2014

Beyond prostate-specific antigen: alternatives for prostate neoplasm screening

Yu, Kevin Kuo-Han

http://hdl.handle.net/2144/15307
Boston University
BEYOND PROSTATE-SPECIFIC ANTIGEN:
ALTERNATIVES FOR PROSTATIC NEOPLASM SCREENING

by

KEVIN K. YU
B.A., Cornell University, 2011

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science
2014
ACKNOWLEDGMENTS

I would like to take the time to thank all of those who have helped me through the past two years as I pursued my goal. Dr. J. Fernando Garcia-Diaz for his steadying guidance that has been invaluable in my two years with the GMS program and on this thesis. Without his limitless patience and guidance, I wouldn’t have accomplished as much as I have. I will always remember his fantastic sense of humor, without which I wouldn’t have been able to succeed during the renal section in physiology.

Throughout the past two years, Dr. Gwynneth D. Offner has been the rock at the center of the MAMS program. Under your guidance, I learned not only the science of human medicine, but also about myself. Thank you for giving me this opportunity and helping me succeed in both the program and on this thesis.

Thank you to those friends who have accompanied me in the program. We struggled together, we supported each other, and we succeeded together. Keep going, we are almost there!

To my brother and sister: Ken and Karen, you are my guidance and my closest friends. Even though we are separated by large distances, you have always been there for me. To my parents, thank you for being my constant source of encouragement. I am grateful beyond words for the love and support you have given me throughout the years despite being so far away from me.

Finally to my fiancé Vivian, thank you for being there for me throughout the past 5 and a half years. Your unwavering support never failed to keep me going even during the most difficult hardships. I can’t imagine my life without your love and friendship.
BEYOND PROSTATE-SPECIFIC ANTIGEN

ALTERNATIVES FOR PROSTATIC NEOPLASM SCREENING

KEVIN K. YU

ABSTRACT

Prostate adenocarcinoma (PCa) is one of the most prevalent cancers in the world. Second only to lung cancer, the key to its successful treatment is in its early detection. With the introduction of prostate-specific antigen in the early 1990s, a screening test involving measuring levels of this protein was developed to detect PCa in asymptomatic individuals. This test is also known as the PSA test. PCa-specific mortalities have been in decline since the test’s introduction.

Despite this decline, recent studies have called the efficacy of the PSA test into question. Two large randomized controlled trials conducted in the US and Europe reveal contradicting results as to PSA’s accuracy and usefulness. Concerns of overdiagnosis and overtreatment as the result of using PSA screening has led to many national organizations recommending caution or even recommending against its use. Through a thorough review of a large collection of current PCa literature, this study reviews the flaws of using PSA to screen for PCa and investigates alternative approaches currently being pursued through active research to make PCa early detection more accurate. These approaches include improving the accuracy of the PSA screen using PSA-derived testing methods, using PCa-induced epigenetic modifications as a new target for PCa screening, and using urine biomarkers. All of these methods were compared using area under the curve (AUC) values obtained via receiver operating characteristic analysis.
Each method has its own flaws but by comparing each of the different approaches, I was able to conclude that out of the currently available screening methods, screening for Engrailed-2 protein in urine is the most promising screening method with the highest AUC values compared to the other methods. Although this method has been introduced in the UK, it has not been introduced in the US yet. Epigenetic screening methods hold the most promise for accurate PCa screening in the future as it confers the highest accuracy in detecting PCa. However, as it hasn’t been shown that epigenetic modifications can be easily obtained in the urine or blood serum for easy and accurate screening, I believe more work has to be done in order for it to be successful in being applied as a screening test. By determining the most promising screening type, we can focus resources and efforts towards finding a way to detect PCa early, allowing for successful treatment.
# TABLE OF CONTENTS

- TITLE .............................................................................................................................................. i
- COPYRIGHT PAGE ......................................................................................................................... ii
- READER APPROVAL PAGE ............................................................................................................ iii
- ACKNOWLEDGMENTS .................................................................................................................... iv
- ABSTRACT ......................................................................................................................................... v
- TABLE OF CONTENTS ................................................................................................................... vii
- LIST OF TABLES ............................................................................................................................. ix
- LIST OF FIGURES ........................................................................................................................... x
- LIST OF ABBREVIATIONS .............................................................................................................. xi

## INTRODUCTION

1. The Anatomy of the Prostate: ........................................................................................................ 1
2. Prostate Adenocarcinoma: .............................................................................................................. 3
3. Prostate-Specific Antigen: .............................................................................................................. 9
4. Concerns of PSA Screen Efficacy in Screening for PCa: ............................................................... 11

## PUBLISHED STUDIES

1. Comparing PSA Alternatives - Receiver Operating Characteristic and Area Under the Curve: ................................................................. 22
2. PSA-derived Alternative Screening Methods: .............................................................................. 24
III. PCa Screening Methods Beyond PSA: ................................................................. 37

DISCUSSION/CONCLUSION ......................................................................................... 50

REFERENCES ............................................................................................................... 55

VITA .............................................................................................................................. 71
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gleason Scores and Corresponding Risks to Patients</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Costs of Treatment for Prostate Adenocarcinomas</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>The Ability of [-2]proPSA to Predict GS of PCa</td>
<td>34</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Prostate Anatomy</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Prostate Adenocarcinoma Adjacent to Normal Prostate Tissue</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Gleason’s Pattern</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Prostate Cancer Incidence Rates by Race and Ethnicity in the US from 1999 to 2010</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Prostate Cancer Death Rates by Race and Ethnicity in the US from 1999 to 2010</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Age-specific PCa Incidence Rates Relative to 1986</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>Sample Receiver Operating Characteristics Curves</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>Immunohistochemical Staining of [-2]proPSA in PCa Tissue</td>
<td>32</td>
</tr>
<tr>
<td>9</td>
<td>[-2]proPSA Differentiation Between PCa of Different GS</td>
<td>36</td>
</tr>
<tr>
<td>10</td>
<td>Maintenance Methylation Versus de novo Methylation</td>
<td>39</td>
</tr>
<tr>
<td>11</td>
<td>Biology of <em>TMPRSS2:ERG</em> Fusion</td>
<td>47</td>
</tr>
</tbody>
</table>
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS</td>
<td>American Cancer Society</td>
</tr>
<tr>
<td>APC</td>
<td>Adenomatosis polyposis coli</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>AUA</td>
<td>American Urological Association</td>
</tr>
<tr>
<td>BPH</td>
<td>Benign prostatic hyperplasia</td>
</tr>
<tr>
<td>BPSA</td>
<td>Benign PSA</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>CMSP</td>
<td>Conventional MSP</td>
</tr>
<tr>
<td>DRE</td>
<td>Digital rectal examination</td>
</tr>
<tr>
<td>DNMT</td>
<td>DNA methyltransferases</td>
</tr>
<tr>
<td>EWP</td>
<td>Early Warning PSA</td>
</tr>
<tr>
<td>EN2</td>
<td>Engrailed-2 protein</td>
</tr>
<tr>
<td>ERG</td>
<td>Erythroblast Transformation-Specific-related gene</td>
</tr>
<tr>
<td>ERSPC</td>
<td>European Randomized study of screening for prostate cancer</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FHCRC</td>
<td>Fred Hutchinson Cancer Research Center</td>
</tr>
<tr>
<td>%fPSA</td>
<td>Free-to-total PSA</td>
</tr>
<tr>
<td>GS</td>
<td>Gleason Score</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Glutathione-s-transferase P1 enzyme</td>
</tr>
<tr>
<td>iPSA</td>
<td>Intact PSA</td>
</tr>
<tr>
<td>MSP</td>
<td>Methylation-specific PCR</td>
</tr>
</tbody>
</table>
NCI................................................................. National Cancer Institute

PPV .................................................................. Positive predictive value

PTGS2............................................................. Prostaglandin-endoperoxide synthase 2

PCa................................................................. Prostate Adenocarcinoma

PCA3.............................................................. Prostate Cancer Antigen 3

PCPT............................................................... Prostate cancer prevention trial

PHI..................................................................... Prostate Health Index

PLCO.................................................................. Prostate, lung, colorectal, and ovarian

PSA.................................................................. Prostate-specific antigen

RTC.................................................................. Randomized control trials

ROC................................................................. Receiver operating characteristics

TMPRSS2........................................................ Transmembrane-serine protease 2

USPSTF......................................................... United States Preventive Services Task Force

UMICH............................................................... University of Michigan
INTRODUCTION

Prostate cancer is one of the most prevalent cancers worldwide. It has been estimated in various developed countries that the number of new prostate cancer cases eclipsed that of lung and bronchus cancer in 2011 and is one of the leading cancer-related causes of mortality and morbidity in the United States. According to the American Cancer Society (ACS), 217,730 cases are diagnosed each year with 63% of prostate cancer cases occurring in men over age 65 (“What is prostate cancer?,” 2014). One in seven men will be diagnosed with prostate cancer during his lifetime (“What is prostate cancer?,” 2014). It is predicted by the ACS that in 2014, 233,000 new cases of prostate cancer will be diagnosed and about 29,480 men will die of prostate cancer (“What is prostate cancer?,” 2014). With the aging population of the US, the number of elderly men who are affected by prostate cancer will likely increase (Wiener & Tilly, 2002). Like in most other neoplasms, the key to the successful treatment of prostatic neoplasms is in their early detection and treatment. As such, there has been a push to develop screening methods in an effort to detect prostate cancer early.

I. The Anatomy of the Prostate:

The prostate gland is a dense organ that surrounds the urethra distal to the urinary bladder and ventral to the rectum. Slightly larger than a walnut at around 2 cm x 3 cm x 4 cm and weighing around 20 grams, it is part of the accessory sex gland system in human males that synthesizes and secretes both organic and inorganic components of the seminal fluid believed to be important for sperm survival in the female reproductive tract.
The anatomy of the prostate is divided into 4 distinct zones that were first described by Dr. John E. McNeal in 1968 (Costello & Corcoran, 2013). The four zones that comprise the prostate are the peripheral zone, the central zone, the transitional zone, and the periurethral glands. The main glands of the prostate are found within the peripheral zone whereas the submucosal and mucosal glands are within the central and transitional zones respectively. Each of the zones occupy 75%, 25%, 5-10%, and <1% of the prostate respectively (Costello & Corcoran, 2013).

The majority of the diagnosed cases (95%) of prostate cancer are adenocarcinomas resulting from the glands from the peripheral zone (Shevchuk & Robinson, 2013). Although there are other types of prostate cancers that originate from other cell types, they are a minority of prostate cancer cases and are out of the scope of this particular study.
II. **Prostate Adenocarcinoma:**

Prostate Adenocarcinoma (PCa) is a cancer originating from the glands of the prostate that predominantly affects men older than 50 years of age. Histologically, it is characterized by changes in the nucleus of glandular cells with increases in cell density and nuclear heterochromativity (Shevchuk & Robinson, 2013). Physically, it is characterized by changes in size, shape, or texture of the prostate. Symptoms of PCa include urinary urgency, hematuria, impotence, frequent need to urinate at night (nocturia), and loss of bladder or bowel control due to advance stages of the cancer applying pressure on the spinal cord (Mayo Clinic Staff, 2013). More advanced stages of PCa are often characterized by pain in the hips, spine, and ribs due to metastasis and possible weakness or numbness in the legs or feet (“What is prostate cancer?,” 2014).

![Figure 2 – Prostate Adenocarcinoma Adjacent to Normal Prostate Tissue](image)

This figure depicts the histological differences between PCa and normal prostate tissue. The left side of the figure is abnormal prostate tissue typical of PCa while the right side of the figure shows normal prostate tissue. Note the increased cell density in the PCa tissue where glandular spaces are small compared to normal tissue.

A. Gleason Scoring System for Prostate Cancer:

In order to assess the prognosis of PCa, the cancers are graded based on a scoring system called the Gleason scale. It utilizes a 2 to 10 scale system where lower grade cancers are less dangerous to patients while higher grade cancers are more deadly. The score is determined based on the appearance of the cells in histological sections obtained during prostate biopsies. These patterns are graded on a 1 to 5 system where a well-differentiated pattern is given the score of 1 and the most poorly-differentiated pattern is given the score of 5 (Figure 3). Pathologists first assign a primary grade that reflects the dominant pattern observed (>50% of the tissue) then add a secondary grade that reflects the pattern observed in the remainder of the tissue (<50%) (Humphrey, 2004). This number is the final Gleason score (GS) that physicians use to determine treatment modalities. GS and their respective risks to the patient can be found in the table below ("What is prostate cancer?," 2014).

<table>
<thead>
<tr>
<th>Gleason Score</th>
<th>Description of PCa</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6</td>
<td>Low-grade, well differentiated, and slow growing</td>
<td>Low risk</td>
</tr>
<tr>
<td>7</td>
<td>Intermediate grade and moderately differentiated. Need to be assessed with PSA level with tumor volume.</td>
<td>Moderate risk</td>
</tr>
<tr>
<td>&gt;7</td>
<td>High grade and poorly differentiated. Aggressive cancers that are hard to treat and have a higher chance of recurrence.</td>
<td>High risk</td>
</tr>
</tbody>
</table>

*This table displays the risk stratification of PCa using Gleason scores important in aiding physicians in deciding whether or not treatment is warranted. A Gleason score of 7 can fall in either moderate or high risk depending on the primary pattern.
B. Prostate Cancer Risk Factors:

There are a multitude of risk factors associated with PCa. The most significant of these is age with an increased incidence of PCa in men older than 50. According to the Center for Disease Control (CDC), the percentage of 40-year old men who develop PCa within 10 years is around 0.34%. On the other hand, the percentage of men at an age of 50 who develop PCa within the next 10 years increases 6.8 fold to 2.31%. It has been
shown that 50% of men in the 70-80 age group show histological signs of PCa (“CDC - Prostate Cancer Risk by Age,” 2013).

PCa has also been characterized to run in families with a more than doubled increased risk if another male in the immediate family have or have had prostate cancer. This finding hints at possible genetic factors that lead to the development of PCa that are passed down through generations. It is suggested that changes in the BRCA1 or BRCA2 gene can increase risk of PCa but they only account for a small subset of cases overall (“What is prostate cancer?,” 2014).

Ethnicity and race are also significant risk factors (Figures 4 and 5). It has been suggested in 2011 through the combined data published by the CDC and the National Cancer Institute (NCI) in 2009, the incidence rate was highest in African American followed by Caucasian and Hispanic men with similar trends for mortality rates (“CDC - Prostate Cancer Rates by Race and Ethnicity,” 2013). The reason for the ethnic and racial discrepancies in the likelihood of having PCa is currently unknown.
Figure 4 – Prostate Cancer Incidence Rates by Race and Ethnicity in the US from 1999 to 2010:

The incidence rate of prostate cancer in the U.S. formulated from the combined data of the CDC and NCI. The incidence of prostate cancer is highest among Black Americans followed by Caucasians, Hispanics, Asian and Asian Pacific Islanders, and American Indian/Alaskan Natives. Note the higher prostate cancer incidence rates in the Black population and the lower prostate cancer incidence rates in Asians and Pacific Islanders compared to the other races and ethnicities.

Figure 5 – Prostate Cancer Death Rates by Race and Ethnicity in the US from 1999 to 2010:

The death rates of prostate cancer are highest among Black Americans followed by Caucasians, Hispanics, American Indian/Alaskan Natives, and Asian and Asian Pacific Islanders.

III. Prostate-Specific Antigen:

Prostate-Specific Antigen (PSA) was first discovered in 1979. A member of the tissue kallikrein family, PSA is a serine protease that is secreted into the lumen of prostate glands by the ductal and acinar epithelium. Its function is to cleave semenoglin I and II in the seminal coagulum to liquefy semen for greater sperm movement (Balk et al., 2003). PSA is a histiotypic product of the human prostate and, as implied by its function, is primarily found in semen. Small quantities of PSA is also found in the serum of healthy men.

The fundamental concept underlying screening tests for diseases differs from that of diagnostic tests. While diagnostic tests seek to identify a specific disease based on the symptoms that a patient is already presenting, a screen seeks to identify diseases in symptom-free individuals (Ilic et al., 1996). Prior to the discovery of PSA, PCa was primarily screened for via digital rectal examination (DRE), first proposed by Dr. Hugh Hampton Young in 1905 (Hilton, & Parekh, 2013). In a DRE a physician inserts a gloved finger into the rectum of the patient and physically feels for changes to the prostate anatomy (“What is prostate cancer?,” 2014). If a palpable change is discovered, the patient would then undergo a needle biopsy that obtains 4 or fewer tissue samples of his prostate to definitively diagnose PCa. This was associated with significant procedure-related morbidity. In addition to the morbidity associated with biopsies, due to the nature of the DRE, not all PCa can be felt and discovered as the DRE was limited to the surface of the dorsal side of the prostate (Ilic et al., 2011). As a result, significant numbers of PCa cases were left undetected and untreated until the diseased progressed to a more advanced
stage. At this point, patient prognoses were grim. There was a need for a more definitive way to screen for PCa.

In 1981, increased circulating levels of PSA were found in patients with PCa as the result of prostate neoplasm development (Kuriyama et al., 1981). This discovery and the tissue specific nature of PSA led to the Food and Drug Administration (FDA) approval of its use in 1986 to evaluate response to treatment of PCa that was screened via DRE and diagnosed via biopsies (“Prostate-Specific Antigen (PSA) Test - National Cancer Institute,” 2010). Ultimately, due to the relative simplicity of the test and its deemed usefulness at the time, the PSA blood test was approved by the FDA for use in PCa screening in conjunction with DRE in 1994 for asymptomatic men (“Prostate-Specific Antigen (PSA) Test - National Cancer Institute,” 2010).

As PSA screening helped physicians with the early diagnosis of PCa where active treatment may reduce PCa specific mortality, PCa specific mortality declined steadily since PSA’s introduction in the early 1990s. Two independent models from the Fred Hutchinson Cancer Research Center (FHCRC) and the University of Michigan (UMICH) have shown a decrease in PCa mortality that coincide with the introduction of PSA screening in the early 1990s (Etzioni et al., 2008). According to the FHCRC and UMICH models, PSA screening contributed 45% to 70% respectively of the decrease in PCa-specific mortalities up to the year 2000 since the start of its widespread use as a screening test (Etzioni et al., 2008). To this day, the PSA blood test measuring circulating PSA levels in a patient’s serum used in conjunction with the DRE remain the predominant screening method for PCa. Currently, PSA tests use a classical 4.0 ng/ml cutoff for
normal PSA levels and it is suggested men who have PSA levels above 4.0 ng/ml undergo biopsy to definitively determine PCa presence. The test is done every few years for normal-risk men above the age of 40 if requested by patients.

IV. Concerns of PSA Screen Efficacy in Screening for PCa:

The majority of the recent literature seems to be in agreement that while PSA testing is a good first step in the detection of the early signs of PCa, the test is limited in its specificity and sensitivity for the diagnosis of PCa and there is currently a lack of consensus about using the PSA test to screen for PCa in asymptomatic men (Thompson et al., 2004). A multitude of efficacy concerns and limitations has led many major urological and cancer organizations in the United States to advise caution when testing for PCa through PSA screening.

A. Unknown benefit in reducing PCa-specific mortalities:

In an effort to examine the usefulness of PSA testing in reducing PCa-specific mortalities for patients, various national organizations performed long-term randomized control trials (RCT) to determine whether PSA tests are truly able to decrease the chances of a man dying from PCa. According to the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer screening trial done in the United States by NCI, while the PSA test increased the incidence of PCa in a group of men randomly selected to undergo the PSA test versus the control group in the RTC, the rates of death from PCa between the two groups were the same (Andriole et al., 2012). Specifically, Andriole et al. (2012) found a statistically significant relative increase of 12% in the incidence of PCa detected while there was a statistically insignificant decrease in PCa-specific mortalities. The authors
attribute this slight decrease in PCa-specific mortalities to improvements of treatment modalities rather than directly arising from PSA screening.

On the other hand, the European Randomized Study of Screening for Prostate Cancer (ERSPC), a similar large scale RCT, showed a decreased rate of death from PCa in the group that was screened versus the control group with its 11-year follow up results (Schröder et al., 2012). Schröder et al. (2012) showed a 20% reduction in the risk of patients dying from PCa following PSA testing in the 55-69 age group who are most at risk for developing PCa. Although the finding seems positive, it comes with an important caveat: the same RCT in 2009 estimated 75.9% of men who underwent biopsy as a result of an elevated PSA value had a false positive PCa result due to the PSA test (Schröder et al., 2009). The contradicting evidence regarding the true effect of PSA testing on reducing PCa-specific mortalities and the high probability of false positive readings has called the efficacy of the PSA screen into question.

B. Other Prostate Abnormalities leading to Increases in PSA levels

Despite its primary use of detecting PCa, it is also important to note that PSA is prostate-specific but not PCa-specific. Levels of PSA are also found to rise as the result of various other prostate diseases (Van Neste et al., 2012). One of such diseases is Benign Prostatic Hyperplasia (BPH). This is an extremely common condition in older men with 1 in 4 men experiencing signs by the age of 55. By the age of 75, this increases to 1 in 2 men experiencing symptoms and signs (Mayo Clinic Staff, 2011). Like PCa, men with BPH exhibit an enlarged prostate. Patients suffering from BPH also experience urinary urgency and nocturia (Mayo Clinic Staff, 2011). Notwithstanding certain similarities
between the two conditions, it is important to differentiate between the two conditions as PCa can be aggressive and life-threatening whereas BPH is not. Because BPH is also characterized with an increase in PSA levels, it can be sometimes difficult to differentiate between PCa and BPH simply through a PSA blood test and a DRE without the aid of biopsies.

Prostatitis is another prostate condition that can cause a rise in levels of circulating PSA in a patient’s serum (Van Neste et al., 2012). Prostatitis covers a series of disorders that include bacterial infections to chronic conditions that are characterized by inflammation of the prostate. Prostatitis is a common condition that occurs in as much as 9% of men in their lifetimes and its symptoms vary and are hard to pinpoint (Roberts & Jacobsen, 2000). Patients suffering prostatitis often have enlarge prostates and can have elevated PSA levels that can confound PSA tests for PCa (Mayo Clinic Staff, 2014).

C. Possibility of overdiagnosis and overtreatment:

Since the introduction of PSA in the monitoring of patients treated for PCa in 1986, there has been a dramatic increase in the incidence of PCa. From 1986 to 2005, the incidence of PCa has increased by 26% relative to the incidence rates prior to 1986 (Welch & Albertsen, 2009). Of different age groups, the highest increases in incidence rates relative to 1986 occurred in the 20-49, 50-59, and 60-69 age groups with the 20-49 age group increasing more than 7-fold relative to incidence rates prior to 1986 with 50500 more men diagnosed with PCa (Figure 6). One might question why the authors chose to use 1986 as the base year when FDA approved of PSA screening in 1994. The
authors justify this choice through the argument that investigations into PSA screening begun in 1987 thus increasing the amount of PCa detected (Welch & Albertsen, 2009).

This increase in PCa detection through the implementation of PSA can be a double-edged sword. While the increased PCa detection can lead to a decrease in PCa-specific mortality, one must also recognize the possibility of increased overdiagnosis along with the increase in incidence. In fact, the ERSPC study estimate that the rate of overdiagnosis can be as high as 50% that may lead to overtreatment of indolent cancers that are found through the PSA screen (Schröder et al., 2009). These indolent cancers are currently difficult to differentiate from aggressive and malignant cancers. It was noted by Schröder et al. (2012) in the 11-year follow-up that in order to save one patient from a
clinically significant case of PCa, 1055 men would have to be screened and 37 cases of PCa would need to be detected. This leads to a potentially staggering amount of overtreatment for indolent cancers that are otherwise clinically insignificant and will not adversely affect the patient at which point the treatment will cause more harm than good. As a result, it is a concern that the widespread use of PSA testing will lead to increased amount of false-positives and cause unnecessary treatment-associated morbidities such as sepsis, bleeding, hospitalization, and anxiety in patients who are otherwise healthy (Truong et al., 2013).

**D. Concerns about accuracy of PSA tests:**

Even though PSA blood tests are the predominant way to screen for PCa, the exact ranges for normal and abnormal PSA levels that are indicative of PCa are still debated. Currently, the most commonly accepted upper bound normal PSA circulation levels is 4.0 ng/ml (Hayes & Barry, 2014). PSA circulation levels above 4.0 ng/ml is considered abnormal and a biopsy may be considered to definitively determine the presence of PCa.

Recently there have been a number of studies in the literature calling this upper bound into question. It has been found in the Prostate Cancer Prevention Trial (PCPT) that biopsies conducted on men who never had PSA levels above 4.0 ng/ml, some had malignant PCa when their PSA levels measured as low as 0.5 ng/ml (Thompson et al., 2004). This presents a particularly challenging hurdle for PSA screening as the currently accepted 4.0 ng/ml upper boundary level causes many locally contained but moderately differentiated cancers to be missed. According to the PCPT, 15% of men with PSA of 0
ng/ml to 4.0 ng/ml have PCa and of those, 15% have high Gleason grade disease (Thompson et al., 2004).

However, one cannot just arbitrarily lower the accepted 4.0 ng/ml boundary to simply increase the sensitivity of the screen as this will increase the number of people who are overdiagnosed with the disease. Because many other noncancerous prostate conditions like BPH and prostatitis as well as benign tumors can cause an increase in PSA levels, lowering the upper limit of normal PSA levels from 4.0 ng/ml may result in increasing number of false-positives as well as possible overdiagnosis and overtreatment. The United States Preventive Services Task Force (USPSTF) has estimated that if the lower bound was decreased to 2.5 ng/ml in an attempt to increase the sensitivity of the PSA screening test, it would result in a doubling of men in the 40-69 age group with abnormal PSA levels with the majority being false-positives (Moyer, 2012). This increase is undesirable as too many indolent cancers and noncancerous conditions will be falsely categorized as PCa and the resultant procedures to definitively diagnosis PCa will increase healthcare costs in addition to causing unnecessary harm to the patients. To this date, there is no PSA level cutoff that can guarantee any patient that they are free of PCa.

Additionally, if the PSA cutoff was lowered, one cannot reliably use biopsies to make up for the decrease in specificity of the PSA screening test. Because biopsy detection rates vary with the amount of samples taken per biopsy, there is a possibility that if too few tissue samples were taken that critical cancers can be missed. On the other hand, if a saturation biopsy procedure was employed, the problem with an increase in the
detection of indolent cancers would once again occur. As a result, the accuracy of PSA tests cannot be precisely determined (Moyer, 2012).

E. Costs associated with overdiagnosis and overtreatment:

The costs associated with overdiagnosis and overtreatment that result from the PSA blood test are not trivial. As positive PSA screen results are usually followed by a biopsy to definitively determine the presence of PCa, there are associated side effects that can negatively affect a patient. Side effects of needle biopsy following a positive PSA screen include hematospermia, hematuria, pain, fever, urinary retention, and prostatitis or urosepsis (Ilic et al., 2011). The percentage of men affected by these side effects are 50.4%, 22.6%, 7.5%, 3.5%, 0.4%, and 0.5% respectively (Ilic et al., 2011). About 20% of the men who have had needle biopsies view future biopsies negatively and 1.4% were hospitalized for complications that have resulted from needle biopsies (Moyer, 2012).

Beyond these immediate side effects, it has been shown that as many as 90% of the patients who tested positive in the PSA test have early treatment that includes radiation therapy and/or surgery despite the fact that the tumor may be indolent and not cause them any harm (Moyer, 2012). These patients are then subject to the side effects of these treatments including erectile dysfunction, urinary incontinence, and bowel dysfunction (“Prostate-Specific Antigen (PSA) Test - National Cancer Institute,” 2010). Since PSA screening has the high potential to overdiagnose (as much as 50%), it is reasonable to infer that many of the patients unnecessarily suffer these side effects as a result of interventions targeting PCa.
Other than physical costs associated with overdiagnosis and overtreatment, monetary costs incurred after a positive PSA screening test are also major factors that need to be considered in regards to using PSA to screen for PCa. In the year 2000, the cost associated with PCa diagnosis and treatment accounted for $1.3 billion in American health expenditure (Shteynshlyuger & Andriole, 2011). Treatment costs range from $20,000 to $50,000 depending on the procedure and the patient’s risk level and include radiation treatments and surgeries (Table 2) (Cooperberg et al., 2013). Since the inception of PSA screening tests, it has been estimated that the number of men diagnosed with PCa has increased by 1.3 million. Of these 1.3 million, 56500 deaths had been averted due to PCa screening (Welch & Albertsen, 2009). In Europe, the estimated cost of overdiagnosis and overtreatment amounts to 39% of the total cost of screening and treatment. This results in over €23 million ($33 million) spent on screening and treatment of patients that do not benefit from the screening or the treatments (Heijnsdijk et al., 2009). In the U.S., the amount of money spent as a result of overtreatment of PCa was estimated to be around $32 million per annum, which is comparable to the estimate in Europe (Aizer et al., 2013).
Beyond the tangible costs of PSA screening leading to false positives, overdiagnosis, and overtreatment, there are also intangible costs of PSA screening. Namely, negative psychological effects on patients and their family members as a result of a positive PSA screening result. It has been found up to 49% of the patients who have had a positive screening outcome that ultimately resulted in a benign biopsy reported thinking about PCa more than a control group that had a normal PSA test result (Ford et al., 2005). These patients have a higher perceived risk of PCa and have reported problems with sexual function for up to a year (Moyer, 2012). Also, it has been reported that patients are less likely to return to be screened again after a false-positive result. This

<table>
<thead>
<tr>
<th>Radiation Procedures</th>
<th>Low Risk</th>
<th>Moderate Risk</th>
<th>High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>External Beam Radiation Therapy + Brachytherapy</td>
<td>$40,588</td>
<td>$43,566</td>
<td>$50,276</td>
</tr>
<tr>
<td>Brachytherapy</td>
<td>$25,067</td>
<td>$32,553</td>
<td>$43,952</td>
</tr>
<tr>
<td>3D Conformal Radiation Therapy</td>
<td>$27,626</td>
<td>$30,838</td>
<td>$42,397</td>
</tr>
<tr>
<td>Intensity-Modulated Radiation Therapy</td>
<td>$37,718</td>
<td>$44,639</td>
<td>$53,539</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surgical Procedures</th>
<th>Low Risk</th>
<th>Moderate Risk</th>
<th>High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Radical Prostatectomy</td>
<td>$20,245</td>
<td>$28,589</td>
<td>$36,279</td>
</tr>
<tr>
<td>Robot-Assisted Radical Prostatectomy</td>
<td>$19,901</td>
<td>$28,017</td>
<td>$35,014</td>
</tr>
<tr>
<td>Laparoscopic Radical Prostatectomy</td>
<td>$20,497</td>
<td>$29,041</td>
<td>$35,118</td>
</tr>
</tbody>
</table>

*This table lays out the costs of various procedures that are used in the treatment for PCa. Depending on the assessed risk level of the patients, different treatment modalities are employed. Low risk to moderate risk patients are preferentially treated with surgical procedures while those of higher risk are preferentially treated with radiation. Because of possible overdiagnosis and overtreatment due to PSA screening, a lot of costs associated with the treatment of PCa using these procedures do not confer benefits to a vast majority of the patients treated.

could result in the failure to diagnose PCa that may develop and progress to more advanced stages leading to higher costs for treatments and more adverse physical consequences (Ford et al., 2005).

F. Current Recommendations for PSA testing:

As a result of the disadvantages discussed earlier, many large American medical organizations currently caution the use of PSA screening in men. Recanting on its 2008 recommendation of screening on in men between 45 and 75 years old, the USPSTF now recommends against PSA screening in men of all ages (Moyer, 2012). The American Academy of Family Physicians follows in the USPSTF’s footsteps in recommending against the use of PSA-based screening in all age groups (“Prostate Cancer -- Clinical Recommendations -- AAFP,” 2014).

Other organizations are less absolute in their recommendations. The American Urological Association (AUA) recommends PSA screening in men older than 40 only if the patients request to be screened (Carter et al., 2013). In such cases, the doctor and patient need to discuss the benefits and the harms related to screening. The ACS recommends informed decision making when it comes to PCa screening and men with average risk should be presented with the relevant information at 50 (Wolf et al., 2010). The American College of Preventative Medicine’s (ACPM) recommendation is similar to that of the ACS. The ACPM concludes that there is insufficient evidence that PSA-based screening supported by DRE to recommend routine screening. It advises informed decision making between doctors and patients when determining the need for PSA screening (Lim, Sherin, & ACPM Prevention Practice Committee, 2008). The American
College of Physicians states that men between the age of 50 and 69 years should be informed for the limitations of the PSA-based screening test for PCa and recommends against screening in average-risk men below the age of 50, above the age of 69, or have a life expectancy of less than 10-15 years (Qaseem et al., 2013).
While PSA-based screening test was a great first step in detecting PCa early, the test is limited by a number of considerable drawbacks and the true benefits incurred by its use are uncertain. It is also important to realize that while PSA is a prostate-specific marker, it is not PCa-specific and can be elevated during a whole host of other non-neoplastic diseases. From previous studies, it can be concluded that the test alone is considered inadequate in the early screening and detection of PCa. This conclusion is supported by the recommendations of various national organizations that caution physicians in the use of PSA test or outright recommend against it. However, despite these recommendations, PCa remains one of the most prevalent cancers affecting older American men. As a result, there is a need to either improve upon the PSA-based screening or to find alternatives for screening for PCa.

I. Comparing PSA Alternatives - Receiver Operating Characteristic and Area Under the Curve:

In order to assess the ability to predict clinically relevant PCa of different PSA-derived screening methods, one needs a way to compare the ability for a specific test to predict the presence of PCa with those of other screening methods. A receiver operating characteristics (ROC) graph is the best way to achieve this as it assesses a screening test’s ability to differentiate between normal conditions and abnormal conditions. A ROC curve plots the sensitivity (true positive rate) vs 1-specificity (false positive rate) of a particular screening test (Figure 7). The area under the curve (AUC) is then calculated to measure
the accuracy of the screening test. An AUC of 1.0 represents a perfect test that can
differentiate between normal and abnormal conditions every time. An AUC of 0.5
represents a worthless test, one that has as good of a chance at predicting disease
presence as random chance. In essence, AUC measures the probability that a screen test
will rank a randomly chosen positive test higher than a randomly chosen negative test
(Thomas G. Tape, n.d.). It also shows the tradeoff between the sensitivity and the
specificity of a particular test. As it takes all possible sensitivity and specificities
combinations into account, possible optimal cutoff points can be deduced for specific
tests. These factors make ROC curves and the AUC values the ideal statistic for
interpreting the accuracy of various PCa screening tests.

Figure 7 – Sample Receiver Operating Characteristics Curves:

The three curves depicted in this figure demonstrates what is considered excellent, good, or worthless tests. As the curve approaches a true positive rate of 1 and a false positive rate of 0, its area-under-the-curve (AUC) increases and the performance of the test increases. A large AUC is indicative of a good test. The maximum AUC value attainable is 1.

Another statistic that is frequently used in the assessment of a test’s performance is the positive predictive value (PPV). PPV is the fraction of positive results of screening tests that are true positive results:

\[
PPV = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false positives}}
\]

While this may seem like an attractive test to use in the assessment and comparison of various screening methods, it is actually not as accurate as AUC in assessing performance of various tests. This is due to the fact that PPV is not an intrinsic statistic in tests and is heavily influenced by the prevalence of the disease in the test population that is being screened (Lengerich, 2014). As such, varying conditions and test parameters can result in different PPV values. Because of the heterogeneous nature of PCa, the AUC value obtained from ROC analysis is a more attractive statistic to use when comparing different screening methods because it is not influenced by the different prevalence of the disease in different study cohorts across various studies and is a stable characteristic.

II. **PSA-derived Alternative Screening Methods:**

Currently, there are multiple avenues being investigated for improving PSA-based screening methods. These methods include proposing alternative PSA cutoff ranges, measuring free-to-total PSA (also known as %fPSA), proPSA, PSA density, PSA velocity, PSA acceleration, and PSA doubling time. Out of these various methods to improve upon the traditional PSA test, alternative PSA cutoff ranges, %fPSA, and proPSA are the most promising avenues for improving the PSA screening test.
A. Establishing an Early Warning PSA Zone:

As previously mentioned, although the currently accepted upper bound for normal PSA levels is 4.0 ng/ml, this cutoff has been shown to miss potentially life-threatening cancers that have lower PSA levels (Thompson et al., 2004). This presents a conundrum when considering the use of PSA screening to screen for PCa. If the cutoff level is lowered below 4.0 ng/ml, the sensitivity of the test is increased at the risk of decreasing the specificity of the test. This could result in an increase in the amount of false-positives that lead to a surge of overdiagnosis and overtreatment that would in turn result in considerable harm to patients who otherwise have indolent tumors or noncancerous prostate conditions. This presents a fundamental flaw in the screening test in that it is extremely difficult to determine the aggressiveness of the cancer purely relying on the PSA test.

Instead of using the classical cutoff of 4.0 ng/ml and beginning screening at around age 50, some researchers advocate for the establishment of an Early-Warning PSA (EWP) zone between 1.5 ng/ml to 4.0 ng/ml for a patient’s first test at the age of 45 (Crawford et al., 2011). Crawford et al.’s (2011) reasoning behind this change is that the baseline PSA value measured at an earlier age may have some predictive value that can be used to predict the development of PCa and be used to stratify patients into different risk categories. In essence, by decreasing the age and the baseline PSA threshold, a new risk stratification strategy is introduced that relies on the predictive value of the baseline PSA value (Crawford et al., 2011). It has been demonstrated that Caucasian men with a baseline of greater than 1.5 ng/ml PSA levels develop a 15-fold increase in PCa risk over
men who have lower baseline levels for their initial PSA screening result. This risk is increased 19-fold in black men. When assessed with ROC analysis, it was found that using EWP zone to predict future diagnosis of cancer within 4 years of the test results in an AUC value of 0.87 (Crawford et al., 2011). The AUC values for a standard PSA screening test across all PSA levels range from 0.50-0.66 (Filella & Giménez, 2012). The high AUC value of using EWP zone compared to traditional PSA suggests that the establishment of a baseline EWP zone can be a more powerful tool than simply screening using the 4.0 ng/ml cutoff.

A recent study conducted by Vertosick et al. takes this concept of EWP zones further. The researchers suggest that EWP zones are a better predictor for the risk of developing PCa than race or family history (Vertosick et al., 2014). According to a study conducted by Vickers et al. in 2013, it was determined that around 44% of PCa deaths by the age of 75 were in men who had a higher baseline PSA level of greater than or equal to 1.6 ng/ml at ages between 45 and 49 (Vickers et al., 2013). Vertosick et al. (2014) determined that patients with family history represent 25% of PCa deaths and that patients of Black descent represented 28% of PCa deaths. Thus, they argue that EWP zones offer better risk stratification than relying on race and family history and can be used to determine patients who need more frequent and earlier screening.

Regardless of the advantages shown by Crawford et al. and Vertosick et al., lowering PSA cutoff ranges could result in an increase in overdiagnosis, overtreatment, and medical financial burden for patients and the US healthcare system. In order to combat this potential issue, Crawford et al. (2011) suggest that men with lower baseline
PSA levels be screened less frequently than men with higher baseline PSA levels. The researchers argue that by targeting high risk individuals identified by the EWP zones, the total number of PSA tests conducted in the US can be reduced as much as 70% and save around $1 billion annually (Crawford et al., 2011). However, even with these assurances, the true effect of using EWP zones have yet to be tested and while this change to PSA screening seems promising, more studies are needed to determine its true effectiveness.

**B. Free-to-total PSA:**

Another promising improvement to the traditional serum PSA screening test is the measurement of free-to-total PSA (%fPSA) in addition to traditional PSA measurements. As the name suggests, %fPSA is a simple ratio between the measured levels of free PSA divided by the total serum PSA. The basis for using %fPSA is the fact that not all PSA molecules are free in the serum. Of the total PSA levels, around 70% are bound to the protease inhibitor α1-antichymotrypsin while the other 30% are free (Roddam et al., 2005). In men with PCa, the level of %fPSA is lower than those who do not have PCa (Lee et al., 2006). As a stand-alone test, %fPSA performs comparably to using the traditional PCa screening test measuring total serum PSA. According to a meta-analysis that analyzed the performance of %fPSA, as an independent test %fPSA has an ROC AUC value of 0.70 across all PSA levels (Lee et al., 2006). Comparatively, the AUC of PSA has a range from 0.50 to 0.66 (Filella & Giménez, 2012).

The usefulness of %fPSA comes in when it is being used as a reflex test. A reflex test is used as a confirmatory test when measured serum PSA levels are within the 4.0 ng/ml to 10 ng/ml, a range that is considered a diagnostic gray zone (Lee et al., 2006). It
is relatively difficult to definitively say whether a patient has PCa when his PSA levels fall into this range due to the fact that other prostate diseases can raise serum PSA to this level even in the absence of PCa. Since BPH and prostatitis have been shown to increase PSA levels but not change %fPSA, the difference in %fPSA levels allows for differentiation between the cancerous and noncancerous conditions in men who have borderline levels of serum PSA (Lee et al., 2011). This increases the accuracy of the PSA blood screening and can help avoid unnecessary biopsies.

%fPSA has also been suggested to be useful in circumstances when serum levels fall below 4.0 ng/ml. Although it is relatively rare for patients to develop PCa with PSA levels lower than 4.0 ng/ml, the PCPT has shown that there is no definite PSA level below which one can definitively say there is an absence of PCa (Thompson et al., 2004). In a study conducted in Brazil, it was demonstrated that the PPV of %fPSA in finding PCa in situations where PSA levels were less than 4.0 ng/ml was around 26.4% at %fPSA levels ≤15% (Faria et al., 2012). This finding suggests %fPSA’s usefulness in detecting PCa in PSA levels below 4.0 ng/ml.

These studies suggest %fPSA as a viable companion to the traditional serum PSA test that increases the overall performance of PSA screening. However, the test is not without its disadvantages. One of the major difficulties facing physicians when using %fPSA as a tool for PCa screening is that it is currently unknown what the optimal cutoff point is. Previous studies have suggested various cutoff points that correspond with various levels of serum PSA levels that range from 10% to 25% (Catalona et al., 1998; Lee et al., 2011). As a result, there is currently no standardized cutoff point for %fPSA.
use in PCa screening. In addition, as screening tests dip into PSA levels below 4.0 ng/ml, it is necessary to take into account the increased probability of overdiagnosis. Thus, although %fPSA seem to be able at detecting PCa at lower PSA levels, it is unknown whether %fPSA can differentiate between cancers of differing stages and aggressiveness accurately enough to be a viable screening tests for PCa at lower PSA ranges.

There have been efforts to help determine the optimal %fPSA level. One idea behind improving %fPSA is the use of a classification system based on genetics to enhance the clinical performance of %fPSA used alongside traditional PSA serum tests. It is argued that variability with serum PSA levels depend on genetic polymorphisms of certain genes. Zambon et al. (2012) tested this idea for the first time. The authors suggested that KLK3 polymorphisms could be a target for genetic categorization to enhance %fPSA performance (Zambon et al., 2012). The KLK3 gene encodes for PSA and has been previously implicated in changing serum PSA levels. The authors tested various different polymorphisms that included three SNPs in the promoter region and one polymorphism at the 3’ boundary of the KLK3 locus. It was found that the polymorphisms had statistically significant effects on the %fPSA level only and not on measured total serum PSA level in the context of screening for PCa (Zambon et al., 2012). Of the four groups that varied genetically, two groups had the optimal %fPSA cutoff of 11% while the other two groups had the optimal cutoff of 14.5% (Zambon et al., 2012). Since lower %fPSA levels were shown to correlate with a higher risk for PCa, it was reasonable to conclude that patients with less than 11% %fPSA be considered for further screening while those above 14.5% be considered negative for PCa. Those men
who fall within the 11%-14.5% range can then be genetically assessed to determine whether to undergo further testing based on the results (Zambon et al., 2012). While this result from Zambon et al. (2012) is encouraging, it is the first study to try to determine %fPSA cutoff points using genetic testing. More research will need to be conducted before the optimal cutoff point can be determined.

C. PSA Proenzymes:

Despite limitations of using %fPSA in screening for PCa, more recently it has been found that fPSA is actually comprised of various isoforms of PSA. These isoforms include Benign PSA (BPSA), intact PSA (iPSA), and proPSA (Le et al., 2010). These isoform show great promise for use in the screening for PCa. It has been shown previously that BPSA and iPSA are associated with noncancerous prostate conditions while proPSA is associated with PCa (Le et al., 2010). This finding exhibits the potential for using proPSA as a biomarker for screening for PCa. One of the main issues that plagued traditional PSA screening was that PSA was prostate-specific and not PCa-specific. As a result, it was sometimes difficult to differentiate between noncancerous prostate conditions like BPH and prostatitis with PSA screening alone. It was found by Heidegger et al. (2014) that [-2]proPSA levels, a proPSA isoform, was significantly higher in cancerous prostates than in benign prostates 4 years prior to PCa diagnosis (Heidegger et al., 2014). The introduction of a PCa specific biomarker that can distinguish between noncancerous and malignant prostate conditions addresses one of the greatest drawbacks of PSA screening: the inability to differentiate between PCa and BPH or prostatitis based on PSA levels alone.
ProPSA is found in three different forms and these vary by the length of the pro-leader peptide. Cleaved by kallikrein 2 and trypsin, [-7]proPSA yields active PSA. The other forms are [-5, -7]proPSA, [-4]proPSA, and [-2]proPSA (Le et al., 2010). Of all these different isoforms of proPSA, [-2]proPSA has been implicated in PCa. Upon histological staining of prostate tissue, [-2]proPSA was shown to be preferentially concentrated in PCa tissue (Figure 8) (Chan et al., 2003). Consequently, [-2]proPSA is regarded as a promising marker for PCa as it can help physicians identify PCa tissues even in presence of BPH or prostatitis.
In a multitude of multicenter trials, it has been shown that $[-2]\text{proPSA}$ can be used as a screening measure for PCa. $[-2]\text{proPSA}$ is calculated using the following equation:

$$\left(\frac{[-2]\text{proPSA}}{f\text{PSA}}\right) \times 100$$

Figure 8 – Immunohistochemical Staining of $[-2]\text{proPSA}$ in PCa Tissue:

Staining of PCa tissue using monoclonal antibody against $[-2]\text{proPSA}$. The preferential staining of PCa tissue for $[-2]\text{proPSA}$ can be seen on the left of the figure. Normal prostate tissue does not stain for $[-2]\text{proPSA}$.


In a multitude of multicenter trials, it has been shown that $[-2]\text{proPSA}$ can be used as a screening measure for PCa. $[-2]\text{proPSA}$ is calculated using the following equation:
In a meta-analysis, the performance of %-2proPSA had an AUC of 0.64-0.78 (Filella & Giménez, 2012). AUC values for a traditional PSA test range from 0.50-0.66 (Filella & Giménez, 2012). Although the range of AUC values for %-2proPSA suggests that %-2proPSA outstrips PSA screening alone in testing for PCa, it is debated whether %-2proPSA outperforms %fPSA. A study conducted by Loeb et al. showed that %-2proPSA significantly outperformed %fPSA (p<0.005) while Lazzeri et al. found that although %-2proPSA had a higher AUC value, it did not significantly outperform %fPSA (p>0.300) (Lazzeri et al., 2013; Loeb et al., 2013). A similar result by Sokoll et al. substantiates Lazzeri et al.’s finding (Sokoll et al., 2008). This difference could be due the heterogeneous nature of the tests performed and the population of the test cohort (Filella & Giménez, 2012).

Based on these results, a new test called the Prostate Health Index (PHI) was developed by the company Beckman Coulter and was recently approved by the FDA in 2012 for use to screen for PCa. The PHI is calculated using the following equation:

$$\%[-2]proPSA \times \sqrt{PSA}$$

The PHI has very similar AUC levels compared to %-2proPSA (0.67 to 0.77 and 0.64 to 0.78 respectively) but also takes into account of the total serum PSA levels (Filella & Giménez, 2012). The PHI test employs a blood test that is simple to conduct and is ideal for a clinical screening situation.

In addition to the promising predictive nature of %-2proPSA for PCa, absolute values of [-2]proPSA is also potentially useful in PCa screening tests. The absolute levels of [-2]proPSA was shown to not be an effective predictor for PCa by itself with. In a
study conducted by Lazzeri et al. (2013), the AUC of the ROC curve for absolute levels of [-2]proPSA was 0.51, which confers no useful screening information. A similar result was also found by Catalona et al. who found the AUC levels to be 0.56 (Catalona et al., 2011). Despite these findings, absolute values of [-2]proPSA could be useful in determining the aggressiveness of the cancer detected, a previously difficult issue to resolve based on screening alone. Heidegger et al. (2014) found that absolute levels of [-2]proPSA was associated with the GS of PCa. In this study, it was found that highly aggressive cancers with a GS≥8 could be distinguished from moderately aggressive cancers with a GS≤7 three years prior to PCa diagnosis via biopsy using absolute levels of [-2]proPSA (Heidegger et al., 2014). The [-2]proPSA values were significantly higher (p<0.001) for cancers classified as GS≥8 than those classified as GS≤7 as early as 3 years prior to diagnosis (Figure 9) (Heidegger et al., 2014). Heidegger et al.’s (2014) findings also suggested that [-2]proPSA was better than PSA alone in predicting the GS of PCa up to 4 years prior to diagnosis (Table 3).

Table 3 – The Ability of [-2]proPSA to Predict GS of PCa*:

<table>
<thead>
<tr>
<th>Years before PCa diagnosis</th>
<th>AUC of [-2]proPSA</th>
<th>AUC of PSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 years</td>
<td>0.80</td>
<td>0.61</td>
</tr>
<tr>
<td>3 years</td>
<td>0.78</td>
<td>0.55</td>
</tr>
<tr>
<td>2 years</td>
<td>0.74</td>
<td>0.60</td>
</tr>
<tr>
<td>1 years</td>
<td>0.85</td>
<td>0.53</td>
</tr>
</tbody>
</table>

*Absolute levels of [-2]proPSA has higher AUC levels when used to predict GS of PCa diagnosed via biopsy compared to that of just using PSA. This suggests that absolute [-2]proPSA can be used to differentiate between PCa of different aggressiveness.

Furthermore, [-2]proPSA levels can be used to distinguish between organ-confined PCa and non-organ-confined PCa. Organ-confined PCa is not as detrimental to the patient compared to non-organ-confined disease. As early as 4 years prior to diagnosis, there was a significant increase in [-2]proPSA for non-organ-confined PCa compared to the levels in organ-confined PCa (Heidegger et al., 2014). As such, absolute levels of [-2]proPSA can be extremely useful when screening for PCa in that it can help physicians identify aggressive cancers from those that are more unlikely to harm the patient. This can decrease the amount of overdiagnosis and overtreatment of patients who, instead of undergoing invasive procedures that can cause considerable harm to the patients, can be carefully observed to assess the risk level of indolent PCa cases.
One distinct disadvantage to the PHI test is that it is more costly to perform and is currently not covered by health insurance like Medicare. Based on the estimates from Beckman Coulter and Medicare Fee Schedule, one PHI test costs approximately $71.95 while PSA combined with %fPSA costs $53.70 (Nichol et al., 2012). However, in a study funded by Beckman Coulter, Nichol et al. (2012) argued that despite the higher costs, the overall screening for PCa could cost less when using PHI compared to PSA-fPSA.
combination through a reduction in overdiagnosis and overtreatment. While this claim may hold some validity, it still needs to be verified via independent studies.

Regardless of the promise of the [-2]proPSA biomarker for use in PCa screening, one must understand that the test is not perfect. Due to its relatively recent introduction as a test compared to PSA, the cutoff points for normal %[-2]proPSA and PHI levels are still debated (Filella & Giménez, 2012). Although approved by the FDA, its effectiveness over PSA at curbing overdiagnosis and overtreatment is still uncertain despite promising results from previous studies. With all these caveats in mind, the [-2]proPSA biomarker and its derivative index, PHI, are still promising avenues to improve the traditional PSA serum screening test due to its ability to not only improve upon PSA’s ability to predict the presence of PCa but also potentially differentiate between different grades of PCa.

III. **PCa Screening Methods Beyond PSA:**

As a result of the lack of agreement in the efficacy of the PSA test within the scientific literature, in addition to possibly improving PSA screens, there has been an interest in finding alternative screening methods for PCa. The recent literature highlights various possible avenues for pursuing new screening methods that offer alternatives to PSA screening. These alternative avenues include the screening for PCa with epigenetic markers and biomarkers present in urine.

**A. Epigenetic markers for use in PCa screening:**

Epigenetics involve the investigation of the meiotically and mitotically heritable changes in gene activity that occur independently of the changes in an organism’s primary DNA sequence (Choi et al., 2013). These changes include histone modifications
and DNA hyper- and hypo-methylation. All of these epigenetic modifications have been found to occur in cancer. However, of these three different types of epigenetic changes, DNA hypermethylation is best characterized in various types of cancers including PCa (Costa et al., 2007). DNA hypermethylation primarily occurs in mammals at cytosines within sequences of repeating cytosine and guanine nucleotides (Jerónimo et al., 2011). These repeating C and G sequences are called CpG islands and are frequently found in the 5’ regulatory regions of genes (Jerónimo et al., 2011). The methylation process is carried out by DNA methyltransferases (DNMTs) that add methyl groups to the fifth carbon of the cytosine residue ring and it has been shown that genes that are silenced often have hypermethylated CpG islands (Choi et al., 2013). The mechanisms through which methylated CpG islands silence genes range from preventing the binding of regulatory factors to affecting chromatin structure.

There are two distinct types of DNA methylations that occur during the course of a person’s life (Figure 10). One that is passed on during cell division and is passed down from one generation of cells to their daughter cells called maintenance methylation. As its name suggests, this type of DNA methylation maintains the DNA methylation profile of cells during cell division in order to maintain the cells’ specific function (Jones & Liang, 2009). The other type of methylation is where new methyl groups are introduced into CpG islands of genes by DNMTs via de novo methylation. The type of epigenetic change that occurs throughout development, aging, and in carcinogenesis are de novo methylation changes (Henrique & Jerónimo, 2004). Often times, the methylation silencing in cancer cells affects genes involved in key cellular processes that when
disrupted, can cause progression into cancer cells.

![Diagram of methylation changes](image)

**Figure 10 – Maintenance Methylation Versus de novo Methylation:**

Cytosine methylation in the promoter regions is a regulatory mechanism that is employed by cells. Cytosine methylation occurs on cytosine residues within CpG islands and fall into two categories: De novo methylation or maintenance methylation. Maintenance methylation occurs during cell division that maintains methylation patterns of genes while de novo methylation occurs on cytosine molecules that were not previously methylated. Both types of methylation are carried out by DNA methyltransferases.


Methylation changes are found via methylation-specific PCR (MSP) techniques that utilizes bisulfite-treated DNA. The use of bisulfite converts any unmethylated cytosine residues into uracil while keeping 5-methylcytosine residues unaffected. The DNA can then be probed with two sets of primers that are designed to amplify
methylated or unmethylated sequences of the DNA (Henrique & Jerónimo, 2004). This result is then run on a gel in the conventional MSP (CMSP). The results from CMSP can only determine if the DNA section in question is methylated but not the extent of the methylation. In order to measure the extent of the methylation, a real-time quantitative MSP can used. In this case, the DNA is amplified with two sets of primers while a fluorescent probe that can anneal to methylated sections are used to measure the extent of the methylation by comparing to reference genes such as MyoD1 or β-actin (Henrique & Jerónimo, 2004).

A relatively new field compared to the study of PSA, the study of PCa epigenetics has resulted in a number of promising epigenetic biomarkers that can be used in screening for PCa in the future. Of the more than 50 common aberrant DNA hypermethylation changes, three genes show particular promise in being used for PCa screening: GSTP1, APC and PTGS2.

1. Glutathione-s-transferase P1 gene:

GSTP1 encodes the glutathione-s-transferase P1 enzyme (GSTP1) that is part of a family of enzymes that protect DNA from carcinogens and reactive oxygen species by conjugating glutathione to these species (Henrique & Jerónimo, 2004). It is a caretaker gene that acts to protect the prostate from insults that could result in carcinogenesis. Loss of function of GSTP1 could result in the predisposition of prostate cells for DNA damage (Phé et al., 2010). In PCa, loss of expression of GSTP1 was found and is the result of the silencing of its gene through promoter hypermethylation. This is one of the most PCa-
specific epigenetic changes because it is seldom found in BPH and other urogenitary cancers (Henrique & Jerónimo, 2004).

In one study conducted on tissue samples of patients with PCa, it was found that GSTP1 promoter hypermethylation could accurately predict the presence of PCa. In PCa tissue samples, more than 90% had GSTP1 promoter methylation (Nelson et al., 2013). In a ROC analysis using tissue samples, it was found that GSTP1 hypermethylation had an AUC value of close to 0.96 with high sensitivity (90.6%) and specificity (100%) exhibiting its high predictive ability for PCa (Bastian et al., 2005). There have been a multitude of other studies that were also conducted on tissue samples that have shown that GSTP1 hypermethylation exhibits high sensitivity and specificity for detecting PCa (Van Neste et al., 2012).

One of the reasons that GSTP1 hypermethylation is one of the ideal epigenetic targets for PCa screening is that it can be detected in bodily fluids such as blood serum and urine. Like PSA, this characteristic lends itself to the easy acquisition of samples during routine physical exams. In one study conducted by Goessl et al. (2001), the researchers have measured GSTP1 promoter hypermethylation in the urine of 78% of the patients who were diagnosed with locally advanced or metastatic disease, 68% in the urine of patients with early PCa. Furthermore, GSTP1 promoter hypermethylation was detected in 72% of the plasma samples of patients being treated for PCa (Goessl et al., 2000). In the light of this finding, it is important to note that the results obtained by Bastian et al. (2005) and those included in Van Neste et al.’s (2012) study were from tissue samples of a previously diagnosed prostate that is positive for PCa. In fact,
although GSTP1 detected in urine has a specificity (86.8-100%), it has a low sensitivity (18.8-38.9%). As such, the same conclusion from tissue samples may not apply to GSTP1 promoter methylation detection in urine and/or blood samples, samples that are more desirable for a screening scenario. Further research will need to be conducted in order to assess the viability of using urine or blood serum to measure GSTP1 promoter hypermethylation as a screening test for PCa.

2. Adenomatosis polyposis coli gene

In addition to GSTP1, APC, which encodes the tumor suppressor adenomatosis polyposis coli (APC), shows promise as a possible epigenetic biomarker for PCa. First implicated in colorectal cancer, APC has also been shown to be inactivated in PCa through methylation (Bastian et al., 2005). Similar to GSTP1, when analyzed with ROC, the AUC value was 0.90 (Bastian et al., 2005). This points to the predictive value of APC promoter methylation in detecting PCa. However, unlike GSTP1, since APC promoter methylation is not specific for PCa but is also present in other cancers, its sensitivity (83%) and specificity (92.9%) were lower than that of GSTP1 (Bastian et al., 2005). Despite this, an important fact is that APC promoter methylation is not detected in conditions like BPH (Bastian et al., 2005). As such, it still potentially has value to be used in PCa screening.

Since this result with APC methylation was obtained via tissue samples, it does not guarantee that the same result will be found in urine or serum samples that are preferred for screening test due to their minimally invasive nature. However, it has been previously described that APC promoter methylation have been detected in urine samples
so its application in screening tests cannot be completely ruled out (Jerónimo et al., 2011). Like GSTP1, further research will need to be conducted in order to definitively ascertain whether the DNA hypermethylation changes in APC promoter regions as a result of PCa can be a viable biomarker for use in PCa screening.

3. Prostaglandin-endoperoxide synthase 2 gene

The last gene that has shown potential to be used in PCa screening is the PTGS2 gene that encodes prostaglandin-endoperoxide synthase 2 (PTGS2). PTGS2 has been implicated in various cellular responses that include regulation of inflammation and carcinogenesis (Bastian et al., 2005). Similar to both GSTP1 and APC, the AUC value for PTGS2 was 0.91 with a sensitivity of 71.7% and a specificity of 100% (Bastian et al., 2005). Like the other two promising genes, PTGS2 promoter methylation only occurs in PCa and not noncancerous prostate conditions, lending itself potentially useful in discriminating between the conditions so as to avoid misdiagnosis leading to overtreatment (Bastian et al., 2005).

Although PTGS2 promoter hypermethylation shows potential for predicting PCa in tissue samples, it has not been reported to be detectable in urine or serum samples. As such, despite its high AUC value, PTGS2 may be limited in its clinical value in the early detection of PCa through minimally invasive procedures. Future investigations into whether PTGS2 can be detected in urine or serum samples will elucidate whether this particular epigenetic marker can be used in screening tests for PCa.
B. Non-epigenetic Urine Biomarkers:

Other than epigenetic alterations, other biomarkers detected in urine can be used for PCa screening. Biomarkers that are PCa specific and can be detected in urine make for promising PCa screening targets because of the ease in obtaining samples for analysis without the use of invasive procedures that may cause stress or harm to patients. These types of biomarkers include non-coding RNA, gene fusion transcripts, and proteins.

1. Non-Coding RNA: Prostate Cancer Antigen 3

Prostate Cancer Antigen 3 (PCA3) was first described in 1999. It is a prostate-specific non-coding RNA from chromosome 9q22-21 that is highly overexpressed in PCa tissues (Challacombe et al., 2013). Compared to non-neoplastic prostate tissue, PCA3 expression is increased by 66 fold in 95% of PCa tissues (Crawford et al., 2012). This piqued the interests of researchers searching for possible PCa-specific biomarkers that can be used in PCa screening efforts that can exclude conditions such as BPH.

PCA3 is measured using samples of whole urine from patients after undergoing DRE. It is important for patients to undergo DRE prior to urine collection as the prostate massage from DRE results in the shedding of cells from the prostate into the urine that can be collected to detect PCA3 levels (Chevli et al., 2014). This characteristic of PCA3 makes it a highly viable target as a possible urine biomarker for PCa screening as it does not require invasive procedures like previously reported epigenetic markers. After the sample has been collected, a real-time polymerase chain reaction called Progensa® PCA3 is used quantify PCA3. A PCA3 score is generated by establishing a ratio between PCA3 and PSA mRNA (Crawford et al., 2012):
\[
\frac{PCA3 \text{ mRNA}}{PSA \text{ mRNA}} \times 1000
\]

Currently, the FDA approved cutoff point for the PCA3 score is 25 (Crawford et al., 2012). A score above 25 means that there is an increased probability for a biopsy positive for PCa and vice versa for a score below 25. This cutoff point is still being debated as studies have argued that a cutoff of 35 offers better balance between test sensitivity and specificity (Challacombe et al., 2013; Chevli et al., 2014; Crawford et al., 2012).

PCA3’s predictive potential for PCa has been studied in great detail. In multiple studies, it has been shown through ROC analysis that the AUC value for PCA3 tests significantly outperform that of the traditional PSA screening test. PCA3 tests had an estimated AUC of 0.70-0.74 compared to that of the PSA test value of 0.50-0.66 (Chevli et al., 2014; Crawford et al., 2012; Ferro et al., 2013; Filella & Giménez, 2012; Stephan et al., 2013).

Disadvantages of PCA3 use in the screening of PCa include the cost of the test and the discomfort for patients. Compared to the traditional PSA test or even the PHI test, the Progensa® PCA3 test is more expensive. Although there are no data for cost in the US, the test costs $385.00 CAD in Canada (“Cost of the Test: PCA3 Urine Test for Prostate Cancer Detection,” 2013). Comparatively, the PSA test costs $30 CAD which is comparable to the costs in the US (Sher, 2012). Additionally, the DRE needed in order to collect urine samples may cause discomfort for patients compared to the standard blood tests and adds a layer of complexity to the screening procedure. One final caveat regarding the use of Progensa® PCA3 is that although it is currently approved by the
FDA, it is only approved for use in patients that have had one or more negative biopsies and is not approved for use with patients that have yet to undergo an initial biopsy (Stephan et al., 2013).

2. Gene Fusion Transcripts: TMPRSS2-ERG

Currently, the majority of research efforts in detecting fusion transcript biomarkers in urine is focused on the feasibility of using TMPRSS2:ERG fusion to help detect PCa presence in urine. TMPRSS2:ERG is a gene fusion between an androgen-regulated transmembrane-serine protease 2 (TMPRSS2) gene and the Erythroblast Transformation-Specific-related gene (ERG). Described in 2005 by Tomlins et al., TMPRSS2:ERG fusion transcripts were found in 50% of PCa (Tomlins et al., 2005). By itself, TMPRSS2 codes for a prostate-specific, androgen-responsive transmembrane serine protease (St. John et al., ). ERG is part of the Erythroblast Transformation-Specific family of genes that are responsible for regulating embryonic development, cell proliferation, differentiation, angiogenesis, inflammation and apoptosis (“ERG - v-ets avian erythroblastosis virus E26 oncogene homolog,” 2014; Oikawa & Yamada, 2003). Since its discovery it has been shown that TMPRSS2:ERG gene fusions are characteristic of PCa that have poorer outcomes and lower survival rates (St. John et al., 2012). This finding suggests that TMPRSS2:ERG detection could be a potential prognostic marker during PCa screening. It is thought that the TMPRSS2:ERG gene fusion allows for the cells to bypass regulation resulting in the over expression of ERG (St. John et al., 2012). This will allow the cell to undergo incorrect differentiation forming unregulated and unorganized tissue leading to PCa. In a recent study conducted by Hessels et al. it was
found that TMPRSS2:ERG fusion transcripts could be detected after DRE (Hessels et al., 2007). Similar to the PCA3 test, this characteristic of the TMPRSS2:ERG fusion transcripts makes it a candidate for PCa screening. Moreover, because of the possible application of TMPRSS2:ERG in distinguishing more aggressive and dangerous cancers from those that are unlikely to affect a patient, it can be used as a means to reduce the overdiagnosis and overtreatment that limit the traditional PSA serum test.

Figure 11 – Biology of TMPRSS2:ERG Fusion:

The fusion of TMPRSS2 with ERG results in ERG overexpression. Because ERG is implicated in a variety of cellular processes involving growth, the overexpression of ERG could open the gates and predispose prostate cells harboring this fusion to undergo unregulated growth leading to PCa.


By itself, the screening capabilities of TMPRSS2:ERG transcripts were not much better than that of a standard PSA test. When analyzed with the ROC curve, TMPRSS2:ERG gene had an AUC of 0.66 compared to that of the traditional PSA screening test (0.50-0.66) (Stephan et al., 2013). Although independently it does not confer a large increase in accuracy over PSA screening, recently there has been an
increased amount of interest in the use of \textit{TMPRSS2:ERG} in conjunction with the Progensa® PCA3 test as a means to increase the sensitivity of the two tests as both biomarkers can be obtained in urine after DRE. Together, PCA3 with \textit{TMPRSS2:ERG} have an AUC value of 0.77 whereas PCA3 by itself has an AUC value of 0.70 (Tomlins et al., 2011). Currently, a combination test called Mi-Prostate Score combining PCA3 score with \textit{TMPRSS2:ERG} fusion transcript levels and serum PSA levels are being offered by the UMICH Health System as a means to screen for PCa (“U-M offers new early detection test for prostate cancer,” 2013). This screening method was only recently introduced and has yet to be approved by the FDA is under further testing.

\textbf{C. Urine Protein Biomarker: Engrailed-2}

The genes for homeodomain-containing transcription factors, also known as \textit{HOX genes}, are a set of genes that function in early embryonic development where they determine the identity of cells and tissues and regulate the proliferation of cells (Javed & Langley, 2014). It was shown in mouse models that they play an important role in the normal development of the prostate gland (Javed & Langley, 2014). A member of a the \textit{HOX genes}, \textit{En}-2 codes for the Engrailed-2 protein (EN2) that has a multitude of functions that range from developmental regulation to survival of dopaminergic neurons within the nervous system (Javed & Langley, 2014). Recently implicated in breast cancer, it was shown that EN2 is expressed and secreted by PCa cells but not in normal prostate tissue (Morgan et al., 2011). As a result, it has become a possible target for use in PCa screening.
In order for a marker to be useful in a screening test situation, it needs to be present in easily obtainable samples to be measured. Blood serum or urine are often considered the ideal samples in the case of PCa screening. In 2011, Morgan et al. showed that it was indeed possible to detect EN2 in urine (Morgan et al., 2011). The researchers collected urine samples from 194 patients pre-biopsy and not immediately after DRE (Morgan et al., 2011). Using a western blot with anti-EN2 antibodies, they were able to show the presence of EN2 in urine. To substantiate this finding, they conducted an ELISA assay and obtained the concentrations found in the urine samples collected. Using ROC curve analysis, the AUC values were calculated and found to be 0.81 using an EN2 cutoff of 42.5 ng/ml (Morgan et al., 2011; Pandha et al., 2012). The high AUC value lends to EN2’s value as a potential biomarker that is usable for screening for PCa.

Tumor volume is considered an important factor when PCa are assessed for their clinical significance. In a recent study, Pandha et al (2012) showed that EN2 levels significantly correlated with PCa tumor size (p = 0.006). Also, it was found EN2 levels were significantly higher in higher stage tumors (Pandha et al., 2012). These findings point to the possible use of EN2 levels in urine as a means to differentiate between benign PCa cases from those that are malignant and aggressive, a crucial differentiation that traditional PSA tests failed to do. As such, due to the ease of measuring its levels in urine, its PCa specific nature, its high predictive potential for the presence of PCa, and its ability to help differentiate between different PCa cancer stages, EN2 is a very promising urine biomarker that can be used for PCa screening.
DISCUSSION/CONCLUSION

PCa is one of the most prevalent cancers in men worldwide and its early detection is essential for treatment success and increased quality of life for patients. The introduction of the PSA screening test is a good first step in this direction. However, PSA is limited by a variety of factors that include low sensitivity and specificity and its inability to differentiate between indolent and aggressive PCa that result in potential overdiagnosis and overtreatment of otherwise clinically insignificant cancers. Large clinical trials like the PLCO and ERSPC have put the efficacy of the PSA test into question and has highlighted the possibility that as much as 50% of the diagnosed PCa cases are results of overdiagnosis, many of which undergo treatment (Schröder et al., 2009). The overdiagnosis and overtreatment leads to not only financial burdens for patients and the country’s healthcare system, but also negative physical and psychological affects that can adversely affect a patient and his family’s lives. In addition to overdiagnosis and overtreatment, the PSA test can also miss clinically significant PCa that have PSA levels below the currently accepted 4.0 ng/ml upper normal boundary. Currently, PSA is still the preferred PCa screening method. However, many national organizations like the USPSTF and the AUA either recommend against its use or recommend exercising caution due to possible overdiagnosis and overtreatment issues associated with the test. As a result, there is an increased need for alternative methods for screening for PCa that will lower the probability of overdiagnosis and overtreatment while maintaining a high rate of PCa detection.
There are two approaches to increasing the accuracy of future PCa screening tests. One of these is to improve PSA screening itself by exploring the possibility of using PSA-derived screening methods. These include altering the age at which to begin offering PCa screening to men using the PSA test, using %fPSA, proPSA markers like [-2]proPSA, or combining PSA with %fPSA and [-2]proPSA in a composite screening test called PHI. The tests’ respective performance were acquired via AUC values of ROC curves. Of these different methods, creating a EWP zone and using the PHI composite test to screen for PCa are the most promising as they had the highest AUC values. Of particular note, PHI has the potential to distinguish between aggressive and benign PCa with its use of [-2]proPSA which has been shown to be expressed at different levels in PCa cases with different GS. %fPSA modified by genetic screening also shows promise, but Zambon et al.’s study using KLK3 genetic profiles was the first of its kind and further investigation into genetic profiling’s application in PSA screening is needed (Zambon et al., 2012).

Despite these promising results, it is important to understand that these modifications to the traditional PSA screening test have their respective disadvantages. In the case of establishing EWP zones to further stratify patients based on risk, there is a chance that the lowering of the PSA cutoff point from 4.0 ng/ml to 1.5 ng/ml will result in an increase in overdiagnosis and overtreatment. As such, a large scale trial will have to be conducted in order to assess both the EWP zone’s efficacy in risk stratification and its effects on the rates of overdiagnosis and overtreatment.
In the case of PHI, the optimal cutoff points for the test are still being debated. Also, even though the test is shown to be more accurate than traditional PSA screening tests, the cost of the test is more expensive than that of the traditional serum PSA screening. It is argued that despite this fact, the added accuracy of the test will offset the cost of the test by minimizing costs associated with overdiagnosis and overtreatment. However, the fact that PHI is still a relatively new test means that whether or not it can decrease the overdiagnosis and overtreatment rates is still unknown. As such, investigations into the test’s cost versus benefit are needed.

The other way of improving PCa screening is to utilize biomarkers other than PSA and those derived from it. One of the various types of biomarkers are epigenetic modifications in PCa cells in the form of DNA methylation changes in the promoter regions of different genes. The genes that have been shown to be PCa-specific and undergo promoter hypermethylation as a consequence of PCa are \textit{GSTP1}, \textit{APC}, and \textit{PTGS2}. These epigenetic markers are both highly specific and highly sensitive for PCa. Their AUC values are higher than any of the other tests that have been introduced so far. The use of the epigenetic markers is limited despite this fact. The high AUC, sensitivity, and specificity values were measured using tissue samples acquired from patients. This is not ideal for screening tests as it is invasive and difficult to do compared to simple blood and urine test. It has been shown that \textit{GSTP1} and \textit{APC} methylation status can be measured in urine, albeit resulting in a less accurate prediction of PCa. This limits their usefulness in a screening scenario. On the other hand, \textit{PTGS2} has not been found to be detectable in urine yet, which precludes it from screening tests for PCa. Currently, some
researchers suggest the use of gene panels that evaluate the epigenetic profiles of a number of genes to increase the specificity of epigenetic screening efforts (Jerónimo et al., 2011; Phé et al., 2010). More research is needed to determine the feasibility of using urine measurements of epigenetic changes to accurately predict the presence of PCa. Also, the discovery of GSTP1, APC, and PTGS2 methylation changes opens the door to the discovery of other possible epigenetic biomarkers for use in PCa screening in the future.

The other urine biomarkers for PCa are the PCA3 non-coding mRNA, TMPRSS2:ERG gene fusion transcript, and EN2 protein. PCA3 was found to have a higher AUC than the currently available PSA-derived screening methods. However, in two independent studies, it was found that the AUC values among the %[-2]proPSA, PHI, and PCA3 tests were not significantly different. With the discovery of TMPRSS2:ERG fusion transcripts in the urine, which by itself confers no real advantage in PCa screening over PSA-derived screening tests, researchers have begun to investigate the possibility of enhancing PCA3 tests with TMPRSS2:ERG transcript detection. When used in conjunction with TMPRSS2:ERG, PCA3 test’s AUC increased with the added benefit of potentially differentiating between indolent disease and malignant cancers. Whether or not this increase in AUC is significant has yet to be investigated.

EN2 protein is the final urine biomarker investigated in this study. It is one of the most recently described urine biomarkers that has potential to be used in PCa screening. The predictive value of EN2 is the highest amongst the currently available tests for early PCa detection with an AUC of 0.81 (Morgan et al., 2011; Pandha et al., 2012). EN2
levels have also been shown to increase with increasing tumor sizes lending it as a possible method to differentiate between indolent disease and one that requires treatment.

As the result of this investigation into various proposed PSA screening improvements and alternatives, it has become clear that no perfect method to screen for PCa is currently available. Of all the tests that are presently offered, PHI seem to be the most promising. Compared to the other PSA-derived screening tests, PHI has the added advantage of being able to distinguish between more clinically advanced cancers from less advanced PCa, making it more suitable in a screening scenario to reduce overdiagnosis. Even though EN2 based screening seems to have a higher AUC compared to that of PHI and thus suggesting it is a better screening method, it is a relatively new test that only began testing in the UK and is not yet introduced in the US. Both EN2 and PHI have been shown to possess the potential to be also prognostic markers that can differentiate between different stages and grades of PCa.

There are some promising biomarkers such as epigenetic changes in GSTP1 and APC genes that have much higher AUC values compared to that of other test and have been shown to be in urine samples. However, there have not been many studies yet that measure the biomarker’s accuracy in predicting PCa in those samples. As such, epigenetics is not a viable strategy for screening PCa currently. However, with more research into detecting epigenetic changes in urine and combining multiple genes into a single gene panel, epigenetic markers can prove to be the best way to screen for cancer in the future due to their high sensitivities and specificities for PCa despite present limitations.
REFERENCES


http://www.mayoclinic.org/diseases-conditions/prostatitis/basics/complications/con-20020916


VITA

KEVIN K. YU

kky6@bu.edu • 607-220-4324 • DOB: 1989
Permanent Address: 5077 Watling Street • Burnaby, BC • V5J 1W8 • Canada
Current Address: 1007 Alexander Street • Houston, TX • 77008

EDUCATION

Boston University School of Medicine • Boston, MA
Division of Graduate Medical Sciences
Candidate for Master of Arts of Medical Science • 2012 (current)

Cornell University • Ithaca, NY
College of Arts and Sciences
Bachelor of Arts in Biological Sciences • May 2011
GPA: 3.43/4.0

RESEARCH EXPERIENCE

July 2011 – Present
Saban Research Institute • Children’s Hospital Los Angeles
Volunteer Research Associate • Los Angeles, CA

- Discovered novel DNA methylation changes occurring in the urothelium as a result of CYP-induced chronic bladder cystitis in CD-1 mice.
- Investigated possible application of Low Level Laser Therapy (LLLT) for treatment of CYP-induced hemorrhagic bladder cystitis in CD-1 mice.
- Investigated the role of FGF-10 in bladder urothelium wound healing in FGFrs attenuated mice with CYP-induced hemorrhagic cystitis.

June 2008 – April 2011
Weill Institute of Cell and Molecular Biology • Cornell University
Undergraduate Research Assistant • Ithaca, NY

- Adapted and determined optimal conditions for activating and controlling fluorescence of Photoactivatable mCherry for protein localization assays in Saccharomyces cerevisiae under a confocal light microscope.
- Investigated the effect of anchoring PtdInsP’s to the plasma membrane on S. cerevisiae on phosphatidylinositol phosphate metabolism.

PUBLICATIONS

ABSTRACTS


POSTER PRESENTATIONS


SHADOWING EXPERIENCE
July 2011 – July 2012

Dr. Chester J. Koh, MD (70Hrs.) Children’s Hospital Los Angeles Los Angeles, CA

- Observed Dr. Koh 8-10 hours every few weeks in the Operating Room (OR)
- Operated mobile X-Ray machine during operations
- Prepared operation rooms for surgery

VOLUNTERING EXPERIENCE
March 2013 – August 2013

Massachusetts General Hospital Volunteer Office Boston, MA

- Help in the coordination of volunteers in various volunteering assignments
- Maintain profiles of volunteers in an effort to help the office stay up to date with document and health regulations compliance of individual volunteers
- Answer any general phone calls directed to the volunteer office
- Answer any inquiries about various volunteer programs at MGH
- Help respond to urgent requests for volunteers by various departments within MGH

MGH Bone Marrow Transplant Clinic Volunteer Boston, MA

- Maintain food and refreshment stocks at the BMT clinic for patient use
- Offer food and drinks to patients within the clinic
- Engage in friendly conversation with any patients who desire to talk in an effort to make patients and significant others feel welcome and comfortable
- Support staff on an as-needed basis

PREPARE International Orientation Group Leader Cornell University
Ithaca, NY

- Advised a group of 15-20 international students in the College of Arts and Sciences about academic options and living a foreign country

**LEADERSHIP AND ORGANIZATIONS**

**SASE Professional Chapter Boston**

- Co-founder of SASE Professional Chapter Boston
- Organized interest meetings
- Organized initial leadership team
- Oversee the planning of various social/networking, community service/mentoring, and professional development events by focus groups

**June 2009 – May 2011**

**SASE Cornell Chapter Co-President & Treasurer**

- Founded SASE Cornell with three other undergraduates
- Allotted and maintained organization’s budget and reimbursements
- Organized fall and spring volunteering events, fundraisers and workshops
- Planned first Annual SASE Northeast Regional Conference at Massachusetts Institute of Technology
- Advised members from the College of Arts and Sciences regarding academics, development of interpersonal skills and advancement in academic skills
- Helped create and maintain SASE Cornell’s website and Facebook page

**April 2008 – April 2009**

**Cornell Taiwanese-American Society Culture Chair**

- Orchestrated and planned CTAS Taiwanese culture show
- Created and organized culture show’s budget and reimbursements
- Penned and directed music videos for culture show
- Assisted in devising CTAS’s participation in Asia Night 2009, a university-wide showcase of Asian organizations

**TECHNICAL SKILLS**

**Laboratory:**

- Maintaining S. cerevisiae and E. coli cultures
- Gene cloning through plasmid amplification • Media preparation
- Gel Electrophoresis • Western Blotting • Comassie Assays • Northern Blotting
- DNA extraction and purification • PCR • RT-PCR
- DNA sequencing • Enzyme assays • Protein localization assays • Dialysis
- Chromatography • Pyrosequencing • Mouse genotyping • Mouse breeding
- Murine Bladder • Extraction • PCNA expression detection
- Preparation of Histological Sections • H&E staining Luminex cytokine assay
- TUNEL assay • Quantitative Methylation-Sensitive Real Time PCR (MethyLight)

**Laboratory Instruments:** Light microscopes • Centrifuges • RT-PCR equipment • Luminex

**Computer:**

- Microsoft Office Suites • Knowledge of Microsoft Windows and Mac OS X

**LANGUAGES**

- Bilingual in English and Mandarin