CI: 0.99-4.11, Table 3-8).

No associations were observed between regular NA-NSAID use pre-surgery (HR: 0.96, 95% CI: 0.64-1.45) or post-surgery (HR: 1.20, 95% CI: 0.84-1.71) and recurrence. Joint use of NA-NSAIDs both pre- and post-surgery was also not associated with recurrence (HR: 1.23, 95% CI: 0.80-1.88). No effect modification by stage/grade or year of surgery was observed.

Null associations were observed for all exposures and the secondary outcome of prostate cancer metastasis (pre-surgical aspirin: HR: 0.89, 95% CI: 0.43-1.82; post-surgical aspirin: HR: 1.17, 95% CI: 0.56-2.43); pre-surgical NA-NSAIDs: HR: 0.73, 95% CI: 0.30-1.77; post-surgical NA-NSAIDs: HR: 0.65, 95% CI: 0.30-1.41, Table 3-11).

Aspirin and NA-NSAID use pre- and post-surgery and prostate cancer recurrence, with time at risk beginning in 2007

There were 1363 men still at risk for prostate cancer recurrence in 2007 (90% of survey respondents, and 58% of the full cohort). Age-adjusted characteristics of these men by aspirin and NA-NSAID use pre-surgery are shown in Table 3-12. Among these men, there were 57 recurrences and 9 metastases.

In this analysis, aspirin use pre-surgery was positively associated with prostate cancer recurrence (HR: 2.05, 95% CI: 1.13-3.72, Table 3-13). Similar results were observed among all categories of duration of use pre-surgery. Aspirin use post-surgery was also positively associated with recurrence (HR: 2.22, 95% CI: 1.18-4.16). The positive association was strongest among men who reported aspirin use both pre- and post-surgery, compared to never users of aspirin (HR: 2.61, 95% CI: 1.31-5.19). When stratified by year of surgery, the positive association was most apparent among men treated with surgery after 2000 (for aspirin use pre-surgery: HR: 3.08, 95% CI: 1.31-7.26; for aspirin use post-surgery: HR: 2.47, 95% CI 1.02-6.01, Table 3-14), similar to the analyses with time at risk beginning one-year post-surgery.

Null associations were observed for NA-NSAID use pre-surgery (HR: 0.92, 95% CI: 0.46-1.82), post-surgery (HR: 1.19, 955 CI: 0.66-2.15), and both pre- and post-surgery (HR: 1.16, 95% CI: 0.56-2.41) and prostate cancer recurrence (Table 3-15). There were not enough events among NA-NSAID users to conduct stratified analyses. There were also not enough metastatic events in this subset to examine time to prostate cancer metastasis.

Discussion

In this study, neither aspirin nor NA-NSAID use was inversely associated with prostate cancer recurrence, as was hypothesized. Instead, our results suggest null, or possibly positive, associations between aspirin use pre- and post-surgery and recurrence, even after adjustment for key confounders. For both pre- and post-surgical aspirin use, positive associations were most evident when the analysis was restricted to men undergoing surgery after 2000, who were recalling medication use over a shorter time frame and who thus may have been less like to misremember their medications history. Positive associations were also observed in the analyses with time at risk beginning in 2007, in which only events occurring prospectively in relation to the survey were included. We did not observe associations between NA-NSAID use

at any time point and recurrence, or between aspirin or NA-NSAID use and prostate cancer metastases, though power in these analyses was limited.

Our results for aspirin were somewhat surprising given our hypotheses, our findings on aspirin use pre-diagnosis and prostate cancer case-fatality in Chapter 2, and the prior literature, which has most often reported null (46-50, 53, 79) or inverse (44, 52, 78) associations between aspirin use pre- and post-diagnosis and prostate cancer outcomes. One previous population-based study using a national prescription-drug database in the United Kingdom did find a positive association between post-diagnostic aspirin use and prostate cancer case-fatality, but the increased risk was only observed among men initiating aspirin use after diagnosis, and it is possible that these men initiated aspirin to combat adverse side effects of treatments for prostate cancer that had already recurred or progressed (51). Prior studies of aspirin use and prostate cancer outcomes have also shown evidence of effect modification by cancer stage and grade. Specifically, studies have reported inverse associations that were more pronounced (47, 52) or only observed (48) among men with advanced stage or grade prostate cancer. These findings were not replicated in this study, as our results for aspirin were consistent across strata of pathologic stage and grade. Our results are however consistent with the few preceding studies of NA-NSAID use and prostate cancer outcomes, which have also reported null associations (50, 53).

There are several possible reasons why aspirin use might appear positively correlated with recurrence. In the analyses of pre-surgical aspirin use, pre-surgical use may have prevented some cancers from developing or from being diagnosed. Aspirin use has been associated with a slight to moderate decreased risk of prostate cancer in observational studies (42) as well as decreased PSA (95), which could lower the likelihood of prostate cancer detection. Prostate cancers that do develop or that release enough PSA to prompt detection, despite aspirin use, may thus be more aggressive and likely to recur, which could make pre-surgical aspirin use appear harmful. It is also possible that aspirin use during these time

intervals, instead of reducing inflammation, may have actually impeded existing inflammation from resolving, as has been shown in some animal models (17). Finally, due to the observational nature of this study, we cannot rule out confounding by unmeasured factors related to both indications for aspirin use and prostate cancer outcomes, or other sources of bias as discussed below.

This study included three distinct analyses, each with its own strengths and potential biases:

Aspirin and NA-NSAID use at the time of the preoperative consultation and prostate cancer recurrence

The first analysis, with exposure data collected via medical record abstraction, had both a clearly-defined cohort (men undergoing surgery for prostate cancer by a single surgeon between 1993-2006) and exposure (aspirin and NA-NSAID use at the time of the pre-surgical consultation). This analysis also had the advantage of being fully prospective, as aspirin and NA-NSAID use were recorded in the medical records in real-time, prior to prostate cancer treatment and outcomes. However, there may have been exposure misclassification due to possible underreporting of aspirin and NA-NSAID use. The prevalence of aspirin and NA-NSAID use in this analysis was low compared to the prevalence of aspirin and NA-NSAID use reported via the 2007 survey. In part, the lower prevalence may be due to aspirin and NA-NSAID use being ascertained at a very specific time point, as opposed to the survey, which asked about regular aspirin use more generally at any time prior to surgery. Due to the prospective nature of this analysis, any misclassification of exposure should have been non-differential with respect to prostate cancer recurrence, and would bias the results predictably towards the null.

Aspirin and NA-NSAID use pre- and post-surgery and prostate cancer recurrence, with time at risk beginning one-year post-surgery

The analysis of NSAID use pre- and post-surgery based on the survey data, with time at risk beginning one-year post-surgery, had the advantage of using the more detailed information on aspirin and NA-NSAID use collected via the survey. As a result, this analysis was able to examine pre- and post-surgical NSAID use separately, as well as duration of use pre-surgery. However, this analysis was not fully prospective, as the 2007 survey was administered after men had already been followed for several years and after some had already recurred. Results from this analysis may have thus been affected by recall bias. Specifically, biased positive associations between NSAID use and recurrence could have arisen if men who recurred before 2007 were more likely to remember and/or report NSAID use than the men who remained recurrence-free at the time of the 2007 survey.

This analysis was also subject to selection bias due to the fact that men had to survive to 2007 and respond to the mailed survey in order to be included. Men who returned the survey may have differed in key ways from the men who died prior to 2007 (from prostate cancer or from other causes) or who failed to respond to the survey request. Some differences between survey respondents and non-respondents were observed (Table 3-5) and could be accounted for in multivariable models, but survey respondents and non-respondents may have differed in unmeasured ways as well. For additional discussion of selection bias, see Appendix 3-1.

Finally, in the analysis of post-surgical NSAID use and recurrence with time at risk beginning one-year post-surgery, there was possible misallocation of person-time. Post-surgical NSAID use was treated as a time-fixed covariate in this analysis due to the lack of information on start and stop dates of medication use. However, men who reported post-surgical NSAID use may not have used NSAIDs for the entirety of their post-surgical follow-up, and so it is likely that person-time at risk, and possibly even events, were misallocated to the exposed group instead of the unexposed.

Aspirin and NA-NSAID use pre- and post-surgery and prostate cancer recurrence, with time at risk beginning in 2007

Our third analysis, with time at risk beginning in 2007, was designed to maintain the strengths and circumvent some of the biases of the previous analysis. This analysis was restricted to men who were still at risk for recurrence in 2007; as a result, all outcomes included in this analysis occurred prospectively in relation to the 2007 survey and recall bias was avoided. Misallocation of person-time was also not an issue, as ever use of NSAIDs post-surgery, but pre-2007 survey, could appropriately be treated as time-fixed. However, the time origin in this analysis was 2007, which is a less meaningful origin than the origin used in the first two analyses (year of surgery). This analysis also included an even more selected group of men, and selection bias was consequently more likely. In this analysis, men not only had to survive to complete the 2007 survey, but they also had to remain recurrence-free in 2007. The median time from surgery to recurrence in the full cohort was 4 years, and so men who had not yet experienced a recurrence in 2007, 1-14 years after their prostate cancer surgery, may have had much more favorable-risk prostate cancer than the men who recurred prior to 2007 and were excluded. Ideally, we would have been able to restrict this analysis to men treated in the few years prior to 2007 to limit the potential impact of this survival bias, but power was too low to conduct the analysis in this manner.

For all three analyses, generalizability of the results may be limited. First, the source population was narrowly defined as men undergoing surgical treatment for clinically-localized prostate cancer by a single surgeon at JHH between 1993 and 2006. The results may thus only be applied to men with prostate cancer who undergo surgery or are good candidates for surgical treatment, i.e. men with non-metastatic disease and few to no comorbidities. The patient population seeking care at JHH is also a selected population, consisting primarily of white men

of high socioeconomic status. Generalizability to other racial groups or socioeconomic strata is thus unknown.

At the same time, the homogeneity of the study population is an important study strength. Because all men in the cohort received the same prostate cancer treatment at the same hospital by the same surgeon, there should be little to no confounding by access to medical care or variability in healthcare quality. There was consistent recording of medication use in the preoperative consultation medical records of all patients, and consistent capturing of prostate cancer outcomes. Though it is likely that both methods for ascertaining medications use (the medical record abstraction and the mailed survey) had some measurement error, and though our three analyses were subject to different potential biases, the relatively consistency in findings across exposure ascertainment and analytic methods provide credence to our results.

In summary, our study does not provide support for an inverse association between aspirin and NA-NSAID use pre- or post-surgery and prostate cancer recurrence after surgery. These results suggest that aspirin and NA-NSAIDs should not be recommended to patients to improve prostate cancer outcomes, but given the conflicting findings for aspirin across study, additional large, well-designed observational studies or randomized controlled trials are still warranted.

Grant Support and Acknowledgments

Lauren Hurwitz was supported by an NCI National Research Service Award (T32 CA09314). This study was also supported by the following NIH grants: NCI P50 CA58236 and P30 CA006973. Thank you to the colleagues from JHU who contributed to this work.

Chapter 3 Tables

Table 3-1. Age-adjusted characteristics of the full cohort at the preoperative consultation, by aspirin and NA-NSAID use at the preoperative consultation, Johns Hopkins Hospital Radical Prostatectomy Cohort

	Aspirin Use			NA-NSAID Use		
	No	**Yes**	***p*-value**	**No**	**Yes**	***p*-value**
N	2135	229		2230	134	
Age, years, mean	56.0	58.7	<0.01	56.3	57.0	0.26
Year of surgery, mean	1999.1	1999.1	1.0	1999.1	1999.7	0.05
PSA value, ng/mL, mean	6.9	6.9	0.9	6.9	7.4	0.2
Pathologic stage, %						
<T3	72	68	0.2	72	69	0.4
≥T3	28	32		28	31	
Pathologic grade (Gleason sum), %						
<4+3	90	89	0.6	90	83	<0.01
≥4+3	10	11		10	17	
Race, %						
White	93	92	0.3	93	93	0.3
Black	3	2		3	4	
Other/Missing	4	7		5	3	
BMI, %						
<25 kg/m^2	32	33	0.08	33	30	0.2
25 - <30 kg/m^2	52	58		53	52	
≥30 kg/m^2	11	7		11	12	
Missing	4	2		4	6	
Family history of PCa, %						
Yes	35	33	0.8	35	40	0.6
No	60	60		60	56	
Missing	5	7		5	4	
Other NSAID use, %						
Yes	6	7	0.4	10	12	0.4
No	94	93		90	88	
Statin use, %						
Yes	14	34	<0.01	16	18	0.6
No	86	66		84	82	
Type II diabetes, %						
Yes	2	2	1.0	3	0	0.05
No	98	98		98	100	
Cardiovascular disease, %						
Yes	1	24	<0.01	4	1	0.2
No	99	76		96	99	

Arthritis, %						
Yes	2	4	0.05	1	19	<0.01
No	98	96		99	81	

PSA, prostate-specific antigen; BMI, body mass index; PCa, prostate cancer; NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

Table 3-2. Aspirin and NA-NSAID use at the time of the preoperative consultation and prostate cancer recurrence, Johns Hopkins Hospital Radical Prostatectomy Cohort

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Aspirin Use					
No	265 / 23136	1.00	--	1.00	--
Yes	35 / 2486	1.14	(0.80, 1.62)	1.16	(0.79, 1.69)
NA-NSAID Use					
No	278 / 24305	1.00	--	1.00	--
Yes	22 / 1317	1.37	(0.89, 2.11)	1.06	(0.64, 1.75)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug*

Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), BMI at the time of surgery (<25, 25-30, ≥30 kg/m^2, missing), use of statins at the time of surgery, cardiovascular disease, arthritis

Table 3-3. Aspirin and NA-NSAID use at the time of the preoperative consultation and prostate cancer recurrence, stratified by stage & grade at surgery and year of surgery, Johns Hopkins Hospital Radical Prostatectomy Cohort

	Low Stage & Low Grade					High Stage or High Grade				
		Age-Adjusted		Multivariable-Adjusted*			Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	HR	95% CI	HR	95% CI	Events/ Person-years	HR	95% CI	HR	95% CI
Aspirin Use										
No	4 / 16722	1.00	--	1.00	--	220 / 6414	1.00	--	1.00	--
Yes	3 / 1713	0.64	(0.20, 2.07)	0.61	(0.17, 2.20)	32 / 773	1.14	(0.78, 1.65)	1.23	(0.82, 1.83)
NA-NSAID Use										
No	43 / 17550	1.00	--	1.00	--	235 / 6755	1.00	--	1.00	--
Yes	5 / 885	2.31	(0.92, 5.85)	2.35	(0.86, 6.42)	17 / 432	1.01	(0.62, 1.66)	0.89	(0.50, 1.58)

	Year of Surgery <2000					Year of Surgery ≥2000				
		Age-Adjusted		Multivariable-Adjusted**			Age-Adjusted		Multivariable-Adjusted**	
	Events/ Person-years	HR	95% CI	HR	95% CI	Events/ Person-years	HR	95% CI	HR	95% CI
Aspirin Use										
No	186 / 14752	1.00	--	1.00	--	79 / 8384	1.00	--	1.00	--
Yes	23 / 1644	1.05	(0.68, 1.62)	1.11	(0.69, 1.78)	12 / 842	1.33	(0.72, 2.46)	1.32	(0.69, 2.51)
NA-NSAID Use										
No	194 / 15712	1.00	--	1.00	--	84 / 8593	1.00	--	1.00	--
Yes	15 / 684	1.69	(1.00, 2.86)	1.35	(0.72, 2.52)	7 / 633	1.08	(0.50, 2.33)	0.68	(0.29, 1.58)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for age, year of surgery, race (white, non-white), BMI at the time of surgery (<25, 25-30, ≥30, missing), use of statins at the time of surgery, cardiovascular disease, arthritis

**Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), BMI at the time of surgery (<25, 25-30, ≥30 kg/m^2, missing), use of statins at the time of surgery, cardiovascular disease, arthritis

Table 3-4. Aspirin and NA-NSAID use at the time of the preoperative consultation and prostate cancer metastases, Johns Hopkins Hospital Radical Prostatectomy Cohort

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Aspirin Use					
No	70 / 23540	1.00	--	1.00	--
Yes	11 / 2574	1.28	(0.68, 2.43)	1.00	(0.48, 2.07)
NA-NSAID Use					
No	75 / 24772	1.00	--	1.00	--
Yes	6 / 1342	1.44	(0.63, 3.32)	1.16	(0.48, 2.79)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), BMI at the time of surgery (<25, 25-30, ≥30 kg/m^2, missing), use of statins at the time of surgery, cardiovascular disease, arthritis

Table 3-5. Comparison of men who did and did not complete the mailed survey in 2007, Johns Hopkins Hospital Radical Prostatectomy Cohort

	Did Not Complete Survey	Completed Survey	*p*-value
N	859	1505	
Age, years, mean	55.7	56.6	<0.01
Year of surgery, mean	1998.6	1999.3	<0.01
PSA value, ng/mL, mean	7.43	6.59	<0.01
Pathologic stage, %			
<T3	67	74	<0.01
≥T3	33	26	
Pathologic grade (Gleason sum), %			
<4+3	89	90	0.2
≥4+3	11	10	
Race, %			
White	89	95	<0.01
Black	4	2	
Other/Missing	7	3	
BMI, %			
<25 kg/m^2	30	34	0.1
25 - <30 kg/m^2	53	53	
≥30kg/m^2	12	10	
Missing	5	4	
Family history of PCa, %			
Yes	35	38	0.06
No	59	57	
Missing	6	5	
Aspirin use at Sx, %			
Yes	9	10	0.2
No	91	90	
NA-NSAID use at Sx, %			
Yes	5	6	0.5
No	95	94	
Statin use at Sx, %			
Yes	15	17	0.1
No	85	83	
Type II diabetes, %			
Yes	3	2	0.4
No	97	98	
Cardiovascular disease, %			
Yes	5	3	0.04
No	95	97	
Arthritis, %			
Yes	3	2	0.9
No	98	98	

PSA, prostate-specific antigen; BMI, body mass index; PCa, prostate cancer; Sx, Surgery; NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

Table 3-6. Age-adjusted baseline characteristics of men who completed the survey, by aspirin and NA-NSAID use pre-surgery*, Johns Hopkins Hospital Radical Prostatectomy Cohort

	Aspirin Use			NA-NSAID Use		
	No	**Yes**	***p*-value**	**No**	**Yes**	***p*-value**
N	841	604		1082	353	
Age, years, mean	55.8	57.9	<0.01	56.9	56.0	0.03
Year of surgery, mean	1999.1	1999.7	<0.01	1999.2	1999.7	0.03
PSA value, ng/mL, mean	6.7	6.3	0.09	6.6	6.1	0.06
Pathologic stage, %						
<T3	76	75	0.7	75	75	1.0
≥T3	24	25		25	25	
Pathologic grade (Gleason sum), %						
<4+3	91	92	0.6	91	91	0.8
≥4+3	9	8		9	9	
Race, %						
White	94	95	0.9	94	96	0.1
Black	2	1		2	1	
Other/Missing	3	4		4	2	
BMI, %						
<25 kg/m^2	38	35	0.2	37	35	0.4
25 - <30 kg/m^2	51	53		52	52	
≥30 kg/m^2	8	9		8	10	
Missing	2	3		2	2	
Weight change >2.2 kg, %**						
Yes	29	26	0.7	27	28	0.9
No	69	71		70	70	
Missing	2	3		2	2	
Smoking status 1-year post-surgery, %						
Never	54	52	0.9	54	49	0.3
Former	43	47		43	49	
Current	2	1		2	2	
Missing	1	1		1	0	
Family history of PCa, %						
Yes	38	34	0.1	37	35	0.5
No	57	62		58	60	
Missing	5	4		5	4	
Other NSAID use pre-surgery, %						
Yes	17	34	<0.01	36	58	<0.01

No	83	64		64	41	
Missing	0	2		0	0	
Statin use pre-surgery, %						
Yes	15	36	<0.01	23	25	0.2
No	85	64		77	74	
Missing	0	1		0	1	
Type II diabetes, %						
Yes	1	3	<0.01	2	3	0.3
No	99	97		98	97	
Cardiovascular disease, %						
Yes	1	6	<0.01	3	3	0.8
No	99	94		97	97	
Arthritis, %						
Yes	3	1	0.02	2	4	<0.01
No	97	99				

*Excludes men with who experienced the event of interest or who were lost to follow-up within one year of surgery, and men missing data on aspirin and NA-NSAID use pre-surgery

**From five-years pre-surgery to one-year post-surgery

Table 3-7. Aspirin use pre- and post-surgery and prostate cancer recurrence among men who completed the 2007 survey, with time at risk beginning one-year post-surgery, Johns Hopkins Hospital Radical Prostatectomy Cohort

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Aspirin Use Pre-Surgery					
Ever Use					
No	78 / 9563	1.00	--	1.00	--
Yes	69 / 6619	1.19	(0.86, 1.65)	1.29	(0.91, 1.82)
Duration of Use					
<1 year	78 / 9770	1.00	--	1.00	--
1-4 years	15 / 1487	1.18	(0.68, 2.06)	1.45	(0.82, 2.56)
5-9 years	21 / 1557	1.53	(0.94, 2.49)	1.28	(0.77, 2.12)
≥10 years	31 / 3179	1.15	(0.76, 1.75)	1.27	(0.82, 1.96)
Aspirin Use Post-Surgery					
Ever Use					
No	57 / 7070	1.00	--	1.00	--
Yes	90 / 9032	1.16	(0.83, 1.63)	1.37	(0.96, 1.96)
Aspirin Use Pre- & Post-Surgery					
Ever Use					
Never	52 / 6464	1.00	--	1.00	--
Pre- Only	5 / 582	1.00	(0.40, 2.50)	0.75	(0.30, 1.90)
Post- Only	25 / 3020	1.00	(0.62, 1.61)	1.09	(0.66, 1.78)
Pre- and Post-	64 / 5941	1.23	(0.85, 1.79)	1.47	(0.99, 2.19)

*Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

Table 3-8. Aspirin use pre- and post-surgery and prostate cancer recurrence among men who completed the 2007 survey, with time at risk beginning one-year post-surgery, stratified by stage & grade and year of surgery, Johns Hopkins Hospital Radical Prostatectomy Cohort

	Low Stage & Low Grade					High Stage or High Grade				
		Age-Adjusted		Multivariable-Adjusted*			Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	HR	95% CI	HR	95% CI	Events/ Person-years	HR	95% CI	HR	95% CI
Aspirin Use Pre-Surgery										
No	17 / 7097	1.00	--	1.00	--	61 / 2466	1.00	--	1.00	--
Yes	14 / 4851	1.20	(0.59, 2.46)	1.40	(0.66, 2.98)	55 / 1768	1.21	(0.84, 1.75)	1.33	(0.89, 1.97)
Aspirin Use Post-Surgery										
No	11 / 5203	1.00	--	1.00	--	46 / 1867	1.00	--	1.00	--
Yes	21 / 6646	1.48	(0.71, 3.11)	1.52	(0.71, 3.24)	69 / 2386	1.17	(0.80, 1.70)	1.37	(0.92, 2.06)

	Year of Surgery <2000					Year of Surgery ≥2000				
		Age-Adjusted		Multivariable-Adjusted**			Age-Adjusted		Multivariable-Adjusted**	
	Events/ Person-years	HR	95% CI	HR	95% CI	Events/ Person-years	HR	95% CI	HR	95% CI
Aspirin Use Pre-Surgery										
No	62 / 6296	1.00	--	1.00	--	16 / 3267	1.00	--	1.00	--
Yes	42 / 3972	1.02	(0.69, 1.51)	1.04	(0.68, 1.59)	27 / 2647	1.97	(1.05, 3.70)	2.39	(1.20, 4.77)
Aspirin Use Post-Surgery										
No	41 / 4340	1.00	--	1.00	--	16 / 2730	1.00	--	1.00	--
Yes	63 / 5878	1.07	(0.72, 1.58)	1.18	(0.79, 1.79)	27 / 3154	1.38	(0.74, 2.59)	2.02	(0.99, 4.11)

*Adjusted for age, year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

**Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

Table 3-9. NA-NSAID use pre- and post-surgery and prostate cancer recurrence among men who completed the 2007 survey, with time at risk beginning one-year post-surgery, Johns Hopkins Hospital Radical Prostatectomy Cohort

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
NA-NSAID Use Pre-Surgery					
Ever Use					
No	115 / 12246	1.00	--	1.00	--
Yes	30 / 3829	0.85	(0.57, 1.26)	0.96	(0.64, 1.45)
Duration of Use					
<1 year	118 / 12520	1.00	--	1.00	--
1-4 years	4 / 833	0.50	(0.19, 1.36)	0.72	(0.24, 2.13)
5-9 years	4 / 87	0.50	(0.18, 1.35)	0.57	(0.21, 1.55)
≥10 years	17 / 1653	1.16	(0.69, 1.93)	1.14	(0.70, 1.92)
NA-NSAID Use Post-Surgery					
Ever Use					
No	97 / 11456	1.00	--	1.00	--
Yes	47 / 4561	1.23	(0.87, 1.75)	1.20	(0.84, 1.71)
NA-NSAID Use Pre- & Post-Surgery					
Ever Use					
Never	96 / 10733	1.00	--	1.00	--
Pre- Only	1 / 653	**	**	**	**
Post- Only	18 / 1388	1.50	(0.91, 2.49)	1.08	(0.64, 1.81)
Pre- and Post-	29 / 3121	1.04	(0.69, 1.58)	1.23	(0.80, 1.88)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

**Not enough events to calculate stable HRs and 95% CIs

Table 3-10. NA-NSAID use pre- and post-surgery and prostate cancer recurrence among men who completed the 2007 survey, with time at risk beginning one-year post-surgery, stratified by stage & grade and year of surgery, Johns Hopkins Hospital Radical Prostatectomy Cohort

	Low Stage & Low Grade					High Stage or High Grade				
		Age-Adjusted		Multivariable-Adjusted*			Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	HR	95% CI	HR	95% CI	Events/ Person-years	HR	95% CI	HR	95% CI
NA-NSAID Use Pre-Surgery										
No	24 / 9032	1.00	--	1.00	--	91 / 3214	1.00	--	1.00	--
Yes	7 / 2832	0.92	(0.40, 2.14)	0.97	(0.42, 2.27)	23 / 997	0.82	(0.52, 1.29)	0.91	(0.57, 1.48)
NA-NSAID Use Post-Surgery										
No	20 / 8509	1.00	--	1.00	--	77 / 2947	1.00	--	1.00	--
Yes	11 / 3281	1.42	(0.68, 2.97)	1.40	(0.67, 2.94)	36 / 1280	1.09	(0.73, 1.63)	1.13	(0.75, 1.70)

	Year of Surgery <2000					Year of Surgery ≥2000				
		Age-Adjusted		Multivariable-Adjusted**			Age-Adjusted		Multivariable-Adjusted**	
	Events/ Person-years	HR	95% CI	HR	95% CI	Events/ Person-years	HR	95% CI	HR	95% CI
NA-NSAID Use Pre-Surgery										
No	80 / 7917	1.00	--	1.00	--	35 / 4329	1.00	--	1.00	--
Yes	22 / 2270	0.97	(0.61, 1.56)	1.07	(0.66, 1.74)	8 / 1559	0.65	(0.30, 1.40)	0.61	(0.26, 1.42)
NA-NSAID Use Post-Surgery										
No	67 / 7299	1.00	--	1.00	--	30 / 4157	1.00	--	1.00	--
Yes	35 / 2872	1.35	(0.90, 2.03)	1.42	(0.93, 2.18)	12 / 1689	0.99	(0.51, 1.94)	0.62	(0.31, 1.27)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for age, year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

**Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

Table 3-11. Aspirin and NA-NSAID use pre- and post-surgery and prostate cancer metastases among men who completed the 2007 survey, with time at risk beginning one-year post-surgery, Johns Hopkins Hospital Radical Prostatectomy Cohort

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Aspirin Use Pre-Surgery					
No	24 / 9717	1.00	--	1.00	--
Yes	20 / 6574	1.12	(0.60, 2.10)	0.89	(0.43, 1.82)
Aspirin Use Post-Surgery					
No	18 / 7009	1.00	--	1.00	--
Yes	25 / 9199	0.97	(0.51, 1.84)	1.17	(0.56, 2.43)
NA-NSAID Use Pre-Surgery					
No	34 / 12462	1.00	--	1.00	--
Yes	8 / 3701	0.81	(0.35, 1.83)	0.73	(0.30, 1.77)
NA-NSAID Use Post-Surgery					
No	32 / 11461	1.00	--	1.00	--
Yes	10 / 4657	0.79	(0.37, 1.66)	0.65	(0.30, 1.41)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), BMI at the time of surgery (<25, 25-30, ≥30 kg/m^2, missing), use of statins at the time of surgery, cardiovascular disease, arthritis

Table 3-12. Age-adjusted baseline characteristics of men who completed the survey and were still at risk for recurrence in 2007, by aspirin and NA-NSAID use pre-surgery, Johns Hopkins Hospital Radical Prostatectomy Cohort

	Aspirin Use			NA-NSAID Use		
	No	Yes	*p*-value	No	Yes	*p*-value
N	774	552		994	324	
Age, years, mean	55.7	57.8	<0.01	56.8	56.0	0.05
Year of surgery, mean	1999.3	1999.9	0.01	1999.4	1999.9	0.04
PSA value, ng/mL, mean	6.5	6.1	0.06	6.4	6.0	0.09
Pathologic stage, %						
<T3	79	79	0.9	79	78	0.8
≥T3	21	21		21	22	
Pathologic grade (Gleason sum), %						
<4+3	93	94	0.5	93	93	0.9
≥4+3	7	6		7	7	
Race, %						
White	94	95	0.7	94	97	0.08
Black	2	1		2	1	
Other/Missing	4	4		4	2	
BMI, %						
<25 kg/m^2	38	34	0.1	37	35	0.4
25 - <30 kg/m^2	52	54		53	53	
≥30 kg/m^2	7	9		7	10	
Missing	2	2		2	2	
Weight change >2.2 kg[*], %						
Yes	28	26	0.6	27	28	0.9
No	70	72		70	70	
Missing	2	3		2	2	
Smoking status 1-year post-surgery, %						
Never	54	52	0.7	55	51	0.4
Former	42	46		43	47	
Current	2	1		2	2	
Missing	1	1		1	0	
Family history of PCa, %						
Yes	39	34	0.08	37	35	0.3
No	57	62		58	61	
Missing	5	4		5	4	
Other NSAID use pre-surgery, %						
Yes	18	33	<0.01	36	57	<0.01

No	82	65		64	43	
Missing	0	2		0	0	
Statin use pre-surgery, %						
Yes	16	37	<0.01	24	26	0.2
No	84	63		76	73	
Missing	0	1		0	1	
Type II diabetes, %						
Yes	1	3	0.01	2	3	0.6
No	99	97		98	97	
Cardiovascular disease, %						
Yes	1	6	<0.01	3	3	0.7
No	99	94		97	97	
Arthritis, %						
Yes	3	1	0.04	2	5	<0.01
No	97	99		98	95	

PSA, prostate-specific antigen; BMI, body mass index; PCa, prostate cancer; NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*From five-years pre-surgery to one-year post-surgery

Table 3-13. Aspirin use pre- and post-surgery and prostate cancer recurrence among men who completed the 2007 survey, with time at risk beginning in 2007, Johns Hopkins Hospital Radical Prostatectomy Cohort

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Aspirin Use Pre-Surgery					
Ever Use					
No	25 / 4157	1.00	--	1.00	--
Yes	31 / 2971	1.67	(0.98, 2.85)	2.05	(1.13, 3.72)
Duration of Use					
<1 year	25 / 4248	1.00	--	1.00	--
1-4 years	7 / 722	1.60	(0.69, 3.70)	2.27	(0.93, 5.56)
5-9 years	10 / 682	2.38	(1.14, 4.99)	2.00	(0.92, 4.34)
≥10 years	13 / 1379	1.55	(0.79, 3.04)	2.09	(0.99, 4.40)
Aspirin Use Post-Surgery					
Ever Use					
No	18 / 3189	1.00	--	1.00	--
Yes	39 / 3908	1.69	(0.96, 2.97)	2.22	(1.18, 4.16)
Aspirin Use Pre- & Post-Surgery					
Ever Use					
Never	17 / 2922	1.00	--	1.00	--
Pre- Only	1 / 256	**	**	**	**
Post- Only	8 / 1203	1.11	(0.48, 2.57)	1.44	(0.59, 3.53)
Pre- and Post-	30 / 2677	1.84	(1.01, 3.36)	2.61	(1.31, 5.19)

*Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

**Not enough events to calculate stable HRs and 95% CIs

Table 3-14. Aspirin use pre- and post-surgery and prostate cancer recurrence among men who completed the 2007 survey, with time at risk beginning in 2007, stratified by stage & grade and year of surgery, Johns Hopkins Hospital Radical Prostatectomy Cohort

	Low Stage & Low Grade					High Stage or High Grade				
		Age-Adjusted		Multivariable-Adjusted*			Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	HR	95% CI	HR	95% CI	Events/ Person-years	HR	95% CI	HR	95% CI
Aspirin Use Pre-Surgery										
No	5 / 3214	1.00	--	1.00	--	20 / 943	1.00	--	1.00	--
Yes	8 / 2303	2.15	(0.70, 6.65)	2.54	(0.77, 8.37)	23 / 668	1.64	(0.90, 3.00)	1.91	(0.97, 3.78)
Aspirin Use Post-Surgery										
No	3 / 2435	1.00	--	1.00	--	15 / 754	1.00	--	1.00	--
Yes	11 / 3035	2.78	(0.77, 10.04)	2.93	(0.79, 10.88)	28 / 873	1.65	(0.87, 3.11)	1.92	(0.95, 3.87)

	Year of Surgery <2000					Year of Surgery ≥2000				
		Age-Adjusted		Multivariable-Adjusted**			Age-Adjusted		Multivariable-Adjusted**	
	Events/ Person-years	HR	95% CI	HR	95% CI	Events/ Person-years	HR	95% CI	HR	95% CI
Aspirin Use Pre-Surgery										
No	14 / 2146	1.00	--	1.00	--	11 / 2011	1.00	--	1.00	--
Yes	12 / 1336	1.33	(0.61, 2.88)	1.55	(0.67, 3.57)	19 / 1635	2.07	(0.97, 4.40)	3.08	(1.31, 7.26)
Aspirin Use Post-Surgery										
No	8 / 1473	1.00	--	1.00	--	10 / 1716	1.00	--	1.00	--
Yes	19 / 1998	1.67	(0.73, 3.84)	1.75	(0.74, 4.18)	20 / 1911	1.73	(0.80, 3.73)	2.47	(1.02, 6.01)

*Adjusted for age, year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

**Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

Table 3-15. NA-NSAID use pre- and post-surgery and prostate cancer recurrence among men who completed the 2007 survey, with time at risk beginning in 2007, Johns Hopkins Hospital Radical Prostatectomy Cohort

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
NA-NSAID Use Pre-Surgery					
Ever Use					
No	45 / 5335	1.00	--	1.00	--
Yes	11 / 1748	0.76	(0.39, 1.48)	0.92	(0.46, 1.82)
Duration of Use					
<1 year	45 / 5446	1.00	--	1.00	--
1-4 years	0 / 390	**	**	**	**
5-9 years	2 / 397	**	**	**	**
≥10 years	9 / 750	1.53	(0.75, 3.13)	1.63	(0.78, 3.41)
NA-NSAID Use Post-Surgery					
Ever Use					
No	38 / 5082	1.00	--	1.00	--
Yes	17 / 1971	1.16	(0.65, 2.05)	1.19	(0.66, 2.15)
NA-NSAID Use Pre- & Post-Surgery					
Ever Use					
Never	37 / 4743	1.00	--	1.00	--
Pre- Only	1 / 311	**	**	**	**
Post- Only	7 / 535	1.65	(0.74, 3.70)	1.15	(0.50, 2.67)
Pre- and Post-	10 / 1413	0.92	(0.46, 1.85)	1.16	(0.56, 2.41)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

**Not enough events to calculate stable HRs and 95% CIs

Appendix 3-1. Methods to explore and control for selection bias

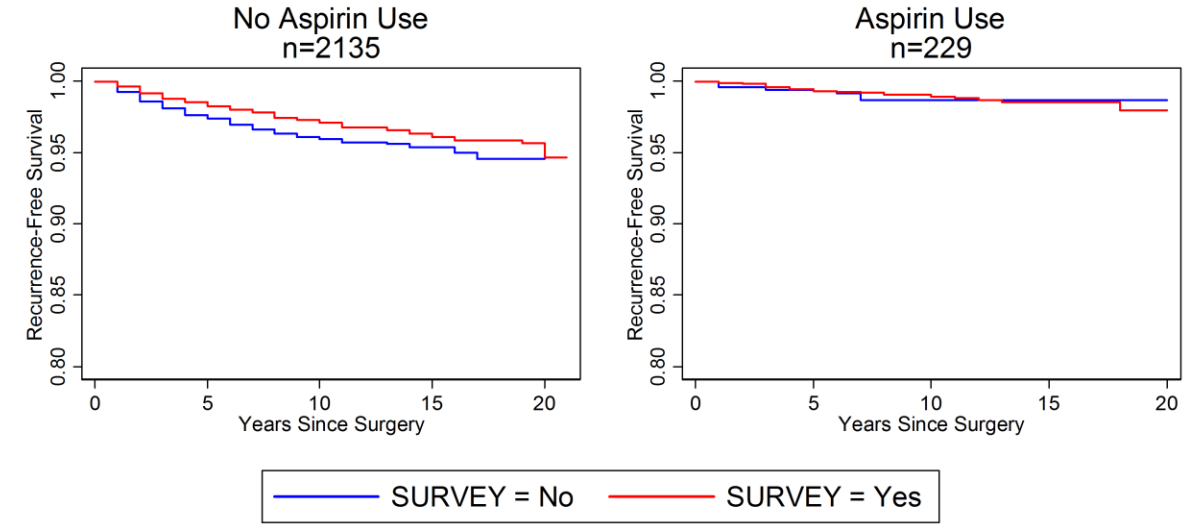
In the analyses utilizing data from the 2007 survey, there was potential for selection bias due to the fact that only men who completed the survey (64% of the original cohort) were included. Men excluded from the analyses included those who died from prostate cancer prior to 2007 (n=22), those who died from other causes prior to 2007 (n=51), those who were lost to follow-up prior to 2007 (n=218), and those who were either mailed the survey and did not respond, or who were not mailed the survey due to residency outside of the U.S. (n=568). Exclusion of these men from the analysis could have led to selection bias if selection was associated with both NSAID use and prostate cancer recurrence, either via direct relationships or common causes, or if the association between selection and recurrence differed by level of NSAID use (96, 97).

We were fortunate to have data on the men who did not complete the 2007 survey, allowing us to explore potential for selection bias. We compared characteristics of men who completed vs. did not complete the survey in Table 3-5. Aspirin and NA-NSAID use at the time of the preoperative consultation did not differ significantly by survey completion, but the percentage of reported aspirin and NA-NSAID was low, and minor differences between survey non-responders and responders (9 vs. 10% for aspirin, 5 vs. 6% for NA-NSAIDs) may have still been meaningful. Survey completion was strongly associated with prostate cancer recurrence, even after adjusting for age, race, and pathologic stage (HR: 1.53, 95% CI: 1.21-1.93). The association between selection and recurrence also appeared to vary by level of aspirin use (Supplemental Figure **3-1**), suggesting that selection bias was possible.

To account for possible selection bias, as a sensitivity analysis, models using the 2007 survey data were re-run using inverse probability of selection weights (IPSW). Predicted probabilities of selection were calculated from a logistic regression model in which survey completion was regressed onto the following covariates: age, year of surgery, age*year of

surgery, race, ethnicity, BMI, family history of prostate cancer, pathologic stage, pathologic Gleason, diagnostic PSA value, medications used at the time of the preoperative consultation (including aspirin, NA-NSAIDs, statins, angiotensin-converting enzyme inhibitors, beta blockers, diuretics, hypertension medications, CVD medications, asthma medications, gastroesophageal reflux disease medications, gout medications, diabetes medications, insulin, and psychiatric medications), comorbidities (CVD, arthritis, hypertension, type II diabetes, other cancers), age*CVD, and year of surgery*CVD. Weights were constructed from the inverse of the predicted probabilities and stabilized using the marginal probability of selection. The weights were then incorporated into Cox proportional hazards regression with robust variance estimation.

As shown in Supplemental Table 3-1, when models were weighted using IPSW, results were very similar to those from the primary analyses presented in Table 3-7 and Table 3-9. This suggests that our results may not have been heavily influenced by selection bias. Alternatively, this could suggest that our model for selection did not sufficiently discriminate between men who did and did not complete the survey (AUC: 0.63) or that the IPSW did not block all backdoor pathways induced by selection. The varying reasons for selection (i.e. death, change of residence out of the U.S., or non-response) made it difficult to model the probability of selection well, and as a result, the analysis with IPSW may still be impacted by selection bias.



Adjusted for age, race, stage, cvd

Supplemental Figure 3-1. Association between survey completion and prostate cancer recurrence, by level of aspirin use at the preoperative consultation

Supplemental Table 3-1. Aspirin and NA-NSAID use pre- and post-surgery and prostate cancer recurrence among men who completed the 2007 survey, with time at risk beginning one-year post-surgery and models weighted using inverse probability of selection weights, Johns Hopkins Hospital Radical Prostatectomy Cohort

		Age-Adjusted		Multivariable-Adjusted*	
	Events/ Person-years	Hazard Ratio	95% CI	Hazard Ratio	95% CI
Aspirin Use Pre-Surgery					
No	78 / 9563	1.00	--	1.00	--
Yes	69 / 6619	1.14	(0.82, 1.58)	1.26	(0.89, 1.78)
Aspirin Use Post-Surgery					
No	57 / 7070	1.00	--	1.00	--
Yes	90 / 9032	1.13	(0.81, 1.59)	1.33	(0.92, 1.94)
NA-NSAID Use Pre-Surgery					
No	115 / 12246	1.00	--	1.00	--
Yes	30 / 3829	0.86	(0.58, 1.29)	1.02	(0.68, 1.52)
NA-NSAID Use Post-Surgery					
No	97 / 11456	1.00	--	1.00	--
Yes	47 / 4561	1.26	(0.89, 1.78)	1.29	(0.91, 1.82)

NA-NSAID, non-aspirin nonsteroidal anti-inflammatory drug

*Adjusted for age, stage (<T3, ≥T3), grade (<4+3, ≥4+3), year of surgery, race (white, non-white), smoking status one-year after surgery, BMI one-year after surgery (<25, 25-30, ≥30 kg/m^2), weight change >2.2 kg, ever use of statins pre-surgery, cardiovascular disease, and arthritis

Chapter 4. Aspirin Use and Inflammation and Immune Cells in Benign Prostate Tissue

Abstract

Background: Regular aspirin use is inversely associated with prostate cancer risk in meta-analyses of observational studies, and is hypothesized to act by reducing inflammation within the prostate. However, a direct link between aspirin use and intraprostatic inflammation has not been established. This study investigated the association between aspirin use and the presence and extent of intraprostatic inflammation, and the abundance of specific immune cell types, in benign prostate tissue of men from the placebo arm of the Prostate Cancer Prevention Trial (PCPT).

Methods: This cross-sectional study included a subset of men from the placebo arm of PCPT, sampled for a previous case-control study of inflammation and lower urinary tract symptoms (LUTS). Aspirin use was ascertained at trial entry (1993-1997). The presence and extent of inflammation was assessed, and markers of specific immune cell types (CD4, CD8, FoxP3, CD68, c-KIT) were scored, in slides from end-of-study prostate biopsies seven years later. Logistic regression was used to estimate associations between aspirin use and each outcome, adjusted for potential confounders.

Results: There were 357 men included in this study. Forty-three percent reported aspirin use at trial entry. The prevalence of intraprostatic inflammation was similar for aspirin users and non-users (66% vs. 67%, respectively), but aspirin users were less likely to have inflammation in all of their biopsy cores (OR: 0.31, 95% CI: 0.11-0.91). Using the median score as the reference, aspirin users were also more likely to exhibit low levels of FoxP3, a marker of T regulatory cells (Tregs) (OR: 3.39, 95% CI: 1.06-10.91). Other immune cell types did not markedly differ in prostate tissue of aspirin users and non-users. Similar results were observed when analyses were restricted to LUTS controls (N=86), men without prostate cancer detected at the end-of-study biopsy (N=295), and men with a PSA <4 ng/mL prior to the end-of-study biopsy (N=317).

Conclusions: In this study, aspirin use was inversely associated with the extent of inflammation and the abundance of Tregs within the prostate. This study provides suggestive evidence that intraprostatic inflammation may mediate the relationship between aspirin use and prostate cancer risk.

Introduction

A growing body of evidence supports that chronic inflammation contributes to prostate carcinogenesis (29). Intraprostatic inflammation is highly prevalent in older men with elevated prostate-specific antigen (PSA), abnormal digital-rectal examination (DRE), or benign prostatic hyperplasia (98-100), and in older men without prostate symptoms (101-103). Chronic inflammation in the prostate could arise through exposure to infectious agents, environmental toxins, dietary factors, or hormones, and could contribute to carcinogenesis via release of mutagenic reactive oxygen species or pro-proliferative and angiogenic cytokines (29).

Despite biological plausibility, establishing a direct epidemiologic link between chronic intraprostatic inflammation and prostate cancer has been challenging, in part due to the difficulty of measuring inflammation in benign prostate tissue. Inflammation can be assessed in tissue collected for clinical indication (i.e. elevated PSA, an indicator of possible prostate cancer), but intraprostatic inflammation may also contribute to rising PSA levels (65). As a result, tissue collected for indication is enriched for inflammation, regardless of prostate cancer status. Of the few studies that have examined inflammation in men without indication for biopsy, one case-control study reported a positive association between inflammation in at least biopsy core and overall and high-grade prostate cancer (102), while a prospective study reported a positive trend between increasing mean percentage area of tissue with inflammation and odds of subsequent prostate cancer diagnosis (103).

If intraprostatic inflammation is causally associated with prostate cancer, then interventions to decrease inflammation, such as use of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs), could plausibly reduce prostate cancer risk. In fact, regular use of aspirin and other non-aspirin NSAIDs are inversely associated with prostate cancer risk according to meta-analyses of observational studies (41, 42). Evidence of a relationship between NSAID 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 use and the prevalence and extent of inflammation, as well as the abundance of specific immune cell types, in benign prostate tissue collected without indication from men in the placebo arm of the Prostate Cancer Prevention Trial (PCPT). We hypothesized that aspirin use would be associated with a decreased prevalence and extent of intraprostatic inflammation and a differing abundance of specific immune cells.

Methods

Study Population

This study included 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 (104). 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 ≥4 ng/mL 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 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 (101). Case-control sets for LUTS incidence and progression were developed based on the IPSS 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 the prior study (101).

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.

Measurement of Aspirin Use and Other Covariates

At enrollment, baseline demographics, medical, and lifestyle factors were collected via questionnaire. Current medication use was assessed with both closed (i.e. “Do you use aspirin?”) and open-ended questions, and responses were used to categorize individuals as aspirin users or non-users at trial entry. Baseline weight and height were measured using standardized protocols, and weight was re-measured annually. From these measurements, 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, and serum PSA was measured in samples collected from baseline and annual follow-ups at a central laboratory.

Measurement of Intraprostatic Inflammation and Immune Cell Markers

This study used data previously collected for the LUTS nested case-control study (101). 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 by two pathologists. In biopsy cores with both tumor and benign tissue, only benign tissue was reviewed. The 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 by immunologists and pathologists as cell types that were expected to be observed within the prostate, based on prior studies from tissue collected for indication. Each slide was visually reviewed and scored by a pathologist on a scale of 0-4, with 0 indicating no cells identified and 4 indicating an extensive number of cells.

Statistical Analysis

Characteristics of current users and non-users of aspirin at trial entry 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 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 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 use and inflammation/immune cells after adjusting for potential confounders. The presence of inflammation was modeled using logistic regression, and the extent of inflammation and abundance of immune cell markers were modeled using nominal polytomous logistic regression. Ordinal logistic regression was also attempted, but the proportional odds assumption did not hold. Multivariable models were adjusted for age (continuous), race (white, non-white), 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, restricted to the LUTS controls, including 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 (N=86). Analyses were also repeated after restricting to men who were not diagnosed with prostate cancer on the end-of-study biopsy (N=295), and men with a PSA <4 ng/mL immediately prior to the end-of-study biopsy (N=317). Men with a PSA ≥4 ng/mL could have been included in the primary analysis 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. 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 stricter 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, 155 (43%) reported aspirin use at trial entry. Other baseline characteristics of the

study population, by aspirin use at trial entry, are displayed in Table 4-1. Of note, aspirin users were less likely than non-users to have a college education (47% vs. 57%), less likely to be never smokers (25% vs. 40%), and more likely to be very active (16% vs 9%). At the end-of-study biopsy, 21% of aspirin users and 15% of non-users were diagnosed with prostate cancer.

A median of 4 biopsy cores per person 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 (66% vs. 67% for aspirin users and non-users, respectively, Table 4-2). However, the extent of inflammation appeared lower among users. Specifically, only 4% of aspirin users compared to 10% of non-users had inflammation in all biopsy cores examined, (Table 4-2). Consistent with these univariable results, aspirin use was not associated with the presence of inflammation (OR: 0.91, 95% CI: 0.57-1.47), but was inversely associated with odds of having all versus no biopsy cores with inflammation (OR: 0.31, 95% CI: 0.11-0.91, Table 4-3) after multivariable adjustment.

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: 0.5-14) were stained for CD4, CD8, FoxP3, and CD68 cells, and a median of 2.5 cores per person (range: 0.5-10) were stained for c-Kit cells. For all markers, the median and mode weighted average score were 1. In univariable analyses, aspirin users appeared more likely to have a low abundance (i.e. scores <1) of CD4, CD8, FoxP3, and CD68 cells, and high abundance (i.e. scores >1) of c-KIT cells compared to non-users (Table 4-2, Figure 4-1). The difference between users and non-users in FoxP3 abundance was statistically significant. Compared with medium abundance, aspirin use remained positively associated with odds of low FoxP3 abundance (OR: 3.39, 95% CI: 1.06-10.91) and inversely associated with odds of high FoxP3 abundance (OR: 0.62, 95% CI: 0.35-1.08, Table 4-4) after multivariable adjustment.

Similar patterns were observed for all outcomes in sensitivity analyses restricted to LUTS controls (Supplemental Table 4-1), men without prostate cancer (Supplemental Table

4-2), and men with a PSA below 4 ng/mL at the end of PCPT follow-up (Supplemental Table 4-3).

Discussion

This study examined associations between aspirin use and the overall presence and extent of inflammation, as well as markers of specific immune cells, in benign prostate tissue. We found that the proportion of men with at least one biopsy core with inflammation was similar among aspirin users and non-users, but that aspirin users were less likely to have inflammation in all biopsy cores examined. The abundance of certain immune cell markers also differed by level of aspirin use. Specifically, FoxP3, a marker of Tregs, appeared less abundant in benign prostate tissue of aspirin users as compared to non-users.

To our knowledge, this is the first study to examine the relationship between aspirin use and inflammation in the prostate of men without biopsy indication. Other studies have examined aspirin use in relation to circulating markers of inflammation (54-64), but circulating markers are not necessarily indicative of inflammation within the prostate, where inflammation pertaining to prostate cancer is most etiologically relevant. The magnitude of the association was modest, with 4% of aspirin users vs. 10% of non-users exhibiting inflammation in all biopsy cores examined, and the same percentage of users and non-users having 0% of cores with inflammation. Whether these differences are clinically important is unknown, but this study provides early evidence that anti-inflammatory effects of aspirin may be observable in the prostate.

We also observed a lower abundance of Tregs in aspirin users as compared to non-users. This finding is plausible given that aspirin, via inhibition of COX-2, inhibits synthesis of prostaglandin E_2, which has been shown to promote development of Tregs (105). Tregs downregulate the immune system and may block T cells from mounting an effective anti-tumor

response (70, 106, 107). In accordance with this proposed pro-tumorigenic role, studies have found Tregs to be more prevalent in tumor vs. benign prostate tissue from the same patients, and in peripheral blood of prostate cancer vs. non-prostate cancer donors (108). Greater numbers of epithelial Tregs have also been positively associated with Gleason sum and pathologic stage (109). On the other hand, Tregs could also reduce cancer-promoting inflammation, which could inhibit cancer development (110). Thus, while our study suggests that aspirin 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.

Limitations of this study include the small sample size and cross-sectional study design. Aspirin use was ascertained at trial entry, seven years prior to the end-of-study biopsy, but whether inflammation was already present at baseline was unknown. Information was not available on history of aspirin use prior to trial enrollment, on aspirin use initiated during the trial period, or on whether men stopped taking aspirin before the end-of-study biopsy, and so some misclassification of exposure may have occurred. Furthermore, data were not collected on aspirin dose, frequency of use, or duration of use, and we were thus unable to examine how these variations in aspirin use influenced inflammation or immune cell markers. Finally, for each immune cell marker, IHC was unsuccessful for some individuals (range: 9-17%) due to unavailability of slides, insufficient tissue on slides, or problems with staining. As a result, the sample size varied across analyses of each marker.

This study also has several notable strengths. There was detailed assessment of multiple outcomes, including both the presence and extent of inflammation and markers of innate and adaptive immune cells. Such detailed assessment allowed us to not only quantify 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 using validated, standardized protocols, thereby minimizing opportunities for errors.

Importantly, 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 elevated PSA and suspected prostate cancer are included.

This study provides direct population-based evidence that aspirin use may influence inflammation and the immune cell milieu within the prostate of men without indication for biopsy. Further research is needed to confirm these observational findings, but results from this study support our hypothesis that intraprostatic inflammation may mediate the relationship between regular aspirin use and prostate cancer risk.

Grant Support and Acknowledgments

Lauren Hurwitz was supported by an NCI National Research Service Award (T32 CA09314). This study used data previously collected and funded by the following NIH grants: NIDDK P50 DK082998, NCI U10 CA37429, NCI UM1 CA182883, and P50 CA58236. The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We thank the PCPT investigators and JHU colleagues who were involved in the research that gave rise to this data.

Chapter 4 Tables

Table 4-1. Characteristics[*] of a subset[**] of men from the placebo arm of PCPT, by aspirin use at trial entry

	No Aspirin Use	Aspirin Use
N	202	155
Age at biopsy, median	69	70
Race, %		
White	96	96
Non-white	4	4
College education, %	57	47
Diabetes, %	5	6
BMI (kg/m^2), median	26.8	27.4
Smoking status, %		
Current	8	7
Former	51	68
Never	40	25
Pack-years of smoking, median[*]	21.3	23.9
Physical activity, %		
Sedentary	23	22
Light	13	16
Moderate	54	46
Very active	9	16
Missing	1	0
Daily intake, median		
Energy (kcal)	2062	2016
Vegetables (servings/day)	1.9	2.0
Total fat (g)	71.9	69.1
Polyunsaturated fatty acids (g)	14.6	14.7
Total protein (g)	83.7	84.4
Red meat (servings/week)	3.5	3.9
Alcoholic beverages (drinks/day)	0.3	0.2
PSA at biopsy (ng/mL), median	1.6	1.4
Prostate cancer diagnosis, %	15	21

BMI, body mass index; PSA, prostate-specific antigen

[*]All variables were assessed at trial entry, with the exception of age, PSA, and prostate cancer diagnosis

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

Table 4-2. Presence and extent of intraprostatic inflammation[*] and abundance of immune cell markers[**] by aspirin use at baseline, in a subset[***] of men from the placebo arm of PCPT

	No Aspirin Use	Aspirin Use	*p*-value[****]
≥1 core with inflammation, %	67	66	0.8
Percent of cores with inflammation, %			
None	34	34	0.3
Some	55	62	
All	10	4	
CD4, %			
Low	16	21	0.2
Medium	42	42	
High	42	37	
CD8, %			
Low	9	12	0.3
Medium	69	70	
High	22	18	
FoxP3, %			
Low	3	8	**0.01**
Medium	66	70	
High	31	21	
CD68, %			
Low	13	15	0.2
Medium	75	76	
High	13	9	
c-KIT, %			
Low	11	7	0.1
Medium	74	72	
High	15	21	

[*]Extent of inflammation categorized as: none: 0% of cores with inflammation, some: >0% but <100% of cores with inflammation, all: 100% of cores with inflammation

[**]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)

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

[****]p-value from the chi-square test (for dichotomous variables) or Cochran-Armitage trend test (for ordinal variables)

Table 4-3. Associations between aspirin use and the presence and extent of intraprostatic inflammation in a subset* of men from the placebo arm of PCPT

	At least one core with inflammation		
	No	Yes	
# aspirin users / non-users	53 / 67	102 / 135	
Model 1 OR (95% CI)	Ref	0.96 (0.61-1.49)	
Model 2 OR (95% CI)	Ref	0.87 (0.55-1.37)	
Model 3 OR (95% CI)	Ref	0.91 (0.57-1.47)	
	Percent of cores with inflammation		
	None	Some	All
# aspirin users / non-users	53 / 69	96 / 112	6 / 21
Model 1 OR (95% CI)	Ref	1.12 (0.71-1.75)	**0.37 (0.14-0.99)**
Model 2 OR (95% CI)	Ref	1.02 (0.64-1.63)	**0.31 (0.11-0.84)**
Model 3 OR (95% CI)	Ref	1.04 (0.64-1.69)	**0.31 (0.11-0.91)**

*From a case-control study of LUTS nested in the placebo arm of PCPT. 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)

Bolded values are statistically significant

Table 4-4. Associations between aspirin use at baseline and the abundance of immune cell markers[*] in a subset[**] of men from the placebo arm of PCPT

	CD4 (n=321)		
	Low	Medium	High
# aspirin users / non-users	31 / 28	61 / 73	55 / 73
Model 1 OR (95% CI)	1.33 (0.72-2.45)	Ref	0.90 (0.55-1.47)
Model 2 OR (95% CI)	1.38 (0.74-2.56)	Ref	0.87 (0.53-1.43)
Model 3 OR (95% CI)	1.43 (0.75-2.74)	Ref	0.90 (0.54-1.51)
	CD8 (n=326)		
	Low	Medium	High
# aspirin users / non-users	18 / 16	104 / 122	27 / 39
Model 1 OR (95% CI)	1.32 (0.64-2.72)	Ref	0.81 (0.47-1.42)
Model 2 OR (95% CI)	1.34 (0.65-2.77)	Ref	0.78 (0.44-1.36)
Model 3 OR (95% CI)	1.18 (0.55-2.51)	Ref	0.73 (0.41-1.31)
	FoxP3 (n=315)		
	Low	Medium	High
# aspirin users / non-users	12 / 5	100 / 115	30 / 53
Model 1 OR (95% CI)	2.76 (0.94-8.10)	Ref	0.65 (0.39-1.10)
Model 2 OR (95% CI)	**3.30 (1.10-9.89)**	Ref	0.60 (0.35-1.03)
Model 3 OR (95% CI)	**3.39 (1.06-10.91)**	Ref	0.62 (0.35-1.08)
	CD68 (n=325)		
	Low	Medium	High
# aspirin users / non-users	23 / 22	113 / 132	13 / 22
Model 1 OR (95% CI)	1.22 (0.65-2.31)	Ref	0.69 (0.33-1.43)
Model 2 OR (95% CI)	1.16 (0.61-2.21)	Ref	0.67 (0.32-1.39)
Model 3 OR (95% CI)	1.05 (0.53-2.06)	Ref	0.64 (0.30-1.38)
	c-Kit (n=297)		
	Low	Medium	High
# aspirin users / non-users	9 / 18	93 / 125	27 / 25
Model 1 OR (95% CI)	0.67 (0.29-1.56)	Ref	1.45 (0.79-2.66)
Model 2 OR (95% CI)	0.63 (0.27-1.49)	Ref	1.44 (0.78-2.66)
Model 3 OR (95% CI)	0.51 (0.20-1.26)	Ref	1.44 (0.76-2.71)

[*]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)

[**]From a case-control study of LUTS nested in the placebo arm of the PCPT. 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, black), BMI (continuous), smoking status (current, former, never), physical activity (sedentary, light, moderate, active), education (college, no college), diabetes (yes, no)

Bolded values are statistically significant

Chapter 4 Figures

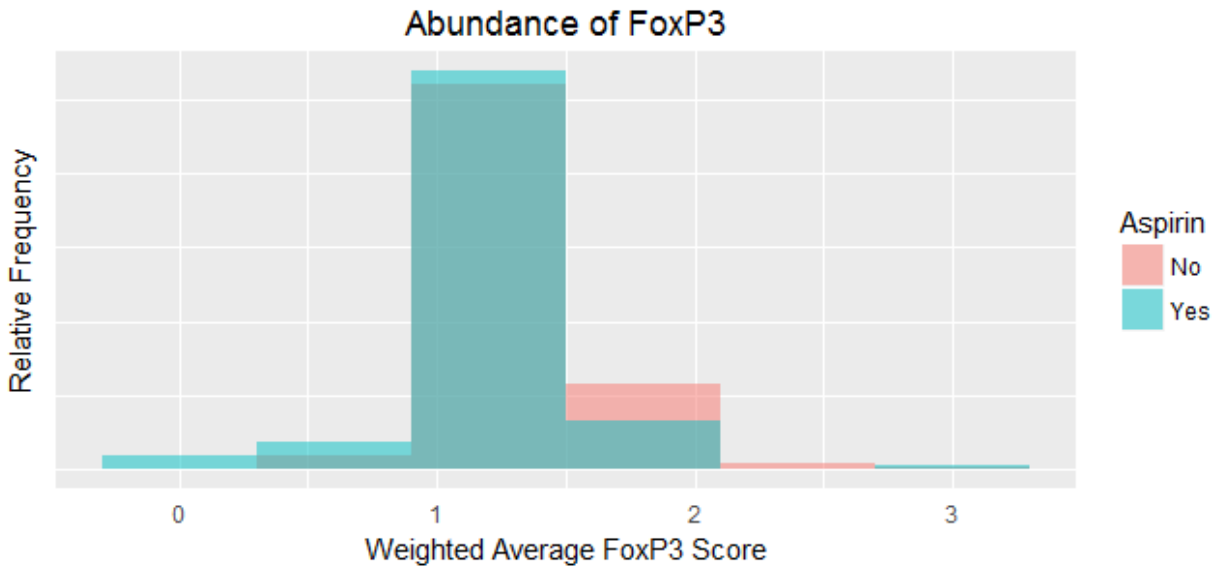


Figure 4-1. Abundance of FoxP3 in aspirin users and non-users. As shown in the overlaid histograms above, the distribution of FoxP3 was similar overall across level of aspirin use. However, there was more weight in the lower end of the distribution for aspirin users, and more weight in the upper end of the distribution for aspirin non-users.

Appendix 4-1. Supplemental Tables

Supplemental Table 4-1. Presence and extent of intraprostatic inflammation[*] and abundance of immune cell markers[**] by aspirin use at baseline, in a subset[***] of men from the placebo arm of PCPT who did not have LUTS[****], n=86

	No Aspirin Use	Aspirin Use	*p*-value[*****]
≥1 core with inflammation, %	67	63	0.8
Percent of cores with inflammation, %			
None	33	37	0.2
Some	56	63	
All	11	0	
CD4, %			
Low	11	20	0.4
Medium	50	46	
High	39	34	
CD8, %			
Low	8	11	0.8
Medium	77	73	
High	15	16	
FoxP3, %			
Low	3	9	0.2
Medium	71	74	
High	26	17	
CD68, %			
Low	13	14	0.8
Medium	77	78	
High	10	8	
c-KIT, %			
Low	11	3	0.1
Medium	82	80	
High	8	17	

[*]Extent of inflammation categorized as: none: 0% of cores with inflammation, some: >0% but <100% of cores with inflammation, all: 100% of cores with inflammation

[**] 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)

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

[****]LUTs controls included men with IPSS<8 at baseline and at year 7 (n=41) and men with IPSS<8 at baseline and baseline to year 7 slope <25th percentile (n=45)

[*****]p-value from the chi-square test (for dichotomous variables) or Cochran-Armitage trend test (for ordinal variables)

Supplemental Table 4-2. Presence and extent of intraprostatic inflammation[*] and abundance of immune cell markers[**] by aspirin use at baseline, in a subset[***] of men from the placebo arm of PCPT without cancer detected at the end-of-study biopsy, n=295

	No Aspirin Use	Aspirin Use	*p*-value[****]
≥1 core with inflammation, %	69	64	0.4
Percent of cores with inflammation, %			
None	32	36	0.1
Some	57	60	
All	11	4	
CD4, %			
Low	16	23	0.2
Medium	43	40	
High	41	37	
CD8, %			
Low	7	12	0.3
Medium	72	71	
High	20	18	
FoxP3, %			
Low	3	9	**<0.01**
Medium	66	74	
High	31	18	
CD68, %			
Low	14	18	0.2
Medium	76	74	
High	11	8	
c-KIT, %			
Low	11	8	0.3
Medium	74	73	
High	15	19	

[*]Extent of inflammation categorized as: none: 0% of cores with inflammation, some: >0% but <100% of cores with inflammation, all: 100% of cores with inflammation

[**]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)

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

[****]p-value from the chi-square test (for dichotomous variables) or Cochran-Armitage trend test (for ordinal variables)

Supplemental Table 4-3. Presence and extent of intraprostatic inflammation* and abundance of immune cell markers by aspirin use at baseline, in a subset*** of men from the placebo arm of PCPT without clinical indication for biopsy, n=317**

	No Aspirin Use	Aspirin Use	*p*-value****
≥1 core with inflammation, %	67	66	0.8
Percent of cores with inflammation, %			
None	34	34	0.4
Some	56	62	
All	10	4	
CD4, %			
Low	15	23	0.2
Medium	46	41	
High	39	36	
CD8, %			
Low	9	13	0.3
Medium	70	69	
High	22	19	
FoxP3, %			
Low	3	8	**0.05**
Medium	69	72	
High	28	20	
CD68, %			
Low	12	16	0.3
Medium	77	75	
High	11	9	
c-KIT, %			
Low	10	8	0.1
Medium	77	72	
High	13	21	

*Extent of inflammation categorized as: none: 0% of cores with inflammation, some: >0% but <100% of cores with inflammation, all: 100% of cores with inflammation

**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)

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

****p-value from the chi-square test (for dichotomous variables) or Cochran-Armitage trend test (for ordinal variables)

Chapter 5. Conclusion

Key findings, implications, and future directions

The purpose of this dissertation was to address research gaps pertaining to the role of aspirin and non-aspirin nonsteroidal anti-inflammatory drugs (NA-NSAIDs) in prostate cancer prevention and control. Specifically, this dissertation aimed to determine whether aspirin or NA-NSAID use might protect against prostate cancer development, and particularly development of lethal prostate cancer. This dissertation also investigated whether NSAID use among men already diagnosed with prostate cancer might reduce the risk of prostate cancer recurrence or case-fatality. We hypothesized that chronic inflammation within the prostate might mediate these effects, and tested the biological plausibility of this mechanism by comparing intraprostatic inflammation and markers of immune cells in aspirin users and non-users. The overarching goal of these studies was to inform strategies for the primary and tertiary prevention of lethal prostate cancer.

Aspirin and NA-NSAID use and prostate cancer incidence and mortality

The first aim investigated the relationship between aspirin and NA-NSAID use and prostate cancer incidence, including incidence of lethal disease, and prostate cancer mortality in the Atherosclerosis Risk in Communities (ARIC) study. While NA-NSAID use was not associated with total, lethal, or fatal prostate cancer, aspirin use was inversely associated with both lethal and fatal prostate cancer. These results are consistent with two previous cohort studies, which also reported inverse associations between aspirin use and lethal prostate (43, 44). In ARIC, the magnitude of the associations was striking, with aspirin users exhibiting a 42% (95% confidence interval [CI]: 5-65%) lower risk of lethal prostate cancer relative to non-users, even after adjusting for potential confounders. Importantly, the magnitude of the association was similar for both white and black men, suggesting that aspirin may protect against lethal prostate

cancer regardless of race and the underlying sociocultural and genetic factors that contribute to current prostate cancer racial disparities.

While these results are promising, the number of events in our analysis was small, and larger studies are needed to confirm our results and produce more precise point estimates. Given the observational nature of our study and similar studies, replication of these findings across multiple study populations is also needed to build support for a causal relationship. With additional research, increasingly specific questions should be addressed, such as how aspirin dose, frequency of use, duration of use, and age at initiation of use affect risk of lethal prostate cancer. Our study suggests that aspirin may only be protective against lethal prostate cancer if used regularly for cardiovascular disease (CVD) prevention (i.e. daily use of low-dose aspirin), but this finding is preliminary and requires further investigation. Answering these questions may require pooling of data from existing observational studies or randomized clinical trials (RCTs) of aspirin and other disease outcomes, if sufficient data are available, or creation of entirely new, large-scale cancer epidemiology cohorts, which could take several decades. Answering these finer questions is necessary, however, before results can be translated into clinical practice.

Additional research could also help to determine precisely who might benefit most from aspirin chemoprevention. For example, genetic factors that influence aspirin absorption, distribution, metabolism, and excretion might make individuals more likely to benefit from aspirin use, or more susceptible to its harms (81). For colorectal cancer (CRC), certain single nucleotide polymorphisms (SNPs) have been found to modify associations between NSAID use and CRC risk (111); these SNPs might modify the association between aspirin use and lethal prostate cancer as well. Aspirin might also be more effective among individuals with other lethal prostate cancer risk factors. Aspirin use has been shown to lessen the effect of obesity on CRC among individuals with Lynch syndrome (112) and to reduce risk of gastric cancer among individuals treated for *Helicobacter pylori* infection (113). For lethal prostate cancer, aspirin might help counteract effects of obesity or cigarette smoking, as these risk factors are

hypothesized to contribute to lethal prostate cancer, in part, by generating chronic inflammatory states. Given the known harms of regular aspirin use, including risk of gastrointestinal bleeding and hemorrhagic stroke, identifying finer subgroups of men most likely to benefit would help to better tailor recommendations and minimize harms.

If additional studies confirm the inverse association between aspirin use and lethal prostate cancer, these findings may eventually help inform guidelines for regular aspirin use. In the general population, regular aspirin use is currently recommended for certain individuals at increased risk of CVD (39). Specifically, in 2016, the United States Preventive Services Task Force (USPSTF) recommended low-dose aspirin (grade: B) for individuals ages 50-59 with a 10% or greater 10-year risk of CVD, a life expectancy of at least 10 years, no increased risk of bleeding, and willingness to take aspirin daily for at least 10 years (39). These guidelines considered benefits of aspirin pertaining to CVD and CRC, but not other cancer types due to limitations in the evidence (114). Eventually, if studies consistently observe inverse associations between aspirin use and lethal prostate cancer, and if these findings are incorporated into risk-benefit calculations, the benefits of regular aspirin use may begin to outweigh the harms for additional segments of the general population. Moreover, even if the risk-benefit balance for the general population is not meaningfully altered, the balance may change for certain high-risk individuals. Ideally, any new or updated guidelines would be based on evidence from randomized controlled trials (RCTs), but given issues with feasibility and lack of clinical equipoise, an RCT of aspirin for primary prevention of lethal prostate cancer is unlikely, and well-designed observational studies are needed to develop this burgeoning evidence base.

Aspirin and NA-NSAID use and prostate cancer recurrence and case-fatality

The second aim of this dissertation investigated the relationship between aspirin and NA-NSAID use and prostate cancer outcomes after prostate cancer diagnosis and treatment.

Among men diagnosed with prostate cancer in the ARIC study, current aspirin use at the visit prior to diagnosis was inversely associated with risk of dying of prostate cancer (HR: 0.45, 95% CI: 0.22-0.94). In contrast, in the Johns Hopkins Hospital (JHH) study, aspirin use pre- and post-surgery were not inversely associated with prostate cancer recurrence, and may have been associated with an increased risk. Findings for NA-NSAID use were null in both study populations. Other studies that have examined pre- or post-diagnostic aspirin and NA-NSAID use and prostate cancer case-fatality have also reported mixed results (44, 46-51, 53, 78, 79, 115).

There are several possible explanations for these seemingly conflicting results. First, the ARIC and JHH studies included different measures of aspirin use. In ARIC, the exposure of interest was current aspirin use at the study visit prior to prostate cancer diagnosis (median = 5.7 years prior to diagnosis). In JHH, aspirin use was assessed at the time of the preoperative consultation (immediately after diagnosis), pre-surgery (which may have been before or after diagnosis), and post-surgery. It is possible that aspirin may only be protective at certain stages in the natural history of prostate cancer, and that measurement of aspirin in ARIC overlapped the etiologically relevant time window of exposure while measurement of aspirin in JHH did not. The two studies also examined different outcomes. In ARIC, the primary outcome was prostate cancer case-fatality, while in JHH, the primary outcome was prostate cancer recurrence, which was defined as a composite outcome but included mostly biochemical recurrences (62%) and local recurrences (8%). Biochemical recurrence is an early indicator of poor outcomes, but not all biochemically recurrent prostate cancers become metastatic and/or fatal. The inverse association in ARIC, contrasted with the null associations in JHH, could thus be explained if aspirin reduces risk of distant metastasis but not local recurrence or regional spread.

There were also key differences in the study populations. In ARIC, the study population skewed older (mean age at diagnosis = 69) and included white and black men diagnosed with prostate cancer of any stage or grade. In contrast, men in the JHH study were mostly white, and

all were good surgical candidates, i.e. younger (mean age at diagnosis = 56) with clinically localized disease at diagnosis. Differences in results could thus be driven by potential differences in the effect of aspirin by age, race, or disease stage at diagnosis. The two study populations also received different prostate cancer treatments. In ARIC, men could have received any type of treatment from any healthcare provider. Because treatment is associated with survival, at least for advanced prostate cancer, and because factors such as socioeconomic status may be associated with both aspirin use and access to of or quality of treatment, the association between aspirin use and case-fatality may have been confounded in ARIC. In contrast, in the JHH study, all men underwent surgery by the same surgeon at the same hospital, and so while generalizability was more limited, confounding by access to care was not a concern. Instead, the null findings in this study could indicate that aspirin does not confer any added benefit for men with localized prostate cancer treated surgically, who already have a good prognosis.

Due to these lingering uncertainties, additional research is still needed to elucidate whether aspirin used pre-diagnostically or post-diagnostically may reduce risk of poor prostate cancer outcomes. Studies of pre-diagnostic aspirin use are needed to replicate our findings from ARIC and determine whether aspirin may act early in the disease pathogenesis to prevent progression to more lethal disease. Studies of post-diagnostic aspirin use are needed to confirm our findings from JHH and discern whether recommending aspirin to men already diagnosed with prostate cancer may improve prostate cancer outcomes, make outcomes worse, or have no impact. In comparison to studies of aspirin use for primary prevention of lethal prostate cancer, studies of post-diagnostic aspirin use and prostate cancer recurrence are more amenable to an RCT, given the uncertain risk-benefit balance for this population, the higher rate of recurrence events, and the shorter duration of follow-up needed to observe these events.

Enrollment for one such trial, Add-Aspirin, is currently underway (116). For this trial, four parallel cohorts of individuals undergoing treatments for breast, colorectal, gastro-esophageal,

or prostate cancer are being randomized in a 1:1:1 ratio to 100 mg aspirin, 300 mg aspirin, or placebo, to be taken daily for at least five years. The prostate cancer arm of this trial includes men treated with surgery or radiotherapy for early-stage prostate cancer in the United Kingdom and India, and will examine biochemical recurrence-free survival as its primary endpoint. The recruitment goal for the prostate cancer arm is 2,120 participants; as of February 2018, 865 participants had been registered and 696 had been randomized (117). Though it will be several years before results are released, and though this trial will not be able to speak to the effects of aspirin on advanced-stage disease, or on outcomes worse than biochemical recurrence, this trial may at least provide more definitive answers as to whether post-diagnostic aspirin use reduces risk of biochemical recurrence for men with early-stage prostate cancer. For now, however, there is insufficient evidence to recommend aspirin or NA-NSAIDs for the tertiary prevention of lethal prostate cancer.

Aspirin use and inflammation and immune cell profiling in benign prostate tissue

For the third aim of this dissertation, associations between aspirin use and the presence and extent of inflammation in benign prostate tissue, as well as abundance of specific immune cells, were assessed in a subset of men from the placebo arm of the Prostate Cancer Prevention Trial (PCPT). Aspirin use at trial entry was not associated with the presence of any inflammation in benign tissue from the end-of-study biopsy, but the extent of inflammation was moderately lower in aspirin users as compared to non-users. Aspirin users also had moderately lower scores for FoxP3 cells, indicating lower abundance of Tregs. Importantly, these associations were observed among men without a clinical indication for prostate biopsy, and in sensitivity analyses, among men without prostate cancer, with low PSA, and with low or very low LUTS, suggesting that the presence of preexisting prostate conditions was not biasing results. This study provides evidence that aspirin use may alter inflammation and the immune

cell milieu within the prostate, which could be a possible mechanism linking aspirin use to prostate cancer development and progression.

This research is ongoing and will be updated as additional data become available. Specifically, we will soon be able to incorporate data on aspirin use initiated during the trial, which will allow us to better classify individuals as aspirin users or non-users and examine the impact of duration of use during the trial period. Information on use of statins during the trial is also forthcoming. Statins are commonly used concurrently with aspirin, including in PCPT (118), and may also alter the extent of inflammation and abundance of immune cell types within the prostate, and so future analyses will adjust for use of statins to reduce potential confounding.

Ideally, our findings will also be replicated in additional study populations. To obtain unbiased estimates, these additional studies will need access to prostate tissue collected without clinical indication, since indications for prostate tissue removal (i.e. elevated PSA, benign prostatic hyperplasia, prostate cancer) are associated with intraprostatic inflammation (65, 102, 103) and also possibly aspirin use. Replication in observational studies may thus not be possible, as prostate tissue is not typically collected without clinical indication. A small trial could be designed to assess the effect of randomization to aspirin on inflammation and immune cell markers, assuming men would agree to undergo a not-for-cause biopsy. Such a trial would face several barriers, including high costs and challenges with recruitment, but would help to bolster support for a causal relationship between aspirin use and intraprostatic inflammation. A much larger trial could also continue to follow men for incident total or lethal prostate cancer so that a formal mediation analysis could be conducted. However, this trial would need to biopsy participants at baseline to exclude men with prevalent prostate cancer and again after aspirin treatment, to follow participants for several years to observe incident events. This trial would also need to enroll huge numbers of participants to be adequately powered, and as a result, such a trial is likely unfeasible.

Understanding the relationship between aspirin use and intraprostatic inflammation and immune cells might also help inform interventions at other stages of the prostate cancer care continuum. For example, for treatment of advanced prostate cancer, there is currently great interest and promise in cancer immunotherapies, include vaccines such as Provenge and checkpoint inhibitors. The response rate for immunotherapies has been low for many cancer types, and particularly for prostate cancer. However, there is pre-clinical evidence from melanoma mouse models that aspirin might enhance the efficacy of checkpoint inhibitors (119). Pre-clinical work also suggests that aspirin may act synergistically with other therapies for advanced cancer, such as kinase inhibitors used to treat RAS-mutant cancers (120). These findings are not directly applicable to prostate cancer, since prostate cancers respond poorly to checkpoint inhibitors and are rarely RAS-positive. However, while still preliminary, these studies suggest that there may be a role for aspirin in improving certain advanced cancer treatments. For prostate cancer, elucidating how aspirin impacts immune cells in the prostate could eventually help to propel similar studies forward.

Conclusion

This dissertation provides evidence that aspirin but not NA-NSAID use may reduce risk of lethal prostate cancer in both black and white men. Whether aspirin also reduces risk of disease recurrence and/or progression among men diagnosed with prostate cancer remains unclear, but this question warrants further investigation given the strong inverse association between pre-diagnostic aspirin use and case-fatality observed in the ARIC study. Biological plausibility of a relationship between aspirin use and lethal prostate cancer is supported by observed differences in the extent of inflammation and abundance of specific immune cells in benign prostate tissue of aspirin users and non-users. Collectively, these studies suggest that there may be a role for aspirin use in the primary prevention of lethal prostate cancer.

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86. Mondul AM, Joshu CE, Barber JR, et al. Lipid-lowering drug use and risk of fatal prostate cancer in black and white men in the Atherosclerosis Risk in Communities (ARIC) Study. (submitted).
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113. Cheung KS, Chan EW, Wong AYS, et al. Aspirin and Risk of Gastric Cancer After Helicobacter pylori Eradication: A Territory-Wide Study. *J Natl Cancer Inst* 2018;19(4817430).
114. Chubak J, Whitlock EP, Williams SB, et al. Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the U.S. Preventive Services Task Force. *Ann Intern Med* 2016;164(12):814-25. doi: 10.7326/M15-2117. Epub 016 Apr 12.
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119. Zelenay S, van der Veen AG, Bottcher JP, et al. Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. *Cell* 2015;162(6):1257-70. doi: 10.016/j.cell.2015.08.015. Epub Sep 3.
120. Hammerlindl H, Ravindran Menon D, Hammerlindl S, et al. Acetylsalicylic Acid Governs the Effect of Sorafenib in RAS- Mutant Cancers. *Clin Cancer Res* 2017;1:1078-0432.

Curriculum Vitae

Lauren M. Hurwitz, MHS

615 N. Wolfe Street, Room E6139
Baltimore, Maryland 21205
lhurwit3@jhu.edu
(240) 277-2274

EDUCATION

PhD	(*2018*)	**Epidemiology, Johns Hopkins Bloomberg School of Public Health** Baltimore, MD Track: Cancer Epidemiology Advisor: Elizabeth Platz, ScD, MPH Dissertation Title: NSAIDs for the Prevention and Control of Lethal Prostate Cancer
MHS	2013	**Epidemiology, Johns Hopkins Bloomberg School of Public Health** Baltimore, MD Track: Cancer Epidemiology Advisor: Elizabeth Platz, ScD, MPH Thesis Title: Telomere Length as a Risk Factor for Hereditary Prostate Cancer
BA	2011	**Public Health Studies, Johns Hopkins University** Baltimore, MD Minor: Psychology

PROFESSIONAL EXPERIENCE

2015-present (part-time) 2013-2015 (full-time)	**Junior Epidemiologist, Center for Prostate Disease Research** Uniformed Services University of the Health Sciences, Department of Defense, Rockville, MD
2012	**Summer Student, Division of Cancer Epidemiology and Genetics** National Cancer Institute, Rockville, MD
2010	**Summer Student, Division of Cancer Epidemiology and Genetics** National Cancer Institute, Rockville, MD
2009	**Summer Student, Division of Cancer Control and Population Sciences** National Cancer Institute, Rockville, MD

HONORS AND AWARDS

2018	**Scholar-in-Training Travel Award** 2018 American Association for Cancer Research Annual Meeting, Chicago, IL
2017	**Student Dissertation Workshop** 2017 Society for Epidemiologic Research Annual Meeting, Seattle, WA
2017	**Jean Coombs Award** Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2015	**Cancer Epidemiology, Prevention, and Control Training Fellowship** Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2012	**Phi Beta Kappa Honor Society** Johns Hopkins University, Baltimore, MD

PEER-REVIEWED PUBLICATIONS

1. Wojciechowska, U; **Hurwitz, LM**; Helicki, G; Cullen, J, McLeod, DG; Sosnowski, R; Didkowska, J. Decade-long Trends in Prostate Cancer Incidence and Mortality in Poland, 1999-2012. *Polish Annals of Medicine*. (accepted).

2. **Hurwitz, LM**; Cullen, J; Kim, DJ; Elsamanoudi, S; Hudak, J; Colston, M; Travis, J; Kuo, HC; Rice, KR; Porter, CR: Rosner, IL. Longitudinal regret after treatment for low- and intermediate-risk prostate cancer. *Cancer*. 2017 Nov; 123(21): 4252-4258.

3. Hammerich, KH; Donahue, TF; Rosner, IL; Cullen, J; Kuo, HC; **Hurwitz, L**; Chen, Y; Bernstein, M; Coleman, J; Danila, DC; Metwalli, AR. Alkaline phosphatase velocity predicts overall survival and bone metastasis in patients with castration-resistant prostate cancer. *Urologic Oncology: Seminars and Original Investigations*. 2017 July; 35(7): 460.e21-460.e28.

4. Banerji, JS; **Hurwitz, LM**; Cullen, J; Wolff, EM; Levie, KE; Rosner, IL; Brand, TC; L'Esperance, JO; Sterbis, JR; Porter, CR. A prospective study of health-related quality of life outcomes for patients with low-risk prostate cancer managed by active surveillance or radiation therapy. *Urologic Oncology: Seminars and Original Investigations*. 2017 May; 35(5): 234-242.

5. Pham, KN; Cullen, J; **Hurwitz, LM**; Wolff, EM; Levie, KE; Odem-Davis, K; Banerji, JS;

Rosner, IL; Brand, TC; L'Esperance, JO; Sterbis, JR; Porter, CR. Prospective quality of life in men choosing active surveillance compared to those biopsied but not diagnosed with prostate cancer. *Journal of Urology*. 2016 Aug; 196(2): 392-398.

6. **Hurwitz, LM**; Cullen, J; Elsamanoudi, S; Kim, DJ; Hudak, J; Colston, M; Travis, J; Kuo, HC; Porter, CR; Rosner, IL. A prospective cohort study of treatment decision-making for prostate cancer following participation in a multidisciplinary clinic. *Urologic Oncology: Seminars and Original Investigations*. 2016 May; 34(5): 233.e17-25.

7. Kim, DJ; Hawksworth, DJ; **Hurwitz, LM**; Cullen, J; Rosner, IL; Lue, TF; Dean, RC. A prospective, randomized, placebo-controlled trial of on-Demand vs. nightly sildenafil citrate as assessed by Rigiscan and the international index of erectile function. *Andrology*. 2016 Jan; 4(1): 27-32.

8. Jeldres, C; Cullen, J; **Hurwitz, LM**; Wolff, EM; Levie, K; Odem-Davis, K; Johnston, RB; Pham, KN; Rosner, IL; Brand, TC; L'Esperance, JO; Sterbis, JA; Etzioni, RB; Porter, CR. Prospective quality of life outcomes for low-risk prostate cancer: active surveillance versus radical prostatectomy. *Cancer*. 2015 July; 121(14): 2465-73.

9. **Hurwitz, LM**; Heaphy, CM; Joshu, CE; Isaacs, WB; Konishi, Y; De Marzo, AM; Isaacs, SD; Wiley, KE; Platz, EA; Meeker, AK. Telomere length as a risk factor for hereditary prostate cancer. *Prostate*. 2014 April; 74(4): 359-364.

10. Andreotti, G; Karami, S; Pfeiffer, RM; **Hurwitz, L**; Liao, LM; Weinstein, SJ; Albanes, D; Virtamo, J; Silverman, DT; Rothman, N; Moore, LE. LINE1 methylation levels associated with increased bladder cancer risk in pre-diagnostic blood DNA among US (PLCO) and European (ATBC) cohort study participants. *Epigenetics*. 2014 March; 9(3): 404-15.

PRESENTATIONS

Oral Presentations

04/2018	"Aspirin use and risk of lethal prostate cancer in the Atherosclerosis Risk in Communities cohort" Minisymposium session, American Association for Cancer Research Annual Meeting, Chicago, IL
12/2017	"From prostate cancer prevention to survivorship: A synopsis of projects from CPDR and JHU" Center for Prostate Disease Research Weekly Seminar, Rockville, MD
06/2017	"Regular aspirin and non-aspirin NSAID use and prostate cancer recurrence after radical prostatectomy"

Concurrent Contributed Session, Society for Epidemiologic Research Annual Meeting, Seattle, WA

11/2016 "NSAIDs for the prevention and control of lethal prostate cancer"
Doctoral Proposal Seminar
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

05/2016 "Longitudinal regret and health-related quality of life following treatment for low- and intermediate-risk prostate cancer"
Moderated Poster Session, American Urological Association Annual Meeting, San Diego, CA

05/2015 "A prospective cohort study of treatment decision-making for prostate cancer following participation in a multidisciplinary clinic"
Moderated Poster Session, American Urological Association Annual Meeting, New Orleans, LA

04/2015 "Prostate cancer treatment decision-making and quality of life: an overview of CPDR prospective cohort studies"
Center for Prostate Disease Research Weekly Seminar, Rockville, MD

Poster Presentations

12/2017 "Aspirin use and risk of lethal prostate cancer in the Atherosclerosis Risk in Communities cohort"
Johns Hopkins University Prostate Research Day, Baltimore, MD

05/2017 "Aspirin and Non-Aspirin NSAID Use and Prostate Cancer Recurrence after Radical Prostatectomy"
Sidney Kimmel Comprehensive Cancer Center Fellow Research Day,
Johns Hopkins University School of Medicine, Baltimore, MD

05/2017 "Aspirin and Non-Aspirin NSAID Use and Prostate Cancer Recurrence after Radical Prostatectomy"
Cancer Epidemiology, Prevention, and Control Trainee Day,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

10/2016 "NSAID use and prostate cancer recurrence after radical prostatectomy: preliminary results"
Johns Hopkins University Prostate Research Day, Baltimore, MD

05/2016 "Longitudinal regret and health-related quality of life following treatment for low- and intermediate-risk prostate cancer"
Cancer Epidemiology, Prevention, and Control Trainee Day,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

05/2013	"Telomere length as a risk factor for hereditary prostate cancer" Cancer Epidemiology, Prevention, and Control Trainee Day, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
04/2013	"Telomere length as a risk factor for hereditary prostate cancer" Masters Research Symposium Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
08/2012	"*EPAS1*germline variants and modification of HIF-1α/2αprotein expression in ccRCC tumor tissue microarray" NIH Summer Research Day, Bethesda, MD
08/2010	"*VHL* somatic inactivation and *EPAS1* germline variation among clear cell renal cell carcinoma cases" NIH Summer Research Day, Bethesda, MD
03/2010	"Distress, mood, and quality of life characteristics in African American breast cancer survivors." American Psychosomatic Society Annual Meeting, Portland, OR
08/2009	"Distress, mood, and quality of life characteristics in African American breast cancer survivors." NIH Summer Research Day, Bethesda, MD

MEMBERSHIPS

American Association for Cancer Research, Associate Member, 2016-2018

Society for Epidemiologic Research, Student Member, 2017-2018

TEACHING, MENTORING, & SERVICE

2017	Teaching Assistant, Advanced Methods for the Design and Analysis of Cohort Studies Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2017	Teaching Assistant, Multilevel Statistical Modeling Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2017	Teaching Assistant, Longitudinal Data Analysis Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2016	Teaching Assistant, Epidemiologic Methods II Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

2016-2017	Co-organizer, Cancer Epidemiology, Prevention, and Control Research in Progress Seminars Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2016-2017	Doctoral Student Representative, Epidemiology Student Organization Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2015-2016	Mentor to Johns Hopkins University undergraduates, Public Health Connection Mentoring Program, Baltimore, MD
2015	Mentor to two interns in the CPDR HBCU Summer Undergraduate Training Program, Rockville, MD
2012-2013	Mentor to new tutors, Johns Hopkins University Learning Den, Baltimore, MD
2011-2013	Statistics Tutor, Johns Hopkins University Learning Den, Baltimore, MD
2010-2013	Mentor to Baltimore City high school students, Thread (formerly Incentive Mentoring Program), Baltimore, MD