UC Irvine
UC Irvine Electronic Theses and Dissertations

Title
Biomarker assays and MRI-fusion biopsy in the detection of Prostate Cancer

Permalink
https://escholarship.org/uc/item/2n72r7z5

Author
JANI, SUNILKUMAR GIRISHKUMAR

Publication Date
2016

Supplemental Material
https://escholarship.org/uc/item/2n72r7z5#supplemental

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

UNIVERSITY OF CALIFORNIA,
IRVINE

Biomarker assays and MRI-fusion biopsy in the Detection of Prostate Cancer

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE
In
BIOMEDICAL AND TRANSLATIONAL SCIENCE

By
Sunilkumar Jani

Thesis Committee:
Professor Dr. Sheldon Greenfield, Chair
Associate Clinical Professor, Dr. Edward Uchio
Assistant Professor Dr. John Billimek

2016

© 2016 SUNILKUMAR G JANI

TABLE OF CONTENTS

LIST OF ABBREVIATIONS ..... **IV**

LIST OF TABLES ..... **VI**

LIST OF FIGURES ..... **VII**

ACKNOWLEDGEMENTS ..... **VIII**

ABSTRACT OF THE THESIS ..... **IX**

1. INTRODUCTION **1**

2. BACKGROUND **4**

2.1. BIOMARKERS IN PROSTATE CANCER DIAGNOSIS AND RISK ASSESSMENT 4

2.1.1. PSA AND PSA DERIVATIVES *7*

2.1.2. PCA3 ..... *7*

2.1.3. OPKO 4KSCORE *8*

2.1.4. ConfirmMDx: *8*

2.1.5. OncotypeDX: *9*

2.1.6. PROLARIS *9*

2.1.7. GLEASON SCORE *11*

2.2. MRI IN DETECTION OF PROSTATE CANCER 11

2.3. MRI IN PROSTATE RISK STRATIFICATION: 13

2.4. METHODS OF MRI-GUIDED PROSTATE BIOPSY: 15

2.5. MRI-ULTRASOUND FUSION-GUIDED BIOPSY IN PROSTATE CANCER DIAGNOSIS 16

2.6. AIMS OF THIS PROJECT 17

3. METHODS **19**

3.1. STUDY RATIONALE 19

3.2. STUDY DESIGN 20

3.3. STUDY OBJECTIVE AND OUTCOMES 20

3.3.1. Study Objective *20*

3.3.2. Study Outcome *21*

3.4. STUDY PARTICIPANTS 21

3.5. WAIVER OF HIPAA AUTHORIZATION AND STUDY CONSENT 21

3.6. DATA COLLECTION PROCEDURES 23

3.6.1. Outcome Variables *23*

3.6.2. Independent Variables *24*

3.6.3. Covariates *27*

3.7. STATISTICAL ANALYSIS 27
3.7.1. Demographics and Baseline characteristics *28*
3.7.2. Primary Objective Analyses *28*
4. RESULTS 29
5. DISCUSSION 42
6. CONCLUSION 47
7. REFERENCES 49
8. APPENDICES 53
8.1. PROTOCOL NARRATIVE 53
8.2. CASE REPORT FORM 53
8.3. IRB APPROVAL 53

LIST OF ABBREVIATIONS

AJCC	:	American Joint Committee on Cancer
CFR	:	Code of Federal Regulations
CI	:	Confidence Interval
DRE	:	Digital Rectal Examination
FTB	:	Fusion Targeted Biopsy
GPS	:	Genomic Prostate Score
HIPAA	:	Health Insurance Portability and Accountability Act
IRB	:	Institutional Review Board
mpMRI	:	Multi Parametric Magnetic Resonance Imaging
MRN	:	Medical Record Number
NCI	:	National Cancer Institute
NCCN	:	National Comprehensive Cancer Network
NND	:	Number Needed to Diagnose
NNT	:	Number Needed to Treat
NNI	:	Number Needed to Invite
NPV	:	Negative Predictive Value
PCa	:	Prostate Cancer
PCA3	:	Prostate CAncer gene 3
PI-RADS	:	Prostate Imaging Reporting and Data System;
PSA-DT	:	Prostate Specific Antigen Doubling Time
PSAV	:	Prostate Specific Antigen Velocity

PPV	:	Positive Predictive Value
PSA	:	Prostate Specific Antigen
RP	:	Radical Prostatectomy
SB	:	Systematic Biopsy
sPCa	:	Significant Prostate Cancer
SPSS	:	Statistical Package for Social Science
START	:	Standards of Reporting for MRI- targeted Biopsy Studies
TB	:	Targeted Biopsy
TRUS	:	Transrectal ultrasound
UCIMC	:	University of California, Irvine Medical Center

LIST OF TABLES

Table 1	:	Population Characteristics	25
Table 2	:	Association of recent Biomarkers and MRI findings with recent biopsy results	27
Table 2a	:	Association of Biomarkers and Biopsy core positivity	30
Table 3	:	Comparison of biopsy results from standard 12-core biopsy versus MRI/US-fusion targeted biopsies	30
Table 3a	:	Additional Utility of targeted biopsy over 12-core biopsy alone, and of 12 -core biopsy over targeted biopsy alone	31
Table 4	:	Association of Patient Parameters with Gleason score upgrading on targeted biopsy over 12-core biopsy	34

LIST OF FIGURES

Figure 1	:	Tests for Prostate cancer diagnosis and Prognosis	5
Figure 2	:	Distribution of GS on targeted biopsy for standard biopsy diagnosis	32
Figure 3	:	Distribution of GS on standard biopsies for targeted biopsies	33
Figure 4	:	Distribution of cases missed by 12-core biopsy and targeted biopsy	34
Figure 5	:	ROC analysis	35

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my sincere thanks and gratitude to my thesis committee Chair, Dr. Sheldon Greenfield for his valuable guidance, and encouragement and for making the requisite guidelines to enable me to complete my dissertation work.

I would like to express my deep sense of gratitude to Dr. Edward Uchio, for providing me an opportunity to join him in the University of California Irvine Medical center, which gave me the platform for developing my thesis. I have no words to express how valuable this opportunity was, as it not only helped to me to develop my thesis, but also help sustain my life here in California. Dr. Edward Uchio's guidance and inputs on the data elements, interpretation and analysis was the crucial for fundamental appropriateness of this thesis. His review and comments on each point and level of this thesis enabled me to maintain the objective and flow of this thesis.

I would also like to thank, Dr. John Billimek, for teaching me methods for statistical analysis. It would have not been possible to complete the statistical analysis without his input. He has been patiently supporting me since the beginning, patiently answering my queries and giving me the valuable guidance for each micro detail. I was lucky to have Dr. Billimek on my thesis committee.

I would also like to mention my colleague Mr. Steven Bereta, who is more like a friend to me, for his daily assistance in data collection and interpretation. His conceptual understanding for this disease is excellent.

I express my deep thanks to my parents, my wife and my sister for the motivation, inspiration and support in boosting my moral without which I would have been in vain.

"Expression of feelings by words makes them less significant when it comes to make statement of gratitude"

ABSTRACT OF THE THESIS

Biomarker assays and MRI-fusion biopsy in the Detection of Prostate Cancer

By
SUNILKUMAR G JANI

Master of Science in Biomedical and Translational Science
University of California, Irvine, 2016

Dr. Sheldon Greenfield, Chair

Background: Prostate cancer over-diagnosis and over treatment for indolent disease is an area of concern. Various biomarkers and MRI findings have been utilized to improve the predictability of the prognosis.

Objective: To determine the correlation between commercially available biomarkers (PSA, PCA3, Neogenomics Assay, OPKO4K, etc.) with findings on multi-parametric MRI and prostate biopsy specimens. 1. To Predict the probability of PCa diagnosis and behavior prior to performing an invasive prostate biopsy. 2. Describe the rate of disagreement in Gleason scores determined from 12-core biopsy versus targeted biopsy. 3. Identify predictors of "Gleason Score Upgrading".

Design, setting, and participants: A retrospective chart review was performed to collect the data for the patients seen in the Urology Clinic of the Medical center of the University of California, Irvine. The data from 331 patients were collected for this review.

Outcome measurements and statistical analysis: The assay results of all biomarkers and MRI findings, as available, was compared to the recent pathological diagnosis of prostate biopsy. The highest Gleason score noted on pathology was considered for positive biopsy.

Results: The mean patient age was 65 years and mean PSA level was 7.72ng/ml. The overall cancer detection rate was 49.52% (119 of 226 patients). PCA3 was found to have better predictability for a positive biopsy results (OR: 1.04(1.01-1.07); *p*=0.004) and. PI-RADS score 3 predicted against positive biopsy outcomes (OR: 1.43(1.01-2.02); *p*=0.019) and PI-RADS score 5 predicted positive biopsy outcomes (OR: 7.69(3.04-19.41); *p*=<0.001). MRI/US-fusion-guided biopsies resulted in Gleason score upgrading in 26% of cases, and among those upgraded cases, 30% were upgraded to clinically significant disease. We also found that the standard 12-core biopsy method resulted in Gleason score upgrading of 28% of cases, and among those upgraded cases, 21% were upgraded to clinically significant disease. On multivariate analysis, MP-MRI suspicion, Age, and PSA above 10 were associated with Gleason score upgrading in the targeted lesions.

Conclusion: PCA3 and MP-MRI findings can be considered to predict the outcomes of prostate biopsies and patients who are likely to get benefit from prostate biopsy can be selected and sparing the patients harboring indolent disease. MRI/US-fusion-guided biopsies resulted in Gleason score upgrading in 26% cases, among those upgraded cases, 30% were upgraded to clinically significant disease. MRI/US-fusion-guided biopsy missed more cases of less aggressive disease and fewer cases of highly aggressive disease as compared to standard 12-core TRUS biopsy.

1. INTRODUCTION

According to the American Cancer Society, prostate cancer is the most common cancer in males (26%) in 2015 [1]. Despite its high frequency, the mortality rate for PCa was estimated to be only 9%. Despite this, many patients elect to undergo curative treatments that have the potential for side effects (such as incontinence and impotence) which may impact a patient's quality of life. Therefore, it is desirable to identify and treat only significant prostate cancer that may impact mortality and minimizing treatment for indolent disease. Prospective identification of malignant behavior presents a fundamental advantage for patients, in particular for men who harbor low risk disease. This would be a significant benefit in the current concern in the over diagnosis and over treatment of PCa. Therefore, patients harboring indolent PCa are spared the side effects of aggressive treatment, managing the social, emotional, psychological and financial burden with compromised quality of life, and risk of potential morbidity of treatment without a survival benefit over non-treatment.

Suspicion for prostate cancer is traditionally based on Digital Rectal Examinations (DRE) and Prostate-Specific Antigen (PSA) results, followed by a transrectal ultrasound-guided prostate biopsy. Transrectal Ultrasound (TRUS) prostate biopsy has been the diagnostic standard for PCa diagnosis for many years which involves systematic, strategically mapped biopsy samples of the prostate. However, the absence of distinguishing features of PCa on ultrasound images limits the use of TRUS for only

directing biopsies into different quadrants of the prostate. These traditional (systematic) TRUS biopsies are essentially "blind" by today's imaging standards. Multiparametric Magnetic Resonance Imaging (mpMRI) offers an advantage over such limitations and is now recognized as a useful and accurate modality to detect, characterize, and stage PCa. With the advent of MRI-fusion biopsy technology (Artemis, Uronav, etc.), we are able to utilize this information rich MRI and direct needle biopsies in areas with MRI characteristics of PCa.

Current clinical assessments, such as PSA and DRE, lack the predictive accuracy for a negative prostate biopsy. Several recent studies investigated the ability of new biomarkers to improve PCa diagnosis and to discriminate between aggressive and slow growing cancers. These new assays may assist in avoiding over-detection and over-treatment. Many other pre-treatment risk assessment models have also been developed based on clinicopathologic factors, such as biopsy Gleason Score (GS), PSA density, and clinical T Stage (cT). These risk assessment models often aid in developing treatment plans for patients with prostate cancer.

In our proposed study, we are planning to conduct a retrospective review of patient's demographic data and the results of commercially available pre-biopsy biomarkers (e.g.PSA and its derivatives, PCA3, OPKO4K, Neogenomics assay, etc.) of serum and urine, and post-biopsy biomarkers or assays (Prolaris, ConfirmMDx, etc.) used for screening, diagnosis, and assessment of biologic behavior of their PCa. We will correlate these results with the findings of their prostate MRI, standard TRUS prostate

biopsy, MRI-fusion guided biopsies, and treatment outcomes. With this data, we plan to develop screening/diagnostic tools and nomograms to predict the probability of PCa diagnosis and behavior prior to performing an invasive prostate biopsy.

2. BACKGROUND

The National Cancer Institute, Survival, Epidemiology and End Result Program estimated 180,890 new cases of prostate cancer in the year 2016 and noted that approximately 10.7% of men will be diagnosed with prostate cancer at some point during their lifetime [1].

2.1. Biomarkers in Prostate Cancer Diagnosis and Risk Assessment

Prostate gland cells produce a protein called prostate-specific antigen (PSA). The PSA test is a measures the level of PSA in a man's blood. The blood level of PSA was found to be elevated in men with prostate cancer [2]. The FDA approved PSA test to monitor the progression of prostate cancer in men who had already been diagnosed with the disease in 1986. In 1994, the FDA approved the evaluation of the PSA test in combination with a Digital Rectal Exam (DRE) to test asymptomatic men for prostate cancer [3]. Men who have prostate cancer specific symptoms often undergo PSA testing (along with a DRE) to help doctors determine the extent of the disease.

The use of PSA as a screening test for prostate cancer resulted in early prostate cancer diagnosis, allowing for earlier disease detection at a curable stage. However, because the PSA screening test has a low specificity, patients with a positive PSA screening test must undergo a prostate biopsy for diagnosis of prostate cancer. The prostate biopsy itself has the potential for false negatives, with a reported false negative rate of up to 30 percent [4]. Furthermore, in patients with an elevated or consistently

increasing PSA value and a negative initial biopsy, repeat biopsies did not significantly improve the detection rate of prostate cancer; on the contrary, the detection rate decreased with each subsequent biopsy [5]. A prostate biopsy has inherent risks (such as the risk of procedure-related serious infectious complications, bleeding, and acute urinary retention), and may have psycho-social impacts and potential financial burden [6].

Although PSA screening increases the detection of prostate cancer at earlier stages, it also increases the detection of low-grade prostate cancer, which could be non-significant and not require active treatment [7]. A systematic review by Loeb et al., analyzed epidemiological, clinical, and autopsy studies, and found that the over-diagnosis of prostate cancer ranges from 1.7% to 67 %. In turn, over-diagnosis has led to overtreatment of indolent disease, with the potential for unnecessary side effects from invasive treatment [7].

Various clinical practice guidelines have recommended Active Surveillance (AS) as an alternative to immediate therapy. Active Surveillance involves routine monitoring of the PSA and prostate biopsy (when indicated) in order to monitor for potential disease progression; patients who do have progression of their disease still receive treatment with a curative intent, but have the benefit of delaying the development of the associated morbidities common with invasive treatment. Despite this clear evidence-based recommendation, AS is underutilized, and more than 90% of patients presenting with low-grade disease undergo invasive surgery or radiation therapy for immediate treatment [8]. This aggressive treatment of patients who harbor indolent cancer is considered

suboptimal, as the risks associated with treatment (such as urinary incontinence and erectile dysfunction) may outweigh the benefits of treating prostate cancer with a low potential for progression [9]. To overcome this issue, various biomarkers and genomic-based tests have been developed to assist in identifying the aggressiveness of a patient's disease. Therapeutic decision-making has improved over time with enhanced diagnostic accuracy, improved pretreatment and post-treatment risk stratification, and distinct identification of indolent versus aggressive disease.

Pre-diagnostic tests are performed to ascertain the patient's risk of having prostate cancer and are utilized before definitive pathological diagnoses via prostate biopsy. The intent of these pre-biopsy tests is to reduce the negative effect of unnecessary and repeated biopsies.

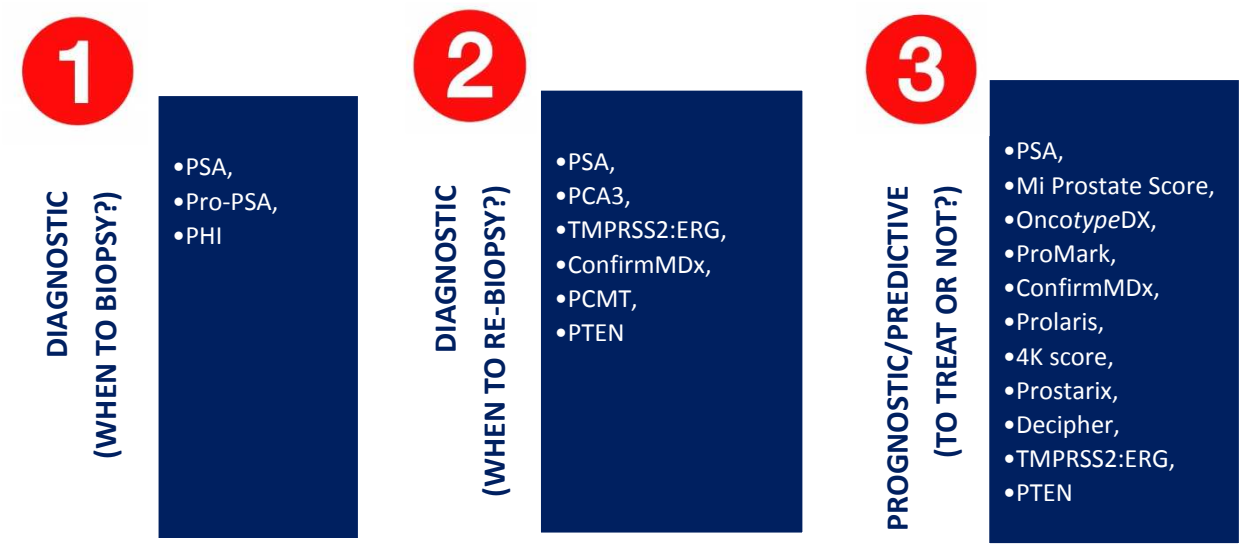


Figure1: Tests for prostate cancer diagnosis and Prognosis

2.1.1. PSA AND PSA DERIVATIVES

PSA is an organ-specific marker, but not a cancer-specific marker [10]. Hyperplastic cells produce more PSA than neoplastic cells, and patients with benign prostatic hyperplasia or prostatitis may also have an elevated PSA level [10]. Therefore, PSA levels alone cannot distinguish between aggressive versus indolent disease. In addition, yearly fluctuations may affect the interpretation of PSA values, making it difficult to predict the prognosis of prostate cancer based on a single assessment or without also evaluating other assessments such as digital rectal exams and prostate biopsies [10].

To improve the outcome assessments and operating characteristics, PSA derivatives are often measured. The ratio of PSA concentration to prostate Volume (PSA Density), the rate of PSA change over a period of time (PSA Velocity), the number of months it takes for PSA values to double (PSA Doubling time), free PSA (fPSA) and the Free/Total PSA (f/t PSA) ratio are some PSA derivatives used in clinical practice. Variations in these derivatives are a measure of aggressiveness and prognosis of prostate cancer in clinical settings.

2.1.2. PCA3

Prostate Cancer Antigen 3 (PCA3) is the and is upregulated in prostate cancer cells. The PCA3 score is generated by comparing the concentration of PCA3 mRNA levels to PSA mRNA levels in post-DRE urine specimens. A low PCA3 score was shown to be a

strong predictor of low volume, clinically insignificant prostate cancer, suggesting a promising application in patient selection for active surveillance [11].

2.1.3. OPKO 4KSCORE

The 4KScore test measures four prostate-derived kallikrein proteins (total PSA, Free PSA, intact PSA and human kallikrein 2) in a patient's blood. The result of the 4KScore test is derived from the quantitative values of the kallikrein proteins, along with the clinical factors of patient's age and digital rectal exam findings, to determine a patient's risk for high-grade, aggressive prostate cancer [12].

2.1.4. ConfirmMDx:

ConfirmMDx uses formalin-fixed paraffin embedded (FFPE) prostate tissues collected during a 12-core prostate biopsy. This is a quantitative methylation assay, identifying an epigenetic field effect supplementary to tumor progression centered on DNA methylation in cells contiguous to PCa foci. Even though the tissue is observed to be normal on microscopic examination, epigenetic field effects can be detected in those tissues, identifying patients who are at risk of a false negative result. Stewart et al., reported 88-90% Negative Predictive Value (NPV) for ConfirmMDx test in detecting methylation in surrounding non-neoplastic tissues. Other validation studies in US and European populations have also been conducted, confirming that epigenetic changes well predict the malignant changes missed by prostate needle biopsy [13].

2.1.5. Onco*type*DX:

Genomic Health, Inc. developed a multi-gene Reverse Transcription Polymerase Chain Reaction expression assay using fixed paraffin embedded tissues collected during prostate biopsies. The test detects 12 oncogenes and 5 reference genes. These genes represent androgen pathways, cellular organization pathways, cellular proliferation pathways, and stromal response pathways of prostate oncogenesis. The Genomic Prostate Score (GPS) is calculated based on the expression of reference-normalized 12 oncogenes and reported from 0 (low) to 100 (high), where a high score represents aggressive disease [14].

Cullen et al., established the assay of a 17-gene Genomic Prostate Score as a measurement of PCa aggressiveness. The GPS was observed to be significantly predictive of both high- grade and non-organ confined disease, and was found to be associated with adverse pathology upon controlling for clinic-pathologic factors. The GPS was also observed to be significantly associated with distant metastases [14]. Klein et al., observed and reported 2.3 times increased risk of having high-grade disease and a 1.9-times increase of non-organ confined disease with each 20-point increase in GPS [14].

2.1.6. PROLARIS

PROLARIS is an RNA expression based cell cycle progression assay, using a prognostic RNA signature, directly measuring tumor cell growth characteristics in order to stratify patients with localized disease per disease aggressiveness. Prostatectomy and prostate biopsy specimens are evaluated to determine and combine expression of 31 cell

cycle progression genes (selected based on their correlation with PCa proliferation) normalized to 15 housekeeper genes.

The PROLARIS study result, reported as a CCP score, was assessed for its usefulness in predicting prostate cancer outcome in numerous studies. A retrospective cohort study was performed to measure the expression of 31 genes in Cell Cycle Progression (CCP) with Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) on RNA extracted from formalin-fixed-paraffin-embedded tumor samples. The gene signature was tested in 366 men treated with Radical Prostatectomy, the operation to remove the prostate gland and some of the tissue around it (RP-Cohort) and another cohort consisted of 337 men diagnosed with clinically localized PCa on Transurethral Resection of the Prostate (TURP), a prostate surgery done to relieve moderate to severe urinary symptoms caused by an enlarged prostate (TURP- Cohort). The CCP score was a strong predictor of biochemical recurrence in the RP-Cohort in both univariate and multivariate analyses. In the TURP cohort, the CCP score was a strong predictor of death due to prostate cancer, as compared to any other Clinicopathologic prognostic factor (GS, PSA and stage) [16].

A meta-analysis study reported a significant association of CCP, derived from prostate biopsy samples of 582 patients, with biochemical recurrence. In a validation study, the CCP score showed significant prognostic accuracy and helped to clearly identify both low-grade (candidates for active surveillance) and high-grade patients (candidates

for definitive treatment) when performed on biopsy tissue. For the RP candidates, the test provided evidence for post-operative risk of adverse pathology outcomes [17].

2.1.7. GLEASON SCORE

Gleason grading system is used to evaluate the prostate cancer prognosis from biopsy specimens. Based on the microscopic appearance of prostate cancer, the Gleason score is attributed on a scale of 1 to 5, for both primary and secondary patters. Higher Gleason score predicts worst prognosis [18].

2.2. MRI in detection of Prostate Cancer

Conventional diagnosis of Prostate cancer is based on systematic (generally 12 core samples) TRUS-guided biopsy prompted by elevated PSA and an abnormal digital rectal examination. Unfortunately, poor sampling in the anterior, midline, and apex regions of the prostate leads to under-diagnosis of clinically significant disease. Up to one in three low-risk biopsies are upgraded on repeat biopsy, and studies have reported false negative results up to 49%. Cohen et al., have reported that TRUS-guided biopsy failed to precisely predict not only the prostate cancer burden, but also prostate cancer aggressiveness. Furthermore, a low agreement was observed and reported between biopsy-derived and surgical Gleason score (a system of grading a prostate cancer) (weighted kappa: 0.37) in this study [19].

Considering the above issues, there is a need for an alternative approach for precisely predicting prostate cancer burden and aggressiveness, along with accurately

predicting the Gleason score. The MRI-targeted prostate biopsy is an emerging as a potential option to supplement for 12-core systematic biopsy. As compared to the standard TRUS-guided biopsy technique, the MRI-guided fusion targeted biopsy technique offers improved diagnostic accuracy by precisely targeting suspicious lesions.

Puech et al., reported a high degree of accuracy in the detection of intra-prostatic cancer foci identified on Dynamic Contrast Enhanced-MRI (DCE-MRI) compared with radical prostatectomy histology. Sensitivity and specificity of DCE-MRI were reported up to 81% and 82% respectively for detection of Gleason grade 4 /5 in more than 10% of cases [20].

Moore et al., conducted a systematic review and pooled data analysis of studies on comparing the accuracy of MRI-targeted biopsy with standard TRUS biopsy, in biopsy naïve patients or patients with at least one negative standard TRUS biopsy. The MRI was directed in 599 biopsy naïve patients in pooled data analysis, of which, 374 (62%) had suspicious findings. Prostate cancer was detected in 242 (66%) of these 374 patients. Furthermore, in a pooled analysis of patients with a previously negative standard TRUS biopsy (n=479), 328 (69%) had a suspicious finding; prostate cancer was detected in 228 (70%). Pooled results also indicated that the MRI-guided targeted biopsies revealed a 16% greater detection of Gleason grade 4 and Gleason grade 5 than the systematic standard TRUS biopsies. Finally, fewer patients and fewer biopsies per patient were required to achieve TRUS-equivalent detection of clinically significant prostate cancer [21].

Siddiqui et al., conducted a prospective clinical study comparing a targeted and standard biopsy approaches for detection of high risk (Gleason Grade ≥ 4+3) prostate cancer (n=1003) and reported 30% more high-grade and 17% less low-grade prostate cancer detection with MRI targeted biopsies as compared to standard TRUS-guided biopsies. Furthermore, significantly higher diagnostic accuracy of MRI-guided biopsy (AUC 0.73) was observed against standard TRUS biopsy (AUC 0.59) for the detection of intermediate or high-risk patients [23].

2.3. MRI in Prostate Risk Stratification:

Recent PSA level, DRE, clinical cancer stage, TRUS biopsy results, and the Gleason Score are the most predictive and prognostic biomarkers for prostate cancer risk stratification [24]. The objective of these assessments is to select the risk level and treatment options for a particular patient. The recent NCCN guideline directs risk assessment by incorporating the above procedure outcomes and life expectancy of patients. Currently, the evidence suggests that patients with a Gleason score 7 or above are ineligible for active surveillance for their prostate cancer [24].

Numerous studies have clearly reported significant discrepancies between biopsy-derived Gleason score and Prostatectomy derived Gleason Scores. Gleason Score upgrading on radical prostatectomy is a well-known and well-documented issue and is now supported by a recent meta-analysis study that incorporated results of more than 15

studies and 14,000 prostate cancer patients. The study reported that 38% of prostate biopsy-derived Gleason scores (≤6) are updated after radical prostatectomy [25].

The MRI has shown a potential to precisely assess tumor aggressiveness before treatment and help improve the selection of appropriate treatment for patients. In a study of 388 patients, presenting low-risk prostate cancer, after initial biopsy who were candidates for active surveillance, the T-2 weighted MRI visualized tumor was a strong predictor of tumor upgrading on confirmatory biopsy. The Gleason score was upgraded in 20% (79 of 388) of the study population on confirmatory biopsy [26].

Diffusion-weighted (DW)-MRI, Dynamic Contrast Enhanced (DCE)-MRI imaging and Magnetic Resonance Spectroscopic imaging (MRSI) are additional techniques for MRI imaging that improved prognostic ability. The prostate cancer signal intensity observed on DW-MRI significantly correlated with a radical prostatectomy derived Gleason score, and significant improvement in the pre-treatment risk stratification was observed through DW-MRI-targeted biopsy. In a study of 154 patients, T2-weighted and Diffusion Weighted –MRI was performed before prostatectomy. A multivariate analysis was performed after adjusting for age, PSA, biopsy Gleason score, and number of positive scores on histology, and it was observed that an MRI was a strong and significant predictor of tumor of a Gleason score ≥ 4 [27].

2.4. Methods of MRI-Guided Prostate Biopsy:

Contrary to other imaging procedures, magnetic resonance imaging techniques offers improved spatial and contrast resolution, resulting in excellent prostate gland anatomic imaging.

Three different approaches can be used in MRI-targeted fusion biopsy.

A. **MRI-MRI Fusion (In-bore Biopsy):**

Performing Biopsy within the MR magnet.

Biopsy needle localization is confirmed with contemporaneous MRI image fusion with a prior MRI identified lesion. After collecting each biopsy sample (few targeted cores only) through a transrectal route, the patient is re-scanned to confirm tissue localization.

- Advantages:
  - Targets a limited number of cores,
  - Reduced detection of insignificant tumors
- Disadvantages:
  - NOT a time and cost effective exercise

B. **MRI-US fusion biopsy:**

A method of combining relevant data from two or more images into a single image, which can be more informative than the individual images. Computer software is used for fusing MRI and ultrasound images, allowing an MRI observed lesion to be targeted with ultrasound.

Using the conventional ultrasound techniques, the prostate is imaged, and the images are concurrently superimposed on to prior collected MRI-derived images using a

digital overlay, resulting in a three-dimensional reconstruction of the prostate. Biopsy sites are aimed and tracked on the reconstructed model.

- Advantages:
    - Very short procedure duration
    - Can be performed in out-patient clinics employing local anesthesia
    - Promising results
- Disadvantages:
    - Indirect procedure
    - Usage of additional devices
    - Requires specialized operation training and skills

C. **Cognitive fusion:**

Performing ultrasound guided biopsy after reviewing MR images.

- Advantages:
    - A simple and quick method
    - Requires MRI and Ultrasound facilities- no additional equipment
    - No specialized training required
- Disadvantages:
    - Potential for human error while extrapolating MRI to TRUS

2.5. MRI-Ultrasound Fusion-Guided Biopsy in Prostate Cancer Diagnosis

It has been studied and reported well that MRI ultrasound fusion-guided biopsy detects a greater number of clinically significant/ high-risk tumors and a smaller number of clinically low-risk tumors. In a prospective study of 164 patients with elevated PSA, MRI/ US guided biopsy was performed. The study reported 97.6% sensitivity and 85.0% specificity of the MRI ultrasound fusion-guided biopsy procedure. PPV and NPV were also reported as 74.6% and 96.3% respectively [28].

Furthermore, numerous studies have reported Gleason score upgrading between 21% to 54% in prostatectomy specimens, where initial grading was done using standard TRUS biopsy. A prospective study was conducted with 582 patients comparing systematic TRUS biopsies to targeted MRI/US-fusion-guided prostate biopsies. Prostate cancer was diagnosed in 54% (315 of 582) of patients and Gleason score upgrading was observed in 32% (81 of 315) of patients with targeted biopsy. As compared to standard TRUS biopsy, the targeted biopsy reported 67% more high-grade tumor (Gleason ≥ 4+3) and missed 36% of low-grade tumor (Gleason <3+4) [29].

2.6. Aims of this Project

We have conducted a retrospective review of patient demographic data and the results of commercially available pre-biopsy biomarkers (e.g. PSA and its derivatives, PCA3, OPKO4K, Neogenomics assay, etc.) of serum and urine, used for screening, diagnosis, and assessment of biologic behavior of their PCa. We also have collected data of MP-MRI findings of prostate cancer. We have generated a test database to develop a correlation of biomarker results and the findings of prostate MRI, standard TRUS prostate biopsy, MRI-fusion guided biopsies, and treatment outcomes.

With this data, we planned to develop screening/diagnostic models to predict the probability of PCa diagnosis and behavior prior to performing an invasive prostate biopsy. We aimed to predict the probability of having positive prostate biopsy, clinically insignificant disease (Gleason Score ≤ 3+4=7) and clinically significant disease (Gleason

Score ≥ 3+4=7) utilizing the biomarkers and MP-MRI data we have collected from the similar patient populations. A validation dataset will be used to validate the developed models.

3. METHODS

3.1. Study Rationale

The FDA has approved Prostate Specific Antigen (PSA) assessment for the screening of PCa. However, ubiquitous application of PSA screening has led to over-detection and over-treatment of PCa. Patients with elevated PSA, and a suspicion for prostate cancer, undergo prostate biopsy for further examination. However, these biopsies often miss the cancerous tissue and sample only benign tissue. This results in false negatives, and the prostate cancer remains undetected.

Transrectal ultrasound (TRUS) is used to help physicians properly guide the biopsy needle, which is projected through the tip of an ultrasound probe inserted into the rectum and directed at the prostate. Recent studies revealed that Magnetic Resonance Imaging/ultrasound fusion guided biopsy significantly improves (non-indolent) prostate cancer detection compared to the standard systematic 12-core transrectal ultrasound prostate biopsy. Recently, numerous commercially available blood/tissue tests and imaging techniques have been introduced to assist in the detection of prostate cancer and to characterize its behavior (indolent vs. aggressive).

Hence, we reviewed the medical records of patients receiving prostate imaging and subsequent prostate biopsies, and correlated these findings with newly available biomarkers in order to observe their relationship to prostate cancer diagnosis and predict

the underlying disease characteristics to stratify clinically insignificant and significant diseases.

3.2. Study Design

This study was a retrospective observational study using chart review to study the outcomes of patients who have undergone diagnostic procedures for prostate cancer. We hypothesized that the results of commercially available pre-biopsy biomarkers of serum and urine, and post-biopsy biomarkers or assays have a correlation with the results and the findings of prostate MRI, standard TRUS prostate biopsy, MRI-fusion guided biopsies, and treatment outcomes. The study protocol narrative was submitted to the UCI Institutional Review Board and was approved on May 6th 2016 with HS# 2016-2781 (*refer appendix 8.3*).

We reviewed the charts of patients who have had an assessment for prostate cancer performed between the dates of January 1st, 2012 and the day before the date IRB approval was granted for this study (May 5th, 2016).

3.3. Study Objective and Outcomes

3.3.1. Study Objective

We aimed to determine the correlation between commercially available biomarkers (PSA, PCA3, Neogenomics Assay, OPKO4K, etc.) with findings on multi-parametric MRI and prostate biopsy specimens.

1. To Predict the probability of PCa diagnosis and behavior prior to performing an invasive prostate biopsy.
2. Describe the rate of disagreement in Gleason scores determined from 12-core biopsy versus targeted biopsy.
3. Identify predictors of “Gleason Score Upgrading”.

3.3.2. Study Outcome

The recent biopsy result noted as positive or negative and the biopsy Gleason score for positive biopsy results were considered as a study outcome measure. Furthermore, number and type of lesions observed on mp-MRI, which revealed the cancer suspicion score of each lesion was also considered for correlation analysis against biomarkers. The PI-RADS version 2.0 was used to denote the cancer suspicion score for each lesion. Lesions score 1 to 5, noted on mp-MRI was considered as an outcome measure for this study.

3.4. Study Participants

Adults (aged 18 years or older) and patients who have available PCa biomarker assessments and/or Imaging studies for prostate cancer diagnosis were included in the study. Patients not meeting the above criteria were excluded from the study.

3.5. Waiver of HIPAA Authorization and Study Consent

This study qualified for expedited review, under ‘Category 5’ of 45 CFR 46.101(b): Categories of Exempt Human Subjects Research, because this research

entailed a review of medical information, collected as part of clinical care, and not collected specifically for this protocol.

The HIPAA Privacy Standard [45 CFR 164.512(h)(i)(2)(ii)] was followed to request a total waiver of HIPAA authorization for research uses of Personal (Protected) Health Information. The waiver justification was documented in the study protocol during submission of an IRB application. The complete HIPAA waiver was approved by the IRB.

For the retrospective data collection process, it was impossible to obtain consent from former patients or patients that have completed their treatment as they may not return to UCIMC. Since the purpose was to be comprehensive and include all patients who underwent diagnostic procedures of prostate cancer in last few years, the study could not practicably be carried out without the waiver of consent. Hence, we requested a waiver of the consent in the study protocol.

The study consent waiver and HIPAA authorization waiver did not affect privacy rights and welfare of individuals whose records were used or disclosed because only those research personnel listed on the protocol were allowed to view the charts and any personal health information. Furthermore, all the study personnel had completed the HIPAA research tutorial so they were all aware of the rules and regulations surrounding the use of private health information for research.

3.6. Data Collection Procedures

The laboratory data-base (Quest) of the UCI medical center was used for data collection activities. Clinical and Pathology laboratory reports, MRI reports, biopsy reports, and progress notes and/or procedure notes were accessed for each patient. The IRB approved protocol number was entered into the system for accessing each patient's information every time the details were accessed. In order to acquire the results of commercial biomarkers, the websites of specific service providers were accessed by the authorized study personnel.

The details of PCA3 tests, Neogenomics tests, onco*type*DX tests, ConfirmMDx tests, Prolaris tests, and OPKO 4k tests were accessed from commercial website. All patients who met the inclusion and exclusion criteria were selected as per the time frame defined in the study protocol and approved by the UCI IRB. The Data Collection tool/Case Report Form was generated to standardize the elements of data collection. The Medical Record Number (MRN) was used as a patient identifier and all data elements are traceable to MR number. Later, MRNs were replaced with a code, and the code key is stored in a secured electronic file, only accessible to the study team. Physical paper charts were also accessed, as and when required. No photographs or audio or video recordings were accessed, viewed, or collected for this research protocol.

3.6.1. Outcome Variables

Gleason Score: The NCI defines the Gleason score as a system of grading prostate cancer tissue based on how it looks under a microscope [18]. Gleason scores range from 2 to 10

and indicate how likely it is that a tumor will spread. A low Gleason score means the cancer tissue is similar to normal prostate tissue and the tumor is less likely to spread; a high Gleason score means the cancer tissue is very different from normal and the tumor is more likely to spread. Low Gleason score 2, 3 and 4 are observed in localized disease found in the transitional zone. Gleason 5, 6 and 7 are generally detected by Biopsy post abnormal DRE and PSA evaluation. The Gleason score 3+4=7 and 4+3=7 tend to differ in disease prognosis. Patients who harbor PCa with Gleason 4+3=7 observe poor prognosis than patients who harbor PCa with Gleason score 3+4=7. The later Gleason 8, 9 and 10 are considered as advanced neoplasms and difficult to cure [18]. We classified Gleason score into three categories clinically insignificant (GS below 6 and 7 (3+4)), and clinically significant (7 (4+3) and 8-10) [30].

Gleason score upgrading was defined as any increase in the Gleason score identified either on Standard TRUS guided biopsy or MRI/TRUS -fusion-guided biopsy.

Core %: the positive percentage of cancerous cores out of the total cores obtained in a biopsy will be noted from the biopsy results.

3.6.2. Independent Variables

PSA: The results of all PSA assessment within the approved time frame was extracted. The free PSA and % free PSA was also extracted, as available. The PSA results were classified as in to four groups as below 4, 4-10, 10-20 and ≥ 21. However, during final analysis, the PSA was grouped as above 10 or below 10.

All subjects were required to have at least two PSA measurements for assessment of PSA Velocity and PSA doubling time.

PSA Doubling time: PSA doubling time is the time required for PSA value to get doubled. It is an indicator of biochemical progression [31]. PSA DT was calculated using the ratio of ln(2) and the rate of change in log (PSA + 1). *(The most recent two PSA values obtained before recent biopsy was considered as for calculation. PSADT was not calculate for patients who did not meet this criterion) [31].* Since, for some patients the subsequent PSA assessment resulted in lower PSA value than former, the resultant PSADT was observed to be negative. The negative value of PSA Doubling time was coded as "0" (zero) to facilitate the analysis.

PSA velocity: For men with only two measurements, PSAV was calculated as the simple rate of change between the index and previous visit divided by the elapsed duration. When ≥3 PSA measurements were available, PSAV was calculated as the running average of the rate of change over three consecutive visits (the index visit and the two preceding visits).

PCA3: Quantitative results of the PCA3 assessment were recorded and qualitative results were dichotomized as positive=1 and negative=0.

OPKO 4K: Results of the OPKO-4K test was noted as a percent of chance of having a Gleason score 7 or greater.

Neogenomics Assay results: The results were dichotomized for *(a) risk of having any grade of prostate cancer* (Positive=1, Negative=0), and *(b) risk of having high-grade prostate cancer* (Positive=1, Negative=0). Furthermore, Neogenomics' recommendation was also noted as 0= biopsy not required, 1= Careful watching, 2=biopsy needed, 3=biopsy needed to confirm.

DRE results: *The Digital Rectal Examination is the rectal examination by doctor for assessment of prostate gland.* The DRE result was noted for this study. Any abnormality in the nodules was recoded as positive and an absence of abnormality was noted as negative. The results were dichotomized (Positive=1 and Negative=0).

ConfirmMDx*:* This is a quantitative methylation assay, identifying an epigenetic field effect supplementary to tumor progression centered on DNA methylation in cells contiguous to PCa foci. The results were dichotomized (Positive=1 and Negative=0).

Onco*type*DX *score:* The results were extracted from the report as GPS score (ranges from 0 to 100), a likelihood of favorable pathology (denoted as a percentage) and GPS interpretation (Very low, Low, and intermediate- coded as 0, 1 and 2 respectively).

Biopsy Methods: The type of biopsy procedure performed for specimen collection was extracted and recorded as standard Transrectal Ultrasound biopsy (TRUS) or MRI-guided fusion biopsy. The data were dichotomized (0=standard TRUS biopsy and 1= MRI-guided fusion biopsy) for ease of analysis.

PROLARIS SCORE: results were extracted from the company websites and noted as a continuous variable.

3.6.3. Covariates

Age is defined in years and will be compared between low, moderate and high-risk groups. It is also categorized into 2 different groups for a more accurate investigation. Since the mean age was noted as 65 years, we dichotomized the age as above mean and below mean.

Race: was categorized in to the following groups: White, Black or African American, Asian or Pacific Islander, unknown or not reported. The data was dichotomized for ease of analysis for each race separately.

3.7. Statistical Analysis

The Statistical Package for Social Science® (SPSS), Version 24.0 (IBM Corp., Armonk, New York) was used for data analysis. All variables were examined for normality distribution. The correlation between biomarkers and the Gleason score was evaluated. Odds ratio, 95% Confidence intervals (CIs) and *p*-values are used as the key measures of the association in the current assessment. Odds ratio below 1 indicated a negative correlation between the variables and an odds ratio above 1 indicated a positive correlation between variables. The *p* value of ≤ 0.05 was considered as a statistically significant outcome for individual analyses. The Hosmer-Lemeshow test is a measure of goodness of fit for logistic regression models and p values of 0.05 is considered as a good

fit for the model under investigation. Receiver operating characteristics (C-Statistics) were used to measure the ability of the model to discriminate 1's from 0's at different threshold values plotting sensitivity (x- axis) on specificity (Y-axis). C= 0.5 indicates no discrimination whereas 1 refers to perfect discrimination.

3.7.1. Demographics and Baseline characteristics

Means and standard deviations were calculated for continuous variables and frequencies and percentages were calculated for categorical variables.

3.7.2. Primary Objective Analyses

Univariate logistic regression models were used to identify the predictors of prostate cancer pathologic results systematic TRUS biopsy and MRI-guided biopsy. A significance level (α) of 0.05, and 95% confidence intervals were calculated for each outcome variable. Association of MRI findings with recent biopsy findings, reported as a Gleason Score, were evaluated using univariate regression models. The detailed association with clinically significant disease and insignificant disease was also evaluated. The Gleason score, observed on the standard 12-core biopsy was compared against those observed on the targeted biopsy. The disagreement was evaluated for each individual Gleason scores (6, 3+4=7, 4+3=7, 8 and above). The predictors of Gleason score upgrading were evaluated by performing univariate and multivariate logistic regression analysis. Models was tested for identification of Gleason score. The performance of these model was assessed using ROC and AUC analysis.

4. RESULTS

4.1. Patient Characteristics

Patient demographics of the study are shown in Table 1. The data were collected from a total 331 patients. The mean age for the patient population was 65 years. A total of 94 (28%) patients were biopsy naïve. The mean PSA level was 7.72± 7.2 ng/ml. The Qualitative and Quantitative PCA3 assessment results were available for 71 patients, including 29 (41%) qualitative PCA3 positive patients. The mean PCA3 score was found to be 30.41 ±21.0. The OPKO 4K score was available for 41 patients. The mean OPKO 4K Score was 14.73%±7.0%. The Onco*type*DX GPS score was available for 82 patients with a mean score 24.70±9.03. The ConfirmMDx Score was available for 64 patients. A total of 30 patients were found to be positive on ConfirmMDx assessments. The Prolaris test result was available for 54 patients and the mean was found to be 0.67± 2.0.

The neogenomics assay was available for 170 patients, out of which, 168 patients were found to be positive for any grade of the disease and 47 were positive for risk of having high grade disease.

Biopsy results for all patients, as available, was recorded. A total 77 patients had a history of previous positive biopsy results and 120 patients had a history of previous negative biopsy results. Recent biopsy reports were available for 226 patients. Total 107 patients had a negative recent biopsies and 119 (114 on MRI guided biopsies 5 on TRUS guided biopsies only) patients were observed positive in recent biopsies.

Table 1: Population Characteristics

	n	Biopsy Naïve	PREVIOUS BIOPSY		RECENT BIOPSY		
			Positive	Negative	Negative	Clinically Insignificant	Clinically Significant
Men, No.	331	94	77	120	107	82	37
Age (years), Mean (SD)	65(9.0)	63.29(8.4)	65.88(9.5)	65.63(8.6)	63.62(7.6)	65.76(8.5)	68.70(9.0)
<65		48(51.1%)	31(40.3%)	51(42.5%)	64(52.5%)	30(38.0%)	9(25.0%)
≥65		46(48.9%)	46(59.7%)	69(57.5%)	58(47.5%)	49(62.0%)	27(75.0%)
DRE, No.	225	72	51	94	104	64	32
POSITIVE	51	22(30.6%)	8(15.7%)	18(19.1%)	17(16.3%)	15(23.4%)	13(40.6%)
NEGATIVE	174	50(69.4%)	43(84.3%)	76(80.9%)	87(83.7%)	49(76.6%)	19(59.4%)
MRI FINDINGS							
Prostate Volume, Mean (SD)	65.74(54.8)	57.84(32.4)	56.43(48.7)	75.93(42.7)	74.79(48.6)	55.82(27.0)	56.10(42.2)
Cancer Suspicion on MP-MRI	209	58	55	76	77	61	29
Intermediate (PI-RADS-3)	70	19(32.8%)	16(29.1%)	27(35.5%)	30(39.0%)	14(23.0%)	5(17.2%)
High (PI-RADS-4)	81	19(32.8%)	21(38.2%)	33(43.4%)	36(46.8%)	25(41.0%)	8(27.6%)
Very High (PI-RADS-5)	54	18(31.0%)	18(32.7%)	14(18.4%)	7(9.1%)	22(36.1%)	16(55.2%)
MRI-Bony lesion, No.	18	2	5	9	6	4	4
Lymphoadenopathy, No.	22	3	9	7	5	3	7
PSAD on MRI, Mean (SD)	0.175 (0.3)	0.13(0.1)	0.20(0.3)	0.16(0.2)	0.11(0.1)	0.18(0.1)	0.20(0.2)
BIOMARKERS							
PSA, No.	238	79	54	104	106	80	37
PSA(ng/ml), Mean (SD)	7.72(7.2)	6.0(5.3)	5.9(3.3)	9.8(9.2)	7.1(5.1)	8.6(10.3)	7.8(5.5)
PSADT (Months), Mean (SD)	71	51.88(93.5)	38.27(48.0)	60.42(115.8)	63.84(119.1)	56.66(83.7)	12.39(8.4)
PSAV(ng/ml/month), Mean (SD)	71	0.45(0.7)	0.52(1.0)	0.57(0.9)	0.42(0.8)	0.57(1.0)	0.52(0.3)
PCA3, No.	71	31	2	35	33	15	6
PCA3 Quantitative, Mean(SD)	30.41 (21.0)	31.94(25.5)	--	30.91(36.6)	20.0(16.67)	43.27(25.35)	68.83(64.9)
PCA3 Qualitative-Positive (No.)	29	15(46.9%)	--	13(37.1%)	11(28.2%)	8(61.5%)	5(83.3%)
PCA3 Qualitative-Negative (No.)	43	17(53.1%)	--	22(62.9%)	22(71.8%)	7(32.5%)	1(16.7%)
OPKO4K, No.	41	10	--	25	15	8	4
OPKO4K. Score, Mean(SD)	14.73%(7.0%)	22.0%(21.7%)	--	10.60%(13.1%)	12.93%(15.1%)	8.1%(9.7%)	29.00%(17.0%)
Onco*type*DX, No.	82	19	40	20	--	48	12
Onco*type*DX GPS Score, Mean(SD)	24.70 (9.0)	28.37(11.5)	22.98(8.0)	24.50(8.3)	--	22.79(7.4)	32.25(11.06)
Onco*type*DX %,Mean(SD)	67.46%(12.5%)	63.63%(14.5%)	69.85%(12.1%)	67.50%(11.5%)	--	70.16%(11.0%)	58.00%(12.6%)
ConfirmMDx, No.	64	15	--	47	56		
ConfirmMDx-Positive, n(%)	30	8(53.3%)	--	21(44.7%)	27(48.2%)	--	--
ConfirmMDx-Negative, n(%)	34	7(46.7%)	--	26(55.3%)	29(51.8%)	--	--
NEOGNOMICS, No.	170	63	7	86	77	34	17
Risk of Any grade-	168	62(98.4%)	7(100.0%)	85(98.8%)	76(98.6%)	33(97.1%)	17(100.0%)
Risk of High Grade-Positive, n(%)	47	13(20.6%)	1(14.3%)	30(34.9%)	21(27.7%)	12(35.3%)	4(23.5%)
Risk of High Grade-Negative, n(%)	123	50(79.4%)	6(85.7%)	56(65.1%)	56(72.7%)	22(64.7%)	13(76.5%)
PROLARIS, No.	54	17	24	10	5	29	12
Score, Mean(SD)	0.67(2.0)	1.41(2.2)	-0.013(1.4)	-0.2(1.2)	1.70(1.8)	-0.24(1.3)	1.76(2.1)

Continuous variables are presented as Mean(SD) and dichotomized variables are presented as percentage. *Clinically Insignificant disease:GS≤3+4, Clinically Significant Disease: GS≥4+3*

The recent biopsy positive cohort was divided in to two sub-cohorts based on the Gleason Score. The biopsy Gleason Scores ≥ 4+3 were classified as a Clinically Significant high-grade disease and a biopsy Gleason Scores ≤ 3+4 were classified as a *Clinically Insignificant* low-grade disease as per the recommendation defined by the Standard for reporting MRI-targeted Biopsy Studies (START) working group [33]. Of the 119 positive biopsies, the study included 82 (69%) and 37 (31%) of clinically insignificant and Clinically significant biopsies, respectively.

The data from more than 200 patients were collected for Multi-Parametric Magnetic Resonance Imaging. The cancer suspicion score was noted for 209 patients and is tabulated under patient characteristics. Very high cancer suspicion (PI-RADS score 5) was noted on 54 patients, while 81 patients were found to have high (PI-RADS score 4) cancer suspicion during MP-MRI. 18 patients were found to have bony lesions and 22 patients were found to have lymphoadenopathy during MP-MRI.

4.2. Predicting the probability of PCa diagnosis and behavior prior to performing an invasive prostate biopsy (Study Objective#1).

The association of recent biopsy results and biomarkers from bivariate analysis are tabulated in Table 2. PCA3 was found to have a strong positive association (odds ratio[OR]: 1.04 (1.01-1.07); p=0.004) with recent positive biopsy. Furthermore, the PCA3 had a strong positive association (OR: 1.02(1.00-1.05); p=0.035) with clinically significant disease. The OPKO-4K score also had a strong positive association (OR: 1.07(0.99-1.14);

Table 2: Association of recent Biomarkers and MRI findings with recent biopsy results

	POSITIVE BIOPSY (All Gleason Score)		Clinically Insignificant (GS ≤3+4=7)		Clinically Significant (GS≥4+3=7)	
	OR (95% CI)	**p Value**	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value**
BIOMARKERS						
PSA	1.02(0.98-1.06)	0.205	1.02(0.98-1.06)	0.216	1.00(0.95-1.05)	0.919
PCA3-Quantitative	1.04(1.01-1.07)	**0.004**	1.01 (0.99-1.071)	0.156	1.02(1.00-1.05)	**0.035**
PCA3-Qualitative	7.81(2.26-26.91)	**0.001**	3.86(1.11-13.38)	**0.033**	8.25(0.89-75.78)	**0.062**
OPKO-4K	1.01(0.95-1.06)	0.709	0.95(0.88-1.02)	0.218	1.07(0.99-1.14)	**0.053**
NEOGENOMICS-HIGH GRADE	1.12(0.52-2.42)	0.768	1.09(0.50-2.34)	0.823	1.16(0.47-2.85)	0.740
MRI FINDINGS						
PI RADS SCORE (3-5)	1.48(1.15-1.90)	**<0.001**	1.43(1.12-1.67)	**0.019**	1.43 (1.01-2.02)	**0.040**
Score 3	0.46(0.23-0.98)	**0.030**	0.66(0.32-1.34)	0.258	0.46(0.17-1.29)	0.144
Score 4	0.82(0.45-1.49)	0.527	1.08(0.58-2.00)	0.789	0.55(0.23-1.30)	0.175
Score 5	7.69(3.04-19.41)	**<0.001**	2.42(1.22-4.79)	**0.011**	4.48(2.01-9.99)	**<0.001**
MRI-PSAD	128.00(7.62-2148.45)	**0.001**	7.58(1.05-54.47)	**0.044**	9.49(1.22-73.89)	**0.032**
LYMPHOADENOPATHY	2.09(0.69-6.33)	0.19	0.46(0.12-1.70)	0.249	6.19(2.07-18.43)	**<0.001**
BONY LESION	1.35(0.45-4.04)	0.583	0.784(0.23-2.58)	0.689	2.42(0.71-8.21)	0.155
PREVIOUS BIOPSY						
BIOPSY NAÏVE	1.13(0.66-1.93)	0.648	0.62(0.34-1.13)	0.122	2.29(1.11-4.69)	**0.024**
PREVIOUS POSITIVE BIOPSY	3.53(1.76-7.09)	**0.001**	2.38(1.21-4.70)	**0.012**	2.75(1.01-7.45)	**0.046**
PREVIOUS NEGATIVE BIOPSY	0.28(0.14-0.56)	**0.001**	0.41(0.21-0.82)	**0.012**	0.36(0.13-0.98)	**0.046**
OTHER						
AGE	1.49(1.01-1.08)	**0.003**	1.01(0.97-1.04)	0.525	1.06(1.02-1.11)	**0.005**
DRE	2.12(1.07-4.17)	**0.029**	1.06(0.52-2.15)	0.85	2.86(1.28-6.37)	**0.01**

The odds ratio was calculated using Binary logistic regression method. *Clinically Insignificant disease:GS≤3+4, Clinically Significant Disease: GS≥4+3*

p=0.053) with clinically significant disease. The PSA and Neogenomics assay failed to demonstrate statistical significance with recent biopsy results.

The association of results of Multi Parametric Magnetic Resonance Imaging (MP-MRI) was evaluated for its relevance with recent biopsy results. The Prostate Imaging Report and Data System (PI-RADS) score was found to have a positive association (OR: 1.48(1.15-1.90); *p*=<0.001) with recent positive biopsy outcomes. Additionally, a stronger and more significant association (OR: 1.43(1.12-1.67); *p*=0.040) was observed with clinically significant biopsy pathology as compare to clinically insignificant pathology (OR: 1.43(1.01-2.02); *p*=0.019). Furthermore, PI-RADS score 3 had a positive and strongest association (OR: 2.14; *p*=0.030) with recent negative pathology negative association (OR: 0.46(0.23-0.98); *p*=0.030) with recent positive biopsy outcomes and contrary PI-RADS score 5 resulted in a strong and positive association (OR: 7.69(3.04-19.41); *p*=<0.001) with recent positive biopsy outcomes. Furthermore, the PI-RADS score was evaluated for its association with sub cohorts of positive biopsy results. It was observed that PI-RADS score 5 had a strong positive association (OR: 4.48(2.01-9.99); *p*=<0.001) with clinically significant disease and a relatively less positive association (OR: 2.42(1.22-4.79); *p*=0.011) with clinically insignificant pathological outcomes on prostate biopsy. Other MP-MRI parameters such as PSA density and lymphadenopathy noted on MRI, were congruent with the noted suspicion score and their association with recent biopsy outcomes.

Previous positive biopsy results were assessed for its association with recent biopsy results. It was noted that a previous positive biopsy had a strong positive

association (OR: 3.53(1.76-7.09); *p*=0.001) with the recent positive biopsy. It was noted that a previous negative biopsy had completely inverse and similar odds ratios for recent biopsy pathology.

Age had a positive association (OR: 1.49(1.01-1.08); p=0.003) with recent positive biopsy pathology. The trend was also similar with clinically significant disease pathology (OR: 1.06(1.02-1.11); p=0.005). Furthermore, positive DRE results were found to have a positive association (OR: 2.12(1.07-4.17); p=0.029) with positive pathological outcomes on recent biopsies.

The association of Biomarkers was evaluated for biopsy core-positivity as tabulated in Table 2a and it was noted that PCA3 had a strong positive association (OR: 6.22(1.48-26.11); p=0.013) with >50% core positivity.

Table 2a: Association of Biomarkers and Biopsy core positivity

		<50%		>50%	
	n	OR (95% CI)	p Value	OR (95% CI)	p Value
PSA	223	1.01(0.97-1.05)	0.493	1.02(0.98-1.06)	0.231
PCA3-Qualitative	56	4.09(0.39-42.00)	0.236	6.22(1.48-26.11)	**0.013**
OPKO-4K	25	1.04(0.98-1.11)	0.156	1.00(0.95-1.06)	0.804
NEOGENOMICS-HIGH GRADE	133	1.30(0.41-4.11)	0.656	1.16(0.47-2.85)	0.740
PROLARIS	45	1.30(0.92-1.81)	0.123	0.97(0.71-1.32)	0.848
Onco*type*DX-GPS- Score	70	0.97(0.91-1.03)	0.396	1.07(1.01-1.14)	**0.017**
ConfirmMDx	-	-	-	-	-

The odds ratio was calculated using Binary logistic regression method.
Biopsy core positivity: The percentage of biopsy cores, found to have cancerous tissues.

4.3 Evaluating the rate of disagreement in Gleason scores determined from 12-core biopsy versus targeted biopsy (Study Objective # 2).

The Biopsy Gleason score derived from standard 12-core biopsies were compared against the biopsy Gleason score observed on MRI/US-fusion guided biopsies for the same patient in table 3. Biopsy Gleason Scores derived from MRI/US-fusion guided biopsies were also compared against Gleason scores derived from standard 12-core biopsies for the same patient in table 3.

Table 3: Comparison of biopsy results from standard 12-core biopsy versus MRI/US-fusion targeted biopsies

		Standard 12-Core Biopsy	
MRI/US-Fusion biopsies	**No Cancer**	**Clinically Insignificant Disease (GS≤3+4=7)**	**Clinically Significant Disease (GS≥4+3=7)**
No Cancer	109	20	2
Clinically Insignificant Disease	10	49	5
Clinically Significant Disease	2	7	20

The distribution of the Gleason scores identified on the standard 12-core biopsy versus MRI/US-fusion guided biopsies can be seen in table 3a. MRI/US-fusion guided biopsies resulted in overall Gleason score upgrading was observed in 30 cases (26%) of the 114 cases identified by the standard 12-core biopsy method. Among those upgraded, 9 cases (30% of the 30 cases) were upgraded from no disease or clinically insignificant disease to clinically significant disease.

Table 3a: Additional Utility of targeted biopsy over 12-core biopsy alone, and of 12 -core biopsy over targeted biopsy alone			
	PCa Clinical Significance		
	Insignificant, No.	Significant, No.	Total
PCa Cases Diagnosed by 12 Core Biopsy	75	27	102
Additional PCa cases diagnosed by targeted biopsy	10	9	19
Additional Gleason upgrading within group	6	5	11
Total Gleason upgrading by targeted Biopsy	16	14	30
PCa Cases diagnosed by targeted biopsy	63	29	92
Additional PCa cases diagnosed by 12-Core biopsy	20	7	27
Additional Gleason upgrading within group	4	2	6
Total Gleason upgrading by standard biopsy	24	9	33

Gleason upgrading: GS 0 to GS≥6, GS 6 to GS ≥3+4, GS 3+4 to GS ≥4+3, GS 4+3 to GS≥8, GS8 to GS≥9

Contrary, the standard 12-core biopsy method resulted in Gleason score upgrading of 33 cases (28%) of the 114 cases identified by MRI/US-fusion guided biopsies. Among those upgraded, only 7 cases (21% of 33 cases) were upgraded to clinically significant disease.

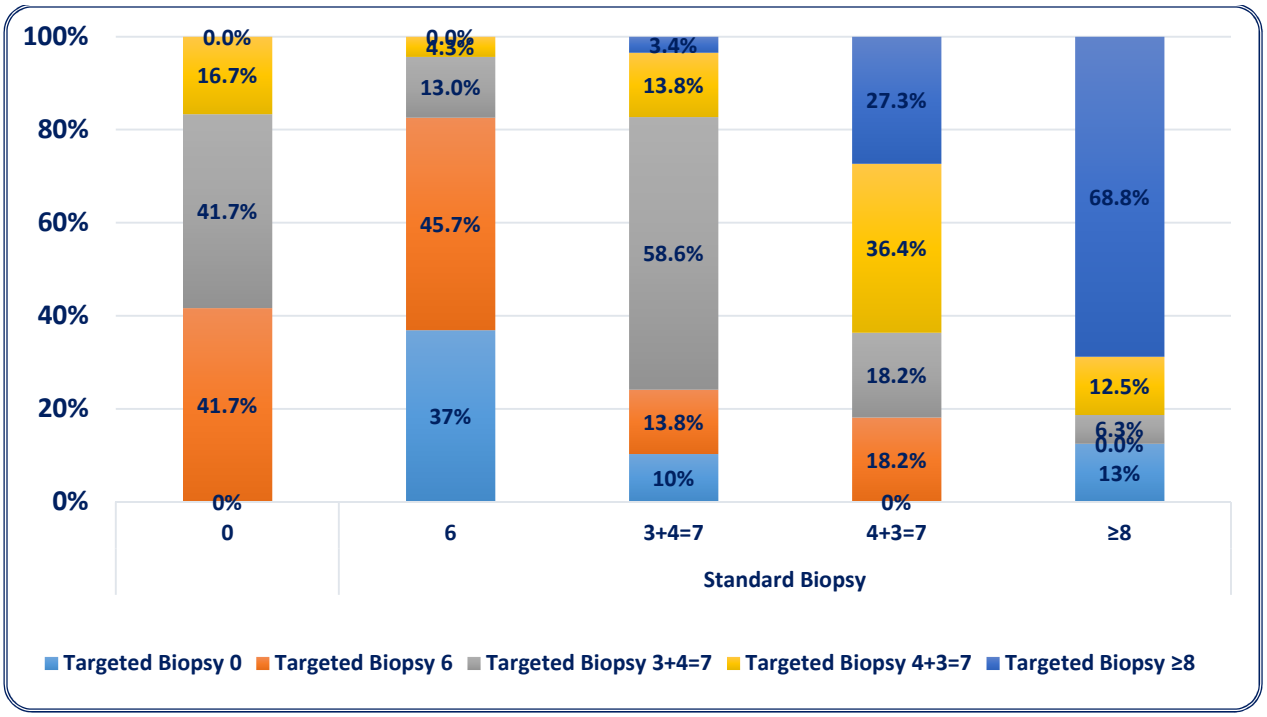


Figure 2: DISTRIBUTION OF GS ON TARGETED BIOPSY FOR STANDARD BIOPSY DIAGNOSIS

Data Table:

Standard Biopsy	Targeted Biopsy					
	0	**6**	**3+4=7**	**4+3=7**	**≥8**	**Total**
0	0	5	5	**2**	0	12
6	17	21	6	**2**	0	46
3+4=7	3	4	17	**4**	**1**	29
4+3=7	0	2	2	4	3	11
≥8	2	0	1	2	11	16
Total	22	32	31	14	15	114

Note: The Gleason Score, observed for each patient on Standard biopsy are compared with the Gleason score observed for the same patient on Targeted biopsy. The percentage disagreement of each biopsy Gleason score observed on Standard biopsy and Targeted biopsy is charted above.

A detailed distribution of each Gleason score observed on standard 12-core biopsy and the corresponding Gleason score identified for the same patient on MRI/US-fusion guided biopsy is presented in Figure 1 with a data table. It was noted that the standard

biopsy method completely missed 12 cases and two of those were found to be clinically significant.

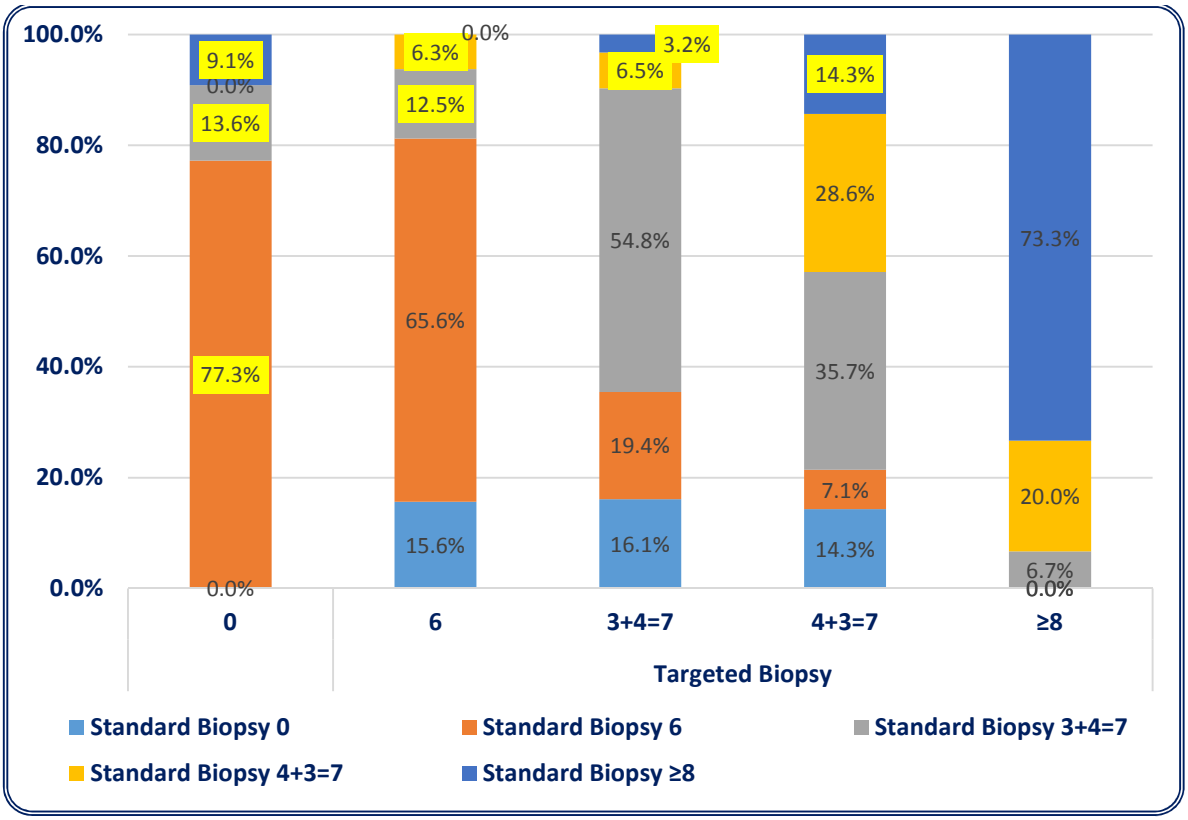


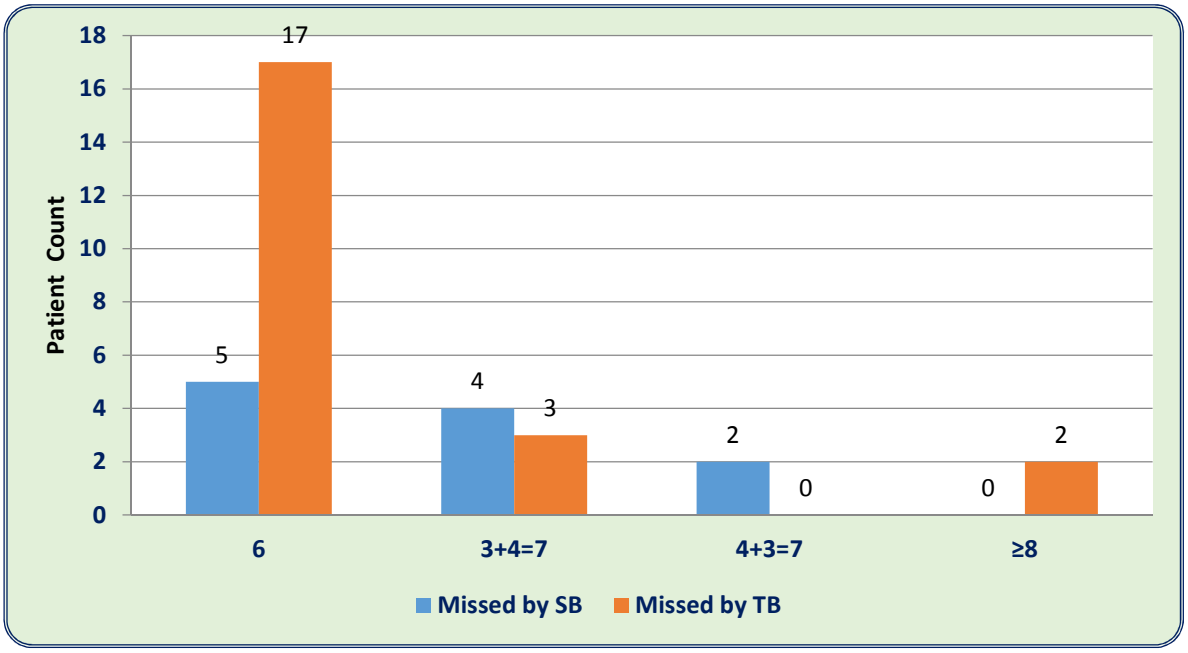
Figure 3: DISTRIBUTION OF GS ON STANDARD BIOPSIES FOR TARGETED BIOPSIES

Data Table:

Targeted Biopsy	Standard Biopsy					
	0	**6**	**3+4=7**	**4+3=7**	**≥8**	**Total**
0	0	17	3	0	**2**	22
6	5	21	4	**2**	0	32
3+4=7	5	6	17	**2**	**1**	31
4+3=7	2	1	5	4	2	14
≥8	0	0	1	3	11	15
Total	12	45	30	11	16	114

Note: The Gleason Score, observed for each patient on Targeted biopsy are compared with the Gleason score observed for the same patient on Standard biopsy. The percentage disagreement of each biopsy Gleason score observed on Targeted biopsy and Standard biopsy is charted above.

A detailed distribution of each Gleason score observed on the MRI/US-fusion guided biopsy and the corresponding Gleason score identified for the same patient on the standard 12-core biopsy is presented in Figure 2 with a data table. It was noted that no cancer was observed on targeted biopsy specimens for a total 22 patients, out of which 2 cases were found to have clinically significant disease on the standard 12-core biopsy.



Note: The number of cases which were reported as "No Cancer (negative biopsy results)" on Standard and Targeted biopsies are compared with the positive biopsy results (Gleason score) identified on Targeted and standard biopsies respectively.

Figure 4: Distribution of cases missed by 12-core biopsy and targeted biopsy

The distribution of the cases missed by 12-core biopsy and targeted biopsies are presented above. It was noted that MRI/US-fusion guided biopsy missed a total 17 cases of Gleason 6 disease, which were identified on the Standard 12-core biopsy. It also missed 3, 0 and 2 cases of a Gleason score 3+4=7, 4+3=7 and ≥8 respectively.

4.4 Identification of predictors of "Gleason Score Upgrading"

Potential predictors of Gleason score upgrading on targeted biopsy were assessed as referred in Table 4.

Table 4: Association of Patient Parameters with Gleason score upgrading on targeted biopsy over 12-core biopsy

	Univariate Analysis			Multivariate Analysis (N=167)		
	OR	95%CI	p Value	OR	95%CI	p Value
Age	1.08	1.02-1.13	**0.003**			
Age Above 65	5.82	1.95-17.35	**0.002**	5.14	1.41-18.68	**0.013**
PSA	1.09	1.02-1.16	**0.006**	--	--	--
PSA Above 10	4.36	1.90-10.00	**0.001**	2.60	0.94-7.19	0.065
MRI Suspicion Score	1.32	0.85-2.05	0.203	--	--	--
MRI Suspicion Score-5 (PI-RADS-5)	3.23	1.30-8.02	**0.011**	2.98	1.13-7.84	**0.026**
DRE	1.79	0.73-4.36	0.198	--	--	--
PCA3-QUANTITATIVE	1.06	1.01-1.10	**0.005**	--	--	--
PCA3-QUALITATIVE	17.35	1.99-151.181	**0.01**			
OPKO-4K	1.08	0.99-1.17	0.064	--	--	--

1. The odds ratio was calculated using Binary logistic regression method.
2. PCA3 was not included in the multivariate analysis due to absence of sufficient data.
3. Dichotomized variables were considered for developing multivariate analysis models.

Potential predictors of Gleason score upgrading on targeted biopsy were assessed. Using univariate analysis, Age, PSA, PI-RADS score 5 and PCA3 test results were found to have an association with Gleason score upgrading by MRI/US-fusion guided biopsy. The OPKO-4K score was approaching its significance on Gleason upgrading by MRI/US-fusion guided biopsy, however, it warrants more data to substantiate the claims. On multivariate

analysis, the age above 65 years, PSA above 10 and PI-RADS score 5 was found to remain associated with Gleason score upgrading.

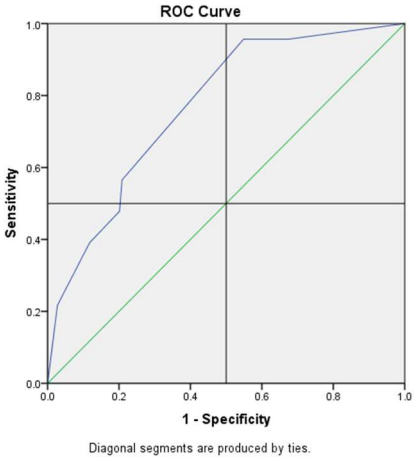


Figure 5: ROC analysis

Furthermore, the validity of this model was tested by a Receiver Operating Characteristics (ROC) curve, which is the plot of the sensitivity versus 1-specificity reporting Area Under the Curve (AUC) as a measure of accuracy. The model resulted in an AUC of 0.768 (95% CI: 0.672-0.864; p=<0.001).

5. DISCUSSION

There has been considerable concern regarding over-diagnosis and over-treatment of Prostate cancer patients who harbor indolent cancer, demanding better characterization of disease severity [5]. Identification and assessment of molecular markers not only help predict the pathological stage of the disease but has also been utilized for selection of therapeutic target and modalities per disease severity. We conducted a retrospective chart review and identified the association of biomarker results and the findings of prostate MRI, standard TRUS prostate biopsy and MRI-fusion guided biopsies. We also have identified the association of these biomarkers and findings of MRI on upgrading of the Gleason score derived from MRI/US-fusion-guided prostate-biopsy as compared to a standard 12-core TRUS biopsy alone.

The positive association of biomarkers and recent biopsy findings indicates the major decisive role of biomarkers in the prediction of biopsy outcomes, and even biopsy Gleason score up to some extent. The findings of the positive PCA3 association with clinically significant disease and a negative association with clinically insignificant disease indicates better predictability of disease severity with confidence. Poppel et al noted that higher PCA3 results were statistically and significantly higher in patients with aggressive disease [35]. However, the present study failed to identify clinically and statistically significant associations of OPKO-4K, Neogenomics test results and ConfirmMDx tests for their predictability of clinically meaningful outcomes of biopsy results. Hence, PCA3 can

be further investigated to select the patients who need to undergo biopsy and reduce the need for unnecessary biopsies.

The MRI findings have been studied well to precisely assess tumor aggressiveness before treatment. The MRI findings helped improve the selection of appropriate treatment for patients [21]. In a study of patients harboring low-risk prostate cancer, who were candidates for active surveillance in initial biopsy, the MRI lesions were a strong predictor of tumor upgrading on confirmatory biopsy. The Gleason score upgrading was observed in 20% (79 of 388) of the study population on confirmatory biopsy [21].

We also evaluated the association of MRI findings to biopsy outcomes. The results were found to be in congruence with the available literature. The PI-RADS score 3 was found to be helpful in deriving clinically and statistically meaningful outcomes for negative biopsy results. It was evident from the study results that the chances of having a negative biopsy outcome increases to 101% if the cancer suspicion score is reported as 3 (PI-RADS 3). Similarly, a positive and strong association of PI-RADS “score- 5” with positive biopsy outcomes and a negative association of PI-RADS score 5 with negative biopsy outcomes gives a strong indication for and great confidence in interpretation and predictability of disease prognosis.

There has been considerable concern regarding over-diagnosis and over-treatment of prostate cancer patients who harbor indolent cancer, demanding better

characterization of disease severity [5]. MRI/US-fusion-guided targeted biopsies offer a considerable advantage. A prospective clinical trial has demonstrated that MRI/US-fusion-guided targeted biopsies significantly improved cancer detection rates as compared to the 12-core TRUS standard biopsy alone [25]. Cohen et al conducted a clinical study with patients who had prior negative biopsies and persistent clinical suspicion. They identified prostate cancer in 37% of the patients using MRI/US-fusion-guided biopsy with a prior negative TRUS biopsies. Of those patients who were found to have a prostate cancer, 11% were found to have a high-grade Gleason score ≥8[32]. The study reported 38.9% pathological upgrading of Gleason score identified by MRI/US-fusion-guided biopsies. Siddiqui et al noted that targeted MRI/US-fusion guided biopsy increased detection of high-risk prostate cancer (30%) and decreased detection of low-risk prostate cancer (17% lower) [19].

In this study, use of targeted biopsy as an adjunct to the standard 12-core biopsy resulted in Gleason upgrading of 26% of the cases identified on the standard 12-core biopsy alone. Among those patient who were upgraded, we found that 30% of the patient were harboring clinically significant disease. We also assessed the utility of the standard 12-core biopsy in conjunction with the MRI/US-fusion-guided biopsies, and examined the rate of Gleason score upgrading by 12-core biopsy compared with the Gleason score identified by targeted biopsy only. The Standard 12-core TRUS biopsy resulted in Gleason upgrading of 28% of the cases, however, among those upgraded, 79% were found to have clinically insignificant disease that was completely missed on a

targeted biopsy. A larger number of patients were upgraded to clinically significant disease by MRI/US-fusion-guided biopsy compare to standard 12-core TRUS biopsy method and on the contrary, a large number of patients were upgraded to clinically insignificant disease by standard 12-core biopsy compared to MRI/US-fusion-guided biopsy. We also found that 22 patients (20 clinically insignificant diseases and 2 clinically significant disease) would have been found to have no cancer based solely on the targeted biopsy data. Since standard 12-core biopsies detected some tumors that were not picked-up by targeted biopsies which is consistent with previous published literatures, our current practice to perform targeted biopsy in addition to standard 12-core biopsy, rather than replacing the standard 12-core biopsy.

These results favor the use of the MRI/US-fusion-guided biopsy method in addition to the standard 12-core TRUS biopsy method in the diagnosis of clinically significant (GS ≥ 4+3) high-grade prostate cancer, without significantly adding to the diagnosis of low-grade (GS≤ 3+4), indolent prostate cancer. Our finding of the association of the high suspicion level on MP-MRI was a useful assessment for the fusion platform. Furthermore, the MRI/US-fusion-guided biopsy was able to upgrade more cases of clinically significant disease as compared to the standard 12-core TRUS guided biopsies, which picked up/upgraded a larger number of clinically insignificant disease, which may be undesirable result.

This raise a question of whether the current method of standard 12-core TRUS guided biopsy can be completely substituted by MRI/US-fusion-guided biopsies.

Although, it seems that MRI/US targeted biopsy could be the method of choice over standard 12-core TRUS biopsy, further research is needed to reach the conclusion of whether to perform only targeted biopsies. However, adding the fusion biopsy to the standard 12-core biopsy remains our current practice in most patients until the emergence of additional data.

The major limitation of the present study was the retrospective study design, which limited data availability for all patients and it was impossible to access to data for some patients which were regulated by UCIMC regulations as a matter of confidentiality. Absence of the biomarkers assessment for the majority of the patients was a limiting step in data analysis and was a reason to suspect that we fail to reach and derive a statistical multivariate model for predicting disease severity. The other limitation of the present study was an absence of confirmatory pathology through prostatectomy results for some patients.

Future studies could be conducted to include more cases with biomarker results and confirmatory disease pathological results in the analysis, to substantiate the claims and develop a predictive model.

6. CONCLUSION

The present study demonstrated an association of various biomarkers to recent biopsy results for patients with clinical suspicion or a history of prostate cancer. The study was able to derive statistically significant and clinically meaningful interpretations of the association of biomarkers and disease severity.

Furthermore, in this study we observed that PCA3 can be considered predict prostate cancer disease severity and select the patients who are likely to get benefit from prostate biopsy and spare the patients harboring indolent disease. Mp-MRI findings, especially, PI-RADS score 3 and PI-RADS score 5 can also be considered to predict the prostate cancer disease severity and thereby possible biopsy outcome. A We also observed 26% Gleason score upgrading with MRI/US-fusion-guided biopsy when compared with the standard 12-core TRUS biopsy alone. We also noticed that a majority of these upgraded cases resulted in clinically significant disease. The converse relationship was also examined for Gleason score upgrading through the standard 12-core biopsy as compared with Gleason score noted on MRI/US-fusion-guided targeted biopsy alone. We noticed that the converse scenario resulted in a 28% Gleason score upgrading rate as compared to the Gleason scores identified by targeted biopsy. However, it was interesting to note that a majority of the cases (79% of 28%) were upgraded to clinically insignificant disease.

We noticed that Age above 65, PSA above 10, and a very high MP-MRI suspicion level had a strong association with Gleason score upgrading by targeted biopsy over 12-core biopsy alone. MRI/US-fusion-guided biopsy missed more cases of less aggressive disease and fewer cases of highly aggressive disease, which highlights the potential usefulness of MRI/US-fusion-guided targeted biopsies for identification of high-risk (clinically significant-high grade) disease otherwise missed by the standard 12-core biopsy method alone.

7. REFERENCES

1. Ref: SEER Cancer Statistics Factsheets: Prostate Cancer. National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/statfacts/html/prost.html.
2. Catalona WJ, Richie JP, Ahmann FR, Hudson MA, Scardino PT, Flanigan RC, Dekernion JB, Ratliff TL, Kavoussi LR, Dalkin BL. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. The Journal of urology. 1994 May;151(5):1283-90.
3. Cary KC, Cooperberg MR. Biomarkers in prostate cancer surveillance and screening: past, present, and future. Therapeutic advances in urology. 2013 Dec 1;5(6):318-29.
4. Jones JS, Patel A, Schoenfield L, Rabets JC, Zippe CD, Magi-Galluzzi C. Saturation technique does not improve cancer detection as an initial prostate biopsy strategy. The Journal of urology. 2006 Feb 28;175(2):485-8.
5. Djavan BO, Ravery V, Zlotta A, Dobronski P, Dobrovits M, Fakhari M, Seitz C, Susani M, Borkowski A, Boccon-Gibod L, Schulman CC. Prospective evaluation of prostate cancer detected on biopsies 1, 2, 3 and 4: when should we stop? The Journal of urology. 2001 Nov 30;166(5):1679-83.
6. Loeb S, Vellekoop A, Ahmed HU, Catto J, Emberton M, Nam R, Rosario DJ, Scattoni V, Lotan Y. Systematic review of complications of prostate biopsy. European urology. 2013 Dec 31;64(6):876-92.
7. Loeb S, Bjurlin MA, Nicholson J, Tammela TL, Penson DF, Carter HB, Carroll P, Etzioni R. Overdiagnosis and overtreatment of prostate cancer. European urology. 2014 Jun 30;65(6):1046-55.
8. Cooperberg MR, Broering JM, Carroll PR. Time trends and local variation in primary treatment of localized prostate cancer. Journal of Clinical Oncology. 2010 Mar 1;28 (7):1117-23.
9. Miller DC, Sanda MG, Dunn RL, Montie JE, Pimentel H, Sandler HM, McLaughlin WP, Wei JT. Long-term outcomes among localized prostate cancer survivors: health-related quality-of-life changes after radical prostatectomy, external radiation, and brachytherapy. Journal of Clinical Oncology. 2005 Apr 20;23(12):2772-80.
10. Catalona WJ, Richie JP, Ahmann FR, Hudson MA, Scardino PT, Flanigan RC, Dekernion JB, Ratliff TL, Kavoussi LR, Dalkin BL. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. The Journal of urology. 1994 May;151(5):1283-90.
11. Auprich M, Chun FK, Ward JF, Pummer K, Babaian R, Augustin H, Luger F, Gutschi S, Budäus L, Fisch M, Huland H. Critical assessment of preoperative

urinary prostate cancer antigen 3 on the accuracy of prostate cancer staging. European urology. 2011 Jan 31; 59(1):96-105.

12. Carlsson S, Maschino A, Schröder F, Bangma C, Steyerberg EW, van der Kwast T, van Leenders G, Vickers A, Lilja H, Roobol MJ. Predictive value of four kallikrein markers for pathologically insignificant compared with aggressive prostate cancer in radical prostatectomy specimens: results from the European Randomized Study of Screening for Prostate Cancer section Rotterdam. European urology. 2013 Nov 30;64(5):693-9.
13. Stewart, Grant D., et al. "Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies: results of the MATLOC study." The Journal of urology 189.3 (2013): 1110-1116.
14. Cullen J, Rosner IL, Brand TC, Zhang N, Tsiatis AC, Moncur J, Ali A, Chen Y, Knezevic D, Maddala T, Lawrence HJ. A biopsy-based 17-gene genomic prostate score predicts recurrence after radical prostatectomy and adverse surgical pathology in a racially diverse population of men with clinically low-and intermediate-risk prostate cancer. European urology. 2015 Jul 31;68(1):123-31.
15. Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, Chan JM, Li J, Cowan JE, Tsiatis AC, Cherbavaz DB. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. European urology. 2014 Sep 30; 66 (3):550-60.
16. Cuzick J, Swanson GP, Fisher G, Brothman AR, Berney DM, Reid JE, Mesher D, Speights VO, Stankiewicz E, Foster CS, Møller H. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. The lancet oncology. 2011 Mar 31;12(3):245-55.
17. Cooperberg MR, Simko JP, Cowan JE, Reid JE, Djalilvand A, Bhatnagar S, Gutin A, Lanchbury JS, Swanson GP, Stone S, Carroll PR. Validation of a cell-cycle progression gene panel to improve risk stratification in a contemporary prostatectomy cohort. Journal of Clinical Oncology. 2013 Apr 10;31(11):1428-34.
18. Cary KC, Cooperberg MR. Biomarkers in prostate cancer surveillance and screening: past, present, and future. Therapeutic advances in urology. 2013 Dec 1;5(6):318-29.
19. Cohen MS, Hanley RS, Kurteva T, Ruthazer R, Silverman ML, Sorcini A, Hamawy K, Roth RA, Tuerk I, Libertino JA. Comparing the Gleason prostate biopsy and Gleason prostatectomy grading system: the Lahey Clinic Medical Center experience and an international meta-analysis. European urology. 2008 Aug 31;54 (2):371-81.
20. Puech P, Potiron E, Lemaitre L, Leroy X, Haber GP, Crouzet S, Kamoi K, Villers A. Dynamic contrast-enhanced–magnetic resonance imaging evaluation of intraprostatic prostate cancer: correlation with radical prostatectomy specimens. Urology. 2009 Nov 30;74(5):1094-9.

21. Moore CM, Robertson NL, Arsanious N, Middleton T, Villers A, Klotz L, Taneja SS, Emberton M. Image-guided prostate biopsy using magnetic resonance imaging–derived targets: a systematic review. European urology. 2013 Jan 31;63(1):125-40.
22. Moore CM, Robertson NL, Arsanious N, Middleton T, Villers A, Klotz L, Taneja SS, Emberton M. Image-guided prostate biopsy using magnetic resonance imaging–derived targets: a systematic review. European urology. 2013 Jan 31;63(1):125-40.
23. Siddiqui MM, Rais-Bahrami S, Turkbey B, George AK, Rothwax J, Shakir N, Okoro C, Raskolnikov D, Parnes HL, Linehan WM, Merino MJ. Comparison of MR/ultrasound fusion–guided biopsy with ultrasound-guided biopsy for the diagnosis of prostate cancer. Jama. 2015 Jan 27;313(4):390-7.
24. Schmid HP, Riesen W, Prikler L. Update on screening for prostate cancer with prostate-specific antigen. Critical reviews in oncology/hematology. 2004 Apr 30;50(1):71-8.

25. Cohen MS, Hanley RS, Kurteva T, Ruthazer R, Silverman ML, Sorcini A, Hamawy K, Roth RA, Tuerk I, Libertino JA. Comparing the Gleason prostate biopsy and Gleason prostatectomy grading system: the Lahey Clinic Medical Center experience and an international meta-analysis. European urology. 2008 Aug 31;54 (2):371-81.
26. Vargas HA, Akin O, Afaq A, Goldman D, Zheng J, Moskowitz CS, Shukla-Dave A, Eastham J, Scardino P, Hricak H. Magnetic resonance imaging for predicting prostate biopsy findings in patients considered for active surveillance of clinically low risk prostate cancer. The Journal of urology. 2012 Nov 30;188(5):1732-8.
27. Borofsky MS, Rosenkrantz AB, Abraham N, Jain R, Taneja SS. Does suspicion of prostate cancer on integrated T2 and diffusion-weighted MRI predict more adverse pathology on radical prostatectomy?. Urology. 2013 Jun 30;81(6):1279-83.
28. FERDA J, Kastner J, Hora M, HES O, FÍNEK J, TOPOLČAN O, Kreuzberg B. A role of multifactorial evaluation of prostatic 3T MRI in patients with elevated prostatic-specific antigen levels: prospective comparison with ultrasound-guided transrectal biopsy. Anticancer research. 2013 Jun 1;33(6):2791-5.
29. Siddiqui MM, Rais-Bahrami S, Truong H, Stamatakis L, Vourganti S, Nix J, Hoang AN, Walton-Diaz A, Shuch B, Weintraub M, Kruecker J. Magnetic resonance imaging/ultrasound–fusion biopsy significantly upgrades prostate cancer versus systematic 12-core transrectal ultrasound biopsy. European urology. 2013 Nov 30;64(5):713-9.
30. Pinto PA, Chung PH, Rastinehad AR, Baccala AA, Kruecker J, Benjamin CJ, Xu S, Yan P, Kadoury S, Chua C, Locklin JK. Magnetic resonance imaging/ultrasound fusion guided prostate biopsy improves cancer detection following transrectal

ultrasound biopsy and correlates with multiparametric magnetic resonance imaging. The Journal of urology. 2011 Oct 31;186(4):1281-5.

31. Vickers AJ, Brewster SF. PSA velocity and doubling time in diagnosis and prognosis of prostate cancer. British Journal of Medical and Surgical Urology. 2012 Jul 31;5(4):162-8.
32. Vourganti S, Rastinehad A, Yerram NK, Nix J, Volkin D, Hoang A, Turkbey B, Gupta GN, Kruecker J, Linehan WM, Choyke PL. Multiparametric magnetic resonance imaging and ultrasound fusion biopsy detect prostate cancer in patients with prior negative transrectal ultrasound biopsies. The Journal of urology. 2012 Dec 31;188(6):2152-7.
33. Moore CM, Kasivisvanathan V, Eggener S, Emberton M, Fütterer JJ, Gill IS, Grubb III RL, Hadaschik B, Klotz L, Margolis DJ, Marks LS. Standards of reporting for MRI-targeted biopsy studies (START) of the prostate: recommendations from an International Working Group. European urology. 2013 Oct 31;64(4):544-52.
34. Stewart, Grant D., et al. "Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies: results of the MATLOC study." The Journal of urology 189.3 (2013): 1110-1116.
35. Epstein JI, Allsbrook Jr WC, Amin MB, Egevad LL, ISUP Grading Committee. The 2005 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma. The American journal of surgical pathology. 2005 Sep 1;29(9):1228-42.
36. Wibmer AG, Vargas HA, Hricak H. Role of MRI in the diagnosis and management of prostate cancer. Future Oncology. 2015 Oct;11(20):2757-66.
37. van Poppel H, Haese A, Graefen M, de la Taille A, Irani J, de Reijke T, Remzi M, Marberger M. The relationship between Prostate CAncer gene 3 (PCA3) and prostate cancer significance. BJU international. 2012 Feb 1;109(3):360-6.

8. APPENDICES

8.1. Protocol Narrative

8.2. Case Report Form

8.3. IRB Approval