Prostate Specific Antigen Dynamics and Prostate Cancer Risk:

A Population-Based Study

by

Amanda Elizabeth Hird MD

A thesis submitted in conformity with the requirements for the degree of:

Master of Science

in

Clinical Epidemiology and Health Care Research
Institute of Health Policy, Management, and Evaluation
University of Toronto

2021

Toronto, Ontario

Prostate Specific Antigen Dynamics and Prostate Cancer Risk: A Population-Based Study

Amanda Elizabeth Hird MD

Master of Science in Clinical Epidemiology and Health Care Research

Institute of Health Policy, Management, and Evaluation

University of Toronto

2021

Abstract

The objective was to determine the association between prostate specific antigen (PSA) predictors and prostate cancer diagnosis. This was a retrospective, unmatched nested case-control study of men 40-75 years of age undergoing opportunistic PSA testing. PSA levels of patients diagnosed with prostate cancer were compared to patients who were not using logistic regression analysis. Overall, 508,238 patients were included (12,444 cases and 495,794 controls) over a median 8.2 years (IQR: 7.0-9.1). First PSA, final PSA, and percentage change in PSA per 365 days were highly predictive of prostate cancer. The final multivariable model preformed well when predicting any prostate cancer and clinically significant prostate cancer diagnosis. Together, the identified variables can be used to better select those who should undergo prostate biopsy. Due to inherit selection and verification bias, prospective validation studies are needed. Further studies are needed to determine the association with metastatic and lethal prostate cancer.

Keywords: prostatic neoplasms, prostate cancer, prostate-specific antigen, baseline prostate-specific antigen, risk assessment

Acknowledgements

There are many people who have had a significant influence on my academic journey. I am grateful for the advice, expertise, and support from all of these individuals. They provided the inspiration to pursue and the support to complete this dissertation.

I would like to specifically thank my husband, Dr. David Parente, for his encouragement. He has been a reliable sounding board, offering unwavering support and reassurance.

I would like to thank my Supervisor, Dr. Robert Nam. As a constant source of support, enthusiasm and inspiration, his mentorship has helped to shape me into a successful clinician and academic researcher.

To Drs. Kulkarni and Del Giudice, who generously gave of their time to guide me through the design and conduct of this project.

To Refik Saskin, who provided exceptional statistical and methodological guidance throughout this process.

Finally, I would like to thank my colleagues, Drs. Diana Magee and Douglas Cheung. Our “think tank” has not only been a source of camaraderie and friendship, but also academic support and growth.

Table of Contents

List of Tables

List of Figures

List of Appendices

Nomenclature

5-alpha reductase inhibitor (5-ARI)

Aggregate disease group comorbidity score from the Johns Hopkins system (ADG)

Akaike Information Criterion (AIC)

Area under the curve (AUC)

Bayesian Information Criterion (BIC)

Confidence interval (CI)

Canadian Institute for Health Information (CIHI)

Canadian Task Force on Preventative Health Care (CTFPHC)

Canadian Urological Association (CUA)

Discharge Abstract Database (DAD)

Digital rectal exam (DRE)

European Randomized Study of Screening for Prostate Cancer (ERSPC) trial

Federal Drug Administration (FDA)

International Society of Urological Pathology (ISUP)

Interquartile range (IQR)

Local health integration network (LHIN)

Magnetic resonance imaging (MRI)

Memorial Sloan Kettering Cancer Center (MSKCC)

National Ambulatory Care Reporting System (NACRS)

Number needed to diagnose (NND)

Number needed to screen (NNS)

Odds ratio (OR)

Ontario Cancer Registry (OCR)

Ontario Laboratory Information System (OLIS)

Ontario Health Insurance Plan (OHIP)

Ontario Registrar General – Death (ORGD)

Positive predictive value (PPV)

Prostate, Lung, Colorectal, and Ovarian (PLCO) screening trial

Prostate Cancer Prevention Trial (PCPT)

Prostate Health Index (PHI)

Prostate specific antigen (PSA)

Prostate specific antigen doubling time (PSADT)

Randomized controlled trial (RCT)

Receiver Operating Characteristic (ROC) curve

Registered Persons Database (RPDB)

Relative risk (RR)

Resource utilization band (RUB)

Same Day Surgery (SDS)

Standardized difference (SD)

Transrectal ultrasound (TRUS)

United States (US)

United States Preventative Services Task Force (USPSTF

Chapter 1:

Introduction to Prostate Specific Antigen Testing for Prostate Cancer

1.1 Background

Prostate cancer is the most commonly diagnosed cancer among Canadian men and it will affect one in seven men during his lifetime[1]. Although curable when detected early, prostate cancer remains the second leading cause of cancer death among Canadian men[1]. Symptoms are rarely present early in the disease and typically develop in locally advanced or metastatic cases due to local mass effect or symptomatic deposits elsewhere in the body, for example obstructive voiding symptoms or pain from bone metastasis.

Most prostate cancers are adenocarcinomas of the prostate that produce prostate specific antigen (PSA). PSA, also known as human kallikrein 3, is a serine protease produced almost exclusively by the epithelial cells of the prostate[2, 3]. Men with prostatic diseases, including adenocarcinoma of the prostate, may have high serum PSA levels due to architectural distortions in the gland that allow PSA greater access to the circulation[2].

Serum PSA was initially approved by the Federal Drug Administration (FDA) in 1986[2] as an indicator of disease recurrence among men who had previously undergone curative primary therapy. However, PSA soon emerged as an objective, quantitative, and minimally invasive screening test that could serve as an early marker of prostate cancer.

In 1991, Catalona et al. published the first results of a prospective non-randomized study of 1653 men who underwent serum PSA measurement as a first-line screening test for prostate cancer compared to 300 controls who underwent ultrasound-directed biopsy because of symptoms or abnormal findings on digital rectal exam (DRE)[3]. PSA testing combined with DRE significantly increased the predictive ability to identify prostate cancer compared to rectal examination alone. A serum PSA level ≥4.0 ng/mL (odds ratio [OR]: 6.2) predicted cancer better than age, suspicious DRE, and suspicious ultrasound result. An elevated PSA also had the highest positive predictive value (PPV) (40%) compared to DRE (33%) and ultrasonography (28%). Soon after the publication of this landmark trial, PSA testing was approved by the FDA as a screening test for prostate cancer when combined with DRE in asymptomatic men[2].

This approval was quickly followed by wide-spread uptake of PSA screening, resulting in an initial increase in disease incidence. Population-level data from the United States (US) reported an 82% increase in the age-adjusted incidence of prostate cancer from 1986 to 1991 among men 65 years of age and older[4]. This observed peak has been referred to as a harvest effect: a depletion of previously undiagnosed and accumulated cases from the pool of prevalent preclinical cases[5].

A large prospective observational study subsequently reported a dramatic stage migration among patients 50 years of age and older who underwent PSA screening compared to controls who were referred based on ultrasonography and DRE[6]. In this analysis, the proportion of cancers that were clinically or pathologically advanced was 57% in the control group compared to 29% among those who underwent serial PSA screening (p=0.002) and this advantage was observed primarily in patients 70 years and younger[6]. Subsequent epidemiological studies demonstrated a dramatic decrease in the rate of distant disease[7, 8]. This was consistent with a

population-based, case-control study reporting that the frequency of PSA-testing was significantly lower among men with metastatic prostate cancer[9].

Despite this, there was conflicting evidence regarding the beneficial effect of routine PSA screening on prostate cancer specific mortality in the US[10, 11] and Europe[10, 12]. In Canada, two studies investigated the impact of routine PSA screening on prostate cancer mortality in Saskatchewan[13] and British Columbia[14]. Both studies found a transient increase in incidence of prostate cancer coinciding with the routine uptake in screening, followed by a decline in the relative risk of prostate cancer death by 60%[13] and 8-29%[14]. However, one of the major criticisms of a time series analysis is whether concomitant advancements in prostate cancer treatment could cause this decline in mortality rather than the adoption of widespread PSA screening. Indeed, one study analyzed the regional intensity of PSA screening and the magnitude of change in prostate cancer mortality[14]. No relationship was found, which brings into question whether there was a beneficial screening effect.

Furthermore, observational analyses are subject to both lead-time bias (where earlier diagnosis of disease makes it appear as though patients are surviving longer) and length-time bias (where detection of more slowly progressing disease results in an overestimation of the survival benefit)[2]. Ultimately, randomized trials were needed to confirm whether or not PSA screening resulted in a reduction in cancer-specific mortality.

1.2 Clinical Trials Evaluating Routine PSA Screening

Three landmark randomized controlled trials (RCTs) were completed to answer this question in the US and Europe enrolling a total of over 270,000 men: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, the European Randomized Study of

Screening for Prostate Cancer (ERSPC) and the Göteborg randomized population-based prostate-cancer screening trial (Table 1).

Table 1. Randomized clinical trials evaluating PSA screening

	PLCO[15, 16]	**ERSPC**[17-20]	**Göteborg**[21, 22]
Sample size	Screened: 38,343 Control: 38,350	Screened: 82,816 Control: 99,184	Screened: 9,952 Control: 9,952
Patients	Men 55-74 years of age	Men 50-74 years of age	Men 50-64 years of age
Site	10 centers across the US	8 European countries	Goteborg, Sweden
Intervention	Annual PSA testing for 6 years, annual DRE for 4 years	PSA screening once every 4 years with or without DRE	PSA screening every 2 years until age 70
Median follow-up	15 years	16 years	18 years
Definition of positive test	PSA >4.0ng/mL Suspicious DRE	PSA >3.0ng/mL (Finland and Italy >4.0ng/mL)	PSA >3.0ng/mL (2.9 ng/mL in 1999-2004; 2.5ng/mL in 2005+)
Prostate cancer deaths	Screened: 255 Control: 244	Screened: 520 Control: 793	Screened: 79 Control: 122
Rate ratio for prostate cancer specific survival (intention to treat)	1.04 (95%CI: 0.87-1.24)	0.80 (95%CI: 0.72-0.89)	0.58 (95%CI: 0.46-0.72)
NNS to prevent one prostate cancer death	--	570	139
NND to prevent one prostate cancer death	--	18	13
Other notes	Significant contamination of the study control group, low biopsy compliance (50-64%)	Dose response relationship: patients with at least one (RR 0.75, 95%CI: 0.66-0.75) and two screening events (RR 0.52, 95%CI: 0.42-0.63)	Increased opportunistic screening in the control group did not result in any significant difference between observed and expected mortality

Adapted from CUA Guideline: PSA Screening and Early Diagnosis[23]

Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, ERSPC: European Randomized Study of Screening for Prostate Cancer, PSA: prostate specific antigen, DRE: digital rectal exam, RR: relative risk, CI: confidence interval, NNS: number needed to screen, NND: number needed to diagnose

1.3 Paradigm Shift

Ultimately, the two European studies[17-22] demonstrated a survival benefit in the intention to treat analysis with a number needed to diagnose between 13 and 18 to prevent one prostate cancer death. One study demonstrated an interesting dose response relationship where the risk of

prostate cancer mortality was lower among men with at least two screening events compared to those who had only one[20].

The positive results in the two European trials were overshadowed by the US study, which did not show a decrease in the risk of cancer-specific mortality with PSA screening. However, it is well known that there were two significant methodological flaws in the US study. First, there was significant contamination of the control group. More than 50% of patients in the control group had a PSA test within the past year and 70% within two years prior to study enrollment. In addition, more than 80% of patients in the control group underwent PSA screening during the study period[24]. Secondly, biopsy compliance was markedly lower in the PLCO trial compared with the other two screening trials[15]. Despite these clear limitations of the PLCO trial, the survival benefit associated with routine PSA screening has been questioned[25, 26].

Adding to the argument against routine PSA screening is the lack of specificity of the test given that other benign conditions can also elevate PSA and the only way to confirm the diagnosis is with a prostate biopsy[27]. While studies report that a PSA value above 4.0 ng/mL should prompt a prostate biopsy, as many as 25% of prostate cancers may present with a PSA less than this threshold[28-30] suggesting that other factors must be considered to evaluate prostate cancer risk beyond absolute PSA value alone. The lack of a concrete cut-off value and the poor specificity of the test has introduced debate regarding the clinical utility of PSA for prostate cancer screening[27, 31]. Furthermore, the harms associated with prostate biopsy and the transition away from active treatment of many low-grade prostate cancers has prompted many preventative health regulatory bodies to change their recommendations regarding routine PSA screening[27, 32].

Indeed, PSA screening has remained one of the most controversial topics in Urology. In 2012, the United States Preventative Services Task Force (USPSTF) recommended against

routine PSA screening for all men[32]. A similar recommendation was released by the Canadian Task Force on Preventative Health Care (CTFPHC) in 2014[27]. International urological societies responded with significant concern, stating this decision counters decades of research showing detection of prostate cancer with PSA screening when in its most manageable state saves lives[33]. Updated long-term follow-up from randomized screening trials[20, 22] and population-based analyses from the US suggesting a possible increase in high risk disease after the change in screening recommendations[34, 35] indicate that PSA testing still has an important role to play in the early detection of prostate cancer.

1.4 Free-to-total PSA Ratio

Given the limitations of total serum PSA as a screening test, other related predictors have been studied in the context of prostate cancer prediction, including free-to-total PSA, rate of change in PSA, and baseline PSA.

PSA exists in the serum in free and protein-bound states. Approximately 70-80% of serum PSA is complexed to either alpha-1-antichymotripsin, alpha-2-macroglobulin, alpha-1-protease inhibitor. PSA produced by prostate cancer escapes proteolytic processing more frequently, resulting in a greater fraction of circulating protein-bound PSA. Therefore, low percentage of free PSA relative to the total PSA is a marker of prostate cancer and can be an indicator of aggressive disease[36, 37].

A prospective study of 773 men with a PSA level between 4.0 and 10.0 ng/mL and a negative DRE reported that free-to-total PSA ratio may be more predictive of prostate cancer than total PSA alone (average area under the curve [AUC] 0.72, 95%CI 0.68-0.75 versus AUC 0.53, 95%CI: 0.49-0.57) with a 10-point decline in free-to-total PSA ratio associated with a three-fold increased likelihood of prostate cancer (OR 3.2, 95%CI: 2.5-4.1, $p<0.001$)[37].

Multiple studies have reported clinically useful free-to-total PSA ratio cut-off values[37, 38]. Catalona et al. reported that free-to-total PSA ratio below 25% detected 98% of cancers in subjects 50-59, 94% of cancers in subjects 60-69, and 90% of cancers in subjects 70-75 years of age and could spare approximately 20% of patients from prostate biopsy[37]. However, it has been reported that, like PSA, free-to-total PSA ratio can fluctuate over time. In a study of 1210 men with two or more measures, as many as 40% of patients who never underwent a prostate biopsy or who had one or more negative biopsies were found to have passed below the 25% threshold at least once during the observation period[38], stressing the need for confirmatory testing prior to clinical decision making. In this study, using a free-to-total PSA ratio cut-off value of 15% resulted in fewer false positive results (21%)[38]. It also has been reported that cut-offs may be influenced by patient age, prostate size, and the total PSA level[37].

1.5 Rate of Change of PSA

Rate of change of PSA could be a marker of cancer and aggressiveness of disease[39]. There are two primary metrics to assess PSA dynamics: PSA velocity and PSA doubling time (PSADT) with the latter demonstrating a beneficial predictive effect in the post-treatment setting[39, 40]. PSA velocity is defined as the change in PSA over time, often quantified as nanograms per millilitre per year. PSADT refers to the number of months required for a given PSA level to increase by a factor of two[39].

In the pre-treatment setting, PSA dynamics are related in part to the underlying cancer growth rate but also influenced by benign prostate tissue[39]. For prostate cancer detection, a PSA velocity greater than 0.35mg/mL/year has been considered a potential useful threshold[41].

In a systematic review by Vickers et al. of 64 articles assessing PSA velocity, most studies found PSA to be a more accurate predictor of prostate cancer diagnosis than PSA

velocity, although some reported the AUC was higher when velocity was included in the model[42, 43] and among men with a negative biopsy[39, 44, 45]. Many of these studies analyzed PSA velocity according to predefined categories. However, the risk is unlikely to be homogenous on either side of a specific threshold. While it is preferable for PSA velocity to be assessed as a continuous variable, it is possible that it has a non-linear relationship with prostate cancer diagnosis[39, 46]. Vickers et al. also highlighted the inherit practical limitation of PSA velocity, including the complexity of its calculation whereby the motivation to compute this value will be directly related to its clinical benefit[39].

1.6 Baseline PSA

While evidence has been mixed regarding the predictive utility of PSA velocity, recent studies have documented the value of baseline PSA as a prognostic marker of future prostate cancer[47], particularly among young men[48, 49]. In an analysis of 10,968 men 55-60 years of age enrolled in the PLCO study, the 13-year incidence of any prostate cancer and clinically significant prostate cancer increased with each baseline PSA category (3.2% and 1.5% among men with a baseline PSA between 0.55-0.99 ng/mL, 11.2% and 5.4% among men with a baseline PSA of 1.0-1.9 ng/mL, 24.0% and 10.6% among men with a baseline PSA of 2.0-2.9 ng/mL, and 36.9% and 15.3% among men with a baseline PSA of 3.0-3.9 ng/mL)[50].

Baseline PSA has also been shown to be a strong predictor of prostate cancer death. Among 945 men aged 40-59 years from the Physicians' Health Study, the risk of lethal prostate cancer was strongly associated with baseline PSA[49]. Compared to patients with a baseline PSA less than or equal to the median, patients with a baseline PSA in the highest decile were seven- to twelve-times more likely to experience lethal prostate cancer (40-49 years: OR 8.7 [95%CI: 1.0-7.8]; 50-54 years: OR 12.6 [95%CI: 1.4-110.4], and 55-59 years: OR 6.9 [95%CI: 2.5-19.1])[49].

Nearly three quarters of patients with lethal prostate cancer who had a baseline PSA measured between 40-59 years had a baseline value above the median for their age group[49]. Similarly, a nested case-control study from Sweden suggested that 44% of prostate cancer deaths could be predicted based on a baseline PSA in the highest decile among 21,277 men 45-49 (PSA ≥1.6 ng/mL) and 51-55 years of age (PSA ≥2.4 ng/mL)[48]. Thus, considering baseline PSA levels when developing a prostate cancer prediction tool may be important.

1.7 Other Potential Screening Tests

Several PSA-derived biomarkers have emerged as potential adjuncts to PSA testing with the goal of reducing unnecessary biopsies. The 4K and Prostate Health Index (PHI) tests are blood tests that measure several PSA derivatives, including total PSA, free PSA, intact PSA, and human kallikrein 2 in combination with age, DRE, and prior biopsy status (4K)[51]; and total PSA, free PSA, and pro PSA (PHI)[52, 53], respectively. The tests have been shown to have an association with tumour aggressiveness[54, 55]. Both tests have demonstrated improved diagnostic performance compared to total PSA alone[56]; however, their widespread adoption as screening tests is limited by the need to process these tests at select laboratories and their significant cost[23]. For the above reasons, widespread use of these tests is not currently recommended by the Canadian Urological Association (CUA)[23].

The PCA3 test is a molecular (mRNA) biomarker measured in the urine after DRE using quantitative polymerase chain reaction that can be used to predict risk of prostate cancer with the strongest evidence among men with a prior negative prostate biopsy[57]. The utility of this test among men with no history of a prior biopsy remains uncertain[23]. The most appropriate cut-off also remains undetermined. Lastly, circulating tumour cells and the use of microRNAs are

additional potential screening tools although there is insufficient evidence at present to recommend their routine use[58].

Chapter 2:

Prostate Cancer Risk Factors

2.1 Age

There are several clinical risk factors that have been shown to be associated with prostate cancer diagnosis. Age is perhaps the most important risk factor for prostate cancer development. This has been demonstrated in two ways. Epidemiological prostate cancer studies have demonstrated that few men under the age of 50 develop prostate cancer and 85% of men are diagnosed after the age of 65 years[59]. Secondly, the increase in incidence of prostate cancer with age has been demonstrated by several autopsy studies evaluating incidental prostate cancer among post-mortem men. Meta-analysis of data from 22 studies revealed that the likelihood of incidental prostate cancer increases 1.7-fold with each increasing decade[60]. The estimated mean cancer prevalence was 5% (95% confidence interval [CI]: 3-8%) and 59% (95%CI: 48-71%) at <30 and >79 years of age, respectively[60].

2.2 Ethnicity

Race is also an established risk factor for prostate cancer. The lowest prostate cancer rates are consistently seen in Asian countries and the highest in North America and Scandinavia[59]. These differences are multifactorial, relating to both lifestyle and health care access in the country of origin, as well as genetic predisposition[59]. Within the US population, there are 20- to 30-fold differences in the risk of prostate cancer among patients from African compared to Asian descent[61, 62]. Migration studies have shown that men who immigrate from Japan, a country with low incidence, to the US, a country with a high incidence, are at increased

risk of prostate cancer; however, the increase is only to about 50% of the rate seen among Caucasians and to 25% of that for African-American people in the US[63].

2.3 Genetic Predisposition

The role of heritable genetic factors in the development of prostate cancer is supported by retrospective studies demonstrating an increased risk of the disease among men with a positive family history[61]. Approximately 10-15% of all patients diagnosed with prostate cancer will have at least one relative who is affected[61]. First degree relatives of patients with prostate cancer will have a two- to three-fold increased risk of developing the disease[59, 61]. Furthermore, the risk of developing prostate cancer in relatives rises as the number of affected individuals increases in the family and with a decrease in the age at diagnosis of the index prostate cancer case[59, 61]. However, such retrospective studies are limited by recall bias, whereby cases may be more likely to report prostate cancer diagnoses in relatives compared to controls. If present, this source of bias would tend to inflate the risk associated with family history. Furthermore, the exact amount of risk that is attributable to genetics rather than shared lifestyle and environmental factors is uncertain.

Comparisons of the concordance of cancer between monozygotic and dizygotic twins provide insight as to whether the familial pattern is due to hereditary or environmental factors. If cancer incidence rates are similar in dizygotic twins (who share 50% of their genes) and monozygotic twins (who share all genes), then shared environmental effects are likely important[64]. However, if rates are higher among monozygotic twins, then genetic effects are likely important[64]. In a meta-analysis including 44,788 pairs of twins from three registries in Sweden, Denmark, and Finland, the estimated heritability of prostate cancer was 42% (95% CI:

29-50)[64]. Absolute risk of prostate cancer in a monozygotic twin of an affected person was 18% compared to 3% among dizygotic twins[64].

The most established genetic mutations linked to the development of prostate cancer include BRCA1 and BRCA2 as well as HPC1. However, such mutations represent only a minority of prostate cancer cases[65]. Genome-wide association studies have identified 76 susceptibility loci associated with prostate cancer risk, which occur commonly but are of low penetrance. Currently, approximately 30% of the familial risk is due to such variants[65]. While genetic testing is playing a role in targeted therapy for advanced prostate cancer[66], there is still much to be clarified before genetic testing can be integrated into screening practices[65].

Chapter 3:

Existing Prostate Cancer Risk Prediction Tools

To improve the predictive utility of PSA testing, different risk prediction tools or risk calculators have been developed incorporating not only serum PSA, but also other clinical factors including patient age, ethnicity, and family history. Using these tools during the patient encounter, clinical variables can be entered into the online calculator to allow improved explanation of prostate cancer risk in real time[67]. The four most widely used tools are described below (Table 2). Two of these risk calculators were developed based on post-hoc analyses of landmark prospective trials[68-70] while the other two were generated based on prospective multi-center data[30,67].

3.1 Prostate Cancer Prevention Trial

The Prostate Cancer Prevention Trial (PCPT) was a phase III double-blind RCT evaluating whether treatment with finasteride (a 5-alpha reductase inhibitor [5-ARI]) for seven years could prevent prostate cancer development. The investigators enrolled over 18,000 men who were 55 years of age or older with a PSA less than or equal to 3.0 ng/mL. Men underwent a prostate biopsy during the trial if their PSA reached 4.0 ng/mL or if their DRE was considered abnormal. From the control group, 5519 men were included in the cohort used to develop the predictive model. The strengths of this study included the use of many patients with complete data, including family history, DRE results, and biopsy data[68].

This study found that increasing PSA, operationalized as logPSA (OR=2.34, 95%CI: 2.13-2.56, p<0.001), positive family history (OR=1.31, 95%CI: 1.11-1.55, p=0.002), abnormal DRE result (OR=2.47, 95%CI: 2.03-3.01, p<0.001) and having one or more negative biopsy (OR=0.64, 95%CI: 0.53-0.78, p<0.001) predicted risk of prostate cancer. Using these variables, the AUC for the multivariable prediction model was 0.70 (SD=0.006). Notably, PSA velocity did not predict prostate cancer development after adjusting for the above items. African American race reached marginal statistical significance when predicting overall risk prostate cancer (OR=1.42, 95%CI: 1.0-2.01, p=0.051), but was a highly significant predictor of high risk disease (OR=2.61, 95%CI: 1.55-4.41, p<0.001)[68]. The results of the study were formatted into an online risk calculator available at: http://www.compas.fhcrc.org/edrnnci/bin/calculator.main.asp.

3.2 European Randomized Study of Screening for Prostate Cancer Rotterdam Cohort

A subset of the ERSPC trial (the Rotterdam, Netherlands) cohort was used to develop a graphical device to predict prostate cancer risk based on *a-priori* criteria, including PSA, prostate volume, DRE and transrectal ultrasound (TRUS) outcome. This prediction tool demonstrated a high AUC of 0.79. (http://www.prostatecancer-riskcalculator.com)[69, 70].

3.3 Sunnybrook Prostate Cancer Prediction Tool

In Canada, the Sunnybrook prostate cancer prediction tool was developed among 3108 subjects, including 408 men with PSA values <4.0 ng/mL. Patients were recruited from two tertiary centers in Toronto. Patients were excluded if they had a history of prostate cancer or if their presenting PSA was more than 50.0 ng/mL. All patients underwent TRUS biopsy (6 to 15 cores). Overall, 42% of men were diagnosed with prostate cancer and more than half of these patients had a clinically significant cancer (Gleason score of 7 or higher). Age, ethnicity, family history, prostate volume, free-to-total PSA ratio, DRE, and urinary symptom score were included

in the model. The AUC was 0.74 (95%CI: 0.71-0.81) and 0.77 (95% CI: 0.74-0.81) for predicting overall and clinically significant prostate cancer, respectively. The model outperformed PSA and DRE alone and performed well when stratified by PSA less than 4.0 and greater than or equal to 4.0 ng/mL[30].

3.4 Memorial Sloan Kettering Cancer Center Prediction Tool

The Prostate Biopsy Collaborative Group published a prediction tool developed using more than 15,000 patients across eleven international centers (eight North American and three European) using a combination of prospective and retrospectively collected patient data. The exact inclusion criteria are unclear and the indications for prostate biopsy as well as prostate biopsy technique varied across centers.

Data from the eight North American centers (5992 biopsies) was used to develop the model and data from the three European centers (10,377 biopsies) was used to validate the model. Patient age, PSA, DRE, African ethnicity, first-degree family history and prior negative biopsy history were collected. Study outcomes included diagnosis of any prostate cancer and high-risk prostate cancer. Median age of the cohort was 65 years and median presenting PSA was 6.0 ng/mL. There was significant variation in the positive DRE rate between centers. As well, many centers were missing more than 30% of the data for DRE, race, and family history. Nevertheless, the developed model demonstrated high external validity with AUC of 0.73 (95% CI: 0.72-0.74) compared to 0.70 (95%CI: 0.69-0.71) using the PCPT risk calculator ($p<0.0001$). The model was better able to predict prostate cancer risk at high PSA levels compared to PCPT[67].

Table 2. Existing prostate cancer prediction tools

	PCPT[68]	**ERSPC**[69, 70]	**Sunnybrook**[30]	**MSKCC**[67]
Patients	Prospective (control arm of PCPT) USA	Prospective (Rotterdam cohort) Netherlands	Prospective, two academic centers Canada	Prospective and retrospective, eleven international centers (8 centers in NA, 3 in Europe)
Sample size	5519	8621 screened (1923 biopsied)	3108	15611
Inclusion criteria	55 years or older Negative DRE PSA </=3	55-74 years of age	PSA >/=4 or abnormal DRE: 2700 PSA<4: 408	Unclear
Biopsy indications	Abnormal DRE PSA>4 End of study biopsy at 7 years	Suspicious DRE, TRUS, or PSA >4 (changed mid-study to PSA>3)	All	Variable
Biopsy technique	TRUS 6+ cores	TRUS 6-7 cores	TRUS 6-15 cores	TRUS 10-12 cores
Mean age (years)	NR 62 (PCPT) Majority (47%) 70 years of age or older at end of study biopsy	NR 62 (ERSPC)	Cancer: 65.9 No cancer: 63.5	65 (median)
Ethnicity	Caucasian 96% African 3%	NR	Cancer: Caucasian 84% African 22% No cancer: Caucasian 77% African 7%	Development: Other 69% Unknown 18% African 13%
Median baseline PSA (ng/mL)	1.5 ng/mL (end of study PSA)	PSA <3 ng/mL in 79%	5.7 ng/mL	6.0 ng/mL
Included variables	Age, PSA, family history, DRE, previous biopsy	Age, PSA, DRE, prostate volume, TRUS result	Age, race, DRE, family history, PSA, free to total PSA, urinary symptom score	Age, race, DRE, family history, previous biopsy
AUC	Overall: 0.70 (SD: 0.006) High-risk*: 0.70 (SD: 0.103)	Overall: 0.79	Overall: 0.74 (95% CI: 0.71-0.81) High-risk*: 0.77 (95% CI: 0.74-0.81)	Overall: 0.76 (95%CI: 0.74-0.77)

*High-risk defined as Gleason score ≥7.

PCPT: Prostate Cancer Prevention Trial, ERSPC: European Randomized Study of Screening for Prostate Cancer, MSKCC: Memorial Sloan Kettering Cancer Center, PSA: prostate specific antigen, DRE: digital rectal exam, TRUS: transrectal ultrasound, AUC: area under the receiver operating characteristic curve, NR: not reported

3.5 Application of Published Risk Prediction Tools in a Canadian Population

The above risk calculators have been evaluated prospectively among Canadian patients. Nam et al. directly compared the predictive ability of the PCPT tool to the Sunnybrook tool among 2130 men across five centers in Canada (four in Ontario, one in Nova Scotia). The authors found that the Sunnybrook risk calculator was associated with a significantly higher concordance index for both overall prostate cancer risk (AUC 0.67 vs 0.61, p=0.001) and high-risk prostate cancer risk (AUC 0.72 vs 0.67, p=0.001) compared with the PCPT tool (Table 3).

Similarly, Trottier el al. directly compared the predictive ability of the PCPT tool to the ERSPC tool among 982 men at a single tertiary oncology center in Ontario. Age, prior biopsy, positive DRE, TRUS nodule, and prostate volume significantly predicted overall and high-risk prostate cancer diagnosis on multivariable logistic regression analysis. The ERSPC tended to outperform the PCPT risk calculator for predicting both overall and high-risk prostate cancer (Table 3).

Table 3. Validation of existing risk prediction tools at Canadian centers

	Nam et al. 2011	**Trottier et al. 2011**
Patients	Prospective, five centers across Canada	Prospective, single tertiary center
Sample size	2130 men with PSA > 2.6 ng/mL and < 50 ng/mL who underwent prostate biopsy	982 men referred with an elevated PSA, biopsy at discretion of treating urologist
Biopsy technique	10-12 cores	11 cores
Median age (years)(IQR)	63 (58-69)	64 (58-69)
Positive family history	466 (22%)	175 (18%)
Ethnicity	Caucasian 1848 (80%) African 103 (4%)	Caucasian 801 (82%) African 57 (6%)
Median baseline PSA (ng/mL) (IQR)	5.7 (4.2-8.1)	5.93 (4.2-8.3)
Positive DRE	331 (14%)	265 (29%)
All patients with prostate cancer	867 (41%)	454 (46%)
Patients with high-risk prostate cancer	403 (17%)	225 (23%)
Risk calculators compared	PCPT vs Sunnybrook	PCPT vs ERSPC vs PSA alone
Predictive ability (AUC, 95%CI)	Overall: PCPT: 0.61 (0.59-0.64) Sunnybrook: 0.67 (0.65-0.69) High-risk: PCPT: 0.67 (0.64-0.70) Sunnybrook: 0.72 (0.70-0.75)	Overall: PCPT: 0.63 (0.60-0.67) ERSPC: 0.71 (0.68-0.74) PSA: 0.55 (0.52-0.59) High-risk: PCPT: 0.68 (0.65-0.72) ERPSC: 0.78 (0.74-0.81) PSA: 0.61 (0.56-0.65)

PCPT: Prostate Cancer Prevention Trial, ERSPC: European Randomized Study of Screening for Prostate Cancer, PSA: prostate specific antigen, DRE: digital rectal exam, IQR: interquartile range, AUC: area under the receiver operating characteristic curve

3.6 Limitations of Existing Prediction Tools

There are many limitations to the existing risk calculators. Some were developed in context of a clinical trial and somewhat limits the generalizability of the findings. In the PCPT trial, the mean age at study entry was 62 years, more than 95% of the study population was Caucasian, all men were required to have a PSA less than 3.0 ng/mL at enrollment and very few patients diagnosed with prostate cancer had a PSA greater than 4.0 ng/mL (less than 25% of all detected prostate cancers, with fewer than 1% of patients with a PSA >10.0 ng/mL). Therefore, the applicability of the model for younger patients or those with a higher PSA at study entry is not clear.

In the Rotterdam analysis of the ERSPC data, the exact methodology behind variable selection is unclear and the chosen predictors not uniformly assessed on all patients. Specifically, TRUS is not uniformly performed on all patients and clinical estimation of prostate size based on DRE is known to be inaccurate[71]. Furthermore, the overall AUC estimations for the Canadian validation studies for PCPT and ERSPC are lower than other validation studies and thus the applicability of these risk calculators to our population may not be as robust as elsewhere.

Although the Sunnybrook risk calculator appears to perform the best, the transition away from routine PSA screening in Canada questions the applicability of existing prediction tools to the current PSA-tested population. While end-of-study biopsies were required in some trials[30, 68], the indications for biopsy in others were less clear and, therefore, there is risk for verification bias. Lastly, none of the existing models incorporated baseline PSA, which may have clinically important prognostic utility.

Chapter 4:

Study Rationale and Research Question

While other adjuncts to PSA testing have emerged, such as serum and urinary biomarkers, the utility of these tests is limited by many factors, including cost[72] and limited accessibility in Canada[23, 73]. Therefore, PSA remains the most widely available and economical population-based opportunistic test for prostate cancer[27].

Following the expressed concerns from the Urologic community and the emergence of long term cancer-specific survival data from the randomized screening trials, the USPSTF has reversed their recommendations to suggest shared decision making regarding periodic PSA-based screening for men 55 to 69 years of age after a discussion of the potential harms and benefits[74]. However, the practice of many physicians has already changed. PSA is now predominantly preformed as an opportunistic test with 45.6% of surveyed Ontario family physicians reporting that they preform PSA screening on fewer patients[75].

Other predictors have emerged as potentially important variables to include in a contemporary risk prediction model. As mentioned, recent studies have documented the predictive utility of a baseline PSA measurement on future prostate cancer diagnosis[47-49]. Studies reporting the association between baseline PSA and lethal prostate cancer reported concordance statistics greater than 0.70[49], which is similar to the predictive performance of many existing risk calculators that were developed using both total PSA and patient variables. Therefore, the

combination of contemporary predictors with current serum PSA level may improve risk stratification and better identify who should receive a prostate biopsy.

No existing studies have developed a prostate cancer prediction model using real world population-level data. The linkage of laboratory data in Ontario to established population-based administrative databases presents a unique opportunity to evaluate PSA dynamics and prostate cancer risk in a contemporary cohort of men undergoing PSA testing. We believe the resulting model will be highly generalizable to a contemporary cohort of men undergoing opportunistic PSA testing.

The objective of this study was to assess the association between first and final PSA predictors with prostate cancer diagnosis in a large population-based cohort of men 40 to 75 years of age in Ontario who had at least two total serum PSA tests. We hypothesized that first PSA in combination with final PSA and other PSA predictor variables will demonstrate a strong association with the diagnosis of prostate cancer and clinically significant prostate cancer.

Chapter 5:

Materials and Methods

5.1 Study Design and Setting

We conducted an unmatched nested case-control study of men 40 to 75 years of age between January 1, 2010 and October 31, 2019 in Ontario using laboratory and administrative data from the ICES. ICES is an independent, non-profit research institute whose legal status under Ontario's health information privacy law allows it to collect and analyze health care and demographic data, without consent, for health system evaluation and improvement. Ontario is the most heavily populated province in Canada, occupied by more than 14.5 million people (https://www150.statcan.gc.ca).

PSA testing was completed between January 1, 2010 and September 30, 2015. Hospital and community-based laboratory data was sequentially linked to administrative data beginning in 2007 with most community-based labs linked by 2010. This accrual period was chosen to allow a three-year look back period to identify men who underwent incident PSA testing. Men were followed from index date (date of first PSA test) until death or date of last contact.

5.2 Data Sources

Within ICES, individual patient data is linked to existing databases using an encrypted version of their unique provincial health card number. With ICES as a data repository, several administrative databases were utilized. All permanent residents of Ontario are eligible for publicly funded health care through the Ontario Health Insurance Plan (OHIP). The OHIP

database contains inpatient and outpatient physician billing data. The Canadian Institute for Health Information (CIHI) database contains diagnostic and procedure data for both inpatient (Discharge Abstract Database [DAD]) and same-day (Same Day Surgery [SDS]) hospital admissions since 1988. The CIHI National Ambulatory Care Reporting System (NACRS) contains information for ambulatory and emergency room visits. The Ontario Cancer Registry (OCR) was used to identify patients with prostate cancer and is known to capture more than 95% of all malignancies[76]. The Registered Persons Database (RPDB) contains validated demographic information[77] and together with the Ontario Registrar General – Death (ORGD) database was used to determine cancer-specific mortality. The Ontario Laboratory Information System (OLIS) was used to capture laboratory data. We used the Ontario Drug Benefit (ODB) database, which captures all prescription data for patients 65 years of age and older, to capture 5-ARI use. The OHIP and CIHI data sources have been validated for capture of diagnostic and procedural data and found to have very good sensitivity and specificity[78, 79] (Appendix 1).

5.3 Study Population

We identified a population of men 40 to 75 years of age who had at least one PSA test during the observation period. We excluded patients with a missing identification number, with a previous diagnosis of prostate or any other invasive cancer, those who underwent PSA testing within three years prior to study initiation (2007-2010), and those whose first PSA was >20.0 ng/mL. Patients with invalid or missing data were excluded (Figure 1).

To better understand how men who undergo PSA testing compare to those who do not, we compared the demographic characteristics of patients included in the PSA cohort to all men not included in the cohort from the general population of Ontario (Figure 1).

To define our nested case-control population from this large population of men who underwent PSA testing, we identified all patients with a subsequent diagnosis of prostate cancer (cases) and those without (controls) who had at least two PSA tests during the study period. We further required all controls have at least three years of follow-up to minimize the potential for verification bias whereby the outcome of the test (in this case, the PSA result) will directly affect the likelihood that the patient undergoes a diagnostic prostate biopsy. Further, any cases with an interval of more than one year from their final PSA test to prostate cancer diagnosis were excluded as it was felt that this PSA would not be an accurate representation of the true PSA at the time of prostate cancer diagnosis (Figure 1).

5.4 Outcomes

Our primary outcome was prostate cancer diagnosis, which was ascertained using the OCR (ICD-O-C61). Our secondary outcome was the diagnosis of clinically significant prostate cancer (International Society of Urological Pathology [ISUP] grade group 2-5) based on histological diagnostic codes from the OCR. Although unvalidated, the ORDG has been used to ascertain cause-specific cancer mortality[80].

5.5 PSA Variables

We captured PSA values from the OLIS using their unique laboratory codes (2857-1, 35741-8, and 19197-3; Appendix 2). We collected all total PSA values, the number of PSA tests, and the percentage change in PSA from baseline per 365 days. Change in PSA was operationalized as a categorical variable (<0% change, 0-19.9% change, 20-99.9% change, and ≥100% change per 365 days)[68]. Continuous total PSA variables were logarithmically transformed to optimize fit within the model[39, 68].

We also created predictor variables using the first total PSA relative to the population distribution for each baseline age. Specifically, we captured whether a patients' PSA was above the 25th percentile, median, 75th percentile and 90th percentile based on the patient's baseline age decade (40-49 years, 50-59 years, 60-69 years, and 70 years or older) as described in previous studies[48, 49].

We were interested in identifying easy-to-apply clinical cut-offs that may prompt prostate biopsy. Therefore, we studied different first and final PSA values that may be associated with prostate cancer diagnosis, specifically first PSA >1.0 ng/mL, >1.5 ng/mL, and >2.0 ng/mL as well as final PSA >2.0 ng/mL, >3.0 ng/mL, >4.0 ng/mL, and >5.0 ng/mL. These specific cut-off values were chosen based on previous literature reporting prognostic utility of PSA values above and below these first and final PSA thresholds[3, 47]. We also operationalized final PSA into the following categories: 0-3.9 ng/mL, 4.0-9.9 ng/mL, and 10.0-20.0 ng/mL.

5.6 Other Covariates

We collected patient demographic characteristics, including age, geographic region based on local health integration network (LHIN), comorbidity of disease using the John Hopkins aggregate disease group (ADG) and resource utilization band (RUB) score, income quintile, and rural versus urban residence. Age as a continuous variable was assessed as a polynomial and logarithmically to optimize fit within the model. An individuals' ADG score was calculated based on identified medical conditions from both inpatient and outpatient health care data sources and according to the duration, severity, etiology, diagnostic certainty, and subspecialty involvement for the condition[81, 82]. A patient could be assigned as few as none and as many as 32 ADGs. The RUB score was generated as a measure of overall burden of morbidity (0=nonuser, 1=healthy user, 2=low morbidity, 3=moderate morbidity, 4=high morbidity, 5=very high

morbidity)[81, 82]. We used a look-back window of two years to ascertain comorbidity score. Rurality was ascertained using the RPDB.

5.7 Cohort Creation

We initially identified 1,125,295 patients who underwent incident PSA testing and 2,575,593 patients who did not during the study period (Appendix 3). Patients who were less than 40 or greater than 75 years of age at baseline (n=800,798) were excluded. Each variable was evaluated for outliers and missing values. No outliers were found in any of the demographic variables. We then excluded any PSA-tested patients with a missing PSA, invalid PSA value at baseline, and those with an incorrect index date after removal of invalid PSA data. We also excluded those who were missing baseline demographic data or who died prior to their index date (Table 4). Overall, 0.73% of patients were excluded from the cohort using these criteria.

Table 4. Overall study cohort

Exclusion Criteria	**Number of Patients (%)**	**Cumulative Frequency (%)**
Missing baseline PSA	5116 (0.18%)	5116 (0.18%)
Incorrect index date	2971 (0.10%)	8087 (0.28%)
Missing income or rurality	13003 (0.45%)	21090 (0.73%)
Death before index date	99 (<0.01%)	21189 (0.73%)
FINAL COHORT	2,878,901	

To create our unmatched nested case-control cohort, we identified all PSA-tested patients with a diagnosis of prostate cancer as possible cases and all PSA-tested patients without a diagnosis of prostate cancer as controls. We then applied the exclusion criteria described above (See section *5.2 Study Population*; Figure 1). A total of 508,238 patients remained in the cohort (12,444 cases and 495,794 controls).

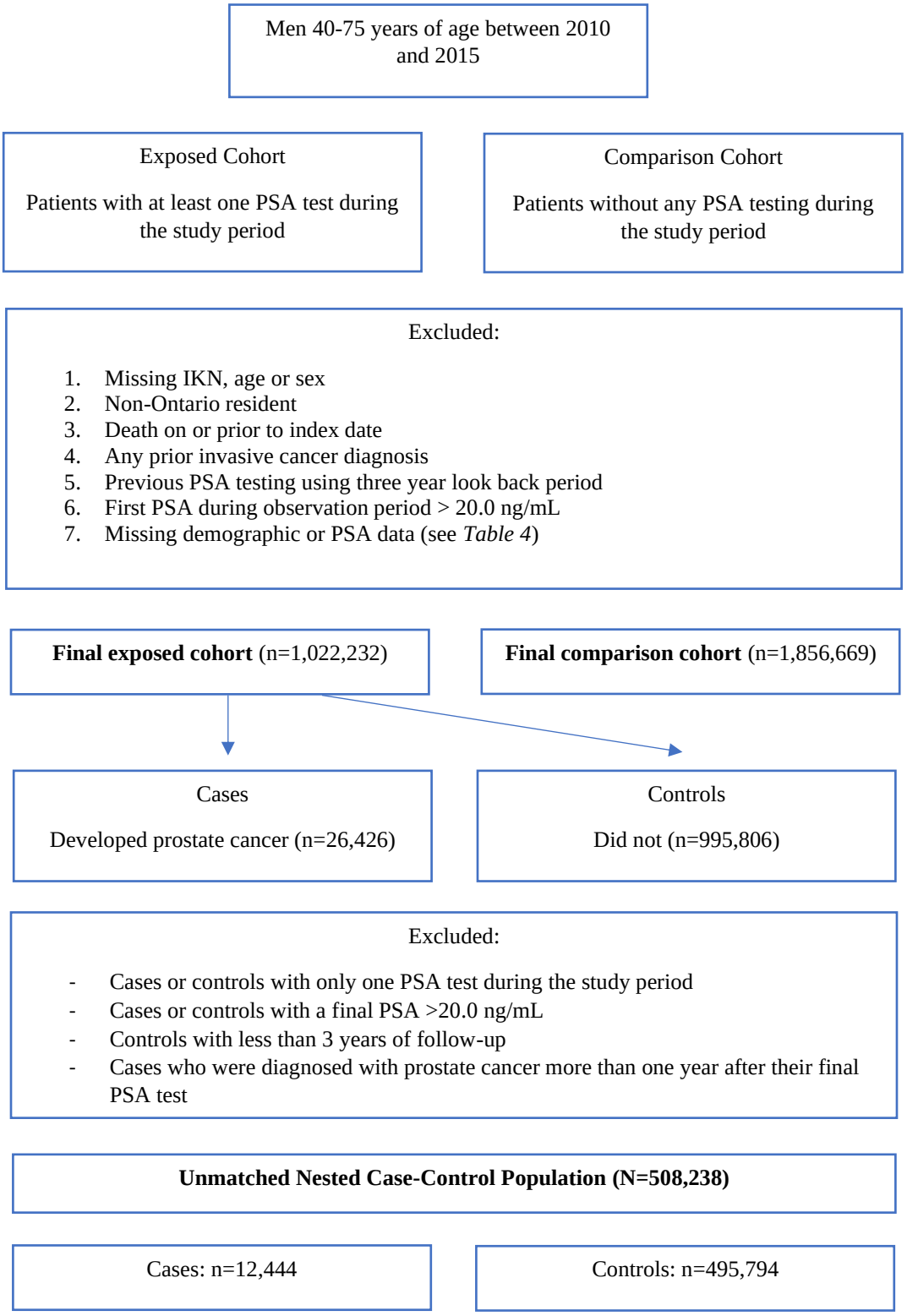

Figure 1. Study flow diagram

5.8 Statistical Analysis

Descriptive statistics were completed on all baseline demographic characteristics. Demographic variables that were continuous and normally distributed were reported as mean with standard deviation. Demographic variables that were continuous and not normally distributed were reported as median with interquartile range (IQR). Normality was assessed graphically using q-q plots and the Kolmogorov-Smirnov statistic for normality with a value above 0.05 suggesting normally distributed data. Categorical variables were presented as numbers with percentages.

Demographic tables were presented to compare patients who underwent PSA testing to those who did not and to compare patients who underwent PSA testing based on age group (as defined using the age groups traditionally presented in PSA screening guideline document recommendations [<55 years, 55-69 years, and 70 years and older]) and separated by time period (pre-guideline recommendation change: 2010-2012, and post-guideline recommendation change: 2013-2015) for the entire study population. From the nested case-control population, patients were separated by the presence or absence of a prostate cancer diagnosis during the study period and compared using standardized differences. Due to missing histology data for 11.1% of our nested case population, demographics were compared among those with and without missing histology data.

We used univariable logistic regression analysis to determine which variables had the strongest association with prostate cancer diagnosis. Age as well as all PSA-specific covariates were analyzed. Multicollinearity was then assessed to determine correlation between the predictor variables before constructing our multivariable models. When variables were collinear,

as indicated by a variance inflation factor greater than 3.0, the variable that had the strongest association with our primary outcome on univariable analysis was retained.

Given the variability of first and final PSA values by baseline age of the patient, stratified univariable analyses were completed by age group to determine which variables were most strongly associated with prostate cancer diagnosis. We found that the thresholds that were most strongly associated with prostate cancer diagnosis were consistent across the age groups. Therefore, multivariable analysis was completed on the entire cohort and not stratified by age.

Several *a-priori* multivariable models were constructed. Model 1 included the optimal first PSA cut-off and final PSA (categorical); Model 2 included the optimal first PSA cut-off and final PSA (categorical) as well as change in PSA relative to baseline; Model 3 contained all variables from Model 2 plus number of PSA tests; Model 4 included final PSA (categorical) and change in PSA relative to baseline; and finally Model 5 included first PSA and change in PSA relative to baseline. Model fit was assessed using the concordance statistic or area under the curve (AUC), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), r-squared value, max rescaled r-squared, and Brier score (higher AUC, lower AIC and BIC, and large r-squared, and lower Brier score suggest better model fit).

AIC assesses goodness of fit of the model and includes a penalty that is a function of the number of estimated parameters, which discourages overfitting[83]. The explained variation (r-squared) and Brier score are important measures of overall model performance. R-squared is a statistical measure that represents the proportion of the variance for a dependent variable that is explained by an independent variable or variables in a regression model. The Brier score is a quadratic scoring rule, where the squared differences between actual binary outcomes and predictions are calculated[84].

Discrimination is an important performance measure to consider when evaluating a prediction model[84]. Accurate predictions discriminate between those with and without the outcome. Area under the Receiver Operating Characteristic (ROC) curve, or AUC, is a measure of discrimination or how well the model can separate the two outcomes of interest by plotting the sensitivity (true positive rate) against one minus the specificity (false positive rate) for consecutive cutoffs for the probability of an outcome. The value of the AUC ranges from 0 (no discriminate ability) to 1 (perfect discrimination)[84]. The model that demonstrated the best performance was chosen.

We verified the assumptions underlying the model, including overspecification and influential observations using the deviance residual (removed only where biologically implausible). Each model was then used to assess our secondary outcomes.

We used a p-value of <0.05 to indicate statistical significance for a two-tailed comparison. All analyses were performed using SAS® version 9.4 (SAS Institute, Inc., Cary, NC, USA).

5.9 Subgroup and Sensitivity Analyses

Because of the potential for selection bias in our analysis with respect to the patients who underwent PSA testing before and after the change in the PSA screening guideline recommendations (which recommend against routine PSA screening for all men)[27], we repeated our primary analysis in two separate stratified before and after analyses (2010-2012 versus 2013-2015). Given that 5-ARI can affect the PSA level, we repeated our primary analysis among a subgroup of patients 65 years of age and older without documented 5-ARI use during the study period. To further limit potential verification bias in our analysis, we limited our controls to those who had at least five years of follow-up and repeated our primary and secondary analyses.

5.10 Estimating the Effect of Verification Bias

A statistical method to estimate the potential effect of verification bias on sensitivity and specificity based on PSA screening data has been described[85, 86]. Using this method, the nested case-control cohort was divided into two groups, "verified" (i.e. patients who did receive a prostate biopsy to confirm the presence or absence of prostate cancer) and "nonverified" (i.e. patients who did not undergo a prostate biopsy during the study period). The empirical probability of verification was calculated for each first and final PSA category. Within each test result category, the observed frequency counts among verified patients were divided by the empirical probability to obtain unbiased estimates of the frequency counts encountered had all patients received a prostate biopsy. Several assumptions must be made when using this method; first, that all prostate cancers are diagnosed using this diagnostic test and second, the decision to undergo a prostate biopsy is based solely on the PSA value alone and thus all patients with missing confirmatory data are missing at random[85].

To further understand the potential impact of verification bias on our model performance and parameter estimates, we limited our primary analysis to cases and controls who underwent any prostate biopsy during the study period.

5.11 Ethics Approval

We obtained ethics approval from the Sunnybrook Health Sciences Centre Research Ethics Board (#189-2019).

Chapter 6:

Results

6.1 Complete Cohort Patient Demographics

Our complete cohort consisted of 1,022,232 patients who underwent incident PSA testing during the study period and 1,856,669 patients who did not. Patients who underwent PSA testing were older (median 55 years [IQR: 50-63] versus 49 years [IQR: 44-57], standardized difference: 0.61), had a higher comorbidity score (median 4 [IQR: 2-7] versus 1 [IQR: 0-4], standardized difference: 0.91), and were more likely to be from the highest income quintile. The two groups were similar with respect to geographic region (Table 5).

Among the 1,022,232 patients who underwent PSA testing, most patients were younger than 70 years of age (92%) and most who underwent PSA testing did so prior to the task force guideline recommendation changes (2010-2012: 64.6% versus 2013-2015: 35.4%) (Table 6). The median first PSA for the entire tested cohort was 0.92 ng/mL (IQR: 0.57-1.60) and did not differ between patients tested before versus after the change in guideline recommendations (2010-2012: 0.94 [IQR: 0.58-2.65] versus 2013-2015: 0.90 [IQR: 0.57-1.53, standardized difference: 0.05). However, patients who underwent PSA testing after the change in guideline recommendations tended to be younger and have fewer comorbidities than the patients tested prior to this change (Appendix 4).

There were important age-related differences in the distribution of the first total PSA. For example, among patients in the youngest age group (40-49 years), the median PSA at baseline

was 0.74 ng/mL compared to 1.54 among patients 70 to 75 years of age (Table 7). Patients who were in the older age groups were more likely to undergo a prostate biopsy (1.9%, 5.5%, and 6.0% among patients who were 40-54, 55-69, and 70 years and older, respectively) (Table 8).

A total of 26,446 (2.6%) patients were diagnosed with prostate cancer (Appendix 5) after a median 2.4 years (IQR: 0.5-4.8). Among patients with available histology data, 13,422/20,838 (64.4%) were diagnosed with clinically significant prostate cancer, including 10,184 (48.9%), 1681 (8.1%), and 1557 (7.5%) with grade group 2-3, 4, and 5 disease, respectively. Median age at prostate cancer diagnosis was 65 years (IQR: 59-70). A total of 54,975 patients (5.4%) died during the study period. Very few patients experienced a prostate cancer death (384/1,022,232; <0.1%) (Table 8).

Table 5. Overall patient demographics, by exposure group

Variable	Untested n=1,856,669	Tested n=1,022,232	SD
Age (years)			
Median (IQR)	49 (44-57)	55 (50-63)	0.61
Age group			
40-54 years	1275853 (68.7%)	478788 (46.8%)	0.45
55-69 years	492368 (26.5%)	461858 (45.2%)	0.40
70+ years	88448 (4.8%)	81586 (8.0%)	0.13
Index year			
2010	574184 (30.9%)	270623 (26.5%)	0.10
2011	415939 (22.4%)	207223 (20.3%)	0.05
2012	314082 (16.9%)	182937 (17.9%)	0.03
2013	201229 (10.8%)	155477 (15.2%)	0.13
2014	198394 (10.7%)	130979 (12.8%)	0.07
2015	152841 (8.2%)	74993 (7.3%)	0.03
ADG score			
Median (IQR)	1 (0-4)	4 (2-7)	0.91
RUB			
0	779575 (42.0%)	30903 (3.0%)	1.05
1	83762 (4.5%)	52161 (5.1%)	0.03
2	255347 (13.8%)	185893 (18.2%)	0.12
3	572556 (30.8%)	578938 (56.6%)	0.54
4	105872 (5.7%)	120497 (11.8%)	0.22
5	59557 (3.2%)	53840 (5.3%)	0.10
Income quintile			
1 (lowest)	421229 (22.7%)	161270 (15.8%)	0.10
2	379212 (20.4%)	190591 (18.6%)	0.04
3	357042 (19.2%)	206842 (20.2%)	0.03
4	350031 (18.9%)	228427 (22.4%)	0.09
5 (highest)	349155 (18.8%)	235102 (23.0%)	0.10
LHIN			
1	108593 (5.9%)	34325 (3.4%)	0.12
2	121359 (6.5%)	68554 (6.7%)	0.01
3	100865 (5.4%)	50960 (5.0%)	0.02
4	178555 (9.6%)	115034 (11.3%)	0.05
5	111261 (6.0%)	67853 (6.6)	0.03
6	155878 (8.4%)	98484 (9.6%)	0.04
7	212062 (11.4%)	87184 (8.5%)	0.10
8	237940 (12.8%)	153114 (15.0%)	0.06
9	193253 (10.4%)	129360 (12.7%)	0.07
10	69607 (3.8%)	35922 (3.5%)	0.01
11	186979 (10.1%)	89595 (8.8%)	0.04
12	54450 (2.9%)	37198 (3.6%)	0.04
13	85962 (4.6%)	41327 (4.0%)	0.03
14	39905 (2.2%)	13367 (1.3%)	0.06
Rural			
N	1645048 (88.6%)	906390 (88.7%)	0.00
Y	211621 (11.4%)	115842 (11.3%)	
Death (all-cause)			
N	1765567 (95.1%)	967257 (94.6%)	0.02
Y	91102 (4.9%)	54975 (5.4%)	

ADG: John Hopkins aggregate disease group comorbidity score; LHIN: local health integration network; IQR: interquartile range; RUB: resource utilization band; SD: standardized difference

Table 6. Overall PSA-tested patient demographics, by age group

Variable	Age Group 1: 40-54 years n=478,788	Age Group 2: 55-69 years n=461,858	Age Group 3: 70-75 years n=81,586	Overall N=1,022,232
Index year				
2010	112218 (23.4%)	132142 (28.6%)	26263 (32.2%)	270623 (26.5%)
2011	97530 (20.4%)	93284 (20.2%)	16409 (20.1%)	207223 (20.3%)
2012	87579 (18.3%)	81569 (17.7%)	13789 (16.9%)	182937 (17.9%)
2013	75188 (15.7%)	68762 (14.9%)	11527 (14.1%)	155477 (15.2%)
2014	66436 (13.9%)	55536 (12.1%)	9007 (11.0%)	130979 (12.8%)
2015	39837 (8.3%)	30565 (6.6%)	4591 (5.6%)	74993 (7.3%)
Time period				
2010-2012	297327 (62.1%)	306995 (66.5%)	56461 (69.2%)	660783 (64.6%)
2013-2015	181461 (37.9%)	154863 (33.5%)	25125 (30.8%)	361449 (35.4%)
ADG score				
Median (IQR)	4 (2-6)	5 (3-7)	6 (4-9)	4 (2-7)
RUB				
0	16446 (3.4%)	13246 (2.9%)	1211 (1.5%)	30903 (3.0%)
1	31392 (6.6%)	19294 (4.2%)	1475 (1.8%)	52161 (5.1%)
2	102633 (21.4%)	75495 (16.4%)	7765 (9.5%)	185893 (18.2%)
3	269511 (56.3%)	263973 (57.2%)	45454 (55.7%)	578938 (56.6%)
4	42908 (9.0%)	61225 (13.3%)	16364 (20.1%)	120497 (11.8%)
5	15898 (3.3%)	28625 (6.2%)	9317 (11.4%)	53840 (5.3%)
Income quintile				
1 (lowest)	72801 (15.2%)	74376 (16.1%)	14093 (17.3%)	161270 (15.8%)
2	86572 (18.1%)	87757 (19.0%)	16262 (20.0%)	190591 (18.6%)
3	98060 (20.5%)	92380 (20.0%)	16402 (20.1%)	206842 (20.2%)
4	111201 (23.2%)	100128 (21.7%)	17098 (21.0%)	228427 (22.4%)
5 (highest)	110154 (23.0%)	107217 (23.2%)	17731 (21.7%)	235102 (23.0%)
LHIN				
1	14193 (3.0%)	16935 (3.7%)	3167 (3.9%)	34295 (3.4%)
2	28556 (6.0%)	33865 (7.3%)	6133 (7.5%)	68554 (6.7%)
3	22543 (4.7%)	24389 (5.3%)	4028 (4.9%)	50960 (5.0%)
4	50626 (10.6%)	54430 (11.8%)	9978 (12.2%)	115034 (11.3%)
5	35437 (7.4%)	27639 (6.0%)	4777 (5.9%)	67853 (6.6%)
6	52264 (10.9%)	39650 (8.6%)	6570 (8.1%)	98484 (9.6%)
7	43822 (9.2%)	37008 (8.0%)	6354 (7.8%)	87184 (8.5%)
8	78748 (16.5%)	63159 (13.7%)	11207 (13.7%)	153114 (15.0%)
9	61740 (12.9%)	57878 (12.5%)	9742 (11.9%)	129360 (12.7%)
10	12697 (35.4%)	19463 (4.2%)	3762 (4.6%)	35922 (3.5%)
11	42817 (8.9%)	40153 (8.7%)	6610 (8.1%)	89580 (8.8%)
12	16154 (3.4%)	17684 (3.8%)	3360 (4.1%)	37198 (3.6%)
13	14119 (3.0%)	22535 (4.9%)	4673 (5.7%)	41327 (4.0%)
14	5072 (1.1%)	7070 (1.5%)	1225 (1.5%)	13367 (1.3%)
Rural				
N	436605 (91.2%)	400034 (86.6%)	69751 (85.5%)	906390 (88.7%)
Y	42183 (8.8%)	61824 (13.4%)	11835 (14.5%)	115842 (11.3%)
First PSA (ng/mL)				
Median (IQR)	0.78 (0.52-1.20)	1.09 (0.63-1.97)	1.54 (0.81-2.95)	0.92 (0.57-1.60)
Number of PSAs				
Median (IQR)	1 (1-2)	2 (1-3)	2 (1-3)	2 (1-3)
Follow-up (years)				
Median (IQR)	7.0 (5.4-8.4)	7.4 (5.7-8.8)	7.2 (5.5-8.8)	7.2 (5.6-8.6)

ADG: John Hopkins aggregate disease group comorbidity score; LHIN: local health integration network; IQR: interquartile range; PSA: prostate specific antigen; RUB: resource utilization band

Table 7. Distribution of first PSA values, by age group

Age Group	25th Percentile PSA (ng/mL)	50th Percentile PSA (ng/mL)	75th Percentile PSA (ng/mL)	90th Percentile PSA (ng/mL)
Age Group 1 (40-54 years)	0.52	0.78	1.20	1.88
Age Group 2 (55-69 years)	0.63	1.09	1.97	3.50
Age Group 3 (70-75 years)	0.81	1.54	2.95	5.17
Decade				
40-49 years	0.50	0.74	1.10	1.64
50-59 years	0.56	0.88	1.44	2.42
60-69 years	0.68	1.20	2.22	3.94
70-75 years	0.81	1.54	2.95	5.17

PSA: prostate specific antigen

Table 8. Overall PSA-tested patient outcomes, by age group

Variable	Age Group 1: 40-54 years n=478,788	Age Group 2: 55-69 years n=461,858	Age Group 3: 70+ years n=81,586	Overall N=1,022,232	p-value
Any prostate biopsy	9160 (1.9%)	25464 (5.5%)	4869 (6.0%)	39493 (3.9%)	<0.001
Any prostate or pelvic MRI	6973 (1.5%)	9585 (2.1%)	1579 (1.9%)	18137 (1.8%)	<0.001
Any free-to-total PSA	37343 (7.8%)	38183 (8.3%)	7971 (9.8%)	83497 (8.2%)	<0.001
Any 5-ARI use					
Unknown	478788 (100%)	335482 (72.6%)	0	814270 (79.7%)	<0.001
N	0	99636 (21.6%)	66252 (81.2%)	165888 (16.2%)	
Y	0	26740 (5.8%)	15334 (18.8%)	42074 (4.1%)	
Prostate cancer					
N	473973 (99.0%)	444487 (96.2%)	77418 (94.9%)	995878 (97.4%)	<0.001
Y	4853 (1.0%)	17419 (3.8%)	4174 (5.1%)	26446 (2.6%)	
PSA at prostate cancer diagnosis (ng/mL)					
Median (IQR)	4.61 (3.08-6.67)	5.54 (3.80-8.01)	6.80 (4.40-10.20)	5.50 (3.68-8.13)	<0.001
Death (all-cause)					
N	470011 (98.2%)	430619 (93.2%)	66627 (81.7%)	967257 (94.6%)	<0.001
Y	8777 (1.8%)	31239 (6.8%)	14959 (18.3%)	54975 (5.4%)	
Death (prostate cancer)					
N	478738 (>99.9%)	461651 (>99.9%)	81459 (99.8%)	1021848 (>99.9%)	
Y	50 (<0.1%)	207 (<0.1%)	127 (0.2%)	384 (<0.1%)	<0.001

IQR: interquartile range; PSA: prostate specific antigen, 5-ARI: 5-alpha reductase inhibitor

6.2 Nested Case-Control Population

A total of 508,238 patients were included in the nested case-control population (12,444 cases and 495,794 controls). The two groups were similar with respect to comorbidity score, income quintile, geographic region, and rurality. Compared to patients who did not develop prostate cancer, those who did were older (median age 62 years [IQR: 56-67] versus 56 years [IQR: 50-63], standardized difference: 0.61). Patients with prostate cancer had a higher first PSA than patients who did not develop prostate cancer (median 4.79 ng/mL [IQR: 3.29-6.96] versus 0.96 ng/mL [IQR: 0.58-1.69], standardized difference: 1.93). The median follow-up time was 8.2 years (IQR: 7.0-9.1) and was similar between groups (Table 9). The time between first and final PSA was highly variable, ranging from 0-5.7 years (median 2.8 years [IQR: 1.8-4.0]). Patients in both groups had a median of three PSAs over the study period (IQR: 2-4).

Fewer than 0.1% (248/261,463) of patients with a first PSA less than 1.0 ng/mL, 0.1% (609/352,247) of patients with a first PSA less than 1.5 ng/mL, and 0.2% (1147/400,121) of patients with a first PSA less than 2.0 ng/mL were diagnosed with prostate cancer during the study period.

Among men younger than 60 years of age, an initial PSA level of less than 1.0, 1.0-1.9, 2.0-2.9, 3.0-3.9, 4.0-4.9, 5.0-9.9, and 10.0-20.0 ng/mL was associated with a prostate cancer rate of 0.05%, 0.5%, 3.1%, 15.9%, 22.6%, and 27.6%, respectively, and a clinically significant prostate cancer rate of 0.01%, 0.2%, 1.5%, 5.0%, 7.0%, 12.6%, 19.4%, respectively.

Patients with a first PSA less than 1.0, between 1.0-1.9, 2.0-2.9, 3.0-3.9, 4.0-4.9, 5.0-9.9 and 10.0-20.0 ng/mL represented 2.0%, 7.1%, 11.4%, 16.4%, 16.5%, 36.4%, and 10.3% of all patients diagnosed with prostate cancer, respectively.

The median final PSA among patients who developed prostate cancer was significantly higher than those who did not (6.50 [IQR: 4.92-9.00] versus 1.00 [IQR: 0.61-1.81], $p<0.001$). From the overall cohort, 0.3% (1495/467,649) of patients with a final PSA less than 4.0 ng/mL were diagnosed with prostate cancer and 0.1% were diagnosed with clinically significant prostate cancer. Among patients with a final PSA between 4.0-9.9 ng/mL and between 10.0-20.0 ng/mL, 24.0% and 49.3% were diagnosed with any prostate cancer while 13.8% and 36.1% were diagnosed with clinically significant prostate cancer, respectively (Table 10).

In men younger than 60 years of age at baseline, a final PSA level of less than 4.0, 4.0-9.9, and 10.0-20.0 ng/mL was associated with a prostate cancer rate of 0.2%, 27.7%, and 50.5%, respectively, and a clinically significant prostate cancer rate of 0.1%, 14.5%, 36.1%, respectively.

Patients with a final PSA less than 4.0, between 4.0-9.9 and 10.0-20.0 ng/mL represented 12.0%, 68.9%, and 19.1% of all patients diagnosed with prostate cancer, respectively (Table 10).

Table 9. Nested case-control population demographics

Variable	Controls n=495,794	Cases n=12,444	Overall N=508,238	SD
Age				
Median (IQR)	56 (50-63)	62 (56-67)	57 (50-63)	0.61
Index year				
2010	195120 (39.4%)	5012 (40.3%)	200132 (39.4%)	0.02
2011	125075 (25.2%)	2893 (23.3%)	127968 (25.2%)	0.05
2012	95794 (19.3%)	1988 (16.0%)	97782 (19.2%)	0.09
2013	57548 (11.6%)	1303 (10.5%)	58851 (11.6%)	0.04
2014	20081 (4.1%)	918 (7.4%)	20999 (4.1%)	0.14
2015	2176 (0.4%)	330 (2.7%)	2506 (0.5%)	0.18
ADG score				
Median (IQR)	5 (3-7)	5 (3-7)	5 (3-7)	0.00
RUB				
0	10191 (2.1%)	325 (2.6%)	10516 (2.1%)	0.04
1	22043 (4.5%)	508 (4.1%)	22551 (4.4%)	0.02
2	79952 (16.1%)	1925 (15.5%)	81877 (16.1%)	0.02
3	294458 (59.4%)	7209 (57.9%)	301667 (59.4%)	0.03
4	63435 (12.8%)	1758 (14.1%)	65193 (12.8%)	0.04
5	25715 (5.2%)	719 (5.8%)	26434 (5.2%)	0.03
Income quintile				
1 (lowest)	71771 (14.5%)	1830 (14.7%)	73601 (14.5%)	0.01
2	90130 (18.2%)	2312 (18.6%)	92442 (18.2%)	0.01
3	100893 (20.4%)	2488 (20.0%)	103381 (20.3%)	0.01
4	114273 (23.1%)	2671 (21.5%)	116944 (23.0%)	0.04
5 (highest)	118727 (24.0%)	3143 (25.3%)	121870 (24.0%)	0.03
LHIN				
1	15118 (3.1%)	375 (3.0%)	15493 (3.1%)	0.00
2	30989 (6.3%)	994 (8.0%)	31983 (6.3%)	0.07
3	21979 (4.4%)	616 (5.0%)	22595 (4.5%)	0.02
4	54802 (11.1%)	1431 (11.5%)	56233 (11.1%)	0.01
5	33629 (6.8%)	788 (6.3%)	34417 (6.8%)	0.02
6	49921 (10.1%)	1068 (8.6%)	50989 (10.0%)	0.05
7	43728 (8.8%)	1085 (8.7%)	44813 (8.8%)	0.00
8	82189 (16.6%)	1633 (12.9%)	83822 (16.5%)	0.10
9	66018 (13.3%)	1606 (12.9%)	67624 (13.3%)	0.01
10	15948 (3.2%)	487 (3.9%)	16435 (3.2%)	0.04
11	41700 (8.4%)	1109 (8.9%)	42809 (8.4%)	0.02
12	16904 (3.4%)	508 (4.1%)	17412 (3.4%)	0.04
13	17029 (3.4%)	573 (4.6%)	17602 (3.5%)	0.06
14	5840 (1.2%)	171 (1.4%)	6011 (1.2%)	0.02
Rural				
N	443542 (89.5%)	10826 (87.0%)	454368 (89.4%)	0.08
Y	52252 (10.5%)	1618 (13.0%)	53870 (10.6%)	
First PSA (ng/mL)				
Median (IQR)	0.96 (0.58-1.69)	4.79 (3.29-6.96)	0.98 (0.59-1.78)	1.75
Number of PSAs				
Median (IQR)	3 (2-4)	3 (2-4)	3 (2-4)	0.23
Follow-up time (years)				
Median (IQR)	8.2 (7.0-9.1)	8.2 (6.6-9.1)	8.2 (7.0-9.1)	0.04

ADG: John Hopkins aggregate disease group comorbidity score; LHIN: local health integration network; IQR: interquartile range; PSA: prostate specific antigen; RUB: resource utilization band; SD: standardized difference

Table 10. Proportion of cases and controls with each potential predictor

Variable	Controls n=495,794	Cases n=12,444	Overall N=508,238	p-value
First PSA (ng/mL)				
Median (IQR)	0.96 (0.58-1.69)	4.79 (3.29-6.96)	0.98 (0.59-1.78)	<0.001
First PSA category				
<4.0	464759 (93.7%)	4584 (36.8%)	469343 (92.4%)	
4.0-9.9	27930 (5.6%)	6583 (52.9%)	34513 (6.8%)	<0.001
10.0-20.0	3105 (0.6%)	1277 (10.3%)	4382 (0.9%)	
First PSA > 25th percentile				
N	125095 (25.2%)	93 (0.8%)	125188 (24.6%)	
Y	370699 (74.8%)	12351 (99.2%)	383050 (75.4%)	<0.001
First PSA > 50th percentile				
N	247195 (49.9%)	330 (2.7%)	247525 (48.7%)	
Y	248599 (50.1%)	12114 (97.4%)	260713 (51.3%)	<0.001
First PSA > 75th percentile				
N	369488 (74.5%)	1147 (9.2%)	370635 (72.9%)	
Y	126306 (25.5%)	11297 (90.8%)	137603 (27.1%)	<0.001
First PSA > 90th percentile				
N	444033 (89.6%)	3488 (28.0%)	447521 (88.1%)	
Y	51761 (10.4%)	8956 (72.0%)	60717 (11.9%)	<0.001
First PSA > 1.0 ng/mL				
N	261215 (52.7%)	248 (2.0%)	261463 (51.4%)	<0.001
Y	234579 (47.3%)	12196 (98.0%)	246775 (48.6%)	
First PSA > 1.5 ng/mL				
N	351638 (70.9%)	609 (4.9%)	352247 (69.3%)	<0.001
Y	144156 (29.1%)	11835 (95.1%)	155991 (30.7%)	
First PSA > 2.0 ng/mL				
N	398974 (80.5%)	1147 (9.2%)	400121 (78.7%)	<0.001
Y	96820 (19.5%)	11297 (90.8%)	108117 (21.3%)	
Final PSA (ng/mL)				
Median (IQR)	1.00 (0.61-1.81)	6.50 (4.92-9.00)	1.04 (0.62-1.91)	<0.001
Final PSA category				
<4.0	466154 (94.0%)	1495 (12.0%)	467649 (92.0%)	
4.0-9.9	27197 (5.5%)	8575 (68.9%)	35772 (7.0%)	<0.001
10.0-20.0	2443 (0.5%)	2374 (19.1%)	4817 (1.0%)	
Final PSA > 2.0 ng/mL				
N	388496 (78.4%)	410 (3.3%)	388906 (76.5%)	<0.001
Y	107298 (21.6%)	12034 (96.7%)	119332 (23.5%)	
Final PSA > 3.0 ng/mL				
N	440996 (89.0%)	765 (6.2%)	441761 (86.9%)	<0.001
Y	54798 (11.0%)	11679 (93.8%)	66477 (13.1%)	
Final PSA > 4.0 ng/mL				
N	466908 (94.2%)	1531 (12.3%)	468439 (92.2%)	<0.001
Y	28886 (5.8%)	10913 (87.7%)	39799 (7.8%)	
Final PSA > 5.0 ng/mL				
N	478318 (96.5%)	3293 (26.5%)	481611 (94.8%)	<0.001
Y	17476 (3.5%)	9151 (73.5%)	26627 (5.2%)	
Change in PSA from baseline (per 365 days, %)				
Median (IQR)	2.4 (-5.3-11.5)	23.7 (4.4-54.0)	2.6 (-5.2-12.0)	<0.001

IQR: interquartile range; PSA: prostate specific antigen

6.3 Primary Outcome

On univariable analysis, all variables were associated with prostate cancer diagnosis (Table 11). When assessing easy to apply clinical cut-offs, first PSA above 2.0 ng/mL and final PSA as a categorical variable had the strongest association with the diagnosis of prostate cancer (AUC: 0.86 and 0.91, respectively). An initial PSA greater than 2.0 ng/mL was strongly associated with the development of prostate cancer (unadjusted OR 40.59, 95%CI: 38.18-43.14, $p<0.001$) (Table 11). When stratified by baseline age, first PSA above 2.0 ng/mL continued to perform the best across all four age groups (Appendix 6).

Compared to patients with a final PSA less than 4.0 ng/mL, patients with a final PSA between 4.0 and 9.9 ng/mL had a higher odds of being diagnosed with prostate cancer (unadjusted OR 98.31, 95%CI: 92.93-103.99, $p<0.001$) as did patients with a final PSA between 10.0-20.0 ng/mL (unadjusted OR 302.99, 95%CI: 280.83-326.90, $p<0.001$) (Table 11).

Table 11. Univariable logistic regression analysis

Variable	OR (95%CI)	p-value	AIC	C-statistic	R^2
Age	1.07 (1.06-1.07)	<0.001	113129	0.663	0.007
First PSA	1.51 (1.51-1.52)	<0.001	92671	0.921	0.047
Log First PSA	7.85 (7.65-8.05)	<0.001	81204	0.921	0.068
Baseline PSA category					
<4.0 (reference)	1.00	<0.001	90489	0.786	0.051
4.0-9.9	23.90 (22.97-24.86)				
10.0-20.0	41.70 (38.83-44.79)				
First PSA above the 25th percentile	44.80 (36.53-54.94)	<0.001	110666	0.622	0.012
First PSA above the 50th percentile	36.50 (32.72-40.73)	<0.001	103046	0.736	0.027
First PSA above the 75th percentile	28.82 (27.11-30.63)	<0.001	93670	0.827	0.045
First PSA above the 90th percentile	22.03 (21.16-22.93)	<0.001	91619	0.808	0.049
First PSA > 1.0 ng/mL	54.76 (48.28-62.10)	<0.001	101087	0.753	0.031
First PSA > 1.5 ng/mL	47.37 (43.65-51.39)	<0.001	92754	0.830	0.046
First PSA > 2.0 ng/mL	40.59 (38.18-43.14)	<0.001	88127	0.856	0.055
Final PSA	1.78 (1.77-1.79)	<0.001	73189	0.959	0.082
Log Final PSA	19.24 (18.56-19.94)	<0.001	63514	0.959	0.100
Final PSA category					
<4.0 (reference)	1.00	<0.001	66250	0.914	0.095
4.0-9.9	98.31 (92.93-103.99)				
10.0-20.0	302.99 (280.83-326.90)				
Final PSA > 2.0 ng/mL	106.27 (96.28-117.29)	<0.001	84472	0.875	0.062
Final PSA > 3.0 ng/mL	122.86 (114.13-132.26)	<0.001	73056	0.914	0.083
Final PSA > 4.0 ng/mL	115.21 (109.07-121.70)	<0.001	67341	0.909	0.093
Final PSA > 5.0 ng/mL	76.05 (72.88-79.36)	<0.001	73666	0.850	0.082
Change in PSA from Baseline per 365 days					
<0% (reference)	1.00	<0.001	104585	0.711	0.024
0-19.9%	1.00 (0.95-1.05)				
20-99.9%	6.53 (6.23-6.85)				
>=100%	19.80 (18.50-21.19)				
Number of PSA tests	1.18 (1.17-1.20)	<0.001	115961	0.563	0.002

PSA: prostate specific antigen; Akaike Information Criterion (AIC)

The variables that were included in our multivariable model included dichotomized first PSA, final PSA as a categorical variable, percentage change in PSA from baseline per 365 days, and total number of PSA tests (Table 12). The model that resulted in the best fit and overall performance included first PSA >2.0 ng/mL, final PSA as a categorical variable, and change in PSA relative to baseline.

Table 12. Multivariable logistic regression models

Model	R^2	Max rescaled R^2	AIC	BIC	Brier score	C-statistic
First PSA >2 + Final PSA category	0.097	0.470	65251	65295	0.018	0.938
First PSA >2 + Final PSA category + Change in PSA relative to baseline	**0.104**	**0.507**	**61038**	**61116**	**0.017**	**0.951**
First PSA >2 + Final PSA category + Change in PSA relative to baseline + PSA count	0.105	0.511	60569	60658	0.017	0.951
Final PSA category + Change in PSA relative to baseline	0.100	0.487	63325	63392	0.017	0.932
First PSA >2 + Change in PSA relative to baseline	0.082	0.398	73534	73589	0.019	0.923

PSA: prostate specific antigen; Akaike Information Criterion (AIC); Bayesian Information Criterion (BIC)

From our final multivariable logistic regression model, patients with a first PSA above 2.0 ng/mL had a higher odds of being diagnosed with prostate cancer (adjusted OR 6.64, 95%CI: 6.13-7.20, p<0.001) than those with a first PSA below this threshold. Compared to a final PSA less than 4.0 ng/mL, a final PSA between 4.0-9.9 ng/mL and 10.0-20.0 ng/mL was also strongly associated with prostate cancer diagnosis (adjusted OR 22.09, 95%CI: 20.58-23.71, p<0.001 and adjusted OR 47.46, 95%CI: 43.28-52.05, p<0.001, respectively). Change from first to final PSA per 365 days of 20.0-99.9% was associated with a three-times increased odds of prostate cancer diagnosis (adjusted OR 3.40, 95%CI: 3.21-3.60, p<0.001) whereas a change of more than 100% was associated with a nearly seven-fold increased odds of prostate cancer (adjusted OR 6.91, 95%CI: 6.29-7.59, p<0.001) (Table 13). The overall discriminative performance of the model was excellent with an AUC of 0.95 (Figure 2).

When age was added to the final multivariable model, there was no significant change in the model fit or discrimination (AUC=0.95). When stratified by baseline age group, the model continued to perform well (Appendix 7).

Table 13. Final multivariable logistic regression model predicting any prostate cancer diagnosis

Variable	OR (95%CI)	p-value
First PSA above 2.0 ng/mL	6.64 (6.13-7.20)	<0.001
Final PSA category		
<4.0	1.00	
4.0-9.9	22.09 (20.58-23.71)	<0.001
10.0-20.0	47.46 (43.28-52.05)	<0.001
Change in PSA from baseline per 365 days		
<0% (reference)	1.00	
0-19.9%	0.84 (0.79-0.89)	<0.001
20-99.9%	3.40 (3.21-3.60)	<0.001
>=100%	6.91 (6.29-7.59)	<0.001

PSA: prostate specific antigen; OR: odds ratio; CI: confidence interval

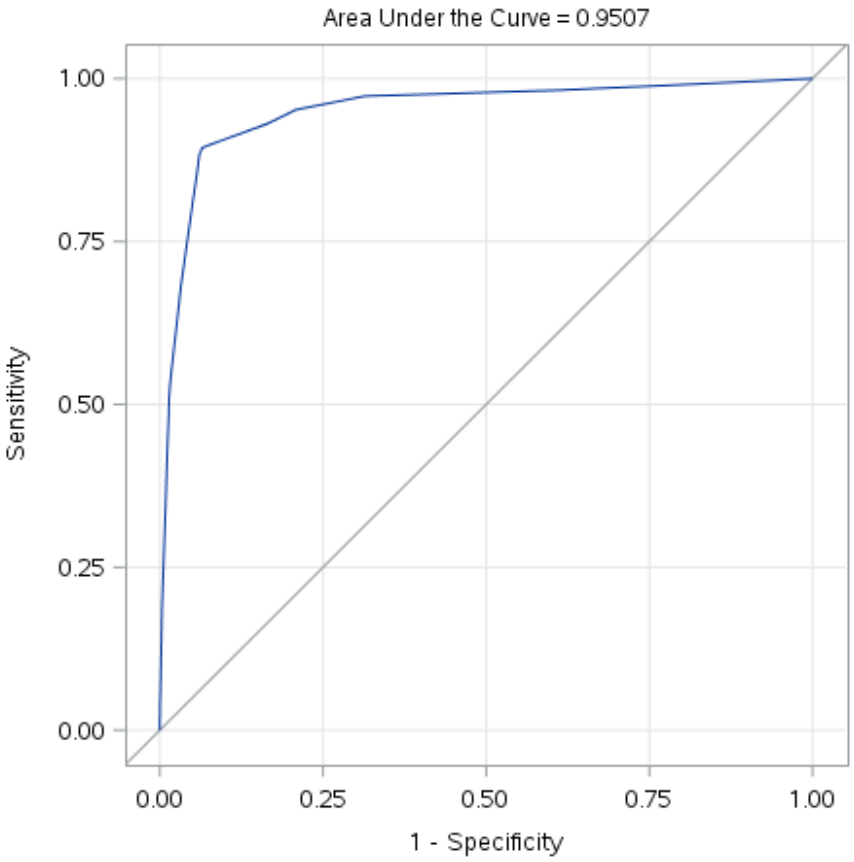

Figure 2. Receiver operating characteristic curve for the final multivariable logistic regression model predicting any prostate cancer diagnosis

6.4 Secondary Outcome

Among patients with available histology data, a total of 7039/11,065 (63.6%) patients were diagnosed with clinically significant prostate cancer. Overall, 5572/11,065 (50.4%) had grade group 2-3 disease, 820/11,065 (7.4%) had grade group 4 disease, and 647/11,065 (5.8%) had grade group 5 disease. Histology data was missing for 1379/12,444 (11.1%) patients.

Comparing patients with missing histology data to those without missing data, patients were similar with respect to age, index year, comorbidity score, income quintile, and rurality. However, missing data was more common in certain geographic regions. For example, patients from LHIN 5 (Central West), 7 (Toronto Central), and 9 (Central East) were more likely to have missing histology data. Patients with missing histology data had a lower first PSA (median 4.33 [IQR: 2.85-6.45] versus 4.83 [IQR: 3.35-7.02], standardized difference: 0.19) and final PSA (5.90 [IQR: 4.30-8.34] versus 6.60 [IQR: 5.00-9.08], standardized difference: 0.23) compared to patients without missing data. The median follow-up was similar between groups. Although patients with missing data were more likely to have died during the study period (11.6% versus 7.8%, standardized difference: 0.13), there was no difference in prostate cancer-specific mortality between groups (Table 14).

Table 14. Comparing baseline characteristics and outcomes of patients with prostate cancer included in the nested case-control cohort, by missing histology data

Variable	Gleason score known n=11,065	Gleason score missing n=1,379	Prostate cancer n=12,444	SD
Age				
Median (IQR)	62 (57-67)	61 (56-67)	62 (56-67)	0.07
Age group				
40-54 years	1984 (17.9%)	280 (20.3%)	2264 (18.2%)	0.06
55-69 years	7319 (66.2%)	889 (64.5%)	8208 (66.0%)	0.04
70+ years	1762 (15.9%)	210 (15.2%)	1972 (15.9%)	0.02
Index year				
2010	4447 (40.2%)	565 (41.0%)	5012 (40.3%)	0.02
2011	2568 (23.2%)	325 (23.6%)	2893 (23.3%)	0.01
2012	1729 (15.6%)	259 (18.8%)	1988 (16.0%)	0.08
2013	1191 (10.8%)	112 (8.1%)	1303 (10.5%)	0.09
2014	834 (7.5%)	84 (6.1%)	918 (7.4%)	0.06
2015	296 (2.7%)	34 (2.5%)	330 (2.7%)	0.01
ADG score				
Median (IQR)	5 (3-7)	5 (3-7)	5 (3-7)	0.02
RUB				
0	291 (2.6%)	34 (2.5%)	325 (2.6%)	0.01
1	454 (4.1%)	54 (3.9%)	508 (4.1%)	0.01
2	1715 (15.5%)	210 (15.2%)	1925 (15.5%)	0.01
3	6401 (57.9%)	808 (58.6%)	7209 (57.9%)	0.02
4	1553 (14.0%)	205 (14.9%)	1758 (14.1%)	0.02
5	651 (5.9%)	68 (4.9%)	719 (5.8%)	0.04
Income quintile				
1 (lowest)	1595 (14.4%)	235 (17.0%)	1830 (14.7%)	0.07
2	2055 (18.6%)	257 (18.6%)	2312 (18.6%)	0.00
3	2190 (19.8%)	298 (21.6%)	2488 (20.0%)	0.04
4	2391 (21.6%)	280 (20.3%)	2671 (21.5%)	0.03
5 (highest)	2834 (25.6%)	309 (22.4%)	3143 (25.3%)	0.08
LHIN				
1	360 (3.3%)	15 (1.1%)	375 (3.0%)	0.15
2	933 (8.4%)	61 (4.4%)	994 (8.0%)	0.16
3	589 (5.3%)	27 (2.0%)	616 (5.0%)	0.18
4	1316 (11.9%)	115 (8.3%)	1431 (11.5%)	0.12
5	629 (5.7%)	159 (11.5%)	788 (6.3%)	0.21
6	938 (8.5%)	130 (9.4%)	1068 (8.6%)	0.03
7	920 (8.3%)	165 (12.0%)	1085 (8.7%)	0.12
8	1449 (13.1%)	184 (13.3%)	1633 (13.1%)	0.01
9	1327 (12.0%)	279 (20.2%)	1606 (12.9%)	0.23
10	424 (3.8%)	63 (4.6%)	487 (3.9%)	0.04
11	1052 (9.5%)	57 (4.1%)	1109 (8.9%)	0.21
12	436 (3.9%)	72 (5.2%)	508 (4.1%)	0.06
13	533 (4.8%)	40 (2.9%)	573 (4.6%)	0.10
14	159 (1.4%)	12 (0.9%)	117 (1.4%)	0.05
Rural				
N	9616 (86.9%)	1210 (87.7%)	10826 (87.0%)	0.03
Y	1149 (13.1%)	169 (12.3%)	1618 (13.0%)	
First PSA				
Median (IQR)	4.83 (3.35-7.02)	4.33 (2.85-6.45)	4.79 (3.29-6.96)	0.19

Number of PSAs				
Median (IQR)	3 (2-4)	3 (2-4)	3 (2-4)	0.01
Final PSA				
Median (IQR)	6.60 (5.00-9.08)	5.90 (4.30-8.34)	6.50 (4.92-9.00)	0.23
Death (all-cause)				
N	10203 (92.2%)	1219 (88.4%)	11422 (91.8%)	0.13
Y	862 (7.8%)	160 (11.6%)	1022 (8.2%)	
Death (prostate cancer)				
N	10958 (99.0%)	1360 (98.6%)	12319 (99.0%)	0.04
Y	107 (1.0%)	19 (1.4%)	126 (1.0%)	
Follow-up time (years)				
Median (IQR)	8.2 (6.6-9.1)	8.1 (6.7-9.1)	8.2 (6.6-9.1)	0.05

ADG: John Hopkins aggregate disease group comorbidity score; LHIN: local health integration network; IQR: interquartile range; PSA: prostate specific antigen; RUB: resource utilization band; SD: standardized difference

An initial PSA above 2.0 ng/mL was associated with the development of clinically significant prostate cancer (adjusted OR 4.90, 95%CI: 4.37-5.48, p<0.001). Patients with a final PSA between 4.0 ng/mL-9.9 ng/mL (adjusted OR 35.41, 95%CI: 31.68-39.57, p<0.001) and patients with a final PSA between 10.0-20.0 ng/mL (adjusted OR 89.07, 95%CI 78.36-101.26, p<0.001) had a higher odds of being diagnosed with clinically significant prostate cancer. Patients who demonstrated a change in PSA from baseline of 20.0-99.9% and more than 100% had a three and five-fold increased odds of being diagnosed with clinically significant prostate cancer, respectively (Table 15).

The multivariable model performed well on the overall nested cohort (AUC=0.96) (Figure 3) and when stratified by age group (Appendix 7).

Table 15. Multivariable logistic regression model predicting the diagnosis of clinically significant prostate cancer

Variable	OR (95%CI)	p-value
First PSA above 2.0 ng/mL	4.90 (4.37-5.48)	<0.001
Final PSA category		
<4.0	1.00	
4.0-9.9	35.41 (31.68-39.57)	<0.001
10.0-20.0	89.07 (78.36-101.26)	<0.001
Change in PSA from baseline per 365 days		
<0% (reference)	1.00	
0-19.9%	0.78 (0.72-0.84)	<0.001
20-99.9%	3.03 (2.82-3.25)	<0.001
>=100%	4.83 (4.34-5.38)	<0.001

PSA: prostate specific antigen; OR: odds ratio; CI: confidence interval

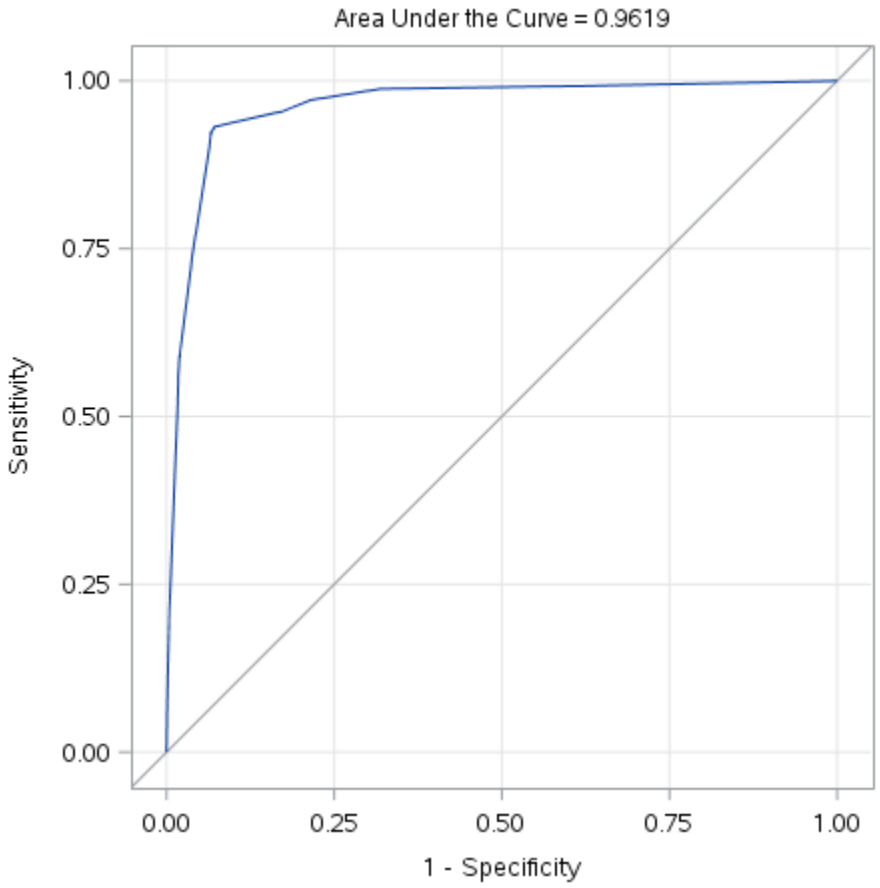

Figure 3. Receiver operating characteristic curve for the final multivariable logistic regression model predicting the diagnosis of clinically significant prostate cancer

6.5 Clinical Application of the Models

The predicted risk of any prostate cancer diagnosis and any clinically significant prostate cancer diagnosis can be generated for a variety of different clinical scenarios (Figure 4). For example, the lowest risk patient (first PSA less than or equal to 2.0 ng/mL, final PSA less than 4.0 ng/mL and 0% change in PSA from first to final per 365 days) had a risk of any prostate cancer diagnosis and clinically significant prostate cancer diagnosis of 3% and 2%, respectively. In contrast, the highest risk patient (first PSA greater than 2.0 ng/mL, final PSA between 10.0-20.0 ng/mL and at least a 100% change in PSA from first to final per 365 days) had a risk of any prostate cancer diagnosis and clinically significant prostate cancer diagnosis of 77% and 58%, respectively.

First PSA <= 2.0 ng/mL												First PSA > 2.0 ng/mL											
Final PSA <4.0 ng/mL				Final PSA 4.0-9.9 ng/mL				Final PSA 10.0-20.0 ng/mL				Final PSA <4.0 ng/mL				Final PSA 4.0-9.9 ng/mL				Final PSA 10.0-20.0 ng/mL			
Change in PSA <0%*	Change in PSA 0-19.9%*	Change in PSA 20.0-99.9%*	Change in PSA >=100%*	Change in PSA <0%*	Change in PSA 0-19.9%*	Change in PSA 20.0-99.9%*	Change in PSA >=100%*	Change in PSA <0%*	Change in PSA 0-19.9%*	Change in PSA 20.0-99.9%*	Change in PSA >=100%*	Change in PSA <0%*	Change in PSA 0-19.9%*	Change in PSA 20.0-99.9%*	Change in PSA >=100%*	Change in PSA <0%*	Change in PSA 0-19.9%*	Change in PSA 20.0-99.9%*	Change in PSA >=100%*	Change in PSA <0%*	Change in PSA 0-19.9%*	Change in PSA 20.0-99.9%*	Change in PSA >=100%*
8%	**3%**	**12%**	**21%**	**--**	**7%**	**22%**	**37%**	**--**	**13%**	**38%**	**56%**	**18%**	**8%**	**26%**	**41%**	**32%**	**16%**	**43%**	**60%**	**50%**	**28%**	**62%**	**77%**
Probability of any prostate cancer																							
4%	**2%**	**6%**	**9%**	**--**	**4%**	**14%**	**20%**	**--**	**9%**	**28%**	**39%**	**8%**	**4%**	**13%**	**19%**	**18%**	**8%**	**26%**	**36%**	**35%**	**19%**	**47%**	**58%**
Probability of clinically significant prostate cancer																							

*Percentage change from first to final PSA, per 365 days

Figure 4. Predicted risk of prostate cancer diagnosis based on the final multivariable logistic regression model

6.6 Subgroup and Sensitivity Analyses

When stratified by time period, the primary model continued to perform well for patients enrolled in 2010-2012 and 2013-2015 (Appendix 8). The primary model also performed well when limited to patients 65 years of age and older without documented 5-ARI use during the study period (Appendix 8). When our nested control group was limited to those with at least five years of follow-up, our primary and secondary outcome models generated similar parameter estimates with excellent overall model performance (Table 16).

Table 16. Multivariable logistic regression model predicting the diagnosis of any prostate cancer and clinically significant prostate cancer, limiting to controls with at least five years of follow-up

	Any prostate cancer		**Clinically significant prostate cancer**	
Variable	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
First PSA above 2.0 ng/mL	7.17 (6.62-7.77)	<0.001	5.20 (4.64-5.82)	<0.001
Final PSA category				
<4.0	1.00		1.00	
4.0-9.9	22.17 (20.67-23.79)	<0.001	35.41 (31.71-39.55)	<0.001
10.0-20.0	49.00 (44.63-53.79)	<0.001	90.52 (79.62-102.91)	<0.001
Change in PSA from baseline per 365 days				
<0% (reference)	1.00		1.00	
0-19.9%	0.79 (0.74-0.83)	<0.001	0.74 (0.68-0.79)	<0.001
20-99.9%	3.33 (3.14-3.53)	<0.001	2.92 (2.72-3.14)	<0.001
>=100%	8.23 (7.45-9.09)	<0.001	5.30 (4.75-5.92)	<0.001

PSA: prostate specific antigen; OR: odds ratio; CI: confidence interval

6.7 Estimating the Effect of Verification Bias

There are different statistical methods that can be used to estimate the potential effect of verification bias on observational data. To explore this, the sensitivity and specificity of different first and final PSA cut-offs were adjusted based on the probability of having a positive or negative prostate biopsy (see section *5.10: Estimating the Effect of Verification Bias*).

From our original analysis, the sensitivity of the various first and final PSA thresholds on our overall study cohort ranged from 0.74-0.98 and specificity from 0.53-0.96. However, when adjusted based on the probability of having a positive or negative biopsy, the sensitivities and specificities of these different cut-offs ranged from 0.12-0.76 and 0.62-0.97, respectively. To account for the potential confounding effect of 5-ARI use, when restricted to patients over the age of 65 years at baseline who did not receive a 5-ARI during the study period, the sensitivities ranged from 0.19-0.84 and specificities from 0.56-0.96 (Appendix 9).

When our primary outcome model was limited to cases and controls who had a prostate biopsy during the study period (26,349/508,238 [5.2%]), we found that our parameter estimates were similar, but tempered. In addition, the performance of the model decreased with an AUC of 0.88, suggesting that verification bias has likely played an important role in our primary and secondary outcome results (Table 17).

Table 17. Multivariable logistic regression model predicting the diagnosis of any prostate cancer, limiting to cases and controls who had a prostate biopsy during the study period

	Patients who underwent a prostate biopsy		**Original model**	
Variable	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
First PSA above 2.0 ng/mL	4.92 (4.12-5.88)	<0.001	6.64 (6.13-7.20)	<0.001
Final PSA category				
<4.0	1.0		1.0	
4.0-9.9	10.18 (8.61-12.05)	<0.001	22.09 (20.58-23.71)	<0.001
10.0-20.0	30.18 (24.01-37.94)	<0.001	47.46 (43.28-52.05)	<0.001
Change in PSA from baseline per 365 days				
<0% (reference)	1.0		1.0	
0-19.9%	0.83 (0.71-0.97)	<0.001	0.84 (0.79-0.89)	<0.001
20-99.9%	2.77 (2.37-3.23)	<0.001	3.40 (3.21-3.60)	<0.001
>=100%	5.64 (4.48-7.11)	<0.001	6.91 (6.29-7.59)	<0.001
	AUC=0.88		*AUC=0.95*	

PSA: prostate specific antigen; OR: odds ratio; CI: confidence interval

Chapter 7:

Discussion

In this large, nested case-control study of more than 500,000 men who underwent incident PSA testing in Ontario, we found that first and final PSA were highly associated with prostate cancer diagnosis. We identified important and easy-to-apply clinical cut-offs to generate probabilities of being diagnosed with any prostate cancer and clinically significant prostate cancer. Our multivariable model demonstrated excellent performance when discriminating patients with prostate cancer and those with clinically significant prostate cancer from those without.

Among all men who underwent PSA testing during the study period, the median first PSA was 0.92 ng/mL and varied depending on the baseline age of the patient (0.74 ng/mL, 0.88 ng/mL, 1.20 ng/mL, and 1.54 ng/mL for patients 40-49, 50-59, 60-69, and 70-75 years of age, respectively). In 2008, Capitanio et al. published a population-based cohort study of 3,222 patients invited to participate in a PSA screening event in Montreal[87]. The reported distribution of first PSA values was very similar to the current study. Among patients 40-49, 50-59, 60-69 and 70-79 years of age, the median baseline PSA was 0.7 ng/mL, 0.9 ng/mL, 1.3 ng/mL and 1.9 ng/mL, respectively. Although this study was conducted and published prior to the change in PSA screening recommendations, we found that the overall PSA distribution was consistent with the current study. Similarly, in our study, we did not notice a significant change in the median first total PSA among patients enrolled in 2010-2012 versus 2013-2015 although patients screened after the guideline change tended to be younger and have fewer comorbidities.

In our study, first PSA was highly predictive of subsequent prostate cancer diagnosis. Similar to a post-hoc analysis of the PLCO data[50], increasing baseline PSA was associated and an incremental increase in the crude rate of any prostate cancer and clinically significant prostate cancer diagnosis. This is also consistent with several other studies[88, 89], including findings from the Baltimore Longitudinal Study of Aging[90]. In this prospective cohort study of 795 men, a baseline total PSA greater than the median was associated with a 3.6 (95%CI: 1.6-8.6) to 3.5 (95%CI: 2.0-6.2)-fold increase in the relative risk of prostate cancer among patients 40-49 and 50-59 years of age, respectively. This association was even stronger in a study by Loeb et al. involving 13,943 men enrolled in a PSA screening study[89]. In this study, men with a baseline PSA above the median at age 40-49 had a 31-fold increased odds of prostate cancer of any grade (OR 30.9, 95%CI: 4.2-229.4). In that study, baseline PSA was a stronger predictor than race, family history, and DRE. In our study, the association between baseline PSA and subsequent prostate cancer diagnosis was strongest in the younger age groups (Appendix 6). The magnitude of these associations suggest that the pathological process leading to prostate cancer development has begun in early middle age[90].

These data not only highlight the predictive utility of a patient's first PSA, but also suggest the clinical cut-offs needed to interpret a first-ever PSA with respect to risk stratification are different than traditional cut-offs of 3.0 or 4.0 ng/mL. When we explored risk stratification based on baseline PSA level at various easy to apply clinical cut-offs (1.0 ng/mL, 1.5 ng/mL and 2.0 ng/mL), we found that the association between first PSA above 2.0 ng/mL and subsequent prostate cancer diagnosis was the strongest across all age strata, although PSA above 1.5 ng/mL also performed well for patients between the ages of 40-49 and 50-59 at baseline (Appendix 6). A study by Carlsson et al. demonstrated that among men who had an initial screening PSA by

age 60, the benefit of ongoing screening was highest for patients with a baseline PSA >2.0 ng/mL with a number needed to screen of 23 to avoid one prostate cancer death at 15 years[47]. This suggests that a baseline PSA of 2.0 ng/mL may be an appropriate cut-off to recommend regular ongoing testing.

Many previous studies have sought to identify optimal clinical cut-offs to prompt prostate biopsy. Historically, prostate cancer screening trials have required biopsy for any PSA above a designated threshold, typically 3.0 ng/mL or 4.0 ng/mL[68-70]. Similarly, we found that a final PSA cut-off of 3.0 ng/mL or 4.0 ng/mL were optimal with respect to accurately discriminating patients with and without prostate cancer, with the lower threshold favored for patients less than 60 years of age at baseline.

However, perhaps the reason there are few prostate cancers diagnosed below these traditional cut-offs highlights the potential for verification bias whereby the result of the test dictates the need for a confirmatory biopsy[85, 91]. Thompson et al. reported the risk of any prostate cancer among men enrolled in the placebo arm of the PCPT trial with a normal DRE and PSA less than or equal to 4.0 ng/mL who had a study biopsy after seven years. Approximately 15% of these men were diagnosed with clinically significant prostate cancer[68]. This is significantly more than what was seen in the current study where less than 1% of men with a final PSA below that threshold were diagnosed with prostate cancer during the study period.

We attempted to control for the potential effect of verification bias by requiring a minimum of three years of follow-up for patients in the control group. As a sensitivity analysis, when we limited to controls with five years, the performance of the model remained very strong. We also attempted to minimize the impact of this bias by using clinically significant prostate cancer as a secondary outcome - it is less likely that a patients with a clinically significant lesion

would remain undiagnosed for more than three years. However, when we repeated our primary analysis only among those cases and controls who had a biopsy during the study period, we found that our parameter estimates were tempered, and the overall performance of our model decreased. This suggests that verification bias had an important impact on our primary and secondary results.

To try to quantify the potential effect of verification bias, we also compared our computed sensitivity and specificity adjusted for the likelihood of having a positive or negative confirmatory biopsy at different first and final PSA thresholds. We found that the sensitivity estimates reported in this study are likely overestimated. However, this method to estimate the potential impact of verification bias is based on two critical assumptions. Most importantly, this method assumes that patients with missing diagnostic biopsy data are missing at random; however, this is unlikely to be the case. The decision to biopsy a patient with a final PSA less than 2.0 ng/mL, for example, is likely influenced by other clinical factors, including DRE result, family history, and 5-ARI use, among others. Limiting to patients over the age of 65 years at baseline without 5-ARI use during the study period creates estimates that more closely approximate the unadjusted estimates reported in this study but does not eliminate this potential for verification bias. Secondly, this method of adjustment requires that the gold standard test (in this case prostate biopsy) detects all cancers. However, it is well established that standard prostate biopsy is associated with an estimated 10% false negative rate[85, 92].

There are other methods to correct for verification bias. For example, by building logistic regression models that can estimate the likelihood of a positive or negative diagnostic test modeled on other clinical predictors. However, without individual patient-level data, this was not possible. In the absence of mandated random biopsies among patients with a low PSA, it is not

possible to quantify the true impact of verification bias using population-level data. Based on the results of our exploratory analyses, verification bias has resulted in an overestimation of the performance of our models. The true sensitivity and specificity of the PSA thresholds explored in this analysis likely fall somewhere in between the original and adjusted estimates and this highlights the need for the findings of this study to be confirmed with prospective data.

In our multivariable model, adjusting for baseline PSA and final PSA, we found that patients who had a PSA decrease over the study period had a higher odds of prostate cancer diagnosis compared to patients who demonstrated a slight increase in their PSA of 0-19.9% per 365 days. We explored why this may be the case. First, there was more 5-ARI use among patients with a less than 0% change in total PSA compared to the other groups, suggesting the total PSA in this group may be misrepresented given this medication is known to decrease the PSA by as much as 50%. In the absence of prescription data for men less than 65 years of age, when designing the study, we elected not to exclude men over 65 years who received 5-ARI medications during the study period to minimize selection bias and generate a more representative population undergoing PSA testing. As a sensitivity analysis including only patients 65 years of age or older who did not receive a 5-ARI medication during the study period, the confidence interval for the odds ratio estimate for change in PSA 0-19.9% versus <0% was very close to 1, suggesting while this may play a role in the findings, there may be other effects at play.

This increased risk among patients with a PSA change of less that 0% could also be explained by the use of prostate surgery (e.g. transurethral resection of the prostate or simple prostatectomy) during the study period or the off-label use of other anti-androgen agents prior to prostate cancer diagnosis. Likely the group who had a decrease in their PSA over the study

period is heterogeneous and should be separated from the other groups who demonstrated an increase in their PSA from baseline in the multivariable model. The clinical application of the prediction scores generated for the group who saw a decrease in their PSA over the study period may be limited in the absence of individual-level patient data that can shed light on the exact reason for this observed effect.

The strengths of this analysis include the use of a large, population-based cohort representative of a contemporary group of patients undergoing opportunistic PSA testing. Given the challenges associated with the application of isolated clinical cut-offs, the combination of first PSA, final PSA, and change over time improved the predictive performance of our model and generated probabilities that may be easy to apply in clinical practice to guide decision making regarding selecting which patients should go on to receive prostate biopsy.

Nevertheless, there are limitations to this analysis. The conversion of continuous PSA variables into dichotomized or categorized variables can reduce statistical strength. However, we chose to identify easy-to-apply clinical cut-offs that could be more readily incorporated into clinical practice to risk stratify patients. While this cohort represents a contemporary cohort of PSA-tested men, the non-random selection of patients who receive a PSA test, the frequency at which they underwent PSA testing, and the decision to receive a prostate biopsy was at the discretion of the care provider and thus, this may introduce selection, observation, and verification bias. Further, different PSA assays were used across the province as well as different biopsy techniques, including approach and number of cores, which may introduce additional observation bias. MRI is becoming an increasingly useful tool in the identification of patients at higher risk of clinically significant prostate cancer. Due to limitations within the existing administrative datasets, we are only able to identify patients who underwent pelvic MRI, and not

prostate MRI specifically. Moreover, the results of these tests are not recorded in administrative databases and given the strict indications for prostate MRI in Ontario, incorporating MRI into the model would limit the generalizability of the findings to PSA tested populations in the US and Europe where prostate MRI is used more widely.

Overall, we were missing little demographic data, eliminating approximately 0.7% of patients from the original study cohort. However, approximately 11% of patients included in the nested case-control population had missing histology data. These patients tended to have a lower PSA and tended to reside in the Toronto area. Therefore, this may introduce an element of observation bias in the analysis of our secondary outcomes. Furthermore, not all laboratories and hospitals have linked their laboratory data to OLIS. We attempted to minimize this potential for selection bias by starting our study in 2010, when the majority of community laboratories were linked. Despite this, there is potential for misclassification of a patient's first PSA if they in fact had undergone PSA testing in the past that was not captured in the early years of OLIS linkage. Due to the limited use of free-to-total PSA testing in the study cohort, we could not explore the additional prognostic utility of this variable. Other potentially important patient-level data were also missing from this analysis, including race, DRE findings, and family history, the later of which has been shown to be a significant predictor after adjusting for baseline PSA[89].

Finally, given that PSA data was not linked to administrative data sources until 2007, our follow-up time was limited, and we were unable to examine prostate cancer death as an outcome due to few events. Data from the ERSPC screening study suggest that PSA testing starts to have an effect on mortality only after eight years or longer of follow-up[69, 70]. Therefore, this is an outcome that can be examined in future studies.

Prospective studies are needed to confirm our findings, to systematically address potential verification bias using retrospective data, and to explore the utility of adding additional prognostic variables (such as DRE, race, family history, and free-to-total PSA ratio) that are not readily available in population-based datasets to optimize the performance of a prediction model. Contemporary prediction models could also further pinpoint which patients would benefit most from the addition of a screening prostate MRI, particularly in the context of a health care system with significant resource constraints where uniform prostate MRI screening is neither realistic nor cost-effective[93].

Chapter 8:

Conclusions

This study suggests that baseline PSA in combination with final PSA and rate of change can be used to predict prostate cancer and better select those who should go on to receive a prostate biopsy. The multivariable model was able to discriminate between those with and without prostate cancer with a high degree of accuracy but may be at significant risk of verification bias. Therefore, the findings of this study need to be verified prospectively with exploration of the value of adding other potentially important clinical and biochemical prognostic factors to the model before it can be integrated into practice as a clinical decision-making tool. Further studies are also needed to determine the association between our predictors and metastatic and lethal prostate cancer.

References

1. Government of Canada. Canadian Cancer Statistics: A 2018 Special Report on Cancer Incidence by Stage. Accessed July 10, 2018,
2. Barry MJ. Clinical practice. Prostate-specific-antigen testing for early diagnosis of prostate cancer. *N Engl J Med*. May 2001;344(18):1373-7. doi:10.1056/NEJM200105033441806
3. Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Engl J Med*. Apr 1991;324(17):1156-61. doi:10.1056/NEJM199104253241702
4. Potosky AL, Miller BA, Albertsen PC, Kramer BS. The role of increasing detection in the rising incidence of prostate cancer. *JAMA*. Feb 1995;273(7):548-52.
5. Fleshner K, Carlsson SV, Roobol MJ. The effect of the USPSTF PSA screening recommendation on prostate cancer incidence patterns in the USA. *Nat Rev Urol*. Jan 2017;14(1):26-37. doi:10.1038/nrurol.2016.251
6. Catalona WJ, Smith DS, Ratliff TL, Basler JW. Detection of organ-confined prostate cancer is increased through prostate-specific antigen-based screening. *JAMA*. Aug 1993;270(8):948-54.
7. Bartsch G, Horninger W, Klocker H, et al. Tyrol Prostate Cancer Demonstration Project: early detection, treatment, outcome, incidence and mortality. *BJU Int*. Apr 2008;101(7):809-16. doi:10.1111/j.1464-410X.2008.07502.x
8. Helgstrand JT, Røder MA, Klemann N, et al. Trends in incidence and 5-year mortality in men with newly diagnosed, metastatic prostate cancer-A population-based analysis of 2 national cohorts. *Cancer*. 07 2018;124(14):2931-2938. doi:10.1002/cncr.31384
9. Kopec JA, Goel V, Bunting PS, et al. Screening with prostate specific antigen and metastatic prostate cancer risk: a population based case-control study. *J Urol*. Aug 2005;174(2):495-9; discussion 499. doi:10.1097/01.ju.0000165153.83698.42
10. Roberts RO, Bergstralh EJ, Katusic SK, Lieber MM, Jacobsen SJ. Decline in prostate cancer mortality from 1980 to 1997, and an update on incidence trends in Olmsted County, Minnesota. *J Urol*. Feb 1999;161(2):529-33.
11. Agalliu I, Weiss NS, Lin DW, Stanford JL. Prostate cancer mortality in relation to screening by prostate-specific antigen testing and digital rectal examination: a population-based study in middle-aged men. *Cancer Causes Control*. Nov 2007;18(9):931-7. doi:10.1007/s10552-007-9031-7
12. Post PN, Kil PJ, Crommelin MA, Schapers RF, Coebergh JW. Trends in incidence and mortality rates for prostate cancer before and after prostate-specific antigen introduction. A registry-based study in southeastern Netherlands, 1971-1995. *Eur J Cancer*. Apr 1998;34(5):705-9.
13. Skarsgard D, Tonita J. Prostate cancer in Saskatchewan Canada, before and during the PSA era. *Cancer Causes Control*. Jan 2000;11(1):79-88.
14. Coldman AJ, Phillips N, Pickles TA. Trends in prostate cancer incidence and mortality: an analysis of mortality change by screening intensity. *CMAJ*. Jan 2003;168(1):31-5.
15. Andriole GL, Crawford ED, Grubb RL, et al. Mortality results from a randomized prostate-cancer screening trial. *N Engl J Med*. Mar 2009;360(13):1310-9. doi:10.1056/NEJMoa0810696

16. Pinsky PF, Prorok PC, Yu K, et al. Extended mortality results for prostate cancer screening in the PLCO trial with median follow-up of 15 years. *Cancer*. Feb 2017;123(4):592-599. doi:10.1002/cncr.30474
17. Schröder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med*. Mar 2009;360(13):1320-8. doi:10.1056/NEJMoa0810084
18. Schröder FH, Hugosson J, Roobol MJ, et al. Prostate-cancer mortality at 11 years of follow-up. *N Engl J Med*. Mar 2012;366(11):981-90. doi:10.1056/NEJMoa1113135
19. Schröder FH, Hugosson J, Roobol MJ, et al. Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up. *Lancet*. Dec 2014;384(9959):2027-35. doi:10.1016/S0140-6736(14)60525-0
20. Hugosson J, Roobol MJ, Månsson M, et al. A 16-yr Follow-up of the European Randomized study of Screening for Prostate Cancer. *Eur Urol*. Feb 2019;doi:10.1016/j.eururo.2019.02.009
21. Hugosson J, Carlsson S, Aus G, et al. Mortality results from the Göteborg randomised population-based prostate-cancer screening trial. *Lancet Oncol*. Aug 2010;11(8):725-32. doi:10.1016/S1470-2045(10)70146-7
22. Arnsrud Godtman R, Holmberg E, Lilja H, Stranne J, Hugosson J. Opportunistic testing versus organized prostate-specific antigen screening: outcome after 18 years in the Göteborg randomized population-based prostate cancer screening trial. *Eur Urol*. Sep 2015;68(3):354-60. doi:10.1016/j.eururo.2014.12.006
23. Rendon RA, Mason RJ, Marzouk K, et al. Recommandations de l'Association des urologues du Canada sur le dépistage et le diagnostic précoce du cancer de la prostate. *Can Urol Assoc J*. Oct 2017;11(10):298-309. doi:10.5489/cuaj.4888
24. Shoag JE, Mittal S, Hu JC. Reevaluating PSA Testing Rates in the PLCO Trial. *N Engl J Med*. May 2016;374(18):1795-6. doi:10.1056/NEJMc1515131
25. Hayes JH, Barry MJ. Screening for prostate cancer with the prostate-specific antigen test: a review of current evidence. *JAMA*. Mar 2014;311(11):1143-9. doi:10.1001/jama.2014.2085
26. Ilic D, Djulbegovic M, Jung JH, et al. Prostate cancer screening with prostate-specific antigen (PSA) test: a systematic review and meta-analysis. *BMJ*. Sep 2018;362:k3519. doi:10.1136/bmj.k3519
27. Bell N, Connor Gorber S, Shane A, et al. Recommendations on screening for prostate cancer with the prostate-specific antigen test. *CMAJ*. 11 2014;186(16):1225-34. doi:10.1503/cmaj.140703
28. Heidenreich A, Bastian PJ, Bellmunt J, et al. EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. *Eur Urol*. Jan 2014;65(1):124-37. doi:10.1016/j.eururo.2013.09.046
29. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. *N Engl J Med*. May 2004;350(22):2239-46. doi:10.1056/NEJMoa031918
30. Nam RK, Toi A, Klotz LH, et al. Assessing individual risk for prostate cancer. *J Clin Oncol*. Aug 2007;25(24):3582-8. doi:10.1200/JCO.2007.10.6450
31. Carter HB. American Urological Association (AUA) guideline on prostate cancer detection: process and rationale. *BJU Int*. Sep 2013;112(5):543-7. doi:10.1111/bju.12318

32. Moyer VA, Force USPST. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*. Jul 2012;157(2):120-34. doi:10.7326/0003-4819-157-2-201207170-00459
33. AUA. USPSTF Reform: What You Should Know. Accessed March 28, 2019,
34. Banerji JS, Wolff EM, Massman JD, Odem-Davis K, Porter CR, Corman JM. Prostate Needle Biopsy Outcomes in the Era of the U.S. Preventive Services Task Force Recommendation against Prostate Specific Antigen Based Screening. *J Urol*. Jan 2016;195(1):66-73. doi:10.1016/j.juro.2015.07.099
35. Gejerman G, Ciccone P, Goldstein M, et al. US Preventive Services Task Force prostate-specific antigen screening guidelines result in higher Gleason score diagnoses. *Investig Clin Urol*. 11 2017;58(6):423-428. doi:10.4111/icu.2017.58.6.423
36. Catalona WJ, Smith DS, Wolfert RL, et al. Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening. *JAMA*. Oct 1995;274(15):1214-20.
37. Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA*. May 1998;279(19):1542-7. doi:10.1001/jama.279.19.1542
38. Ankerst DP, Gelfond J, Goros M, et al. Serial Percent Free Prostate Specific Antigen in Combination with Prostate Specific Antigen for Population Based Early Detection of Prostate Cancer. *J Urol*. 08 2016;196(2):355-60. doi:10.1016/j.juro.2016.03.011
39. Vickers AJ, Savage C, O'Brien MF, Lilja H. Systematic review of pretreatment prostate-specific antigen velocity and doubling time as predictors for prostate cancer. *J Clin Oncol*. Jan 2009;27(3):398-403. doi:10.1200/JCO.2008.18.1685
40. Oudard S, Banu E, Scotte F, et al. Prostate-specific antigen doubling time before onset of chemotherapy as a predictor of survival for hormone-refractory prostate cancer patients. *Ann Oncol*. Nov 2007;18(11):1828-33. doi:10.1093/annonc/mdm332
41. Carter HB, Pearson JD, Metter EJ, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. *JAMA*. 1992 Apr 22-29 1992;267(16):2215-20.
42. Loeb S, Roehl KA, Catalona WJ, Nadler RB. Prostate specific antigen velocity threshold for predicting prostate cancer in young men. *J Urol*. Mar 2007;177(3):899-902. doi:10.1016/j.juro.2006.10.028
43. Loeb S, Metter EJ, Kan D, Roehl KA, Catalona WJ. Prostate-specific antigen velocity (PSAV) risk count improves the specificity of screening for clinically significant prostate cancer. *BJU Int*. Feb 2012;109(4):508-13; discussion 513-4. doi:10.1111/j.1464-410X.2011.10900.x
44. Eggener SE, Roehl KA, Catalona WJ. Predictors of subsequent prostate cancer in men with a prostate specific antigen of 2.6 to 4.0 ng/ml and an initially negative biopsy. *J Urol*. Aug 2005;174(2):500-4. doi:10.1097/01.ju.0000165203.40973.0f
45. Elshafei A, Li YH, Hatem A, et al. The utility of PSA velocity in prediction of prostate cancer and high grade cancer after an initially negative prostate biopsy. *Prostate*. Dec 2013;73(16):1796-802. doi:10.1002/pros.22718
46. Vickers AJ, Wolters T, Savage CJ, et al. Prostate-specific antigen velocity for early detection of prostate cancer: result from a large, representative, population-based cohort. *Eur Urol*. Nov 2009;56(5):753-60. doi:10.1016/j.eururo.2009.07.047

47. Carlsson S, Assel M, Sjoberg D, et al. Influence of blood prostate specific antigen levels at age 60 on benefits and harms of prostate cancer screening: population based cohort study. *BMJ*. Mar 2014;348:g2296. doi:10.1136/bmj.g2296
48. Vickers AJ, Ulmert D, Sjoberg DD, et al. Strategy for detection of prostate cancer based on relation between prostate specific antigen at age 40-55 and long term risk of metastasis: case-control study. *BMJ*. Apr 2013;346:f2023. doi:10.1136/bmj.f2023
49. Preston MA, Batista JL, Wilson KM, et al. Baseline Prostate-Specific Antigen Levels in Midlife Predict Lethal Prostate Cancer. *J Clin Oncol*. 08 2016;34(23):2705-11. doi:10.1200/JCO.2016.66.7527
50. Kovac E, Carlsson SV, Lilja H, et al. Association of Baseline Prostate-Specific Antigen Level With Long-term Diagnosis of Clinically Significant Prostate Cancer Among Patients Aged 55 to 60 Years: A Secondary Analysis of a Cohort in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. *JAMA Netw Open*. Jan 2020;3(1):e1919284. doi:10.1001/jamanetworkopen.2019.19284
51. Vickers AJ, Cronin AM, Aus G, et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Göteborg, Sweden. *BMC Med*. Jul 2008;6:19. doi:10.1186/1741-7015-6-19
52. Jansen FH, van Schaik RH, Kurstjens J, et al. Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate cancer detection. *Eur Urol*. Jun 2010;57(6):921-7. doi:10.1016/j.eururo.2010.02.003
53. Catalona WJ, Partin AW, Sanda MG, et al. A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. *J Urol*. May 2011;185(5):1650-5. doi:10.1016/j.juro.2010.12.032
54. Parekh DJ, Punnen S, Sjoberg DD, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. *Eur Urol*. Sep 2015;68(3):464-70. doi:10.1016/j.eururo.2014.10.021
55. Loeb S, Sanda MG, Broyles DL, et al. The prostate health index selectively identifies clinically significant prostate cancer. *J Urol*. Apr 2015;193(4):1163-9. doi:10.1016/j.juro.2014.10.121
56. Nordström T, Vickers A, Assel M, Lilja H, Grönberg H, Eklund M. Comparison Between the Four-kallikrein Panel and Prostate Health Index for Predicting Prostate Cancer. *Eur Urol*. Jul 2015;68(1):139-46. doi:10.1016/j.eururo.2014.08.010
57. Gittelman MC, Hertzman B, Bailen J, et al. PCA3 molecular urine test as a predictor of repeat prostate biopsy outcome in men with previous negative biopsies: a prospective multicenter clinical study. *J Urol*. Jul 2013;190(1):64-9. doi:10.1016/j.juro.2013.02.018
58. Filella X, Fernández-Galan E, Fernández Bonifacio R, Foj L. Emerging biomarkers in the diagnosis of prostate cancer. *Pharmgenomics Pers Med*. 2018;11:83-94. doi:10.2147/PGPM.S136026
59. Grönberg H. Prostate cancer epidemiology. *Lancet*. Mar 2003;361(9360):859-64. doi:10.1016/S0140-6736(03)12713-4
60. Bell KJ, Del Mar C, Wright G, Dickinson J, Glasziou P. Prevalence of incidental prostate cancer: A systematic review of autopsy studies. *Int J Cancer*. Oct 2015;137(7):1749-57. doi:10.1002/ijc.29538

61. Whittemore AS, Wu AH, Kolonel LN, et al. Family history and prostate cancer risk in black, white, and Asian men in the United States and Canada. *Am J Epidemiol*. Apr 1995;141(8):732-40. doi:10.1093/oxfordjournals.aje.a117495
62. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer*. Jun 1993;54(4):594-606. doi:10.1002/ijc.2910540413
63. LAG R, M E, CL K, al. e. *SEER cancer statistics review, 1973–1999*. National Cancer Institute; 2002.
64. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med*. Jul 2000;343(2):78-85. doi:10.1056/NEJM200007133430201
65. Eeles R, Goh C, Castro E, et al. The genetic epidemiology of prostate cancer and its clinical implications. *Nat Rev Urol*. Jan 2014;11(1):18-31. doi:10.1038/nrurol.2013.266
66. Mateo J, Carreira S, Sandhu S, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *N Engl J Med*. Oct 2015;373(18):1697-708. doi:10.1056/NEJMoa1506859
67. Ankerst DP, Straubinger J, Selig K, et al. A Contemporary Prostate Biopsy Risk Calculator Based on Multiple Heterogeneous Cohorts. *Eur Urol*. 08 2018;74(2):197-203. doi:10.1016/j.eururo.2018.05.003
68. Thompson IM, Ankerst DP, Chi C, et al. Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. *J Natl Cancer Inst*. Apr 2006;98(8):529-34. doi:10.1093/jnci/djj131
69. Kranse R, Roobol M, Schröder FH. A graphical device to represent the outcomes of a logistic regression analysis. *Prostate*. Nov 2008;68(15):1674-80. doi:10.1002/pros.20840
70. Kranse R, Beemsterboer P, Rietbergen J, Habbema D, Hugosson J, Schröder FH. Predictors for biopsy outcome in the European Randomized Study of Screening for Prostate Cancer (Rotterdam region). *Prostate*. Jun 1999;39(4):316-22. doi:10.1002/(sici)1097-0045(19990601)39:4<316::aid-pros14>3.0.co;2-o
71. Roehrborn CG, Girman CJ, Rhodes T, et al. Correlation between prostate size estimated by digital rectal examination and measured by transrectal ultrasound. *Urology*. Apr 1997;49(4):548-57. doi:10.1016/s0090-4295(97)00031-9
72. Mottet N, Bellmunt J, Bolla M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. *Eur Urol*. 04 2017;71(4):618-629. doi:10.1016/j.eururo.2016.08.003
73. CIHI. Analysis in Brief: Wait Times for Priority Procedures in Canada 2017. Accessed March 28, 2019,
74. Grossman DC, Curry SJ, Owens DK, et al. Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA*. 05 2018;319(18):1901-1913. doi:10.1001/jama.2018.3710
75. Akerman JP, Allard CB, Tajzler C, Kapoor A. Prostate cancer screening among family physicians in Ontario: An update on attitudes and current practice. *Can Urol Assoc J*. Feb 2018;12(2):E53-E58. doi:10.5489/cuaj.4631
76. Robles SC, Marrett LD, Clarke EA, Risch HA. An application of capture-recapture methods to the estimation of completeness of cancer registration. *J Clin Epidemiol*. 1988;41(5):495-501.
77. Iron K, Zagorski BM, Sykora K, DG M. Living and dying in Ontario: an opportunity for improved health information. Toronto: ICES; 2008.

78. D J, C P, R C, et al. Canadian Institute for Health Information Discharge Abstract Database: A Validation Study. Institute for Clinical Evaluative Sciences Toronto2006.
79. Goel V WJ, Anderson G, et al. *Patterns of Health Care in Ontario, Canada: The ICES Practice Atlas. A summary of studies on the quality of health care administrative databases in Canada.* 1996:339.
80. Baxter NN, Goldwasser MA, Paszat LF, Saskin R, Urbach DR, Rabeneck L. Association of colonoscopy and death from colorectal cancer. *Ann Intern Med*. Jan 2009;150(1):1-8. doi:10.7326/0003-4819-150-1-200901060-00306
81. Health Services Research & Development Center. The Johns Hopkins ACG Case-Mix System Reference Manual Version 7.0. Baltimore, USA: Johns Hopkins University Bloomberg School of Public Health; 2005.
82. Reid RJ, Roos NP, MacWilliam L, Frohlich N, Black C. Assessing population health care need using a claims-based ACG morbidity measure: a validation analysis in the Province of Manitoba. *Health Serv Res*. Oct 2002;37(5):1345-64. doi:10.1111/1475-6773.01029
83. Harrell FE. Regression Modelling Strategies. Department of Biostatistics, Vanderbilt University School of Medicine. Accessed April, 2020.
84. Steyerberg EW, Vickers AJ, Cook NR, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. *Epidemiology*. Jan 2010;21(1):128-38. doi:10.1097/EDE.0b013e3181c30fb2
85. Cronin AM, Vickers AJ. Statistical methods to correct for verification bias in diagnostic studies are inadequate when there are few false negatives: a simulation study. *BMC Med Res Methodol*. Nov 2008;8:75. doi:10.1186/1471-2288-8-75
86. Begg CB, Greenes RA. Assessment of diagnostic tests when disease verification is subject to selection bias. *Biometrics*. Mar 1983;39(1):207-15.
87. Capitanio U, Perrotte P, Zini L, et al. Population-based analysis of normal Total PSA and percentage of free/Total PSA values: results from screening cohort. *Urology*. Jun 2009;73(6):1323-7. doi:10.1016/j.urology.2008.10.026
88. Gann PH, Hennekens CH, Stampfer MJ. A prospective evaluation of plasma prostate-specific antigen for detection of prostatic cancer. *JAMA*. Jan 1995;273(4):289-94.
89. Loeb S, Roehl KA, Antenor JA, Catalona WJ, Suarez BK, Nadler RB. Baseline prostate-specific antigen compared with median prostate-specific antigen for age group as predictor of prostate cancer risk in men younger than 60 years old. *Urology*. Feb 2006;67(2):316-20. doi:10.1016/j.urology.2005.08.040
90. Fang J, Metter EJ, Landis P, Chan DW, Morrell CH, Carter HB. Low levels of prostate-specific antigen predict long-term risk of prostate cancer: results from the Baltimore Longitudinal Study of Aging. *Urology*. Sep 2001;58(3):411-6. doi:10.1016/s0090-4295(01)01304-8
91. Punglia RS, D'Amico AV, Catalona WJ, Roehl KA, Kuntz KM. Effect of verification bias on screening for prostate cancer by measurement of prostate-specific antigen. *N Engl J Med*. Jul 2003;349(4):335-42. doi:10.1056/NEJMoa021659
92. Van der Kwast TH, Lopes C, Martikainen PM, et al. Report of the Pathology Committee: false-positive and false-negative diagnoses of prostate cancer. *BJU Int*. Dec 2003;92 Suppl 2:62-5. doi:10.1111/j.1465-5101.2003.04400.x
93. Cheung DC, Finelli A. Magnetic resonance imaging diagnosis of prostate cancer: promise and caution. *CMAJ*. Oct 2019;191(43):E1177-E1178. doi:10.1503/cmaj.190568

Appendices

Appendix 1. Data sources

Datasets – Health Services	**Years**
CIHI Discharge Abstract Database (DAD)	January 2007 – October 2019
CIHI Same Day Surgery (SDS)	January 2007 – October 2019
Ontario Health Insurance Plan (OHIP)	January 2007 – October 2019
National Ambulatory Care Reporting System (NACRS)	January 2007 – September 2015
Registered Persons Database (RPDB)	January 2010 – October 2019
Ontario Census Area Profiles (CENSUS)	January 2010 – September 2015
Vital Statistics – Death (ORGD)	January 2007 – October 2019
Local Health Integration Network (LHIN)	January 2010 – September 2015
Postal Code Conversion File (PCCF)	January 2010 – September 2015
Ontario Cancer Registry (OCR)	January 1964 – October 2019
Cancer Care Ontario (CCO)	January 2010 – October 2019
Ontario Laboratories Information System (OLIS)	January 2007 – September 2015
Ontario Drug Benefit Claims (ODB)	January 2007 – October 2019

Appendix 2. Prostate specific antigen variable coding in the Ontario Laboratory Information System (OLIS)

Prostate-Specific Antigen	
2857-1	Prostate specific antigen
35741-8	Prostate specific antigen
19197-3	Prostate specific antigen
12841-3	Prostate specific antigen free/Prostate specific antigen total
10886-0	Prostate specific antigen free

Appendix 3. Cohort creation

Exclusion Criterion	**Cases**		**Controls**	
	Number of observations	Number excluded	Number of observations	Number excluded
Missing age	1,714,687	0	3,046,064	0
Missing sex	1,714,687	0	3,046,064	0
Non-Ontario resident	1,713,709	978	2,739,588	306,476
Died prior to index date	1,713,677	32	2,739,248	340
Prior cancer diagnosis	1,569,278	144,399	2,652,704	86,544
Prior PSA test	1,133,075	436,203	2,575,593	77,111
PSA >20ng/mL on index date	1,125,295	7,780	2,575,593	N/A

Appendix 4. PSA-tested patient demographics, separated by screening era (pre- and post-change to guideline recommendations)

Variable	2010-2012 n=660,783	2013-2015 n=361,449	Overall N=1,022,232	SD
Age (years)				
Median (IQR)	56 (50-63)	54 (50-62)	55 (50-63)	0.11
Age group				
40-54 years	297327 (45.0%)	181461 (50.2%)	478788 (46.8%)	0.10
55-69 years	306995 (46.5%)	154863 (42.9%)	461858 (45.2%)	0.07
70-75 years	56461 (8.5%)	25125 (6.9%)	81586 (8.0%)	0.06
Age decade				
40-49 years	148213 (22.4%)	87797 (24.3%)	236010 (23.1%)	0.04
50-59 years	272783 (41.3%)	159301 (44.1%)	432084 (42.3%)	0.06
60-69 years	183326 (27.7%)	89226 (24.7%)	272552 (26.7%)	0.07
70-75 years	56461 (8.5%)	25125 (7.0%)	81586 (8.0%)	0.06
ADG score				
Median (IQR)	5 (3-7)	4 (2-7)	4 (2-7)	0.14
RUB				
0	15705 (2.4%)	15198 (4.2%)	30903 (3.0%)	0.10
1	34530 (5.2%)	17631 (4.9%)	52161 (5.1%)	0.02
2	115425 (17.5%)	70468 (19.5%)	185893 (18.2%)	0.05
3	380094 (57.5%)	198844 (55.0%)	578938 (56.6%)	0.05
4	80387 (12.2%)	40110 (11.1%)	120497 (11.8%)	0.03
5	34642 (5.2%)	19198 (5.3%)	53840 (5.3%)	0.00
Income quintile				
1 (lowest)	100136 (15.2%)	61134 (16.9%)	161270 (15.8%)	0.05
2	122627 (18.6%)	67964 (18.8%)	190591 (18.6%)	0.01
3	134714 (20.4%)	72128 (20.0%)	206842 (20.2%)	0.01
4	150264 (22.7%)	78163 (21.6%)	228427 (22.4%)	0.03
5 (highest)	153042 (23.2%)	82060 (22.7%)	235102 (23.0%)	0.01
LHIN				
1	23248 (3.5%)	11047 (3.1%)	34295 (3.4%)	0.03
2	44637 (6.8%)	23917 (6.6%)	68554 (6.7%)	0.01
3	32823 (5.0%)	18137 (5.0%)	50960 (5.0%)	0.00
4	75184 (11.4%)	39850 (11.0%)	115034 (11.3%)	0.01
5	45086 (6.8%)	22767 (6.3%)	67853 (6.6%)	0.02
6	63306 (9.6%)	35178 (9.7%)	98484 (9.6%)	0.01
7	55903 (8.5%)	31281 (8.7%)	87184 (8.5%)	0.01
8	99252 (15.0%)	53862 (14.9%)	153114 (15.0%)	0.00
9	87005 (13.2%)	42355 (11.7%)	129360 (12.7%)	0.04
10	24015 (3.6%)	11907 (3.3%)	35922 (3.5%)	0.02
11	55402 (8.4%)	34178 (9.5%)	89580 (8.8%)	0.04
12	23481 (3.6%)	13717 (3.8%)	37198 (3.6%)	0.01
13	22844 (3.5%)	18483 (5.1%)	41327 (4.0%)	0.08
14	8597 (1.3%)	4770 (1.3%)	13367 (1.3%)	0.00
Rural				
N	585914 (88.7%)	320476 (88.7%)	906390 (88.7%)	0.00
Y	74869 (11.3%)	40973 (11.3%)	115842 (11.3%)	
Baseline PSA				
Median (IQR)	0.94 (0.58-1.65)	0.90 (0.57-1.53)	0.92 (0.57-1.60)	0.05

ADG: John Hopkins aggregate disease group comorbidity score; LHIN: local health integration network; IQR: interquartile range; PSA: prostate specific antigen; RUB: resource utilization band; SD: standardized difference

Appendix 5. PSA-tested patient demographics, by prostate cancer diagnosis

Variable	No prostate cancer n=995,806	Prostate cancer n=26,426	Overall N=1,022,232	SD
Age				
Median (IQR)	55 (50-62)	62 (56-67)	55 (50-63)	0.64
Age group				
40-54 years	473940 (47.6%)	4848 (18.4%)	478788 (46.8%)	0.65
55-69 years	444454 (44.6%)	17404 (65.9%)	461858 (45.2%)	0.44
70+ years	77412 (7.8%)	4174 (15.8%)	81586 (8.0%)	0.25
Index year				
2010	260941 (26.2%)	9682 (36.6%)	270623 (26.5%)	0.23
2011	201261 (20.2%)	5962 (22.6%)	207223 (20.3%)	0.06
2012	178629 (17.9%)	4308 (16.3%)	182937 (17.9%)	0.04
2013	152431 (15.3%)	3046 (11.5%)	155477 (15.2%)	0.11
2014	128691 (12.9%)	2288 (8.7%)	130979 (12.8%)	0.14
2015	73853 (7.4%)	1140 (4.3%)	74993 (7.3%)	0.13
Time period				
2010-2012	640831 (64.4%)	19952 (75.5%)	660783 (64.6%)	0.24
2013-2015	354975 (35.7%)	6474 (24.5%)	361449 (35.4%)	
ADG score				
Median (IQR)	4 (2-7)	5 (3-7)	4 (2-7)	0.09
RUB				
0	30218 (3.0%)	685 (2.6%)	30903 (3.0%)	0.03
1	51087 (5.1%)	1074 (4.1%)	52161 (5.1%)	0.05
2	181790 (18.3%)	4103 (15.5%)	185893 (18.2%)	0.07
3	563621 (56.6%)	15317 (58.0%)	578938 (56.6%)	0.03
4	116802 (11.7%)	3695 (14.0%)	120497 (11.8%)	0.07
5	52288 (5.3%)	1552 (5.9%)	53840 (5.3%)	0.03
Income quintile				
1 (lowest)	157313 (15.8%)	3957 (15.0%)	161270 (15.8%)	0.02
2	185656 (18.6%)	4935 (18.7%)	190591 (18.6%)	0.00
3	201559 (20.2%)	5283 (20.0%)	206842 (20.2%)	0.01
4	222724 (22.4%)	5703 (21.6%)	228427 (22.4%)	0.02
5 (highest)	228554 (23.0%)	6548 (24.8%)	235102 (23.0%)	0.04
LHIN				
1	33247 (3.3%)	1048 (4.0%)	34295 (3.4%)	0.03
2	66466 (6.7%)	2088 (7.9%)	68554 (6.7%)	0.05
3	49621 (5.0%)	1339 (5.1%)	50960 (5.0%)	0.00
4	111951 (11.2%)	3083 (11.7%)	115034 (11.3%)	0.01
5	66191 (6.7%)	1662 (6.3%)	67853 (6.6%)	0.01
6	96376 (9.7%)	2108 (8.0%)	98484 (9.6%)	0.06
7	84925 (8.5%)	2259 (8.6%)	87184 (8.5%)	0.00
8	149686 (15.0%)	3428 (13.0%)	153114 (15.0%)	0.06
9	126104 (12.7%)	3256 (12.3%)	129360 (12.7%)	0.01
10	34830 (3.5%)	1092 (4.1%)	35922 (3.5%)	0.03
11	87285 (8.8%)	2295 (8.7%)	89580 (8.8%)	0.00
12	36080 (3.6%)	1118 (4.2%)	37198 (3.6%)	0.03
13	40009 (4.0%)	1318 (5.0%)	41327 (4.0%)	0.05
14	13035 (1.3%)	332 (1.3%)	13367 (1.3%)	0.00
Rural				
N	883546 (88.7%)	22844 (86.5%)	906390 (88.7%)	0.07
Y	112260 (11.3%)	3582 (13.6%)	115842 (11.3%)	
First PSA				

Median (IQR)	0.90 (0.56-1.53)	4.32 (2.70-6.69)	0.92 (0.57-1.60)	1.84
Number of PSAs				
Median (IQR)	2 (1-3)	2 (1-4)	2 (1-3)	0.54
Follow-up time (years)				
Median (IQR)	7.2 (5.6-8.6)	8.0 (6.4-9.0)	7.2 (5.6-8.6)	0.33

ADG: John Hopkins aggregate disease group comorbidity score; LHIN: local health integration network; IQR: interquartile range; PSA: prostate specific antigen; RUB: resource utilization band; SD: standardized difference

Appendix 6. Univariable logistic regression analysis, stratified by baseline age

Univariable Analysis (DECADE 1: 40-49 years)

Variable	OR (95%CI)	p-value	AIC	C-statistic	R^2
Baseline PSA>p25	107.45 (26.82-430.55)	<0.001	7322	0.625	0.004
Baseline PSA>median	88.56 (42.07-186.39)	<0.001	6877	0.744	0.008
Baseline PSA>p75	91.22 (57.77-144.02)	<0.001	6192	0.854	0.015
Baseline PSA>p90	101.57 (75.19-137.21)	<0.001	5440	0.908	0.022
Baseline PSA >1	104.77 (60.49-181.46)	<0.001	6353	0.833	0.013
Baseline PSA >1.5	99.36 (71.54-137.99)	<0.001	5594	0.902	0.021
Baseline PSA >2	102.61 (80.07-131.50)	<0.001	5179	0.908	0.025
Final PSA >2.0	307.04 (202.37-465.86)	<0.001	4816	0.942	0.942
Final PSA >3.0	293.90 (227.52-379.65)	<0.001	4148	0.933	0.035
Final PSA >4.0	238.77 (198.17-287.69)	<0.001	4399	0.863	0.032
Final PSA >5.0	168.24 (141.23-200.42)	<0.001	5398	0.752	0.023

Univariable Analysis (DECADE 2: 50-59 years)

Variable	OR (95%CI)	p-value	AIC	C-statistic	R^2
Baseline PSA>p25	67.27 (43.34-104.39)	<0.001	37503	0.624	0.010
Baseline PSA>median	59.60 (46.64-76.16)	<0.001	34849	0.740	0.022
Baseline PSA>p75	55.49 (48.07-64.05)	<0.001	30942	0.846	0.040
Baseline PSA>p90	47.50 (43.50-51.87)	<0.001	28056	0.872	0.054
Baseline PSA >1	62.98 (50.60-78.39)	<0.001	33812	0.774	0.027
Baseline PSA >1.5	54.38 (47.42-62.36)	<0.001	30640	0.852	0.042
Baseline PSA >2	48.55 (43.84-53.77)	<0.001	28879	0.873	0.050
Final PSA >2.0	148.38 (123.87-177.74)	<0.001	27512	0.897	0.056
Final PSA >3.0	184.46 (161.53-210.64)	<0.001	23073	0.931	0.076
Final PSA >4.0	167.30 (152.22-183.89)	<0.001	21236	0.915	0.084
Final PSA >5.0	96.98 (90.15-104.33)	<0.001	24901	0.833	0.068

Univariable Analysis (DECADE 3: 60-69 years)

Variable	OR (95%CI)	p-value	AIC	C-statistic	R^2
Baseline PSA>p25	39.78 (30.03-52.71)	<0.001	46828	0.622	0.018
Baseline PSA>median	34.56 (29.61-40.34)	<0.001	43239	0.737	0.041
Baseline PSA>p75	25.29 (23.26-27.49)	<0.001	39230	0.823	0.066
Baseline PSA>p90	18.16 (17.16-19.23)	<0.001	39480	0.783	0.064
Baseline PSA >1	35.09 (29.23-42.11)	<0.001	44553	0.700	0.033
Baseline PSA >1.5	31.04 (27.47-35.08)	<0.001	41634	0.778	0.051
Baseline PSA >2	25.90 (23.65-28.36)	<0.001	39855	0.814	0.062
Final PSA >2.0	64.19 (55.65-74.05)	<0.001	38466	0.828	0.070
Final PSA >3.0	80.96 (72.44-90.47)	<0.001	33442	0.887	0.100
Final PSA >4.0	81.03 (74.50-88.14)	<0.001	30254	0.900	0.119
Final PSA >5.0	55.46 (52.00-59.14)	<0.001	31924	0.854	0.109

Univariable Analysis (DECADE 4: 70-75 years)

Variable	OR (95%CI)	p-value	AIC	C-statistic	R^2
Baseline PSA>p25	29.20 (19.17-44.48)	<0.001	15254	0.618	0.020
Baseline PSA>median	20.16 (16.34-24.87)	<0.001	14263	0.725	0.042
Baseline PSA>p75	16.55 (14.62-18.74)	<0.001	13206	0.798	0.064
Baseline PSA>p90	11.43 (10.40-12.56)	<0.001	13816	0.727	0.051
Baseline PSA >1	26.55 (18.99-37.11)	<0.001	14950	0.653	0.027
Baseline PSA >1.5	21.90 (17.54-27.34)	<0.001	14266	0.723	0.042
Baseline PSA >2	20.24 (17.02-24.08)	<0.001	13717	0.768	0.053
Final PSA >2.0	40.72 (32.00-51.81)	<0.001	13347	0.781	0.061
Final PSA >3.0	43.68 (36.47-52.33)	<0.001	12105	0.844	0.087
Final PSA >4.0	50.14 (43.23-58.16)	<0.001	11023	0.874	0.109
Final PSA >5.0	42.57 (37.78-47.97)	<0.001	10868	0.862	0.112

Appendix 7. Final multivariable models, stratified by baseline age group

Primary outcome	**R^2**	**Max rescaled R^2**	**AIC**	**BIC**	**C-statistic**
40-49 years	0.039	0.532	3678	3744	0.975
50-59 years	0.091	0.532	19485	19556	0.959
60-69 years	0.134	0.487	27476	27546	0.938
70-75 years	0.132	0.436	9833	9894	0.919
Secondary outcome					
40-49 years	0.020	0.527	1825	1892	0.986
50-59 years	0.051	0.489	12193	12265	0.969
60-69 years	0.088	0.456	18723	18793	0.948
70-75 years	0.097	0.424	7023	7083	0.933

Akaike Information Criterion (AIC); Bayesian Information Criterion (BIC)

Appendix 8. Subgroup and sensitivity analyses

A) Multivariable logistic regression model predicting the diagnosis of any prostate cancer diagnosis, limited to patients enrolled between 2010 and 2012 (AUC=0.95)

Variable	OR (95%CI)	p-value
First PSA above 2.0 ng/mL	6.94 (6.37-7.57)	<0.001
Final PSA category		
<4.0	1.0	
4.0-9.9	20.94 (19.38-22.62)	<0.001
10.0-20.0	40.90 (36.85-45.40)	<0.001
Change in PSA from baseline per 365 days		
<0% (reference)	1.0	
0-19.9%	0.85 (0.80-0.91)	<0.001
20-99.9%	3.96 (3.70-4.23)	<0.001
>=100%	11.14 (9.92-12.52)	<0.001

PSA: prostate specific antigen; OR: odds ratio; CI: confidence interval

B) Multivariable logistic regression model predicting the diagnosis of any prostate cancer diagnosis, limited to patients enrolled between 2013 and 2015 (AUC=0.95)

Variable	OR (95%CI)	p-value
First PSA above 2.0 ng/mL	12.84 (9.91-16.65)	<0.001
Final PSA category		
<4.0	1.0	
4.0-9.9	20.42 (17.28-24.15)	<0.001
10.0-20.0	56.30 (46.06-68.82)	<0.001
Change in PSA from baseline per 365 days		
<0% (reference)	1.0	
0-19.9%	0.85 (0.75-0.97)	<0.001
20-99.9%	2.14 (1.91-2.40)	<0.001
>=100%	3.01 (2.55-3.56)	<0.001

PSA: prostate specific antigen; OR: odds ratio; CI: confidence interval

C) Multivariable logistic regression model predicting the diagnosis of any prostate cancer diagnosis, limited to patients 65 years of age and older without 5-ARI use (AUC=0.94)

Variable	OR (95%CI)	p-value
First PSA above 2.0 ng/mL	4.55 (3.88-5.35)	<0.001
Final PSA category		
<4.0	1.0	
4.0-9.9	20.67 (17.95-23.80)	<0.001
10.0-20.0	59.06 (49.80-70.05)	<0.001
Change in PSA from baseline per 365 days		
<0% (reference)	1.0	
0-19.9%	0.83 (0.75-0.93)	<0.001
20-99.9%	3.69 (3.32-4.10)	<0.001
>=100%	7.66 (6.42-9.14)	<0.001

PSA: prostate specific antigen; OR: odds ratio; CI: confidence interval

Appendix 9. Exploring the potential impact of verification bias on the sensitivity and specificity estimates

	Original Dataset		**Adjusted for Verification Bias**	
Predictor	*Sensitivity*	*Specificity*	*Sensitivity*	*Specificity*
OVERALL POPULATION				
First PSA > 1.0 ng/mL	0.98	0.53	0.76	0.62
First PSA > 1.5 ng/mL	0.95	0.71	0.52	0.77
First PSA > 2.0 ng/mL	0.91	0.80	0.34	0.84
Final PSA > 2.0 ng/mL	0.97	0.78	0.69	0.85
Final PSA > 3.0 ng/mL	0.94	0.89	0.49	0.92
Final PSA > 4.0 ng/mL	0.88	0.94	0.25	0.96
Final PSA > 5.0 ng/mL	0.74	0.96	0.12	0.97
<60 YEARS OF AGE AT BASELINE				
First PSA > 1.0 ng/mL	0.98	0.61	0.72	0.68
First PSA > 1.5 ng/mL	0.94	0.79	0.42	0.83
First PSA > 2.0 ng/mL	0.89	0.88	0.24	0.89
Final PSA > 2.0 ng/mL	0.97	0.86	0.62	0.90
Final PSA > 3.0 ng/mL	0.93	0.94	0.32	0.95
Final PSA > 4.0 ng/mL	0.85	0.97	0.14	0.98
Final PSA > 5.0 ng/mL	0.66	0.98	0.06	0.98
≥65 YEARS OF AGE AT BASELINE WITHOUT 5-ARI USE DURING THE STUDY PERIOD				
First PSA > 1.0 ng/mL	0.98	0.39	0.84	0.56
First PSA > 1.5 ng/mL	0.96	0.57	0.67	0.71
First PSA > 2.0 ng/mL	0.93	0.69	0.49	0.78
Final PSA > 2.0 ng/mL	0.97	0.65	0.78	0.81
Final PSA > 3.0 ng/mL	0.95	0.80	0.59	0.90
Final PSA > 4.0 ng/mL	0.91	0.89	0.36	0.94
Final PSA > 5.0 ng/mL	0.81	0.93	0.19	0.96