BASELINE AND LONGITUDINAL CHANGES OF BENIGN PROSTATE SPECIFIC ANTIGEN AND [-2]PROPROSTATE SPECIFIC ANTIGEN IN COMMUNITY-DWELLING BLACK AND WHITE MEN

By

THOMAS RHODES

A dissertation submitted to the

School of Public Health

University of Medicine and Dentistry of New Jersey and

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Graduate Program in Epidemiology

written under the direction of

Kitaw Demissie, MD, PhD

and approved by

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

New Brunswick, New Jersey

May 2011
ABSTRACT OF THE DISSERTATION

BASELINE AND LONGITUDINAL CHANGES OF BENIGN PROSTATE SPECIFIC ANTIGEN AND [-2]PROPSROSTATE SPECIFIC ANTIGEN IN COMMUNITY-DWELLING BLACK AND WHITE MEN

By THOMAS RHODES

Dissertation Director:

Kitaw Demissie, MD, PhD

Context: Prostate cancer (CaP) is the second leading cause of cancer deaths among men in the United States, with a projected 217,730 incident cases and 32,050 deaths in 2010. Prostate specific antigen (PSA) has been used extensively in the screening and detection of prostate cancer. However, PSA is not specific to malignant tissue. For example, benign and inflamed prostatic tissues also secrete PSA. More recently, subforms of PSA have been identified which may be more specific to benign and malignant prostatic tissue.

Benign prostate specific antigen (BPSA) is a subform of free PSA and [-2]proPSA is a precursor form of PSA circulating freely in the serum. BPSA is associated primarily with benign transition zone tissue exhibiting nodular hyperplasia but not with nonhyperplastic transition zone tissue or benign or cancerous peripheral zone prostatic tissue. BPSA is elevated in the prostate transition zone and is associated with pathologic benign prostatic hyperplasia (BPH), while [-2]proPSA is associated with prostate tumor. Preliminary studies suggest that [-2]proPSA increases the specificity for detecting prostate cancer over PSA, particularly in the 2-4 ng/ml range. The increased specificity held through the 2-10 ng/ml range.
Previous studies investigating the BPSA and [-2]proPSA were done using cross-sectional data with clinic or convenience samples. However, little is known about the distribution of these novel biomarkers in a randomly selected cohort of community-based men. Furthermore, there does not appear to be any reports of longitudinal data. The objective of this dissertation is to describe the distribution and clinical correlates of BPSA and [-2]proPSA in an age-stratified, randomly selected sample of men followed prospectively over time. In addition, this dissertation estimates the change over time in these biomarkers and describes the relationship of estimated change over time with baseline urologic measures and risk of urologic outcomes (i.e., progression of benign disease resulting in acute urinary retention and treatment for BPH, as well as histologically confirmed prostate cancer).

**Specific Aims:** The aims of this dissertation were to: (1) describe the distribution of BPSA in a community-based sample of black and white men and examine the association of BPSA with baseline urologic measures, acute urinary retention treatment for BPH and CaP, (2) describe the distribution of [-2]proPSA in a community-based sample of black and white American men and examine the association of [-2]proPSA with baseline urologic measures and CaP, (3) describe the distribution of longitudinal changes in BPSA and [-2]proPSA and how these changes vary over levels of baseline urologic conditions.

**Design, setting and subjects:** Aims 1 and 2 utilized clinical data from two population-based cohort studies established to characterize the natural history and risk factors for progression of prostate disease in white and black male residents of Olmsted County,
Minnesota and Genesee County, Michigan, respectively. Aim 3 utilized longitudinal measurements of BPSA and [-2]proPSA from biennial clinical evaluations in the Olmsted County cohort over a median follow-up interval of 7 years.

For Aims 1 and 2, descriptive statistics and cumulative distribution function plots were used to describe the distributions of both BPSA and [-2]proPSA. Spearman correlations (r_s) and logistic regression were used to investigate the cross-sectional relationships between BPSA, [-2]proPSA and other baseline urologic measures. Multivariable logistic regression was used to describe the association of BPSA with clinically meaningful diagnostic measures, which are presented as odds ratios (ORs) and 95% confidence intervals (CIs). Moderate-to-severe LUTS was defined as AUASI score >7 while depressed urinary flow rate was defined as maximum urinary flow rate <12 mL/s. Prostatic enlargement was defined as prostate volume >30 cc. Due to availability of follow-up data, Cox proportional hazards regression was used in the OCS cohort only to investigate the association of baseline values of BPSA with the risk of a subsequent diagnosis of AUR, biopsy confirmed CaP, and treatment for BPH which are presented as hazard ratios (HRs) and 95% CIs. Similarly for [-2]proPSA, longitudinal follow-up data was used to assess the association between baseline [-2]proPSA level and risk of a subsequent diagnosis of biopsy-confirmed CaP using Cox proportional hazards regression. Hazard ratios (HRs) and 95% CIs were used to describe the associations between these measures.

For Aim 3, mixed-effects regression models were used to estimate longitudinal changes (i.e., annual percent change over time) in BPSA and [-2]proPSA, as well as the other urologic variables. As BPSA and [-2]proPSA measures were only available at the
4th, 6th, and 8th rounds of data collection, the 4th round of follow-up was considered as baseline. Transition zone volume and free PSA were available beginning at the 5th round of data collection. To assess the natural history, observations were censored after the point when subjects either received medical therapy (α-blocker, 5-α reductase inhibitor) or surgical treatment for BPH or were diagnosed with CaP. Each measure was regressed on time from baseline measurement and 10-year age groups. An interaction term was included to allow different slopes across age groups. This method estimates group average longitudinal change (fixed effects) while still allowing each individual subject longitudinal change to deviate from the group average curve (random effects).

Additional models included terms indicating an enlarged prostate (prostate volume >30 cc) or diagnosis of CaP and an interaction of this term with time from baseline to allow for comparison of slopes in men with and without an enlarged prostate or CaP diagnosis. Prevalent cases of CaP or enlarged prostate were removed from these analyses which resulted in 43 and 65 men with incident CaP and enlarged prostate, respectively.

Spearman correlations were used to describe the relationship between annual percent change in BPSA and [-2]proPSA with baseline and annual changes over time of other urologic measures.

**Results:** The distribution of BPSA levels was similar in blacks (median (25th, 75th percentiles) =32.9 (17.3, 68.0) pg/mL) and whites (median=32.2 (16.6, 68.9) pg/mL), p=0.71, but much lower than previous reports. For Olmsted County men in the upper quartile of BPSA, there was a 15-fold increased risk of prostate cancer (CaP; hazard ratio (HR): 14.6 95% confidence interval (CI): 3.1, 68.6) and a 2-fold higher risk of treatment
for benign prostatic hyperplasia (BPH; HR: 2.2, 95% CI: 1.2, 4.2) after adjusting for age. After additional adjustment for baseline PSA, the association between BPSA and CaP risk was attenuated, but remained nearly 9-fold higher for men in the upper BPSA quartile (HR: 8.7, 95% CI: 1.8, 42.4). The 2-fold higher risk of treatment for BPH also remained after adjustment for baseline PSA for men in the upper BPSA quartile (HR: 1.9, 95% CI: 0.9, 4.0).

Baseline [-2]proPSA level was slightly higher among black men (median (25th, 75th percentiles) =6.3 (4.1, 8.9) pg/mL) than among white men (median=5.6 (3.9, 7.7) pg/mL), p=0.01. Baseline [-2]proPSA level was highly predictive of biopsy-confirmed prostate cancer (CaP) in the OCS cohort. Relative to men in the lower quartile of [-2]proPSA, men in the upper quartile had almost an 8-fold increase in the risk of CaP (hazard ratio (HR): 7.8, 95% confidence interval (CI): 2.2, 27.8) after adjustment for age and baseline PSA.

Median (25th, 75th percentiles; Q1,Q3) annual percent change for [-2]proPSA and BPSA were 3.7% (2.5%, 5.2%) and 7.3%(6.8%, 7.7%), respectively. Both were correlated with baseline and annual changes in PSA and prostate volume measures. Annual percent change in [-2]proPSA increased with increasing age decade. The median (Q1, Q3) rate of increase in [-2]proPSA was greater for men who developed enlarged prostates (3.5% (2.6%, 4.4%)) or prostate cancer (8.1% (6.6%, 9.8%)) compared to those who did not develop enlarged prostates (1.9% (0.9%, 3.0%)) or prostate cancer (3.5% (2.3%, 4.8%)).
**Conclusion:** These population-based data provide useful reference ranges to researchers for future studies examining the utility of BPSA, with similar distributions of BPSA for white and black men. Elevated levels suggest some prostate disease, benign or malignant. Further work is needed to develop our understanding of how this might be used in a diagnostic workup.

These data suggest that levels of [-2]proPSA may help identify prostate cancer in men with serum PSA levels in an indeterminate range, although the reference ranges for white and black men may differ slightly.

Overall these results demonstrate that BPSA and [-2]proPSA increase over time. Furthermore, the annual percent change in [-2]proPSA increases with age and is greater in men who develop enlarged prostates and CaP. These data suggest that rapid increases in [-2]proPSA levels over time may help identify men with CaP.
ACKNOWLEDGEMENT

I would like to sincerely thank my advisor, Kitaw Demissie, for his support and guidance through this memorable journey. I am also extremely grateful to Steve Jacobsen for his guidance and for allowing me to use data from the Olmsted County study. It all started as a talk over dinner at a sushi restaurant during an APHA convention. I want to thank George Rhoads and Pamela Ohman Strickland for their guidance and support throughout my academic experience. In addition to my committee, I would like to thank the faculty and staff at the departments of Health Sciences Research and Biostatistics at the Mayo Clinic for their support in not only providing me with the data, but also being available to answer questions. Thank you, Deb Jacobson, Michaela McGree and Jenny St. Sauver.

To my loving wife, Michele, thank you for your unwavering support. To my mom, thank you for believing in me. Ben and Julian, I want this to be an example that you can accomplish anything that you set your mind to.

I would like to thank students, staff and faculty at the UMDNJ-School of Public Health for giving me such a great experience. I always saw my class time as a refuge from the insanity of work. I really enjoyed the daily interactions both on an academic and social level. I have many cherished memories.

Finally, I am indebted to the men in the Olmsted County Study of Urinary Symptoms and Health Status Among Men and the Flint Men's Health Study. Without their dedication to the respective study goals, none of this research would have been possible.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>viii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>ix</td>
</tr>
<tr>
<td>List of tables</td>
<td>x</td>
</tr>
<tr>
<td>List of figures</td>
<td>xi</td>
</tr>
<tr>
<td>PREFACE</td>
<td>xii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>DETAILED SUBJECTS AND METHODS</td>
<td>12</td>
</tr>
<tr>
<td>Olmsted County Study</td>
<td>12</td>
</tr>
<tr>
<td>Flint Men’s Health Study</td>
<td>15</td>
</tr>
<tr>
<td>Manuscript #1</td>
<td>23</td>
</tr>
<tr>
<td>Manuscript #2</td>
<td>44</td>
</tr>
<tr>
<td>Manuscript #3</td>
<td>64</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>89</td>
</tr>
<tr>
<td>Bibliography</td>
<td>94</td>
</tr>
<tr>
<td>Appendix</td>
<td>95</td>
</tr>
<tr>
<td>Curriculum Vita</td>
<td>98</td>
</tr>
</tbody>
</table>
List of tables

**Manuscript #1**

Table 1. Baseline characteristics of Olmsted County Study of Urinary Symptoms and Health Status among Men and Flint Men’s Health Study. 36

Table 2. Spearman correlations between baseline BPSA and baseline clinical urologic measures. 37

Table 3. Crude and age-adjusted associations between BPSA and urologic measures. 38

Table 4. Crude and age- and PSA-adjusted association of baseline BPSA levels with occurrence of urologic outcomes in the Olmsted County Study. 39

**Manuscript #2**

Table 1. Baseline characteristics of Olmsted County Study of Urinary Symptoms and Health Status among Men and Flint Men’s Health Study. 55

Table 2. Selected empirical quantiles of [-2]proPSA (pg/mL) in the OCS, FMHS and combined FMHS and OCS cohorts. 56

Table 3. Crude and age-adjusted associations between [-2]proPSA and urologic measures. 57

Table 4. Crude and age- and PSA-adjusted association of baseline [-2]proPSA levels and future diagnosis of prostate cancer in the Olmsted County Study. 58

**Manuscript #3**

Table 1. Baseline characteristics of Olmsted County Study of Urinary Symptoms and Health Status among Men 78

Table 2. Spearman correlations of annual percent change in BPSA and [-2]proPSA with baseline and annual changes in urologic measures 79

Table 3. Distribution of annual percent changes in BPSA and [-2]proPSA stratified by age and urologic conditions 80
List of figures

Manuscript #1

Figure 1. Cumulative distribution function plots of BPSA by age decade in the combined Olmsted County Study and Flint Men's Health Study Sample. 40

Manuscript #2

Figure 1a. Cumulative distribution function plot of [-2]proPSA by age decade in the Olmsted County Study. 59

Figure 1b. Cumulative distribution function plot of [-2]proPSA by age decade in the Flint Men's Health Study. 60

Manuscript #3

Figure 1a. Observed longitudinal changes of BPSA by age 81

Figure 1b. Predicted longitudinal changes of BPSA by age 82

Figure 2a. Observed longitudinal changes of [-2]proPSA by age 83

Figure 2b. Predicted longitudinal changes of [-2]proPSA by age 84
PREFACE

I started in the Merck Epidemiology department in the beginning of 1993 as a statistician. This was during the early days of the finasteride clinical program. In the early 90's the epidemiology of benign prostatic hyperplasia was not well understood. As part of the finasteride clinical development program, a community-based observational study was initiated in collaboration with the Mayo Clinic to investigate the natural history of prostate disease in randomly selected community men. This study was named the Olmsted County Study of Urinary Symptoms and Health Status among Men.

My initial role was that of a statistician on many of the early manuscripts. I conducted analyses in collaboration with the study investigators. As my experience with the Olmsted County study progressed, I became more involved in the conduct of the study. For example, I was involved (e.g., protocol development) in the design of studies to assess the reliability of urologic measures collected during the clinic rounds such as the uroflowmeter and transrectal ultrasound measurements. In addition, I was involved in discussions of what data should be collected in subsequent rounds and what measures would be used to collect the data. An example of the latter was the collection of male pattern hair loss data that was collected in the Olmsted County study using the Norwood-Hamilton Classification Scale and the Savin Density Scale. Overall, the collaboration was a very fruitful one often leading to coauthorship as well as opportunities for primary authorship of abstracts for presentation at scientific meetings in addition to manuscripts for publication in peer-reviewed journals.
INTRODUCTION

Prostate cancer is the second leading cause of cancer deaths among men in the United States, with a projected 217,730 incident cases and 32,050 deaths in 2010. Prostate specific antigen (PSA) has been used extensively in the screening and detection of prostate cancer. However, PSA is not specific to malignant tissue. This is particularly true for PSA values in the 4.0 to 10.0 ng/ml range. This is often called the "gray zone" since the reported specificity of PSA in this range is about 25%. Both benign and inflamed prostatic tissues also secrete PSA. PSA is an androgen-regulated serine protease and member of the kallikrein family. It is produced by the prostate in ductal epithelial cell and prostatic acini. PSA is found in normal, hyperplastic and malignant prostate tissues. PSA is secreted into seminal plasma and its function is to liquefy semen to enable sperm motility. PSA reaches the serum via diffusion from luminal cells through the basement membrane of the epithelium and stroma of the prostate. In healthy men, release of PSA into the bloodstream is not a common event resulting in nearly undetectable levels of PSA in men without prostate disease. However as benign or malignant prostate disease progresses, serum levels of PSA begin to rise and may be detected as a result of testing. More recently, subforms of PSA have been identified which may be more specific to benign and malignant prostatic tissue.

Benign prostate specific antigen (BPSA) is a subform of free PSA and [-2]proPSA is a precursor form of PSA circulating freely in the serum. Like PSA, BPSA also contains 237 amino acids, however BPSA is clipped at amino acid residues lysine 145-146 and lysine 182-183. BPSA is associated primarily with benign transition zone tissue exhibiting nodular hyperplasia but not with nonhyperplastic transition zone tissue.
or benign or cancerous peripheral zone prostatic tissue. BPSA is elevated in the prostate transition zone and is associated with pathologic benign prostatic hyperplasia (BPH), while [-2]proPSA is associated with prostate tumor. After removal of the 17-amino-acid leader sequence from the 261 amino-acid of PSA, an inactive precursor protein consisting of 244 amino-acids (7 amino acid leader plus the 237 amino acids of PSA) results. The resultant protein was called proPSA or ([7]proPSA). Originally, [-7]proPSA was thought to be the only precursor form. Subsequent studies have reported other truncated forms of proPSA such as [-1]proPSA, [-2]proPSA, [-4]proPSA, and [-5]proPSA that contain one, two, four, and five amino acids, respectively, in the propeptide leader. Preliminary studies suggest that [-2]proPSA increases the specificity for detecting prostate cancer over PSA, particularly in the 2-4 ng/ml range. The increased specificity held through the 2-10 ng/ml range.

**BPSA**

In a 2004 editorial published in Urology, Roehrborn stated that BPSA is the best clinical predictor (above either PSA or free PSA) of clinically significant enlargement of transition zone volume referring to the study by Canto et al. Canto referred to this result as evidence of the potential of BPSA to be a novel predictor of outcomes or response to therapy in patients with BPH. Canto et al. in a study of 261 serum samples obtained from men who underwent TRUS or a TRUS-guided biopsy (10 cores or greater) at an institutional urology department. The eventual study population consisted of 91 patients who had undergone at minimum a 10-core biopsy and were found to be free of CaP. Median age was 64 years (range: 42-85). Median PSA, free PSA, and BPSA was
4.9 ng/ml (range: 0.9-20.9), 0.70 ng/ml (range: 0.1-5.7) and 220 pg/ml (range: 20-1840) respectively. Median prostate volume and transition zone volume were 57 cc (range: 21-259) and 31 cc (range: 6-185) respectively. In analyses of area under the curve from receiver operator characteristic (ROC) curves predicting transition zone enlargement (i.e., >20cc, >30cc, >40cc) the study authors reported that BPSA outperformed both PSA and free PSA in specificity across all levels of sensitivity. Area under the curve (AUC) was high for the various levels of TZ volume ranging from 0.84 to 0.87. These results suggested that BPSA may be a good serum marker for TZ enlargement.

Archived sera from a consecutive series of 161 men participating in the Early Detection Research Network prostate cancer early detection biomarker program was assayed for [-2]proPSA and BPSA. Mean age was 62 years, range 46 to 80. Men with a percent free PSA <15% were enrolled in the program. All men were volunteers presenting to the institution for prostate cancer screening. Serum was obtained prior to digital rectal exam (DRE) or transrectal ultrasound (TRUS) biopsy. Biopsy results indicated that 66 (41%) of subjects had prostate cancer while 95 (59%) had no evidence of prostate cancer. Mean (± S.D.) of BPSA (ng/ml) for men with noncancer and cancer was (0.4 (± 0.4) vs. 0.2 (± 0.2), p<0.001). Receiver operator characteristic (ROC) curves were used to determine how well BPSA and functions of BPSA performed in distinguishing men with and without prostate cancer. The area under the curve (AUC) for both BPSA and the [-2]proPSA/BPSA ratio was 0.72. However, with the sensitivity fixed at 90%, the specificity was greater for [-2]proPSA/BPSA ratio than for BPSA alone (46% vs. 20% respectively).
Based on an artificial neural network model (ANN), Stephan et al, found that BPSA may improve prostate cancer detection. This study used archived sera from 541 white men who were referred to the urology department or the affiliated outpatient department. These patients consisted of 287 men with prostate cancer (mean age: 62 years; mean prostate volume: 40 cc) and 254 with no evidence of malignancy (NEM) (mean age: 67 years; mean prostate volume: 56 cc). CaP/NEM status was confirmed by histologic examination tissue collected from TRUS-guided biopsy (8-12 cores). ROC curves were used to assess diagnostic accuracy of BPSA in distinguishing men with and without prostate cancer. Median age for CaP and NEM groups was 63 years (range: 44-73) and 67 years (range: 43-88) respectively (p<0.0001). Median values of BPSA were significantly lower for men with CaP compared to men in the NEM group (151 pg/ml (range: 12.1-1800) and 192 pg/ml (range: 11.7-2156) respectively (p=0.003)). AUC (± standard error (SE)) of BPSA alone in was 0.55 (±0.025) with specificities of 18% and 9.5% for fixed sensitivities of 90% and 95% respectively. The AUC of the ratio of BPSA to total PSA resulted in an improved AUC of 0.69 (±0.022) with specificities of 30% and 15% for fixed sensitivities of 90% and 95% respectively. Evaluation of %free PSA yielded an AUC of 0.77 with specificities of 41% and 27% for fixed sensitivities of 90% and 95% respectively. The ANN analysis of BPSA also included the ratio BPSA/total PSA resulting in an AUC of 0.81 with specificities of 54% and 45% for fixed sensitivities of 90% and 95% respectively. Similar AUC results to the ANN analysis were found using logistic regression model adjusting for total PSA, %free PSA, age and prostate volume. The ANN model demonstrated greater improvement in specificity over the % free PSA of 13% and 17%. In this study, BPSA was better able to distinguish men with
biopsy confirmed prostate cancer from men with nonmalignancy after adjustment for age, total PSA, % free PSA and prostate volume. This result seems to support the potential utility of BPSA in further distinguishing men with or without prostate cancer in the presence of a PSA test.

In another study de Vries, et al reported data from 61 men participating in the screen arm (n=21,210) of a large cancer screening study. The median age was 68 years (range: 57-75 years). Men were categorized into two groups based on an arbitrary definition of cancer aggressiveness. Men were classified as having favorable CaP (n=44) or unfavorable CaP (n=17) based on prognosis. Patients with favorable prognosis were classified based on sextant biopsy-derived criteria: T1c disease, PSA density <0.15 ng/ml/cm³, no Gleason pattern 4, <50% invasion per core, and 2 cores maximum with CaP invasion. In contrast, patients classified in the unfavorable group had a Gleason score of 4+4 or greater and more than 4 biopsy cores with invasion or pathologic stage T3c. Using Mann-Whitney U test to test the differences between medians, men with a favorable prognosis had lower median BPSA level than men with an unfavorable prognosis (172 pg/ml vs. 264 pg/ml respectively, p=0.043). In addition to the individual biomarker, functions of BPSA were also investigated. The [-2]proPSA/BPSA ratio was lower for men with a favorable prognosis (0.181 vs. 0.228 respectively, p=0.043). However, there was no difference in BPSA/free PSA ratio by prognosis group (p=0.52).

In multivariate logistic regression predicting CaP prognosis, BPSA did not enter into the model. As a secondary analysis the analyses were repeated for PSA levels in the 4 to 10 ng/ml range. Thirty patients remained following the restriction with 19 in the favorable group and 11 in the unfavorable group. Univariate analyses of BPSA suggested no
differences between the favorable and unfavorable prognosis groups (median BPSA 0.234 vs. 0.287 respectively, p=0.813). However, the ratio of [-2]proPSA/BPSA was lower for the favorable group when compared to the unfavorable group (medians: 0.196 vs. 0.308 respectively, p=0.033). There was no difference between groups for the ratio of BPSA/free PSA (p=0.175).

Linton et al\textsuperscript{20} reported BPSA levels in 79 men with BPH (defined as elevated PSA (1.5-1.7 ng/ml) with negative biopsy), 91 men with CaP (PSA, 2.6-9.7 ng/ml), 57 male urologic outpatients who were not suspected to have BPH or CaP (PSA, 0.17-2.3 ng/ml), 37 young men (PSA, 0.21-0.9 ng/ml), 20 women<30 years of age (PSA, undetectable) and 10 post-radical prostatectomy subjects (PSA, 0-0.34 ng/ml). Median BPSA levels were 220 pg/ml and 170 pg/ml in men with BPH and CaP, respectively (Wilcoxon rank-sum test, p=0.53). BPSA in the other groups were below the level of detection (i.e., <0.02 ng/ml). Results suggest that men with BPH in the absence of CaP may have higher levels of BPSA compared to men with CaP.

Jansen et al\textsuperscript{21} evaluated BPSA in 405 serum samples from the Rotterdam arm of the European Randomized Study of Screening for Prostate Cancer and 351 samples from the Urology Department of Innsbruck Medical University. Of the 405 men in the screening study, 226 were CaP cases and 179 were non-CaP cases. There were 174 CaP cases and 179 non-CaP cases out of the 351 men from the urology department. ROC analyses using the AUC as well as the sensitivity and specificity were calculated to assess the ability of BPSA to distinguish men with and without CaP. In addition, nonparametric tests were used to investigate the median values of BPSA by biopsy and pathologic Gleason scores $\geq 7$, a measure of tumor aggressiveness. Median (range) age of men in the
screening study and the urology clinic were 66 (55-75) and 60 (50-77) respectively. Total PSA levels for both study sites ranged from 2.0 to 10.0 ng/mL. For the screening sample, median BPSA levels for the CaP and non-CaP groups were 157.30 pg/ml and 180.52 pg/ml respectively. For the urology department sample, the median BPSA levels for the CaP and non-CaP groups were 137.50 pg/ml and 135.84 pg/ml respectively. Using multivariate logistic regression analyses BPSA did not distinguish between men with and without prostate cancer.

[-2]PROPSA

In a two-site study of 1,091 men enrolled in prostate cancer screening studies who underwent prostate biopsy were divided into 2 groups based on PSA ranges of 2 to 4 ng/ml and 4 to 10 ng/ml. For the low PSA group, there were 320 men classified with benign prostate disease and 235 cancers. For the high PSA group there were 315 men with benign disease and 221 cancers. Catalona et al reported median [-2]proPSA levels of 18 pg/ml and 21 pg/ml in men with benign prostate disease and prostate cancer respectively who had total PSA levels in the 2-4 ng/ml range. For men with total PSA level in the 4-10 ng/ml range, median [-2]proPSA levels of 29 pg/ml and 34 pg/ml were reported for men with benign prostate disease. This study investigated various functions of [-2]proPSA and results indicated that the ratio of [-2]proPSA to free PSA (i.e., % [-2]proPSA) gave the highest specificity with respect to prostate cancer detection when compared to free PSA and complexed PSA. Using a fixed sensitivity of 90% in the PSA range from 2 to 4ng/mL, %[-2]proPSA would have prevented 19% unnecessary biopsies compared to 10% for free PSA and 11% for complexed PSA. Similar results were found for the PSA ranges 4 to 10 ng/mL and 2 to 10 ng/mL.
De Vries et al also reported median [-2]proPSA levels of 32 pg/ml and 65 pg/ml for the less aggressive and aggressive tumor groups respectively.\textsuperscript{19}

In addition to evaluating BPSA described above, Jansen et al\textsuperscript{21} also evaluated [-2]proPSA in 405 serum samples from the Rotterdam arm of the European Randomized Study of Screening for Prostate Cancer and 351 samples from the Urology Department of Innsbruck Medical University. Of the 405 men in the screening study, 226 were CaP cases and 179 were non-CaP cases. There were 174 CaP cases and 179 non-CaP cases out of the 351 men from the urology department. ROC analyses using the AUC as well as the sensitivity and specificity were calculated to assess the ability of [-2]proPSA to distinguish men with and without CaP. In addition, nonparametric tests were used to investigate the median values of [-2]proPSA by biopsy and pathologic Gleason scores \( \geq 7 \), a measure of tumor aggressiveness. Median (range) age of men in the screening study and the urology clinic were 66 (55-75) and 60 (50-77) respectively. Jansen et al reported that [-2]proPSA, %[-2]proPSA and Prostate Health Index (PHI) consisting of a function [-2]proPSA of the and the prostate health index (PHI=\(-2\)proPSA/free PSA)* square root(total PSA)), significantly increased the predictive value and specificity of CaP in the 2 to 10 ng/mL range of total PSA. In both cohorts, analyses adding [-2]proPSA to separate models consisting of free PSA and total PSA demonstrated a significant increase in AUC from 0.67 to 0.75 and from 0.58 to 0.70 respectively. Fixing specificity at 95%, the addition of [-2]proPSA increased sensitivity from 8% to 24% for and 7% to 23% respectively for free PSA and total PSA.

In a recent prospective, multicenter study of 566 patients enrolled in a prostate cancer detection study at four National Cancer Institute Early Detection Research
Network centers, Sokoll et al reported that %[-2]proPSA was a better predictor of CaP over PSA and % free PSA, particularly in the 2 to 10 ng/ml PSA range where 25% of men may have CaP.\textsuperscript{23,24} Mean (±S.D.) ages of the cancer and noncancer group was 63.3 (±9.3) years and 60.5 (±7.9) years respectively. Using analyses of receiver operator characteristic curves, %[-2]proPSA had an area under the curve of 0.73 compared to AUCs of 0.58 and 0.61 for PSA and % free PSA respectively in the 2.0 to 4.0 ng/mL PSA range. Using a fixed sensitivity of 80%, %[-2]proPSA had a higher specificity of 51.6% compared to 29.9% and 28.9% for PSA and % free PSA respectively. Results were similar when repeated for PSA levels ranging from 2.0 to 10.0 ng/mL. Another noteworthy finding is that %[-2]proPSA increased with increasing Gleason score and was higher in aggressive cancers. The study authors concluded that %[-2]proPSA improved the ability to detect prostate cancer as was related to the risk of aggressive disease.

In a population of 2,034 men undergoing screening for CaP, Le et al also investigated the performance the prostate health index (PHI) in discriminating men with CaP from men with benign prostate disease.\textsuperscript{25} Median patient age was 57 years. Le et al compared PHI with PSA, % free PSA and %[-2]proPSA as predictors of prostate cancer in men undergoing prostate biopsy with PSA values of 2.5 to 10 ng/mL and nonsuspicious digital rectal exam. Using analysis of area under the curve from receiver operator characteristic curves, the study authors reported that % free PSA and %[-2]proPSA distinguished men with positive and negative biopsy results. PHI and %[-2]proPSA outperformed total PSA and % free PSA with area under the curves of 0.77, 0.76, 0.50 and 0.68 respectively. Fixing the sensitivity at 88.5%, the specificity of total PSA, % free PSA, %[-2]proPSA and PHI was 24.3%, 40.5%, 48.6% and 64.9%. %[-}
2]proPSA and PHI were better able to discriminate men with CaP and benign prostate disease with PSAs in the 2.5 to 10 ng/mL range and negative DRE.

Makarov et al identified 71 men with existing CaP who were enrolled in a watchful waiting program and had frozen serum and tissue available from diagnosis. Serum total PSA, free PSA, and [-2]proPSA were measured. The objective of the study was to investigate the association of serum and tissue [-2]proPSA measurements in men participating in the active surveillance program. Study participants were biopsied annually as part of the follow up program. Survival analysis methods were used to investigate the association of serum and tissue measurements with unfavorable biopsy conversion. Unfavorable biopsy was defined as: Gleason score ≥ 7gleason pattern 4/5, ≥ 3 cores involved with cancer, >50% of any core involved with cancer. Of the 71 men (mean age: 65 years) in the cohort, 39 developed unfavorable biopsies and 32 maintained favorable biopsies during almost 4 years of follow-up. The ratio of [-2]proPSA/% free PSA was significantly predictive of unfavorable biopsy conversion (Hazard Ratio (HR)=2.65, 95% CI: 1.36-5.16). Tissue levels of other isoforms of proPSA were also predictive of unfavorable biopsy conversion. The study authors conclude that serum and tissue levels of proPSA at diagnosis are associated with subsequent need for treatment. They hypothesized that the increase in the ratio of serum levels of [-2]proPSA/ % free PSA might be driven by the increased production of [-2]proPSA by "premalignant" cells in the prostate.

These previous studies investigating BPSA and [-2]proPSA were often done using cross sectional data with clinic, screening or convenience samples. However, little is known about the distribution of these novel biomarkers in a randomly selected cohort of
community-based men. What are the distributions of BPSA and [-2]proPSA in randomly selected men in the community and how do they compare with what has been previously reported? Furthermore, there are no reports of longitudinal data. How does BPSA and [-2]proPSA change over time? How do these changes correlate with changes in other urologic measures? The answers to these questions are the subject of this dissertation.

The aims of this dissertation were to: (1) describe the distribution of BPSA in a community-based sample of black and white men and examine the association of BPSA with baseline urologic measures and need for medical therapy for BPH as well as CaP, (2) describe the distribution of [-2]proPSA in a community-based sample of black and white American men and examine the association of [-2]proPSA with baseline urologic measures and CaP, (3) describe the distribution of longitudinal changes in BPSA and [-2]proPSA and how these changes vary over levels of baseline urologic conditions.
DETAILED SUBJECTS AND METHODS

Olmsted County Study

A total of 14,944 men aged 40-79 years were enumerated from all patient encounters in the clinics, hospitals and private practices in Olmsted County, Minnesota from 1986 through part of 1989. This enumeration represented about 96% of the census estimates. From this listing, 5,135 men between the ages of 40 and 79 years of age on January 1, 1990 were randomly selected within 5-year age strata, proportionally to the underlying population demographics. Telephone interviews and data collection interviews were used to exclude subjects based on medical history. Deceased and moribund subjects were excluded. Men were also excluded if they had a prior history of prostate surgery, prostate cancer, bladder surgery, bladder cancer, other bladder disorders, urethral surgery or urethral strictures. Subjects with neurological conditions that might interfere with normal voiding, such as lower back surgery, Parkinson's disease requiring medication, diabetic neuropathy leading to lower limb amputation and other progressive neurological conditions were also excluded. In addition, men were excluded if they could not void while in standing position, had any cognitive disorder that prevented them from completing questionnaires, needed renal dialysis or had anteroposterior rectum resection. Finally, prisoners, residents out of the county during the study period, non-English speaking subjects, patients with disputes with Mayo Clinic and other administrative criteria were excluded. The study protocol was approved by the Mayo Clinic and the Olmsted Medical Group institutional review boards.

At baseline, participants in the community cohort (n=2,115; 55% response rate) were visited in their homes and completed a previously validated self-administered
questionnaire with a field research assistant present. An approximate 25% random subsample was taken from eligible men (n=537) and invited to participate in the clinic component of the study resulting in a subset of 475 men in the clinic cohort. These men were selected for initial clinical evaluation, including peak urinary flow rate assessment, transrectal ultrasonography, digital rectal examination, and a serum prostate-specific antigen (PSA) determination. Aliquots of serum were banked at -70°C.

Follow-up evaluations were performed every 18-24 months after the initial assessment. All data that could be used were included in analyses. For men who were diagnosed with prostate cancer, or had a medical procedure or took medical therapy for BPH, all data up to the point of diagnosis, or initiation of medical therapy were included in the analysis. Because procedures and therapy could influence prostate volume and thus BPHA and [-2]proPSA readings, data at the time of and following such procedures of therapy were excluded from the time to event analysis.

Clinical measures of urologic function

Urinary symptoms

Lower urinary tract symptoms (LUTS) were assessed with a questionnaire analogous to the International Prostate Symptom Score (IPSS). The IPSS is also known as the American Urological Association Symptom Index. The IPSS was developed to assess frequency of seven urinary symptoms associated with BPH. It has shown good test-retest reliability and good psychometric properties (e.g., construct validity, internal consistency, etc).28

Urinary flow rates
Urinary flow rates were measured using a Dantec 1000 uroflowmeter calibrated by trained study assistants.\textsuperscript{29} Electronically measured maximum urinary flow rates, average flow rates, and voided volume were determined. Measurements were repeated if voided volume was less than 150 mL. The highest rate with a voided volume $\geq$ 150 mL was used in analyses where multiple measurements existed.

**Prostate volume / transition zone volume**

Prostate dimensions were measured by transrectal ultrasound (Bruel & Kjaer type 8551, 7.0 Mhz endosonic multiplane transducer). The anteroposterior diameter and transverse diameter were measured on the largest transverse image of the prostate, while the horizontal distance between the proximal and most distal points of the prostate on a midline sagittal scan was considered the longitudinal diameter. Total prostate volume and transition zone volume in cubic centimeters (cc) were calculated assuming a prolate ellipsoid shape using the formula (volume = $\pi/6 \times$ anteroposterior $\times$ transverse $\times$ longitudinal).\textsuperscript{30} Transrectal ultrasound has been shown to have good test-reliability among experienced observers using standardized methods.\textsuperscript{31,32}

Transition zone (also known as central zone) has been shown to be highly correlated to the transurethral resection volume.\textsuperscript{33} In addition, the transition zone to total prostate volume ratio has been reported to correlate well with prostate weight\textsuperscript{34} as well as response to 5-alpha reductase therapy.\textsuperscript{35} The transition zone is thought to be the part of the prostate that experiences the greater growth with increasing age and consequently responsible for LUTS.
**PSA/BPSA/proPSA**

PSA, BPSA and [-2]proPSA measurements were performed on banked serum. Due to freezer failure, the initial round of blood collection was lost. This study utilizes serum data from rounds 4 (n=479), 6 (n=367) and 8 (n=421) of data collection for analysis. PSA was assayed using the Tandem-R Hybritech Assay (Beckman Coulter, Inc., Fullerton, CA). BPSA and [-2]proPSA were assayed using research immunoassays (Beckman Coulter, Inc., San Diego, CA). These assays are microtiter plate immunoassays with sensitivity less than 0.025 ng/ml and cross reactivity with one another or mature PSA of less than 0.2%.

**Flint Men’s Health Study**

The Flint Men's Health Study is a cross-sectional study initiated in 1996 to estimate the prevalence of lower urinary tract symptoms in African-American men. Potential study subjects were selected for participation using a 2-stage probability sample of households or group dwelling units located in the city of Flint, Michigan and in select census tracts in Genesee County. Similar to the Olmsted County study, eligible black men were stratified into 10-year age categories—40-49, 50-59, 60-69 and 70-79 years. Older men (60-79 years) were oversampled to increase the eligible number of subjects since it was anticipated that the older men would have a higher rate of nonparticipation. The study protocol was approved by the Institutional Review Board of the University of Michigan.

Letters describing the study were mailed to housing units a week prior to being contacted for study participation. Interviews were conducted by a trained interviewer in order to collect demographic information and to determine study eligibility. Nine
hundred forty-three men were identified by probability sampling as residents in the targeted areas of interest. Of these, 819 completed the in-home interview. Subjects with a prior history of prostate cancer or prostate surgery (n=87), or who did not complete clinical evaluation (n=357) were excluded from the study. In addition, men subsequently diagnosed with prostate cancer upon screening (n=11) were also excluded resulting in a total of 364 study subjects.

**Clinical measures of urologic function**

**Urinary symptoms**

Lower urinary tract symptoms (LUTS) are assessed with the International Prostate Symptom Score (IPSS).\(^{28}\)

**Urinary flow rates**

Peak urinary flow rates were measured electronically by a portable urinary flow meter. Urinary flow rates were measured in all subjects. Subjects with a voided volume <100cc were excluded from analyses due to the unreliability of peak flow rates at that volume.

**Prostate volume**

In the FMHS, prostate volume was calculated from TRUS measurements (prostate length, height and width) performed independently in all subjects by 2 experts from static images. A third expert was only employed if the difference between any dimensional measurement was >50% for the 2 experts. Final prostate volume was
computed as the mean of the 2 experts or the mean of the 2 closest volume measurements if a third expert was utilized.

**PSA/BPSA/proPSA**

Stored blood samples were used to measure BPSA and [-2]proPSA levels using serum samples drawn in 1996 for both the OCS and FMHS (n=328) cohorts. Serum samples were obtained prior to any prostatic manipulations (e.g., DRE or TRUS) and were frozen at -70°C. The Tandem-E PSA assay (Hybritech, Incorporated, San Diego, CA) and Abbott AxSYM polyclonal-monoclonal immunoassay (Abbott Diagnostics, Abbott Park, IL) were used to measure serum PSA levels for men in the FMHS cohort. In an earlier report, we showed that PSA determinations were consistent across assays and laboratories. In this study, [-2]proPSA levels were assessed in the same laboratory using an automated, sequential, two-step immunoenzymatic (“sandwich”) assay developed for use on the Beckman Coulter (Brea, CA) Access® instrument using investigational-use-only two-site immunoenzymatic reagents provided by Beckman Coulter, Inc. In this laboratory, the intra-assay variation in [-2]proPSA levels ranged from 2.0% to 3.2% while the inter-assay variation ranged from 3.0% to 9.9%.

The details of subject selection and methods are presented more briefly in the three manuscripts that follow due to the word constraints imposed by the scientific journals. The first two manuscripts report the distribution of BPSA and [-2]proPSA in the Olmsted County and Flint Men's Health Study cohorts. Since follow-up data is available in the Olmsted County study, baseline values of these biomarkers are used to predict subsequent urologic outcomes. The third manuscript describes the distribution of the
longitudinal changes in BPSA and [-2]proPSA in the Olmsted County study.


antigen, is found predominantly in the transition zone of patients with nodular benign prostatic hyperplasia. Urology, 55: 41, 2000.


Manuscript #1

Benign Prostate-Specific Antigen Distributions and Associations in Community-Dwelling Black and White Men

Thomas Rhodes\textsuperscript{1,4}, Debra J. Jacobson\textsuperscript{2}, Michaela E. McGree\textsuperscript{2}, Jennifer L. St. Sauver\textsuperscript{2}, Aruna V. Sarma\textsuperscript{3}, Cynthia J. Girman\textsuperscript{4}, Michael M. Lieber\textsuperscript{5}, George G. Klee\textsuperscript{6}, Kitaw Demissie\textsuperscript{1}, Steven J. Jacobsen\textsuperscript{7}

\textsuperscript{1}Department of Epidemiology, University of Medicine and Dentistry in New Jersey, Piscataway, NJ
\textsuperscript{2}Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN
\textsuperscript{3}Departments of Epidemiology and Urology, University of Michigan, Ann Arbor, MI
\textsuperscript{4}Epidemiology Department, Merck Research Laboratories, North Wales, PA
\textsuperscript{5}Department of Urology, Mayo Clinic College of Medicine, Rochester, MN
\textsuperscript{6}Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN
\textsuperscript{7}Department of Research and Evaluation, Kaiser Permanente, Southern California, Pasadena, CA
Abstract

Objectives. To describe the cross-sectional associations of benign prostate-specific antigen (BPSA) with clinical urologic measures, and to examine the risk of future urologic outcomes in two population-based cohorts of black and white men.

Methods. Two population-based, cohort studies were established to characterize the natural history and risk factors for progression of prostate disease in white and black male residents of Olmsted County, Minnesota and Genesee County, Michigan, respectively.

Results. The distribution of BPSA levels was similar in blacks (median (25th, 75th percentiles) =32.9 (17.3, 68.0) pg/mL) and whites (median=32.2 (16.6, 68.9) pg/mL), p=0.71, but much lower than previous reports. For Olmsted County men in the upper quartile of BPSA, there was a 15-fold increased risk of prostate cancer (CaP; hazard ratio (HR): 14.6 95% confidence interval (CI): 3.1, 68.6) and a 2-fold higher risk of treatment for benign prostatic hyperplasia (BPH; HR: 2.2, 95% CI: 1.2, 4.2) after adjusting for age. After additional adjustment for baseline PSA, the association between BPSA and CaP risk was attenuated, but remained nearly 9-fold higher for men in the upper BPSA quartile (HR: 8.7, 95% CI: 1.8, 42.4). The 2-fold higher risk of treatment for BPH also remained after adjustment for baseline PSA for men in the upper BPSA quartile (HR: 1.9, 95% CI: 0.9, 4.0).

Conclusions. These results suggest that elevated BPSA level may help to identify men at risk of treatment for BPH and identify men with prostate cancer.
Introduction

Prostate-specific antigen (PSA) is a widely used serum marker for the early
detection of prostate cancer (CaP) and is recommended in men over 50 years or at high
risk of CaP by the National Cancer Institute/American Urological Association
guidelines.[National Cancer Institute. Prostate cancer screening / Prostate-specific
Antigen (PSA) test 2011 1/04/2011 [cited; Available from:
Available from: http://www.auanet.org/content/media/psa09.pdf] However, elevated
PSA levels are not specific to CaP, since PSA can also be elevated in benign conditions
such as benign prostatic hyperplasia (BPH) or prostatitis.

Benign prostate-specific antigen (BPSA) is an inactive form of PSA that has been
cleaved at lysine residues 145 and 182.1,2 This form of free PSA has been shown to be
elevated in nodular BPH tissue and is also correlated with transition zone volume and
prostatic enlargement.3-5 Additionally, BPSA was found to be significantly higher in men
with an elevated PSA level and a percent free PSA level less than 15% who were also
biopsy negative for CaP.6 Together, these results suggest that BPSA may be associated
with benign disease.

The characteristics of BPSA have been described in clinical and convenience
samples. 3-5,7 These studies provide initial support for the usefulness of BPSA as a
marker for benign prostate disease; however, they may not reflect the full spectrum of
men with and without benign prostate disease. The objective of this paper is to provide
normative data in a population-based, biracial sample of men and to describe the cross-
sectional associations between baseline values of BPSA and common clinical urologic measures. In addition, we examined associations between baseline values of BPSA and risk of clinically relevant urologic outcomes such as acute urinary retention (AUR), CaP, and treatment for BPH.

**Methods**

Details on subject selection for both the Olmsted County Study of Urinary Symptoms and Health Status among Men (OCS) and Flint Men’s Health Study (FMHS) have been published previously.\(^8,9\) Briefly, the OCS and FMHS are population-based, cohort studies established to characterize the natural history and risk factors for progression of prostate disease in white and black male residents of Olmsted County, Minnesota and Genesee County, Michigan, respectively. In the OCS, 2,115 of 3,874 (55%) eligible white men aged 40–79 years in 1990 without a history of prostate cancer or surgery or other conditions known to interfere with voiding, completed the self-administered American Urological Association Symptom Index (AUASI).\(^10\) A detailed urologic examination that included uroflowmetry, digital rectal exam, transrectal ultrasound (TRUS), and serum PSA measurement was performed in a 25% random subsample (476 participants of 537 sampled, 89%).\(^8,11\) The cohort was actively followed on a biennial basis for seventeen years using a protocol similar to the initial examination.

Applying similar criteria and procedures as the OCS, the FMHS recruited 730 of 943 (77%) eligible black men to complete an interview-administered questionnaire in 1996.\(^9,12\) Of these, 369 (51%) men were free of prostate cancer and completed a comprehensive urologic examination that included, as in the OCS, uroflowmetry, TRUS,
a serum PSA measurement, and a self-administered AUASI. The selective participation in the clinical examination and potential resulting selection bias have been addressed previously.13,14

Since the FMHS urologic measurements were collected in 1996, BPSA measures were obtained from serum samples collected in 1996 for both studies and the corresponding 1996 OCS measurements were used as the initial/baseline values. Levels of BPSA were measured in 420 OCS men and 329 FMHS men. This study received approval from the Mayo Clinic, Olmsted Medical Center, and University of Michigan Medical School Institutional Review Boards.

**Urinary flow rates**

Urinary flow rates were measured using a Dantec 1000 uroflowmeter calibrated by trained study assistants.15 Electronically measured maximum urinary flow rates, average flow rates, and voided volume were determined. Measurements were repeated if voided volume was less than 150 mL. The highest rate with a voided volume ≥ 150 mL was used in analyses where multiple measurements existed.

**Prostate volume**

Total prostate volume for the OCS and FMHS participants were measured via transrectal ultrasound (TRUS).9,16 In addition to assessing the echogenic pattern of the prostate gland, three measurements were made to calculate the total volume of the prostate assuming a prolate ellipsoid shape.17
Prostate cancer and acute urinary retention

Information on use of medical and surgical LUTS/BPE treatments was obtained through self-report and through passive follow-up of the community medical records for the OCS men. Dates of biopsy-confirmed prostate cancer and acute urinary retention were abstracted from community medical records. Acute urinary retention was defined as medical record documentation of both the inability to urinate and catheterization to empty the bladder. Catheterizations done perioperatively were excluded, but episodes in which a catheter was needed after surgery, secondary to acute retention were included. Data on CaP, AUR and treatment were not available for the FMHS men; therefore analyses examining associations between BPSA and these outcomes were confined to the OCS cohort.

Assays

For both studies, serum samples were obtained prior to any prostatic manipulations, including digital rectal examination and transrectal ultrasound, and were frozen at -70°C for latter assays. Stored blood samples from the OCS fourth biennial round (1996) of follow-up (n=42) and from the FMHS baseline (1996) visit (n=329) were used to measure BPSA levels. Serum PSA levels for men in the OCS were assayed using the Tandem-E PSA assay (Hybritech, Incorporated, San Diego, CA). Serum PSA levels for men in the FMHS were assayed using the Abbott AxSYM polyclonal-monoclonal immunoassay (Abbott Diagnostics, Abbott Park, IL). The lower limit of detection for this Abbott assay is reported to be 0.1 ng/mL.18 We have previously reported that PSA determinations were consistent across different assays and laboratories.19 For both
studies, BPSA levels were assessed using an automated, sequential, two-step immunoenzymatic ("sandwich") assay developed for use on the Beckman Coulter (Brea, CA) Access® instrument. The BPSA measurements were run on an Access 2 Immunoassay analyzer using research-use-only two-site immunoenzymatic reagents provided by Beckman Coulter, Inc. In our hands, the intra-assay variation ranged from 4.3% to 8.1% while the inter-assay variation ranged from 5.1% to 5.2%.

**Statistical analysis**

Descriptive statistics and cumulative distribution function plots were used to describe the distributions of BPSA. Spearman correlations ($r_s$) and logistic regression were used to investigate the relationships between BPSA and other baseline urologic measures. Multivariable logistic regression was used to describe the association of BPSA with clinically meaningful diagnostic measures, which are presented as odds ratios (ORs) and 95% confidence intervals (CIs). Moderate-to-severe LUTS was defined as AUASI score $>7$ while depressed urinary flow rate was defined as maximum urinary flow rate $<12$ mL/s. Prostatic enlargement was defined as prostate volume $>30$ cc. Due to availability of follow-up data, Cox proportional hazards regression was used in the OCS cohort only to investigate the association of baseline values of BPSA with the risk of a subsequent diagnosis of AUR, biopsy confirmed CaP, and treatment for BPH which are presented as hazard ratios (HRs) and 95% CIs.

**Results**
Baseline characteristics of the participants in each cohort are shown in Table 1. Urological characteristics were similar for FMHS and OCS men. The two cohorts were also similar with respect to distribution of BPSA levels. BPSA percentile levels increased across age decade almost uniformly for each percentile shown (Figure 1). The 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles of the combined BPSA distributions were 7.3, 10.1, 16.9, 32.5, 68.8, 157.0, and 267.9 pg/mL, respectively.

BPSA was significantly correlated with age and all urologic measures examined, with Spearman correlations ($r_s$) ranging from 0.11-0.82 (all P values <0.05, Table 2). BPSA was most strongly correlated with PSA levels ($r_s$: 0.82 and 0.80 for OCS and FMHS, respectively; p<0.0001) as well as age ($r_s=-.58$, $r_s=0.4$) and prostate volume ($r_s=0.52$, $r_s=0.5$).

For both cohorts, men in the upper quartile of BPSA were more likely to have moderate-to-severe LUTS, a depressed urinary flow rate, and an enlarged prostate compared to men in the lower quartile (Table 3). The magnitude of the association between baseline values of BPSA and the urologic measures increased with increasing quartile of BPSA; however, it was most pronounced for an enlarged prostate (tests for trend: all P values <0.05).

Overall 46 (11.3%) men in the OCS had AUR, 111 (27.8%) of men were treated for BPH, and 41 (9.8%) men were diagnosed with CaP after the baseline BPSA measurements. Men with higher baseline levels of BPSA (upper quartile) were more likely to have a subsequent event of AUR (age-adjusted HR: 2.1, 95% CI: 0.7, 6.5), biopsy-confirmed CaP (age-adjusted HR: 14.6, 95% CI: 3.1, 68.6), and treatment for BPH (age-adjusted HR: 2.2, 95% CI: 1.2, 4.2) than men in the lowest quartile level of
BPSA (Table 4). For all three outcomes, the magnitude of the association increased with increasing BPSA quartile (tests for trend, age-adjusted P values: 0.17, <0.0001, and 0.003 for AUR, treatment for BPH, and CaP, respectively).

After further adjusting for baseline PSA, the associations between BPSA and all outcomes were attenuated (Table 4). However, the association between BPSA and CaP remained nearly 9-fold higher for men in the upper quartile relative to men in the lowest quartile (HR: 8.7, 95% CI: 1.8, 42.4). Additionally, the association between the highest quartile of BPSA and a two-fold increased risk of BPH treatment also remained after adjustment for baseline PSA (Table 4; HR: 1.9, 95% CI: 0.9, 4.0), however it was no longer statistically significant. To assess the impact of potential multicollinearity of dependent variables, the order of entry of age, baseline PSA and BPSA were varied using forward selection criteria with the sequential option and SENTRY=0.10 in PROC PHREG to assess the effects on standard errors of the parameter estimates. This had no appreciable effect on the standard errors and final model suggesting that BPSA adds additional information in the presence of PSA.

**Discussion**

These results suggest that black and white men have similar distributions of BPSA. Men with higher levels of BPSA were more likely to have a depressed urinary flow rate and an enlarged prostate. For the OCS, men with higher baseline levels of BPSA were also more likely to receive subsequent treatment for BPH (i.e., medical therapy, procedure or surgery) and were more likely to be subsequently diagnosed with biopsy-confirmed CaP even after adjusting for age and baseline PSA.
The median (25th, 75th percentiles) BPSA levels in the OCS and FMHS cohorts were 32.2 (16.6, 68.9) and 32.9 (17.3, 68.0) pg/mL, respectively. These levels are much lower than levels previously reported in patients receiving a biopsy or patients who had a diagnosis of prostate cancer. Canto, et al. in a study of 91 consecutive patients without prostate cancer who underwent a 10-core biopsy, reported a median BPSA level of 220 pg/mL. This observed value of BPSA falls at approximately the 93rd percentile in the combined FMHS and OCS distribution. In another study, de Vries, et al reported data from 61 men participating in the screening arm of a large cancer screening study in which men with less aggressive prostate cancer had a median BPSA value of 172 pg/mL and those men with more aggressive prostate cancer had a median BPSA value of 264 pg/mL, again, falling into approximately the 91st and 95th percentile of the BPSA distribution observed for the combined cohorts. Linton, et al. reported median BPSA levels of 220 pg/mL and 170 pg/mL in 79 men with BPH (defined as elevated PSA with negative biopsy) and 91 men with prostate cancer, respectively. These median values reported by Linton for BPSA were both above the 90th percentile of the BPSA distribution for this study. The higher levels observed in these other studies could be due to differences in the underlying populations or to laboratory measurement/calibration.

BPSA has been associated with clinically significant prostatic enlargement in the transition zone and Canto referred to this result as evidence of the potential of BPSA as a novel predictor of outcomes or response to therapy in patients with BPH. Results from phase II and III studies of the Medical Therapy of Prostatic Symptoms Prostatic Samples Analysis Consortium suggested that BPSA is predictive of BPH progression after accounting for prostate volume. The current study found that BPSA was correlated
with prostate volume and PSA level in both black and white men. BPSA was also modestly correlated with AUASI score and maximum urinary flow rate in both cohorts. In the OCS, men with higher baseline levels of BPSA were also more likely to receive subsequent treatment for BPH. These data suggest that BPSA may have utility in predicting LUTS severity and prostate enlargement.

Based on an artificial neural network model, Stephan, et al also found that BPSA may improve prostate cancer detection. In this study, higher levels of BPSA were associated with risk of biopsy-confirmed CaP after adjustment for age and baseline PSA level. This result seems to support the potential utility of this marker in further distinguishing men with or without prostate cancer in the presence of a PSA test. It has been suggested that BPSA may improve the sensitivity and specificity of detecting prostate cancer in men who have PSA levels in the 4-10 ng/mL range or when percent free PSA is less than 15%.

Due to the restriction in the range of values in both the benign prostate disease group and the prostate cancer group, the study samples from these previous reports may not necessarily be the most appropriate for investigating assay performance in differentiating benign prostate disease or prostate cancer. It appears that both the benign prostate disease group and the prostate cancer groups from the previous reports have high levels of BPSA when compared to the general population. Thus, the previous reports may actually be a conservative estimate of the utility of distinguishing diseased men from healthy men if the higher levels observed in these other studies are due to the underlying study populations and the true differences between “diseased” and “non-diseased” men are greater.
The generalizability of the results to other races or ethnicities may be limited; however, few differences were seen between black and white men in cross-sectional analyses. In addition, the stability of BPSA in frozen samples is currently unknown, however free and total PSA have appeared to be stable in serum samples frozen two years at -70°C.25

Conclusions

These population-based data provide useful reference ranges to researchers for future studies examining the utility of BPSA, with similar distributions of BPSA for white and black men. Elevated levels suggest some prostate disease, benign or malignant. Further work is needed to develop our understanding of how this might be used in a diagnostic workup.
**Acknowledgment.** The authors thank the men who participate in the Olmsted County Study and the Flint Men’s Health Study, the study personnel, and Ms. Kristie Shorter for her assistance in preparation of this manuscript. This project was supported by research grants from the Public Health Service, National Institutes of Health (Grants DK58859, AR30582 and 1UL1 RR024150-01), National Cancer Institute (P50CA69568) and Merck Research Laboratories. Beckman Coulter (Brea, CA) provided the test kits for BPSA free of charge and with no obligation.

**Disclosures.** Dr. Klee has received research grants and royalties for unrelated technologies from Beckman Coulter, Inc. Dr. Jacobsen has received research grants from Beckman Coulter, Inc.
Table 1. Baseline characteristics of Olmsted County Study of Urinary Symptoms and Health Status among Men and Flint Men’s Health Study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OCS N=420</th>
<th>FMHS N=329</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>112 (26.7)</td>
<td>91 (27.7)</td>
<td>0.64</td>
</tr>
<tr>
<td>50-59</td>
<td>143 (34.0)</td>
<td>104 (31.6)</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>92 (21.9)</td>
<td>83 (25.2)</td>
<td></td>
</tr>
<tr>
<td>70+</td>
<td>73 (17.4)</td>
<td>51 (15.5)</td>
<td></td>
</tr>
<tr>
<td>AUASI score (&gt;7)</td>
<td>183 (43.6)</td>
<td>128 (39.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>Maximum urinary flow rate (&lt;12 mL/s)</td>
<td>67 (18.9)</td>
<td>28 (14.4)</td>
<td>0.18</td>
</tr>
<tr>
<td>Prostate volume (&gt;30 cc)</td>
<td>153 (40.7)</td>
<td>111 (35.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>PSA (&gt;1.4 ng/mL)</td>
<td>146 (34.8)</td>
<td>109 (33.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>BPSA (pg/mL)</td>
<td>Median (Q1, Q3)</td>
<td>Median (Q1, Q3)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*Rank-sum P value reported for continuous variables, chi-square P value reported for dichotomous variables
†Percentages based on non-missing observations
AUASI: American Urological Association Symptom Index; Q1: 25th percentile, Q3: 75th percentile
Table 2. Spearman correlations between baseline BPSA and baseline clinical urologic measures.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BPSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OCS</td>
</tr>
<tr>
<td>Age</td>
<td>$0.58^*$</td>
</tr>
<tr>
<td>Maximum flow rate</td>
<td>$-0.18^*$</td>
</tr>
<tr>
<td>Prostate volume</td>
<td>$0.52^*$</td>
</tr>
<tr>
<td>AUASI score</td>
<td>$0.28^*$</td>
</tr>
<tr>
<td>PSA</td>
<td>$0.82^*$</td>
</tr>
</tbody>
</table>

$^P<0.05$
Table 3. Crude and age-adjusted associations between BPSA and urologic measures.

<table>
<thead>
<tr>
<th>BPSA quartile (range, pg/mL)</th>
<th>Moderate-to-severe LUTS (AUASI score &gt;7)</th>
<th>Depressed urinary flow rate (Maximum flow rate &lt;12 mL/s)</th>
<th>Enlarged prostate (Prostate volume &gt;30 cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Olmsted County Study</td>
<td>Flint Men's Health Study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crude OR (95% CI)</td>
<td>Age-adj. OR (95% CI)</td>
<td>Crude OR (95% CI)</td>
</tr>
<tr>
<td>Quartile 1 (min-16.9)</td>
<td>reference</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>Quartile 2 (17.0-32.5)</td>
<td>1.3 (0.7, 2.3)</td>
<td>1.2 (0.7, 2.1)</td>
<td>1.3 (0.7, 2.5)</td>
</tr>
<tr>
<td>Quartile 3 (32.6-68.8)</td>
<td>1.3 (0.8, 2.3)</td>
<td>1.1 (0.6, 2.0)</td>
<td>1.8 (0.97, 3.5)</td>
</tr>
<tr>
<td>Quartile 4 (68.9-max)</td>
<td>4.5 (2.6, 8.1)</td>
<td>3.0 (1.5, 5.9)</td>
<td>2.2 (1.2, 4.3)</td>
</tr>
<tr>
<td>P trend</td>
<td>&lt;0.0001</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

|                              | Olmsted County Study                      | Flint Men's Health Study                                |                                          |
|                              | Crude OR (95% CI)                          | Age-adj. OR (95% CI)                                    | Crude OR (95% CI)                        | Age-adj. OR (95% CI) |
| Quartile 1 (min-16.9)        | reference                                  | reference                                              | reference                                | reference            |
| Quartile 2 (17.0-32.5)       | 1.0 (0.4, 2.3)                             | 0.9 (0.4, 2.1)                                         | 2.4 (0.6, 9.9)                           | 2.1 (0.5, 8.9)      |
| Quartile 3 (32.6-68.8)       | 1.8 (0.8, 4.1)                             | 1.5 (0.7, 3.5)                                         | 1.3 (0.3, 7.0)                           | 0.9 (0.2, 5.1)      |
| Quartile 4 (68.9-max)        | 3.8 (1.8, 8.2)                             | 2.5 (1.0, 6.2)                                         | 10.0 (2.7, 37.7)                         | 5.9 (1.5, 23.8)     |
| P trend                      | 0.0002                                    | 0.02                                                   | 0.0004                                   | 0.01                 |

|                              | Olmsted County Study                      | Flint Men's Health Study                                |                                          |
|                              | Crude OR (95% CI)                          | Age-adj. OR (95% CI)                                    | Crude OR (95% CI)                        | Age-adj. OR (95% CI) |
| Quartile 1 (min-16.9)        | reference                                  | reference                                              | reference                                | reference            |
| Quartile 2 (17.0-32.5)       | 2.3 (1.1, 4.5)                             | 1.9 (0.9, 3.9)                                         | 2.7 (1.1, 6.7)                           | 2.7 (1.1, 6.5)      |
| Quartile 3 (32.6-68.8)       | 3.9 (2.0, 7.6)                             | 2.8 (1.4, 5.7)                                         | 6.2 (2.7, 14.7)                          | 5.6 (2.4, 13.3)     |
| Quartile 4 (68.9-max)        | 15.2 (7.4, 31.2)                           | 7.8 (3.4, 17.6)                                        | 18.8 (7.8, 45.3)                         | 15.2 (6.1, 37.7)    |
| P trend                      | <0.0001                                   | <0.0001                                                | <0.0001                                  | <0.0001              |

OR: odds ratio, CI: confidence interval
Table 4. Crude and age- and PSA-adjusted association of baseline BPSA levels with occurrence of urologic outcomes in the Olmsted County Study.

<table>
<thead>
<tr>
<th>BPSA quartile (range, pg/mL)</th>
<th>Acute Urinary Retention (N=46)</th>
<th>Biopsy-Confirmed Prostate Cancer (n=41)</th>
<th>Treatment for BPH (N=111)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude HR (95% CI)</td>
<td>Age-adj. HR (95% CI)</td>
<td>Age-, PSA-adj. HR (95% CI)</td>
</tr>
<tr>
<td>Quartile 2 (17.0-32.5)</td>
<td>1.7 (0.5, 5.1)</td>
<td>1.5 (0.5, 4.5)</td>
<td>1.4 (0.5, 4.4)</td>
</tr>
<tr>
<td>Quartile 3 (32.6-68.8)</td>
<td>2.6 (0.9, 7.3)</td>
<td>1.8 (0.6, 5.3)</td>
<td>1.7 (0.6, 4.9)</td>
</tr>
<tr>
<td>Quartile 4 (68.9-max)</td>
<td>4.6 (1.7, 12.2)</td>
<td>2.1 (0.7, 6.5)</td>
<td>1.5 (0.4, 5.4)</td>
</tr>
<tr>
<td>P trend</td>
<td>0.0004</td>
<td>0.17</td>
<td>0.48</td>
</tr>
</tbody>
</table>

HR: hazard ratio, CI: confidence interval
Figure 1. Cumulative distribution function plots of BPSA by age decade in the combined Olmsted County Study and Flint Men's Health Study Sample.
References


Manuscript #2

Distribution and Associations of [-2]Pro-Prostate-Specific Antigen in Community-Dwelling Black and White Men

Thomas Rhodes¹,⁴, Debra J. Jacobson², Michaela E. McGree², Jennifer L. St. Sauver², Aruna V. Sarma³, Cynthia J. Girman⁴, Michael M. Lieber⁵, George G. Klee⁶, Kitaw Demissie¹, Steven J. Jacobsen⁷

¹Department of Epidemiology, University of Medicine and Dentistry in New Jersey, Piscataway, NJ
²Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN
³Departments of Epidemiology and Urology, University of Michigan, Ann Arbor, MI
⁴Epidemiology Department, Merck Research Laboratories, North Wales, PA
⁵Department of Urology, Mayo Clinic College of Medicine, Rochester, MN
⁶Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN
⁷Department of Research and Evaluation, Kaiser Permanente, Southern California, Pasadena, CA
Abstract

Objectives. To provide cross-sectional normative data for [-2]pro-prostate-specific antigen ([-2]proPSA) from the Olmsted County Study of Urinary Symptoms and Health Status among Men (OCS) and the Flint Men’s Health Study (FMHS) and to describe the associations with clinical urologic measures and risk of prostate cancer diagnosis.

Methods. Measurements of [-2]proPSA were obtained from n=420 white men from Olmsted County, Minnesota and n=328 black men from Genesee County, Michigan. Cross-sectional associations between [-2]proPSA and prostate enlargement / elevated PSA level were assessed. Cox proportional hazard models were used to assess associations between [-2]proPSA and incident diagnosis of prostate cancer.

Results. Baseline [-2]proPSA level was slightly higher among black men (median (25th, 75th percentiles) = 6.3 (4.1, 8.9) pg/mL) than among white men (median = 5.6 (3.9, 7.7) pg/mL), p = 0.01. Baseline [-2]proPSA level was highly predictive of biopsy-confirmed prostate cancer (CaP) in the OCS cohort. Relative to men in the lower quartile of [-2]proPSA, men in the upper quartile had almost an 8-fold increase in the risk of CaP (hazard ratio (HR): 7.8, 95% confidence interval (CI): 2.2, 27.8) after adjustment for age and baseline PSA.

Conclusions. Levels of [-2]proPSA in these cohorts of community-dwelling black and white men are much lower than seen in previous studies. These data suggest that levels of [-2]proPSA may help identify prostate cancer in men with serum PSA levels in an indeterminate range, although the reference ranges for white and black men may differ slightly.
Introduction

Prostate cancer is the most common non-cutaneous cancer in U.S. men and it is estimated that there will be 217,730 new cases and 32,050 deaths from prostate cancer in the United States in 2010.\(^1\) Prostate-specific antigen (PSA) is widely used as a serum marker for prostate cancer screening. However, the diagnostic value of PSA testing is limited by its lack of specificity,\(^2,3\) particularly in the range of 2-10 ng/mL where elevated PSA levels are often due to benign disease.

Precursor forms of PSA, such as [-2]pro-prostate-specific antigen ([-2]proPSA) have been identified as promising new biomarkers for distinguishing men with prostate cancer.\(^4,5\) Preliminary studies suggest that [-2]proPSA may enhance the sensitivity and specificity for distinguishing benign disease from prostate cancer.\(^6\) This may, in turn, result in fewer biopsies, particularly in men who have PSA levels in the 4-10 ng/mL range, where specificity may be lower. Moreover, this biomarker was reported to distinguish men with less aggressive tumors from men with more aggressive tumors\(^7-10\) as well as to predict prostate cancers requiring treatment.\(^11\) In the 2-10 ng/mL range of PSA level, [-2]proPSA had the highest specificity to detect prostate cancer\(^6\), particularly when the ratio of [-2]proPSA to % free PSA level (%[-2]proPSA) was considered\(^5,9,12,13\). In a prospective study, Le and colleagues reported that %[-2]proPSA provided better discrimination to detect biopsy-confirmed prostate cancer on biopsy than PSA level or % free PSA.\(^12,13\). On immunostaining, [-2]proPSA has shown the most cancer specificity.\(^7\)

These initial studies of [-2]proPSA have, however, been limited to select patient groups including clinical series of patients, men selected for biopsy or radical prostatectomy, or prostate cancer screening studies, which do not reflect the full range of
[-2]proPSA levels. The [-2]proPSA levels in these select samples may not reflect the levels in the general community. In order to gain a broader understanding of the utility of [-2]proPSA, we utilized data from the Olmsted County Study of Urinary Symptoms and Health Status among Men (OCS) and the Flint Men’s Health Study (FMHS) to describe baseline levels of [-2]proPSA and cross-sectional associations between [-2]proPSA and urologic measures in two population-based samples of black and white men. In addition, we examined associations between baseline values of [-2]proPSA and risk of biopsy-confirmed prostate cancer (CaP).

Methods

Subjects

Many of the details regarding the construction of both the OCS and FMHS studies have been described previously. Briefly, the OCS is a cohort of white men 40 to 79 years old who were randomly selected from an enumeration of the 1990 Olmsted County, Minnesota population. Men who had a history of prostate cancer, prostate surgery or other conditions known to interfere with voiding were excluded. After excluding men with these pre-existing conditions, 3,874 men were asked to join the study, and 2,115 (55%) agreed to participate and completed a previously validated questionnaire. A 25% random sub-sample 476/537 (89%) participated in a full urologic exam which included a transrectal ultrasound to measure prostate volume and a blood draw to measure serum PSA level. The cohort has been followed biennially since 1990. Men who died or were lost to follow-up were replaced during rounds 2 and 3. The OCS study
received approval from the Mayo Clinic and Olmsted Medical Center Institutional Review Boards (IRB).

The FMHS cohort consists of a probability sample of black men from households located in Genesee County, Michigan in 1996. Subjects were ineligible if they reported a history of prostate cancer or a prior operation on the prostate gland. A trained interviewer contacted each sample household, identified 730 eligible subjects, and performed a detailed in-home interview which included completion of the American Urological Association Symptom Index (AUASI). All participants were invited to participate in a comprehensive urologic examination, similar to that received by men participating in the OCS study. Of the 730 men, 379 (52%) completed the exam phase of the study. The FMHS study received approval from the University of Michigan Medical School IRB.

The FMHS samples and baseline information were collected in 1996. To make the two data sources comparable, the corresponding 1996 OCS measurements and blood samples were used for the initial/baseline values. Levels of [-2]proPSA were available for 420 OCS men and 328 FMHS men.

**Prostate volume**

During the in-clinic exam, total prostate volume was measured via transrectal ultrasound for the OCS and FMHS participants. Anteroposterior and transverse diameters were measured at the maximal dimensions, and the superior-inferior diameter was measured at the maximal length from the base to the apex of the midline sagittal
plane and combined to calculate the total volume of the prostate assuming a prolate ellipsoid shape.\textsuperscript{21}

**Prostate cancer**

Community medical records were reviewed for all men in the OCS cohort and dates of biopsy-confirmed prostate cancer were recorded. As community medical records were not available for the FMHS men, analyses examining associations between [-2]proPSA and future prostate cancer diagnosis were limited to the OCS cohort.

**Assays**

Stored blood samples were used to measure [-2]proPSA levels using serum samples drawn in 1996 for both the OCS (n=420) and FMHS (n=328) cohorts. Serum samples were obtained prior to any prostatic manipulations and were frozen at -70°C. The Tandem-E PSA assay (Hybritech, Incorporated, San Diego, CA) and Abbott AxSYM polyclonal-monoclonal immunoassay (Abbott Diagnostics, Abbott Park, IL) were used to measure serum PSA levels for men in the OCS and FMHS cohorts, respectively. In an earlier report, we showed that PSA determinations were consistent across assays and laboratories.\textsuperscript{22} In this study, [-2]proPSA levels were assessed in the same laboratory using an automated, sequential, two-step immunoenzymatic (“sandwich”) assay developed for use on the Beckman Coulter (Brea, CA) Access® instrument using investigational-use-only two-site immunoenzymatic reagents provided by Beckman Coulter, Inc. In this laboratory, the intra-assay variation in [-2]proPSA levels ranged from 2.0% to 3.2% while the inter-assay variation ranged from 3.0% to 9.9%.
Statistical analysis

Descriptive statistics were used to describe the distributions of [-2]proPSA and urologic measures. The cumulative distribution function by age decade was plotted for each race. Cross-sectional associations of [-2]proPSA with prostatic enlargement (prostate volume >30 cc) and elevated PSA (PSA level >2.5 ng/mL) were assessed using logistic regression models with results presented as odds ratios (ORs) and 95% confidence intervals (CIs). Multivariable models were used to adjust for age. Longitudinal follow-up data, available only in the OCS cohort, was used to assess the association between baseline [-2]proPSA level and risk of a subsequent diagnosis of biopsy-confirmed CaP using Cox proportional hazards regression. Hazard ratios (HRs) and 95% CIs were used to describe the associations between these measures.

Results

Baseline characteristics of the OCS and FMHS cohorts are shown in Table 1. Men in the FMHS had slightly smaller prostates and lower median PSA levels than OCS men. The median [-2]proPSA level was higher in the FMHS cohort [median (25th, 75th percentiles) = 6.3 (4.1, 8.9) pg/mL] than in the OCS cohort [median= 5.6 (3.9, 7.7) pg/mL], p=0.01. For reference, selected percentiles (e.g., minimum, 5, 10, 25, 50, 75, 90, 95, and maximum) of [-2]proPSA are presented in Table 2 for the individual and combined samples. The 95th percentiles of the [-2]proPSA levels were <14.7 pg/mL and <18.4 pg/mL for men in the OCS and FMHS cohorts, respectively. The percentile levels of [-2]proPSA across the entire distribution increased consistently with age decade (Figures 1a and 1b).
After adjusting for age, \([-2\)proPSA levels were correlated with prostate volume (age-adjusted Spearman correlation\(r_S\): OCS \(r_S=0.46\), FMHS \(r_S=0.47\)) and PSA level (OCS \(r_S=0.63\), FMHS \(r_S=0.71\)), all \(P<0.0001\). For both blacks and whites, men in the upper quartile of \([-2\)proPSA were more likely to have an enlarged prostate and an elevated PSA level than those in the lowest quartiles, even after adjusting for age (Table 3). These associations increased with increasing quartile of \([-2\)proPSA (Table 3, test for trend, \(P<0.0001\)).

There were 41 (9.8\%) incident cases of prostate cancer in the OCS cohort after the baseline \([-2\)proPSA measurements. Men in the upper quartile of the \([-2\)proPSA distribution were more likely to be diagnosed with biopsy-confirmed prostate cancer (age-adjusted HR: 10.8, 95\% CI: 3.1, 36.9), compared to men in the lowest quartile (Table 4). The magnitude of the association with prostate cancer increased with increasing \([-2\)proPSA quartile (tests for trend, \(P<0.0001\)). The association was slightly attenuated after further adjusting for baseline PSA, but remained nearly 8-fold higher for men in the upper quartile of \([-2\)proPSA relative to men in the lowest quartile (HR: 7.8, 95\% CI: 2.2, 27.8). To assess the impact of potential multicollinearity of dependent variables, the order of entry of age, baseline PSA and \([-2\)proPSA were varied using forward selection criteria with the sequential option and SLENTRY=0.10 in PROC PHREG to assess the effects on standard errors of the parameter estimates. This had no appreciable effect on the standard errors and final model suggesting that \([-2\)proPSA adds additional information in the presence of PSA. Similar results were seen when adjusting for baseline prostate volume, and for both baseline PSA and prostate volume (data not shown).
Discussion

These results provide normative values from population-based cohorts of black and white men. Although the levels of [-2]proPSA were slightly higher in black men than in white men in this study, previous studies based on clinical and screening cohorts of [-2]proPSA have reported values that on average were much higher (i.e., >90th percentile) than that observed in this study. Median levels in these previous studies ranged from 18 to 65 pg/mL. These levels are at the 95th percentile or greater of the two cohorts included in this study. As men in these screening studies had higher PSA levels than the general community, these differences are likely due to differences between these groups and men in the general community. Alternatively, differences in laboratory measurements or calibration could also lead to differences in the results.

Interestingly, while PSA levels were slightly lower among black men, [-2]proPSA levels were slightly higher for black men compared to white men. This may suggest that the conversion to PSA is slightly down-regulated in black men. Given that the incidence of CaP differs by race, further studies comparing racial differences in PSA and [-2]proPSA levels may provide important insights.

Several other papers have reported improved prostate cancer detection using [-2]proPSA or %[-2]proPSA, particularly in the PSA range of 2-10 ng/mL, and better differentiation of aggressive disease. In this study, higher baseline levels of [-2]proPSA were associated with future diagnosis of biopsy-confirmed CaP after adjustment for age and baseline PSA level. This result provides additional support to the potential use of this marker in distinguishing men with or without prostate cancer.
Many of these studies report sensitivities and specificities for various thresholds and functions of these biomarkers; however the study samples from these previous reports may not accurately reflect the assay performance in distinguishing benign prostate disease from prostate cancer. Compared to the general population, the previous studies are based on men with elevated serum PSA and [-2]proPSA levels. Because of this, the differences between men with and without cancer in the overall PSA range and therefore the utility of identifying men with prostate cancer may be greater than these previous reports have suggested.15

This study included only black and white men and the generalizability of the results to other races or ethnic groups may be limited; however there were few differences between black and white men in this study. Previous studies investigating the utility of [-2]proPSA in distinguishing men with CaP have reported results specifically in the range of PSA level from 4-10 ng/mL. The prevalence of men with PSA levels in this range was <10% in each of our cohorts. Consequently, it was not possible to analyze the ability of this biomarker in predicting risk of cancer in this subgroup. In addition, the stability of the analytes in samples frozen for several years is currently unknown, however [-2]proPSA levels were stable when frozen up to 12 months at -70°C.25

Conclusions

These data suggest that levels of [-2]proPSA may help identify prostate cancer in men with serum PSA levels in an indeterminate range, although the reference ranges for white and black men may differ slightly.
Acknowledgments

The authors thank the men who participate in the Olmsted County Study and the Flint Men’s Health Study, the study personnel, and Ms. Kristie Shorter for her assistance in preparation of this manuscript. This project was supported by research grants from the Public Health Service, National Institutes of Health (Grants DK58859, AR30582 and 1UL1 RR024150-01), National Cancer Institute (P50CA69568), and Merck Research Laboratories. Beckman Coulter (Brea, CA) provided the test kits for [-2]proPSA free of charge and with no obligation.

Disclosures
Dr. Klee has received research grants and royalties for unrelated technologies from Beckman Coulter, Inc. Dr. Jacobsen has received research grants from Beckman Coulter, Inc.
Table 1. Baseline characteristics of Olmsted County Study of Urinary Symptoms and Health Status among Men and Flint Men’s Health Study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OCS</th>
<th>FMHS</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>420</td>
<td>328</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>58.7 ± 10.4</td>
<td>57.4 ± 10.4</td>
<td>0.24</td>
</tr>
<tr>
<td>Family history of prostate cancer (N (%))</td>
<td>65 (15.5)</td>
<td>55 (16.8)</td>
<td>0.63</td>
</tr>
<tr>
<td>Median (Q1, Q3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate volume (cc)</td>
<td>26.7 (21.7, 35.0)</td>
<td>26.2 (20.1, 33.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>PSA (ng/mL)</td>
<td>1.2 (0.8, 1.8)</td>
<td>1.0 (0.5, 1.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>[-2]proPSA (pg/mL)</td>
<td>5.6 (3.9, 7.7)</td>
<td>6.3 (4.1, 8.9)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

SD: Standard Deviation, Q1: 25th percentile, Q3: 75th percentile
*Rank-sum P value reported for continuous variables, chi-square P value reported for dichotomous variables
Table 2. Selected empirical quantiles of [-2]proPSA (pg/mL) in the OCS, FMHS and combined FMHS and OCS cohorts.

<table>
<thead>
<tr>
<th>Quantile</th>
<th>OCS</th>
<th>FMHS</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (minimum)</td>
<td>1.36</td>
<td>1.28</td>
<td>1.28</td>
</tr>
<tr>
<td>2.5</td>
<td>2.06</td>
<td>1.87</td>
<td>1.99</td>
</tr>
<tr>
<td>5</td>
<td>2.34</td>
<td>2.23</td>
<td>2.31</td>
</tr>
<tr>
<td>10</td>
<td>2.98</td>
<td>2.90</td>
<td>2.95</td>
</tr>
<tr>
<td>25</td>
<td>3.94</td>
<td>4.09</td>
<td>3.99</td>
</tr>
<tr>
<td>50 (median)</td>
<td>5.58</td>
<td>6.26</td>
<td>5.84</td>
</tr>
<tr>
<td>75</td>
<td>7.67</td>
<td>8.91</td>
<td>8.28</td>
</tr>
<tr>
<td>90</td>
<td>10.70</td>
<td>13.46</td>
<td>11.44</td>
</tr>
<tr>
<td>95</td>
<td>14.71</td>
<td>18.36</td>
<td>15.25</td>
</tr>
<tr>
<td>97.5</td>
<td>16.92</td>
<td>21.53</td>
<td>20.21</td>
</tr>
<tr>
<td>100 (maximum)</td>
<td>32.32</td>
<td>42.91</td>
<td>42.91</td>
</tr>
</tbody>
</table>
Table 3. Crude and age-adjusted associations between [-2]proPSA and urologic measures.

<table>
<thead>
<tr>
<th>[-2]proPSA quartile (range, pg/mL)</th>
<th>Olmsted County Study</th>
<th>Flint Men's Health Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enlarged prostate (Prostate volume &gt;30 cc)</td>
<td>Elevated PSA level (PSA &gt;2.5 ng/mL)</td>
</tr>
<tr>
<td></td>
<td>Crude OR (95% CI)</td>
<td>Age-adj. OR (95% CI)</td>
</tr>
<tr>
<td>Quartile 1 (min-3.99)</td>
<td>reference</td>
<td>reference</td>
</tr>
<tr>
<td>Quartile 2 (4.00-5.84)</td>
<td>5.0 (2.4, 10.4)</td>
<td>5.2 (2.4, 11.2)</td>
</tr>
<tr>
<td>Quartile 3 (5.85-8.28)</td>
<td>9.3 (4.4, 19.6)</td>
<td>8.1 (3.7, 17.6)</td>
</tr>
<tr>
<td>Quartile 4 (8.29-max)</td>
<td>17.0 (7.7, 37.9)</td>
<td>11.1 (4.8, 25.5)</td>
</tr>
<tr>
<td>P trend</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

OR: odds ratio, CI: confidence interval
Table 4. Crude and age- and PSA-adjusted association of baseline [-2]proPSA levels and future diagnosis of prostate cancer in the Olmsted County Study.

<table>
<thead>
<tr>
<th>[-2]proPSA quartile (range, pg/mL)</th>
<th>Biopsy-Confirmed Prostate Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>Age-adj. HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>Age-, PSA-adj. HR (95% CI)</td>
</tr>
<tr>
<td>Quartile 1 (min-3.99)</td>
<td>reference</td>
</tr>
<tr>
<td>Quartile 2 (4.00-5.84)</td>
<td>1.2 (0.3, 5.5)</td>
</tr>
<tr>
<td>Quartile 3 (5.85-8.28)</td>
<td>4.0 (1.1, 14.5)</td>
</tr>
<tr>
<td>Quartile 4 (8.29-max)</td>
<td>11.4 (3.4, 38.1)</td>
</tr>
<tr>
<td>P trend</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

HR: hazard ratio, CI: confidence interval
Figure 1a. Cumulative distribution function plot of [-2]proPSA by age decade in the Olmsted County Study.
Figure 1b. Cumulative distribution function plot of [-2]proPSA by age decade in the Flint Men's Health Study.
References


Manuscript #3

Longitudinal changes of benign prostate-specific antigen and [-2]pro-prostate-specific antigen over 7 years in a community-based sample of men

Thomas Rhodes¹,³, Debra J. Jacobson², Michaela E. McGree², Jennifer L. St. Sauver², Cynthia J. Girman³, Michael M. Lieber⁴, George G. Klee⁵, Kitaw Demissie¹, Steven J. Jacobsen⁶

¹Department of Epidemiology, University of Medicine and Dentistry in New Jersey, Piscataway, NJ
²Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN
³Epidemiology Department, Merck Research Laboratories, North Wales, PA
⁴Department of Urology, Mayo Clinic College of Medicine, Rochester, MN
⁵Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN
⁶Department of Research and Evaluation, Kaiser Permanente, Southern California, Pasadena, CA
Abstract

Introduction and Objectives. Longitudinal changes in benign prostate-specific antigen (BPSA) and [-2]pro-prostate-specific antigen ([−2]proPSA) over 7 years of follow-up are estimated in a community-based sample of men. These estimates are compared with levels and changes in urologic measures and for men who do and do not develop enlarged prostates and prostate cancer.

Methods. A 25% subsample from a larger cohort of Caucasian men aged 40-79 years randomly selected from Olmsted County, MN residents completed a detailed clinical exam. BPSA and [-2]proPSA were measured from frozen sera. Subjects were evaluated biennially for a median follow-up interval of 7 years (range: 0-8.8 years). Mixed-effects regression models were used to estimate longitudinal changes in BPSA and [-2]proPSA. Spearman correlations were used to compare these changes with baseline levels and annual changes in urologic measures.

Results. Median (25th, 75th percentiles; Q1,Q3) annual percent change for [−2]proPSA and BPSA were 3.7% (2.5%, 5.2%) and 7.3%(6.8%, 7.7%), respectively. Both were correlated with baseline and annual changes in PSA and prostate volume measures. Annual percent change in [-2]proPSA increased with increasing age decade. The median (Q1, Q3) rate of increase in [-2]proPSA was greater for men who developed enlarged prostates (3.5% (2.6%, 4.4%)) or prostate cancer (8.1% (6.6%, 9.8%)) compared to those who did not develop enlarged prostates (1.9% (0.9%, 3.0%)) or prostate cancer (3.5% (2.3%, 4.8%)).

Conclusions. BPSA and [-2]proPSA levels increase over time. The annual percent change in [-2]proPSA increases with age and may be a useful predictor of development
of prostate cancer.
Introduction

Prostate-specific antigen (PSA) is a widely used serum marker for the early detection of prostate cancer (CaP). However, it is not specific to CaP since PSA can also be elevated in benign prostatic conditions.\textsuperscript{1,2} Benign prostate-specific antigen (BPSA) and [-2]pro-prostate-specific antigen ([-2]proPSA) have been identified as promising new biomarkers for distinguishing men with benign prostate disease and CaP, respectively.\textsuperscript{3-8} Additionally, previous reports in the current study sample have shown that men with higher baseline levels of BPSA are at greater risk of receiving benign prostatic hyperplasia (BPH) treatment and that higher baseline levels of both BPSA and [-2]proPSA are associated with future diagnosis of CaP.\textsuperscript{9,10}

Longitudinal studies of PSA have shown that PSA levels, as well as PSA change over time, are associated with a higher risk of acute urinary retention (AUR), CaP, and BPH progression or treatment.\textsuperscript{11-14} In addition, a previous report from this study\textsuperscript{15} found modest correlations between annual changes in urologic measures and found that more rapid increases in PSA level and prostate volume and more rapid decreases in maximum urinary flow rate were associated with increased odds of more rapid increases in lower urinary tract symptoms. However, little is known about the changes of BPSA and [-2]proPSA over time and the relationship of these changes to longitudinal changes in common urologic measures. The objective of this paper is to describe the distribution of longitudinal changes in BPSA and [-2]proPSA in a community-based sample of men, to investigate the association of these changes with longitudinal changes of common clinical urologic measures, and to compare the rates of longitudinal changes among men who do and do not develop enlarged prostates and CaP.
Methods

The Olmsted County Study of Urinary Symptoms and Health Status among Men is a prospective cohort study begun in 1989 which has been described in detail in previous publications. Briefly, an age-stratified random sample was drawn from an enumeration of Caucasian male Olmsted County, Minnesota residents between the ages of 40 and 79 years. Subjects were excluded if they had prior prostate surgery, CaP, or any of a number of specified medical conditions that would affect normal urinary function (other than BPH). At baseline, 2115 men (of 3874; 55% response rate) in the community were visited in their homes and completed a previously validated self-administered questionnaire which contained questions similar to the American Urological Association Symptom Index (AUASI). A random subset of 476 men (of 537; 89%) participated in a full urologic exam, including maximum urinary flow rate assessment, transrectal ultrasonography, digital rectal examination, and a serum PSA determination.

The cohort was actively followed on a biennial basis for fourteen years using a protocol similar to the initial examination. The study design allowed replacement of men who had left the study (n= 158 in the clinic subset) and this paper presents data on the 443 men who had at least one serum measurement during the 4th, 6th, or 8th rounds of the study. This study received approval from the Mayo Clinic and Olmsted Medical Center Institutional Review Boards.

Assays
Stored blood samples from the 4th, 6th, and 8th biennial rounds (1996, 2000, and 2004) were used to measure BPSA and [-2]proPSA levels. Serum samples were obtained prior to any prostatic manipulations, including digital rectal examination and transrectal ultrasound. Measurements of BPSA and [-2]proPSA levels were made using an automated, sequential, two-step immunoenzymatic (“sandwich”) assay developed for use on an Access 2 Immunoassay analyzer (Access® instrument, Beckman Coulter, Brea, CA). The BPSA measurements were run using research-use-only two-site immunoenzymatic reagents and the [-2]proPSA measurements were run using investigational-use-only two-site immunoenzymatic reagents, both provided by Beckman Coulter, Inc. In our laboratory, the intra-assay variation in BPSA ranged from 4.3% to 8.1% while the inter-assay variation ranged from 5.1% to 5.2%. For [-2]proPSA, the intra-assay variation ranged from 2.0% to 3.2% while the inter-assay variation ranged from 3.0% to 9.9%.

Urologic Outcomes

Prostate volume was measured via transrectal ultrasound. Total and transition zone volume were calculated assuming a prolate ellipsoid shape. Prostatic enlargement was defined as prostate volume >30 cc. Additionally, all men were followed through their community medical records. Information on use of medical and surgical lower urinary tract symptom/benign prostatic enlargement treatments were obtained through self-report and through passive follow-up of the community medical records. Dates of biopsy-confirmed CaP were ascertained through detailed medical record review.
**Statistical analysis**

A natural log transformation was used to normalize variables (all PSA and volume measurements, and maximum urinary flow rate) with skewed distributions. Mixed-effects regression models were used to estimate longitudinal changes (i.e., annual percent change over time) in BPSA and [-2]proPSA, as well as the other urologic variables. As BPSA and [-2]proPSA measures were only available at the 4\(^{th}\), 6\(^{th}\), and 8\(^{th}\) rounds of data collection, the 4\(^{th}\) round of follow-up was considered as baseline. Transition zone volume and free PSA were available beginning at the 5\(^{th}\) round of data collection. To assess the natural history, observations were censored after the point where subjects either received medical therapy (\(\alpha\)-blocker, 5-\(\alpha\) reductase inhibitor) or surgical treatment for BPH or were diagnosed with CaP. Each measure was regressed on time from baseline measurement and 10-year age groups. An interaction term was included to allow different slopes across age groups. This method estimates group average longitudinal change (fixed effects) while still allowing each individual subject longitudinal change to deviate from the group average curve (random effects). Additional models included terms indicating an enlarged prostate (prostate volume >30 cc) or diagnosis of CaP and an interaction of this term with time from baseline to allow for comparison of slopes in men with and without an enlarged prostate or CaP diagnosis. Prevalent cases of CaP or enlarged prostate were removed from these analyses which resulted in 43 and 65 men with incident CaP and enlarged prostate, respectively. Spearman correlations were used to describe the relationship between annual percent change in BPSA and [-2]proPSA with baseline and annual changes over time of other urologic measures.
Results

Subjects were evaluated biennially for a median follow-up interval of 7 years (range: 0-8.8 years). The average age at baseline was 58.7 years and baseline levels of BPSA, [-2]proPSA, and urologic measures are presented in Table 1.

The overall median (25th, 75th percentiles; (Q1, Q3)) annual percent change in BPSA was 7.3% (6.8%, 7.7%) /year (Figures 1a and 1b). The overall median (Q1, Q3) annual percent change in [-2]proPSA was 3.7% (2.5%, 5.2%)/year (Figures 2a and 2b). The age-adjusted correlation between baseline levels of BPSA and [-2]proPSA was 0.45 and between annual changes in BPSA and annual changes in [-2]proPSA was 0.34.

Annual percent changes in BPSA and [-2]proPSA were modestly correlated with baseline levels and annual changes in urinary symptoms and urinary flow rates (age-adjusted Spearman correlations (r_s) range: -0.16 to 0.09; Table 2). Stronger, positive correlations were observed between annual changes in BPSA and [-2]proPSA with baseline levels and annual changes in prostate volume, transition zone volume, PSA level, and free PSA level (age-adjusted r_s range: 0.21 to 0.72, Table 2).

The annual increase in BPSA was consistent across age decades and was slightly higher, but not significantly different for men who developed an enlarged prostate or CaP than among men who did not develop an enlarged prostate or CaP (Table 3). Conversely, the median annual increase in [-2]proPSA increased with increasing age decade (p<0.0001) and ranged from 2.1%/year for men in their forties to 6.7%/year for men who were 70 years of age and older (Table 3). The median (Q1, Q3) annual rate of increase in [-2]proPSA among men who developed an enlarged prostate (3.5% (2.6%, 4.4%)/year)
was nearly double the rate of men who did not develop an enlarged prostate (1.9% (0.9%, 3.0%)/year, \( p=0.048 \); Table 3). The greatest annual rate of increase in [-2]proPSA was observed among men who developed CaP (median (Q1, Q3)=8.1% (6.6%, 9.8%)/year) (Table 3). This was over twice the rate of men who did not develop CaP (3.5% (2.3%, 4.8%)/year, \( p=0.003 \)). Similar results were seen comparing the annual median (Q1, Q3) rate of increase in PSA level for men with (8.3% (7.3%, 9.4%)) and without (3.1% (1.6%, 4.7%)) CaP; however, adjusting the rates for [-2]proPSA for men with and without cancer by baseline PSA levels did not change the results (data not shown).

**Discussion**

Over time, the median annual percent increase in BPSA is greater than the median annual percent increase in [-2]proPSA level. While changes in both measures are correlated with levels and changes in prostate size and PSA measures, these results suggest that annual changes in [-2]proPSA level were significantly greater in men who developed enlarged prostates and men who are diagnosed with CaP.

Annual changes in both BPSA level and [-2]proPSA level were most strongly correlated with other PSA and prostate size measures. The modest age-adjusted correlations between changes in BPSA and [-2]proPSA levels and changes in symptoms and maximum flow rate are consistent with the correlations among annual changes in urologic measures previously reported in this same cohort.\(^ {15,24} \) In addition, the cross-sectional associations between BPSA level and presence of lower urinary tract symptoms or depressed urinary flow rate were much lower than the associations with an enlarged prostate.\(^ 9 \)
The age-adjusted correlation of 0.45 between annual changes in BPSA level and transition zone volume was greater than the age-adjusted correlation of 0.32 previously observed between changes in PSA level and transition zone volume.\textsuperscript{24} This is consistent with other cross-sectional studies that observed stronger correlation between transition zone volume and BPSA level than with PSA level.\textsuperscript{4,25}

There were differences between the unadjusted and age-adjusted correlations, with the greatest difference being with the correlation between change in maximum flow rate and change in [-2]proPSA level. As these two measures both progress with increasing age, the observed unadjusted association may be due to the confounding effect of age. Alternatively, there could be an association between changes in the two measures which is over-adjusted and removed when adjusting for age.

Annual changes in BPSA are consistent across age groups, with an overall annual percent change of 7.3%/year. We had previously found that baseline levels of BPSA were associated with future BPH treatment and diagnosis of CaP.\textsuperscript{9} While changes in BPSA level over time were slightly higher for men who developed an enlarged prostate or CaP, these differences were not significant. It is possible that men with a higher level of BPSA may be at greater risk of developing CaP or seeking treatment, however the continued rate of increase in BPSA level does not differ. In addition, BPH may also be present in men with CaP thus explaining the observed lack of differences.

Annual percent changes in [-2]proPSA increased with increasing age and were greatest among men who developed CaP. These findings suggest that age may be an important predictor of the rate of increase in [-2]proPSA level. This is different than the consistent rate of annual percent change over age decades observed in this cohort for
changes in prostate volume, PSA level, and BPSA level. Factors other than changes in prostate volume and changes in PSA level may potentially play a role in the age-related increase in [-2]proPSA over time. Importantly, establishing reference ranges for [-2]proPSA or change in [-2]proPSA as a marker for CaP should account for the age-related increases in annual changes in [-2]proPSA levels. A single cut-point would lead to decreased sensitivity in younger men and decreased specificity and unnecessary biopsies in older men.

Several papers have reported improved CaP detection using a single measurement of [-2]proPSA or %[-2]proPSA ([−2]proPSA/%free PSA). Additionally, previous reports in the current study sample have shown that men with higher baseline levels of [-2]proPSA are associated with future diagnosis of CaP. In the current study, men who were diagnosed with incident CaP had a median annual rate of increase in [-2]proPSA level over time which was more than twice the rate of men not diagnosed with CaP. This supports the potential utility of [-2]proPSA or rate of change in [-2]proPSA in distinguishing men with or without CaP.

The rate differences for men with and without prostate cancer were similar for PSA and [-2]proPSA; however there are several findings which point toward the potential usefulness of [-2]proPSA in detecting CaP beyond PSA alone. First, both associations between baseline levels of [-2]proPSA and development of CaP and differences in longitudinal rates of change in [-2]proPSA for men with and without CaP remained after adjustment for PSA level. Additionally, age-related increases in [-2]proPSA over time were greater than would be explained by age-related changes in PSA. Further work is
needed to determine if development of CaP could partially explain the age-related increase in [-2]proPSA over time.

It is interesting to note that the overall longitudinal estimates of change in BPSA and [-2]proPSA levels were greater than those observed cross-sectionally in this cohort.\textsuperscript{9,10} At baseline, there was an exponential increase of 6.2% and 1.9% per year of age for BPSA and [-2]proPSA levels, respectively, which is similar to median rates observed in men with prostate volume \(\leq 30\) cc. A diagnosis of CaP and surgical treatment for BPH were two of the baseline study exclusion criteria. This may explain the similarity of cross-sectional rates to rates for men with smaller prostate volumes. Therefore, the slightly higher overall longitudinal estimates may better represent disease progression in the community.

A key strength of this study is the ability to examine longitudinal changes in BPSA and [-2]proPSA levels in a community-based study population. Using a randomly selected community cohort and truncation of observations after treatment or CaP diagnosis provides results which represent a more comprehensive picture of how BPSA and [-2]proPSA change over time in the general population.

With a maximum of up to three measurements per subject for BPSA and [-2]proPSA, the high within-subject variability of the urologic measures may result in less stable estimates of change over time and it was not possible to explore nonlinear relationships. While the long follow-up period of this study leads to unavoidable attrition which could bias the results, previous work in this cohort found that dropout was not associated with baseline urologic measures. This study population is limited to Caucasian men and the generalizability of the results to other races or ethnicities may be limited.
Conclusions

Overall these results demonstrate that BPSA and [-2]proPSA increase over time. Furthermore, the annual percent change in [-2]proPSA increases with age and is greater in men who develop enlarged prostates and CaP. These data suggest that rapid increases in [-2]proPSA levels over time may help identify men with CaP.
Acknowledgment

The authors thank the men who participate in the Olmsted County Study, the study personnel, and Ms. Kristie Shorter for her assistance in preparation of this manuscript. This project was supported by research grants from the Public Health Service, National Institutes of Health (Grants DK58859, AR30582 and 1UL1 RR024150-01), and Merck Research Laboratories. Beckman Coulter (Brea, CA) provided the test kits for BPSA and [-2]proPSA free of charge and with no obligation.

Disclosures

Dr. Klee has received research grants and royalties for unrelated technologies from Beckman Coulter, Inc. Dr. Jacobsen has received research grants from Beckman Coulter, Inc.
Table 1. Baseline* characteristics of Olmsted County Study of Urinary Symptoms and Health Status among Men

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=443</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>58.7 ± 10.3</td>
</tr>
<tr>
<td>Age (years), N (%)</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>115 (26.0)</td>
</tr>
<tr>
<td>50-59</td>
<td>155 (35.0)</td>
</tr>
<tr>
<td>60-69</td>
<td>98 (22.1)</td>
</tr>
<tr>
<td>70+</td>
<td>75 (16.9)</td>
</tr>
<tr>
<td>AUASI score, mean ± SD</td>
<td>7.5 ± 5.7</td>
</tr>
<tr>
<td>Maximum flow rate (mL/s)</td>
<td>18.6 (13.1, 24.7)</td>
</tr>
<tr>
<td>Prostate volume (cc)</td>
<td>26.7 (21.7, 35.0)</td>
</tr>
<tr>
<td>Transition zone volume (cc)</td>
<td>11.2 (7.9, 16.3)</td>
</tr>
<tr>
<td>PSA (ng/mL)</td>
<td>1.1 (0.7, 1.8)</td>
</tr>
<tr>
<td>Free PSA (ng/mL)</td>
<td>0.3 (0.2, 0.4)</td>
</tr>
<tr>
<td>BPSA (pg/mL)</td>
<td>31.6 (16.0, 66.3)</td>
</tr>
<tr>
<td>[-2]proPSA (pg/mL)</td>
<td>5.6 (4.0, 7.7)</td>
</tr>
</tbody>
</table>

*Measurements correspond to the time of the baseline BPSA or [-2]proPSA measurement, except for transition zone volume and free PSA which were first measured at round 5.

*Q1: 25th percentile; Q3: 75th percentile
Table 2. Spearman correlations of annual percent change in BPSA and [-2]proPSA with baseline and annual changes in urologic measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>% Change in BPSA Unadjusted</th>
<th>Age-adjusted</th>
<th>% Change in [-2]proPSA Unadjusted</th>
<th>Age-adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs (p)</td>
<td>rs (p)</td>
<td>rs (p)</td>
<td>rs (p)</td>
</tr>
<tr>
<td>Baseline AUASI score</td>
<td>0.17 *</td>
<td>0.08</td>
<td>0.25 *</td>
<td>0.08</td>
</tr>
<tr>
<td>Baseline maximum flow rate</td>
<td>-0.11 **</td>
<td>-0.04</td>
<td>-0.26 *</td>
<td>-0.16 *</td>
</tr>
<tr>
<td>Baseline prostate volume</td>
<td>0.47 *</td>
<td>0.37 *</td>
<td>0.62 *</td>
<td>0.46 *</td>
</tr>
<tr>
<td>Baseline transition zone volume</td>
<td>0.47 *</td>
<td>0.36 *</td>
<td>0.60 *</td>
<td>0.41 *</td>
</tr>
<tr>
<td>Baseline PSA</td>
<td>0.67 *</td>
<td>0.62 *</td>
<td>0.68 *</td>
<td>0.59 *</td>
</tr>
<tr>
<td>Baseline free PSA</td>
<td>0.53 *</td>
<td>0.46 *</td>
<td>0.73 *</td>
<td>0.72 *</td>
</tr>
<tr>
<td>Change in AUASI score</td>
<td>0.17 *</td>
<td>0.07</td>
<td>0.28 *</td>
<td>0.09</td>
</tr>
<tr>
<td>% Change in maximum flow rate</td>
<td>-0.17 *</td>
<td>0.03</td>
<td>-0.50 *</td>
<td>-0.16 *</td>
</tr>
<tr>
<td>% Change in prostate volume</td>
<td>0.40 *</td>
<td>0.43 *</td>
<td>0.25 *</td>
<td>0.37 *</td>
</tr>
<tr>
<td>% Change in transition zone volume</td>
<td>0.38 *</td>
<td>0.45 *</td>
<td>0.09</td>
<td>0.21 *</td>
</tr>
<tr>
<td>% Change in PSA</td>
<td>0.57 *</td>
<td>0.52 *</td>
<td>0.51 *</td>
<td>0.43 *</td>
</tr>
<tr>
<td>% Change in free PSA</td>
<td>0.49 *</td>
<td>0.36 *</td>
<td>0.70 *</td>
<td>0.51 *</td>
</tr>
</tbody>
</table>

*p-value <0.001
**p-value <0.05
Table 3. Distribution of annual percent changes in BPSA and [-2]proPSA stratified by age and urologic conditions

<table>
<thead>
<tr>
<th>% Change in BPSA</th>
<th>% Change in [-2]proPSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>115</td>
</tr>
<tr>
<td>50-59</td>
<td>155</td>
</tr>
<tr>
<td>60-69</td>
<td>98</td>
</tr>
<tr>
<td>70+</td>
<td>75</td>
</tr>
<tr>
<td>Prostate volume*</td>
<td></td>
</tr>
<tr>
<td>≤30 cc</td>
<td>132</td>
</tr>
<tr>
<td>&gt;30 cc</td>
<td>65</td>
</tr>
<tr>
<td>Prostate cancer*</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>400</td>
</tr>
<tr>
<td>Yes</td>
<td>43</td>
</tr>
</tbody>
</table>

*Prevalent cases occurring before first assay measurement were removed
*Associations adjusted for age
Figure 1a. Observed longitudinal changes of BPSA by age
Figure 1b. Predicted longitudinal changes of BPSA by age
Figure 2a. Observed longitudinal changes of [-2]proPSA by age
Figure 2b. Predicted longitudinal changes of [-2]proPSA by age
References


CONCLUSION

These results suggest that BPSA and [-2]proPSA may be useful in identifying men with benign or malignant prostate disease. The aims of this dissertation were to: (1) describe the distribution of BPSA in a community-based sample of black and white men and examine the association of BPSA with baseline urologic measures and need for medical therapy or CaP, (2) describe the distribution of [-2]proPSA in a community-based sample of black and white American men and examine the association of [-2]proPSA with baseline urologic measures and CaP, (3) describe the distribution of longitudinal changes in BPSA and [-2]proPSA and how these changes vary over levels of baseline urologic conditions.

The role of community-based longitudinal studies has been delineated by Guess, et al.\textsuperscript{1} Community-based studies are necessary to understand appropriateness and effectiveness of medical interventions by studying the natural history of the conditions that interventions are intended to treat or prevent as well as the normal reference ranges of the tests used in the diagnosis and management. Guess, et al point out that often the information gained through clinic-based studies can lead to misleading conclusions that the study authors attribute to a type of selection bias. One of the primary reasons for community-based studies is to establish normal ranges of tests used in disease diagnosis and management. The proper evaluation of any test used in disease management requires a broad spectrum of both diseased and nondiseased people. Evaluation of a test in a narrow spectrum of patients (spectrum bias) seen in referral clinics can often lead to optimistic estimates of sensitivity, specificity and predictive value that are later attenuated when the tests are performed in patients with milder disease.\textsuperscript{2}
In addition to the need for normative ranges for tests, community-based studies allow estimation of disease prevalence and severity in the community and investigation of patient characteristics in those seeking treatment as well as those not seeking treatment. Community-based studies also provide the opportunity to study an unbiased sample of all cases of a condition occurring within a well-defined population instead of only those patients treated at certain health care facilities. Furthermore, community-based studies provide information on the natural history of a disease thus providing information on how to interpret the effects of interventions from the absence of intervention. Lastly, community-based studies allow comparison and insight into representativeness.

These population-based data provide useful reference ranges to researchers for future studies examining the utility of BPSA and \([-2\)proPSA]. Results suggest that there were similar distributions of BPSA for white and black men. Elevated levels of BPSA suggest the presence of some prostate disease, benign or malignant. Further work is needed to develop our understanding of how this might be used in a diagnostic workup. The results also suggest that levels of \([-2\)proPSA] may help identify prostate cancer in men with serum PSA levels in an indeterminate range, although the reference ranges for white and black men may differ slightly.

In addition, the longitudinal results demonstrate that both BPSA and \([-2\)proPSA] increase over time. The change in BPSA was fairly constant across all age groups, whereas change in \([-2\)proPSA] increased with increasing age. Furthermore, the annual percent change in \([-2\)proPSA] increases with age and is greater in men who develop enlarged prostates and CaP. This observation needs to be examined further to provide important insight into the natural history and development of prostate disease. These data
suggest that rapid increases in [-2]proPSA levels over time may help identify men with CaP.

Some limitations of the current study include generalizability of the results to other races and ethnicities. In addition, previous studies have investigated the utility of these biomarkers in the 2 to 10 ng/mL range of PSA while the population in the current study had less than 10% of the baseline PSA values within this range. However, results in the current study have shown that levels lower than previous reports were predictive of increased risk of CaP.

Although the preliminary results from this study and earlier reports are promising, further research is needed evaluating both BPSA and [-2]proPSA in the context of a broad spectrum of disease in order to gain a better understanding of the characteristics of these tests. Indeed, as Djavan\textsuperscript{3} pointed out in a recent editorial, although these new markers are encouraging they are not yet mature enough for general clinical use in a urologic practice. For example, he points out that all of the studies that are investigating these novel biomarkers ultimately base the biopsy decision on PSA values and associated thresholds and not the novel biomarkers themselves. The clinical utility of the predictive models has yet to be determined. Results in the current study predicting prostate cancer using separately baseline BPSA and [-2]proPSA in the presence of baseline PSA suggest that these new biomarkers may have a role in the diagnostic workup of patients suspected of CaP. Clinical studies are needed incorporating BPSA and/or [-2]proPSA into existing protocols (e.g., nomograms) to assess clinical utility. The normative reference ranges provided by this study may prove to be valuable in clinical practice by increasing the
sensitivity and specificity allowing for the detection of CaP while decreasing unnecessary biopsies.
References


Bibliography

<table>
<thead>
<tr>
<th>Page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction 19</td>
</tr>
<tr>
<td>Manuscript #1 41</td>
</tr>
<tr>
<td>Manuscript #2 61</td>
</tr>
<tr>
<td>Manuscript #3 85</td>
</tr>
<tr>
<td>Conclusion 93</td>
</tr>
</tbody>
</table>
Appendix

- Age and study specific cumulative percentile graphs for BPSA and [-2]proPSA.
- Age and study specific reference tables for BPSA and [-2]proPSA.
Appendix 1. Cumulative Percentile Curves of Benign Prostate Specific Antigen (BPSA) by Age Decade in the Flint Men's Health Study (FM) and Olmsted County Study (OC).
Appendix 2. Cumulative Percentile Curves of [-2] pro-Prostate Specific Antigen ([-2]proPSA) by Age Decade in the Flint Men's Health Study (FM) and Olmsted County Study (OC).
Curriculum Vita

Thomas Rhodes

EDUCATION

1992 University of South Carolina – School of Public Health, Columbia, SC
Master of Science in Public Health (M.S.P.H) with an emphasis on
Biostatistics

1985 Augusta State University, Augusta, GA
Bachelor or Science (B.S.), Major: Computer Science

POSITIONS AND APPOINTMENTS

2007 - Present Research Collaborator Appointment
Department of Health Sciences Research
Mayo Clinic, Rochester, MN

2003 - Present Associate Director
Epidemiology Department - Merck Research Laboratories

1999 – 2003 Senior Epidemiologist
Epidemiology Department - Merck Research Laboratories

1996 – 1999 Epidemiologist
Epidemiology Department - Merck Research Laboratories

1992 – 1996 Senior Statistician
Epidemiology Department - Merck Research Laboratories

1986 – 1992 Data Manager / Statistician
Georgia Institute for the Prevention of Human Disease and Accidents
Medical College of Georgia, Augusta, GA

1989 – 1992 Graduate Assistant
Department of Epidemiology and Biostatistics
University of South Carolina, Columbia, SC

1984 – 1986 Programmer
Department of Pharmacology and Toxicology
Medical College of Georgia, Augusta, GA
PUBLICATIONS

A. Refereed Original Articles in Journals


Rhodes T, Girman CJ, Jacobson SJ, Guess HA, Oesterling JE, Lieber MM. Natural History of BPH: Longitudinal Prostate Growth Rates During Five Years in Randomly Selected Community Men 40 to 79 Years Old. Journal of Urology 1999; 161: 1174-1179


**B. Communications (Abstracts and conferences)**


Qasim A, Mehta N, Wolfe ML, McMahon K, Rhodes T, Girman CJ, Rader DJ, Reilly MP. Plasma levels of leptin but not adiponectin are associated with coronary atherosclerosis independent of the metabolic syndrome and c-reactive protein levels. AHA Scientific Session, Nov 13-16, 2005, Dallas, TX


Girman CJ, Rhodes T, Clearfield M, Beere P, Mercuri M. Metabolic syndrome and the risk of cardiovascular outcomes in the placebo groups of two large clinical trials. 43rd Annual Conference on Cardiovascular Disease Epidemiology and Prevention, American Heart Association, Miami FL, March 2003.


Lilly FRW, Chumlea Wm C, Rhodes T and Girman CJ Inter-rater Observations in Assessing Hair Loss in Community Based Men. (Accepted American Academy of Dermatology Annual Meeting, February 1998.)

Girman CJ, Rhodes T, Lilly FRW, and Chumlea Wm C. Degree of Bother and Concern Associated with Self-Perceived Hair Loss in Community Men. (Accepted American Academy of Dermatology Annual Meeting, February 1998.)


Roehrborn CG, Sech SM, Girman CJ, Rhodes T. Correlation between prostate size estimated by digital rectal examination (DRE) and measured by transrectal ultrasonography (TRUS). (American Urological Association annual meetings, 1997)


Urinary Tract Symptoms and Quality of Life in Four International Community Studies. European Association of Urology, September 1996.


