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Prostate Specific Antigen (PSA) Growth Curves: A Method to Improve Prostate Cancer Screening

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PROSTATE SPECIFIC ANTIGEN (PSA) GROWTH CURVES: A METHOD TO IMPROVE PROSTATE CANCER SCREENING

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DEDICATION

To the three people who brought me to this life and raised me with so much love; my dad, mum, and aunt Siham. To my life partners Halla and Gowtham. To my incredible mentors back home, Rana, Abed and Rita and to the holy land I come from and will continue to cherish, Palestine.

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ABSTRACT

Prostate cancer (PrCA) screening aimed at detecting aggressive disease represents a significant public health issue. Development of biomarkers to predict PrCA that is likely to kill if left untreated is a major challenge. This dissertation focused on analyzing existing repeated measures of prostatic specific antigen (PSA) to develop and validate a tool to improve both sensitivity and specificity of the PSA-based screening test to detect high-risk PrCA. We used the Prostate Lung Colorectal and Ovarian trial data (PLCO) for PSA growth model building. Using 6 years of annual PSA measurements we established the PSA growth curves for four groups of men; those who developed high-risk PrCA, those who developed low-risk PrCA, those who developed benign prostatic hyperplasia (BPH) and those who were not diagnosed with either PrCA or BPH. We used these curves to estimate PSA annual rate of change at defined time points; one and two years before diagnosis for each individual in the cohort. We then examined the area under the curve (AUC) to estimate the specificity and the sensitivity of PSA annual rate thresholds. We validated our work by replicating the PSA growth models in a cohort of screened men in The Department of Veterans Affairs. Our results show that PSA annual change rates varied significantly by cancer status in both cohorts. The difference between the means of PSA rate values across the four groups of men was high and robust. Annual individual PSA rates showed substantial variability; however, a distinct range and significantly higher values were observed among men who developed high-risk PrCA. This resulted in high AUC (0.97) in the logistic regression model. A threshold of

0.37ng/ml/year had the best combination of sensitivity and specificity; i.e., of 97.2%, and 97.3% respectively. In the VA validation cohort, the same pattern was observed. However, men in the low-risk PrCA group had higher annual PSA rates as compared to the same group in the PLCO cohort. This resulted in a lower AUC of 93.3 (92.86-93.71) and the threshold of 0.37ng/ml/year predicted high-risk PrCA with a sensitivity and specificity of 95.5% and 86.2 % retrospectively.

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CHAPTER 1: INTRODUCTION

Prostate cancer (PrCA) is a major source of morbidity in the US population [1]. After skin cancer (basal cell and squamous cell) and *in situ* cancers, it is the most common cancer, accounting for 27% of all cancers in men [1]. PrCA has a relatively high incidence that is coupled with racial disparities [2, 3] making it a public health challenge. About 1 in 7 American men are diagnosed with PrCA during their lifetime[1]. However, PrCA is considered relatively indolent, as 83% of those clinically diagnosed are reported to die from something else [6] – in fact, during autopsy many men are found to have incidental PrCA that had never become clinically evident[4].

There is now consensus that most men, if they lived long enough, will develop histological PrCA[5]. Still, most of these men will die due to reasons unrelated to PrCA. Once PrCA is clinically detected the survival is strongly related to its aggressiveness at the time of detection. Men with localized PrCA have a 5-year relative survival rate of 100%, while those with distant metastases have less than one third the relative survival rate (31.9%) [6]. Early detection of men with virulent tumors might reduce PrCA mortality and morbidity, while early detection of men with indolent low-risk PrCA that is less aggressive, may increase iatrogenic morbidity without impacting mortality.

Prostate specific antigen (PSA) was initially identified as a marker for the management of PrCA, and over the past 20 years it had become a routine, inexpensive

PrCA screening tool [7]. Aggressive use of this tool is thought to have led to an increase in the identification of indolent low-risk PrCA as most of the newly diagnosed PrCAs were found to have low prognostic risk (i.e., Prognostic group I, IIA) – a concept called 'PrCA stage migration' [8-10]. In 2011, The American College of Preventive Medicine (ACPM) [11], American Urological Association (AUA) [12], American Cancer Society and the U.S. Preventive Services Task Force (USPSTF) [13] recommended against the use of PSA for routine population-based PrCA screening. The over detection of indolent PrCA coupled with over-treatment and iatrogenic harm became the basis for the recent controversies on PSA-based screening recommendations [14].

PSA-based screening is not thought to be able to differentiate between aggressive PrCA and indolent PrCA. Much of the work to improve PSA-based screening, including PSA kinetics, complexed PSA, PSA density, free PSA, aimed to increase the screening sensitivity and specificity of PrCA [15]. Up until now, there has been no conclusive evidence to suggest that PSA-based screening is able to distinguish aggressive PrCA from other prostate pathologies such as indolent PrCA, benign prostatic hypertrophy (BPH) or even normal prostate.

In 1993 Carter et.al. first proposed a concept of using serial PSA tests over a long-time as a PrCA screening tool [16]. The use of serial PSA measures has been variously described as PSA kinetics, PSA velocity and/or PSA rate. Although PSA velocity was not commonly used during PrCA screening, it was widely used in the management of prostate cancer (i.e., among men who had undergone prostatectomy) [17]. The main idea is that the continuum of PSA values reflects tumor activity and provides additional value over one single PSA value. Until today there is no clinical trial that has examined the

effectiveness of PSA kinetics in predicting PrCA and findings from observational studies have not been conclusive in resolving this issue. Another problem is the lack in methodological homogeny in the PSA kinetics literature.

When taken together, current literature suggests the following three key messages related to PSA kinetics: first, when measured rigorously the rate of PSA change is quantitatively and qualitatively different by prostate pathology such that it may be possible to distinguish aggressive PrCA, non-aggressive PrCA, benign prostatic hyperplasia (BPH) and normal prostate [12, 17-19]. Second, there is no single threshold for a linear PSA rate/PSAV that has been found to significantly enhance the prediction of (any) PrCA over a single recent PSA value [10]. Finally, there is an opportunity to use PSA kinetics in predicting aggressive PrCA [18, 19].

In the absence of a reliable method for PrCA screening, the burden of the disease is expected to grow. The main concern must be aggressive PrCA, a virulent disease associated with high clinical risks that requires medical treatment and is expected to impact the morbidity and mortality of the affected individual. The challenge is to improve screening specificity for clinically high-risk, aggressive disease and to avoid over detection and treatment of insignificant, indolent low-risk PrCA [20].

We conducted this dissertation with the aim of using repeated measures of PSA in order to develop, refine and validate a tool that will improve both sensitivity and specificity of the current PSA-based screening test for high-risk aggressive PrCA – the hypothesis was that the use of multiple repeated PSA tests over time will be able to distinguish virulent, high-risk PrCA from other underlying prostate pathology including

low-risk PrCA, BPH and normal prostate. If results obtained from the study are consistent with our hypothesis, then a significant portion of the unnecessary prostate biopsies and PrCA treatment, for men with low-risk PrCA, may be avoided. This will reduce unwanted expenses while improving quality of life and cost-effectiveness.

In this dissertation, we modeled PSA change to develop PSA growth graphs/curves based on statistical models of repeated PSA measurements from men with high-risk aggressive PrCA, low-risk indolent PrCA, BPH and normal prostate. The data was then used to test our hypothesis that these curves are significantly different, with differences described quantitatively. Through these graphs, estimates of PSA change over time may be derived at an individual level at any time before diagnosis – giving an estimate of an individual patient's probability that their prostate biopsy would identify high-risk PrCA vs. any of the other conditions.

We used an innovative and robust approach to achieve our aims while considering accumulated evidence. We used two independent large population-based data sources: the Prostate Lung Colorectal and Ovarian (PLCO) clinical trial data [21] and the national clinical data from the routine care of patients of The Department of Veterans Affairs (VA). The PLCO clinical trial data was used for model building while the VA data was used for model validation. Our statistical modeling approach allows for broad generalizability by avoiding unrealistic assumptions and restrictions -the goal is to ensure that the tool may be used for individual level decision making at the bed-side. We used nonlinear mixed models [22] that explicitly account for between and within individual variability, while also estimating the overall mean PSA growth trajectory across the two data sets without imposing unrealistic statistical assumptions. This is a 'change-point

model' that assumes that PSA level changes in a time-dependent manner, starting in a linear form and then changing to a non-linear form – called 'change point' – during the natural history of the disease.

We believe that this dissertation work has important clinical implications for early detection and prevention of high-risk PrCA, especially for population subgroups that are at higher-than-average risk of virulent PrCA such as African Americans and young men.

1. A. Objectives

The overall goal of this research work was to identify and refine a means for differentiating "significant" prostate cancer (i.e., virulent/aggressive disease with high potential for causing harm) from any other condition that could be related to an increase in PSA level at any particular point in time. To achieve this, the specific aims of our work are:

1. To describe and establish separate graphical reference growth curves for different patterns of PSA change over time according to disease presence and virulence using longitudinal repeated measures of PSA from patients confirmed to: a) have no clinically detected PrCA, b) have low-risk PrCA, c) have high-risk PrCA. The curves were adjusted for age, race, initial PSA and BMI. The model was built using data from the PLCO Cancer Screening trial [21]. (See chapter 4)

2. To compare the curves from patients with high-risk (clinically significant) PrCA to those without and estimate test characteristics, especially specificity and sensitivity, based on the resulting curves. (See chapter 5)

3. To validate the resulting curves in a different population in which individuals have multiple PSA measures, but measured opportunistically as opposed to on a regular, fixed schedule. To achieve this aim we will apply the same analysis techniques as developed in PLCO using enterprise-wide national electronic health record data from the Veterans Department of Veterans Affairs (VA). (See chapter 6)

CHAPTER 2: BACKGROUND

2. A. Prostate cancer

Globally, Prostate cancer (PrCA) is the 2nd most common cancer and the 6th leading cause of cancer related deaths among men [23]. With the aging world population, global burden of PrCA is on the rise. The number of new cases is expected to increase to about 1.7 million in 2030. Significant variation persists internationally [23]. While it is hard to compare incidence rate across the world, due to differences in screening patterns and cancer registration procedures, PrCA appears to be more common in Western populations such as Europe and the USA. However, in these countries the rates appear to have started to decline.

In the USA , 233,000 new cases and 29,480 deaths are estimated to occur in 2014 [1]. Combined with Lung cancer, PrCA account for about 50% of all cancers among American men [1]. The lifetime probability of being diagnosed with PrCA cancer is 15.3% (1 in 7) and the median age at diagnosis is 66 years old [1]. Trends have been changing since 1992.

Figure 2.1 and .2.2 represent long-term trends in cancer incidence and death rates for PrCA. The graphs show a general decreasing trend of PrCA incidence rates since

2000. However, year to year fluctuation is evident. This fluctuation is due to the variation in the use of prostate specific antigen (PSA) screening across the years. The estimated annual decline between 2006 and 2010 for PrCA incidence rate is about 2%/year, and the decline in mortality rate for the same period is about 3%/year [1]. In the US, the peak of PrCA mortality was around 1992-1993; since then and until 2010, death rates have declined by 45% as a result of enhancement in screening and treatment. There is considerable geographical variation in PrCA occurrence, which might be confounded by racial distribution and some differences in PSA test utilization (figure 2.3); for example, the age -adjusted incidence rate is the highest in the District of Columbia (194.4 per 100,000 men) and the lowest in Arizona (112.7 per 100,000). Also, District of Columbia has the highest age- adjusted death rate of 38.8 per 100,000, while Hawaii has the lowest death rate of only 15.7 per 100,00.

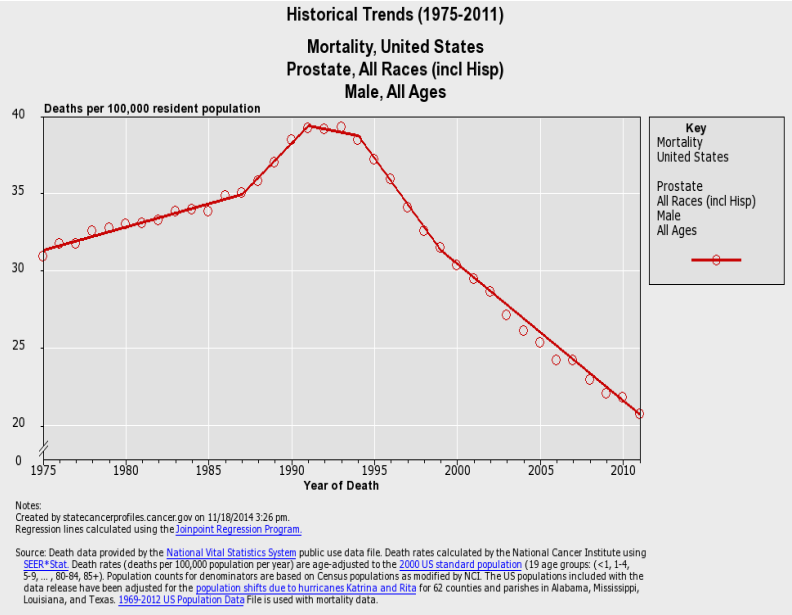


Figure 2.1 Historical mortality trends of Prostate Cancer in the United States

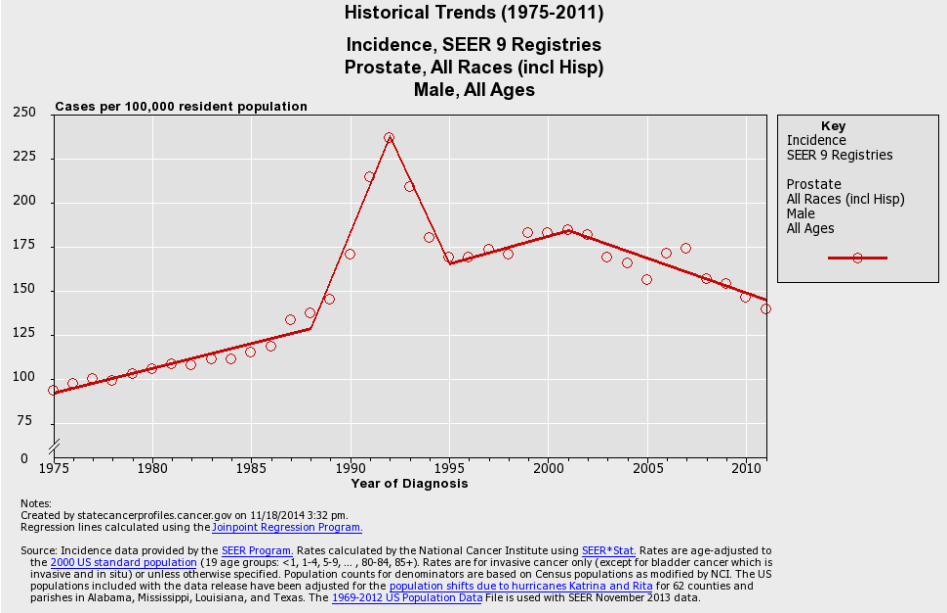


Figure 2.2 Historical Incidence trends of Prostate Cancer in the United States

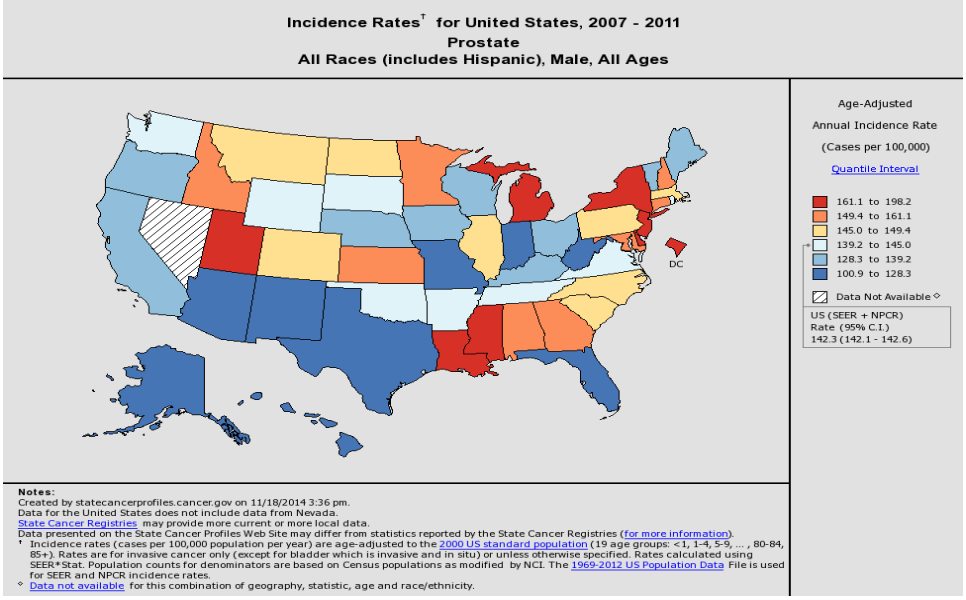


Figure 2.3 Incidence rate of Prostate Cancer (2007-2011) by state

Prostate cancer is a heterogeneous disease with multiple genetic and environmental factors involved in its etiology. However, the main known risk factors are age, race and genetic/family history [24]. The risk of prostate cancer increases with age; it is an extremely rare disease among those under 40 years old (1 in 10,000) and relatively common among those above 60 years of age (1 in every 15) [25]. However, those at younger age are more likely to present with highly aggressive disease [26]. African Americans continue to have some of the highest incidence and mortality rates in the world [25]. They are 1.6 times more likely to be diagnosed with prostate cancer and 2.4 times more likely to die of the disease when compared to European Americans [25]. African Americans tend to present with more aggressive characteristics of PrCA upon detection when compared to others [3]. Disparities also persist in screening, treatment regimens, disease quality of life and survivals [5]. Evidence suggests that the disease has different etiology among African Americans as compared to other races[5]. The determinants of this observed racial disparity across the continuum of PrCA occurrence and management seem to be complex; including genetic/biological, environmental (socio-economic and socio-cultural) and health services factors. Men with immediate relatives who have or had PrCA are twice as likely to have PrCA when compared to those who don't. Five to ten percent of PrCA cases are believed to be mainly caused by high-risk inherited genetic factors or prostate cancer susceptibility genes [27]. There is some evidence that accumulation of genetic risks is also associated with an aggressive PrCA but the findings are not conclusive. Some dietary exposures may also increase the risk of PrCA, these include; fat and/or meat consumption, lycopene, and dairy products/calcium/vitamin D. The evidence regarding these factors is yet not conclusive

[24]. Similarly, there is some evidence that BMI /obesity and endogenous hormones (androgens and estrogens) alter the risk of PrCA.

PrCA screening has been the central focus for cancer research over the past couple of decades. The discovery of PSA is a significant milestone in the history of PrCA prevention and early detection. However, there are other biomarkers, imaging and clinic-based screening methods that have been proposed and studied. In this section we will provide summary information on some of these non-PSA-based screening tools. In the next section, we will provide a more detailed description of the evidence behind PSA-based-diagnostics.

One of the oldest and common methods for PrCA detection is digital rectal examination (DRE). This clinical exam detects abnormalities such as asymmetry, induration or nodules in the posterior and lateral aspects of the prostate gland. Most cases detected via DRE are likely to be very advanced, as lower staged PrCA such as T1 (TNM stage) cancers are by definition non-tangible. The estimated sensitivity, specificity and positive predictive value of DRE to predict PrCA is 59% , 94 % and 28% respectively [28] . Most of PrCA cases detected by DRE are clinically advanced, making the value of the test (stand alone) questionable [29]. Clinical control trials have not shown improvement in PrCA outcome when detected by DRE [29]. The evidence is stronger for benefit when DRE is used in combination with a PSA screening test [30]. Observational studies have shown that PSA test and DRE complement each other's and thus combining the two can improve PrCA screening [7, 31-33]. However, the PLCO clinical trial did not demonstrate significant survival benefits for men who underwent combined PSA and DRE screening [34, 35].

Prostate cancer antigen 3 (PCA3) is a main emerging biomarker for PrCA. It is a noncoding RNA that has been shown to be overexpressed (>90%) in PrCA tissue [36]. This biomarker is both sensitive and specific to PrCA and unlike PSA it is not sensitive to non-cancerous factors like BPH or prostate volume or prostatic infection[36]. Urine samples, that contain cells shed from prostate during urination, can be used as non-invasive tool to determine a PCA3 score. The main limitation though is the sensitivity of urine PCA3 test ranged from 47-69% across different studies. Currently the use of PCA3 is in combination with PSA[36]. The FDA approved the use of PCA3 for PrCA detection for men with persistent high PSA levels and a previous negative biopsy. The studies that investigated PCA3 are insufficient, but there is promising indication that this biomarker may have future role in PrCA screening. In general advances in genetic mapping have shifted biomarkers research to the potential of the "-omics" diagnostics. Other promising biomarkers are; TMPRSS-ERG gene fusions, he enzyme alpha-methylacyl-CoA racemase (*AMACR*), Germline prostate cancer risk loci etc. Also, some imaging-based technologies such as transrectal ultrasound, computed tomography , magnetic resonance imaging and positron emission tomography have been shown to improve PrCA screening.

2. B. Prostate specific antigen (PSA)

PSA discovery has revolutionized PrCA occurrence and treatment. The steep decline of PrCA mortality in the population after the widespread use of PSA based screening can't be a coincidence but does not indicate causality [6, 37]. The other hallmarks of PSA testing are the dramatic increase of PrCA incidence and the migration to a lower stage disease[37]. The approval for adapting PSA based screening at population wide level was made in absence of any evidence from controlled trials,

making it a controversial topic[38]. Today and after the completion of two population based randomized clinical trials, the controversy is still persistent.

2. B.1. What is Prostate specific antigen (PSA)

PSA is a normal, abundant prostate-secreted serine proteinase with a half-life of 2.2 days [39]. It is encoded by the KLK3 gene and is secreted by prostate gland epithelial, normal prostatic acini and abnormal neoplastic prostatic cells. PSA is a major protein in the semen, its main function is to cleave and liquefy semen allowing sperms to swim freely [39]. It also dissolves cervical mucosa to allow the entry of sperm into the uterus [39]. During the secretory process a small fraction (active and inactive form) leaks into the bloodstream through the normal gaps found in the loose prostate basement membrane barrier [40]. Thus it can be measured in the blood and may serve as a marker for prostate activity.

Neoplastic prostatic cells are generally less mature compared to normal secretory cells, and their absolute per-cell PSA secretion is lower compared to normal cells. However, the circulating levels of leaked serum PSA is much higher in PrCA compared to when there is no PrCA. This is because cancer cells disrupt the basement membrane leading to increased leakage of PSA into circulation[41]. This disturbance of the basement membrane is pathognomonic of cancer and its growth. Thus, among patients with confirmed PrCA , the use of PSA has been established to reliably and non-invasively predict the extent of PrCA and estimate the response of PrCA to therapy[41].

PSA test was discovered in 1970's and was originally approved by the Food and Drug Administration (FDA) in 1986 to monitor the progression of prostate cancer in men

already diagnosed with the disease [42]. It was also used to detect recurrence of the tumor after therapeutic intervention of prostate cancer. Later on, some large scale observational studies showed that PSA can also be used for PrCA detection [43]. Eventually the FDA approved its use for early diagnosis of prostate cancer in 1994 [42].Throughout the last 15 years it has been routinely used as an inexpensive screening tool for PrCA.

2. B.2. PSA test performance for detecting PrCA

Recent research studies such as the Prostate Cancer Prevention Trial have been unable to identify a definitive PSA cut off point that has the most optimal sensitivity and specificity for PrCA [44]. It is now universally accepted that there is a continuum of risk for PrCA at all levels of PSA, with higher levels being associated with the highest risk PSA. The cutoff point of 4ng/ml leads to a sensitivity of (70-90%) for prostate cancer, and since PSA is also produced by non-malignant cells the 4ng/ml threshold has a specificity of only 20-40% a positive predictive value (PPP) of about 30%. Further, PSA is not specific to high-risk prostate cancer; about 75% of cases with PSA level of (4-10 ng/ml) are diagnosed with locally confined disease that is considered of low-clinical risk – indolent PrCA. Most patients with such low-risk PrCA are more likely to die from other causes before PrCA becomes clinically advanced enough to cause morbidity and mortality.

Over the years of the "PSA era" a new stage of low-grade, low-stage, low-risk (indolent) prostate cancer widely emerged among the screened populations. The introduction of PSA testing as a population based screening of "normal-risk" asymptomatic men led to the detection of many such new cases. From 1985 to 1995 the trends of prostate cancer completely shifted, 'stage migration', the incidence doubled but

only 1 of 5 detected cases was likely to be virulent[45, 46]. It is argued that diagnosing and treating such low-risk PrCAs is more likely to cause harm to a patient's health than benefit.

Some common benign conditions can also elevate PSA level resulting in many false positive and some false negative results are:

- Age: There is a slight natural increase of PSA with age. It is estimated that PSA increase by 0.2ng/ml/year. This increase in value is attributed to the growth of the prostate with time/aging [47].
- Race/ethnicity: There is some evidence that African American men tend to have naturally higher PSA at a given age while Asian tends to have lower PSA when compared to Caucasians. Some of these differences can be explained by the differences in the prostate gland size or prostate gland volume - others are attributable to genetic factors [47].
- Medications: Finasteride (5 alpha-reductase inhibitor) blocks the conversion of testosterone to dihydrotestosterone which leads to decreases in prostate volume and lower serum PSA. In one study, it was estimated that men who took finasteride had a 50% decrease in serum PSA level after 1 year of treatment [48]
- Prostate gland inflammation/infections: PSA level increase and fluctuate with prostatitis. Changes vary depending on the category of the inflammatory process. Also, PSA seem to increase in men with signs and symptoms of urinary tract infection (UTI) with positive bacterial culture. Among these men PSA level may increase reaching levels of 14.1 ng/mL during the acute phase of an infection [49].

- Benign prostate hyperplasia (BPH): This is the main factor of high PSA levels in non-cancer cases. The increased amount of benign prostatic tissue, increases serum PSA significantly. This is a very common condition among men and the risk increases with age. Men with BPH can have PSA levels as high as 15 ng/ml.
- BMI: There is an inverse relationship between BMI and PSA levels. It is estimated that there is 5% to 21% decrease in PSA value in men with BMI > 30 compared to men with normal BMI. This effect is primarily thought to be due to hemodilution [50].
- Other factors include trauma and lab variability, DRE examination and ejaculation.

2. B.3 The effectiveness of PSA based screening (findings of randomized control trials):

In 2011, The U.S. Preventive Services Task Force (USPSTF) and other medical agencies recommended against the use of PSA for routine population-based PrCA screening[51]. This recommendation was heavily based on the findings of two randomized control trials that investigated the effectiveness of PSA based screening in improving PrCA outcomes[52]. We will discuss below seven relevant randomized clinical trials; the Quebec[53-55], the two Sweden studies - the Norrkoping [56, 57] and Kjellman et [58]; European randomized study of screening for prostate cancer (ERSPC) [59-61] , French ERSPC[62], Gothenburg[63], and the Prostate, Lung , Colorectal and Ovarian (PLCO) cancer screening trial [64-66]. Two of these still ongoing (ERSPC and PLCO) at the time of this review.

The 1988 Quebec prospective randomized controlled trial by Labrie et al. [53-55], was one of the earliest clinical trials. Through 15 years, 31,133 men identified from Quebec City were randomly assigned to PSA and digital rectal examination (DRE) screening with 15,353 men as their controls. Only 24% (7,348 men) of the intervention arm complied (76%) didn't. In the control arm, 7% (1,122 men) performed screening at one point of the follow up. Given this significant contamination, the authors didn't perform an intention to screen analysis. Instead the analysis was done based on whether man actually underwent screening or not. The relative risk for PrCA death among the screened group when compared to the non-screened group was 0.39 (95% CI, 0.21-0.71). There were 11 PrCA deaths among screened group and 217 deaths among the unscreened. The core limitation here is the breaking of the randomization, especially that the authors didn't adjustment for potential confounding. Such analysis essentially represents an observational study. However, in one of their subsequent publications the authors provided data on intention to screen analysis. Using this approach, there was no significant difference in PrCA mortality between the intervention and the control groups (RR= 1.09; 95% CI 0.82- 1.43).

In 2004, Sandblom et al. [56, 57] published their work of 15 years follow-up of a quasi-randomized pilot study. Starting 1987, the investigators used the national population register to randomly assigned 1,494 men into PSA and DRE screening - every three years. 7,532 men served as the control group. Forty three cases (3%) of PrCA were detected in the intervention group compared to 292 (4%) in the control group. There was no difference in PrCA or overall mortality between the two groups. The study was not

powered to detect statistical difference in PrCA mortality, as the detected cases are not big enough to detect significance different between the 2 groups.

Kjellman et.al [58] published another Sweden trial that started in 1988 and compared one-time PrCA screening using a combination of PSA, DRE and transrectal ultrasonography with a control group of no screening. The study included 1,796 men who agreed to participate as an intervention group (out of 2400 men who were between 55 to 70 year of age and living within a defined geographical area in Stockholm - randomly selected by investigators). 24,804 men from the remaining source population served as the control group for the trial. After 15 years of follow-up there was no significance difference in PrCA deaths between the intervention group and control group. There were 53 PrCA deaths in the intervention group (26% of diagnosed) and 506 deaths in the control group (28% of diagnosed) leading to an incidence rate ratio of 1.10; 95% CI, 0.83-1.46. Limitations of this study include; use of high PSA cut off point for biopsy (≥10ng/ml), the screening group simultaneously underwent a combination of screening methods making it hard to isolate the effect of PSA and making the application side challenging and it was not clear whether the committee who assigned the cause of death were blinded to the screening allocation, a potential deferential misclassification bias.

The three aforementioned trials provided an early useful context for PrCA screening using PSA. However, each of them had some critical methodological limitations, especially when it comes to allocation concealment, description of loss of follow up and blinding of assignment to assessors. The recent USPSTF review on the topic considered those three trials as “poor-quality trials” and used their evidence with lower weights towards the overall recommendation[51].

The ERSPC [59-61] is an ongoing cross-national trial that has included eight European countries; France, Sweden, Netherlands, Finland, Belgium, Spain, Italy and Switzerland. The trial was launched in 1990's with 182,160 men between 50 and 74 years who were randomly assigned to screening arm with PSA testing or a control arm without (72,890 screening arm vs. 89,353 as control). The protocol and the implementation varied slightly by country; randomization took place prior to consent in three countries and post consent in five, France joined later in 2001 and thus its data was not included in the first (ERSPC) 2009 publication, instead the French data was published separately. While most countries included PSA measures in the intervention arm, the cut off point for biopsy varied; 3 ng/ml was commonly considered as positive results. The most important source of variation was differences in screening frequency: six countries tested every 4 years, Sweden screened every 2 years and Belgium had one screening every 7-years. Overall, 82.2% of men in the screening group actually underwent screening at least one test with some variation among the 8 countries. The authors reported that the study was designed to have sufficient power to account for a 20% contamination rate; however they did not provide enough information about actual rates of screening in the control arm. The study included age range of 50-74 but the investigators predefines a "core" group of 162,387 men with a narrower age group of 55-69. Men were followed up for an average of 8.8 years (median of 9 years). In the main and overall study population analysis there was some reduction in PrCA mortality in the intervention arm when compared to the control arm but this was not statically significant (rate ratio 0.85; CI 95% 0.73-1.00). However, in the predefined "core" group there was a statistically significant reduction (20%) in PrCA mortality among the intervention arm when compared to the control arm. There

were 214 PrCA deaths in the screened group vs. 326 deaths in the control group: RR of 0.80; 95% CI 0.65-0.98). This reduction started to emerge after about 8 years of the follow up and is translated into an absolute reduction of 0.71 PrCA deaths per 1,000 men. In addition, the investigators performed exploratory analysis on different age groups and reported interesting findings; there was a significant PrCA death reduction among men aged 65 to 69 years (RR of 0.74; 95%CI 0.56-0.99), in contrary there was statistically non-significant trend toward increased risk of PrCA death among younger men (50 to 54 years old) with a RR of 1.47; 95%CI 0.41-5.19. Similar trend was observed among the oldest age group (70 to 74 years old) with a RR of 1.26; 95%CI 0.80-1.99. The overall findings can be interpreted as 1,410 men aged between 55 and 69 years needing to be screened and 48 additional prostate cancers needed to be treated (NNT) to prevent or delay one PrCA death. This is considered a high relatively NNT number, making PSA – if considered useful not efficient. Recently published, is the third and the most updated results for the study [67], these included an extended follow up of 13 years. The rate ratio of prostate cancer mortality was 0.79 (95%CI 0.69-0.91) at 13 years, the non-participation adjusted RR was 0.73 (95% CI 0.61-0.88). The absolute risk reduction of death from prostate cancer at was 0.11 per 1000 person-years which translates into one prostate cancer death averted for every 27 (17-66) additional PrCA detected [67]. These latest findings provide strongest evidence supporting the PSA screening. However, as the authors point out, this trial didn't consider the harm of over detection and over treatment associated with screening, and they concluded that more evidence is still needed.

The French ERSPC [62] is originally part of the ERSPC study, but as mentioned above, was reported separately. The whole study included 84,781 men aged 55 to 69

years; 42 590 men were included in the screened group and 42,191 in the control group. Men in the screening group were repeatedly invited by mail to be screened while nothing was done for the control group. The protocol included randomization before information and consent. Knowledge of any pre-existing PSA test was obtained through the health insurance database. Cumulated incidence of PrCA with a four years follow-up was 2.48% (n=1,053) in screening and 1.99% (n=840) in control group, with a relative risk (RR) of 1.242. Mortality measures were not yet reported due to short period of follow up. The published accessible work does not provide enough information to make conclusion on the effectiveness of PSA screening to reduce mortality. Another published work was released but with limited access [62].

The Gothenburg trial [63] is also related to the ERSPC study as it included men previously reported in the ERSPC study. In the Gothenburg trial 19,904 participants were enrolled in three birth cohorts (1930-1934, 1935-1939, and 1940-1944). The first two, (1930-1934, 1935-1939) are basically the Swedish cohort (n=1,185) of the ERSPC study. For these, the Gothenburg trial provided longer follow up than the ERSPC. Men in the screening group (9,925) were invited for PSA screening and only men with raised PSA concentrations were offered additional tests such as digital rectal examination and prostate biopsies. Men in the control group (9,952) were offered nothing. The absolute cumulative risk reduction of death from PrCA at 14 years was 0.40% (95% CI 0.17-0.64), from 0.90% in the control group to 0.50% in the screening group. The rate ratio for death from PrCA was 0.56 (95% CI 0.39-0.82; p=0.002) in the screening compared with the control group. The rate ratio of death from PrCA for attendees compared with the control group was 0.44 (95% CI 0.28-0.68; p=0.0002). Overall, 293 (95% CI 177-799) men

needed to be invited for screening and 12 needed to be diagnosed to prevent one PrCA death. What is interesting is that the authors of this study were the only ones to conclude that the benefit of PrCA screening compares favorably to other cancer screening programs, namely breast and colorectal cancer despite the high risk of over treatment and over diagnoses. This study reported the highest effect of PSA screening to reduce overall PrCA mortality and had the longest time of follow up. Concerns of the results are related to unclear information from loss of blindness and the contamination of randomization.

The (PLCO) trial [64-66] assigned randomly 76,685 men aged 55 to 74 years at 10 US study centers to annual PrCA screening (38,340 men) or usual-care opportunistic screening (38,345men). The screening included annual PSA measurements for 6 years along with DRE for 4 years. Men with more than one PSA screening in the three years prior to randomization were excluded. A PSA threshold of > 4ng/ml was considered a positive screen and an indication for further diagnostic procedure by subjects private health care providers. Compliance among the control group was an issue; 40%-60% in the control group underwent PSA testing on the 1st and the 6th year respectively. 85% of the screening group complied with PSA testing. All incident PrCA and deaths from PrCA through 13 years of follow-up or through December 31, 2009, were ascertained. Approximately 92% of the study participants were followed for 10 years and 57% for 13 years. There was no statistically significant difference in PrCA mortality. The cumulative mortality rates from PrCA in the screened and control groups were 3.7 and 3.4 deaths per 10,000 person-years, respectively, resulting in a non-statistically significant difference between the two arms (RR = 1.09, 95% CI = 0.87 to 1.36). These results indicate that after 13 years of follow-up, there was no evidence of a mortality benefit for organized

annual screening in the PLCO trial compared with opportunistic screening. The main limitation was that many men in the opportunistic screening arm did indeed screen for PrCA using at least one PSA test.

The PLCO and the ERSPC are considered the best current evidence to the issue of PSA screening and its efficiency to decrease cancer mortality - however with limitations. While they were both considered of "fair" quality in the 2011 USPSTF review, it is important to note here that the up to date findings of the two studies are divergent. The ERSPC considered PSA testing life-saving but with high related costs, the PLCO didn't find any statistically significant mortality gains but with higher mortality trend among the screened group. The discrepancy is what some scientist referred to as "the controversy that refuses to die"[68]. The length of follow up is important to consider here. The lead time for PSA screening and PrCA mortality is estimated around 15 years. Very recently the ERSPC has reached this point of follow up and the updated results indicate stronger and moderate benefits for the PSA screened group. The PLCO study has not yet completed this duration and additional benefits may emerge later. The contamination among the control group is another important consideration; in the PLCO study (44%-50%) of the control group underwent PSA testing. Additionally, more than 40% of the enrolled men have had up to 2 PSA testing before the enrolment, leaving the possibility of detecting a cancer lower than expected in both groups. In the ERSPC the contamination rates are not clearly described for most sites, 20% contamination rate among the control group was reported from Rotterdam site and was extrapolated for the whole study. Another two important methodological differences between the 2 trials are the interval of screening and the engagement of the control group in the trial; in the

PLCO study, men followed an annual PSA testing protocol for 6 years , in the ERSPC trial men were screened either every 2 or 4 years. The PLCO trial reported high rates of over diagnoses of indolent cancer among the screened group and high number of invasive treatment such as prostatectomy among the treated group when compared to the control group - when compared to the ERSPC trial screened group. The more frequent PSA testing may have resulted in relative increase in the number of indolent PrCA diagnosis along with the invasive treatments (which have high mortality rate) might explain the net harm (higher mortality) among the screened group in the PLCO study. Finally, in the ERSPC trial men in the control group were not aware that they are participating in the trial, those who were tested and diagnosed among them received treatment in their own regular place. Those who were diagnosed in the screened group were followed at specific care centers and might have received different level of treatment and medical care/expertise. In the PLCO trial both groups were treated the same and were referred to their own health care providers.

The totality of the evidence described in all the trials above, indicate that PSA based screening for PrCA is associated with over diagnoses, over treatment and high costs; with a possible slight to moderate gain in mortality. It is critical to note here, that none of these studies considered race as a potential effect modifier. That is, we do have enough evidence that PrCA is more invasive among African American, especially those who are also younger age [69]. The majority of participants in these trials were Caucasians; consequently the findings might not be generalizable to African Americas in whom there is a different natural history of PrCA [25].

2. C. Prostate specific antigen kinetics

PSA kinetic can be mathematically defined and referred to by multiple ways; PSA velocity (PSAV) is the change in PSA (ng/ml) per the change in time that is also referred to as PSA rate. If the change in time is fixed on one unit of change (say one year), then it is the annual PSA rate. The change can also be represented as a percentage that would be; PSA annual percentage change (APC). PSA doubling time (PSADT) is the time it takes for one PSA test result to double. The continuum of change across long period of time is referred to as PSA growth curves (longitudinal change over time with a certain trend). Similar to PSA single test, PSA kinetics was initially introduced to monitor PrCA progression after diagnosis. Until today, PSA kinetics were widely used in the management of prostate cancer [70].

2. C.1. The use of PSA kinetics in screening for Prostate cancer (evidence from observational studies)

Carter et al. [16, 71] were the 1st to propose the concept of PSA change and its potential implication in PrCA early detection. In an earlier work, Carter et al. described the long term change of PSA across a group of men who developed PrCA using data from up to 14 years prior to the clinical detection of the disease. They compared the pattern and the magnitude of the change (in what they called PSA growth curves) with a control group of healthy men and others with BPH. They were able to show that there is a transition time at which PSA starts to accelerate among individuals who developed PrCA, while both control groups showed monotonic nonaccelerating patter. They reported that the transition/acceleration take place years prior to the clinical diagnosis. They also showed higher and earlier PSA progression (transition) among those who were diagnosed

with metastatic disease as compared to local disease. This pattern was later confirmed by others [72] [73]. The inflection point represents a clinical change from the slow gradual expansion of prostatic epithelial volume due to normal prostate age-related growth to a rapid increase of peripheral PSA due to rupture in the prostate basement from significant tumor growth[72]. Despite the evidence provided by these early reports, the value PSA kinetics to improve PrCA screening has not been confirmed. This is because data from control trials is lacking and evidence from subsequent observational studies varied. We will discuss below several observational studies that investigated the value of PSA kinetics in PrCA screening

Carter et.al[74] defined three groups of men from subjects in the Baltimore Longitudinal Study of Aging (BLSA) [75] which is an ongoing long-term prospective aging study of the National Institute of Aging. Participants are of community-dwelling volunteers with the continued recruitment. More than 1,400 men and women are study volunteers. They range in age from their 20s to their 90s. Participants in this study return every 2 years for a series of tests and donate blood for current and future studies. Previous analysis of the sub-population of men [75] revealed the age-specific incidence of prostate disease to be similar to that in the general white male population. Carter et.al estimated PSA growth curves from serial PSA measurements using frozen sera from three groups of men: (*a*) 16 men with no prostatic disease by urological history and examination; (*b*) 20 men with a histological diagnosis of benign prostatic hyperplasia (BPH) who had undergone simple prostatectomy; and (*c*) 18 men with a histological diagnosis of PrCA. The median number of repeated PSA measurements over an 8 to 26 year period prior to histological diagnosis or exclusion of prostate disease was 8 and 11

for non-cancer and cancer subjects, respectively. Predicted rates of change in PSA were linear and curvilinear for control and BPH subjects, respectively. Subjects with cancer demonstrated both a linear and an exponential phase of PSA velocity. Based on time to double PSA, Carter et.al showed that the rate of PSA increase was higher among PrCA patients compared to BPH patients or those with normal prostate. They proposed a PSA velocity (PSAV) threshold of 0.75ng/ml/year to differentiate PrCA and BPH in men with elevated PSA. [76] Thus they concluded that it would be expected that "*serum PSA would change faster with time in men with PrCA than in men without prostate cancer*" and that estimates of prostatic growth rate from changes in PSA may be useful clinically in management of men with prostate disease. Following Carter et.al, in 1994 Pearson *et al* [72, 77] conducted similar analysis using updated data on the same cohort. They concluded that the most significant factor affecting change of serum PSA levels with age is the development of prostate disease and that the "*rate of change in PSA levels may be a sensitive and specific early clinical marker for the development of prostate cancer"*. Using the same source of data, Morrell et al., [78] tried a different statistical approach (non-linear mixed models) to estimate the growth curves of PSA with time among men with and without cancer (all before cancer diagnoses) and found that local/regional and advanced/metastatic cancers had similar rates of PSA progression that is significantly different from PSA change in healthy mean, but advanced/metastatic cancers are diagnosed later. [78] . Loeb et al. [79] reported similar results on additional data from the BLSA study. They reported that having a PSAV over the threshold of 0.4 ng/ml/y increased the risk of life threatening PrCA by 13.6% while a PSA threshold of 3ng/ml for PSA incensed the risk by only 3%. Again using the BLSA data, Fang et al. [80] estimated

the sensitivity and specificity of PSAV among 89 men with serial PSA of 2-4 ng/ml for at least 28 month. The sensitivity and specificity of a threshold of 0.1ng/ml/year was 81% and 50%, respectively. The cumulative probability of PrCA during the next 10 years was only 2.9% when PSAV was less than 0.1ng/ml/year and 35.2% when PSAV was more than 0.1ng/ml/year.

Vickeres et. al. [81] used data from 5519 participants in the PCPT trial who underwent biopsy to build a multivariable model predicting PrCA. They compared the area under the curve (AUC) of two models, with and without PSA velocity (used at the threshold of 0.35, 0.5. 0.75). Both models included age, PSA, digital rectal examination, family history and previous biopsy. They also evaluated the number of biopsies avoided and the additional cases detected by using PSA velocity in men with low PSA and negative DRE. They reported a trivial increase in AUC (from 0.702 to 0.709) when PSA was included in the model. Surprisingly, the gains were even smaller for high-grade cancer. Also, using the PSAV thresholds in men with low PSA and negative DRE results led to a large number of additional biopsies. Their PSA thresholds had a better combination of specificity and sensitivity when compared to comparable PSAV thresholds. Ulmert et al.[82] assessed the additive the value of adding PSAV into a model using PSA to predict PrCA. The analysis was done using the Malmo preventive study where participants were 5,722 Swedish men (44-50 years) who underwent 2 PSA screening test, 6 years apart. There measurements were taken 10 to 15 years prior to PrCA diagnosis. The authors reported that PSAV was highly and independently associated with PrCA but didn't improve the predictive value of PSA.

Finally, two recently published studies concluded that PSA change over time does in deed improve prostate cancer detection. Wallner et al. [18] evaluated whether the rate of change in serum PSA levels (represented by annual percent change) accurately detects prostate cancer in a managed population of 219,388 men passively followed from 1998 to 2008. Their results indicated that longitudinal measures of PSA improved the accuracy of aggressive prostate cancer detection when compared to single measurements of PSA. Orsted et al.[19] investigate the same question among 7,455 men in the Copenhagen city heart study. They also concluded that adding long-term PSAV to baseline PSA values improves classification of PrCA risk and morality. The results of these two recent studies provide insight into the potential use of PSA annual rate as a predictive marker for aggressive prostate cancer.

Due to main methodological differences, it is hard to compare the results of all these observational studies. In a recent systematic review, Loughlin [83] defined several problems in the PSA kinetics literature. He emphasizes on the methodological heterogeneity. Further, he showed that many studies on this topic do not conform to the original definition of PSA Velocity. In their systematic review, Vickers et al. [84] concluded that; studies that investigated PSA kinetics either found single PSA to be a better predictor than PSA kinetics, or found trivial differences in favor of PSA kinetics, or had serious methodological shortcomings.

In conclusion, the accumulating evidence indicates that; when measured rigorously the rate of PSA change is quantitatively and qualitatively different among men who developed life-threatening PrCA. These differences may be detected 5-10 years prior to the clinical detection of the disease [12, 17-19]. PSAV as commonly measured in

many studies may be highly correlated with PSA and thus may not improve PSA predictive value. However, some recent publications have shed the light on a significant potential value of PSA kinetics in particularly predicting high-risk prostate cancer. More studies with robust methodologies are required to further accumulate evidence on the value of PSA kinetics in improving PrCA screening.

CHAPTER 3: METHODS

For this dissertation we aim to identify and refine a means for differentiating high-risk prostate cancer (PrCA) from any other condition that could be related to an increased prostate specific antigen (PSA) measure at any particular point in time. In this chapter we will describe the overarching theme behind the methods of this research. Specific descriptions of methods used in each of the three separate objectives will be provided in individual chapters that are designed to be stand-alone manuscripts.

3. A. Overall Epidemiological Design:

We followed a nested case-control study design. The cases were always patients with confirmed diagnoses of high-risk PrCA. Two different control groups were identified; patients without any prostatic disease and patients with low-risk PrCA. Using a classic retrospective approach, we "followed" all patient's repeated PSA measures over time and describe the trajectories at which those measures changed with time. We used repeated measures statistical methods to obtain a description of the mean and individual PrCA growth in the cases and the controls over the study time.

3. B. data sources

The data for this study was obtained from two different data sources; the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening trial [21], and the Veterans affair administration (VA) data

1. The Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening trial: The PLCO trial [21] is a large population-based randomized trial designed and sponsored by the National Cancer Institute (NCI). The overall objective was to determine the effects of screening on cancer-related mortality in men and women aged 55 to 74 participating in 10 screening centers around the country. The 10 screening centers were: the University of Colorado, Georgetown University, Pacific Health Research Institute (Honolulu), Henry Ford Health System, and University of Minnesota, Washington University in St Louis, University of Pittsburgh, University of Utah, Marshfield Clinic Research Foundation, and University of Alabama at Birmingham. The enrolment took place between 1993 and 2001 and screening component of the trial was completed in 2006. Participants are being followed for outcome assessment through 2015. Figure 3.1 illustrates the schematic view of the trial design. Approximately 155,000 participants were enrolled and individually to the control arm or intervention arm equally. Participants in the intervention arm received screening exams for prostate, lung, colorectal and ovarian cancers while those at the control arm received usual medical care. At the time of this analysis the median follow-up time was 12.4 years

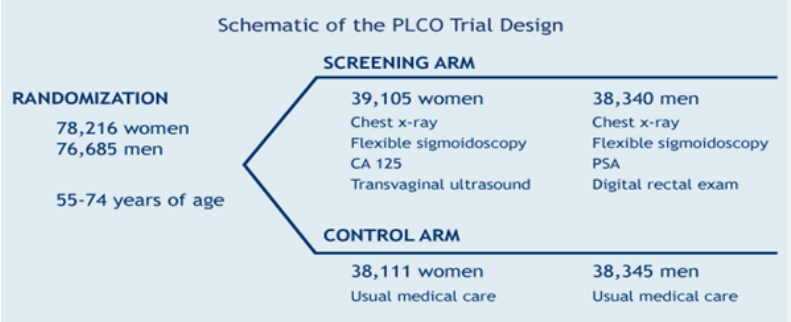


Figure 3.1 schematic of the PLCO trial design

Legend: Source (http://prevention.cancer.gov/plco/background)

For this dissertation, we used data from men who were randomized to the prostate cancer intervention arm and thus received PSA and digital examination screening. For this dissertation we obtained access and used the following items from the wide set of data provided by the PLCO study:

a. The baseline questionnaire which includes demographic, life-style and medical history data for all participants enrolled in the trial at the time of enrolment.
b. Screening data: This includes serial PSA measurements for about 38,000 males who were randomized to the prostate cancer intervention arm. Each participant was expected to comply with 6 six annual blood draws. Each draw was sent to a central lab to assess the level of PSA. In addition to PSA tests results, digital rectal examination results were also obtained. Overall, data on about 177,000 PSA exams and 128,000 DREs were obtained. PSA results are contained in the data in both numeric and qualitative result (i.e., negative, positive, inadequate), where levels above 4.0 ng/ml are considered positive.
c. Diagnostic procedure: Data were collected and obtained on procedures that were part of a diagnostic work-up for prostate cancer and for staging procedures following a diagnosis. Two different types of events triggered the collection of diagnostic procedure information; a positive screening PSA (above 4.0mg/ml) exams or abnormal suspicious DRE or when a participant was diagnosed with prostate cancer within the trial period.
d. Cancer diagnoses: Complete data on PrCA diagnosis were obtained. The PLCO trial confirmed diagnoses of PrCA through medical record abstraction

(MRA) of men suspected/reported by the trial to have prostate cancer. Clinical stage was almost always available (98% of prostate cancers). Pathologic stage was only available for men who had a prostatectomy (37% of prostate cancers). Gleason scores were collected from both biopsies (98%) and prostatectomies (37%) and assessed at pathology labs local to the screening center. Information on Gleason score was captured on a 2 to 10 scale.

2. The Department of Veterans Affairs (VA) data:

We have obtained access to national VA electronics health record (EHR) Corporate Data Warehouse (CDW) data extracts. This included nationwide demographic, administrative claims, vital signs, mortality, laboratory results, pharmacy dispensation and oncology record via the Veterans Affairs Informatics and Computing Infrastructure (VINCI) platform. All these data sources are linkable using a common individual patient-level identifier; i.e., scrambled SSN. The utility, accuracy, validity, and access methodology of the available data is transparently maintained by the VA has been previously described [85-88]. The components of each of the VA data source are listed below:

a) VA medical SAS datasets: contains International Statistical Classification of Diseases and Related Health Problems-9 (ICD9), Current Procedural Terminology (CPT), cost, demographic, socioeconomic, health care utilization information

b) VA Decision support system datasets: contains laboratory, pharmacy, cost, demographic, socioeconomic, health care utilization information

c) VA vital status file: Contains demographic and mortality information

d) VA Corporate data warehouse: Provides vital measures such as height, weight, waist circumference, blood pressure, pharmacy and related information. It also includes raw data extract from oncology files; which includes field on Gleason score and TNM stage.

3. C. Cohort definition:

PLCO trial participants are uncompensated volunteers recruited from the general population in the geographic area of each of the screening centers [21]. A potential participant was considered eligible for the PLCO trial if he did not meet any of the following exclusion criteria:

- Less than 50 or greater than or greater than or equal to 75 years of age at the time of randomization.
- Individuals undergoing treatment for cancer at the time of randomization (excluding basal-cell and squamous-cell skin cancer).
- Individuals with known prior cancer of the colon, rectum, lung, prostate.
- Individuals with previous surgical removal of the entire colon, one lung, or the entire prostate.
- Individuals who were participating in another cancer screening or cancer primary prevention trial.
- Males who had taken Proscar/Propecia/finasteride in the 6 months prior to randomization.
- Males who had more than one PSA blood test in the three years prior to randomization.

- Individuals who had a colonoscopy, sigmoidoscopy, or barium enema in the three years prior to randomization.
- Individuals who were unwilling or unable to sign the informed consent form.

We applied further eligibility specific criteria based on the specific aims of this dissertation. Please see chapter 4 and 5 for details on the PLCO cohort selection and characteristic for aims one and 2.

The VA is one of United States largest integrated health care system consisting of 150 medical centers, nearly 1,400 community-based outpatient clinics, community living centers, Vet Centers and Domiciliaries [89]. These facilities serve more than 8.3 million Veterans each year [89]. To approximate the VA cohort to the PLCO clinical trial cohort - we identified male veterans between 50 and 75 years of age who had their first VA based PSA test in the calendar years of 2002 and 2011, and did not have any prostate cancer diagnosis, BPH diagnosis or prostate procedures such as biopsy, prostatectomy (partial or complete) or other prostate surgeries, orchiectomy or dispensation of 5-alfa reductase inhibitors, any cancer. Chapter 6 describes the details of the VA cohort characteristics.

3. D. Data definition and measurements:

Exposure/biomarker**:** long term PSA annual rate of change (ng/ml/year) derived from PSA growth curves. PSA growth curve is the longitudinal repeated measure of PSA over multiple years of time. The derivation of PSA annual rate depends on the equation that best fits the observed change of PSA over time (PSA growth curve). PSA is a numeric continuous variable measured in certain time intervals over multiple years prior

to any clinical presentation of PrCA. In the PLCO trial, PSA was measured on serum obtained and frozen within 2 hours of blood draw at each of the 10 screening centers. The samples were then shipped to the UCLA Immunogenetics Center on dry ice where the analysis was performed centrally. The quality control measures for the collection and storage of blood samples and tissue is an integral component of the trial and all described in details elsewhere [21]. PSA serum measurements at the VA hospitals labs are all done in compliance with the quality control standards of the of the Clinical Laboratory Improvement Amendments (CLIA). All laboratory testing is subject to onsite inspection and accreditation by a nationally recognized accreditation body, either College of American Pathologists (CAP) or Joint Commission (JC).

Outcome: The outcome is a pathological diagnosis of PrCA. We defined the stage and the extent of PrCA separately for the two cohorts using the VA oncology files and the cancer diagnoses file in the PLCO data. Based on this information we classified the PrCA into clinically high-risk and low-risk PrCA. The definition of high-risk PrCA was based on the prognostic classification of PrCA introduced in 2010 by American Joint Committee on Cancer (AJCC) [90]. The committee considered a PrCA meeting any of the following criteria as a PrCA with high clinical risk; PSA level ≥ 20ng/ml, cancer that invades prostate capsule, PrCA that involves more than one lobe, or Gleason score >7. In the PLCO study all diagnosed cancers, deaths, and causes of death were ascertained by annual follow-up questionnaire and periodic linkage to the National Death Index. Follow-up clinical stage was determined from the clinical assessment of the extent of tumor involvement using the TNM staging system. Tumor stage was categorized according to the fifth edition of the American Joint Committee on Cancer (AJCC) Cancer Staging

Manual (5). Gleason grade was determined using the biopsy Gleason score (range 2-10). The underlying cause of death was determined in a uniform and unbiased manner from the death certificate and relevant medical records, as has been described in detail previously [21].

Covariates: baseline Age, race, body mass index [BMI=weight(kg)/height(m)^2] and clinical history of benign hyperplasia (BPH) are important variables that were controlled for while modeling the PSA growth curves. These baseline measurements were available in both data sets. In the PLCO data, these measurements were available as part of the baseline questionnaire. For the VA data, the VA CDW data extracts in VINCI had reliable measures for these variables.

Study time period: In the VA data we obtained access to all VA data for calendar year 2002 to 2011. The total study period will be 10 years - details on the distribution of the follow up time are provided in chapter 6. For the PLCO data the PSA measurements started in 1993 and cancer outcome were collected up to 2009, median follow-up time was 12.4 years with 6 years of PSA measures completed in 2006.

Startup date: For the VA data that will be defined as the date of 1st PSA measure. For the PLCO data that will be defined as the enrolment date to the study (Randomization date).

Date of end of follow-up (Right censor): For individuals who developed PrCA at any time during the course of the follow up, the exit date was the date they were diagnosed with PrCA either through pathological diagnosis of PrCA or death due to PrCA or received treatment for PrCA (radiotherapy, chemotherapy or surgical) which ever

happened earlier. For those who have no evidence of PrCA diagnosis, the exit date is a predetermined end of follow up date; December 2009 (or up to 13 years from trial entry) and December 2011 for the PLCO and VA respectively.

3. E. Statistical approach

Baseline data was subjected to simple descriptive statistical methods [(i.e., using Proc Univariate, Means, and Freq in SAS® 9.4 (SAS institute N.C.)] to determine mean, median, standard deviation and proportions (as appropriate). For the VA data, all analysis was performed inside the VINCI environment which provided us with SAS/Grid cloud based parallel computing environment with raw data sources provided through Microsoft SQL Server 2014. The PLCO data was processed, cleaned and provided for the purposes of this research project by the NCI Cancer data access system (CDAS). All analytics were carried using standard statistical procedures from SAS® 9.4 (SAS institute N.C.).

3. E.1. Repeated measure analysis (overview)

The term repeated measures refers to data sets with multiple measurements of a response variable on the same experimental unit [91]**.** Multiple measurements of PSA (annually in the case of PLCO) are available for different groups of men. In this basic setup, there are two factors, group/(s) and time. Group is called the **between-subject** factor because levels/categories of groups can change only between subjects and; all measurements on the same subject will represent the same group. Time is called a **within-subject** factor because different measurements on the same subject are taken at different times. In repeated measures studies, where a group effect is hypothesized, main interests are (1) how group(s) means differ (the effect of the group), (2) how group means

change over time (the effect of time), and (3) how difference between group means change over time (slope). In other words, is there a group main effect (difference by group), is there a time main effect, and is there a group-by-time interaction?

What makes repeated measure data analysis distinct is the covariance structure [92], that is, pattern at which the repeated measures (for the same subject) are correlated. For example, two measurements taken at adjacent time points are typically more highly correlated than two measurements taken further apart in time; and such temporal ordering usually violates the independence assumption. Effort will be applied at the beginning of the statistical analysis to assess the covariance structure of the data in order to avoid biases and model assumption violations.

3. E.2. Statistical approaches to repeated measure analysis (mixed-effect models):

There are several approaches to modeling repeated/correlated response data [92]. The two basic approaches are the use of 1) marginal models and 2) mixed-effect models. With marginal models, the emphasis is on *population-average inferences* (i.e., comparing the average change in PSA for the different groups), or the marginal expectation of the response (mean PSA-measures) [93]. As in all longitudinal measure analysis, the within-individual correlation of the response variable – PSA – is accounted for, but this is solely through the specification of a marginal variance-covariance structure. The regression parameters derived from a marginal model only describe the population mean response and do not describe the mean response at a single-individuals level.

In contrast, with a mixed effect model, the regression parameters are able to describe an individual's mean response; i.e., the response is subject-specific. Mixed-

effect models are called “mixed” because they estimate both random and fixed effects. Fixed effects involve only factor effects that are treated as unknown constants. An effect is fixed if the levels in the study represent all possible levels of the factor, or at least all levels about which inferences are to be made. Random effects are used in the study to represent only a sample (usually a random sample) of a larger set of possible levels. For example, in a longitudinal repeated measure study, time and individuals can have a random effect as we have limited number of observed times for each participants. A factor is considered random if its levels reasonably represent a larger population with a probability distribution (usually normal distribution). The ability of mixed-effect models to consider both random and fixed effect make them appropriate for numerous experimental and observational data and study designs including repeated measures designs. Here correlation is accounted for through specification of subject-specific random effects and possibly on intra-subject covariance structure. Unlike marginal models, the fixed-effect regression parameters of mixed-effect models describe the average of an individual’s response and are more informative when advising individuals of their expected outcome.

Additional approaches include semi parametric approaches to mixed models [94]. Penalized splines regressions using a Bayesian approach is one well-known application. These methods are similar to parametric mixed models in terms of their ability to estimate inferences that are more likely to be subject-specific in scope with the focus centering on the individual’s mean response rather than estimating marginal, or population inferences. However, fitting mixed models using spline regressions allow

higher level of flexibility in modeling complicated relationships between the response and covariates in various longitudinal studies.

To effectively achieve the overall goal of this dissertation, the subject oriented approaches are more appropriate as we are interested in predicting individual's outcome rather than only population's means. In fact, the differences in mean PSA rate or velocity among men who develop PrCA as compared to those who don't are known and well documented in all studies discussed in in chapter 2. What this dissertation aims to investigate is whether the individual PSA rate can be used to predict PrCA outcome. We are also interested in accounting for the random effect of the heterogeneity in PSA values and the rate of change in PSA within the population. Mixed-effect models (either parametric or semi parametric) can be used to estimate the pattern of change of PSA with time. Such a model also estimates the influence of individual characteristics of PSA. It can be assumed that each individual has his own true PSA pattern, and that these true patterns vary about the population average. Thus the models allow PSA patterns to differ according to PrCA category (group), even among men with the same characteristics. In addition, the model allows observed PSA for each individual to vary about his true values, because of measurement error and day-to-day variation.

Mixed models include different types/categories of models determined by the mean structure (The relationship between PSA and time/age in our case) and the type of data (The distribution function). Table 3.1 below summarizes five basic types.

Table 3.1 Mixed effect models category

Model	Mean structure	Cumulative distribution function
Linear mixed-effect	Linear	Normal
Generalized Linear mixed-effect	Linear	General
Non-linear mixed-effect models	Non-linear	Normal
Generalized non-linear mixed-effect	Non-linear	General
Semi parametric mixed models	Non-parametric	General

Previous studies and our preliminary analyses indicate that PSA levels increase with age, but that the growth rate may not be constant (i.e., linear-monotonic) with time, especially in those with PrCA or BPH (or possibly other prostatic diseases) [95, 96]. Several previously published studies have modeled long term PSA repeated measures with time among individuals with PrCA; most of them indicated a non-linear change of PSA with time and modeled a longitudinal profile of (log-transformed) PSA with some sort statistical technique to overcome the non-linearity. The log-transformation was likely done to make the distribution less skewed and to allow for normal distribution assumption. Almost all the studies agreed on the following pattern of PSA over time; an observed linear increase of PSA when considering normal patients. However, among individuals with PrCA, PSA trends seemed to be linear up to a certain point (before PrCA

diagnoses) at which both the trend and the rate change appeared to accelerate in an exponential pattern. That point is what statistician refers to as the acceleration point or inflection point [73].

These previous findings suggest that the use of linear mixed-effect models may not be appropriate for our data and not suitable to obtain reasonable statistical inferences. Non-linear mixed models [92] where parameters enter the model individually and nonlinearly can be used to correctly establish PSA growth curves. Another alternative and more stable approach to overcome the linearity assumption is penalized spline regressions and the use of Bayesian approaches to mixed models (semi-parametric approach) [94, 97]. The two approaches are similar in many ways and, in theory, should lead to the similar results, especially when employing large sample sizes.

3. E.3. Non-linear mixed-effect model for modeling PSA growth curves

Statistically speaking, nonlinear models are models whose parameters enter the model individually and nonlinearly. Traditional nonlinear models have the general form

$$\boldsymbol{y} = \boldsymbol{f}(\boldsymbol{x}, \boldsymbol{\beta}) + \boldsymbol{e}$$

Where: f is a nonlinear function of known constants (x) and unknown parameter (β) and the errors (*e*) are additive. In our case, we would like to fit a model that simultaneously accounts for the nonlinear mean structure as well as the variability between and within subjects while taking account for both inter-subject variability and intra-subject correlation and heterogeneity.

We will start by demonstrating the observed longitudinal profiles/trajectories of PSA for all participants as a function of age for each study group. The observed individual trajectories are helpful in determining the suitable statistical model (non-linear function) for the observed data. This is because the inference here focuses on features or mechanisms that underlie individual profiles of repeated measurements of the PSA and how these vary in the population. Non-linear mixed effect models are theoretical or empirical models for individual profiles with parameters that may be interpreted as representing such features or mechanisms. For example, if the observed individual curves display a mildly nonlinear S-shaped growth trend the logistic growth model such as $\boldsymbol{E[y] = \beta_1/(1 + \beta_2 e^{\beta_3 X})}$ would be interpretable and appropriate. However, such assumption cannot be made at this early stage. The link function could be anything that is a good fit for the observed curves in each study group.

NLMIXED [92] procedure in SAS is a recent addition and is currently considered the 1st choice for fitting non-linear mixed-effect models. PROC NLMIXED fits non-linear mixed-effect models by numerically maximizing an approximation to the marginal likelihood - that is, the likelihood integrated over the random effects. Different integral approximations are available [92]. The resulting marginal likelihood can be maximized using a variety of alternative optimization techniques. Successful convergence of the optimization problem results in a maximum likelihood parameters estimates along with their approximate standard errors based on the second derivative matrix of the likelihood function. PROC NLMIXED enables the use of the estimated model to construct predictions of unknown functions using Bayes estimates of the random effect. Also, one can estimate arbitrary functions of the non-random parameters, and PROC NLMIXED

computes their approximate standard errors using the delta method. Nonlinear mixed models have important applications in wide variety of fields and are effective ways to model correlated data with a nonlinear relationship between independent and dependent variables [72, 92].

3. F. Sample size justification

Sufficient sample size is necessary to reliably estimate growth models. However, in such a design determining what is sufficient might be problematic. This is because the power calculation here depends - in part - on other factors of the research design such as complexity of the growth model and the variance expected to be explained by the model [98]. One of the main determinant is relation between the number of individuals and the number of repeated observations per individual; so that , the total number of person*time observations is what ultimately defines the statistical power in a given study [92]. In general , growth models require – on average – three repeated measures per individual, although this requirement can also be ambiguous [92]. For example, in an unbalanced data, some individuals might have just one or two observations, whereas others have three or more and this is usually acceptable. Since three repeated measures over-identify the trajectory, 3 measures are preferred for at least a sizeable portion of the cases. Another consideration is the estimation method; for typical maximum likelihood (ML) method , it is assumed that the repeated measures are continuous and normally distributed [92]. However, there are alternative methods for estimation which allow for measures that are continuous and not normally distributed.

In conclusion, growth models can fit and explain multiple types of data structures; careful selection of proper models and methods of estimation should be done based on the characteristics of the given data set which also allows appropriate sample size calculations. Growth models have successfully been fitted to samples as small as small as 22 [98], although sample sizes approaching at least 100 are often preferred.

Some statistical work has been done to estimate sample size requirements based on different data distribution and mean structures. As in Overall and Doyle (1994), sample size of contrast c of group population means across n time points:

$$N = \frac{2(z_{\alpha+} z_{\beta})^2 \sigma_c^2}{\omega_c^2}$$

$$\omega_c^2 = \sum_{i=1}^{n} c_i(\mu_{1i} - \mu_{2i})$$

$$\sigma_c^2 = \sum_{i=1}^{n} c_i^2 \, \sigma_i^2 + 2 \sum_{i<j}^{n} c_i c_j \sigma_{ij}$$

σ_i^2 = Common variance in the groups at time point

$\sigma_{ij} = common\ covariance\ in\ the\ two\ groups\ between\ time\ points\ I\ and\ j$

$c_i = contrast\ applied\ at\ time\ piont\ i$

In 1999, Hederk et al. [99], extended the above formula for non-balanced data and created tables for sample size required in repeated measures assuming different variance -

covariance structures and random effect. For our study, we are estimating the difference of PSA trajectories in two different data sets with an average of 3 time points in each dataset for each individual.

Based on Hederk et al. [99] published tables for sample size calculations of repeated measure data and assuming the following: at least 3 time points per individual, Power of 0.8 for a 2-tailed 0.5 test, test of a group effect over time, attrition rate of 0.1, a small effect (a between group difference of 0.2 SD unit at each time point) and random effect structure with a random slop and random residual; for this we will need 237 participants in each group.

We are proposing a population-based study that will include all eligible patients receiving care at the VAMC (i.e., > 5 million men). Though not nearly as huge, the PLCO data is large data set of 36,000 men. Both data sets exceed the minimum number needed participants in each study group. We also had access to the technology platform to conduct analysis on such large sample (distributed parallel processing on a Linux operating system based SAS grid platform of the VA VINCI).

CHAPTER 4

Understanding long-term changes in serum Prostate-specific antigen in the PLCO study cohort[1]

[1] Shoaibi , A., Rao, G., Cai, B., Rawl, J., Hebert, J.. **Understanding long-term changes in serum Prostate-specific antigen in the PLCO study cohort.** To be submitted to Aanals of Epidemiology**.**

Introduction:

Prostate cancer (PrCA) is the most common visceral cancer in the United States and the second leading cause of cancer deaths among men [25] . In 2014, about 233,000 new cases of PrCA were diagnosed, and 29,480 men died of the disease [1]. In May 2012, The U.S. Preventive Services Task Force (USPSTF) released a guideline recommending against routine prostate specific antigen (PSA)-based screening [100]. Currently, the American Urological Association and American College of Physicians recommend limited PSA-based screening only in high-risk populations. For non-high-risk men, they recommend individualized informed decision-making [101] . The increased detection of low-risk PrCA based on PSA-based screening is the foundation of the controversy that led to the current recommendations against screening.

PrCA is generally a relatively indolent disease, with the lowest mortality to incidence ratio compared to any other epithelial cancer [102, 103] and a lifetime risk of death of only 2.9% [104]. The unique combination of high incidence and low virulence drives the debate around the value of current screening strategies using a single elevated PSA level or digital rectal exam. The fact that PSA is not an exclusive marker of malignancy is a major shortcoming of this biomarker. While a single elevated PSA measurement is highly sensitive to PrCA it is of low specificity and does not distinguish well between indolent and aggressive PrCA or even non-malignant conditions; thus, burdening patients with biopsies, ineffective and sometimes hazardous treatments and, concomitantly, large and unjustifiable health expenditures [105] .

In response to the need for better screening strategies, especially among those at higher risk of developing aggressive disease, previous studies proposed using PSA change over time to improve detection of PrCA [15, 83, 106] . The use of serial measurements of serum PSA levels represents an attempt to model PSA kinetics or PSA velocity (PSAV). This concept has been widely used in the management of PrCA. Starting in the early 90's, investigators used frozen serum samples collected prior to PrCA diagnosis to provide information about natural history of PSA change or 'growth'[16]. Since then, several researchers have modeled PSA change using different definitions, assumptions, and statistical computation methods on differing populations [83]. The results have varied with some concluding that PSAV did improve PrCA detection [18, 19, 76], while others refuted these suggestions [83, 106, 107]. Many have questioned the incremental value of the PSAV beyond that of a single PSA result, describing the concept of PSA dynamics as a "sticky" concept that further perpetuates the issue of over-detection and over-treatment of an indolent disease [83, 106, 107].

The totality of the evidence indicates that there is major heterogeneity in PSAV/PSA change definitions [83]. No single threshold for PSAV has been found to significantly enhance the clinical value of PSAV over PSA alone [106], but when measured rigorously the rate of PSA change is quantitatively and qualitatively different by various groups such as aggressive PrCA patients, non-aggressive PrCA, benign prostatic hyperplasia (BPH) and healthy men [16, 72, 108, 109]. Finally, PSA change is sensitive to many biological and bio-behavioral characteristics, such as BMI, race, age, medications and smoking. All of these factors have the potential to influence PSA measures and modify its change over time.

Given this controversy and the potential important implications for clinical practice, this study aims to utilize advanced unrestrictive statistical methods to fully describe and quantify PSA change over time in three groups of men: men with no evidence of PrCA, men who have been diagnosed with low-risk PrCA and men who have been diagnosed with high-risk PrCA using data from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) cohort [21]. We aim to quantify and compare PSA growth curves in years prior to the clinical diagnosis of disease or exit from the study while considering baseline factors such as age, race, BMI and initial PSA.

Methods:

We conducted a retrospective analysis using PLCO data. Cases were patients with confirmed diagnoses of high-risk PrCA. Two different control groups were identified: participants without evidence of PrCA and patients with low-risk PrCA. Using a classic retrospective approach, we "followed" all patients' repeated PSA measures over time until they were confirmed to either have PrCA or have exited from the study. For each individual, we describe the trajectories of the repeated PSA measures over time.

Setting:

The PLCO trial [21] is a large population-based randomized trial designed to determine the effects of screening on cancer-related mortality and secondary endpoints in men and women 55 to 74 years of age participating at one of 10 screening centers in 10 cities in the US: Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St Louis, MO; and Washington, DC. For the PrCA component of the trial, participants were enrolled

between November 1993 and July 2001 and were individually randomized to the control arm or intervention/screening arm in equal proportions. We conducted our analysis on 38,340 men who were randomized into the screening arm. Each man was expected to comply with up to 6 six annual blood draws and digital rectal examination (DRE) in the six years of enrollment. Blood samples were sent to a central lab to assess PSA level. Men were enrolled and actively screened (for six years) between 1993 and 2006, after which they were passively followed-up for seven additional years. Data were collected on cancer diagnoses and deaths from all causes that occurred through December 31, 2009 or up to 13 years from trial entry resulting in a median follow-up time of 12.4 years.

Participants:

Trial participants were volunteers recruited from the general population in the geographic area in the ten screening centers. Participants were excluded if they were; " *less than 50 or greater than or equal to 75 years of age at the time of randomization; undergoing treatment for cancer at the time of randomization (excluding basal-cell and squamous-cell skin cancer); individuals with known prior cancer of the colon, rectum, lung or prostate; individuals with previous surgical removal of the entire colon, one lung, or the entire prostate; individuals who were participating in another cancer screening or cancer primary prevention trial; individuals who had taken finasteride in the 6 months prior to randomization; individuals who had more than one PSA blood test in the three years prior to randomization; individuals who had a colonoscopy, sigmoidoscopy, or barium enema in the three years prior to randomization; or individuals who were unwilling or unable to sign the informed consent form*." [21]

To strengthen our statistical models we excluded men with less than four PSA measures. To avoid information bias we excluded 3 groups of participants for potential misclassification: those who were reported to have cancer outcome but was not confirmed ("death certificate unconfirmable", "self/other reported unconfirmable", "erroneous report of cancer", "borderline malignancy"); those who were classified as non-responsive (refusal to continue with study activities or loss of contact before confirming outcome status); those who did not have complete follow-up information in response to a positive screen (had a positive screening result but were not captured in further diagnostic follow-up and were never confirmed to have cancer). We also excluded men who were diagnosed with benign hyperplasia (BPH) at baseline and those with incomplete information on baseline age, BMI and race (these are important covariate in analyses); Figure 4.1 represents the analytical cohort tree.

Definitions measurements and assessments:

The classification of PrCA into high biological risk and low biological risk was based on the prognostic stage introduced by The American Joint Committee on Cancer (AJCC) in 2010 incorporating pretreatment markers [9, 10]. Any PrCA that met even one of these criteria were considered high biological risk: PSA level ≥ 20ng/ml, cancer that has invaded prostate capsule, PrCA that involves more than one lobe, and Gleason score (if available) > 7. Patients with PrCA who did not meet all of these criteria were considered to have PrCA of low biological risk (prognostic group IIa and below). Clinical stage was determined from the clinical assessment using the TNM staging system. Tumor stage was categorized according to the fifth edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual. Gleason grade was determined using the biopsy

Gleason score (range 2–10). All diagnosed cancers, deaths, and causes of death were ascertained by annual follow-up questionnaire and periodic linkage to the Social Security National Death Index. The underlying cause of death was determined in an uniform manner from the death certificate and relevant medical records. Demographic, behavioral and medical data were available as part of the baseline questionnaire. Total (PSA) serum ng/ml testing was performed centrally at the University of California, Los Angeles, Immunogenetics Center [21, 110].

Statistical methods:

First we plotted the observed individual trajectories of PSA for all participants as a function of time for each study group so as to determine the suitable statistical model for the observed data. We used spaghetti plots to illustrate the individual trajectories and the loess option to fit the mean trajectories in each group separately [111]. We defined time as years to exit or diagnosis. Our descriptive observed plots and evidence from previous studies indicate that PSA levels increase with age/time, but that the growth rate may not be constant (i.e., is not linear-monotonic) especially in those with PrCA. To account for this pattern, we used multiphase non-linear mixed models framework to estimate PSA change over time in 2 different models:

1) Linear-exponential piecewise PSA model: In this model, we estimated the individual PSA as a function of time (years to diagnosis/exit). We hypothesized that each individual's PSA trajectory starts with a phase of slow linear change followed by a phase of rapid exponential increase. The transition point from the

linear phase to exponential phase was considered unknown and unique for each individual influenced by random factors.

We built the model in two stages:

a. Because our hypothesis is that the pattern of change in PSA is significantly different among the healthy men and the two cancer groups, so as to allow different coefficient estimates per group, we started with an initial model that used an interaction term between the group type and time. To account for individual level natural heterogeneity in the rate of growth, the transition point and the intercept in each group, we included random effects for their corresponding estimates. The full mixed-effect model for the data is fully described in appendix 1. The most parsimonious model was determined by backwards elimination of non-significant terms. As expected, cancer groups exhibit a significant exponential term that is not significant in the cancer group. Also, the estimate of CP for the no cancer group was significantly low (very close to zero) while for the cancers groups had a significant value of 5-7.

b. We then used our reduced model (allowing a transition to an exponential phase among the cancer groups only) to estimate average and individual PSAV as ng/ml/year per group while adjusting for baseline age , BMI (kg/m2), PSA measure (ng/ml) and race [African American (AA) versus others]. To investigate and account for possible effect-modification of these variables on PSA change over time, we included an interaction term between

all of these variables and time. The simplified presentation of the reduced mixed-effect model is shown in appendix 1 simplified.

2) Linear –Linear piecewise LOG PSA model:

In this model, we estimated the change of PSA over time on the natural log transformed scale of the PSA measures. We regressed individual log [PSA-1] as a function of time (years to diagnosis/exit). This transformation improves the distribution of the data, allows a realistic linear assumption of the time–PSA relationship and represents PSA change over time as an annual percent change instead of an absolute change, replaces the observed linear-exponential relationship by linear-linear and simplifies derivation to allow for a single growth rate for all years post the accelerating point. We also used this model in two stages as follows.

a. We started with an initial model that allowed the same trend for all groups. We modeled a linear-linear multiphase model with unknown continuous change point. Fixed and random effects were included to estimate the mean, and allow for individual variation on the intercept, 1st and 2nd phase time coefficients and the transition point. The full mixed-effect model for log PSA is described in appendix 1. Again, the most parsimonious model was determined by backwards elimination of non-significant terms. The cancer groups exhibit a significant second time coefficient not significant in the no-cancer group.

b. We then proposed the reduced model describe growth of log (PSA + 1) as a function of time to exit while adjusting for all potential confounders allowing

a transition to an exponential phase among the cancer groups only. The reduced mixed-effect model for log PSA is described in appendix 1.

In both models (liner-exponential and linear-linear), we assumed the transition from one phase to another to be continuous so that even though there is a shift in function, the changeover to the new section is steady and incremental . PSA change over time was estimated by taking the 1st derivative of the final equation in each model. The models included a time variable, main effects of baseline characteristics, and corresponding interactions with the time variable. The time variable corresponded to slope, and the interaction of time with baseline characteristics corresponded to the association of these characteristics with PSA slope.

Results:

Cohort demographics:

The baseline characteristics among the three groups comprising the cohort are illustrated in Table 4.1, chi-squared tests for association and two-sided t-tests are used for statistical comparisons. Men with a diagnosis of PrCA (both high- and low-risk) compared with healthy men were older at baseline. They also had fewer years of follow-up, higher PSA measure at baseline, slightly fewer PSA measurements per person and had a shorter period between their last PSA test and study exit. AAs and those with a family history of PrCA were more likely to be diagnosed with PrCA compared to non-AAs or those without a family history. The two cancer groups were comparable with respect to all of these variables. However, men in the low-risk cancer group had shorter

duration of follow-up and shorter time between the last PSA and exit day as compared to men in the high-risk cancer group.

Description of PSA changes over time: Figure 4.2 illustrates the observed trajectory of the three groups separately. The observed patterns are consistent with past studies. For men in the no-cancer group, we observe a linear trend with a slightly increasing pattern. A similar linear pattern is observed among the two cancers groups, but only during the initial years of follow-up. In the low-risk cancer group, an inflection takes place around 2-3 years before diagnosis; as we move closer to the date of cancer diagnosis, the PSA values seem to increase in an accelerating pattern. This linear-exponential pattern is more pronounced among high-risk cancer patients. In the high-risk group, the inflection point leading to exponential pattern seems to take place much earlier, around 4-5 years before diagnosis. Table 4.2 reports the unique change point statistics for the two cancer groups estimated from the final reduced models.

Table 4.3 summaries PSA change/rate over time using different methods, the first method is the commonly used traditional formulas for PSAV, and the other methods are derived from our proposed model. The first estimate, arithmetic velocity is estimated using the arithmetic equation$(1/(n-1)) * (\sum_{i=1}^{n}(p_i - p_{i-1})/(t_i - t_{i-1}))$, where n = total number of PSA tests, p = PSA value, t = time at PSA test. The other four measures were estimated by taking the 1st derivative of our reduced adjusted models and computing PSA rate before and after the change point separately. Men who were diagnosed with high-risk PrCA have a statistically significant higher estimate of absolute PSA change over time across different methods of estimation. The annual percent (%) rate is higher among men who developed PrCA but comparable between high-risk and

low-risk PrCA. PSA annual change estimated by our models illustrates a narrower 95% CI (less variability around the mean values). Also, no other traditional method can capture the 2nd order effects that become evident when PSA growth begins to accelerate past the change point; this might be crucial to differentiate high-risk PrCA from low-risk PrCA.

The relationship with baseline measurements:

We examined the effect of age, race, BMI and PSA at baseline on the PSA rate of change by including an interaction term between each of these variables with time in each phase separately. Table 4.3 illustrates the parameter estimates corresponding to all interaction terms. Values are adjusted for all other variables in the table. Older men (≥65 years) have higher absolute rate of change when compared to younger men. The interaction term between BMI and time was not significant, suggesting that BMI did not have a significant association with PSA change with time. AA and non-AA had comparable PSA rate of change. PSA at baseline seems to be the most influential factor; those with higher PSA value at baseline had slower change with time.

PSA rate of change as annual percent change seemed to be more sensitive to race. AA had a higher PSA % change when compared to non-AA by 0.35% before the change point. Older men seem to have higher PSA % change as compared to those younger only before the change point. Also, as part of the design, the models test the association of the baseline characteristics on single PSA measure (mainly the PSA value at the change point, for those who had a change to exponential phase and the PSA at the exit point for

those who did not). AAs, older men, and those with higher PSA levels at baseline had higher PSA values at the exit/change point. Men with higher BMI had lower PSA values.

PSA growth curves across age, race and study groups:

We used the 1st derivative of the linear-exponential model equation and the linear-linear model equation to calculate PSA change rate and annual % of PSA change respectively at 1 year before exit; tables 4.5. These rates are illustrated for all study groups, stratified by age and race and adjusted for a baseline distribution of BMI and initial PSA value of 1.3 ng/ml. The absolute PSA rate among men in the high-risk cancer group appears to be significantly greater compared to no-cancer and low-risk groups. The annual percent (%) rate is higher among men who developed PrCA but comparable between high-risk and low-risk groups. Figures 4.4 to 4.6 illustrate the estimated PSA growth curves across the three study groups stratified by race and age.

Discussion:

We used non-linear and linear mixed effect models to describe longitudinal data on PSA change among men who were diagnosed with high-risk PrCA, low-risk PrCA or not diagnosed with PrCA. To describe the absolute PSA change over time, we used a linear-exponential piecewise model. To describe the annual relative (percentage) change we used a linear-linear piecewise model. In both sets of analyses, we selected the most parsimonious model that fit the data best. All models included random components which enable the models to account for natural heterogeneity between individuals due to random factors affecting PSA measures, natural (benign) PSA change with time, time of diagnosis, transition time and the progression of the tumors. We accounted for multiple

baseline factors that can explain some variability in PSA change over time. Across all groups of age, race, BMI and initial PSA, patients who ultimately were diagnosed with high-risk PrCA seem to represent a distinct PSA profile starting as early as 4-5 years prior to date of diagnosis when compared to those who did not have high-risk PrCA. Both cancer groups demonstrated an inflection in PSA trajectories, changing from a linear pattern into an exponential one; however, our findings suggest that low-risk cancer has less aggressive progression and a change time closer to the time of diagnosis than high-risk cancer. These findings were consistent when considering both absolute and relative (expressed as a percentage) PSA change across time .When examining rate of PSA change 1 year prior to exit, we found men in the high-risk cancer group to have much higher absolute PSA change rate when compared to those in the other two groups, not only on average but across almost 99% of the distribution within each group.

Several studies have examined PSA change over time; almost all studies reported the same pattern. Carter et al. [16] Pearson et al. [72] and Inoue. et al. [73] described the PSA pattern among multiple longitudinal studies using the non-linear mixed model approach. Similar to our results, they reported a transition time at which PSA starts to accelerate among individuals who developed PrCA. They also reported higher and earlier progression among those who were diagnosed with metastatic disease as compared to local disease. The vast majority of PSAV studies did not consider this pattern of differential PSAV quantification by risk of PrCA. Furthermore, the various studies used differing formulas to compute PSAV. The range of the values we report here is within the range of previously reported values for PSA velocity and PSA annual percent change. The previously suggested thresholds of 0.4 ng/ml/year and 0.75 ng/ml/year to distinguish

high virulent PrCA are within the lower range of value we reported for men in the high-risk cancer.

Multiple researchers have reported that the inflection point represents a clinical change from the slow gradual expansion of prostatic epithelial volume due to normal prostate age-related growth to a rapid increase of peripheral PSA due to rupture in the prostate basement from significant tumor growth [72]. The two extremes are connected through an interval at which the malignant tumor is initiated but its contribution to peripheral PSA level is still minimal due to its small initial size and relatively intact prostate capsule [16, 72, 73, 108]

We found several significant associations of baseline characteristics with PSA relative and absolute change over six years. Age, race, and initial PSA were associated with single PSA and slightly modified PSA change over time while BMI was inversely associated with single PSA but was not significantly associated with PSA change over time. Older men tend to have higher PSA measurements that tend to increase at a higher rate. Men with higher PSA at baseline tend to have higher rate and continued to have higher PSA at the exit or change point. We observed a qualitative difference by race, but this did not reach statistical significance. These results are consistent with results from previous studies. Several studies have reported the inverse relationship between BMI and mean PSA [83, 112-114]. However, the influence of BMI on PSA change is not as evident. Kristal et al. [113] reported an inverse association between BMI and PSA measures, and no association with PSAV. A few studies have reported significant association between BMI and both PSA and PSA change [112, 114]. All of these studies suggest that the effect of BMI on PSA is due to a hemodilution effect, that is, the dilution

of PSA by increased plasma volume. Many studies reported that AAs present with higher PSA serum when diagnosed with PrCA [115]. However, few studies investigated the effect of race on PSA change. Sarma et al. [115] reported that the annual percent change in PSA among AAs was approximately twice as great at that of white men. Also, Kristal et al. [113] and McGreevy et al. [116] described a more rapid change of PSA with age among AAs when compared to with whites and other race/ethnic groups. The magnitude of differences in PSA change across racial and age groups and the influence of initial PSA range from weak to modest, thus these characteristics do not seem to highly affect the clinical interpretation of PSA rate.

Many previous studies investigated PSA change in relation to prostate cancer. Many have tried to use this concept to predict prostate cancer. While almost all studies reported clear distinct summary statistics (mean and median) for PSAV and PSA doubling time among those who ended up with PrCA when compared to those who did not, they also reported high intra-individual variability within the comparison groups (cancer and no cancer) . This natural intra-individual variability (random error, or "noise") between multiple PSA measures made it difficult to find a threshold that can improve PrCA prediction and raised a considerable concern about the concept of PSA change and its clinical implications. We are one of the few to report a clearly distinct range of calculated rates when considering high-risk cancer as compared to the low-risk cancer and no-cancer groups. Most past studies estimated the individual velocities using a linear model (mostly one phase and sometimes 2 phases) within a narrow time frame, using few PSA measures in close intervals. We built our analysis on Carter et al.'s work that was the first to propose the concept of PSA change. In their original work, Carter et

al. suggested a piecewise linear-exponential model to best describe the natural history of PrCA and to best quantify PSA change among those who ended up with PrCA. We used an appropriate method to calculate PSA rate of change at a given time using flexible models that did not assume a monotonic rate of change, used 5-6 PSA measures taken annually across a time frame of 1-14 years prior to exit, accounted for baseline characteristics and had a large enough sample size to control for within-individual variations. We believe that quantifying PSA growth in such a fashion allows for a rigorous definition and calculation of PSAV that captures what Carter et al. describe as "PSA velocity is the PSA variability corrected for the elapsed time between measurements".

Our study has some limitations worth noting. First, this is a retrospective analysis limited by the cohort characteristics of men participating in the PLCO study. The cohort has a limited number of young men and AAs. Including men at younger age and a higher representation of AAs would have strengthened our analysis. Second, the PSA measures were collected over the first six years of enrolment and follow-up continued for up to 14 years, leaving a gap of up to 3-7 years of unknown PSA measures. This gap period was significantly longer among men with no evidence of PrCA, and that may have introduced some bias. However, given the clear slow linear pattern of PSA change in this non-cancer group, it is unlikely that including the unknown measures would have changed our findings. Third, our calculated velocities might be sensitive to the proposed piecewise model. It could be that lower rates among the no-cancer group are underestimated by the linear model that was used for this group. However, to keep the estimated velocities independent of any pre-assumed pattern, we conducted a sensitivity analysis in which we

used our first full model to estimate PSA rate of change. In this way, we allowed all individuals to either deviate into an exponential pattern or stay in a linear pattern, depending on what fit their observed PSA better. The calculated PSA rates did not change, and the magnitude of the differences between the three groups remained the same. Fourth, information bias and misclassification is a threat. This is especially the case among the non-cancer group who did not have a biopsy to confirm their non-cancer status. We limited this bias by restricting our analysis to only those with biopsies or those that never had a positive screening or if ever had positive screening were followed with a diagnostic follow-up procedure that confirmed their outcome status. Finally, those with fewer than 3 PSA measurements in addition to those who were lost during the follow-up might have lower or higher PSA measurements and might be of lower or higher risk of developing PrCA making our findings prone to selection bias.

This PSA growth model is a real mathematical representation of the natural history of PrCA and thus shows clear differences of PSA rates among those who were diagnosed with high-risk PrCA when compared to low-risk PrCA and no-cancer groups. Our main finding is that PSA change rates in men in the low-risk cancer group and those with no cancer overlap across different age and race groups while those who were subsequently diagnosed with high-risk PrCA are significantly different. Moreover, this clear distinction takes place within a window of time relevant to early detection and can be measured and captured at least three years before diagnosis. These growth models can be used to solve the main problems of single PSA screening and have high clinical relevance.

Tables and figures:

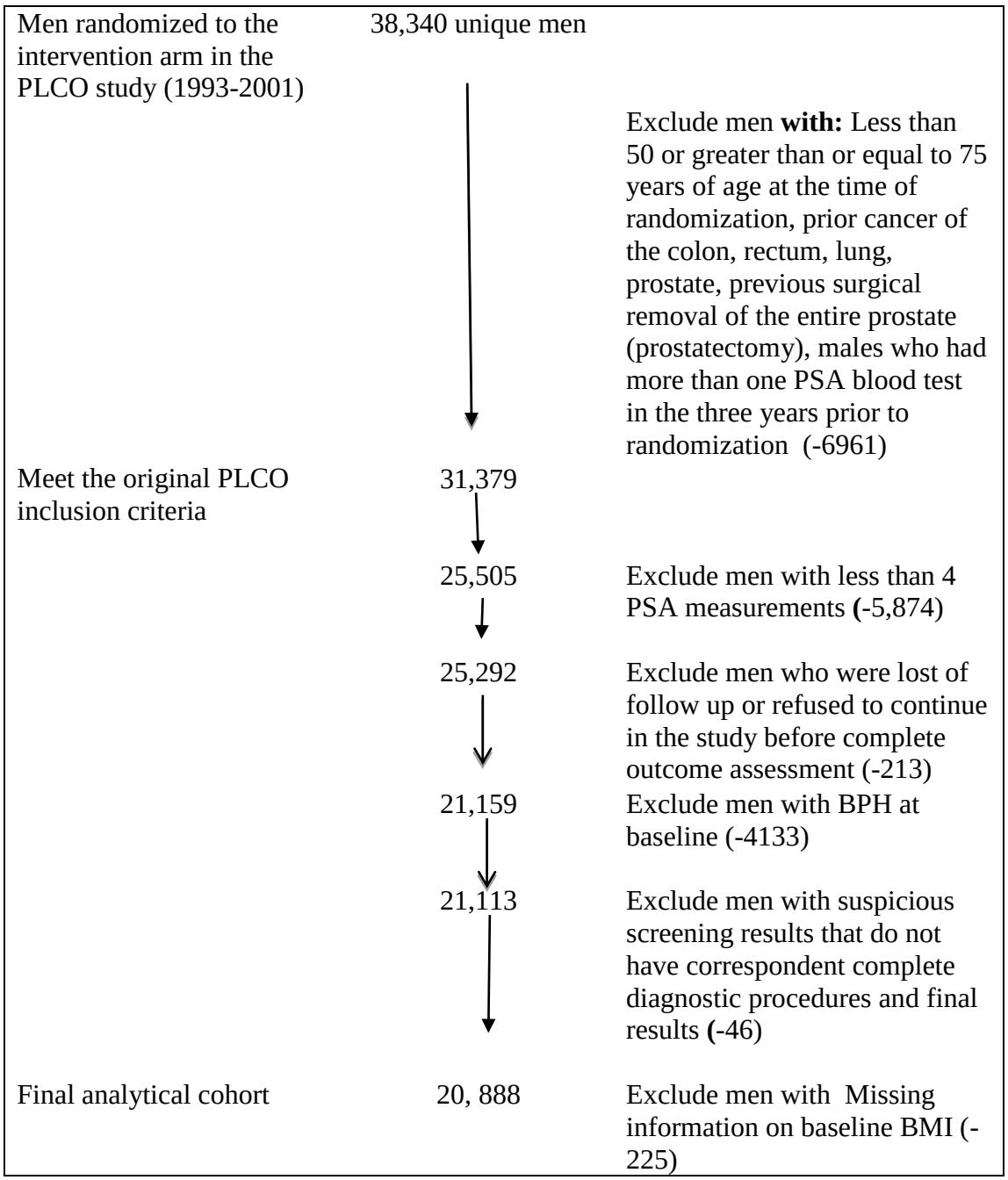


Figure 4.1 PLCO cohort selection tree

Table 4.1 Characteristics of PLCO participants by study groups (n=20,888)

	Men with no cancer (19,196)	Men with LRC* (1368)	Men with HRC** (324)	Comparison (p-value for difference between study groups by characteristic)		
Race, n(%)				NC*** vs. LRC	NC*** vs. HRC	LRC*** vs. HRC
African American	742 (90.05)	62 (7.52)	20 (2.43)	0.098	0.03	0.2
None-African Americans	18454 (91.98)	1306 (6.51)	304 (1.52)			
Ethnicity, n (%) (missing=606)				0.32	0.88	0.74
non-Hispanic	18203 (91.84)	1310 (6.61)	308 (1.55)			
Hispanic	428 (92.84)	26 (5.64)	7 (1.52)			

Family history, n(%) (missing=144)				<0.001	<0.001	0.33
No	17773 (91.33)	1225 (7.06)	284 (1.62)			
Yes, immediate family member	1291 (87.09)	132 (9.96)	39 (2.94)			
Age, n (5) (years)						
<= 55, n=2,228	2096 (94.08)	107 (4.8)	25 (1.12)	0.0004	0.006	0.34
55-65, n= 13,658	12560 (91.96)	898 (6.57)	200 (1.46)			
>65, n=5002	4540 (90.76)	363 (26.54)	99 (1.96)			
Mean (95% CI)	61.42 (61.34-61.49)	62.21 (61.96-62.46)	62.73 (62.17-63.29)	<0.001	<0.001	0.08
BMI, n (%)						
<= 30 kg/m^2	14431 (91.58)	1068 (6.78)	258 (1.64)	0.016	0.0655	0.5399

>30 kg/m^2	4765 (92.87)	300 (5.85)	66 (1.29)			
Mean (95% CI)	27.75 (27.67-27.81)	27.34 (27.14-27.54)	27.63 (27.22-28.05)	<0.001	0.6	0.20
PSA at baseline (ng/ml) mean/median (95% CI)	1.05 /1.06 (1.04-1.06)	2.51 /2.16 (2.42-2.59)	2.91 /1.94 (2.37-3.46)	<0.001	<0.001	0.14
Years of follow up (years) mean/median (95% CI)	11.49 /11.51 (11.46-11.52)	7.52/7.47 (7.37-7.66)	8.24 /7.85 (7.54-8.16)	<0.001	<0.001	0.053
Number of PSA tests mean/median (95% CI)	5.59 /6.00 (5.58-5.60)	5.28 /6.00 (5.24-5.33)	5.21 /5.00 (5.12-5.30)	<0.001	<0.001	0.16
Years from last PSA to exit or diagnosis mean/median (95% CI)	6.56/7.17 (6.54-6.59)	2.92/2.57 (2.79-3.04)	3.36 /3.32 (3.07-3.64)	<0.001	<0.001	0.005
*LRC: Low-risk prostate cancer		**HRC: high-risk prostate cancer		***NC: No Cancer		

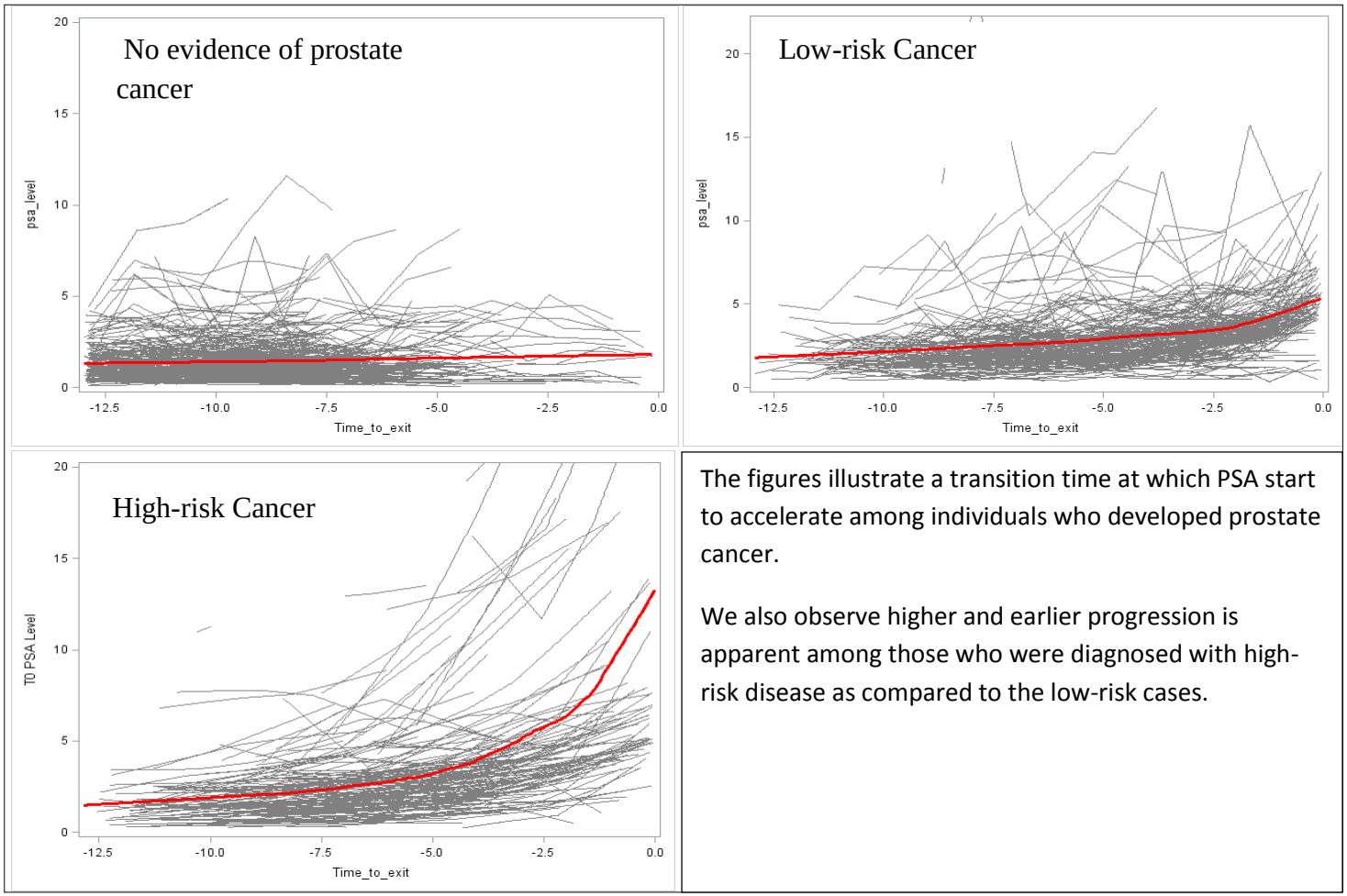


Figure 4.2 Longitudinal trajectories of PSA for all PLCO participants by study group

Table 4.2 Change point mean and median by study groups

Model	**Outcome**	**Function**	**Change point summary**		
Annual PSA rate	PSA	Linear-exponential	Group	Mean (95% CI)	Median (25^{th} ,75^{th})
			Low-risk prostate cancer	2.58 (2.58, 2.58)	2.62(.31,3.02)
			High-risk prostate cancer	5.21 (4.85,5.58)	5.24 (4.75, 5.59)
Annual % PSA rate model	Log PSA	Linear-linear	Low-risk prostate cancer	2.00 (2.00,2.00)	2.00 (2.00,2.00)
			High-risk prostate cancer	3.96 (3.61,4.31)	3.96 (3.70,3.97)

Table 4.3 PSA rate over time (velocity) in three study groups estimated by different methods

Method	Men with NC**(1919 6)	Men with LRC*** (1368)	Men with HRC**** (324)	Comparison (p-valued for difference between study groups)

	mean (95% CI)			NC vs. LRC	NC vs. HRC	LRC vs. HRC
Arithmetic velocity* (ng/ml/year)	0.06 (0.06-0.07)	0.37 (0.34-0.39)	0.79 (0.55-1.03)	<0.01	<0.01	<0.01
Annual rate before change point (ng/ml/year)	0.05 (0.05-0.05)	0.16 (0.15-0.17)	0.13 (0.11-0.16)	<0.01	<0.01	0.21
Annual rate after change point (1 years before diagnosis) ng/ml/year	0.05 (0.05-0.05)	0.59 (0.52-0.66)	2.60 (2.11-3.09)	<0.01	<0.01	<0.01
Annual % PSA rate before change point	1.63 (1.57-1.68)	5.56 (5.33-5.78)	5.06 (4.54-5.57)	<0.01	<0.01	0.31
Annual % PSA rate after change point	1.63 (1.57-1.68)	10.85 (9.02-12.68)	12.10 (10.3-14.17)	<0.01	<0.01	0.09

* using the arithmetic equation $PSAV = (1/(n-1)) * (\sum_{i=1}^{n}(p_i - p_{i-1})/(t_i - t_{i-1}))$,

where n = total number of PSA tests, p = PSA value, t = time at PSA test.

** NC:No cancer *** LRC:Low-Risk Prostate cancer

****HR:High-risk Prostate Cancer

Table 4.4 Associations between baseline characteristics and PSA trajectory, reporting the coefficient estimate in the final reduced models

	PSA rate before CP*	%PSA change before CP	PSA rate after CP	%PSA rate after CP	Single PSA
Age					
≤55	Referent				
55-65	0.005 (0.19)	0.13% (0.12)	-0.051 (<0.001)	-1.42% (0.32)	0.11 (0.009)
65≥	0.009 (0.01)	0.20% (0.04)	0.041 (0.005)	-1.03% (0.50)	0.21 (<0.001)
BMI					
<=30	Referent				
>30	0.001 (0.22)	0.10% (0.23)	0.001 (0.91)	0.10% (0.71)	0.03 (<0.001)
Race					
Non-African Americans	Referent				
African Americans	0.007 (0.22)	0.35% (0.01)	0.005 (0.78)	2.42% (0.14)	0.07 (0.27)
Initial PSA	-0.003 (<0.001)	-0.60% (<0.001)	0.004 (0.005)	1.36% (<0.001)	0.85 (<0.001)
CP= change point					

Table 4.5a Estimated annual PSA rate of 1 year prior to exit stratified by race, age and study groups and fixed at baseline BMI of 25 and initial PSA of 1.3

Race	Age	Group	Mean	(95%CI)	Median	25TH Percentile	75th percentile
Non-African American	**Youngest** (≤55)	No cancer	0.05	(0.04, 0.05)	0.04	0.02	0.06
		Low-risk cancer	0.65	(0.53,0.77)	0.69	0.58	0.88
		High-risk cancer	2.82	(2.08,3.56)	1.95	1.63	3.57
	Middle (55-65)	No cancer	0.05	(0.05,0.05)	0.04	0.02	0.07
		Low-risk cancer	0.47	(0.41,0.54)	0.55	0.42	0.71
		High-risk cancer	2.10	(1.65,2.54)	1.88	1.25	2.68
	Older (65≥)	No cancer	0.06	(0.05,0.06)	0.04	0.02	0.07
		Low-risk cancer	0.92	(0.79,1.06)	1.07	0.81	1.40
		High-risk cancer	4.30	(3.50,5.11)	4.21	2.88	6.33
	Youngest	No cancer	0.05	(0.04,0.07)	0.04	0.03	0.05

Africans American	**(**≤55)	Low-risk cancer	0.69	(0.50,0.88)	1.21	0.81	1.26
		High-risk cancer	3.04	(2.04,4.05)	1.90	1.89	1.91
	Middle (55-65)	No cancer	0.06	(0.05,0.07)	0.04	0.03	0.07
		Low-risk cancer	0.51	(0.36,0.65)	0.70	0.50	0.94
		High-risk cancer	2.26	(1.60,2.93)	2.50	1.75	3.71
	Older (65≥)	No cancer	0.06	(0.05,0.07)	0.04	0.03	0.07
		Low-risk cancer	0.98	(0.73,1.22)	1.00	0.78	1.54
		High-risk cancer	4.62	(3.28,5.95)	3.82	2.11	4.09

Table 4.5b Estimated annual % PSA rate 1 years prior to exit stratified by age, race, study group and fixed at baseline BMI of 25 and initial PSA of 1.3

Race	**Age**	**Group**	**Mean**	(95%CI)	**Median**	**25^{T} Percentile**	**75th percentile**
Non-African	**Youngest (**≤55)	No cancer	1.48%	(1.32%, 1.64%)	11.91%	10.62%	13.77%
		Low-risk cancer	11.67%	(8.96%, 14.38%)	12.20%	11.25%	13.60%

America n		High-risk cancer	12.91%	(10.01%, 15.81%)	13.21%	11.34%	15.39%
	Middle (55-65)	No cancer	1.61%	(1.55%, 1.68%)	11.88%	10.39%	13.76%
		Low-risk cancer	10.53%	(8.64%, 12.42%)	11.52%	10.25%	13.07%
		High-risk cancer	11.79%	(9.66%, 13.91%)	12.56%	10.81%	14.37%
	Older (65≥)	No cancer	1.68%	(1.57%, 1.78%)	11.87%	10.39%	13.76%
		Low-risk cancer	10.93%	(8.61%, 13.26%)	11.81%	10.53%	13.33%
		High-risk cancer	12.18%	(9.68%, 14.68%)	12.80%	11.08%	14.18%
African America ns	**Youngest (**≤55)	No cancer	1.82%	(1.53%, 2.12%)	11.85%	10.62%	13.25%
		Low-risk cancer	14.11%	(10.31%, 17.91%)	15.55%	14.43%	17.81%
		High-risk cancer	15.31%	(11.52%, 19.10%)	9.36%	4.67%	14.04%
	Middle (55-65)	No cancer	1.96%	(1.70%, 2.21%)	11.72%	10.33%	13.53%
		Low-risk cancer	13.00%	(9.62%, 16.39%)	13.92%	12.75%	15.88%
		High-risk cancer	14.22%	(10.87%, 17.57%)	15.93%	10.33%	20.55%
	Older (65≥)	No cancer	2.02%	(1.75%, 2.29%)	11.80%	10.46%	13.90%
		Low-risk cancer	13.40%	(9.78%, 17.01%)	15.77%	13.52%	17.58%

		High-risk cancer	14.61%	(11.04%, 18.18%)	13.64%	4.26%	13.91%

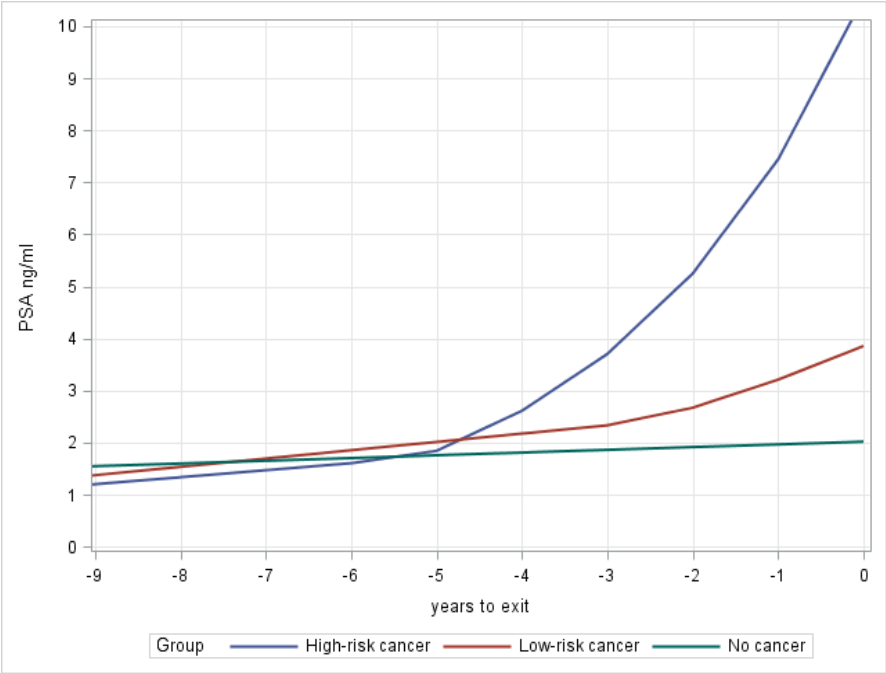


Figure 4.3 Predicted PSA growth curves by study group

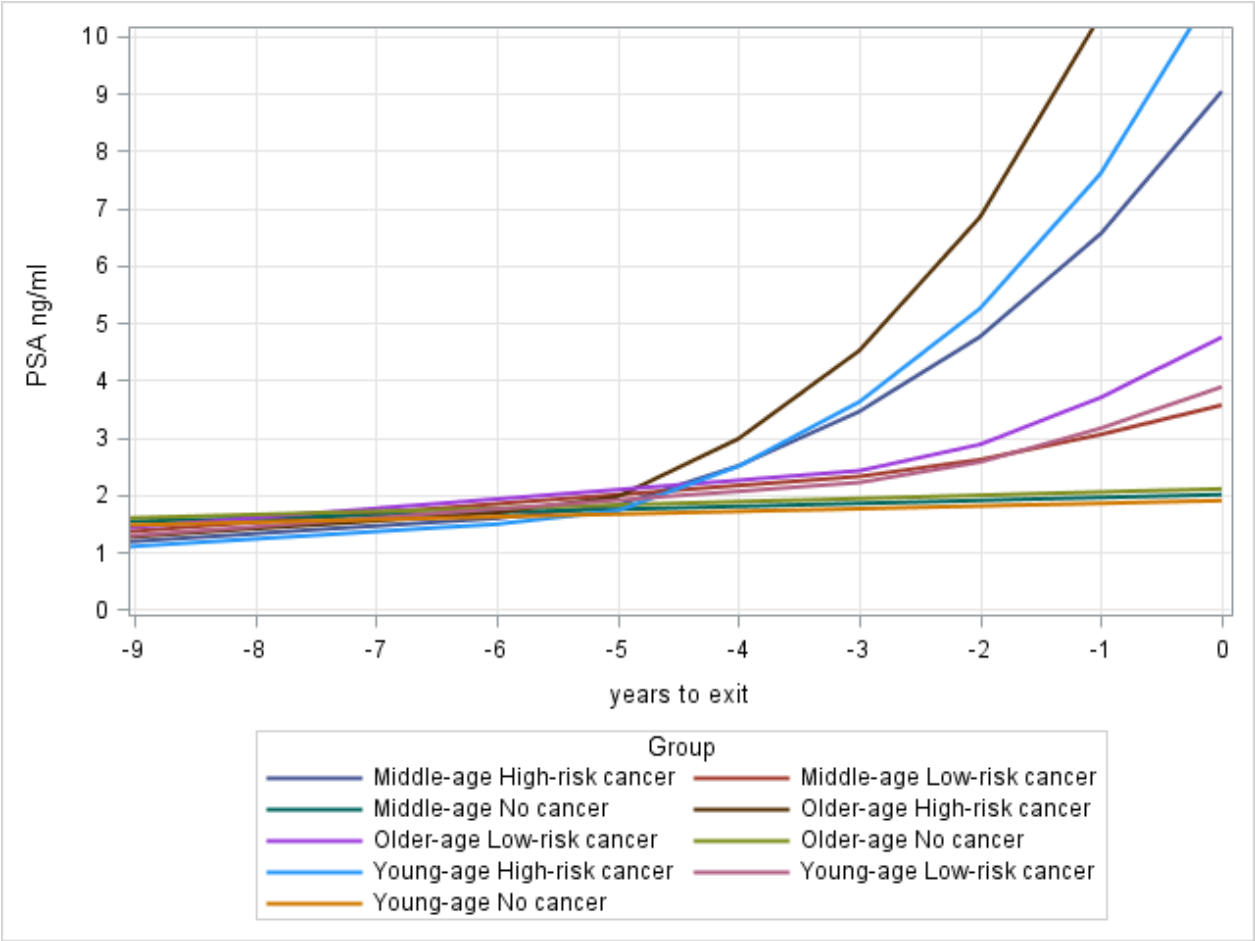


Figure 4.4 Predicted PSA growth curves by study group and age

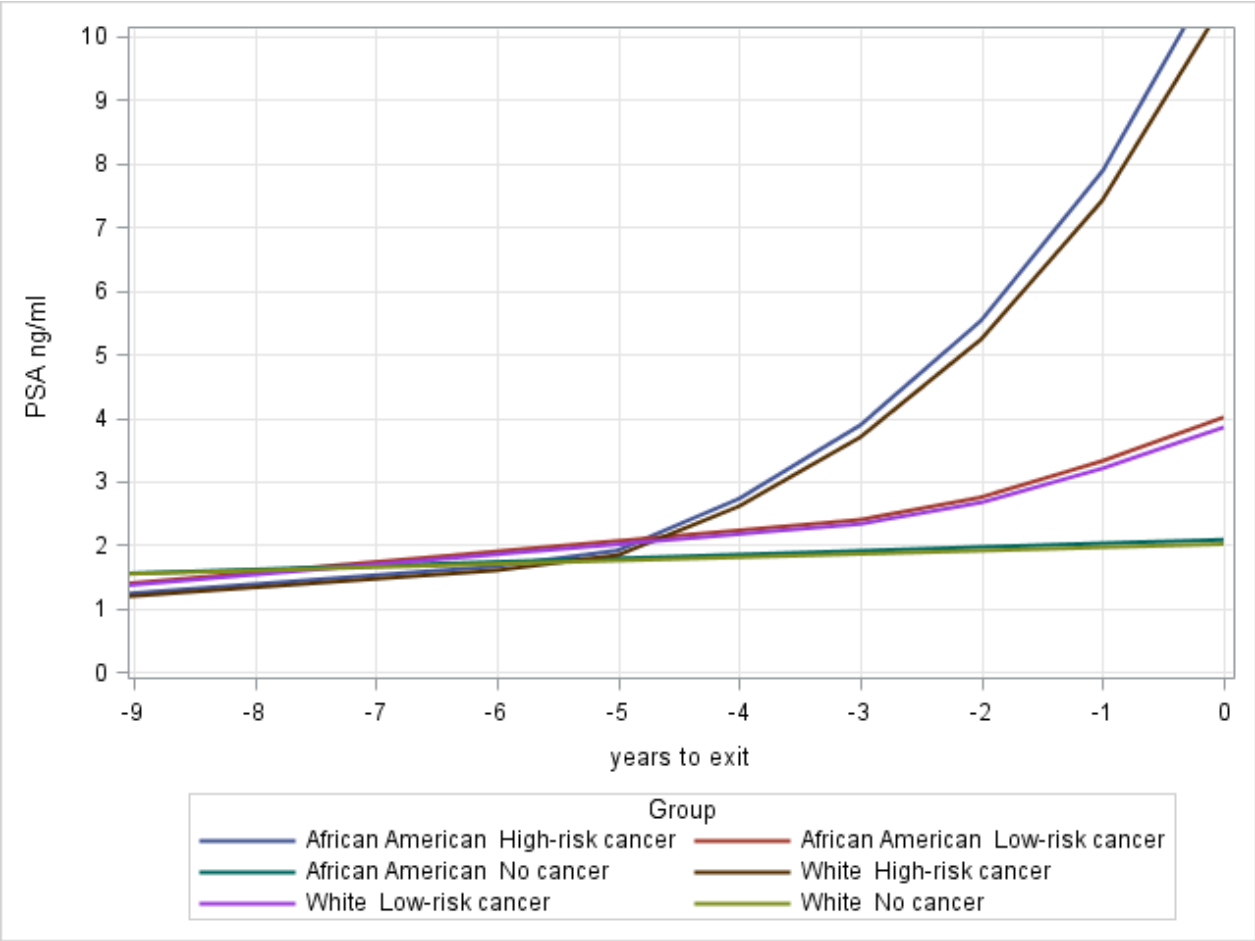


Figure 4.5 Predicted PSA growth curves by study group and race

CHAPTER 5

Prostate Specific Antigen Rate Predicts High-Risk Prostate Cancer in a Large Perspective Screening Trial [2]

[2] Shoaibi , A., Rao, G., Cai, B., Rawl, J., Hebert, J.. Prostate Specific Antigen Rate Predicts High-Risk Prostate Cancer in a Large Perspective Screening Trial**.** To be submitted to the Journal of American Medical Association

Introduction**:**

Because of its relatively high incidence, coupled with large racial disparities in virulence Prostate cancer (PrCA) [25] , represents a major public health challenge in the United States. The unique combination of high incidence but low virulence lies at the heart of this challenge [1] . Although the incidence and mortality rate varies by age and race, the overall lifetime risk of PrCA is approximately 16% and the life time risk of death is between 2 to 3.4% [1] . The value of prostate specific antigen (PSA) in PrCA detection remains a controversy. A single PSA measurement is known to have high sensitivity but poor specificity; and this leads to over-detection and over-treatment of many individuals with indolent disease [117] . A reliable screening for PrCA is needed, because PrCA can still be aggressive, especially among African Americans and younger men.

To improve the performance of PSA-based screening, multiple studies proposed the use of PSA change over time (PSA kinetics or PSA velocity(PSAV)) [83]. The natural serum PSA levels show a more rapid change in PrCA, and PSA velocity is recommended for monitoring the disease progression [118]. Carter et al. [16] proposed the concept of using PSA kinetics for PrCA screening in1993, but we still don't have consistent results confirming or refuting this application of PSA kinetics. In a recent systematic review, Loughlin [83] defined several problems in the PSA kinetics literature. He showed that many studies on this topic do not conform to the original definition to PSAV and the guidelines concerning the number of PSA tests and the interval of time between these tests. The original description of PSAV by Carter et al. [16] was based on a non-linear mixed model with multiple measures of PSA (at least 4) over a long period

of time. In our previous unpublished observations [119], we replicated Carter's work using the data from the Prostate Lung Colorectal and Ovarian Cancer (PLCO) [21] study cohort. We used a flexible piece-wise model with a linear and exponential phase in a manner similar to what Carter had originally proposed to establish PSA growth curves and estimate PSA annual rate 2 and 1 years prior to the study exit. We found that men who developed high-risk prostate cancer had a distinct pattern of PSA change over time (PSA growth curve) both qualitatively and quantitatively.

In this paper we focus on the differences between men who developed high-risk prostate cancer and those who did not. This latter group includes men with low-risk prostate cancer, men with benign hyperplasia (BPH) and men with no evidence of BPH or PrCA. These analyses are focused on answering the following research question: Can PSA change over time (in magnitude and direction) be used to differentiate high-risk PrCA from any other condition that could be related to an increased PSA measure at any particular point in time and across different populations?

We first tested the hypothesis that that the calculated PSA rate 2 and one year prior to diagnosis is highly associated with high-risk PrCA; and that this is above and beyond the association with a single PSA measurement. Second, we evaluated whether the calculated PSA rate accurately detects high-risk prostate cancer and distinguishes these cases from any other outcome.

The definition of high-risk PrCA was based on tissue evaluation through biopsy or surgical samples, or both. We followed the prognostic classification of PrCA introduced in 2010 by American Joint Committee on Cancer (AJCC) [90] who

considered a PrCA meeting any of the following criteria as a disease with high clinical risk; PSA level ≥ 20ng/ml, cancer that invades prostate capsule, PrCA that involves more than one lobe, or Gleason score >7. The overwhelming evidence of the individual and public harm associated with over-detection and over-treatment of indolent prostate cancer provides the rationale behind our focus on high-risk prostate cancer. The increased detection of low-risk prostate cancer by a single PSA testing is the foundation of the controversy that led to the current recommendations against PSA-based screening. Studies suggest that the main harm of PSA testing is the hazardous treatment of many latent prostate cancers, many of which may never have led to harm [120]. Consequently, we aim to evaluate the predictive value of estimated PSA growth curves and their derived PSA annual rate to distinguish high-risk prostate cancer from anything else among a screened population of men 50-75 years of age.

Methods:

Study population: We conducted a case-control study nested within the PLCO trial. The PLCO Participants were men and women (ages 50–74) recruited from ten centers in the United States (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St Louis, MO; and Washington, DC). For the PrCA screening component, men were enrolled between November 1993 and July 2001. We conducted our analysis on 38,340 men who were randomized into the screening arm. These men were offered annual serum PSA screening tests and digital rectal examination (DRE) for 6 and three years respectively. Men with a P and SA test result >4 ng/ml, or a DRE exam suspicious for prostate cancer were referred to their medical-care providers for a diagnostic workout and follow-up. Outcome

data was collected through annual mailed follow-up questionnaires, and medical and pathologic records related to diagnostic follow-up of prostate cancer were obtained by study personnel from medical providers. Linkage to the National Death Index was also conducted, and death certificates and medical and pathology records related to death were obtained. Data were collected on cancer diagnoses and deaths from all causes that occurred through December 31, 2009 or up to 13 years from trial entry, resulting in a median follow-up time of 12.4 years.

For this prostate cancer nested case-control set, we included all men who were randomized to the screening arm and complied with receiving at least 4 PSA screening tests. We initially categorized men into 4 groups; men who developed high-risk PrCA, men who developed low-risk PrCA, men who were diagnosed with BPH during the follow-up and men with no evidence of PrCA or BPH diagnosis. We retrospectively followed men in the four groups and estimated their individual PSA growth curves using PSA measures from enrolment date to exit date (diagnosis of PrCA for men in the cancer groups, or end of the follow-up for men in the no cancer groups). We then used the PSA growth curves equation to calculate PSA annual rate of change 1 and two years prior to exit. To avoid misclassification bias we excluded 3 types of participants; those who were reported to have a cancer outcome that was not confirmed; those who were classified as non-responsive (refusal to continue with study activities or loss of contact) without outcome assessment; and those who did not have complete diagnostic follow-up information in response to a positive screen (PSA above 4ng/ml or a suspicious DRE examination result). We also excluded men who reported ever to have been diagnosed to have BPH at baseline. Men who were diagnosed with BPH during the follow-up and did

not develop prostate cancer were included in the BPH group; those who had a diagnosis of BPH and prostate cancer were classified as prostate cancer. Finally, we excluded data from men with missing information on BMI at baseline (is this is an important covariate in analyses). Figure 4.1 shows the analytical cohort tree and the resulting four cancer status groups (analysis group).

Statistical methods: A non-linear mixed regression approach was used to establish individual and mean PSA growth curves and to estimate summary statistics of PSA annual rate of change. The details of our non-linear mixed model are described elsewhere [119]. In brief, a linear exponential piece-wise function was used with unknown continuous transition and random effect on the intercept, linear coefficient, exponential coefficient and the inflection point. Cancer status group (analysis group), age, race, PSA at baseline and body mass index were all included as main covariates. To allow for PSA rate to vary by cancer status group, age, race and PSA at baseline, interaction terms between time and all of these factors also were included. To estimate the growth curves independent of the cancer status, the linear exponential piece-wise function was used regardless of the cancer status group. The random effects for the inflection point and the time coefficient allows individual-level variability so that every individual can have the best fit line for his own observed PSA measure. It follows that those with constant linear pattern (as expected for men in the no-cancer groups) had individual estimates of an inflection point that was very close to zero (or not different from zero), effectively making their estimated PSA growth line to one linear phase. PSA was used in the model is in its natural scale; therefore PSA rate or velocity (PSAV) was represented by annual change (ng/ml/year). PSA individual rates were calculated at 1 and two years prior to exit

by taking the 1st derivative of the estimated mean model function and adding the individual effects.

A Cox proportional hazards model was used to estimate the association between PSA annual rate and the risk to high-risk prostate cancer. We started with a reduced Cox model that included age, race, family history, BMI, smoking and single PSA measure to predict high-risk prostate cancer. Since we have multiple PSA measures per men, we used the value of closest PSA result to the exit date (i.e., the last PSA screening test result). We then fit a full model that included the estimated PSA rates in addition to all other covariates (including last single PSA measure), we reported the Hazard Ratio (HR) of PSA rate, while adjusting for PSA single measure and compared the goodness-of-fit statistics of the reduced model to those from the full model.

The sensitivity, specificity, area under the curves (AUC) and ROC and their 95% CI for predicting high-risk prostate cancer were estimated for PSA annual rates. We included both PSA annual rate and a single PSA measure in the logistic model and constructed the two ROC curves for comparison; we reported the difference in the AUC between the two curves and the adjusted 95% confidence intervals with p-value.

Results:

Table 5.1 describes baseline characteristic of the analytical cohort and the four analysis groups. The Chi-square test and t-test, for categorical and continuous variables, were used to test for statistical differences between different groups. Of the total analytical cohort (20,888 men); 1,386 (6.55%) men were diagnosed with low-risk prostate cancer, 324 (1.55%) men were diagnosed with high-risk cancer, 7,813 (37.40%)

men were diagnosed with BPH and 11,383 (54.50%) men were right censored from the study for reaching end of follow-up without evidence of BPH or PrCA. Thus, the overall incidence of prostate cancer was 8.10%, and almost 19.00% of these cases were classified as high-risk prostate cancer. African Americans (AA) accounted for 3.49% of the total analytical cohorts. African Americans were more likely to be diagnosed with prostate cancer as compared to other races. Also, 24.39% of the PrCA cases among African Americans were classified as high-risk cancer compared to 18.88% among men of other races, the difference was not statistically significant. Men with BMI higher than 30kg/m2 were 24.56% of the total cohort, and they were not at higher risk of prostate cancer or BPH. Men with family history of prostate cancer in a first-degree relative (7.69% of the total cohort) were at higher risk of prostate cancer when compared to those without. Older men were more likely to have cancer. African Americans, men with family history of prostate cancer in a first-degree relative and those above 65 years of age were at higher risk of being diagnosed with high-risk prostate cancer (as opposed to not) . PSA at baseline was the lowest among men in the no-cancer group (1.28ng/ml (95% CI 1.26-1.30)) and slightly higher among the BPH group (1.32ng/ml (95% CI: 1.29-1.35)). Men in both cancer groups had comparable PSA at baseline; 2.51ng/ml (95% CI: 2.42-2.59) in the low-risk and 2.88ng/ml (95% CI: 2.34-3.42) in the high-risk cancer group. The median follow-up time was the longest among men in the BPH group at 12.49 years, while those in the no-cancer group had a median follow-up of 11.62 years. The follow-up time for the cancer groups was significantly lower at 7.49 years in the low-risk group and 8.26 years in the high-risk cancer group. The median number of PSA tests was 6.00 across no-cancer, BPH and low-risk cancer groups and 5 in the high-risk cancer. Men in

the low-risk cancer group had the lowest number of years between last PSA screening test and the day of diagnosis (gap period) with a median of 2.63 years; men in the high-risk cancer group had a longer median follow-up period of 3.33 years. Men in the no-cancer group had the longest gap period; of 6.75 years.

Figure 5.1 and 5.2 illustrate the mean PSA growth curve as estimated by the non-linear mixed model among different strata of race, age and study group combination. The adjusted mean annual rate among men in the no-cancer group was 0.06 ng/ml/year (95% CI: 0.053-0.061) for African Americans and 0.06ng/ml/year (95% CI: 0.053-0.059) for non-African Americans. The mean rate among men in the BPH group was also 0.06 ng/ml/year (95% CI: 0.056-0.065) for African Americans and 0.06 ng/ml/year (95% CI: 0.057-0.062) for non-African Americans. The mean linear rate among men in the low-risk cancer group was 0.09 ng/ml/year (95% CI: 0.078-0.100) for African Americans and 0.09 ng/ml/year (95% CI: 0.078-0.098) for non-African Americans. The mean rate among men in the high-risk cancer group was 0.13 ng/ml/year (95% CI: 0.096-0.156) for African Americans and 0.13 ng/ml/year (95% CI: 0.095-0.156) for non-African Americans. Among men with prostate cancer, PSA measures start to increase in an exponential phase, the inflection point took place during the 2 to six years prior to diagnosis. Men in the high-risk prostate cancer group demonstrated an earlier inflection point (5.64 years prior to diagnosis) and had a higher exponential coefficient of e^0.34 (e^0.28-e^0.39) as compared to men in the low-risk cancer group, who had a median inflection point of 3 years prior diagnosis and an exponential coefficient of e^0.16 (e^0.11 - e^0.21). All of these estimates were adjusted for mean baseline PSA measure, BMI and age.

After the inflection point (years closer to the exit), the rate of PSA change per year was not constant for the cancer groups. We estimated PSA rate for 1 and two years prior to exit by taking the 1st derivatives of the growth curve equation in each group. Table 5.2 and 5.3 show the age and race-specific mean and median annual PSA rate 1 and two years prior to exit respectively. Overall, men who developed high-risk cancer had higher PSA change rates as compared to other groups, regardless of age and race. Figure 5.2-5.7 show the box-plot of the estimated annual PSA rates for all participants and by different age and race strata. Panel (a) shows annual PSA rate 1 year prior to exit and panel (b) shows annual PSA rate 2 years prior to exit. The high-risk cancer group shows higher values with a slight overlap with any of the other three groups. Among African Americans, there is almost a complete separation.

In a cox regression analysis, increased annual PSA rate was highly associated with increased high-risk prostate cancer risk in the model that adjusted for all other covariates including last screened PSA level. When used as a numeric variable in the Cox proportional hazards model, the adjusted HR for annual PSA rate (one year prior to diagnosis) was 1.06 (1.055-1.072). However, using a threshold of 0.371ng/ml/year, the adjusted hazard ratio was 3229 (1636-6370) for PSA rate one year prior to diagnosis > 0.371 ng/ml per year versus ≤ 0.371. Once PSA rate (as a categorical variable) was included to the Cox model, the fit statistics improved significantly. Table 5.4 shows the fit statistics comparison between the reduced (using all covariates including PSA single test) and the full model (using all covariates, PSA single test and PSA annual rate cut-off).

The sensitivity and specificity for predicting high-risk prostate cancer for selected cut-off points of annual PSA rate are shown in table 5.5. We ran a logistic regression for PSA rate at 1 and two years prior to exit separately. The 1st part of table 5.5 shows the results for PSA rate at one year prior to exit. We selected the thresholds that produced the highest area under the curve in the ROC procedures for AA, non-AA, young (≤55) , middle-age (55-65), old (>65) and in the overall cohort. We started with the threshold that produced the highest sensitivity while keeping specify above 90%. Among AA, a threshold of 0.22 detected 100% of the cases with a specificity of 97.8% (that also corresponds to the threshold with the highest area under the curve in the ROC procedure). Among non-African Americans, a threshold of 0.10 detected correctly 99.7% of the cases but with a lower specificity of 90.8%. We then selected the threshold that had the next highest sensitivity with the highest specificity possible. Among AA, a higher threshold of 1.20 had a perfect specificity of 99.8% but a sensitivity of 95.0%. Among non-African Americans, a higher threshold of 0.31 correctly detected 98.1% of the cases, with higher specificity of 96.7%. Among non-African Americans, a threshold of 0.37 corresponds to the highest point on the ROC curve and thus has the best combination of sensitivity (97.4%) and specificity (97.2%). The thresholds varied by age; men between 55 and 65 years of age had lowest thresholds; a PSA annual rate of 0.130 detected 99.5% of the cases with a lower specificity of 95.6%. A higher threshold of 0.30 had the best combination of almost 98% for both sensitivity and specificity in this group of middle-age men. The younger group (<=55), had an almost perfect performance (100% sensitivity and 99.6 % specificity) at a PSA annual rate threshold of 0.99. Among older men (>65), the best combination of sensitivity and specificity (98% for both) was at the

threshold of 0.93. When considering the PSA rate 2 years prior to exit (part 2 of table 4) the results are similar; but with slightly lower threshold values, given that a high-risk prostate cancer diagnosis is likely to happen in the next 2 years as compared to the next 1 year. Overall, a threshold of 0.11 ng/ml/year is highly sensitive to high-risk prostate cancer but had a lower specificity of 91%. A threshold of 0.37 has the best combination of sensitivity (97.2%) and specificity of (97.3%) to detecting high-risk prostate cancer in a window time of one year. The overall estimates are more likely to reflect the case of non-AA between 55 and 65 years of age, since this group accounts for the majority of the data.

The ROC curves and the AUCs for annual PSA rate 1 year prior to exit are shown in figures 5.8-5.10. The AUC values are displayed on each curve. The curves illustrate high sensitivity and specificity measures obtained by PSA rate thresholds, all groups show high AUC values. The ROC curves and the AUC for a single PSA level (last PSA) alone and the annual PSA rate (1 year prior to exit) alone for all participants are both shown figure 5.11. The ROC curves for comparison clearly show that the PSA rate improved the prediction of high-risk prostate cancer. The AUC for a single PSA measurement is 81.44 (79.25-83.63) while the AUC for PSA rate is 99.50 (99.34-99.66), the added value of PSA rate above the prediction of a single PSA is 18.07 (15.89-20.24) p-value <.0001.

Discussion and conclusion

We used non-linear mixed model to establish individual PSA growth curves among 20,888 PLCO trial participants (aged 50-75) using at least four measurements of

annual PSA measures taken prior to prostate cancer diagnosis. After adjusting for age, race, and baseline PSA and BMI, we confirm that PSA growth curves for men who developed high-risk prostate cancer are significantly different and distinguished them from all other men. On average, the curves start to diverge and accelerate their departure from one another as early as five years prior to the clinical detection of the disease. At a fixed point on the curve, PSA annual change rate can be estimated by taking the 1st derivative of the curve equation at that point. We estimated these values for all participants at two different time points; 1 and two years prior to diagnosis or exit of the study. The estimated PSA rates were highly associated with the risk of high-risk prostate cancer independently of one single recent PSA measurement. Using a logistic regression and ROC procedures we estimated the best threshold to distinguish high-risk prostate cancer from any other condition. A threshold of 0.37ng/ml/year has the best combination of sensitivity (97.2%) and specifies of (97.3%) to detect high-risk prostate cancer time window of 1 year prior to the clinical detection in this cohort of men. When compared with one single PSA measure, the estimated PSA annual rate highly improved the detection of high-risk prostate cancer.

Our work builds on early findings by Carter et al. [16] and Pearson et.al. [121, 122] who estimated PSA growth curves to calculate PSA rate among men with prostate cancer. We used the same non-linear regression function proposed by Carter and Pearson for PSA change over time among men who developed prostate cancer. However, we used the same function for all men in our data regardless of the cancer status, and we allowed every individual to have their own unique PSA growth curve. Thus, the estimated PSA rates were independent of the cancer outcome. In our results, most men on the BPH and

the no-cancer group had a very small estimate of the proposed inflection point and the exponential time coefficient. The reported values of PSA rates for these men might be overestimated as a result of proposing exponential term in the regression function. If the overestimation for the non-cancer groups is true, the results will be slightly biased toward the null. Even though we used a novel approach to define and estimate PSA annual rate, the summary statistics of our PSA rates are close to those reported by previous findings. Carter et al. [123], reported a mean PSA velocity of (0.5 -2 ng/ml/year) for men who developed prostate cancer. Tang et al. [124] reported a mean PSA velocity of 0.02-0.06 ng/ml/year among healthy men. Similar to our findings, studies that investigated potential threshold values for PSA velocity to predict prostate cancer, reported threshold values that ranged around 0.3-0.6ng/ml/year [125] [84] .

There is a large body of literature on the topic of PSA kinetics. The debate on the value of PSA kinetics in improving prostate cancer detection had started 20 years ago and remains a controversial topic today. In their systematic review, Vickers et al. [84] concluded that; studies that investigated PSA kinetics either found single PSA to be a better predictor than PSA kinetics, or found trivial differences in favor of PSA kinetics, or had serious methodological shortcomings. In contrast, two recently published studies concluded that PSA change over time does in deed improve prostate cancer detection. Wallner et al. [18] evaluated whether the rate of change in serum PSA levels (represented by annual percent change) accurately detects prostate cancer in a managed population of 219,388 men passively followed from 1998 to 2008. Similar to ours, their results indicated that multiple measures of PSA improve the accuracy of aggressive prostate cancer detection when compared to single measurements of PSA. Orsted et al.

[19] investigate the same question among 7,455 men in the Copenhagen city heart study. They also concluded that adding long-term PSAV to baseline PSA values improves classification of prostate cancer risk and mortality. Our results and the results of these two recent studies provide insight into the potential use of PSA annual rate as a predictive marker for aggressive prostate cancer.

In a recent systematic review, Loughglin [83] discusses multiple factors contributing to the PSA velocity controversy, many of which can explain why our results may be different from others. First, there are wide range of PSAV/kinetics definitions and estimation methods. We took a conservative approach and used a minimum 4 PSA measurement approach to compute PSA annual rate. Many studies used only two measurements, which does not conform to professional society guidelines. We used balanced data of equal annual intervals across all PSA measures and over a long duration; i.e. of 7 to 11 years. These factors contributed in building a stable statistical regression model that estimates annual PSA rate; it also allowed us to avoid the linear restriction and consider an exponential pattern for PSA change in the years prior to prostate cancer detection. What is common between this study and other studies that reported positive results, is the long-term measurements of PSA that were used to define and estimate PSAV. This observation supports Loughglin argument regarding the need for an uniform definition and sufficient PSA test over a sufficient period. Second, Loughglin brings into attention the high collinearity between PSA and PSAV reported in previous studies ($r=0.70$) that makes the added value of PSAV questionable. Unlike other studies, we did not find a high correlation between the last PSA measure and the calculated PSA rates. Interestingly, Pearson correlation coefficient between the two measures in our data was

only 0.3. This might be due to the novel non-linear mixed method we used to estimate PSA rates.

Lastly, we proposed a different question; unlike other studies we focused on distinguishing high-risk prostate. The increased detection of indolent prostate cancer is main limitation of a single PSA test [14] . It is well established that the challenge is to improve screening specificity of clinically significant disease (i.e., PrCA associated with a high probability of morbidity or death, therefore, should necessitate medical treatment). Cases of low-risk cancers (i.e., Prognostic group I, IIA) are more likely to die from other causes before PrCA becomes clinically advanced enough to cause significant morbidity and mortality. Diagnosing and treating such low-risk PrCA is more likely to cause harm than benefit [126] .

We acknowledge the following limitations; first, this analysis could be strengthened by including a more diverse sample, in terms of age and race. These findings are likely to be applicable for European American men between 55 and 65 years of age. African Americans are at higher risk of aggressive prostate cancer. This racial group might have a distinct natural history to the disease. PSA growth curves have the potential to detect these differences and account for it in establishing screening criteria. We observed some qualitative differences among AA, but the number of AA was small to establish or refute this hypothesis. Second, we conducted this analysis on a screened population; this has the advantage of minimizing outcome misclassification. However, cancers detected by screening as compared to those that are detected clinically tend to have lower PSAs and PSA rates. Also, the time over which the PSA is measured and the time intervals may be different in natural practice and have the potential to change the

calculated PSA rates. A sensitivity analysis with multiple scenarios of PSA number and shorter sampling intervals would be informative. We are unable to conduct such analysis using the PLCO data as the PSA tests are well balanced by design. Third, information bias and misclassification can still be a threat, particularly among men in the non-cancer and BPH group who didn't have a biopsy or a clinical examination to confirm their non-cancer or BPH status. We limited this bias by restricting our analysis to only those with biopsies (positive or negative)/ or those that never had a positive screening. So, individuals who had a positive screening and were not followed with a diagnostic prostate biopsy or BPH diagnosis were excluded.

We are not the first to report that calculated PSA rate is independently associated with prostate cancer risk, but we are the first to propose a non-linear mixed regression model to estimate PSA annual rate across all men (and not only those that developed prostate cancer). So, we are the first to show that these estimated PSA rates significantly increase diagnostic accuracy of a clinically meaningful prostate cancer. While we found an increased risk of high-risk prostate cancer across the continuum of increasing PSA rates, we were still able to define specific threshold to distinguish cases with high probability of having the disease within 1 to 2 years.

In a cohort of 20,888 men, we analyzed existing repeated measures of PSA and developed a regression algorithm that improved both sensitivity and specificity of the PSA-based screening test to detect high-risk prostate cancer. In this study, the estimated PSA growth curves and the derived annual PSA rates are means for differentiating "significant" prostate cancer from any other condition that could be related to an

increased PSA measure at any particular point in time. Further research is required to validate this algorithm and its ability to distinguish high-risk prostate cancer

Tables and Figures

Table 5.1 Characteristics of participants by study group (n= 20888)

Table (1): characteristics of participants by study group (n= 20888)								
	Men with no cancer 11,383 (54.50%)	Men with BPH 7,813 (37.40%)	Men with LRC 1386 (6.55%)	Men with HRC 324 (1.55%)	Comparison			
Race					HRC vs. No-cancer	HRC vs.BPH	HRC vs. LRC	HRC vs. others
African American N=824(3.49)	586 (71.12)	156 (18.93)	62 (7.52)	20 (2.43)	0.41	<.0001	0.22	0.04
Others N=20,064(96.06)	10797 (53.69)	7657 (38.16)	1306 (6.51)	304 (1.52)				

BMI								
<= 30 kg/m^2 N= 15,757 (75.44%)	8419 (53.43)	6012 (38.15)	1068 (6.78)	258 (1.64)	0.02	0.2608	0.54	0.08
>30 kg/m^2 N=5131 (24.56%)	2964 (57.77)	1801 (35.1)	300 (5.85)	66 (1.29)				
Family history of prostate cancer in a first-degree relative (missing=144)								
No N=19,282 (92.31%)	10,547 (54.7)	7226 (37.48)	1225 (6.35)	284 (1.48)	0.0005	0.0015	0.29	0.0012
Yes N=1462 (7.69%)	754 (51.57)	537 (36.73)	132 (9.03)	39 (2.67)				
Age (years)								
<= 55 N=2,228(10.67%)	1203 (53.99)	893 (40.08)	107 (4.8)	25 (1.12)	0.1255	<.0001	0.34	0.0093
55-65 N=13,658 (65.39%)	7131 (52.21)	5429 (39.75)	898 (6.57)	200 (1.46)				
>65 N=5002 (23.95%)	3049 (60.96)	1491 (29.81)	363 (7.26)	99 (1.98)				

PSA at baseline mean/median (95% CI)	1.28 (1.26-1.30) / 0.99	1.32 (1.29-1.35)/ 0.99	2.51 (2.42-2.59)/ 2.16	2.88 (2.34-3.42) /1.95	<.0001	<.0001	0.1832	<.0001
Years of follow up mean/median (95% CI)	11.17(11.13-11.21) / 11.62	11.92 (11.90-12.00)/ 12.49	7.54 (7.39-7.68)/ 7.49	7.86 (7.54-8.17) / 8.26	<.0001	<.0001	0.0589	<.0001
Number of PSA tests mean/median (95% CI)	5.52 (5.52-5.54)/ 6	5.67 (5.65-5.68)/6	5.29(5.24-5.33)/ 6	5.21 (5.12-5.31) /5	<.0001	<.0001	0.1409	<.0001
Years from last PSA to exit or diagnosis	6.30 (6.26-6.33) / 6.75	6.94 (6.91-6.97)/ 7.44	2.93 (2.801-3.05)/ 2.63	3.37 (3.09-3.66/ 3.33	<.0001	<.0001	0.0054	<.0001

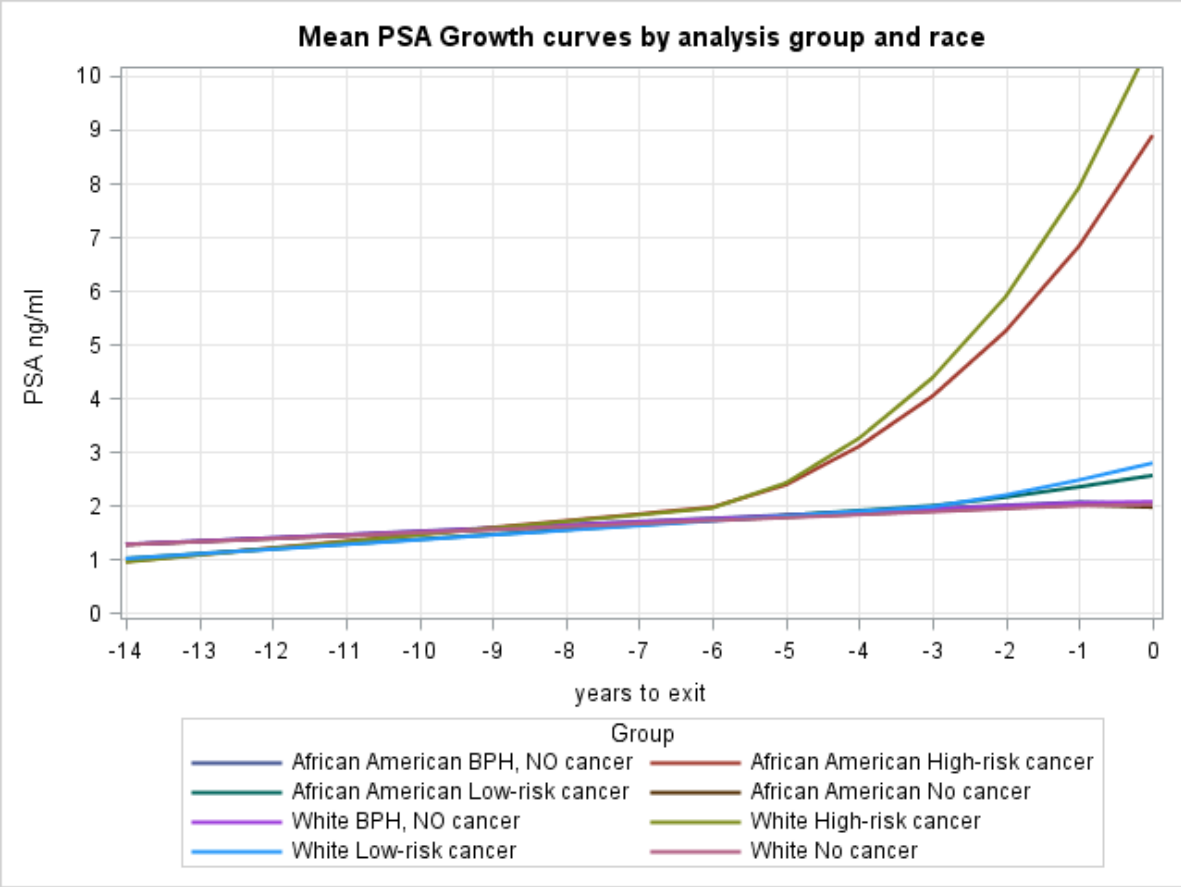


Figure 5.1 Predicted mean PSA growth curve by study group and race

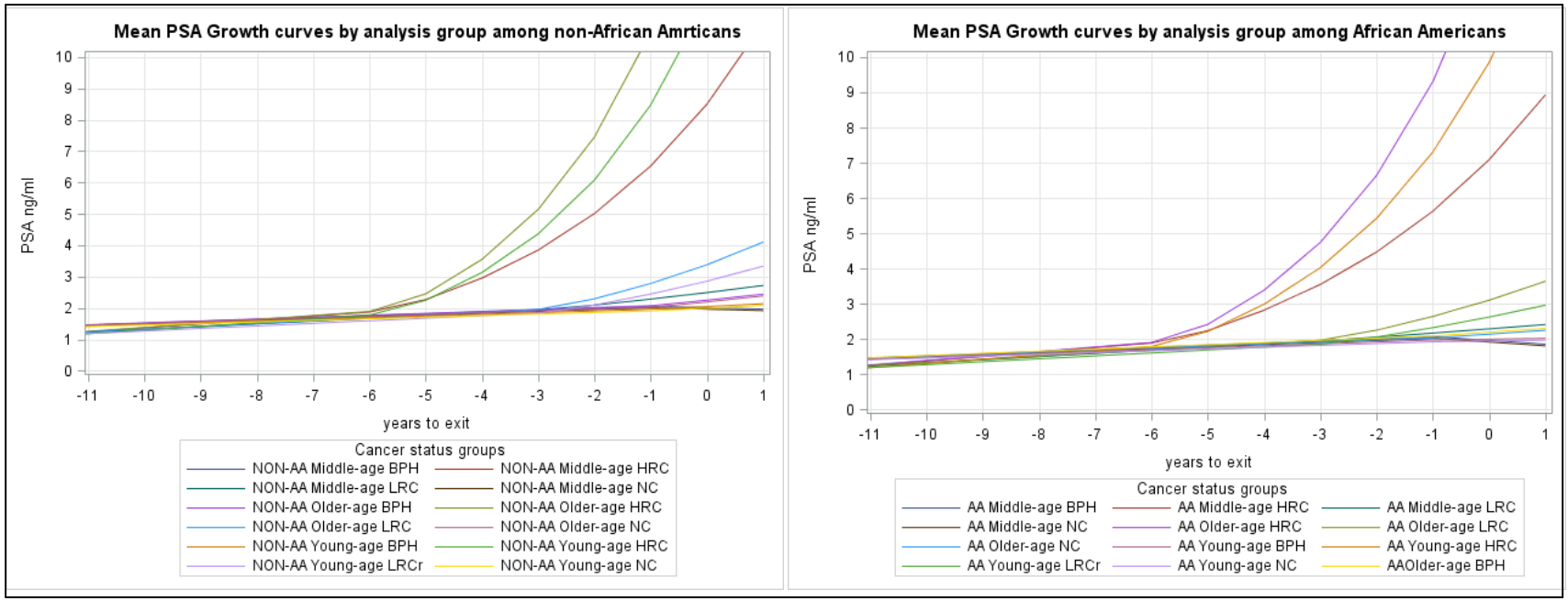


Figure 5.2 Predicted mean PSA growth curve by study group and age for African-Americans and non-African Americans.
AA= African Americans,
NON-AA= none African Americans,
BPH= Benign hyperplasia
HRC= high-risk cancer,
LRC= low-risk cancer,
NC=No evidence of prostate cancer or BPH, young-age>=55, 55>middle-age<=65, older-age>65.

Table 5.2 Estimated Annual PSA rate (ng/ml/year) 1 year prior to exit stratified by race, age and study groups and fixed at baseline PSA of 1.3

Race	Age	Group	mean	(95%CI)	Median	25^{TH} Perc.	75^{th} perc.
Non-African American	Youngest	No cancer	0.05	(0.05,0.06)	0.01	-0.02	0.03
		BPH	0.05	(0.05,0.06)	0.01	-0.02	0.03
		LRC	0.38	(0.31,0.45)	0.36	0.15	0.61
		HRC	2.81	(2.14,3.49)	2.11	1.50	3.55
	Middle	No cancer	0.06	(0.05,0.06)	-0.01	-0.10	0.02
		BPH	0.06	(0.06,0.06)	-0.02	-0.11	0.02
		LRC	0.20	(0.17,0.23)	0.17	0.10	0.35
		HRC	1.73	(1.34,2.12)	1.45	0.99	1.90
	Older	No cancer	0.06	(0.05,0.06)	0.02	-0.02	0.06
		BPH	0.06	(0.06,0.06)	0.01	-0.05	0.06
		LRC	0.54	(0.45,0.63)	0.67	0.42	0.95
		HRC	4.00	(3.32,4.69)	3.60	2.59	5.74
African Americans	Youngest	No cancer	0.05	(0.05,0.06)	0.01	0.00	0.03
		BPH	0.05	(0.05,0.06)	0.01	0.00	0.03
		LRC	0.28	(0.14,0.43)	0.71	0.13	1.19
		HRC	2.18	(1.34,3.02)	1.38	1.19	1.57
	Middle	No cancer	0.06	(0.05,0.06)	0.00	-0.12	0.03
		BPH	0.06	(0.06,0.07)	-0.01	-0.12	0.03

		LRC	0.12	(0.01,0.22)	0.12	0.06	0.18
		HRC	1.30	(0.80,1.79)	1.58	1.32	2.24
	Older	No cancer	0.06	(0.05,0.06)	0.02	-0.01	0.05
		BPH	0.06	(0.06,0.07)	0.01	-0.12	0.03
		LRC	0.42	(0.23,0.62)	0.39	0.10	0.85
		HRC	3.14	(1.94,4.33)	2.36	1.44	3.32
BPH= Benign hyperplasia HRC= high-risk cancer, LRC= low-risk cancer, NC=No evidence of prostate cancer or BPH, young-age>=55, 55>middle-age<=65, older-age>65							

Table 5.3 Estimated Annual PSA rate (ng/ml/year) 2 year prior to exit stratified by race, age and study groups and fixed at baseline PSA of 1.3

Race	Age	Group	mean	(95%CI)	Median	25^{TH} Perc.	75^{th} perc.
Non-African American	Youngest	No cancer	0.05	(0.05,0.06)	0.01	-0.02	0.03
		BPH	0.05	(0.05,0.06)	0.01	-0.02	0.03
		LRC	0.32	(0.27,0.38)	0.23	0.11	0.41
		HRC	2.02	(1.59,2.45)	1.65	1.08	2.54
	Middle	No cancer	0.06	(0.05,0.06)	0.01	-0.08	0.04
		BPH	0.06	(0.06,0.06)	0.00	-0.09	0.04
		LRC	0.18	(0.16,0.21)	0.12	0.09	0.22
		HRC	1.32	(1.06,1.59)	1.16	0.74	1.50
	Older	No cancer	0.06	(0.05,0.06)	0.02	-0.02	0.05

		BPH	0.06	(0.06,0.06)	0.01	-0.05	0.05
		LRC	0.44	(0.38,0.51)	0.43	0.15	0.67
		HRC	2.76	(2.34,3.18)	2.72	1.84	4.08
Non-African American	Younges t	No cancer	0.05	(0.05,0.06)	0.02	0.00	0.04
		BPH	0.05	(0.05,0.06)	0.01	0.00	0.03
		LRC	0.25	(0.14,0.36)	0.13	0.11	0.91
		HRC	1.62	(1.07,2.17)	1.02	0.87	1.18
	Middle	No cancer	0.06	(0.05,0.06)	0.01	-0.05	0.04
		BPH	0.06	(0.06,0.07)	0.01	-0.05	0.05
		LRC	0.11	(0.02,0.20)	0.11	0.06	0.14
		HRC	1.03	(0.68,1.38)	1.25	1.10	1.81
	Older	No cancer	0.06	(0.05,0.06)	0.02	0.00	0.05
		BPH	0.06	(0.06,0.07)	0.01	-0.13	0.05
		LRC	0.36	(0.22,0.51)	0.27	0.10	0.56
		HRC	2.24	(1.49,2.99)	1.73	1.00	2.56

BPH= Benign hyperplasia HRC= high-risk cancer, LRC= low-risk cancer,
NC=No evidence of prostate cancer or BPH, young-age>=55, 55>middle-age<=65, older-age>65

Table 5.4 Cox model fit statistics for high-risk prostate cancer with and without PSA annul rate , calculated at one year prior to exit and used as a categorical variable with a threshold of > 0.371ng/ml/year

	-2 LOG L	AIC	SBC	p-value of the --2 LOG L
Model without PSA rate	6018.42	6040.42	6081.97	<.0001
Model with PSA rate	3679.73	3703.73	3749.06	

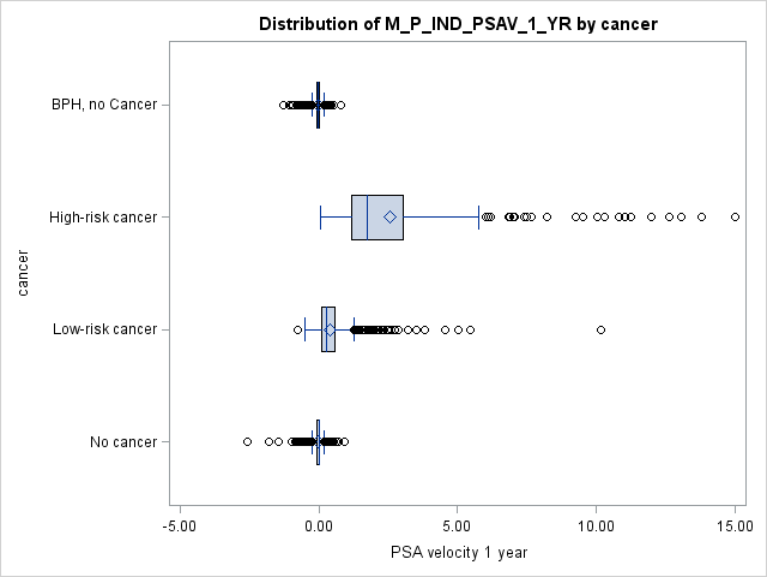


Figure 5.3 Box plot of PSA rate (ng/ml/year) 1 years prior to exit by study group

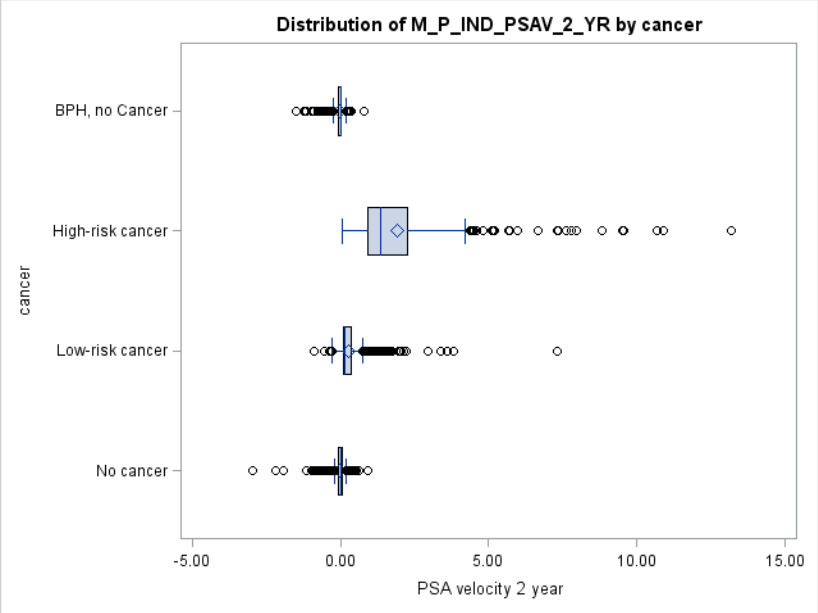


Figure 5.4 Box plot of PSA rate (ng/ml/year) 2 years prior to exit by study group

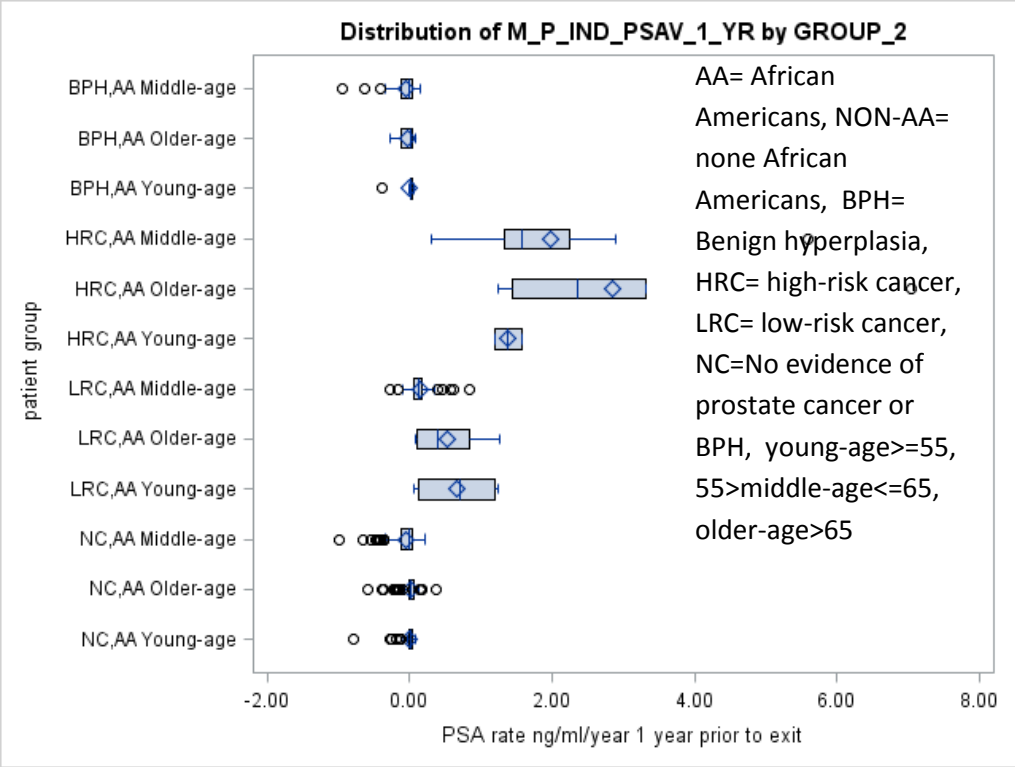


Figure 5.5 Box plot of PSA rate (ng/ml/year) 1 year prior to exit by study group and age among African Americans

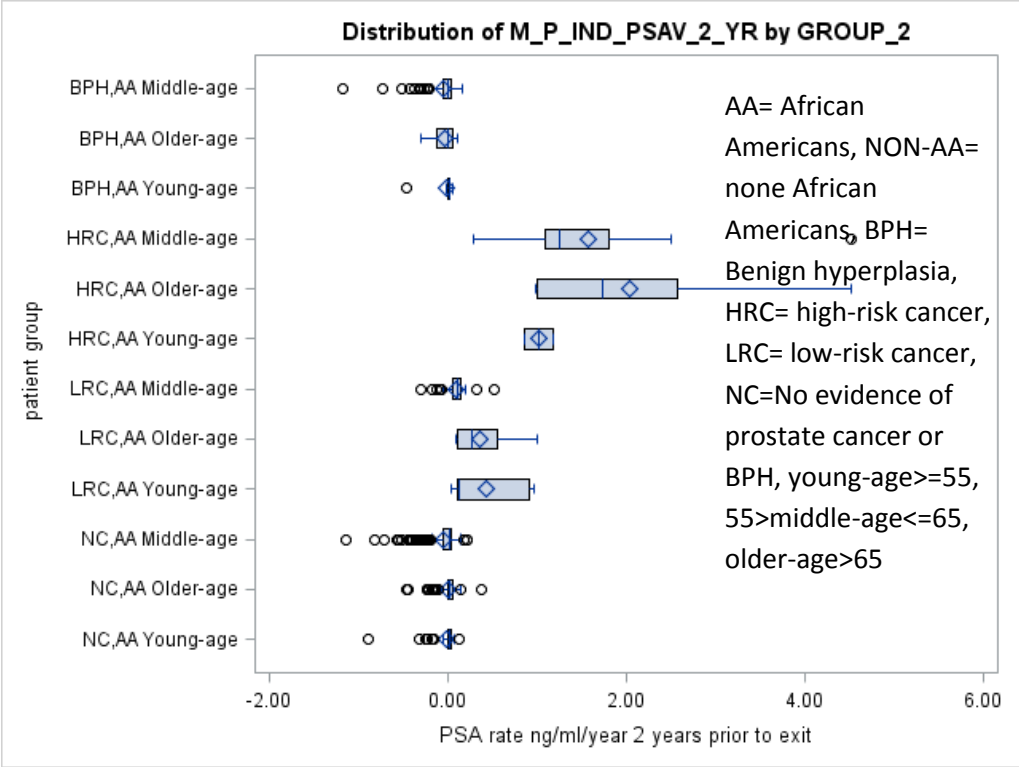


Figure 5.6 Box plot of PSA rate (ng/ml/year) 2 year prior to exit by study group and age among African Americans,

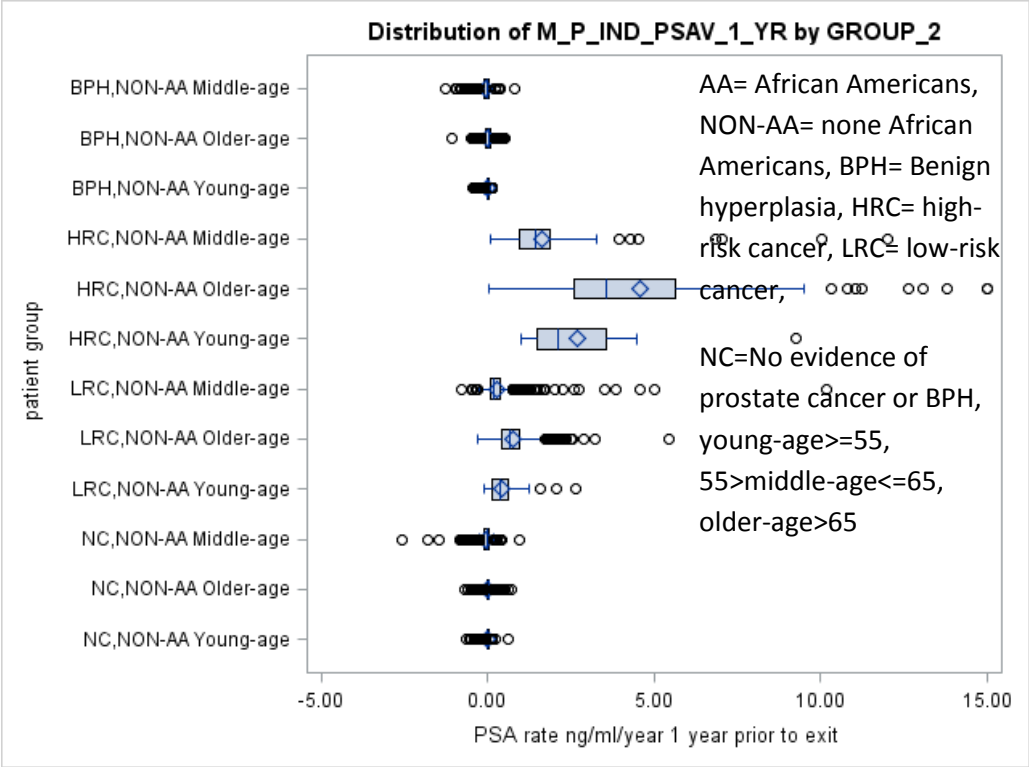


Figure 5.7 Box plot of PSA rate (ng/ml/year) 1 year prior to exit by study group and age among non-African Americans

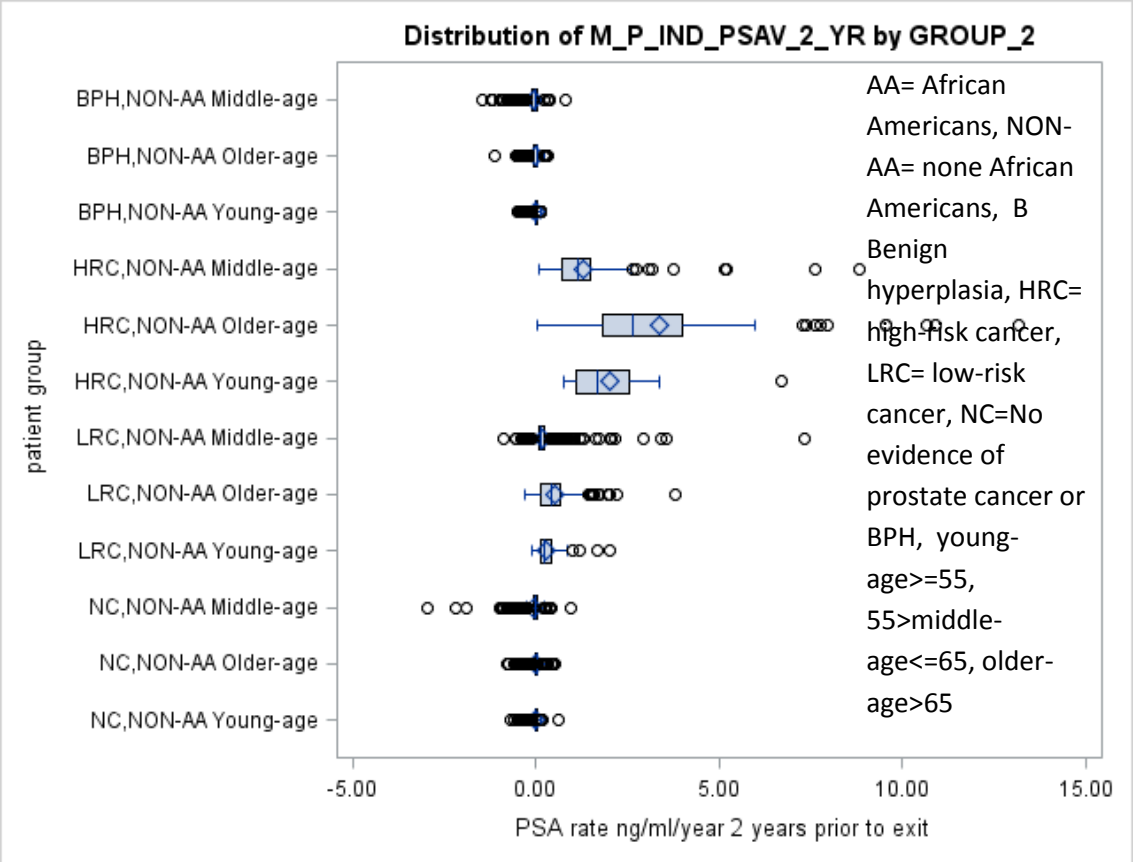


Figure 5.8 Box plot of PSA rate (ng/ml/year) 2 year prior to exit by study group and age among non-African Americans

Table 5.5 Measurements of test performance for the prediction of high-risk prostate cancer by selected anneal PSA rate thresholds stratified by patient population

		PSA rate threshold (ng/ml/year)	Sensitivity	Specificity	True +VE	True -VE	False +VE	False -VE
1	AA	0.22	100.0%	97.8%	20	796	18	0
		1.20	95.0%	99.8%	19	812	2	1
	Non-AA	0.10	99.7%	90.8%	303	18112	1841	2
		0.31	98.0%	96.7%	298	19300	653	6
		0.37	97.4%	97.2%	296	19404	549	8
	Young	0.99	100.00%	99.60%	25	2216	8	0
	Middle age	0.13	99.50%	95.60%	199	12987	603	1
		0.30	98.00%	97.90%	196	13308	282	4
		0.33	97.00%	98.10%	194	13338	252	6
	Old	0.93	98.00%	98.00%	97	4855	98	2
		1.06	97.00%	98.50%	96	4879	74	3
	Overall	0.10	99.7%	90.9%	323	18867	1900	1
		0.29	98.1%	96.7%	318	20081	686	6
		0.37	97.2%	97.3%	315	20208	559	9
2	AA	0.18	100.0%	98.8%	20	804	10	0
		0.87	95.0%	99.6%	19	811	3	1
		0.10	99.7%	92.4%	303	18437	1516	1

	Non-AA	0.19	98.0%	96.7%	298	19288	665	6
		0.27	97.4%	97.6%	296	19481	472	8
	Young	0.91	100.0%	99.6%	25	2216	8	0
	Middle age	0.99	99.5%	95.6%	199	12991	599	1
		0.15	98.0%	96.6%	196	13127	463	4
		0.31	97.0%	98.7%	194	13407	183	6
	Old	0.90	98.0%	98.6%	97	4882	71	2
		1.06	97.0%	98.7%	96	4891	62	3
	Over all	0.11	99.7%	92.4%	323	19187	1580	1
		0.19	98.1%	96.7%	318	20086	681	6
		0.27	97.5%	97.7%	316	20284	483	8
AA= African Americans, NON-AA= none African Americans, young-age>=55, 55>middle-age<=65, older-age>65								

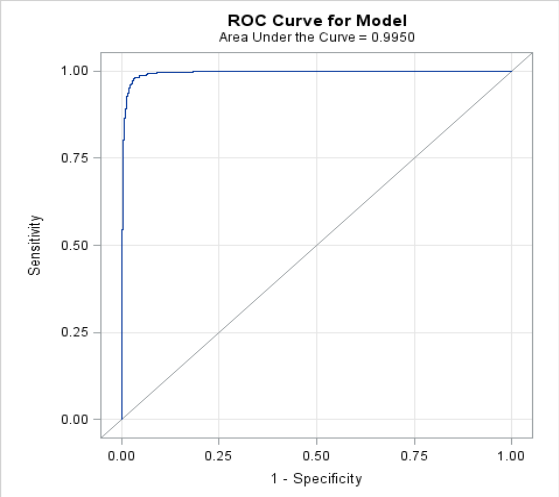


Figure 5.9 AUC curves of PSA rate (ng/ml/year) 1 year prior to exit for all participants

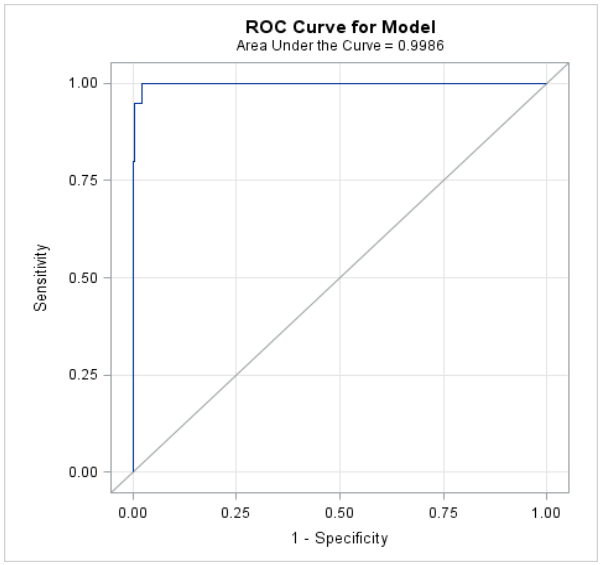


Figure 5.10 AUC curves of PSA rate (ng/ml/year) 1 year prior to exit for African-Americans

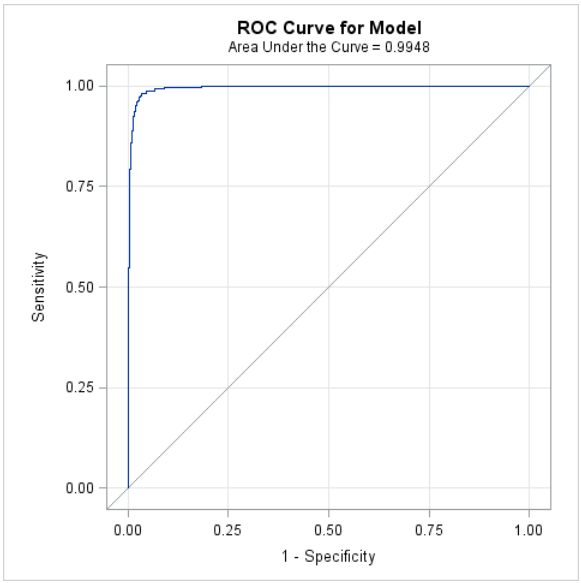


Figure 5.11 AUC curves of PSA rate (ng/ml/year) 1 year prior to exit for non African-Americans

ROC area for PSA rate = 99.50 (99.34-99.66) , ROC area for PSA single measurmnet= 81.44 (79.25-83.63) , the difference = 18.07 (15.89-20.24) p-value<.0001

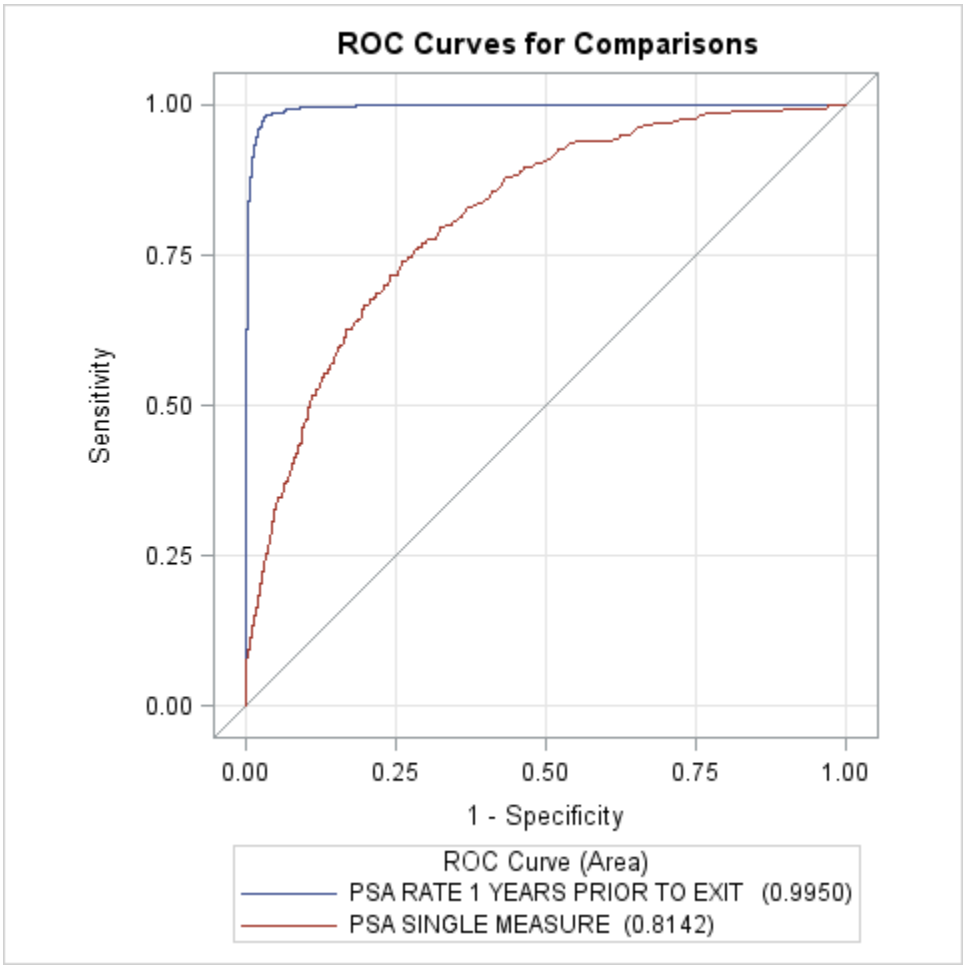


Figure 5.12 ROC curves for PSA rate and single PSA measure (last PSA)

CHAPTER 6

The use of PSA rate to predict high-risk prostate cancer among veterans; a validation study [3]

[3] Shoaibi , A., Rao, G., Cai, B., Rawl, J., Hebert, J.. The use of PSA rate to predict high-risk prostate cancer among veterans; a validation study To be submitted to Urology

Introduction:

Prostate cancer (PrCA) screening, an issue of public health significance, represents a major modern medical controversy. This is because prostate specific antigen (PSA)-based screening was implemented routinely at the population level for several decades (i.e., from the early 1990s until the late 2000). This is thought to have resulted in 'PrCA stage migration' – where most of the newly detected PrCA were indolent and of low risk in terms of both detracting from individuals' quality of life and mortality [127]. The conclusion of several expert panels charged with examining this issue [105, 128, 129] was that detection and treatment of low-risk PrCA caused more iatrogenic harm than benefit, because most of these patients with newly diagnosed low-risk PrCA are unlikely to suffered from PrCA-related morbidity or mortality [24].

An alternative to using a single PSA value for PrCA screening is using multiple serial PSAs over time [130]. PSA is a relatively inexpensive test which, when used serially, can enable computing PSA kinetics or PSA velocity [83]. These parameters are thought to provide insights into the natural history of the process of prostate carcinogenesis [16]. Despite the appeal this theoretical possibility, there is no conclusive evidence, such as through a randomized control trial, that can inform regarding the effectiveness of PSA kinetics in detecting PrCA and the evidence from observational studies have varied [33, 131-135]. Earlier studies showed that there is a significant and independent association between PSA velocity (PSAV) and the risk of PrCA; a threshold of 0.35-0.75 was proposed to indicate prostatic biopsy [16, 73, 118, 121, 136, 137]. Many other studies contradicted these early findings and disproved any additional value of PSAV over a PSA single test in predicting PrCA [83, 84, 117]. Amidst all this

controversy, PSAV is still considered useful, and current guidelines recommend considering PSAV when available along with many other factors to make the decision about the need for prostatic biopsy [101].

In light of the recommendations against routine population level PrCA screening using PSA by the United States Preventive Services Task Force (USPSTF) and other professional organizations [101], individual-level screening, based on shared-decision making between the patient and provider is now more important than ever. In the absence of population-level screening, it may be speculated that over time there will be an under-detection of high risk PrCA and a reverse stage migration towards high-risk PrCA. This has important implications as reductions seen in prostate cancer mortality over the past quarter of a century has, at least in part, reflected aggressive population-based PSA screening. Risk PrCA, is more likely to impact the morbidity and mortality of the affected individual – and there is a need for tool that can specifically predict the occurrence of high-risk PrCA [24].

In our previous work (currently under review) [138], using data from 22,000 participants in the Prostate Lung Colorectal and Ovarian trial (PLCO), we established PSA growth curves to distinguish men who developed high-risk PrCA or either were diagnosed with low-risk PrCA or were found to have no PrCA. Using these growth curves, we estimated an individual's age- and race-adjusted annual PSA rate at one and two years prior to PrCA diagnosis. We found that these age- and race-adjusted PSA rates could accurately detect high-risk PrCA cases and differentiate them, with high sensitivity and specificity, from low-risk PrCA or no PrCA. In this study, we aimed to validate our method of defining PSA growth rate and then use the previously proposed threshold

levels to predict high-risk PrCA. To achieve this aim we used data from enterprise-wide national electronic health record in the Department of Veterans Affairs (VA) to: a) investigate the pattern of PSA change over time in VA cohort; b) estimate annual PSA rate for individuals in the VA cohort; and c) use thresholds derived from our previous work in the PLCO data to predict high-risk PrCA cases. The VA data is different from the clinical trial data of PLCO in that the PSA values were not derived at regular intervals. As such, these data represent what would be available in real-world clinical care delivery (and in a predominantly male cohort of Veterans). These Veterans are known to have higher comorbidity, along with a relatively higher representation of African Americans. The higher African-American representation, a feature not present in PLCO, allows us to further test our model, by stratified analysis by race. This is important as African Americans are known to be diagnosed with PrCA at later stages, and therefore have disproportionately higher morbidity and mortality.

Methods:

Data sources: We extracted data from the VA national electronics health record system; containing demographic, administrative claims, vital signs, mortality, laboratory results, pharmacy dispensation and cancer registry as part of VA Corporate Data Warehouse (CDW). The VA CDW data included detail information on cancer staging (TNM clinical and pathological), histological grade including Gleason score, cancer treatment and dates of diagnosis – all data were stored inside the VA research environment - Veterans Affairs Informatics and Computing Infrastructure (VINCI). All VA data sources are linkable using a common individual patient-level identifier; i.e., scrambled SSN, a unique individual-level identifier. The utility, accuracy, validity, and

access methodology of the available data, including both pharmacological and laboratory-derived, have been described previously [85-88]. PSA serum measurements at the VA hospitals labs are all done in compliance with the quality control standards of the of the Clinical Laboratory Improvement Amendments (CLIA). This study was approved by the IRB of the WJB Dorn VA Medical Center and has received appropriate approvals from VA regulatory entities such as National Data System.

Cohort definition: We identified all men who were between the ages of 50 to 75 years when they had their first VA-based PSA test between January 1st 2002 and December 31st 2011. We chose the cut-off of December 2011, because until 2012 eligible Veterans were more likely to be invited for annual PSA-based screening, and used January 1st 2002 in order to make the total study period 10 years. Any Veteran who, at the time of his first PSA had any coded documentation of the following were excluded: PrCA, Benign prostatic hyperplasia (BPH), any other prostatic pathology or other cancer either in the administrative claims data or in cancer registry or history of any form of surgical intervention of the prostate (such as prostatectomy partial or full, prostatic resection including transurethral resection of prostate, or even prostate biopsy), any history hormonal treatment such as orchiectomy or 5-alfa-reductase inhibitors (Proscar/ Propecia/finasteride or Dutestiride). Also excluded were men who during follow-up were coded for prostate cancer using ICD9 code on claims data but did not have a corresponding entry in the cancer registry or were present in cancer registry, but did not have TNM stage or Gleason score – these patients are more likely to be either misclassified or have incomplete data. All patients were censored on either December 31st 2011, date of diagnosis of PrCA, date of BPH diagnosis (unless they also

subsequently had PrCA, in which case PrCA was used in preference to BPH) or were given hormonal treatment or prostatic surgery – whichever came first. In addition, as our model was dependent on having at least 4 serial PSA measures – we artificially restricted only to those patients who had at-least 4 PSA measures between date of first PSA and date of exit/censor or cancer diagnosis. The 4 PSA measures had to be such that there was at-least a 1-year gap between the first PSA and any subsequent PSA that was not the last PSA, there was a 1-year gap between the last PSA and any previous PSA that was not the first PSA, and there was at-least a 3-year gap between the first and the last PSA – this ensured that cohort represents being screening approximately annually for a minimum of 3 years.

Cohort follow-up: Cases were those who met our PrCA criteria (i.e. classifiable into either low-risk or high-risk PrCA using VA CDW cancer registry data). We then created random sample of controls for the case series. For every case, we randomly selected from the cohort equal numbers of patients with BPH and no cancer or BPH. We then retrospectively followed these patients starting with their first PSA until either outcome or censor. Baseline variables were race (classified as either African American or not African American), age at first PSA, baseline PSA, baseline BMI classified as either obese (≥30kg/m2) or not obese (<30kg/m2).

Statistical analysis:

To investigate the pattern of PSA change, we plotted the observed individual trajectories of PSA as a function of time for the four analysis groups. We then used "spaghetti plots" to illustrate the individual trajectories and the locally weighted scatter plot smoothing regression to fit the mean trajectories in each group separately [24]. We defined time as

number years to censor or outcome. To estimate individual PSA growth curves, we used the non-linear mixed model approach with the same linear-exponential piece-wise function that was established in our previous work [138]. The random effect in the mixed model for repeated measure analysis allows for individual-to-individual variation (between-individual) and account for within-individual variation. By taking the 1st derivative of the PSA growth curve equation we estimated PSA annual rate at a fixed time point of 1 year prior to exit. We then used age- and race-specific thresholds derived previously from the PLCO PSA growth curves to predict high-risk prostate cancer cases in the VA validation cohort using a logistic regression model. The sensitivity, specificity, area under the curves (AUC) and ROC and their 95% CI for predicting high-risk prostate cancer were estimated for these PSA annual rates. We included both PSA annual rate and a single PSA measure in the logistic model and constructed the two ROC curves for comparison. Finally, a logistic regression model was used to estimate the association between PSA annual rate and the risk to high-risk prostate cancer while adjusting for age, race, BM and single PSA measure to predict high-risk prostate cancer. Since we have multiple PSA measures per men, we used the value of closest PSA result to the exit date (i.e., the last PSA screening test result).

Results:

Approximately 2.5 million veterans were screened for PrCA between January 2002 and December 2011; 680,390 of these men met our study criteria. Of those, 7,347 men were diagnosed with PrCA. From the remaining pool of 673,565 men (60,894 with BPH and 611, 581 normal prostate), we randomly selected 7,347 men with normal prostate (no diagnosis of prostate abnormality) and another 7,347 men with BPH. Out of the PrCA

cases, 4,315 (58.73%) were diagnosed with high-risk prostate cancer and 3,032 (41.27%) men were diagnosed with low-risk cancer. Table 6.1 and 6.2 describe baseline characteristic of the analytical cohort and by analytic group, respectively. The Chi-square test for categorical variables was used to test for statistical differences between different analytic groups. Men in the cancer groups were more likely to be African American and of older age compared to men without cancer. A higher proportion of obese men were significantly more likely to be diagnosed high-risk PrCA (44.38%) compared to low-risk PrCA group (40.93%). Similarly, the proportion of older men (>65) in the high-risk PrCA group (26.58%) was significantly higher compared to low-risk PrCA group (18.73%). The proportion of AA in the high-risk PrCA group (22.90%) was slightly higher that their proportion in low-risk PrCA group (21.73%) but the difference was not statistically significant. Men in high-risk PrCA group were more likely to be African American and of older age when compared to all other groups combined.

Men in the cancer groups had, on average, higher number of PSA tests, shorter duration of follow up, higher PSA at baseline and shorter duration between the last PSA and the exit date when compared with men in the other two non-cancer groups (Table 6.2). Men in the low-risk PrCA had a slightly shorter period of follow up when compared to men in the high-risk PrCA group (5.76 years vs. 5.89 years). The two cancer groups had comparable number of PSA tests, PSA level at base line and the number of years - between the last PSA and the diagnosis date. Men in the non-prostate cancer group had the longest duration of follow up; 6.93 years, almost a year longer when compared to the other three groups. The number of years between the last PSA test and the exit date was less than 1 year in all groups and was shortest among men in the high-risk PrCA group

(0.20 years) compared to 0.74 years among men in the normal prostate group. The median of PSA tests ranged between 7 in the cancer groups and 6 in the non-cancer groups. PSA at baseline was the lowest among men in the normal prostate group, with an average of 1.06 ng/ml and a median of 0.80 ng/ml. Men in the BPH group had a baseline PSA that was significantly higher than those in normal prostate group (1.38 ng/ml vs. 1.09 ng/ml) but significantly lower than men in high-risk (2.82 ng/ml) and low-risk PrCA groups (2.66 ng/ml).

Figure 6.1 illustrates the means of PSA measures (ng/ml) at each year before exit for all the four groups. Figure 6.2 shows the longitudinal trajectories of PSA over time as obtained by the locally weighted scatter plot smoothing regression (loess). Figure 6.3 shows the individual trajectories for a random sample for the four groups separately using the same loess method. In the three graphs, the x-axis is on a negative scale representing the number of years before exit (or time to exit). The trend in the two PrCA groups shows higher PSA values across all the years of follow-up as compared to the other two groups. Similar to our earlier observations (in the PLCO cohort), men in the normal prostate and the BPH group follow a slightly increasing linear trend. Closer to the exit point, the BPH group shows higher PSA values (that also corresponds to the time of BPH diagnosis). Men in the cancer groups show an increasing trend; a few years before diagnosis the line seems to evince an exponentially increasing pattern. Men in the high-risk PrCA group show a slightly more rapid change as compared to men in the low-risk PrCA group.

Table 6.3 and 6. 4 show the mean of PSA annual change rate estimated by the piece-wise model across different race, age and the four analytic group and adjusted on baseline PSA of 1.73ng/ml. Table 6.3 shows the mean years prior the change point

across the four groups stratified by race while table 6.4 shows the corresponding means after the change point and one year prior to the exit days. As expected, PSA annual rates are similar among men in the high-risk and the low-risk group prior to the change point, post the change point and at 1 year prior to exit; men at the high-risk group have significantly higher PSA annual change rates as compared to all other groups and across all age and race groups. Also, African Americans had significantly higher rates across different groups, both prior to and after the change point.

Table 6.4 illustrates sensitivity and specificity of the previously reported PSA rate thresholds for predicting high-risk prostate cancer (the first cell in each group). In addition, we reported other PSA values that resulted in the best combination of sensitivity and specify in the logistic regression AUC curves. A threshold 0.37 ng/ml that we had previously found using PLCO data had an overall threshold sensitivity of 95.5% and specificity of 85.2%. The predictive values were fairly homogenous across age and race groups. However, unlike what we observed previously, PSA rates performed better among non-African American versus African Americans. An overall threshold of 0.82 ng/ml had both sensitivity and specificity of about 90%.

In a logistic regression a (Table 6.5), increased annual PSA rate at 1 year prior to exit or diagnosis was strongly associated with high-risk PrCA in the model that adjusted for all other covariates including PSA level at the time of last screening. Using a threshold of 0.37 ng/ml/year, the adjusted odds ratio was 71.43 (83.33-85.82) for PSA rate one year prior to diagnosis > 0.375 ng/ml per year versus ≤ 0.375.

The ROC curves and the AUC for a single PSA level (last PSA) alone and the annual PSA rate (1 year prior to diagnosis) alone for all participants are shown in figure

6.3. The ROC curves for comparison show that the PSA rate improved the prediction of high-risk PrCA. The AUC for a single PSA measurement is 89.99 (89.57-90.41) while the AUC for PSA rate is 93.3 (92.86-93.71) the added value of PSA rate above the prediction of a single PSA is 3.29 (2.82-3.76) p-value <.0001.

Discussion and conclusion:

Using a piece-wise mixed model that used more than a hundred thousand PSA values from 22,041 Veterans belonging to one of the four outcome groups - we were able to establish PSA growth curves and estimate individual-level age- and race-adjusted annual PSA rate at 1 year prior to PrCA diagnosis or exit. Then, using a threshold of 0.37 ng/ml/year, we were able to successfully distinguish high-risk PrCA cases from low-risk PrCA, BPH and normal prostate with a sensitivity of 95.5% and a specificity of 86.2 %. Further, when compared to the predictive value of a single most recent PSA, the performance of our model driven PSAV was significantly improved.

The findings supports that the trends of PSA change over time computed using our piece-wise model performs well to predict high-risk PrCA. Even though the sensitivity and specificity of the PLCO data generated cut-off was found to perform less effectively in the VA validation cohort, it still had a relatively good performance. We had found that men with normal prostate had a (predominantly) linear pattern in the PLCO cohort, and this was confirmed in the VA validation cohort. A significantly higher linear rate among men in the BPH group was observed in the VA data as compared to the PLCO data. This might be explained by the fact that data on BPH was self-reported in the PLCO data but not in the VA data. For the cancer groups we had found a clear change

point in the PLCO data, and this was again found in the VA dataset - where the PSA growth pattern shifted from a linear pattern to an exponential pattern. In the PLCO data, the change point took place around 4-5 years prior to diagnosis. However, in the VA data, a change point was evident 2-3 years prior diagnosis. The mean and median of the PSA annual rate prior the change point was slightly higher for both high-risk and low-risk PrCA in the VA compared to the PLCO data. After the change point, the PSA annual rate was significantly higher in the VA data for low-risk PrCA and was the annual PSA rates for low-risk and high-risk PrCA were very close, with high-risk PrCA having higher rates. This explains why the PLCO driven threshold of 0.375 didn't perform as well in the VA cohort as compared to the PLCO cohort. Nevertheless, compared to traditional single value PSA, the threshold of 0.375 ng/ml/year was still able to significantly improve the specificity and sensitivity to detect high-risk PrCA.

Care delivery patterns and the uniqueness of the cared population in the VA may explain why our model-generated PSAV was not able to predict high-risk PrCA with as much accuracy as it did in the PLCO data. The VA cohort is a health-care system-based population that is known to have distinct characteristics such as higher comorbidity burden along with poor health outcomes. In the VA, we found that the predominant number of the PrCA cases were high-risk, compared to only about 20% in the PLCO data. On its face, indicates that the VA population is at higher-than-population-average risk of prostate cancer, while the PLCO population is at lower-than-population-average risk. There are many potential explanations for this. There may be a real tendency toward higher-risk disease for a tendency to delay diagnosis [63, 67, 139]. Also, Veterans are

known to receive some of medical care outside the VA, but then shift most of their care to a VA medical center upon diagnosis of a serious medical condition.

Additionally, because of prior occupational exposures, such as Agent Orange, VA patients may have a different risk-profile compared to the PLCO population. There also could be a difference in data standards, for example, the PLCO data were collected based on strict clinical trial protocols, while the VA data may be more prone to error as the data collection is based on routine clinical data entered manually at various relatively independent medical centers across the United States. The overall length of the study in the PLCO was longer; i.e., 14 years, and there was a significant gap period between the last PSA and the end of follow-up for individuals without PrCA. This was due to the fact that, in the PLCO, active PSA-based screening occurred only in the first 6 years, followed by a long period of passive follow-up. By contrast, in the VA because Veterans were encouraged to receive PSA screening annually. This resulted in a much shorter gap between end of follow-up and last PSA as compared to PLCO. This interval may have slightly biased the PLCO results away from the null. This is because the calculated PSA rate for men with no cancer was driven from PSA measures taken several years before the end of the study; while for men with PrCA more recent measures were naturally available. Third, while the same definition of low-risk PrCA was applied in both studies, low-risk PrCA in the PLCO study were predominantly at the lower end of the of the case definition (TNM stage 1 and Gleason score of less than 6), the VA cases were likely to be at the higher end (TNM stage 2B and Gleason score of 6) .

In addition to our findings that serial PSA based measures, when appropriately modeled, can predict high-risk PrCA, recently, two other independent research have

reported similar results [18, 19, 138]. Wallner et al. used routine clinical care data from 219,388 members of the Kaiser Permanente Health Plan in Southern California (1998 - 2008). In that study, PSAV accurately predicted PrCA cases, with higher specificity and sensitivity for high-grade PrCA when compared to single PSA test. Similarly, Orsted et al. used data from 7,455 participants in the Copenhagen City Heart Study. Their findings indicated that long-term PSA change may be used to identify men with a low probability of PrCA mortality [140]. Although the overarching message appears to be similar, each study was methodological different, mainly because of heterogeneity in defining and estimating PSA change/PSAV, the number of PSA tests to use, and the time interval between the tests.

The main limitation of this is study is information bias. In biomarker-based studies, a new indicator is compared to what is considered a "gold standard" criteria or the "truth". We used raw oncology files extracted from the VA electronic medical record to classify high-risk and low-risk PrCA. The definition was based on TNM staging and Gleason score documented through different data entry patterns at the point of source that may have led to errors – potentially leading to misclassification. We tried to minimize this bias by restricting our analysis to those with clear, unambiguous information on confirmed stage – it is possible that patients with high risk PrCA are more likely to have higher quality unambiguous data compared to lower risk PrCA. As this is a retrospective analysis, confounding cannot be eliminated. There always is a possibility that the differences we observed in the PSA rates among the four groups are confounded by unknown and unmeasured factors.

In this study, we have applied our previously developed statistical model on an independent and distinct validation cohort. The results of our findings in the validation cohort were comparable to that obtained in the model-building cohort. This evidence of robustness of our original model supports the conclusion that it is possible to use serial measurements of PSA to differentiate, with a high degree of precision, if a patient is likely to develop high-risk PrCA. Patients with suspected high-risk PrCA are the ideal candidates for prostate biopsies as confirmation of PrCA and subsequent treatment may improve the outcomes of these patients. At the same time, our model will be able to avoid unnecessary biopsies among patients who may have a single elevated PSA that is not reflective of high-risk PrCA. This has important public health and policy implications. Next steps will involve further validation in an alternate dataset and subsequent prospective cohort study.

Tables and figure

Table 6.1 Age, Body mass index (BMI) and race by analysis group (n=22, 041)

	Normal prostate 7,347	BPH 7,347	LRC 3,032	HRC 4,315	Comparison				
Race					HRC vs. normal	HRC vs. BPH	HRC vs. LRC	HRC vs. others	PrCA vs. None-PrCA
African American 3,252 (14.75)	861 (11.72)	744(10.13)	659(21.73)	988(22.90)	<0.001	<0.001	0.23	<0.001	<0.001
Others 18,789 (85.25)	6,486 (88.28)	6,603(89.87)	2,373(78.27)	3,327(77.10)					
BMI (kg/m^2)					0.25	0.7	0.00	0.78	0.01

<= 30 kg/m^2 12,303(55.82)	4,006(54.53)	4,106(55.89)	1,791(59.07)	2,400(55.62)					
>30 kg/m^2 9,738(44.18)	3,341(45.47)	3,241(44.11)	1,241(40.93)	1,915(44.38)					
Age (years)									
<= 55 5,088 (22.72)	2,247(27.20)	1,601(21.79)	655 (21.60)	816(18.91)					
55-65 11,621 (52.72)	4,195(50.80)	3,696(50.31)	1,809(59.66)	2,352(54.51)	<0.001	<0.001	<0.001	<0.001	<0.001
>65 5,412 (24.55)	1817(22.00)	2,050(27.90)	568(18.73)	1,147(26.58)					

Table 6.2. Mean and median of follow up duration, number of PSA tests, and PSA at baseline by analysis group (n=22, 041)

	Men with no cancer 7,347	Men with BPH 7,347	Men with LRC 3,032	Men with HRC 4,315
	Mean (95% CI)/Median	Mean (95% CI)/Median	Mean (95% CI)/Median	Mean (95% CI)/Median
Years of follow up	6.93(6.89,6.96)/6.93	6.01(5.98,6.05)/5.82	5.79(5.73,5.84)/5.53	5.89(5.84,5.93)/5.66
Years from last PSA to exit	0.74(0.72,0.76)/0.50	0.39(0.37,0.40)/0.13	0.21(0.20,0.22)/0.16	0.20(0.19,0.21)/0.15
Number of PSA tests	6.54(6.49,6.60)/6.00	6.32(6.26,6.37)/6.00	7.62(7.52,7.73)/7.00	7.47(7.38,7.55)/7.00
PSA at baseline (ng/ml)	1.09(1.07,1.12)/0.80	1.38(1.35,1.41)/1.00	2.82(2.76,2.88)/2.50	2.66(2.61,2.71)/2.30

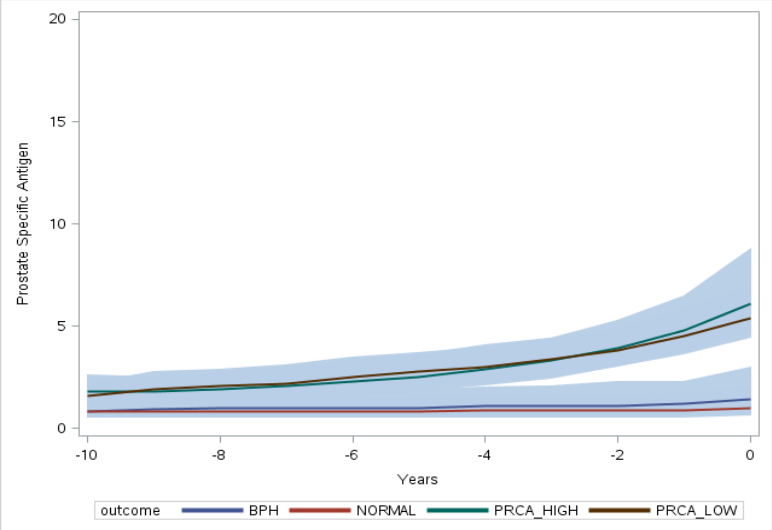


Figure 6.1 Mean and inter-quartile range of PSA values over years of follow up by analysis group

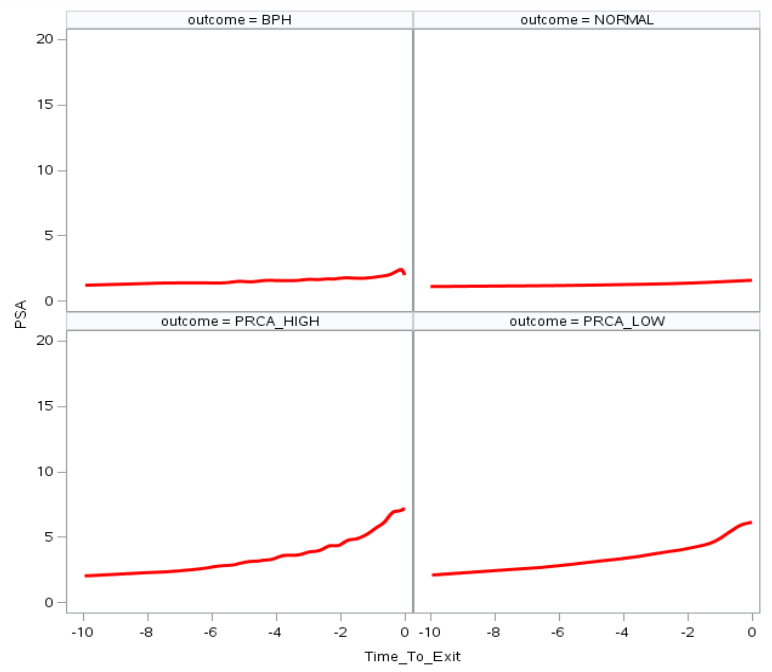


Figure 6.2 Longitudinal trajectories of PSA for the analytical cohort as a function of time in the 4 analytical groups

Table 6.3 Estimated Annual PSA rate of change prior to the change point stratified by race, age and analysis groups and fixed at baseline PSA of 1.73ng/ml

Race	Age	Normal prostate (7,347)	BPH (7,347)	LRC (3,032)	HRC (4,315)
Non-African Americans	<=55	0.05 (0.03, 0.05)	0.08 (0.05, 0.08)	0.23 (0.2, 0.24)	0.24 (0.21,0.25)
	55-65	0.06 (0.04, 0.07)	0.09 (0.06, 0.10)	0.24 (0.21,0.26)	0.25 (0.22,0.27)
	>65	0.06 (0.04, 0.07)	0.09 (0.06,0.10)	0.24 (0.21, 0.26)	0.25 (0.22,0.27)
African American	<=55	0.10 (0.07, 0.10)	0.13 (0.09, 0.13)	0.28 (0.24,29)	0.29 (0.25, 0.30)
	55-65	0.11 (0.08, 0.12)	0.14 (0.10, 0.15)	0.29 (0.25,0.31)	0.30 (0.26,0.32)
	>65	0.10 (0.07,0.10)	0.14 (0.10, 0.15)	0.29 (0.25, 0.31)	0.30 (0.26,0.32)

Table 6.4 Estimated Annual PSA rate (ng/ml/year) of 1 year prior to exit stratified by race, age and analysis groups and fixed at baseline PSA of 1.73 ng/ml

Race	Age(years)	Normal prostate (7,347)	BPH (7,347)	LRC (3,032)	HRC (4,315)
Non-African Americans	<=55	0.05 (0.03, 0.05)	0.08 (0.05, 0.08)	1.38 (1.36,1.38)	2.55 (2.41, 2.57)

	55-65	0.06 (0.04, 0.07)	0.09 (0.06, 0.10)	1.24 (1.17,1.30)	2.26 (2.06, 2.43)
	>65	0.06 (0.04, 0.07)	0.09 (0.06,0.10)	1.27 (1.21,1.34)	2.33 (2.12, 2.5)
African American	<=55	0.10 (0.07, 0.10)	0.13 (0.09, 0.13)	1.62(1.55,1.70)	3.02 (2.78, 3.21)
	55-65	0.11 (0.08, 0.12)	0.14 (0.10, 0.15)	1.46 (1.34,1.61)	2.69 (2.38, 304)
	>65	0.10 (0.07,0.10)	0.14 (0.10, 0.15)	1.49 (1.38,1.66)	2.76 (2.44, 3.13)

Table 6.5 Measurements of test performance for the prediction of high-risk prostate cancer by for PLCO driven and other selected anneal PSA rate thresholds , stratified by race and age

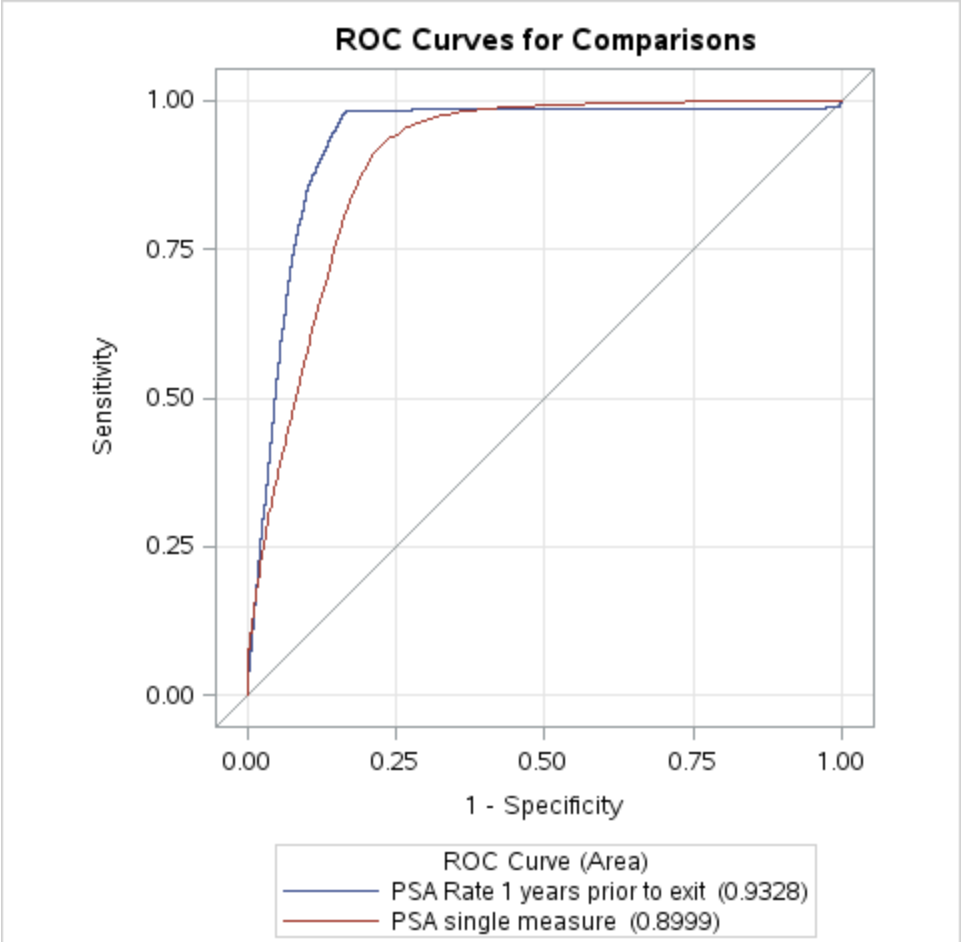
	PSA rate cut off (ng/ml/year)	Sensitivity	Specificity	True +VE	True –VE	False +VE	False -VE
African American	1.20	89.0%	80%	874	1786	446	108
	0.99	89.1%	80.0%	857	1785	447	107
Non-African Americans	0.37	95.5%	86.7%	3081	13345	2043	145
	0.80	90%	89.3%	2886	13747	1641	340

age≤55 years	0.88	89.6%	88.9%	708	3703	461	82
	0.55	95.0%	86.7%	750	3612	553	40
55-65 years	0.33	95.6%	83.0%	2188	7644	1564	102
	0.77	90.0%	86.1%	2059	7929	1279	230
Age >65 years	0.90	89.0	91.1	1005	3871	376	124
	0.42	95.0%	88.8	1072	3771	476	57
	067	92%	90%	1038	3821	426	91
Overall Ages	**0.37**	95.5%	85.2%	4018	15018	2602	190
	0.82	90%	89.0%	3773	15499	2121	435

Table 6.6 Results from a logistic model predicting high-risk prostate cancer

Factor	Odds ratio (95% CI)	P-VALUE
PSA annual rate*	71.43 (58.82-83.33)	<.001

Last PSA single measure** (ng/ml)	1.22 (1.20-1.23)	<.001
Age at baseline**	1.03 (1.02-1.04)	<.001
Race***	1.12 (0.99-1.27)	0.06
Body mass index (kg/m2)****	0.97 (0.91-1.03)	0.32
*used as a dichotomous variable, ≥0.375 ng/ml/year versus <0.375ng/ml/year **used as continues variable *** used as African American versus others *** used as a dichotomous variable, <30 kg/m^2 versus ≥ 30kg/m^2		



ROC area for PSA rate = 93.3 (92.86-93.71) , ROC area for PSA single

Figure6.3. ROC curves for PSA rate and single PSA measure (last PSA):

CHAPTER 7: SUMMARY

This chapter consists of two sections. The first is an overall summary of our scientific query and the interpretation of our results in the overall context of prostate cancer prevention and control. In the second I will shed the light on the personal experience that shaped the journey of perusing this research over the last four years of my life.

7.1. PSA growth curves: an approach for PrCA screening

Prostate cancer (PrCA) screening is a significant public health issue, especially among populations at high risk of an aggressive PrCA. The development and discovery of biomarkers to predict risk of PrCA aggressiveness at the time of detection remains an unmet clinical need in prostate cancer prevention and control [36]. This dissertation analyzed a very large and robust dataset consisting of existing repeated measures of prostatic specific antigen (PSA). Its role was to develop and validate a tool to improve both sensitivity and specificity of the PSA-based screening test to detect PrCA, while, for the first time, differentiating high-risk PrCA from all other prostatic conditions (including indolent PrCA). Using this dataset [from the Prostate Lung Colorectal and Ovarian trial data (PLCO) trial] and an even larger confirmatory/validation dataset through the Veterans Administration, we showed that there is real potential to improve screening specificity for high-risk PrCA through investigating PSA trends over time.

We used the PLCO trial data for PSA growth model building. Using 6 years of annual PSA measurements we established the PSA growth curves for four groups of men; those who developed high-risk PrCA, those who developed low-risk PrCA, those who developed benign prostatic hyperplasia (BPH) and those who were not diagnosed with either PrCA or BPH until the end of the study period. We used these curves to estimate PSA annual rate of change (defined as an absolute and relative change) at pre-specified time points; one and two years before diagnosis for each individual in the cohort. We then used the area under the curve (AUC) method to estimate the specificity and the sensitivity of the prediction of high-risk PrCA using selected PSA annual rate thresholds. After developing the model using the PLCO data, which produced excellent results, we validated our work using the VA data - where we replicated the work done in the PLCO data to estimate PSA growth curves equation in the VA cohort. During this process we further tuned the original model. We then used model derived individual patient curves to estimate individual PSA annual rate at one year prior to diagnosis. Finally, we used the PSA annual rate thresholds derived from the PLCO data analysis to predict high-risk PrCA among the VA cohort.

We built our statistical methods to model the relation between PSA and time/age (PSA growth curves) based on *a priori* knowledge of current consensus evidence that PSA increases with age/time and that this increase (or growth pattern) is linear among patients with normal prostate tissue and becomes non-linear among patients who develop low-risk PrCA and high-risk PrCA [16]. We built on and validated past findings that the pattern of transition from linear PSA growth to a non-linear growth is a harbinger to

high-risk PrCA; i.e., the pattern of transition (rate and curve) is different between high-risk PrCA and low-risk PrCA. To account for this difference in patterns and to estimate individual growth model parameters, we used a non-linear mixed-effect model. These models are very effective in allowing non-linear functions that take into account random effects that, in turn, allow for individual-to-individual variation. In the select models piece-wise function we specified that every individual may have an unknown point in time at which they transition from a linear to an exponential phase. While building and validating these models we accounted for baseline age, race, BMI and initial PSA. We accounted for these variables by including them as source of variation on the intercept and on the slope over both the linear and the exponential phase.

Our results show that PSA annual change rates varied significantly by cancer status (i.e., both in distinguishing PrCA from other conditions and in differentiating virulent PrCA from indolent PrCA) in both cohorts. The differences between the means and medians of PSA rate values across the 4 groups of men (high-risk PrCA, low-risk PrCA, BPH and healthy men) were large and robust across different estimation methods. This is an observation that has been replicates over 20 years of research on PSA kinetics. Nevertheless, what is more important about our findings is that the distribution of the individual PSA annual rates shows substantial variability. A distinct range and significantly higher values were observed among men who developed high-risk PrCA – starting many years prior to diagnosis (versus all others). This resulted in a substantially higher area under the curve; 99.50 (99.34-99.66), in a logistic regression model that used these individual PSA annual rates to predict high-risk PrCA among the PLCO cohort. A threshold of 0.37ng/ml/year had the best combination of sensitivity and specificity; i.e.,

of 97.2%, and 97.3% respectively. In the VA validation cohort, the same pattern was observed. However, men in the low-risk PrCA group had higher annual PSA rates as compared to the same group in the PLCO cohort. This resulted in a lower area under the curve of 93.3 (92.86-93.71) in the logistic regression model and the same threshold of 0.37ng/ml/year predicted high-risk PrCA with a sensitivity of 95.5% and a specificity of 86.2 %. In both cohorts, when compared to the predictive value of a single most recent PSA, the performance of our model driven PSA rate was significantly improved.

Using non-linear mixed models we were able to detect substantial differences in PSA rates among people who developed high-risk cancer and those who did not. The method and the design we adapted is in agreement with the main concept of PSA velocity. As described by Carter [108], velocity (rate) here is not merely the random fluctuation of PSA values across different measuring time (by chance) or across different characteristics such as age, race and BMI. Rather, it is the variability corrected for elapsed time between measurements and for other sources of variability [108]. It is important to note that the PSA rates and the thresholds calculated and reported here may be specific to underlying piece-wise function that we used to establish the growth curves. Thus, we caution that the thresholds reported here to identify high-risk PrCA should not be applied to PSA annual rates or velocity driven by other methods.

The PSA growth curves can be further developed and populated to be used as a true bench-to-bed-side tool: indeed, this would be a research product that combines both bench and applied research. Such a tool would be different from currently available PSA change nomograms (PSA doubling or regression algorithms) – as these traditional tools rely completely on a linear PSA increase assumption and mostly use only two PSA

measurement points. Few studies have modeled PSA growth curves using linear and exponential equations to represent change over time with an acceleration of growth at some point prior to disease onset – but were based on small sample size, lacked sufficient geographical and racial variability along with generalizability, and were not translated to an outcome appropriate to applied research [73]. The traditional measures of PSA, although sometimes applied for PSA screening – were originally developed for post-treatment monitoring of PrCA recurrence. As such, they have not been validated for this new purpose. In contrast, our approach was designed to develop a tool exclusively for PSA-based PrCA screening so as to facilitate shared decision making regarding the need for prostate biopsy between physician and patient – by providing information on prior probability of finding high-risk PrCA.

There are some limitations that should be considered when interpreting our results. First, this work is based on retrospective data and is prone to design-inherent biases. Misclassification is possible; ideally the outcome status is confirmed by a prostatic biopsy, which confirms the existence or the absence of a tumor. However, it is impossible to biopsy every participant, and thus those who were classified in the non-PrCA group might - in reality - have PrCA. This is more of an issue in the VA data rather than in the PLCO data. While not every participant was biopsied to confirm the absence of a tumor in the PLCO study, everyone was screened and closely followed. The VA analysis is based on routine clinical data entered manually at various relatively independent medical centers across the united the states. Misclassification in both cases would represent random error and is likely to attenuate our results. Misclassification could exist among men with a BPH diagnosis, specifically in the PLCO trial where BPH

is not one of the study's main outcomes. Data on BPH was self-reported and collected retrospectively. It is possible that some of the men in the normal prostate group had BPH or *vice versa*. This source of error does not influence our sensitivity and specificity results for distinguishing high-risk PrCA. However, the reported PSA growth curves for men with BPH should be interpreted with caution. Second, selection bias also is possible. In both cohorts baseline PSA measures were slightly higher than what would be expected in the general population. In the VA cohort, the proportion of high-risk cancer was much higher than what we expected or what was observed in the PLCO cohort. These observations indicate that the two cohorts might be different in terms of health status than that of the general population. Third, the scope of this research is establishing the PSA growth curves as they best fits the observed long-term change of PSA over time using at least 4 measures of PSA taken over long periods of time. Thus the derived PSA annual change rates thresholds may not be applicable to other methods defining and estimating PSA velocity. Fourth, in PLCO, where we established our models, the representation of African-America race was limited. By contrast, in the VA analysis there not only was a significant representation of African Americans, but also there was important variability compared to other races with regard to PSA annual rates and their predictive values. Further research is needed to investigate the influence of race in applying the concept of PSA kinetics. Finally, confounding cannot be eliminated. There is a possibility that the differences we observed in the PSA rates among the four groups are confounded by unknown and unmeasured factors.

These findings do not confirm the absolute effectiveness of PSA-based screening using the concept of PSA velocity; rather they shed the light on the potential use of PSA kinetics to distinguish high-risk PrCA with a high degree of precision; this is an important addition to the growing evidence that supports this concept. Patients with suspected high-risk PrCA are the ideal candidates for early detection and subsequent treatment. At the same time, this approach is able to avoid unnecessary biopsies among patients who may have a single elevated PSA that is not reflective of high-risk PrCA. More research is required to refine and validate a decision algorithm incorporating PSA serial measurements in addition to other factors such as digital rectal examination results and other non PSA biomarkers.

7.2. The journey of this dissertation

I joined the doctorate program in epidemiology and biostatistics at the University of South Carolina in fall 2011, as a Fulbright-sponsored scholar all the way from the West Bank/ Palestine. As he was helping me pack for the long-journey away from home, my father asked if I will help "cure" diabetes. I smiled and said "No, I will help prevent cancer." He smiled back. At that time, I don't think I was dreaming of preventing cancer – I think I was dreaming of crossing the Mediterranean, Europe and the Atlantic – more occupied by the excitement and challenges that lay ahead – but the seed for cancer research was there somewhere. At the University of South Carolina orientation, I met Dr. Susan Steck. I told her about this seed inside me for cancer epidemiology, and she introduced me to my mentor, Dr. James Hebert. Our 1st meeting was in that 1st week of my life in Columbia, SC was amazing, I knew I wanted to work with him for the next many years – I knew who would help me with my PhD dissertation.

A month after our first meeting, Dr. Hebert asked me to join him at a urology patient group meeting where he gave a talk about Prostate Cancer screening. This was a follow-up to the recommendation by United States Preventive Services Task Force (USPSTF) against routine population level screening of PrCA using PSA. I clearly remember a wide conference room occupied with middle aged men, mostly of color, expressing their disapproval of the new recommendation. Here my mentor displayed a colorful world map to a captive audience showing an inverse relationship between PrCA screening and PrCA mortality, there were a lot of concerned faces in the audience. As he finished his talk, Dr. Hebert was asked the same question many times in several ways -- "*what is the alternative*?" "*What do we do now?" "Do we just not know until it's too late*?" In every question there was a story of PrCA screening saving someone's life – a brother, a husband, a father or even their own life. I listened as my mentor, tried hard to explain that the result of the recent randomized trial is not an invalidation of their own life stories but rather a bigger absolute perspective of scientific research that may apply for many but not for all. We left the building with a thought that occupied my mind for many months to come; "*what can we do*?"

I am not sure when Dr. Hebert shared the idea of PSA kinetics as a potential solution, but I think it was a seed he planned during our drive back from this meeting. He asked me to meet another graduate student Dr. Gowtham Rao, "*he is a very sharp student from India, he is doing his research using the VA data*" he said "a *few months ago he wrote a grant proposing to improve PSA based screening using serial measure of PSA, you should discuss the idea with him*". I had coincidently met Gowtham – and we had talked about the idea he had written as part of his PhD dissertation under Dr. Hebert. His

enthusiasm, and Dr. Hebert's trust grew my interest in investigating PSA kinetics, in one year, under the supervision of my mentor and my doctorate committee and with prodigious assistance from Gowtham, the research scope, question and design took a shape – leading to my doctorate dissertation proposal.

I proposed my dissertation in May 2013, that day I walked out of the room fully confident of the idea, of my understanding of the problem, and of my familiarity with the statistical methods to be used. In the course of the nineteen months that followed, my confidence level momentously fluctuated. As I pulled through different challenges, I came to realize two main facts about the work we do; first, your knowledge and skills at the beginning of the research work is only the onset of an incremental accumulation of deeper and broader comprehension of the problem in hand. As you go back and forth between your data and the findings of others in the field, you realize how little you knew when you first started. Second, the heart of any research project is the research question. We do everything to better answer a specific scientific query. But the reality is that there is no single research work – not even a randomized control trail – that can fully answer any research question once and for all. The more answers your results indicate, the more questions are to be asked. These two facts combined, make the finish line of a research project hazy, but as one of my mentors told me once *"a good dissertation is a complete dissertation;"* that is precisely why a doctorate degree is not the end of the journey. It is rather the starting point.

In the last four years I learned that your mentors are the ones who make or break your success. I was blessed by mentors back home that are way ahead of their time. They are the reason I started this journey in the 1st place. It is hard to believe how progressive,

scientifically oriented, and influential they are. Their lives are series of success stories of bringing change. In a place like Palestine where scientific research (especially in the field of public health) is not merely an academic process, but it is a systematic approach of documenting and analyzing the political, social and economic injustice that we collectively face and that shape our health outcomes. Because of them, I came here with sufficient baseline training and extremely open mind set to admire science and peruse knowledge, I came here ready to learn and grow. I was blessed again with my mentorship here at the University of South Carolina. I still wonder if it was a matter of pure luck. My mentor Dr. James Hebert, inspired, motivated, supervised, and carefully oriented this dissertation and my whole learning journey. Everything about working with him is exciting; there were times that I really wanted to drop this whole idea of PSA kinetics. Fitting the appropriate mathematical model, figuring out the details of the statistical method and its interpretation were extremely challenging. Each time that I got close to giving up, he faced me with creative solutions and profound excitement about the idea which kept me going. He provided unlimited support every step on the way. He inspired the idea, gave me full lead and ownership, trusted my work and only took over when I needed him to. He has unlimited energy, he thinks big and acts big. This kind of mentorship makes any dissertation project very stimulating and valuable.

I also learned that no matter how smart you are, good work is always a reflection of team work. Fellow students are a great resource to solve many problems along the way. I learned to be always ready to help my colleagues, as there will be a time when I will need their help. They are also the main supporting system, because we all go through this journey, we can easily relate to each other's obstacles. I learned that over a four-

year journey, both my strengths and limitations will surface. It is not wise to mask your limitations to your team or your supervisors. They both need to know, because only they can help you avoid irreversible mistakes. In a fair environment – even when highly competitive – being honest, taking responsibility, and working hard to avoid and correct mistakes do pay off.

My biggest lesson of all is that there is a dynamic relationship between stress, momentum, productivity and quality of the resulting work. The biggest challenge in working toward a PhD is to find the balance between stress and productivity that will keep the momentum and produce good quality of work at the right time. The structure of our program of study is fairly lucid, but every dissertation has its own circumstances and time-limiting steps. One of my fellow students once told me "*doing a PhD feels like being lost in a desert*", this is fairly true. The first eight months after I proposed the dissertation idea, time passed fast and I felt like I accomplished nothing. My productivity was going down and at one point I felt like I lost the momentum. I was struggling with the statistical methods, learning the details was a very slow process and tangible results seemed so far away. I also was easily distracted, and gave other work priority over my dissertation efforts, a pattern commonly described by PhD students. When I realized that I was falling behind my timeline, the stress started to push my productivity, results started to emerge and I gained back my momentum. I was about to catch up with my timeline when I was faced with the biggest challenge of my dissertation work; data access problems. My access to the VA network was disabled, the data source for the third aim of my dissertation proposal. Ironically, I had gained access back in 2011 and used the VA data for all of the preliminary analysis. At that critical time, I lost all my access privileges

and the IRB approval for this particular research was closed. This was a problem that I did not anticipate and, at that point, I had no time to lose. Still, I had to patiently wait almost two months to regain access to the data. With such stress, we stretch our productivity above our limits but we also increase the possibility of irreversible mistakes. I eventually learned that if I had maintained even momentum throughout the four years, I could have adhered to my timeline and thus avoided much of the time stress that was created at the end. Delays in data access and other problems are common; the implication can be minimized by proper time management. The challenge is to find this optimum level of stress that will keep you focused on your dissertation project, maintain your productivity but does not jeopardize the quality of your work.

In December 2014 (one week from today) I am to defend the results of my dissertation work. From the day my father helped me packing until today, many things happened. I did not cure Diabetes nor did I prevent Cancer, but I overcame many of the challenges – those I anticipated and many others. I was stretched beyond of what I thought were my limits. I was exposed to opposite perceptions of mine; all of that contributed to an exponential growth of my character, my values, my academic knowledge and skills and more importantly to my perception of health and wellbeing. At the end of this stage of my training, and at the starting point of my research journey I am fully committed to use what I learned and will further learn to help preventing and controlling Cancer.

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APPENDIX A-DESCRIPTION OF THE STATISTICAL MODELS

1. A. The initial linear-exponential piecewise PSA model used an interaction term between the group type and time. To account for individual level natural heterogeneity in the rate of growth, the transition point and the intercept in each group, we included random effects for their corresponding estimates. The full mixed-effect model for the data can be written as:

$$PSA_{ij} = \begin{cases} \beta_0 + (\beta_g * G) + (\beta_c * C) + b_i + [(\beta_t + \beta_{tg} * G + \beta_{tc} * C + b_{ti}) \\ \quad * ((CP + \beta_{cpg} * G + b_{cpi}) - x)], \ \ x < cp \\ (\beta_0 + (\beta_c * C) + (\beta_g * G) + b_i) * \\ e^{(\beta_{t2} + \beta_{t2g} * G + \beta_{t2c} * C + b_{t2i}) * (CP + \beta_{cpg} * G + b_{cpi} - x)} \end{cases}$$

Where PSA_{IJ} is the PSA measure for i[th] individual j[th] occasion.

Coefficients of the linear phase:

β_0 is the value of PSA at the transition between linear and exponential phase

b_i is the random effect for β_0

β_g is the coefficient corresponding to the group effect

G is a categorical indicator of the group, and is replaced in the model by 2 binary dummy variables as follow: $\begin{cases} g_1 = 1 \ for \ Low - risk \ \ prostate \ cancer \ , else \ g_1 = o, \\ g_2 = 1 \ for \ High - risk \ prostate \ cancer \ , else \ g_2 = 0, \end{cases}$

β_c is a vector of the coefficients corresponding to the effect of the set of covariates

C is a matrix representing the individual covariate values

β_t is the linear coefficient corresponding to the effect of time, i.e. the linear rate of change

β_{tg} is the coefficient corresponding to the effect of the group on the linear rate of change; i.e., interaction between time and group

β_{tc} is the coefficient corresponding to the effect of covariates on the linear rate of change, i.e. interaction between time and covariates

b_{ti} is the random effect on β_t

X is time (years) before exit/diagnosis

CP is the change point (inflection point) between linear and exponential phase

β_{cpg} is the coefficient corresponding to the effect of group on the change point

b_{cpi} is the random effect on cp

Coefficients of the exponential phase:

β_{t2} is the exponential rate constant during the exponential PSA phase

β_{t2g} is the coefficient corresponding to the effect of group on the exponentia l rate constant i.e., interaction between time and group in phase 2

β_{t2c} is a vector of coefficients corresponding to the effect of covariats on the exponential rate constant, ie interaction between time and covariates in the second stage

b_{t2i} is the random effect on β_{t2}

1. B. The reduced linear-exponential piecewise model (allowing a transition to an exponential phase among the cancer groups only) estimates average and individual PSAV as ng/ml/year per group while adjusting for baseline age , BMI (kg/m^2), PSA measure (ng/ml) and race [African American (AA) versus others]. We included an interaction

term between all of these variables and time. The reduced mixed-effect model can be simplified to :

$$PSA_{IJ} = \begin{cases} \beta_0^* + b_i + \beta_t^* + b_{ti} * (cp - x), x < cp \; and \; for \; no \; cancer \; group \\ \beta_0^* * e^{(\beta_{t2}^* + b_{t2i}) * (cp^* + b_{cpi} - x)}, x \geq cp \end{cases}$$

Where the set of (β_0^*, β_t^*, β_{t2}^* , cp^*) is adjusted for group and effect of age, BMI (kg/m^2), PSA measure (ng/ml) , and race (AA versus others).

β_0^*: $PSA \; at \; the \; trsition \; point \; for \; cancer \; groups \; and \; at \; exit \; for \; no \; cancer \; group$

b_i : $random \; effect \; on \; \beta_0^*$

β_t^* : $linear \; time \; coefficient$

b_{ti}: $random \; effect \; on \; \beta_t^*$

β_{t2}^*: $exponential \; time \; coefficient$

b_{t2i}: $random \; effect \; on \; \beta_{t2}^*$

cp^*: $is \; the \; change \; point$ between linear and exponential phase

b_{cpi} : $random \; effect \; on \; CP$

2. A. The full mixed-effect model for log PSA :

$$Log \; (PSA + 1)$$

$$= \begin{cases} \beta_0 + (\beta_g * G) + (\beta_c * C) + b_i + \begin{bmatrix} (\beta_t + \beta_{tg} * G + \beta_{tc} * C + b_{ti}) * \\ \left((CP + \beta_{cpg} * G + b_{cpi}) - x\right) \end{bmatrix}, x < cp \\ \beta_0 + (\beta_c * C) + (\beta_g * G) + b_i + [(\beta_{t2} + \beta_{t2g} * G + \beta_{t2c} * C + b_{t2i}) * \\ \left((CP + \beta_{cpg} * G + b_{cpi}) - x\right)], x \geq cp \end{cases}$$

Where

β_0 is the value of log(PSA)at the trasition between the 1st and the 2nd linear phase

β_g *is the coefficient corresponding to the patinet group effect on* β_0

G is a categorical indicator of the group, and is replaced in the model by 2 binary dummy variables as follow: $\begin{cases} g_1 = 1 \; for \; Low - risk \;\; prostate \; cancer \; , else \; g_1 = o, \\ g_2 = 1 \; for \; High - risk \; prostate \; cancer \; , else \; g_2 = 0, \end{cases}$

β_c*is a vector of* coefficient corresponding *the effect of the set of* covariate on β_0

C is a matrix representing the individual covariate values

b_i *is the random effect for* β_0

β_t is the 1st phase linear coefficient, i. e. the linear rate of change at the 1st phase

β_{tg} *is the coefficient corresponding to the effect of group on the* 1*st linear rate of change ie. interaction between time and group at the* 1*st phase*

β_{tc} *is the coefficient corresponding to the effect of covariatts on the* 1*st linear rate of change*

b_{ti} *is the random effect on* β_t

X *is time* $(years)$*before exit/diagnosis*

CP *is the change point* between the 1st and the 2nd linear phases

β_{cpg} *is the coefficient corresponding to the effect of group on the change point*

b_{cpi} *is the random effect on cp*

<u>Coefficients of the 2nd phase:</u>

β_{t2} is the difference in rate of change between the 1st and the 2nd phase

β_{t2g} *is the coefficient corresponding to the effect of group on* β_{t2} *ie. interaction between time and group in phase* 2

β_{t2c} *is is a vector of coefficients corresponding to the effect of covariats*

$on\ \beta_{t2}$

$b_{t2i}\ is\ the\ random\ effect\ on\ \beta_{t2}$

Based on this model, the rate of change at the second phase is the addition of β_t and β_{t2}

2.B. The reduced mixed-effect model for log PSA :

$$PSA_{IJ} = \begin{cases} \beta_0^* + b_i + \beta_t^* + b_{ti} * (cp - x), & x < cp\ and\ for\ no\ cancer\ group \\ \beta_0^* + (\beta_{t2}^* + b_{t2i}) * (cp^* + b_{icp} - x), & x \geq cp \end{cases}$$

Where the set of (β_0^*, β_t^*, β_{t2}^* , cp^*) is adjusted for group and all other coverlets effect.

β_0^*: log(PSA) $at\ the\ trsition\ point$ b_i : $random\ effect\ on\ \beta_0^*$

β_t^* : $linear\ time\ coeffcient$ b_{ti}: $random\ effect\ on\ \beta_t^*$

β_{t2}^*: $exponential\ time\ coeffcient$ b_{t2i}: $random\ effect\ on\ \beta_{t2}^*$

cp^*: $is\ the\ chamge\ point$ between linear and exponential phase b_{icp} :

$random\ effect\ on\ CP$