AGENT ORANGE AS A RISK FACTOR FOR POSITIVE PROSTATE BIOPSY

By

Nathan J. Ansbaugh

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CERTIFICATE OF APPROVAL

This is certify that the Master's thesis of Nathan Ansbaugh
has been approved

Jackilen Shannon, PhD

Tomi Mori, PhD

Mark Garzotto, MD

TABLE OF CONTENTS

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ABSTRACT

Background: Agent Orange (AO), a defoliate contaminated with the known carcinogen dioxin, has become a prominent concern as veterans of the Vietnam War who were exposed to AO are now reaching the age at which they are at greatest risk of developing prostate cancer. While sufficient evidence has linked AO exposure to many diseases, only limited but suggestive evidence exists to support a positive association between AO and prostate cancer. Despite mixed findings, recent studies have found that the risk of prostate cancer in those exposed to AO was as high as twice the risk in those not exposed. The goal of this study was to examine this association between AO exposure and prostate cancer in a cohort of men referred for a prostate biopsy.

Methods: In this retrospective cohort-design, risk factors were identified using historical clinical data from a population of veterans referred to the Portland VA Hospital for a prostate biopsy between 1993 and 2010. In addition to AO exposure, covariates included prostate specific antigen density (PSAD), results of the digital rectal exam (DRE), age at biopsy, family history, body mass index (BMI), race, and service history. Outcomes of the biopsies were defined as either positive or negative according to the pathology report and risk factors were compared between individuals found to have prostate cancer and those without cancer. A second analysis compared these risk factors between individuals who were found to have high grade cancer (Gleason ≥ 7) and individuals with low grade cancer (Gleason < 7). Multiple logistic regression was used to evaluate the effect of AO on risk of prostate cancer and high grade prostate cancer after adjustment for confounders.

Results: Of the 2720 veterans who underwent prostate biopsy, 896 (32.9%) were found to have prostate cancer and 459 (16.9%) were found to have high grade cancer. After adjustment for significant confounders including PSAD, DRE, age at biopsy, service history, and family history of prostate cancer, veterans with AO exposure were 49% more likely to have prostate cancer compared to those without exposure to AO (aOR = 1.49; 95% CI: 1.05 - 2.10, p=.02). Additionally, amongst those with prostate cancer, individuals with exposure to AO were diagnosed with prostate cancer on average roughly 5 years earlier than individuals not exposed to AO (Mean age of diagnosis for AO exposed = 61.4 years; Mean age of diagnosis for non-exposed = 66.1 years, p<0.0001). Individuals with AO exposure were also 70% more likely to have high grade prostate cancer compared to those without AO exposure (aOR = 1.70, 95% CI: 1.10 – 2.55, p = 0.02). Agent Orange appears to be particularly associated with high grade cancer (aOR = 1.70, 95% CI: 1.08 to 2.70, p =0.02 in the analysis of high grade cancer vs. no cancer; aOR = 1.25, 95% CI: 0.81 to 1.92, p = 0.32 in the analysis of low grade cancer vs. no cancer).

Conclusions: Agent Orange exposure was associated with a significant increase in risk of prostate cancer and, more specifically, high grade cancer among men referred for a prostate biopsy. The limitations in identifying biologically significant levels of AO exposure in this study may suggest potential for underestimation of the true risk. Agent Orange exposure was associated with a significant increase in the risk of high-grade prostate cancer in men referred for an initial prostate biopsy. If validated, these findings could have significant implications in the development of effective prostate cancer screening strategies for male Veterans.

INTRODUCTION

Prostate Cancer Overview

Prostate cancer is the most commonly diagnosed cancer among men in the United States and is the second leading cause of cancer related death in men[1]. Based on prostate cancer rates between 2005 and 2007, approximately 1 out of every 6 men is predicted to develop prostate cancer during their lifetime [2]. In 2010 alone, the National Cancer Institute estimates 217,730 new cases of prostate cancer and 32,050 deaths from prostate cancer [2]. While the incidence of prostate cancer is the highest of all cancers for men across all races and ethnicities, the respective age-adjusted incidence proportions for black and Caucasian individuals are roughly 235 and 150 per 100,000 over one year compared to American Indian/Alaska Native, Hispanic, and Asian/Pacific Islander at 78, 125, and 90 per 100,000 over one year, respectively [2]. In general, the population most prone to prostate cancer is men over the age of 60 with a positive family history of prostate cancer [3, 4].

With the link between increased frequencies of prostate cancer and family history of prostate cancer as well as the pattern seen between different races and ethnicities, it has been suggested that there is a genetic link to prostate cancer. Recently, HPC1, or human prostate cancer gene 1, was mapped to the long arm of the first chromosome [5]. Other genes have also been isolated and shown to lead to increased risk of developing prostate cancer among men of European-American descent. In fact, various analyses of high-risk genotypes have shown over a 2.5 fold increase in the risk of developing prostate cancer [5]. This link between genetics and prostate cancer is especially seen in the younger

population of individuals developing prostate cancer. It has been shown that between 43-65% of prostate cancer cases before the age of 56 can be linked to high-risk autosomal dominant genotypes [6].

In addition to new findings about the potential causes of prostate cancer, improvements in technologies over the last 25 years have made major shifts in trends in prostate cancer. Since 1990, there has been a downward trend in the prostate cancer specific mortality rates along with an increased overall incidence of prostate cancer that is also just recently beginning to trend downward [1]. A major spike in the reported incidence of prostate cancer in the United States in the late 1980s and the early 1990s is likely due to the adoption of the prostate specific antigen (PSA) screening test in 1986 as a highly sensitive tool for detecting prostate cancer. The decrease that has been seen even more recently may be due to the adoption of hormone therapy as a treatment for prostate cancer and most studies suggest that this PSA screening itself does not decrease prostate cancer specific mortality.

With new knowledge regarding the genetic components of prostate cancer, new technologies that expand treatment and screening for prostate cancer, the high incidence and mortality rates for the disease, and ultimately the cost of screening for and treating the disease, it is important that we continually look for other factors that may affect the risk of prostate cancer in order to more effectively treat this disease.

Screening and Treatment

Initial Screening and PSA Testing

Prostate specific antigen (PSA) is a protein produced by epithelial cells of the prostate and is secreted into the bloodstream. Thus, PSA levels can be measured in a simple blood test. When abnormal growth occurs in prostate tissue, PSA levels in the blood change and are detectable using a PSA test that measures the volume of PSA in the blood. For this reason, PSA is known as a tumor marker because a large and/or rapid increase in PSA often indicates the presence of cancer. However, elevations in PSA levels in the blood are not always indicative of cancer. As men age, changes in body chemistry and hormone levels often cause many individuals to experience inflammation of the prostate known as prostatitis or enlargement of the prostate known as benign prostatic hypertrophy (BPH), both of which cause increases in measurable PSA levels but are not cancerous. Thus, it is difficult to determine the presence or absence of prostate cancer based solely on the PSA test.

Current screening methods for prostate cancer are highly sensitive within typically healthy ranges of PSA levels, but specificity at the most sensitive PSA cut-offs for detecting prostate cancer is very poor [7]. For over twenty years, the PSA test has led to increased detection of prostate cancer but has simultaneously led to a large increase in the number of individuals undergoing unnecessary prostate biopsies. Several prominent studies from the early 1990s showed that the percentage of individuals actually found to have prostate cancer among all individuals undergoing prostate biopsy ranged from as low as 11% to 34% [8-11].

In a 2005 study by Thompson et al, when a low PSA cutoff such as 1.1 ng/ml was considered a positive test, this showed 83% sensitivity and 39% specificity as a test for detecting prostate cancer. However, when the cutoff was higher at 4.1 ng/ml, the sensitivity dropped to 21% while specificity rose to 94% [7]. Thus, when a lower cutoff is used, the PSA test is able to detect many more individuals who have prostate cancer. In a 1996 study by Jacobson *et. al.*, a median PSA level of 9.4 ng/ml was found for 177 case patients and a median for 305 control patients was 1.2 ng/ml [12]. As we saw in the later study by Thompson, the highest sensitivity for the PSA test was when the cutoff was around 1.1 ng/ml which is just below the median PSA level for patients without prostate cancer in the Jacobson study. So the question, of course, is where the cutoff should be made or whether the test should even be used at all? If the cutoff is 1.1ng/ml, this screening will detect around 80% of individuals who actually have the disease but there will also be a very high false positive rate due to the low specificity of the test. If we raise the PSA cutoff to around 4.1 ng/ml, we will only detect roughly 21% of individuals who truly have prostate cancer but there will be far less individuals who test positive, pursue further treatment, and do not have the disease. Fortunately, other techniques are used in combination with the PSA test to screen for prostate cancer, but this dilemma with the PSA test brings its validity into question.

Signs and Symptoms

Signs and symptoms of prostate cancer are fairly non-specific and a definitive diagnosis requires more advanced assessment to better evaluate whether or not a patient has prostate cancer. Though there are many different symptoms that may be present due

to prostate cancer, there are currently no known symptoms that are solely specific to the disease. Typically, prostate cancer causes an enlargement of the prostate which not only obstructs the internal urethral orifice, but can also distort the prostatic urethra [5]. The enlargement of the prostate can therefore lead to symptoms such as urgency, frequency, dysuria, weak stream, and urine leakage or incontinence. These symptoms, however, are more typical of prostate enlargement associated BPH. The inability of the above symptoms to provide a definitive diagnosis of prostate cancer requires that different screening techniques like those mentioned previously be used in monitoring one's possible development of the disease. Thus, other screening procedures are used to detect additional signs of prostate cancer.

Digital Rectal Exam

Along with the PSA test and the variations of this test, another technique that is commonly used by physicians as an aid to screening is the digital rectal exam (DRE). By palpating the prostate, a physician can feel for any hypertrophy, firmness or architectural abnormalities that are often signs of prostate cancer. Physicians may also use a transrectal ultrasound which provides the physician with an actual image of the prostate that allows him/her to visually inspect for abnormalities as well as to ascertain a rough estimate of the prostate volume.

Incorporating Risk Factors/Tumor Markers into Screening

Age and PSA

Our inability to rely on a singular biomarker or symptoms for screening and diagnosis of prostate cancer makes understanding other risk factors for prostate cancer essential. Determining an individual's risk can allow physicians to make educated decisions about whether or not to suggest various strategies for monitoring and/or treating prostate cancer. There are many different risk factors that are important in screening and in diagnosing prostate cancer. Some of these have already been mentioned including age, race/ethnicity, and family history.

Age of the patient, as stated above, is a major risk factor for prostate cancer. As individuals age, their likelihood of developing prostate cancer increases dramatically. Age-specific PSA cutoffs are used to make the PSA test more sensitive in younger men and more specific in older men [13]. Often times, individuals who are older and are diagnosed with prostate cancer are monitored and major treatment is avoided due to the slow growing nature of the disease and shorter life expectancy in many cases. In these cases, the symptoms associated with prostate cancer are treated to maximize quality of life, and often times, individuals diagnosed with prostate cancer later in life die from other unrelated illness or injury and not the cancer itself. For this reason, the PSA cutoff is typically higher for older individuals as this not only decreases the number of slowly developing cases that would be treated unnecessarily but it also decreases the false positive rate for individuals in this age group. Older patients who have a more aggressive

cancer and are more at risk for having the cancer lead to their death will often demonstrate higher PSA levels that would be considered a positive test even with the higher PSA cutoffs [14].

For younger individuals in their 40s and 50s, however, a lower cutoff is often used because catching even a non-aggressive cancer of the prostate early in development is essential as the cancer itself is much more likely to be an eventual cause of death if left untreated. As a result of this higher sensitivity, there are many more false positives in this area due to the low specificity associated with a lower PSA cutoff.

Alternatives to the PSA Test

Other strategies utilizing PSA levels in the blood are often used that can be more informative than the basic PSA test alone. One of these strategies is to track the rate of increase in PSA levels over time. This is known as PSA velocity. Using PSA velocity may be helpful for detecting prostate cancer in individuals who have only slightly elevated PSA levels that may not have a positive PSA test because their PSA level falls below the PSA cutoff. In the clinical setting, high PSA velocity may indicate the need for prostate biopsy as studies such as the 2004 study by D'Amico *et al* found that men with a PSA velocity above 2.0 ng/mL per year were at an increased risk of prostate cancer death after surgical treatment compared to men with a PSA velocity of 2.0 ng/mL per year or less in the year before diagnosis [14]. However, even though it has been shown that a consistently rising PSA may be indicative of prostatic growth and cancer, the cumulative evidence for this association is generally weak.

In addition to PSA velocity, clinicians have looked for ways to improve the PSA test to account for a larger healthy prostate which may produce more PSA than a smaller healthy prostate simply due to the total amount of PSA producing tissue. Thus, PSA Density (PSAD) has become a popular measure that describes the amount of PSA in the blood relative to the volume of the prostate. This density measure is intended to improve specificity over the PSA test alone by accounting for elevated PSA that is attributable to increased prostate size but not attributable to abnormal growth.

Other Risk Factors

Elevated levels of insulin-like growth factor (IGF-I) have also been associated with an increased risk of prostate cancer. In a meta-analysis by Roddam *et. al.,* individual patient data from 11 prospective studies analyzed a total of 3,700 men with prostate cancer and 5,200 men without prostate cancer. Those who were in the highest quintile for serum IGF-I had an odds ratio of 1.38 for prostate cancer compare to the lowest quintile ($p < .001$) [15].

History of sexually transmitted diseases has also been noted as a potential risk factor for prostate cancer. In a 2005 study by Taylor et al., meta-analysis of 29 case-control studies comparing 6,022 men with prostate cancer to 7,320 men without prostate cancer found significant odds ratios for prostate cancer in individuals with any history of sexually transmitted diseases (1.48, 95% CI: 1.26-1.73) [16].

While there are many other studies suggesting links between various factors and risk for prostate cancer, few of these factors are widely recognized and sufficiently studied.

Today, the PSA test and the DRE are the primary methods for deciding whether an individual should undergo prostate biopsy. However, recent studies show that, on average, only 25% of individuals undergoing prostate biopsy are actually found to have cancer [17]. Recently, Agent Orange (AO) exposure has arisen as a new potential risk factor for prostate cancer and has gained particular attention among the United States veteran population. Despite the limited body of research, this potential prostate cancer risk factor demands great attention.

Agent Orange

The United States Veteran population includes a cohort of nearly 17 million males between the ages of 45 and 84 with the largest proportion of this population being those who served during the Vietnam era, just under 8 million [18]. With these Vietnam veterans now reaching their mid 60s, the age at which prostate cancer is most prevalent in the general United States population [4], the eye of prostate cancer research has fallen on this population. In particular, research regarding prostate cancer and exposure to Agent Orange, a defoliate that was heavily used in the Vietnam War which was contaminated with the toxin, dioxin, has become a prominent topic as more and more veterans with past exposure to Agent Orange are now being diagnosed with prostate cancer. Clinicians in Veterans Affairs hospitals are being flooded with information regarding Agent Orange exposure and the potential increased risk of prostate cancer in these veterans despite inconclusive evidence for Agent Orange being causally related to prostate cancer. While

no real estimates exist for what percentage of Vietnam veterans were exposed to Agent Orange, roughly three million veterans served in Southeast Asia alone where the highest amounts of Agent Orange were used during the war.

Agent Orange is among the more controversial topics facing the Department of Veterans Affairs due to the fact that individuals with history of Agent Orange exposure currently receive compensation for medical costs associated with treatment of prostate cancer despite inconclusive evidence of a causal association. Agent Orange was the most widely used of various herbicides sprayed in Vietnam to clear foliage and reveal hidden enemy forces, destroy enemy crops, and to kill tall grasses and bush around United States military bases [19]. Mixtures of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), picloram, and cacodylic acid where the ingredients that made up the various herbicides which were code named Agents Pink, Green, Purple, Orange, White, and Blue after the color of the band on the 55 gallon barrels that held each specific chemical. Though herbicides were first sprayed in Vietnam in 1961, Agent Orange, a 50:50 mixture of 2,4-D and 2,4,5-T, was used mainly from 1965 to 1970. Newly revised estimations from 2008 on the amounts of herbicides used from 1965 to 1972 suggest that roughly 27 million gallons of various herbicides where sprayed over 3.6 million acres of land by helicopter and other aircraft [19].

The process of producing 2,4,5-T, a component found in all the herbicides used in Vietnam except for Agents White and Blue, also produced the unwanted contaminate 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), better known as dioxin [19]. In laboratory animals and wildlife species, even extremely small doses of TCDD have proven fatal

leading some to call TCDD “the most toxic man-made chemical” [20]. Elevated levels of TCDD (over 5ppt) in Vietnamese who were exposed to Agent Orange during the Vietnam War were found to be as high as 413ppt thirty years after the end of spraying [21]. An enormous number of diseases, defects, and disorders where found in this population including births defects, liver damage, diabetes, thyroid disease, developmental abnormalities, chloracne, changes in serum testosterone levels, and other soft tissue cancers to name a few, all of which have been linked to TCDD exposure [20]. However, the impact of TCDD exposure on the risk of prostate cancer in US forces is less clear.

One of the main difficulties in studying the effects of Agent Orange as an exposure has been accurately defining and identifying the exposure itself. Congener-specific high-resolution gas chromatography high-resolution mass spectrometry (HRGC-HRMS) is the only method for measuring specific dioxin congener levels and it remains very expensive at around $1200 for a single analysis [20]. While several studies using the HRGC-HRMS method reported elevated levels of TCDD in those who reported the highest Agent Orange exposure levels [22-24]. Further difficulty exists in defining exposure in that other studies have found no evidence for elevated TCDD levels in veterans who were known to be exposed to Agent Orange [25, 26]. Due to the half-life of TCDD in Vietnam veterans which has been estimated to be 7-11 years [19], detecting elevated levels in Vietnam veterans after what has been nearly 40 years since exposure is unlikely not to mention quite expensive as the cost of testing the millions of men who were potentially exposed in the Vietnam War era would be exorbitant.

Despite the difficulties in evaluating Agent Orange exposure in Vietnam Veterans and the inconclusive evidence for its causal association with prostate cancer in this population, the concern surrounding this association and the need for further research has been justified in past models that have demonstrated a positive association between Agent Orange and soft tissue sarcoma. The first study showing the effects of Agent Orange on soft tissue sarcoma was published just five years after the end of herbicide spraying in Vietnam [27]. This marked the beginning of a wave of research using laboratory animals including rats, mice, and hamsters that all provided evidence for the carcinogenic effects of dioxin [28-32]. The interest in dioxin toxicity sparked investigations into farming communities and different occupations with known exposure to dioxin. In 1991, a German study reported a mortality follow-up of 1583 workers (1184 men, 399 women) employed in chemical plant in Germany that produced herbicides contaminated with TCDD. This study showed increased cancer specific mortality rates in the highest exposure groups which supported the hypothesis that TCDD is a human carcinogen [33]. Two years later, a study of farmers spraying herbicides found a weak but statistically significant association between number of acres sprayed with herbicides in 1970 and risk of prostate cancer mortality which further suggested the need for research in this area [34]. A meta-analysis of the association between farming and herbicide use in association with prostate cancer using peer-reviewed journals between Jan 1983 and June 1994 found positive associations once again [35].

Currently, sufficient evidence has linked Agent Orange exposure to soft tissue sarcoma, Non-Hodgkin's lymphoma, Hodgkin's disease, chloracne, and porphyria

cutanea tarda. Limited but suggestive evidence exists for an association with respiratory cancers, prostate cancer, and multiple myeloma [19]. The US Environmental Protection Agency has reported on the plausible biological model for TCDD's association with prostate cancer suggesting that this interaction is mediated through the action of a cellular protein known as an Ah receptor which functions as a transcriptional enhancer by interacting with various regulatory proteins including heat shock proteins, kinases, translocases, and DNA binding species [36].

As the Vietnam veteran cohort was reaching the age at which cases of prostate cancer began to develop, attention turned in this direction. In 2001, a study by Zafar and Terris found no significant relationship between prostate cancer and Agent Orange exposure in patients referred for prostate biopsy [37]. One of the main criticisms of this study was that the Vietnam veteran cohort was at an age where only a very small percentage of prostate cancer cases occur in the general population. Three years later, a cohort study of cancer incidence and mortality rates among Operation Ranch Hand veterans who were responsible for the majority of the spraying of TCDD contaminated herbicides in Vietnam found that incidence of melanoma and prostate cancer were increased among white Ranch Hand veterans relative to national rates [38]. Among veterans who spent at most 2 years in Southeast Asia, the risk of cancer at any site, of prostate cancer and of melanoma was increased in the highest dioxin exposure category [38]. That same year, a case control pilot study found that men with prostate cancer were approximately two times more likely to report previous exposure to Agent Orange [39]. Though this finding was significant, the potential for bias away from the null as a result of men with prostate

cancer being more likely to report Agent Orange exposure as well as the lack of adjustment for risk factors such as family history and other potential confounders suggested the need for caution upon interpretation.

The Operation Ranch Hand cohort gained further attention in a 2006 paper by Pavuk *et. al.* summarizing a cohort study that measured actual serum TCDD levels and found no overall increase in the risk of prostate cancer in Ranch Hand veterans versus the comparison group [40]. They did, however, find a significant positive association in Ranch Hand veterans in the higher TCDD category who served in Southeast Asia before 1969 when AO was used the most heavily. A within-group comparison was also performed and the investigators found that, in the comparison group of veterans who were not exposed to Agent Orange, increased time of service in Southeast Asia was associated with increased risk of prostate cancer. This finding raised the possibility that longer service in Southeast Asia and exposures to other factors not including TCDD may be the reason that past studies saw an increased risk in prostate cancer amongst those exposed to Agent Orange. However, it also suggests that the serum TCDD levels could not detect significant biologic exposure in many individuals and that individuals found to have no measurable TCDD levels may have still had significant exposure to AO and similar elevated risk of prostate cancer as those with measurable levels.

In 2008, a historical cohort study by Chamie et al looking at 6214 individuals exposed to Agent Orange and 6930 who were unexposed found that Agent Orange was the most important predictor not only of developing prostate cancer but also of high-grade and metastatic disease on presentation [41]. Differences in disease characteristics

such as age, race, smoking history, family history, BMI, finasteride exposure, prebiopsy PSA level, clinical and pathological stage, and Gleason score were all assessed along with Agent Orange exposure using multivariate logistic regression. The odds of exposure to AO among those with prostate cancer were found to be over twice the odds of exposure in those who did not develop the disease. This study also found that Agent Orange-exposed men were diagnosed at a younger age (59.7 years; 95% CI 58.9-60.5) compared to the unexposed cases (62.2 years; 95% CI 60.8-63.6). Potential sources for misclassification bias were present in this study because TCDD blood tests were not used to determine exposure status, and bias could exist in the reporting of exposure between cases and non-cases. Once again, it is important to note that even with the use of the TCDD blood tests, misclassification of exposure is likely in that all individuals who were exposed are not likely to be detected using the serum test due to variability in the rate of TCDD metabolism amongst different individuals and differing levels of exposure that may still have been harmful. The limitations of the previous studies regarding the association between AO exposure and prostate cancer suggest the need for additional large studies that will be crucial in evaluating the presence of a causal association.

Primary Objectives

1. Estimate risk of prostate cancer in veterans with AO exposure relative to risk of prostate cancer in those without exposure (as estimated by odds ratio).
2. Estimate risk of high grade prostate cancer (Gleason score ≥ 7) in veterans with AO exposure relative to risk of high grade cancer in those without AO exposure.

METHODS

Overview

This is a historical cohort analysis of 2720 veterans who were referred to the Portland VA Medical Center (PVAMC) for prostate cancer screening and have undergone at least one trans-rectal ultrasound (TRUS) guided prostate biopsy at the PVAMC. Individuals who were determined by their physician to be at elevated risk for prostate cancer were referred for prostate biopsy and were included in this study. Historical information regarding prostate cancer risk factors and AO exposure were collected for each individual undergoing prostate biopsy. Individual biopsy results were then compared to the patient's clinical information prior to prostate biopsy in order to assess possible risk factors for positive prostate biopsy. Unlike prior studies, our study uses the VA hospital electronic medical information to classify AO exposure as this is the information most readily available to physicians in the VA hospital system. Additionally, PSA density as well as service branch information were recorded in an attempt to reduce residual and unmeasured confounding.

Data Management and Collection

All TRUS biopsy parameters were recorded using a standardized form. These forms and printed ultrasound images were retained by the Urology Section of the Portland VA Hospital in locked filing cabinets. These forms served as the source for clinical, laboratory and ultrasound data. Pathology reports were accessed to determine the

presence of cancer in addition to the biopsy grade (represented as Gleason score). All data was recorded on an Excel® spreadsheet.

Patient information in this database was then linked to historical information from the VISN 20 Consumer Health Information Performance Sets (CHIPS) Data Warehouse. The VISN system, or the Veterans Integrated Service Networks, was established in the 1990s by the Veterans Health Association (VHA). When this system was first established, the VISN system included 22 different administrative regions or service networks. VISN 20 services include veterans in Alaska, Idaho, Oregon and Washington. In 1997, VISN 20 initiated CHIPS, a VISN wide information system for decision support, performance measuring and population studies. Linking the TRUS biopsy database with the CHIPS data allowed us to capture additional clinical information and to validate existing information in the TRUS biopsy data. In particular, the CHIPS data was essential for obtaining information on AO exposure.

The process of linking these two data sources involved using Microsoft SQL programs through an Access interface on the VA's password-secured thin client server in order to maintain data security. The cumulative data was then maintained in a table on the VA research SQL server. For statistical analysis, the data, without personal identifiers, was exported to STATA. Quality assurance checks were performed by the study PI using comparisons to the CPRS record.

Risk Factor Information

Information that will be used in the analysis include Agent Orange exposure, family history of prostate cancer, age at biopsy, race, PSA density, body mass index (BMI), and service history. Though smoking information was available for some individuals, there was not enough information to explain any of the variability in the outcome of prostate cancer or high grade cancer in the multivariate analyses. Additionally, while data on finasteride exposure was available as well as information regarding prior vasectomies and inflammation of the prostate, these variables were not examined in the model as information regarding these factors was a mix of available information prior to biopsy as well as post-biopsy. This suggests that some of these factors may have been a consequence of the outcome of prostate cancer and not a risk factor. Thus, this data was not analyzed. Lastly, for purposes of quality assurance, missing data and outlier data was reviewed prior to anonymization of the data to assure that all appropriate variable definitions were applied.

Inclusion/Exclusion Criteria

Patients who were referred to the Portland VA Medical Center for prostate cancer screening and have undergone at least one TRUS guided prostate biopsy.

Outcome Definitions

For the purposes of this study, the outcome is based on the findings of the prostate biopsy:

- Positive vs. Negative biopsy (according to pathology report of initial biopsy)
- Low Grade Cancer (Gleason score ≤ 6) or Negative Biopsy vs. High Grade Cancer (Gleason score > 6)

Primary Exposure Definition

Agent Orange Exposure

Agent Orange exposure status was determined during patient enrollment in the VA hospital system. Each individual was defined as a either "Exposed" or "Unexposed" in accordance with the Portland VA standards for documenting AO exposure. Individuals who did not have available AO exposure status were assumed to not have been exposed. This assumption was deemed appropriate as it is probable that individuals with reported exposure would have this information available in their medical records due to the known harmful effects of AO in many different conditions/diseases. Of the 2720 individuals in the study, only nine individuals did not have explicitly stated information regarding AO exposure status. The exposure status for the remaining 2711 individuals was obtained directly from the VISN 20 data warehouse. This information in the data warehouse classified individuals as exposed if they met either of following criteria:

1. Patient served for any period of time in a location where AO was known to have been used during the time of their service.
2. Patient reported having had AO exposure at the time of their enrollment into the VA hospital system (prior to prostate biopsy).

Additional Risk Factor Definitions

PSA information was collected from laboratory results reported to the referring clinician and was collected in the TRUS biopsy database. The highest observed PSA recorded prior to prostate biopsy was used in this evaluation for each individual. Prostate volume, as estimated during the TRUS-guided biopsy was also recorded for each individual at the time of prostate biopsy. Given that prostate biopsies are often conducted soon after a high PSA finding, PSA levels and prostate volume are generally measured close in time. PSA information and prostate volume estimates were used to calculate PSAD. Since PSAD provides combined information regarding PSA levels as well as prostate volume, this measure was used in place of PSA in order to standardize PSA levels by prostate volume.

Body mass index (BMI) and findings of the DRE were also recorded in the TRUS database. Height and weight information was used for each subject to calculate BMI. This formula is as follows:

$$BMI = Weight\ (lb) / (Height\ (in))^2 \times 703$$

The calculated BMI as well as the results of the DRE were categorized into levels consistent with clinical use. The levels for these categorical variables are listed below:

Code	BMI	DRE	Race	Service History
1•	0 to 18.5 (Underweight)	Normal	White	Air Force
2	18.5 to 24.9 (Normal)	Suspicious	Black	Navy
3	25.0 to 29.9 (Overweight)	Cancer Likely	Hispanic	Army
4	≥ 30.0 (Obese)	*Unknown*	Asian	Marine Corps
5	*Unknown*	-	American Indian	Coast Guard
6	-	-	Other	Merchant Marines
7	-	-	-	*Unknown*

• Indicates reference level

Statistical Analysis

Two separate multiple logistic regression models were built to accomplish the primary objectives of this study. The following is an explanation for the modeling strategies used to construct these models. The same strategies were used for both outcomes of interest.

Step 1: Scaling Continuous Variables & Managing Unknowns/Missing Data

First, all continuous variables were individually plotted against the probability of a positive biopsy or high grade vs low grade or no cancer using Lowess smoother plots transformed for logistic modeling using STATA. Based on observation of trends in each plot as well as known significant categories of clinical relevance, variables were categorized into dichotomous outcomes or multiple levels. For missing or unknown information in continuous variables, missing values were given a score of "999" and were categorized into a separate level of the categorical variable in order to prevent individuals with missing or unknown information from dropping out of the model. For missing or unknown information in categorical variables, an unknown level was simply created for missing information. These missing levels were examined in the model construction to determine whether the missing information was related to the outcomes of interest as any significant association would suggest some sort of selection bias. The following continuous variables were categorized.

Step 2: Univariate Analysis

Univariate analysis of all categorical variables was then performed to analyze the relationship between each independent categorical variable and the probability of a positive biopsy in the first model and high grade cancer in the second model. No continuous variables fit a normal linear model and were therefore not left as continuous variables. All variables (not including AO as the primary predictor of interest) not found to be significantly associated with the prediction of the outcome at the alpha = 0.25 level using the Pearson Chi^2 Test and the Log-Likelihood Ratio tests were excluded from the model.

Step 3: Simple Logistic Regression (Crude Estimates)

Each of the remaining variables after removal of variables that were not significant in univariate analysis were then modeled against the probability of prostate cancer using simple logistic regression to determine the crude coefficient of each independent predictor, the standard error of each predictor, the crude odds ratios and 95% confidence intervals, and the statistical significance for each variable using the Log-Likelihood Ratio test without adjustment for the other variables. Again, any variables not significant at the 0.25 level were excluded from the model.

Step 4: Multiple Logistic Regression (Adjustment) and Collinearity

The variables remaining in the model following simple logistic regression analysis were all incorporated into a multivariable logistic regression model relating all the

covariates. Each variable was once again assessed for significance at the 0.25 level using the Wald test. Odds ratios and 95% confidence intervals were created for each variable. Collinearity was assessed here and any variables found to be collinear were discussed with clinical experts and the more clinically significant variable was left in the model. The remaining model was considered the preliminary main effect model. Two additional models were considered in the analysis of high grade cancer comparing AO exposure between individuals with high grade cancer and no cancer as well as a comparison between individuals with low grade cancer and no cancer. These models were used to determine whether a true association existed between high grade cancer and AO exposure or if this was primarily driven by one of these alternative associations.

Step 5: Effect Modification

Given the biologic plausibility of effect modification existing between AO and the remaining variables as well as effect modification between other significant predictors, relevant interaction terms were independently evaluated using simple logistic regression. Interaction terms that were significant at the 0.10 level were added to the multiple logistic regression model.

Step 6: Final Model

The final model included only variables that were significant at the alpha = 0.05 level.

Step 7: Model Diagnostics

Two similar strategies will be used to assess the fit of the final model. First, the goodness of fit test based on deviance will be calculated to test the null hypothesis that the model was a good fit. The appropriateness of this model was evaluated by determining the number of covariate patterns and comparing this to the number of observations in our study. If the number of covariate patterns is much less than number of observations, the goodness of fit test based on deviance will provide an accurate estimate of the true goodness of fit. However, to ensure the fit of the final model, the Hosmer Lemeshow test for goodness of fit will also be used to account for the presence of continuous variables in our model. An alpha level of 0.05 will be used for the rejection of the null hypothesis that the model is a good fit. STATA software was used to assess the presence of over dispersion and under dispersion in our final model.

Several diagnostic plots were also examined for potential outliers. This includes observational analysis of leverage vs. predicted probability, cooks distance vs. predicted probability, change in chi^2 vs. predicted probability, change in deviance vs. predicted probability, Pearson's standardized residuals vs. predicted probability, and a summary plot of change in chi^2 vs. predicted probability.

The AUC (Area Under the Receiver Operating Characteristic Curve) was used as a measure of the model's overall accuracy and to determine the cutoff point for the predictive probability of positive biopsy. The final logistic regression model was applied to patients in the validation data set and classified patients with the predicted probability

greater than the cut-off point as high or low-risk for prostate cancer. The model's sensitivity and specificity was evaluated by comparing the observed and predicted positive biopsy outcomes.

RESULTS

Population Demographics

White individuals made up 93.6% of the study population with 3.8% black individuals, 1.0% Hispanic, and less than 1% Asian, American Indian, and other as seen in Figure 1. The average age for all veterans that were referred for prostate biopsy was 64.7 years ± 7.4 with the youngest individual being 41 years of age and the oldest being 91 (Distribution seen in Figure 2). As seen in Table 1, individuals exposed to AO underwent prostate biopsy roughly 5 years earlier than individuals not exposed to AO. Additionally, among those with prostate cancer as seen in Table 2, individuals with exposure to AO were found to have cancer, on average, roughly 5 years earlier than individuals not exposed to AO (Mean age of diagnosis for AO exposed = 61.4 years; Mean age of diagnosis for non-exposed = 66.1 years, p-value for difference <.0001). No difference in BMI, PSA, PSAD, or Family History was seen between exposure groups.

TABLE 1
Entire Study Population

	Means and % (95% CI)		
	Agent Orange Exposure (n = 203)	**No Exposure (n = 2517)**	***P***
Age at Biopsy, *y*	60.6 (60.0, 61.2)	65.0 (64.8, 65.3)	<0.0001
BMI	30.0 (29.2, 30.8)	29.3 (29.0, 29.6)	0.117
PSA•, *ng/ml*	11.2 (4.3, 18.2)	12.4 (8.6, 16.2)	0.864
PSAD••, *ng/ml/ml*	0.20 (0.13, 0.28)	0.19 (0.18, 0.21)	0.710
Family History, %	28.1 (21.9, 34.3)	21.8 (20.2, 23.4)	0.038
Positive Biopsy, %	36.5 (29.8, 43.1)	32.7 (30.8, 34.5)	0.268

CI Indicates Confidence Interval, BMI = Body Mass Index (Weight (lb) / (Height (in))^2 x 703), PSA= Prostate Specific Antigen, PSAD = Prostate Specific Antigen Density

• Excluding extreme values above 5000ng/ml

•• Excluding extreme values above 20ng/ml/ml

p-values for means calculated using 2-Sample t-test with equal variances

p-values for proportions calculated using Pearson Chi-Square

TABLE 2
Positive Biopsy Only

	Means and % (95% CI)		
	Agent Orange Exposure (n = 74)	**No Exposure (n = 822)**	***P***
Age at Biopsy, *y*	61.4 (60.5, 62.3)	66.1 (65.6, 66.6)	<0.0001
BMI	30.1 (28.8, 31.4)	29.7 (29.2, 30.2)	0.550
PSA•, *ng/ml*	8.8 (6.1, 11.5)	23.0 (11.3, 34.7)	0.478
PSAD••, *ng/ml/ml*	0.34 (0.14, 0.53)	0.32 (0.27, 0.37)	0.845
Family History, %	28.3 (18.1, 38.7)	25.4 (22.4, 28.4)	0.578

CI Indicates Confidence Interval, BMI = Body Mass Index (Weight (lb) / (Height (in))^2 x 703), PSA= Prostate Specific Antigen, PSAD = Prostate Specific Antigen Density

• Excluding extreme values above 5000ng/ml

•• Excluding extreme values above 20ng/ml/ml

p-values for means calculated using 2-Sample t-test with equal variances

p-values for proportions calculated using Pearson Chi-Square

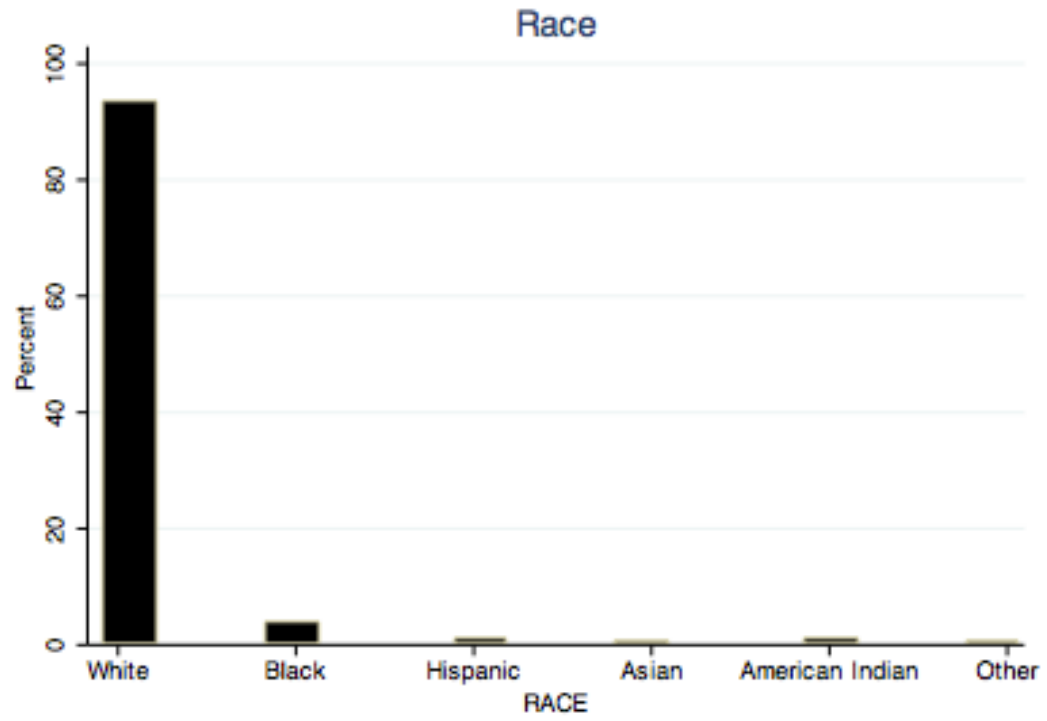

Figure 1: Distribution of race/ethnicity in study population by percent.

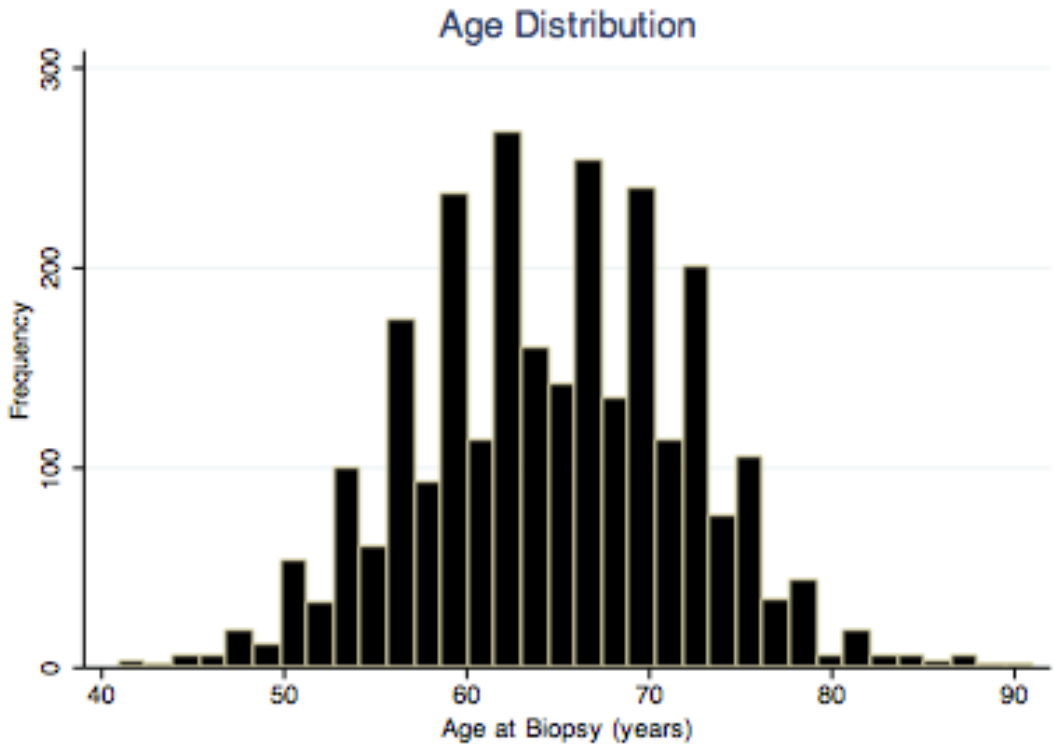

Figure 2: Distribution of age in study population by frequency (total # of individuals).

Of the 2720 individuals referred for prostate biopsy, 896 individuals were found to have prostate cancer. Assuming that this veteran population referred for prostate biopsy is a representative sample of all veteran populations referred for prostate biopsy, this suggests that 32.9% of veterans who are referred prostate biopsy are found to have prostate cancer (95% CI: 31.2% to 34.7%). Of these 896 individuals positive for prostate cancer, 459 were found to have high grade cancer (Gleason score ≥ 7). This suggests that 16.9% of veterans who are referred for prostate biopsy are found to have high grade prostate cancer (95% CI: 15.4% to 18.3%).

Agent Orange Exposure

Of the 2720 individuals referred for prostate biopsy, only 203 met the definition for having exposure to AO. This suggests that roughly 7.5% of veterans referred for prostate biopsy report exposure to AO (95% CI: 6.5% to 8.5%). Crude observations shown in Tables 3 demonstrate that 36.5% of exposed individuals were found to have prostate cancer compared to 32.7% who were not exposed. Additionally, among those with prostate cancer, 54.1% of those exposed to AO

TABLE 3
AO Exposure and Biopsy Results

AO Status	Biopsy Negative, *n*	Biopsy Positive, *n* (%)	TOTAL
Exposed	129	74 (36.5%)	203
Unexposed	1695	822 (32.7%)	2517
TOTAL	1824	896 (32.9%)	2,720

% = Biopsy Positive / (Biopsy Positive + Biopsy Negative)*100

TABLE 4
AO Exposure and Cancer Grade

AO Status	Low Grade, *n*	High Grade, *n* (%)	TOTAL
Exposed	34	40 (54.1%)	74
Not Exposed	403	419 (51.0%)	822
TOTAL	437	459 (51.2%)	896

Low Grade = Gleason Score < 7
High Grade = Gleason Score ≥ 7
% = High Grade / (TOTAL Positive Biopsy)*100

developed high grade cancer compared to 51.0% in those not exposed to AO as seen in Table 4.

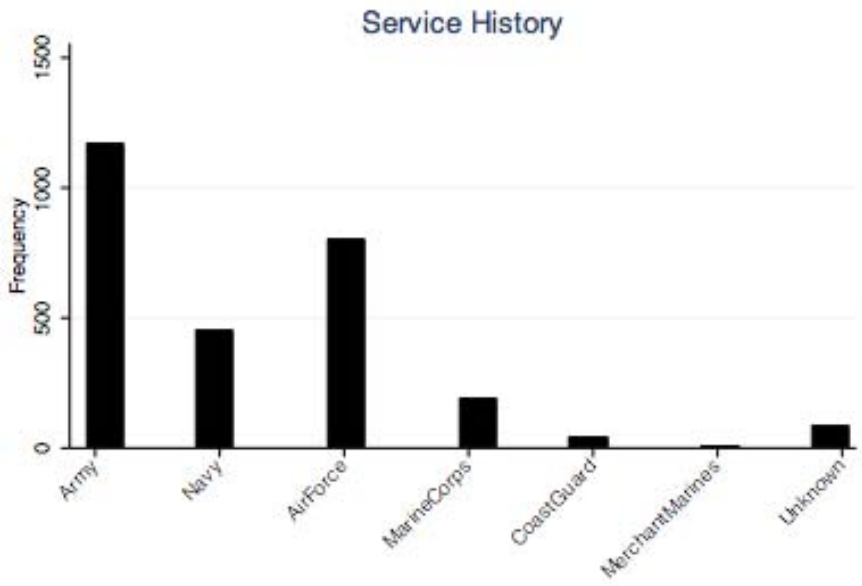

Figure 3: Distribution of service history in study population by frequency (total # of individuals).

Exposure by each branch of the military, as shown in Figure 3, was as follows: 8.9% of the Army veterans referred for prostate biopsy reported exposure to AO compared to 4.2% in the Navy, 6.3% in the Air Force, 14.3% in the Marine Corps, 0% amongst those in the Coast Guard and Merchant Marines, and 3.9% in the unknown group. Additionally, frequency of positive biopsy in exposed individuals was compared over different service branches. Air Force veterans were used as the reference category as the Air Force was responsible for the majority of spraying of AO. This group was chosen as reference because of this is the group that has received the most attention from research in this area. We did not expect this group to have the highest prevalence of exposure. Additionally, this allowed us to compare frequency of positive biopsy and high grade cancer between

exposed individuals who likely served in the air and were responsible for spraying AO versus individuals who served on the ground such as in the Marines and the Army. As seen in Tables 5 and 6, no significant associations were found across service branch and frequency of prostate cancer in exposed individuals upon crude comparison.

TABLE 5
Service Branch and Positive Biopsy in those with AO Exposure

	Total Exposed, *n*	Cancer, *n* (%)	*P*
Air Force *	50	18 (36.0%)	-
Army	104	33 (31.7%)	0.595
Navy	19	10 (52.6%)	0.210
Marine Corps	27	12 (44.4%)	0.492

* Indicates reference category
p-values for proportions calculated using Pearson Chi-Square

TABLE 6
Service Branch and High Grade Cancer in those with AO Exposure

	Total Exposed, *n*	Cancer, *n* (%)	*P*
Air Force *	50	10 (20.0%)	-
Army	104	17 (16.4%)	0.595
Navy	19	6 (31.6%)	0.210
Marine Corps	27	7(25.9%)	0.492

* Indicates reference category
p-values for proportions calculated using Pearson Chi-Square

In the study population for which BMI information was available (as seen in Figure 4), less than 1% of the individuals in the study population were considered underweight with a BMI less than 18.5 while 17.4% were considered at a healthy weight with a BMI within the between 18.5 and 24.9, 39.4% were considered overweight with a BMI between 25.0 and 29.9, and 42.3% were considered obese with a BMI greater than 30. Unfortunately, either height or weight information was missing for 38.2% of this study population, so BMI information was unavailable for this group.

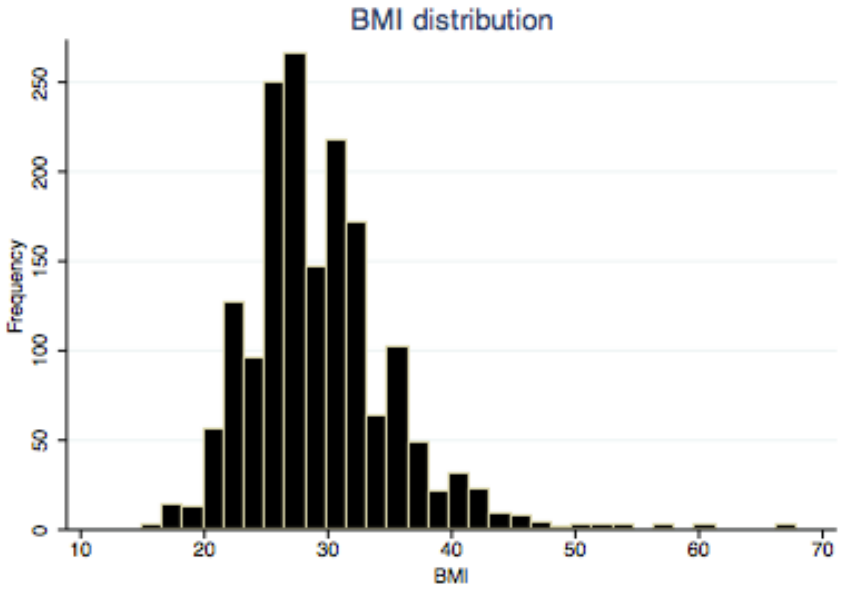

Figure 4: Distribution of BMI in study population by frequency (total # of individuals).

Though information on family history of prostate cancer was unavailable for 50 individuals out of the 2720 total, 18.9% of the remaining 2670 individuals reported family history of prostate cancer. Comparing family history status between individuals with and without prostate cancer, 17.1% of individuals without cancer reported family history of prostate cancer compared to 21.4% of individuals found to have prostate cancer.

Multivariate Analysis for Positive Prostate Biopsy

All continuous variables were examined and were transformed into categorical variables due to either significant deviations from linearity or to maintain consistency with clinical practice. Extreme values in PSA, PSAD, and BMI caused the Lowess plots and summary statistics to be skewed by a very small number of data points. These

extreme points were observed and analyzed for potential error in data entry. None of these extreme data points were determined to be due to data entry and were all within the realm of possible clinical findings. Therefore, these data were left in the model but these variables were categorized to avoid skewing the model based on these few extreme values. The Lowess smoothing plots and summary information can all be found in Appendix A.

TABLE 7
Model Building: Simple Logistic Regression for Positive Prostate Biopsy

	β	se(β)	OR	95% CI	*P*
Agent Orange*	0.168	0.152	1.18	0.88 to 1.59	0.269
Family History	0.282	0.103	1.33	1.08 to 1.62	<.01
Age 60-69, *y*	0.274	0.105	1.32	1.07 to 1.62	<.01
Age ≥ 70, *y*	0.478	0.116	1.61	1.28 to 2.03	<.001
BMI 18.5 - 24.9	-0.234	0.531	0.79	0.27 to 2.24	0.659
BMI 25.0 - 29.9	-0.319	0.523	0.73	0.26 to 2.03	0.542
BMI ≥ 30.0	0.018	0.522	1.02	0.37 to 2.83	0.973
PSAD 0.10- 0.14	0.702	0.13	2.02	1.56 to 2.60	<.001
PSAD 0.15 - 0.19	1.38	0.14	3.95	3.00 to 5.20	<.001
PSAD ≥ 0.20	2.28	0.123	9.75	7.66 to 12.41	<.001
DRE Suspicious	0.473	0.09	1.61	1.35 to 1.91	<.001
DRE Cancer Likely	2.70	0.211	14.87	9.83 to 22.48	<.001
Race = Black	0.223	0.227	1.25	0.80 to 1.95	0.326
Race = Hispanic	-0.334	0.474	0.72	0.28 to 1.81	0.481
Race = Asian	0.071	0.614	1.07	0.32 to 3.57	0.907
Race = American Indian	-0.845	0.634	0.43	0.12 to 1.49	0.183
Race = Other	-1.18	0.112	0.31	0.04 to 2.50	0.270
Service = Navy	0.196	0.125	1.22	0.95 to 1.55	0.116
Service = Army	0.102	0.099	1.11	0.91 to 1.34	0.300
Service = Marine Corps	0.247	0.169	1.28	0.92 to 1.78	0.145
Service = Coast Guard	0.495	0.360	1.64	0.05 to 3.83	0.170
Service = Merchant Marines	-0.809	1.098	0.45	0.39 to 1.18	0.461

CI Indicates Confidence Interval
BMI = Body Mass Index (Weight (lb) / (Height (in))^2 x 703), PSA= Prostate Specific Antigen,
PSAD = Prostate Specific Antigen Density, DRE = Digital Rectal Exam Findings

* Indicates primary predictor
p-values calculated using Wald Test

Upon univariate analysis of categorical variables, all variables were found to be significant at the 0.25 level. In the simple logistic regression comparisons shown in Table 7, AO, all levels of BMI, service in the Army or Merchant Marines, and Race except for

American Indian, were not found to be significant predictors of prostate cancer at the 0.25 level. While AO was left in the model as the primary predictor of interest, the rest of these non-significant variables were dropped.

After adjustment for all variables in the multivariate logistic regression model, all variables except for Race = American Indian remained significant at the 0.25 level and AO became significant at the 0.05 level. Test of collinearity found that no variables in the remaining model had significant collinearity. This produced the preliminary main effect model with the following variables: AO, Family History, Service History (Navy, Marine Corps, or Coast Guard), PSAD ($\geq 0.10 < 0.15$, $0.15 < 0.20$, ≥ 20.0, Unknown), Age (≥ 60 & < 70, Age ≥ 70), and DRE (Suspicious, Cancer Likely).

Interaction terms including AOxAge, AOxFamily History, AOxService History were evaluated due to the possible compounding effects of dioxin and age, varying degrees of exposure to dioxin based on service history, or effects of dioxin in relation to genetic predisposition for prostate cancer on the risk of developing prostate cancer. None of these variables were found to be significantly associated with the outcome at the 0.25 level. At this point, all variables not significant at the 0.05 level were removed to produce the final model.

Final Model

The primary predictor of interest, AO, was significantly associated with an increased risk of a positive prostate biopsies. As seen in Table 8, the risk of prostate cancer in those with AO exposure was 49% greater (aOR = 1.49, 95% CI: 1.05 - 2.10, p=0.02) than the

risk of prostate cancer in those without AO exposure. Odds ratio was used as an estimate of relative risk given that we have incident cases of prostate cancer and incidence of prostate cancer is relatively low. Additional predictors of prostate cancer include family history, age at biopsy, service in the Marine Corps, PSAD, and DRE results.

TABLE 8
Multivariate Analysis for Positive Prostate Biopsy

	OR	**95% CI**	***P***
Agent Orange*	1.49	1.05 to 2.10	0.02
Family History	1.33	1.05 to 1.67	0.02
Age < 60, *y*	1.00	-	-
Age 60-69, *y*	1.42	1.12 to 1.78	<0.01
Age ≥ 70, *y*	1.62	1.24 to 2.11	<0.001
Service = Air Force	1.00	-	-
Service = Marine Corps	1.63	1.12 to 2.37	0.01
PSAD < 0.10, *ng/ml/ml*	1.00	-	-
PSAD 0.10- 0.14, *ng/ml/ml*	2.12	1.63 to 2.77	<0.001
PSAD 0.15 - 0.19, *ng/ml/ml*	4.15	3.12 to 5.54	<0.001
PSAD ≥ 0.20, *ng/ml/ml*	9.05	7.03 to 11.65	<0.001
DRE Normal	1.00	-	-
DRE Suspicious	1.82	1.50 to 2.21	<0.001
DRE Cancer Likely	10.29	6.60 to 16.02	<0.001

CI Indicates Confidence Interval
PSA= Prostate Specific Antigen, PSAD = Prostate Specific Antigen Density, DRE = Digital Rectal Exam Findings

* Indicates primary predictor
p-values calculated using Wald Test

Model Diagnostics

Due to the high number of covariate patterns in this model, the Hosmer and Lemeshow test for goodness of fit using 25 groups was used to assess the fit of the model. There was no evidence to reject the null hypothesis that this model represents a

good fit of the actual data based on this method (p=0.617). Additionally, the area under the ROC curve (see Appendix B for ROC curve) was found toe be 0.769 suggesting excellent discriminative abilities of this model. While the predicted R^2 value for the model (calculated using the first recommended method by Mittlbock and Schemper (1996) [42]) was found to be 0.179, we expect that the R^2 value in logistic regression is slightly lower in logistic regression models than we would expect for a good model in linear regression. Adjustment for dispersion was not needed in this model as the (1/df)* deviance = 1.04 and the (1/df)*Pearson chi-square = 1.01.

After observational analysis of leverage vs. predicted probability, cooks distance vs. predicted probability, change in chi^2 vs. predicted probability, change in deviance vs. predicted probability, Pearson's standardized residuals vs. predicted probability, and a summary plot of change in chi^2 vs. predicted probability (*see Appendix D for plots*), no outliers were found to be of significant concern to the validity of the model.

A classification table was created to determine the sensitivity and specificity of this model in predicting the presence of prostate cancer (*See Appendix C*). This model classified an outcome as positive for prostate cancer if the predicted probability of prostate cancer was greater than or equal to 0.50. In our referred population with a prostate cancer prevalence of 32.9%, this model was found to correctly identify 74.3% of cases with a sensitivity of 45.1% and a specificity of 88.7%. This suggests that this model has relatively poor ability to predict prostate cancer when it is present but has relatively strong ability to predict the absence of prostate cancer when it is not present. Given that the sensitivity in predicting prostate cancer is most important in clinical practice, the

sensitivity of this model can be increased (to 92.1%) by using a predicted probability of 0.15. Consequently, this considerably decreases the specificity of the model to 31.4%. However, this model still correctly classifies 51.4% of individuals upon internal validation of the model when the underlying prevalence of prostate cancer in this referred population is 32.9%.

Multivariate Analysis for High Grade Prostate Cancer

All continuous variables were examined and were transformed into categorical variables in the same fashion as with Model 1 to maintain consistency across models. Upon univariate analysis of categorical variables, AO was the only variable not found to be significant at the 0.25 level but was left in the model as the primary predictor. In the simple logistic regression comparisons seen in Table 9, AO, all levels of BMI except for BMI of 25.0 to 29.9, Race, and service in the Merchant Marines were not found to be significant at the 0.25 level. These variables, except for AO, were dropped from the model.

After adjustment for all variables in the multivariate logistic regression model, BMI was no longer significant at the 0.25 level and AO became significant at the 0.25 level. Test of collinearity found that the no variables in the remaining model had significant collinearity. This produced the preliminary main effect model with the following variables: AO, Family History, Service History (Navy, Marines Corps, Army, Coast Guard), PSAD ($\geq 0.10 < 0.15$, $0.15 < 0.20$, ≥ 20.0, Unknown), Age (≥ 60 & < 70, Age ≥ 70), DRE (Suspicious, Cancer Likely).

Interaction terms including AOxAge, AOxFamily History, and AOxService History were evaluated once again due to the possible compounding effects of dioxin and age or dioxin in related to genetic predisposition to prostate cancer on the risk of developing high grade prostate cancer. None of these variables were found to be significantly associated with the outcome at the 0.25 level.

TABLE 9
Model Building: Simple Logistic Regression for High Grade Prostate Cancer

	β	se(β)	OR	95% CI	*P*
Agent Orange*	0.206	0.184	1.23	0.85 to 1.76	0.264
Family History	0.277	0.126	1.32	1.03 to 1.69	<.05
Age 60-69, *y*	0.373	0.141	1.45	1.10 to 1.91	<.01
Age ≥ 70, *y*	0.683	0.150	1.98	1.48 to 2.66	<.001
BMI 18.5 - 24.9	-0.522	0.558	0.59	0.20 to 1.77	0.349
BMI 25.0 - 29.9	-0.782	0.549	0.46	0.16 to 1.34	0.155
BMI ≥ 30.0	-0.410	0.547	0.66	0.23 to 1.94	0.453
PSAD 0.10- 0.14	0.683	0.203	1.98	1.33 to 2.95	<.01
PSAD 0.15 - 0.19	1.61	0.198	4.99	3.38 to 7.37	<.001
PSAD ≥ 0.20	2.49	0.173	12.01	8.55 to 16.86	<.001
DRE Suspicious	0.566	0.119	1.76	1.39 to 2.22	<.001
DRE Cancer Likely	2.94	0.181	18.89	13.24 to 26.95	<.001
Race = Black	0.167	0.290	1.18	0.67 to 2.09	0.564
Race = Hispanic	0.126	0.551	1.13	0.39 to 3.34	0.819
Race = Asian	0.126	0.777	1.13	0.25 to 5.21	0.871
Race = American Indian	-0.343	0.752	0.709	0.16 to 3.10	0.648
Service = Navy	0.407	0.157	1.50	1.10 to 2.04	<.01
Service = Army	0.280	0.128	1.32	1.03 to 1.70	0.028
Service = Marine Corps	0.340	0.213	1.40	0.93 to 2.13	0.111
Service = Coast Guard	0.841	0.404	2.32	1.05 to 5.12	0.037
Service = Merchant Marines	0.212	1.100	1.24	0.14 to 10.68	0.847

CI Indicates Confidence Interval
BMI = Body Mass Index (Weight (lb) / (Height (in))^2 x 703), PSA= Prostate Specific Antigen, PSAD = Prostate Specific Antigen Density, DRE = Digital Rectal Exam Findings

* Indicates primary predictor
p-values calculated using Wald Test

Final Model

After adjustment for confounders significant at the 0.05 level including PSAD, DRE, service in the Navy, the Marine Corps, or Coast Guard, and age at biopsy, individuals with AO exposure had 70% greater risk of having high-grade cancer compared to those

without AO exposure (aOR = 1.70, 95% CI: 1.10 – 2.55, p = 0.02). As seen in Table 10, AO appears to be particularly associated with high grade cancer (aOR = 1.70, 95% CI: 1.08 to 2.68, p =0.02 in the analysis of high grade cancer vs. no cancer; aOR = 1.23, 95% CI: 0.80 to 1.90, p = 0.35 in the analysis of low grade cancer vs. no cancer). Additional predictors of high grade prostate cancer include age at biopsy, service in the Navy, Marine Corps, and Coast Guard, PSAD, and DRE results.

TABLE 10
Multivariate Analysis for High Grade Prostate Cancer

	OR	**95% CI**	***P***
Agent Orange*	1.70	1.10 to 2.55	0.02
Age < 60, *y*	1.00	-	-
Age 60-69, *y*	1.56	1.15 to 2.13	<0.01
Age ≥ 70, *y*	1.91	1.36 to 2.69	<0.001
Service = Air Force	1.00	-	-
Service = Navy	1.52	1.07 to 2.18	0.02
Service = Marine Corps	1.89	1.17 to 3.06	<0.01
Service = Coast Guard	2.52	1.02 to 6.23	0.05
PSAD < 0.10, *ng/ml/ml*	1.00	-	-
PSAD 0.10- 0.14, *ng/ml/ml*	2.02	1.34 to 3.05	<0.01
PSAD 0.15 - 0.19, *ng/ml/ml*	4.99	3.32 to 7.49	<0.001
PSAD ≥ 0.20, *ng/ml/ml*	10.11	7.10 to 14.41	<0.001
DRE Normal	1.00	-	-
DRE Suspicious	1.93	1.50 to 2.49	<0.001
DRE Cancer Likely	12.84	8.69 to 18.97	<0.001

CI Indicates Confidence Interval
PSA= Prostate Specific Antigen, PSAD = Prostate Specific Antigen Density, DRE = Digital Rectal Exam Findings

* Indicates primary predictor
p-values calculated using Wald Test

Model Diagnostics

The Hosmer and Lemeshow test for goodness of fit using 10 groups was used to assess the fit of the model. This test does not provided significant evidence to reject the

null hypothesis that this model represents a good fit of the actual data based on this method (p=0.132). In addition, dispersion was analyzed in this model and (1/df) deviance = 0.70 and the (1/df)*Pearson chi-square = 1.05. These measures did not differ greatly from 1 suggesting that dispersion was not an issue. While (1/df) deviance was slightly lower than 1, this represents minor under-dispersion which suggests that the results are not inflated. The model adjusted for deviance increased the significance of the predictors, thus, the model without adjustment for deviance was used as the final model since this provides more conservative estimates.

The area under the ROC curve (see Appendix B for ROC curve) was found to be 0.809 suggesting excellent discriminative abilities of this model. Additionally, the predicted R^2 value for the model (calculated using the first recommended method by Mittlbock and Schemper (1996) [42]) was found to be 0.230. Once again, we expect that the R^2 value in logistic regression to be slightly lower in logistic regression models than we would expect for a good model in linear regression.

A classification table was created to determine the sensitivity and specificity of this model in predicting the presence of high grade prostate cancer (*See Appendix C*). This model classified an outcome as positive for prostate cancer if the predicted probability of prostate cancer was greater than or equal to 0.50. This model was found to provide a sensitivity of 27.0% and a specificity of 98.6% as well as correctly identify 86.5% of cases in this referred population with a high grade prostate cancer prevalence of 16.9% . This suggests that this model has minimal ability to predict high grade prostate cancer when it is present but has a very strong ability to predict the absence of prostate cancer

when it is not present. Given that the sensitivity in predicting high grade prostate cancer is most important in clinical practice, the sensitivity and specificity can be roughly equalized by using a predicted probability of .15 giving a sensitivity of 76.5% and specificity of 71.5%. This model still correctly classifies 72.4% of individuals upon internal validation of the model when the prevalence of high grade cancer in this referred population is 16.9%.

DISCUSSION

Our results suggest that AO is positively associated with nearly a 50% increase in risk of prostate cancer. This is in agreement with the findings of recent studies suggesting that AO exposure increases the risk of prostate cancer. Agent Orange exposure was also associated with a significant increase in the risk of high-grade prostate cancer in men referred for an initial prostate biopsy. If these associations are substantiated in larger future studies, these findings could have significant implications in the development of effective prostate cancer screening strategies in Veterans. The following is an evaluation of the observed associations and the possible causal relationship between AO exposure and prostate cancer risk as well as high grade prostate cancer risk.

Does an association exist?

A mix of evidence exists regarding the presence of an association between AO exposure and risk of prostate cancer due to several studies reporting non-significant results. However, since the 2001 study by Zafar *et. al.,* the majority of the reviewed

studies looking at AO exposure and risk of prostate cancer have shown a positive association and none have shown a protective effect. Though the study by Zafar *et al.* found no significant association between AO exposure and risk of prostate cancer, 13 (41%) of the 32 AO exposed individuals were found to have prostate cancer compared to 33 (34.4%) of the 96 non-exposed individuals. The investigators also compared the number of poorly differentiated cancers (Gleason score 7 or greater) between AO exposed and unexposed individuals. There were only 18 total individuals with poorly differentiated cancer and no association was found. The small sample size in this study warrants caution in interpretation as the investigators may have detected a significant association regarding AO exposure and risk of prostate cancer had they used a larger sample. Additionally, this study was limited in that Vietnam veterans exposed to AO had not yet reached an age at which prostate cancer is typically detected.

As mentioned previously, the 2004 pilot case-control study by Giri *et al.* also found a non-significant positive association with an odds ratio of 2.06 comparing odds of AO exposure between those with and without prostate cancer. Additionally, despite finding a slight positive but non-significant association between AO exposure and high grade cancer, a greater proportion of men with reported AO exposure were found to have extraprostatic disease extension compared to men without exposure suggesting that AO may be associated with more aggressive cancer. In either association, this study was also underpowered to detect these moderate associations.

The Operation Ranch Hand cohort study in 2004 reported a significant increase in the incidence of prostate cancer in veterans exposed to high levels of dioxin relative to the

incidence of prostate cancer in the general population [38]. As mentioned previously, a similar increase in prostate cancer incidence was seen in a comparison veteran group who had no exposure to dioxin suggesting the potential for unmeasured confounding which will be discussed later. This study did not evaluate the association between AO and high grade prostate cancer.

The 2008 historical cohort study by Chamie *et al.* compared 6214 individuals who were exposed to AO to 6930 individuals unexposed to AO. With this increase in power, they detected a relative risk estimate of 2.19 (95% CI: 1.75 to 2.75) which was highly significant. Additionally, this study detected a significant association with high grade cancer suggesting that the risk of having a Gleason score greater than 7 in those with AO exposure was roughly twice the risk in those without exposure.

Our study, which evaluated 2720 veterans, 896 of which were found to have prostate cancer and 459 were found to have high grade cancer, found a significant positive association suggesting that the odds of AO exposure in those with prostate cancer was 1.49 times the odds of exposure in those without cancer. A significant association was also found with high-grade cancer suggesting that individuals with cancer were 1.73 times as likely to have exposure to AO as those without cancer.

Overall, the bulk of the evidence supports a positive association between AO exposure and prostate cancer and suggests that this association may be more specific to risk of high grade prostate cancer.

Are these associations due to chance?

Despite the non-significant findings in several of the studies, our study as well as the studies by Chamie *et al.* and Akhtar *et al.* all found positive significant associations and are the only studies with adequate power to detect an odds ratio of less than 2.0. In addition to the significance of the observed associations in these studies, the consistency in the point estimates of the effect measures centering around 1.5 and 2.0 for both the associations of prostate cancer and high grade cancer would not be expected if these associations were due to chance alone. Thus, we conclude that it is not probable that these associations are due to chance.

Could the observed associations be due to bias?

Selection Bias

One of the main concerns regarding selection bias in our study was the possibility that physicians were more likely to refer an individual for prostate biopsy if it was known that an individual was exposed to AO. This would lead to an increase in the number of individuals being diagnosed with cancer that would have otherwise not been detected causing a false inflation of the effect measure. There are, however, several reasons why we do not suspect that this differential selection occurred. First, if this differential selection bias were to have occurred, this suggests that individuals with a history of AO exposure but similar PSA levels, DREs, age, race, and family histories would be referred for prostate biopsy at a higher rate than similar individuals without AO exposure. If this occurred, we would expect to see that AO exposure was associated with increased

detection of prostate cancer due to increased frequency of prostate biopsy, but we would also expect to see a higher proportion of individuals with low grade prostate cancer since individuals with high grade cancer generally display clinical symptoms, significantly elevated PSAs, or abnormal DREs that would have caused them to be referred regardless of AO exposure. In our study, the opposite was observed in that AO exposure was significantly associated with an estimated 70% increase in risk of high grade prostate cancer. This is not consistent with what would be expected if this physician referral bias accounted for the observed association between AO exposure and positive prostate biopsy. In fact, the 2001 study by Zafar *et al.* found that, on a yearly basis, 1.07% of those with a history of AO exposure were referred for prostate biopsy versus 1.33% of unexposed patients. While information regarding AO has become increasingly prevalent in medical practice since 2001, physicians at the Portland VA Hospital reported that this was not something that was taken into account when determining whether a patient should undergo prostate biopsy. This issue was also discussed by Chamie *et al.* who also observed an increase in risk of high grade cancer with exposure to AO.

Lastly, Chamie *et al.* suggested the possibility that AO exposure may have been associated with an increase in PSA which would have led more individuals with AO exposure to be referred for prostate biopsy as a result of having higher PSA levels but not having higher risk of prostate cancer. In our study, patients who were exposed to AO had a mean maximum PSA of 11.2 ng/ml compared to 12.4 ng/ml in those without AO exposure. Furthermore, of the individuals found to have prostate cancer upon biopsy, the average PSA of individuals exposed to AO was 8.8 ng/ml compared to 23.0 ng/ml.

Neither of these differences were statistically significant at the alpha = 0.05 level. These results, however, certainly do not support the hypothesis that AO exposure may be associated with a higher PSA level causing a higher rate of referral and a false association between AO exposure and prostate cancer and high grade prostate cancer.

Overall, there is no reason to suspect that the observed associations in our study or the other studies discussed are a result of selection bias.

Information Bias

A primary limitation of our study is that AO exposure status is subject to misclassification bias for several reasons. First, it is important to consider whether misclassification bias could have occurred due to individuals changing their exposure status through the VA hospital after receiving a diagnosis of prostate cancer. Unlike the study by Chamie *et al.,* we had no way to determine exactly how many individuals may have switched exposure status after being diagnosed with prostate cancer. The most likely reason that individuals would have switched exposure status following diagnosis of prostate cancer was that they simply had no reason to report their true exposure until they had a disease for which they could potentially receive financial compensation. It is possible that many individuals who truly have AO exposure do not report AO exposure to the VA as they wish to avoid the additional time and effort that is involved with the AO meeting that is required to receive compensation through the VA for diseases deemed to be related to AO exposure. The AO meeting involves a full history and physical examination and screening for different AO related conditions. Thus, it is crucial to

consider whether individuals who really did have exposure to AO simply did not indicate their exposure status until after their diagnosis of prostate cancer. If this occurred, we would expect that there are individuals who were misclassified as not having exposure simply because they had not reported this exposure. Thus, this would result in an artificial inflation of the number of prostate cancer patients with AO exposure and would create a differential bias away from the null.

An additional limitation of our study was not being able to record the time at which individuals reported AO exposure relative to the time of their diagnosis. However, if this switching of exposure status did occur, we would expect to see a similar proportion of individuals changing exposure status after diagnosis of cancer to the proportion seen in the study by Chamie *et al.* In their study, only 7 (0.11%) out of the 6214 exposed individuals switched exposure status after diagnosis of cancer. In our study, only 203 individuals reported exposure to AO. Assuming a similar proportion of exposure status changes, , we would expect to see at most one individual switch exposure status following diagnosis of cancer. Even if the proportion of individuals in our study who switched exposure status after prostate cancer diagnosis was 25 times the proportion in the study by Chamie *et al.,* we would still only expect to see 5 to 6 individuals switch exposure status. Given that the collection period of subjects for our study was similar to that of Chamie *et al*., it is not only highly improbable that our population switched exposure status 25 times as often as the population in Chamie's study, but even if this did occur, this would not explain the observed association in either our study or the study by Chamie *et. al.*

The association between AO exposure and high grade cancer seen in both our study and the study by Chamie *et al.* also adds additional evidence against AO exposure classification being a consequence of the diagnosis of prostate cancer or high grade cancer. In other words, if the observed association between AO exposure and positive prostate biopsy is simply an artifact of individuals claiming to have been exposed to AO as a result of their diagnosis of prostate cancer, we would not expect to see any association between AO exposure and high grade cancer. It has been suggested that individuals diagnosed with high grade cancer may be more likely to switch exposure status following diagnosis of cancer than individuals with low grade or no cancer. While this information was not recorded for data analysis, referring physicians in this study reported that they did not observe any discrepancy between high and low grade patients with regard to switching exposure status post-biopsy. The study by Chamie *et al.* who used a similar study population found no evidence of this occurring as well.

Another possible source of misclassification bias could have resulted due to capturing AO status through the VISN 20 data warehouse which includes AO status that is ultimately based on patient self report. Thus, an individual may claim to have a history of AO exposure regardless of his true exposure. Additionally, the duration and dose may have been so minimal in individuals who were exposed that exposure is not biologically significant. This bias, however, is likely non-differential as misclassification of exposure with regard to dose and duration is not related to diagnosis of cancer. Therefore, we expect this misclassification of AO exposure to bias the effect measure toward the null.

Thus, the positive associations that were found in both models are likely to underestimate the true strength of the associations.

While it is difficult to estimate the true magnitude of this potential misclassification bias, AO status was compared across service history to gauge the magnitude of this misclassification bias if present. It is likely that individuals serving in the Marine Corps had the highest prevalence of exposure during the war as these were the individuals on the ground in the areas that were heavily spayed with AO. Additionally, individuals in the Army and Air Force likely had the next highest prevalence of exposure as Air Force was responsible for handling and spraying AO across Vietnam and Army veterans were also on the ground in areas where AO was heavily used. While it is possible that individuals in the Navy had AO exposure due to inland river and river delta operations, handling of the barrels during transport, and time spent off the ships and on land [43], it is likely that individuals in the Navy were not heavily exposed. Lastly, we expected to see little to no exposure amongst individuals in the Coast Guard or the Merchant Marines though it is not well known whether AO was used in certain circumstances by these branches. In our study population, AO exposure patterns were similar to what would be expected in each service branch if minimal misclassification of exposure occurred. The Marine Corps had the highest prevalence of exposure at 14.3% with Army and Air Force veterans coming in next at 8.9% and 6.3%, respectively. Only 4.2% of all Navy veterans in our study population reported exposure while 0% reported exposure amongst the Coast Guard and Merchant Marines.

Unfortunately, information about exposure duration was not available. Shah *et al.*, in their review of the study by Chamie *et al.*, mention similar concerns regarding bias and reverse causation as discussed previously. However, they also suggest the possibility that veterans who served in Korea were included in the ‘‘unexposed’’ group, resulting in a weakening of the correlation of AO exposure and increased risk of prostate cancer. Additionally, roughly 3 million of the 8 million veterans who served in the Vietnam War served in Southeast Asia in areas where AO was heavily used. This suggests that just under 40% of all Vietnam veterans had potential exposure to high levels of AO and it is reasonable to assume that there are individuals who were misclassified as “unexposed” simply because they were unaware of their exposure to AO. This would also create a non-differential bias toward the null.

Overall, the cumulative effect of the bias in this study as well as bias seen in the other studies discussed suggests potential for underestimating the true risk of prostate cancer as well as high grade prostate cancer in association with AO exposure.

Could the observed associations be due to confounding?

Additional limitations of our study included lack of sufficient information regarding smoking status, occupation, alcohol use, dietary factors, co-morbidities, and use of medications such as finasteride. While information on finasteride use was available, it was not possible to determine whether this use occurred before or after prostate biopsy. In preliminary statistical analysis, we found finasteride to be strongly protective against prostate cancer. However, this is likely due to physicians prescribing finasteride to individuals who were found not to have cancer but were subsequently given finasteride to

treat benign prostatic hypertrophy (BPH). In fact, finasteride use has historically been restricted to urology service discretion. In our study population, it was routine practice to screen for prostate cancer prior to initiating its use. Thus, this information on finasteride use was not used in our modeling. In addition, information on smoking history and alcohol use was available but only for a very small percentage of individuals in our study and was therefore not included in the modeling.

While it is possible that smoking, alcohol use, dietary factors, and co-morbidities may be associated with AO exposure, it is not likely that finasteride use or occupation are associated with AO exposure. Given that our study as well as others have provided evidence against physicians referring individuals for prostate biopsy based on AO exposure status, this also suggests that physicians do not preferentially prescribe finasteride to individuals with AO exposure given the possible protective nature of finasteride use against prostate cancer. Thus, finasteride is not considered a potential confounder of the association between AO exposure and risk of prostate cancer or high grade cancer. Additionally, there is no evidence we are aware of to suggest that the underlying prevalence of any occupation such as farming, an occupation with high prevalence of exposure to herbicides and pesticides, is any different between individuals with AO exposure and those without. This suggests that occupation is not a confounder of concern in this association. However, smoking, alcohol use, and dietary factors must be discussed regarding their potential for confounding in this and other studies.

Smoking and Prostate Cancer

Associations have been observed between smoking and risk of prostate cancer, but similar to Agent Orange exposure, causality has not been formally established. While the majority of large studies suggest that smoking is not associated with increased risk of prostate cancer, there is evidence across a number of studies that suggests smoking may act to increase risk of fatal prostate cancer. It is difficult, however, to rule out the possibility of a missed weak association between smoking and risk of prostate cancer. Regardless, if a true small association exists that was missed by past studies, it is possible that smoking could be playing a significant role in a high risk population such as the US veteran population given that age-adjusted prevalence of smoking in US veterans is significantly greater (25%) than the prevalence of smoking in non-veterans (20%) [47]. In our study, it is possible that individuals who reported exposure to AO have different smoking patterns than individuals who did not report exposure to AO whether this is directly related to AO exposure or related to time spent in Vietnam. However, even if a difference was seen such that individuals who were exposed to AO also smoke more than individuals not exposed to AO, studies suggest that this would not increase the risk of prostate cancer but may increase the risk of fatal prostate cancer. The study by Chamie *et al.* did not find smoking status to be significant in their multivariate model suggesting that it was not a significant confounder. We expect that even if smoking is associated with AO exposure, that this would not be a confounder of our observed association between AO exposure and risk of cancer or high grade cancer.

Alcohol Use

Recently, heavy alcohol use has been suggested as a potential risk factor for prostate cancer. A large recent meta-analysis by Fillmore *et. al.* in 2009 suggested that prostate cancer incidence is positively linearly associated with heavier alcohol use [48]. Another recent study by Gong *et. al.* found that individuals who were daily drinkers had twice the risk of high-grade prostate cancer compared to non-drinkers and heavy drinking eliminated the protective effect of finasteride use for reducing prostate cancer risk [49]. In our study population, in it important to consider these findings as alcohol use may have significant implications on our findings due to the high prevalence of alcoholism in the veteran population.

First, if Vietnam veterans who were exposed to AO have higher prevalence of alcoholism and this could partially account for the differences in risk seen between those with and without AO exposure, it is unlikely that it accounts for the full association as the unexposed population in our study consisted of a large proportion of Vietnam veterans based upon the age and timeframe during which these individuals received prostate biopsies. Since there are a large number of Vietnam veterans who were in the unexposed population in this study, this suggests that the difference in risk of prostate cancer due to difference in alcohol use between the AO exposed and AO unexposed groups would be considerably smaller than that seen in the study by Gong *et. al.* While the studies by Akhtar *et. al.* and Chamie *et. al.* also did not control for alcohol use as a potential confounder, their selection of study participants included only Vietnam veterans. Thus, any differences seen between individual alcohol use that may be related to time spent in

Vietnam must not be related to AO exposure status. While it is possible that alcohol could in some other way be associated with AO exposure status, this is not something that has any supported evidence in the population of veterans in our study.

Diet

Similar to smoking and alcohol use, there is no reason to believe that a direct association exists between dietary habits and exposure to AO. However, it is possible that individuals who served in Vietnam have different eating habits than the general population. A wide range of studies discuss of potential risk factors for prostate cancer including intake of foodstuffs rich in fat or red meats as well as many possible protective factors such as selenium, zinc, isoflavones, carotenoids and lycopenes and vitamins E and D. In general, even if slight differences exist between Vietnam veterans and other veterans or the general population, we do not expect that appreciable differences exist in diet such that they are of significant concern as confounders in our study. Once again, the study by Chamie *et. al.* compared exposure amongst Vietnam veterans only which would eliminate the potential for diet as a confounding variable associated with time spent in Vietnam. Additionally, while the veteran population, as seen in our study, tends to have a higher rate of obesity than the general U.S. population, we found no significant differences in BMI between those exposed to AO and those not exposed to AO suggesting that nutritional habits are probably similar as well.

Other Sources of Confounding

The study by Akhtar *et. al.* raised concern regarding the potential for some other unmeasured confounder associated with time spent in Southeast Asia during the Vietnam War. While this study found a significant increase in the incidence of prostate cancer amongst white Ranch Hand veterans who were responsible for spraying AO compared to national incidence of prostate cancer, they also found similarly elevated incidence in the comparison group who were not responsible for spraying AO. The investigators hypothesized that this finding indicated that AO exposure was a surrogate for some other factor associated with being present in Southeast Asia during these years that was causing an increase in risk of prostate cancer. Several of the investigators tested this hypothesis just one year later. In this study, this comparison group had a median TCDD level measured in 1987 of 3.8 pg/g lipid (range 0.4 to 54.8 pg/g lipid), as compared with 5.7, 14.7, and 45.7 pg/g for Ranch Hands in the Background, Low, and High TCDD categories. While there was an increase in prostate cancer incidence related to increasing duration of service in Southeast Asia, no significant association was seen between prostate cancer incidence and level of TCDD. The investigators suggested that these comparison veterans who did not spray AO may have been exposed to other common pesticides or herbicides while in SEA and that AO may simply be a marker for these other exposures. However, while these comparison individuals did not spend as much time in Vietnam as the Ranch Hand veterans and were not responsible for spraying AO, it is possible that they were still exposed to AO at harmful levels during their time spent in the area. Given that the half-life of AO is roughly 7 years, it is possible that these individuals

did have elevated exposure to AO that were harmful, but given that the measurement of TCDD levels was roughly two decades after exposure, this test may have been unreliable for individuals with lower levels of exposure. Additionally, the study by Chamie *et. al.,* which compared only Vietnam veterans with and without exposure still found a significant association between AO exposure and prostate cancer providing evidence against the theory that AO is simply a marker for some other factor associated with time spent in Vietnam. This also provides evidence in support of the possibility that the measurement of TCDD levels in the Operation Ranch Hand studies were not accurate measures of true exposure to AO. Regardless, to our knowledge, no studies have been done to definitively rule out the possibility that AO exposure is simply a marker for some other factor that is truly causing the increased risk of prostate cancer.

Thus, we conclude that it is not possible to definitively rule that the observed associations between AO exposure and prostate cancer as well as AO exposure and high grade prostate cancer are not due to a confounder. However, we feel that the evidence provided from the study by Chamie *et. al.* along with the consistent evidence from our study provide sufficient evidence against this claim.

Overall Evaluation of Association

While it is difficult to rule out the possibility that AO exposure may simply be a marker for some other variable, it is important to consider the consistency seen across studies as well as the strength of the association, the overall ability of the reviewed studies to specifically define AO exposure, the biologic plausibility of such an

association, and other factors such as whether a dose-response relationship has been observed or if additional evidence such as intervention effect exists to support this association. Thus, attention must be given to examining the extent to which this possible confounding can affect the validity of the cumulative evidence for the association of interest and not simply whether or not this confounding exists. Additionally, there is no evidence strong enough to suggest that any of the alternative explanations including chance, reverse causation, and bias could account for the observed association. The only remaining question is whether AO exposure is causally related to the outcome of prostate cancer as the above considerations are not sufficient to support or eliminate cause.

CAUSAL EVALUATION

Strength of Association

The strength of association demonstrated in our study as well as other studies that show a positive association that centers around an estimated risk of prostate cancer in those who are exposed to AO that is roughly 1.5 to 2.0 times the risk in the unexposed. The estimated risk for high grade prostate cancer seems to have similar if not higher strength of association. Given the similar strength of association found for risk of prostate cancer as well as risk of high grade prostate cancer, this suggests that the observed associations between AO exposure and risk of prostate cancer may be driven primarily by an increase in risk of high grade cancer. In our study, two separate multivariate logistic regression models were run comparing AO exposure amongst individuals with high grade cancer and no cancer and a second comparison of AO exposure between those with low

grade cancer compared to no cancer. After adjustment for significant confounders, the odds of AO exposure in those with high grade cancer was significantly higher than the odds of exposure in those without cancer (aOR = 1.70, 95% CI: 1.08 to 2.70, p =0.02). In the second analysis, the odds of AO exposure in those with low grade cancer was not significantly different from the odds of exposure in those without cancer (aOR = 1.25, 95% CI: 0.81 to 1.92, p = 0.32). This further suggests that the strength of association seen in our study and previous studies looking at the association with AO exposure and risk of prostate cancer is driven by the association with high grade cancer. The strength of association for the association between low grade cancer and AO exposure is much smaller than the association observed in those with high grade cancer.

Overall, it is clear that the strength of the association between AO exposure and prostate cancer provide strong support for a causal relationship. Additionally, the strength of association between AO exposure and high grade cancer provide added support for a causal relationship and suggest that the increase in risk of prostate cancer with AO exposure manifests primarily in the form of high grade cancer.

How specific are these association?

While prostate cancer and high grade prostate cancer are clearly and specifically defined outcomes, AO exposure is less specific and is the main limitations of the literature surrounding this association. As previously discussed, there most specific way to define AO exposure is to measure actual levels of TCDD in the body. However, even studies that found some success in measuring TCDD are also limited in their ability to determine whether these tests are actually reflective of true exposure levels that occurred

at least two to three AO half-lives in the past. While studies measuring levels of TCDD in Vietnam veterans are not only very expensive, it has been nearly 45 years since many individuals were exposed. Thus, this test is no longer a practical solution to detecting moderate levels of AO exposure in veterans that may have been harmful.

Additionally, the limitations of self-reported AO exposure status have been discussed previously. In any case, it is clear that the true exposure status with regard to dose, duration, exposure site, etc., of individual veterans is something that will remain illusive. Thus, the specificity of the association between AO exposure and prostate cancer as well as high grade cancer provides only weak evidence for a causal conclusion.

How consistent is the association?

External consistency:

As mentioned, the consistency across the studies looking specifically at AO exposure and prostate cancer with regard to effect measure and positive associations provides a great deal of support for a causal conclusion. Both our study and the study by Chamie *et. al.* found that both prostate cancer risk and risk of high grade prostate cancer were associated with AO exposure. These two studies also found that individuals with AO exposure were diagnosed with prostate cancer at a younger age than individuals not exposed to AO. Other studies found consistent relative risk estimates including studies that may have been underpowered and found no significant association.

Overall, external consistency provides strong support for a causal conclusion.

Internal consistency:

There has been very little evidence in any study to suggest any significant inconsistency within individual studies. The Operation Ranch Hand studies which found similar increases in the incidence of prostate cancer between individuals with AO exposure and the comparison group of Vietnam veterans without known AO exposure are one source of internal inconsistency that has been shown. The same investigators' follow-up study which found a dose-response relationship between time spent in Southeast Asia and risk of prostate cancer in the same Operation Ranch Hand population also found no difference in prostate cancer incidence in individuals who were exposed to AO compared to those who were not. Exposure in this population, however, was estimated based on the TCDD test which we have previously discussed regarding its limitations. As mentioned earlier, this does present the possibility for confounding, however, later studies such as the study by Chamie *et. al.* which included only Vietnam veterans did find a significant difference in prostate cancer risk in relation to AO exposure. It is very possible that the inconsistencies seen in the studies measuring TCDD levels are a consequence of inaccurate estimation of true AO exposure status amongst individuals who had moderate but significant exposure levels.

We are aware of no other internal inconsistencies in the additional studies. In our study, the slightly stronger association seen between AO exposure and high grade prostate cancer than the association with AO exposure and prostate cancer overall is what we would expect if there is a true causal association between AO exposure and prostate cancer that is primarily driven by high grade prostate cancer. Similar internal consistency

was also seen in the study by Chamie *et. al.*

Thus, we conclude that internal consistency provides moderate support of a causal association.

Biologic Plausibility

As discussed previously, the US Environmental Protection Agency has reported on the plausible biological model for the mechanism by which AO could cause prostate cancer in humans. Dioxin, the main toxin associated with AO, is thought to bind with aryl hydrocarbon (AHC) receptors that are responsible for a signal cascade by translocating to the nucleus of the AHC's associated cell and attaching to a second aryl hydrocarbon nuclear translocator protein. This attachment allows the second translocator protein to bind to regulatory regions of target genes and act as a tumor promoter [19]. This suggests that dioxin may not necessarily be associated with the actual initiation of cancer but may cause promotion of cancer that manifests in faster progressing cancer. In other words, the increased risk in prostate cancer that has been observed may simply be a product of AO increasing the severity of non-clinically detectable cancers such that they become detectable while also increasing the severity of cancer that would already be detectable. As a result, AO is associated with increased risk of prostate cancer and high grade cancer likely as a result of cancer promotion and not cancer initiation.

Additionally, while it is difficult to rule out whether AO is simply a confounder of some other factor that is closely associated with AO exposure, AO is already established as a causal agent of many other conditions including various cancers and pesticide

exposure amongst farmers and in other occupational settings also have shown consistent associations with cancer as discussed previously.

The biologic plausibility as well as the coherence between this association and other established associations provides strong support for a causal association.

Dose Response

Observation of a dose response relationship was not possible in studies like our own which use patient self-report of AO exposure. However, it is possible that the 2005 study by Pavuk *et. al.* which found a dose response relationship between prostate cancer incidence and years of service in Southeast Asia is actually representative of the duration of AO exposure. While this same study found no association with levels of TCDD and prostate cancer incidence, it is important to remember that the half life of TCDD in the body 0.4 to 10 years suggests that many individuals who were exposed to AO would not have detectable TCDD levels.

In general, no dose-response relationship has been directly shown between AO exposure and prostate cancer or high grade prostate cancer in humans. Thus, dose response does not add any support to a causal association but also does not detract from this conclusion.

CONCLUSIONS/RECOMMENDATIONS

Despite the lack of an observed dose-response, the lack of a demonstrated intervention effect, and the possibility of some unknown confounder, evidence supports an existing causal association between AO exposure and prostate cancer primarily mediated through cancer promotion thus resulting increased risk of high grade prostate cancer. Unless further evidence is presented that is largely inconsistent with the findings of our study and other recent studies, there is little to no evidence sufficient to suggest that this is not a causal relationship based on the consistency in the results across a range of study designs.

Currently, individuals who are thought to be at increased risk of high grade cancer, such as African-Americans, are screened more aggressively for prostate cancer in order to prevent high grade disease. In fact, prostate cancer incidence amongst African-Americans is roughly 50% higher than the incidence amongst white individuals which is similar to the strength of association we have observed in association with AO [1]. Similarly, patients with a FHx of prostate cancer are recommended to undergo more frequent screening. Based on the evidence in support of a causal relationship between AO exposure and prostate cancer and high grade prostate cancer suggesting that individuals with reported exposure to AO are roughly 50% more likely to develop cancer and 70% more likely to develop high grade cancer, prostate cancer screening may need to be modified for those with a history of AO exposure, such as more frequent screening at younger age.

It is important to acknowledge the fact that our conclusion is based on the evidence at

hand and that there is always the potential for error. However, failure to modify screening recommendations may lead to increased incidence of high grade prostate cancer as late diagnosis of disease may mean that patients with AO exposure will continue to be detected in later stages of this disease. If our conclusion regarding the causal association is not correct and our recommendations are followed, the consequences of more aggressive screening of individuals with AO exposure could lead to unnecessary increased anxiety from the patient perspective and potentially treatments that are overly aggressive. However, the consequences of not screening these individuals, who may harbor higher grade prostate cancers more aggressively, seem to outweigh the consequences associated with increased anxiety and potentially increased frequency of prostate biopsy amongst individuals exposed to AO.

Based on the strong evidence that is available, it does not appear cost effective or appropriate to measure levels of TCDD in Vietnam veterans to look once again for the presence of dose response or to confirm our findings as well as others while using the serum testing. With Vietnam veterans who were exposed to AO already at the point where many are being diagnosed with prostate cancer, it would be ill advised to look for further supportive evidence before making the recommended screening changes known to physicians. The benefits of making prostate cancer screening more aggressive for individuals with a history of AO exposure has the potential to prevent hundreds and even thousands of individuals from developing metastatic disease or from facing serious complications association with high grade prostate cancer.

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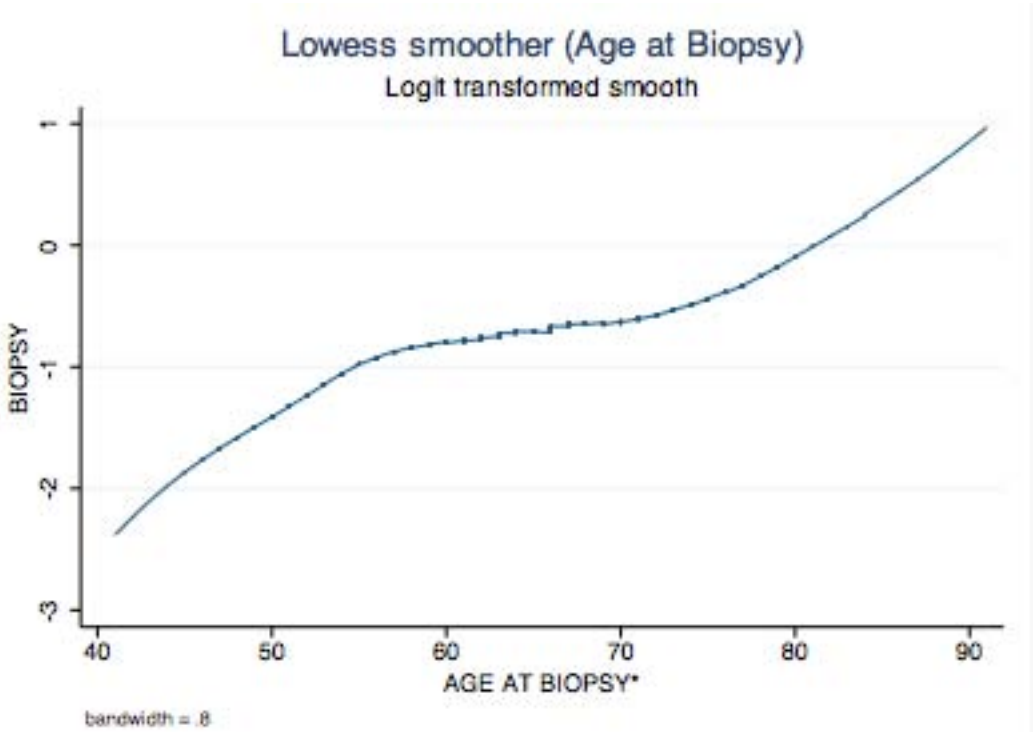
APPENDIX A

Lowess Smoother Plots

Age at Biopsy Summary Statistics and Lowess Smoother Plots

AGE AT BIOPSY*

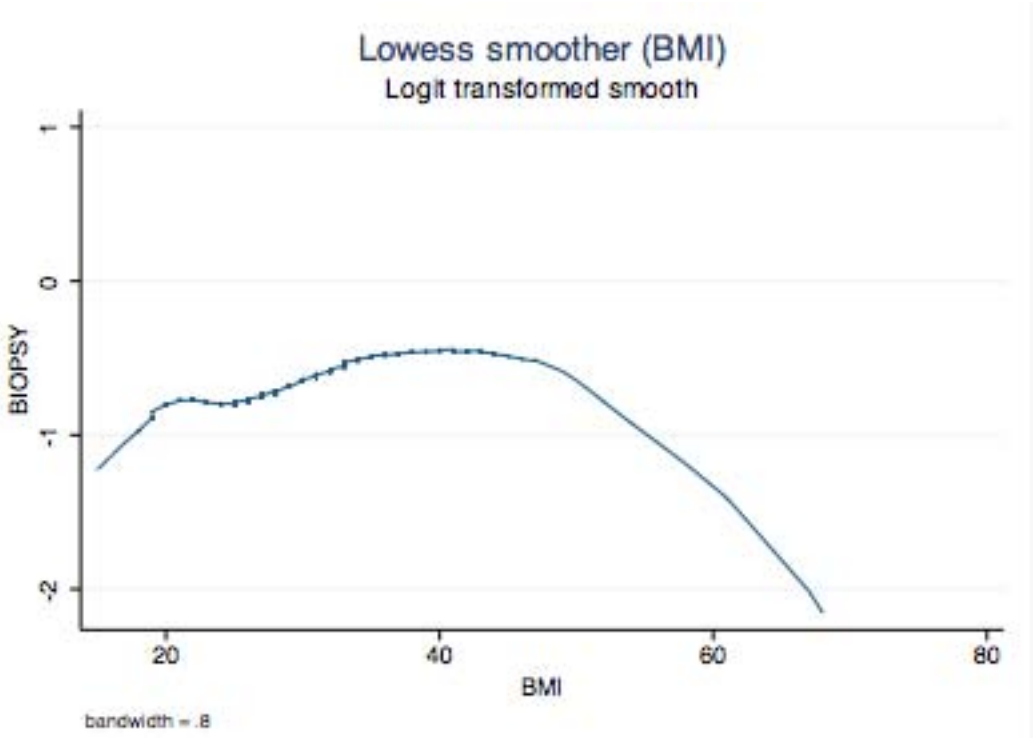
	Percentiles	Smallest		
1%	48	41		
5%	53	42		
10%	55	42	Obs	2720
25%	60	43	Sum of Wgt.	2720
50%	65		Mean	64.71324
		Largest	Std. Dev.	7.344973
75%	70	88		
90%	74	89	Variance	53.94863
95%	76	90	Skewness	-.0002232
99%	82	91	Kurtosis	2.944573



PSA *Summary Statistics and Lowess Smoother Plots*

BMI

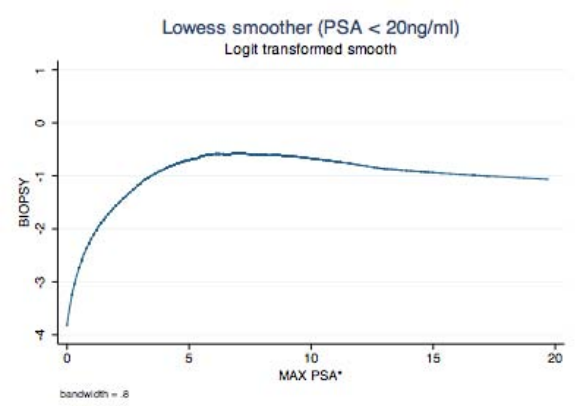
	Percentiles	Smallest		
1%	19	15		
5%	21	16		
10%	23	17	Obs	1682
25%	26	17	Sum of Wgt.	1682
50%	29		Mean	29.33115
		Largest	Std. Dev.	5.81228
75%	32	61		
90%	36	61	Variance	33.7826
95%	40	67	Skewness	1.25359
99%	47	68	Kurtosis	7.132488

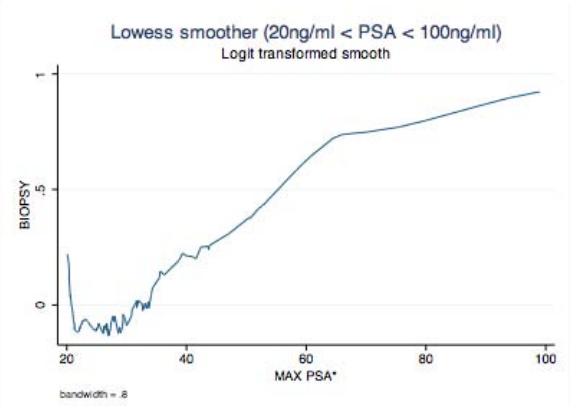


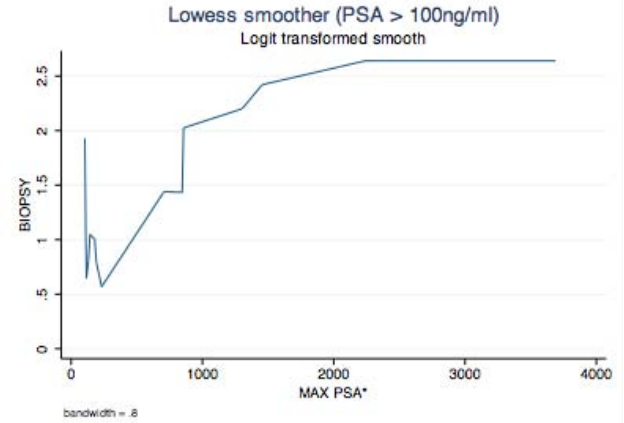
PSA Summary Statistics and Lowess Smoother Plots

MAX PSA*

	Percentiles	Smallest		
1%	.5	0		
5%	1.1	0		
10%	1.9	0	Obs	2712
25%	4.6	.05	Sum of Wgt.	2712
50%	6.2		Mean	12.33427
		Largest	Std. Dev.	95.01214
75%	8.7	1302		
90%	13.5	1455	Variance	9027.307
95%	20.6	2239	Skewness	28.90307
99%	66.2	3683	Kurtosis	971.0513



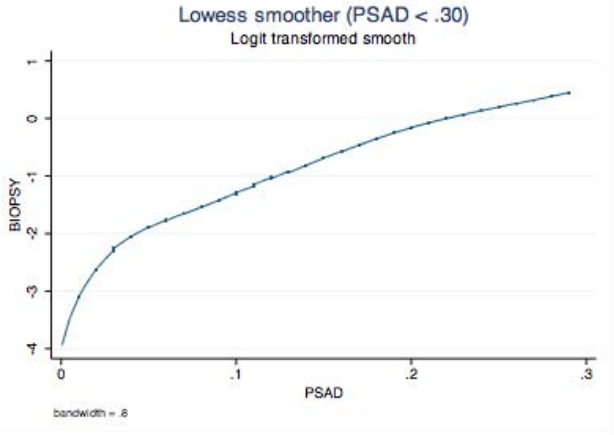


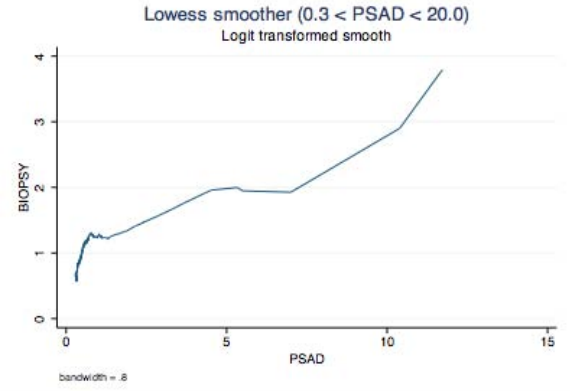


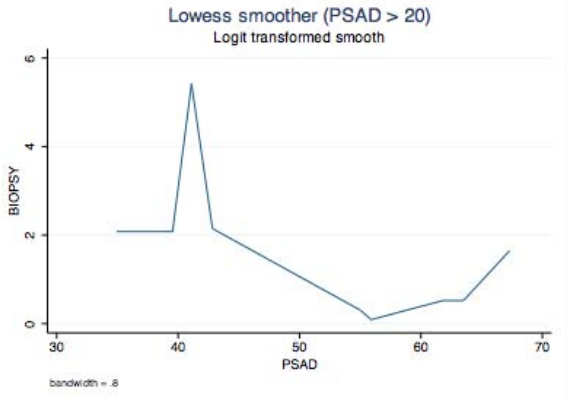
PSAD Summary Statistics and Lowess Smoother Plots

PSAD

	Percentiles	Smallest		
1%	.013	.0005		
5%	.03	.005		
10%	.05	.01	Obs	2686
25%	.08	.01	Sum of Wgt.	2686
50%	.12		Mean	.3648239
		Largest	Std. Dev.	3.057097
75%	.2	55.9		
90%	.32	61.82	Variance	9.345842
95%	.5	63.5	Skewness	17.82446
99%	1.62	67.3	Kurtosis	334.7577

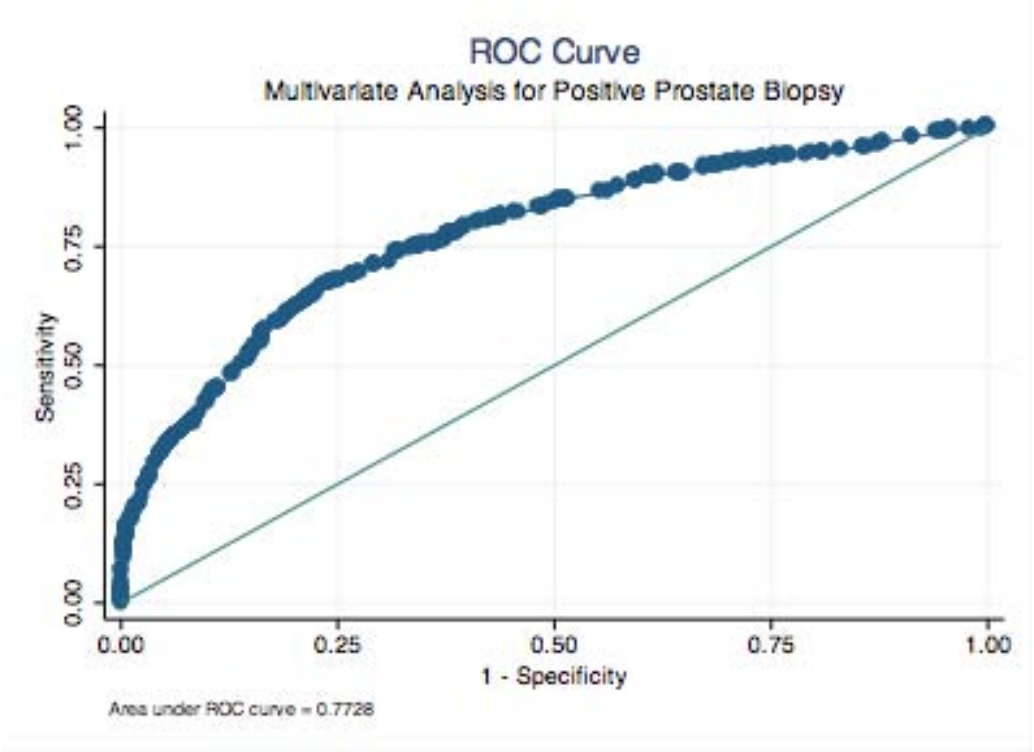


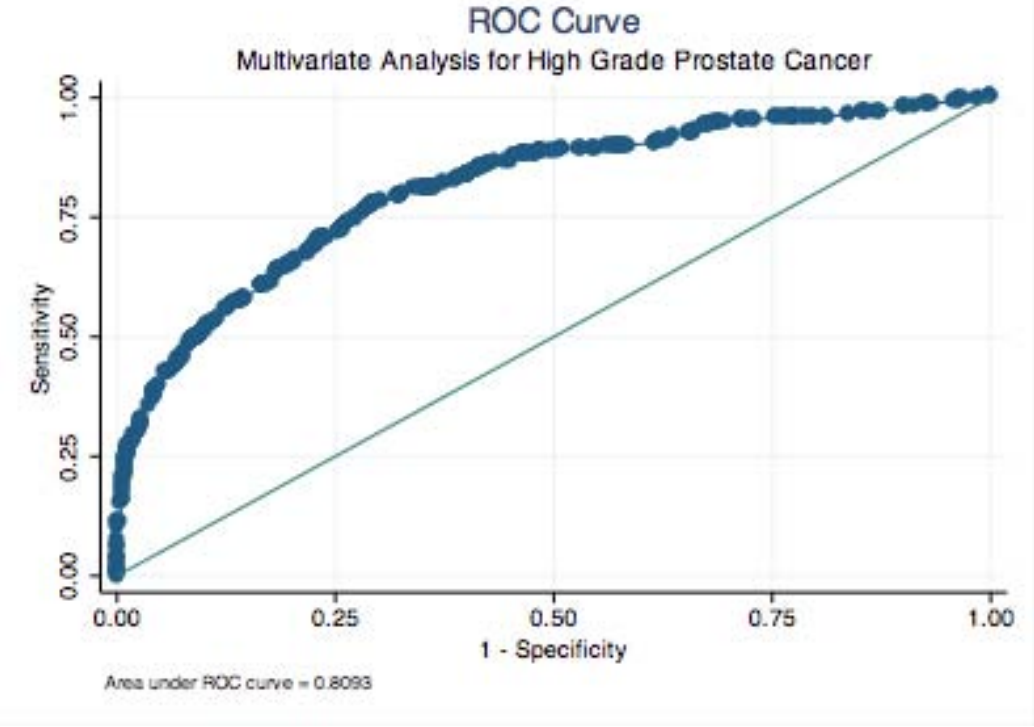




APPENDIX B

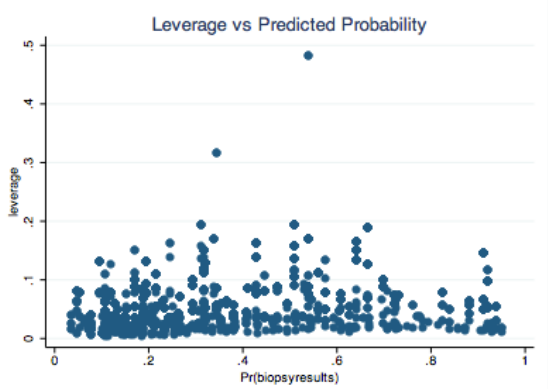
ROC Curves

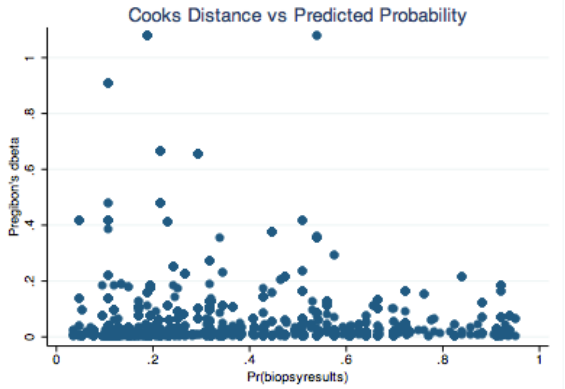


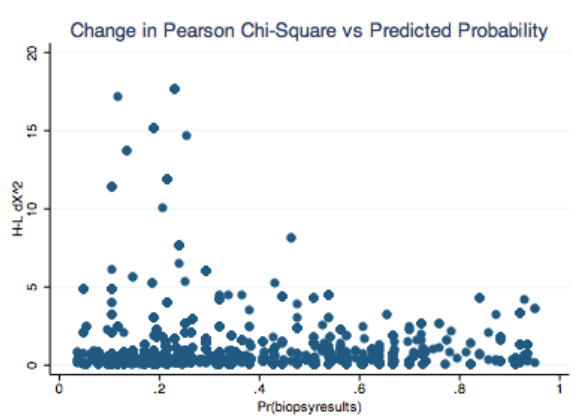


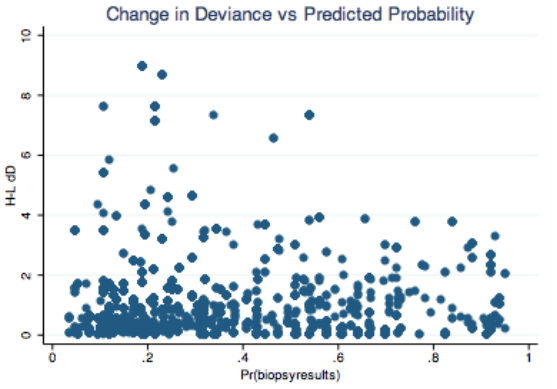
APPENDIX C

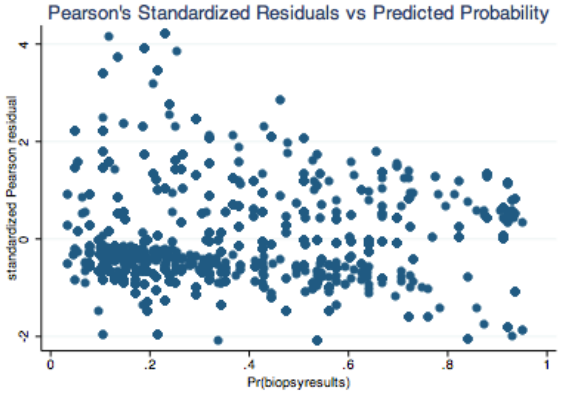
Residual Plots

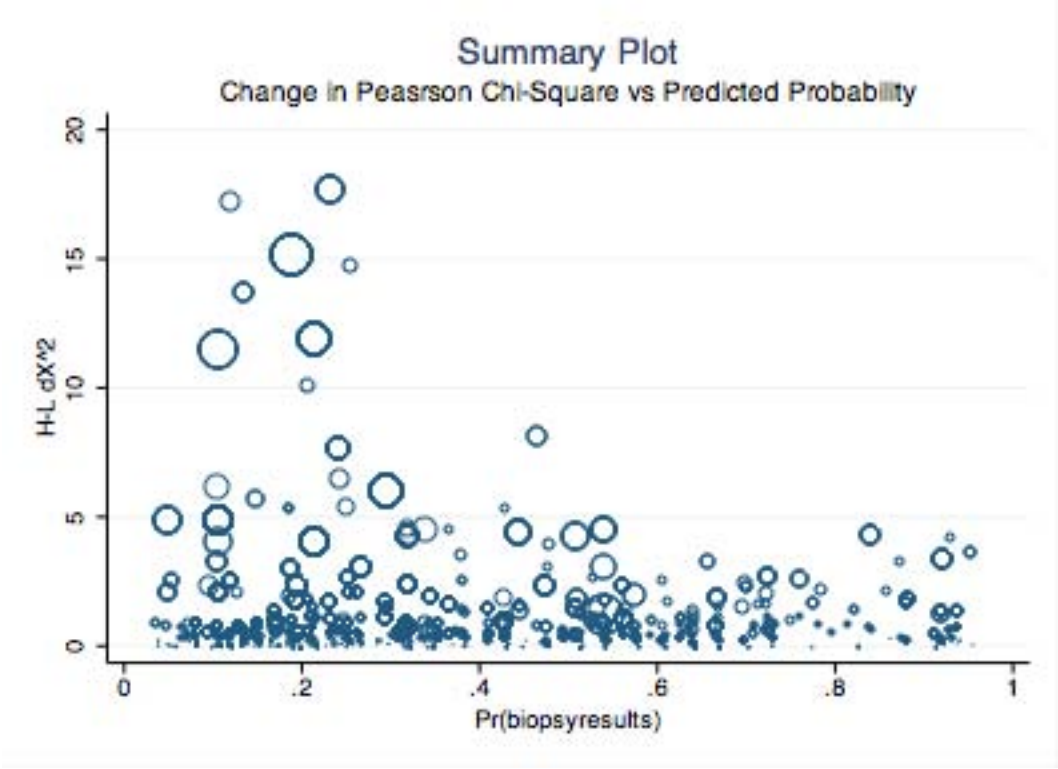











Summary Plot
Change in Peasrson Chi-Square vs Predicted Probability
H-L dX^2
0
5
10
15
20
0
.2
.4
.6
.8
1
Pr(biopsyresults)

APPENDIX D

Classification Tables

Multivariate Analysis for Positive Prostate Biopsy

Predicted Probability = 0.50

Classified	True D	True ~D	Total
+	404	206	610
-	492	1618	2110
Total	896	1824	2720

Classified + if predicted Pr(D) >= .5
True D defined as biopsyresults != 0

Sensitivity	Pr(+| D)	45.09%
Specificity	Pr(-|~D)	88.71%
Positive predictive value	Pr(D| +)	66.23%
Negative predictive value	Pr(~D| -)	76.68%
False + rate for true ~D	Pr(+|~D)	11.29%
False - rate for true D	Pr(-| D)	54.91%
False + rate for classified +	Pr(~D| +)	33.77%
False - rate for classified -	Pr(D| -)	23.32%
Correctly classified		74.34%

Predicted Probability = 0.15

Classified	True D	True ~D	Total
+	825	1251	2076
-	71	573	644
Total	896	1824	2720

Classified + if predicted Pr(D) >= .15
True D defined as biopsyresults != 0

Sensitivity	Pr(+| D)	92.08%
Specificity	Pr(-|~D)	31.41%
Positive predictive value	Pr(D| +)	39.74%
Negative predictive value	Pr(~D| -)	88.98%
False + rate for true ~D	Pr(+|~D)	68.59%
False - rate for true D	Pr(-| D)	7.92%
False + rate for classified +	Pr(~D| +)	60.26%
False - rate for classified -	Pr(D| -)	11.02%
Correctly classified		51.40%

Multivariate Analysis for High Grade Prostate Cancer

Predicted Probability = 0.50

Classified	True D	True ~D	Total
+	124	32	156
-	335	2229	2564
Total	459	2261	2720

Classified + if predicted Pr(D) >= .5
True D defined as gleasonscore != 0

Sensitivity	Pr(+| D)	27.02%
Specificity	Pr(-|~D)	98.58%
Positive predictive value	Pr(D| +)	79.49%
Negative predictive value	Pr(~D| -)	86.93%
False + rate for true ~D	Pr(+|~D)	1.42%
False - rate for true D	Pr(-| D)	72.98%
False + rate for classified +	Pr(~D| +)	20.51%
False - rate for classified -	Pr(D| -)	13.07%
Correctly classified		86.51%

Predicted Probability = 0.15

Classified	True D	True ~D	Total
+	351	644	995
-	108	1617	1725
Total	459	2261	2720

Classified + if predicted Pr(D) >= .15
True D defined as gleasonscore != 0

Sensitivity	Pr(+| D)	76.47%
Specificity	Pr(-|~D)	71.52%
Positive predictive value	Pr(D| +)	35.28%
Negative predictive value	Pr(~D| -)	93.74%
False + rate for true ~D	Pr(+|~D)	28.48%
False - rate for true D	Pr(-| D)	23.53%
False + rate for classified +	Pr(~D| +)	64.72%
False - rate for classified -	Pr(D| -)	6.26%
Correctly classified		72.35%

APPENDIX E

Logistic Regression Model

Model 1: Positive Prostate Biopsy

ghat(Positive Prostate Biopsy) = 0.397(Agent Orange) + 0.486(Marine Corps) + 0.753 (PSAD ≥ 0.10 < 0.15) + 1.42(PSAD ≥ 0.15 < 0.20) + 2.20(PSAD ≥ 0.20) + 0.600 (DRE Suspicious) + 2.33(DRE Cancer Likely) + 0.354(Age ≥ 60 & < 70) + 0.482 (Age ≥ 70) + 0.282(Family History) -2.63

Model 1: High Grade Prostate Cancer

ghat(High Grade Prostate Cancer) = 0.541(Agent Orange) + 0.413(Navy) + 0.630 (Marine Corps) + .939(Coast Guard) + 0.696(PSAD ≥ 0.10 < 0.15) + 1.60(PSAD ≥ 0.15 < 0.20) + 2.31(PSAD ≥ 0.20) + 0.666(DRE Suspicious) + 2.57(DRE Cancer Likely) + 0.439(Age ≥ 60 & < 70) + 0.695(Age ≥ 70) -4.10