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Prostate Cancer: A Review of Current Prostate Cancer Markers and a Novel Model of Organization

CONTENTS

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INTRODUCTION

Accurately diagnosing clinically significant prostate cancer remains a challenge, especially when relying on the current "gold standard:" prostate needle biopsy, DRE, and serum PSA. There have been a number of recent additions to our armamentarium to aid in the identification of localized high-risk prostate cancer. Ideally, limiting biopsies to men identified at risk of high grade disease should be the goal of these tests. At the same time, markers are needed to aid in verifying risk stratification of men diagnosed with a low risk disease. Several risk stratification nomograms based on PSA, Gleason score, and DRE exist, however many men predicted to have low risk disease based on these nomograms are upgraded to higher risk at radical prostatectomy (1). Here, we provide a concise and thorough review of available biomarkers and genomic tests developed to address these issues. Additionally, we provide a discussion of the challenges, implementation, and costs associated with these tests. Finally, we propose an algorithm incorporating the latest data for clinical use.

DEFINING THE CHALLENGE: TO SCREEN OR NOT TO SCREEN?

Prostate cancer diagnosis and staging remains a diagnostic dilemma due to a variety of factors. First, the disease itself is in an anatomically difficult location, with the prostate wedged at the base of the pelvis. Examining or palpating the prostate by digital rectal exam (DRE) is highly provider dependent, and based on experience, as well as patient

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Many indicators and tests tell you where you are. Only Decipher® tells where you will go, and when.

If your patient has prostate cancer there are three things about the Decipher test you should know.

First, Decipher is a new and proven genomic test, developed by analyzing the entire genome of prostate tumors. Decipher has repeatedly demonstrated that it can predict the risk of metastasis more accurately than both traditional diagnostic tools and existing genomic tests.[1, 2, 3]

Second, Decipher can be used after a biopsy diagnosed with prostate cancer and/or after radical prostatectomy. Decipher Biopsy can help guide whether the right path for your patient is active surveillance, definitive local therapy alone (surgery or radiation), or intensification with multi-modal therapy.[1] Decipher Post-Op can help determine if and when radiation is likely to decrease the chance of metastasis after surgery.[4, 5]

Third, the company behind Decipher, GenomeDx, is a leader in prostate cancer testing and is totally committed to helping patients manage their cancer treatment through increasingly accurate, individualized information.

So get the accurate, personal information you need to guide your patient's next step. For immediate information, visit us at DecipherTest.com.

1. Klein, E.A. et al., Decipher Genomic Classifier Measured on Prostate Biopsy Predicts Metastasis Risk. Urology. 2016. Apr;90:148-52. pii: S0090-4295(16)00069-8. doi: 10.1016/j.urology.2016.01.012. **2.** Karnes, R.J. et al., Validation of a Genomic Classifier that Predicts Metastasis Following Radical Prostatectomy in an At Risk Patient Population. The Journal of Urology 2013; 190:2047-2053. **3.** Erho, N. et al.,Discovery and Validation of a Prostate Cancer Genomic Classifier that Predicts Early Metastasis Following Radical Prostatectomy. PLOS ONE 2013; 8(6):e66855. **4.** Den, R.B. et al., A genomic classifier identifies men with adverse pathology after radical prostatectomy who benefit from adjuvant radiation therapy. Journal of Clinical Oncology 2015; 33:944-951. **5.** Freedland S.J. et al., Utilization of a genomic classifier for prediction of metastasis following salvage radiation therapy after radical prostatectomy. European Urology. 2016; pii: S0302-2838(16)00059-2. doi: 10.1016/j.eururo.2016.01.008

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comfort with the exam. Also, the finding of an "abnormality" on exam provides little information regarding grade or stage of disease. Second, the PSA test itself can be highly variable and subject to fluctuations based on various patient activities, including prostate massage and ejaculation. It is commonly elevated in benign conditions, such as BPH and prostatitis. The United States Preventative Task Force (USPTF) issued a grade D recommendation regarding PSA for prostate cancer screening (2), further complicating the picture. In short, they recommended that physicians should not pursue PSA screening unless they are prepared to engage in shared decision making to enable an informed choice by patients, and that the harms of prostate biopsy outweigh potentially finding an aggressive prostate cancer. The American Urological Association (AUA), along similar lines, recommends shared decision making taking into account a patient's preferences when deciding on PSA monitoring (3). Third, various specialties screen for prostate cancer and interpret results of PSA tests, including internal medicine, family medicine, and urology providers. In fact, the majority of the PSA tests ordered (over 90%) in the United States are ordered by internal medicine doctors and family practitioners, likely reflecting confusion over the multitude of guidelines and opinions on CaP screening in current literature (4).

Given these challenges, there has been a large push to develop new diagnostic tests, algorithms, and paradigms to inform prostate cancer risk stratification and guide screening and treatment recommendations. Much of the recent literature has been aimed at uncovering tools that might help select patients whose disease will be more aggressive and benefit from biopsy and subsequent treatment if necessary, including refining the use of the PSA, as well as development of novel prostate cancer markers (PCMs). The ultimate goal in searching for new diagnostics is a reduced number of unnecessary biopsies not just by better distinguishing benign from malignant disease but by identifying men harboring high risk disease (i.e. men harboring ≥ Gleason 4 pattern disease).

PROSTATE CANCER MARKERS

Prostate cancer markers (PCMs), like all biomarkers, are molecules that can be found in blood, tissue or body fluids that are a sign of a normal or abnormal process (5). Both older and novel techniques have been evaluated and explored, including making the PSA test more useful (PSA doubling time, velocity, etc), as well as exploring new fields, such as proteomics. Proteomics takes genomics a step further by evaluating the cell's actual proteins and their function, thus better demonstrating cellular activity (6). Given the rapid advancement of the various subsects within the biomarker development arena, there understandably remains a great deal of confusion regarding these tests, and when to use them. Here, we hope to clarify the nature and role of these biomarkers.

MODEL OF ORGANIZATION

In deriving a model by which to organize and clarify PCMs, several models have been created. Crawford et al have developed the previous "bucket model:" patients are placed into various "buckets," designated as "whom to biopsy?", "whom to rebiopsy?", and "what is the disease aggressiveness in the setting of a positive biopsy or after intervention?" (Fig. 1). Then, various PCMs can be utilized for patients placed in each bucket. For example, one can use the prostate health index (phi) to determine "whom to biopsy." The bucket model can also be combined into a treatment algorithm (Fig. 2), which incorporates PSA cutoffs to help determine when a PCP should refer to urology, as well as which PCMs are useful at various stages of evaluation.

MARKERS TO AID IN DECIDING WHOM TO BIOPSY (TABLE 1)

PSA

Arguably the most utilized marker of prostate disease, prostate specific antigen (PSA), is currently used as a screening test as well as a monitor of response to treatment. Prior the USPTF recommendations against PSA screening, close to 19 million men in 2013 underwent PSA screening tests, leading to over 240,000 new diagnoses of prostate cancer that year (7). 2016 data project approximately 181,000 new cases, likely reflecting decreased screening (8). Despite PSA being specific to the prostate, using PSA as a biomarker of CaP is controversial at this time, especially given that benign diseases such as BPH can cause elevations in PSA (9). Most men with an "elevated PSA" do not have prostate cancer. In fact, of the men biopsied secondary to elevated PSA, only about 25% have a prostate cancer, most of which are low risk (10). PSA is made by prostatic cells, so in the setting of post-prostatectomy follow up, it is useful to determine the adequacy of treatment as well as possible local recurrence or metastatic disease. Many studies have attempted to answer the question: should men be regularly screened for prostate cancer? The large PLCO screening trial found no survival benefit to screening (11), however a large European trial with eleven years of follow up did find a survival benefit (12), as did smaller studies of men with no comorbidities (13). While much of the recent literature has focused on whether or not to screen, other

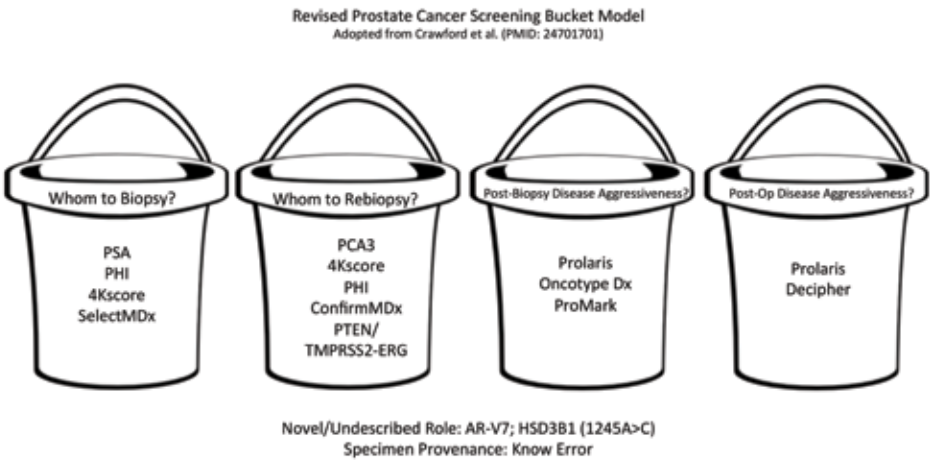

Figure 1. The Prostate Cancer Screening Bucket Model
Patients are evaluated and placed into a certain "bucket." Based off this, a test can be chosen based on patient preference, cost, and specific needs in clinical decision making. The model can be further incorporated in a clinical decision flow pathway. Bucket Clipart: clker.com

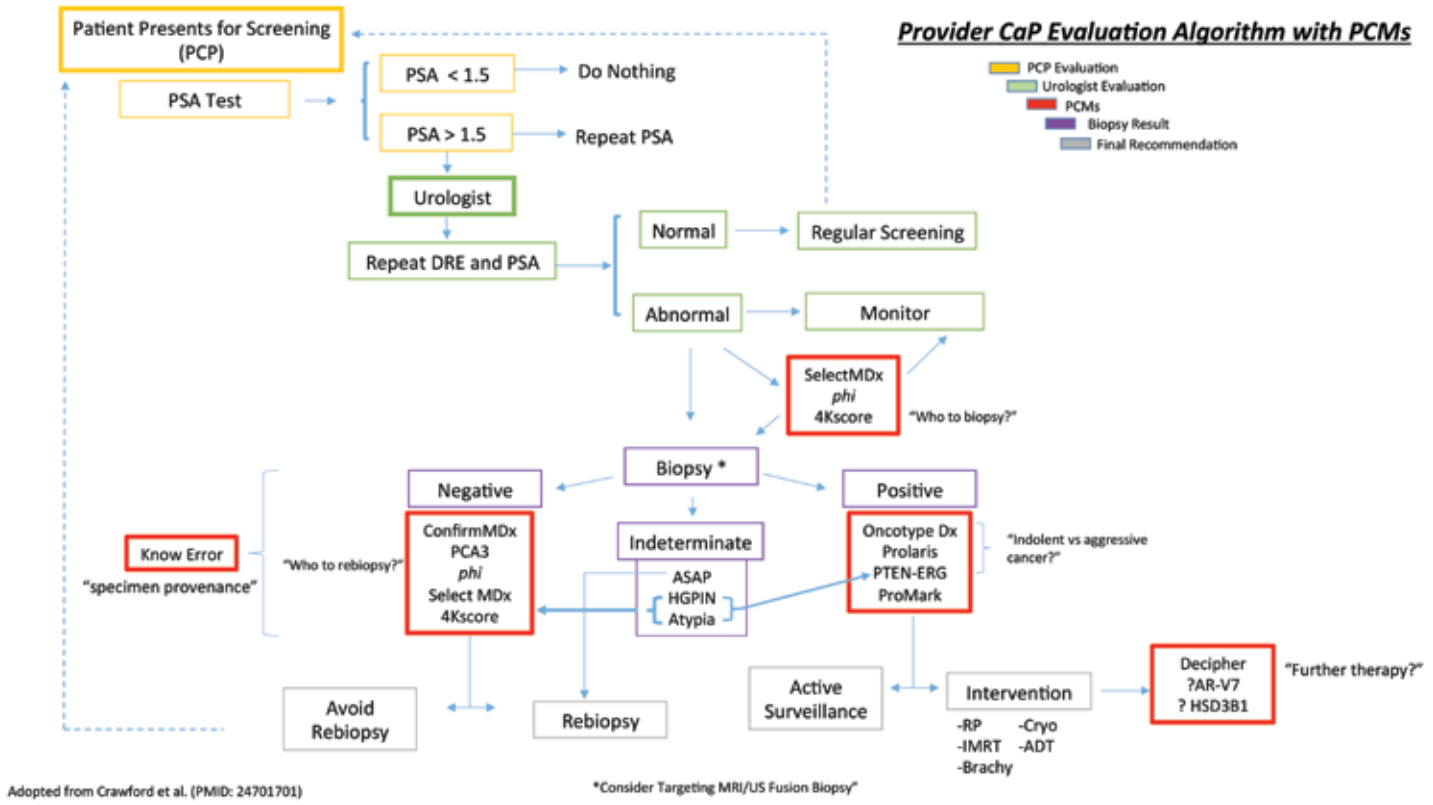

Figure 2. Patient presents to Family Practitioner for routine visit
PSA is obtained with other routine laboratory tests. If PSA is less than 1.5 ng/ml, patient enters the follow-up pool and has a repeat PSA in 5 years. If PSA greater than 1.5 ng/ml informed decision is obtained and consideration of referral to urologist. Urologists will evaluate for possible causes including BPH, prostatitis, and prostate cancer. If there is concern for prostate cancer, then a genomic test such as phi, SelectMDx, 4K, or PCA3 is performed to identify men who are at risk of a significant cancer. If low risk return to Family Practitioner for repeat in one year. If high risk consider TRUS biopsy.

authorities have evaluated how to utilize the PSA test better. Crawford and colleagues evaluated men over 40 years of age with PSA levels between 1.5-4.0 ng/mL, which they deemed the "Early-Warning" PSA zone, and found patients with PSA greater than or equal to 1.5 have an increased risk of developing CaP (14). This study, which included 21,000 men in the Henry Ford System, 29% of them African American, had follow up out at a minimum of 5 years for all patients. Extrapolating this data, a cutoff of 1.5 ng/mL when checking PSA should trigger concern and either evaluation by urologist and/or additional testing (Fig. 2). Further efforts on improving the performance of the PSA have come in a variety of new tests, including the phi, mentioned below. PSA density, velocity, and doubling time have also been used in an effort to improve the use of PSA.

Prostate Health Index (phi)

The Prostate Health Index, or phi, is a test that was designed to fill in the "diagnostic gap" between obtaining a serum PSA test and deciding on observation vs biopsy based on those results. Specifically, phi was approved by the US FDA in 2012 to improve the early detection of prostate cancer in men with PSA in 4-10 ng/mL range. This test takes advantage of the PSA isoform [-2]proPSA, which has been found to be associated with prostate cancer (15). When combining this isoform with both total and free PSA in an algorithm found the Beckman Coulter Access instrument, the phi is thus calculated. A higher number on the phi correlates with a higher probability of CaP, with a phi score of greater than 55 being associated with a 52.1% chance of malignancy (16). Catalona et al. found that the phi was approximately three times more specific than PSA for detection of prostate cancer (17). The phi can also help predict aggressive prostate cancer (GS ≥7), however data supporting it use in this regard is limited (18). The NCCN incorporates the phi into early detection guidelines as a reflex test for both initial biopsy or repeat biopsy (19). As such, its clinical utility is broad and applicable to many patients.

4Kscore

The 4Kscore (OPKO Health, Inc.) takes advantage of our current understanding of human Kallikreins, and is designed to identify men at risk for aggressive prostate cancer. Kallikreins are a collective group of serine proteases, and are involved in various body processes, including semen liquefaction in the case of PSA (human Kallikrein 3). Specifically, human Kallikrein 2 (hK2) has received much attention, as it been found to be highly expressed in high grade prostate cancer (20,21). The 4Kscore is an algorithm that combines results obtained from a serum sample of total PSA, free PSA, intact PSA, hK2, and combines it with patient biopsy history, age, and DRE results (22). 4Kscore is the probability of an individual's percent risk of having aggressive, high grade Gleason 7 or higher prostate cancer if a bi-

Table 1. Markers to Aid in Deciding Whom to Biopsy

TEST	INDICATION	SCIENCE	PERFORMANCE	RESULTS	COST
Prostate Health Index Beckman Coulter	Blood based test for patients with a PSA between 2-10ng/ ml (4-10ng/ ml USFDA) • Reduces negative biopsies • Calculates risk of finding prostate cancer on biopsy	FDA approved index of proteins in the blood that combines the concentrations of PSA, freePSA and pro2PSA in a formula that produces the PHI Score, which has three time the specificity for prostate cancer compared to PSA alone.	AUC 0.73	• PHI results fall into four categories of risk that prostate cancer may be found on biopsy.	• $499 • Prostatehealthindex.us
4Kscore® OPKO	Indicated for men with an abnormal PSA or DRE as a reflex blood test combined with clinical information to determine an individual patient's risk for high-grade prostate cancer on biopsy • For patients who are considering a first biopsy or who have had a prior negative biopsy vs HGPIN on first biopsy	Laboratory test measuring 4 kallikreins in the blood (Total PSA, Free PSA, Intact PSA and hK2) and combined with the patient's clinical information in an algorithm to predict the individual patient's risk of having high-grade prostate cancer versus indolent or no cancer.	AUC 0.82	• 4K results provide percent probability (positive predictive value) of an individual patient's risk of having high-grade prostate cancer on biopsy.	• $595 • Clinical.opko.com • Patient support program available through OPKO
SelectMDx™ MDxHealth	Post DRE first void urine-based test • For patients who are considering first time biopsy with concerning clinical risk factors • Can reduce number of negative biopsies but selecting those with higher likelihood of cancer on biopsy	Uses qPCR, detects KLK3, HOXC6 and DLX1 mRNA biomarkers to predict likelihood for prostate cancer upon biopsy, as well as likelihood of low grade prostate cancer vs high grade prostate cancer.	AUC 0.90	• Percent likelihood of cancer on biopsy • Percent likelihood of high grade cancer • Percent likelihood of low grade cancer	• $500 • Client.Services@MDxHealth.com
Prostate Specific Antigen (PSA)	Obtained in men who, after shared decision making, can screen for potential prostate pathology. Part of the kallikrein family. Organ specific to the prostate, but no disease specific. Useful as makrer of therapeutic response post intervention (i.e. prostatectomy follow up)	Blood test, detects prostate pathology. Can be elevated in benign disease such as BPH or prostatitis. Functions in semen liquefaction.	AUC 0.69	• ng/mL • PSA levels of >1.5 should trigger evaluation by Urologist • PSA levels of 1-4 considered "danger zone"	• $50.00

*Area Under the Curve (AUC) shown without comparator

opsy were to be performed. Per the NCCN, the 4Kscore is useful prior to first biopsy, a first biopsy with focal high grade prostatic intraepithelial neoplasia (HGPIN), or in the setting of a negative first biopsy with persistent clinical concern (19). A low risk result is a 4Kscore of less than 7.5%, with high risk scores being greater than or equal to 20%. Those with intermediate or high risk 4Kscores should be referred to urology for evaluation, as there was a 5.6% chance of CaP distant metastasis at 10 years if left untreated. Low risk scores are useful in that roughly 99% of men studied with PSA >2ng/mL with these scores will not have metastatic prostate cancer at 10 years. As such, men with low risk scores can avoid immediate biopsy, and can be safely followed as they are at very low risk of adverse outcomes 10-20 years later (23). In comparison to other tests, including total PSA and PCA3, the 4kscore outperformed them in predicting aggressive prostate cancers (GS >7) on prostate biopsy when analyzing the AUC for each test (24,25) and it has also been extensively validated (24). The 4Kscore can significantly influence physicians and patients in shared decision making. Konety et al demonstrated both reductions in the number of prostate biopsies being formed, while increasing the probability of detecting aggressive cancers when incorporating the use of the 4Kscore test as a reflex to abnormal DRE and/or PSA levels (26).

SelectMDx

SelectMDx (MDxHealth) is a new urine based molecular test that utilizes a reverse-transcriptase PCR assay based algorithm to determine the likelihood of high grade vs. low grade prostate cancer when considering biopsy in patients with risk factors. Interestingly, this test was developed by the investigators of the PCA3 test. SelectMDx is a useful test in determining disease prognostication, as it provides results of probabilities of both low grade and high grade disease on biopsy. The test combines the biomarkers KLK3, HOXC6 and DLX1 mRNA from urinary sediment with PSA density and DRE (27). HOXC6 and DLX1 are both genes found previously to be expressed in high grade prostate cancer, and have been shown to predict GS >7 on prostate biopsy (28). The SelectMDx test performed well in validation studies, with an AUC of up to 0.9. Validation studies also suggested that when a NPV cut off of 98% was used for high risk prostate cancer, a greater than 50% reduction in unnecessary biopsies could be obtained using the SelectMDx methodology (27). A 'low risk' result lends a 98% probability that there is no high grade cancer present. Taken together, the SelectMDx test results provide useful data in terms of percent probability of either low risk vs high risk disease on biopsy, making shared decision making discussions more objective and ultimately more useful for the patient.

MARKERS TO AID IN DECIDING WHOM TO REBIOPSY (TABLE 2)

4Kscore

As mentioned above, the 4Kscore is also included in the NCCN guidelines for determining which men to rebiopsy (19). Given its ability to predict probability of low vs high risk cancers, it may help men and their physicians in deciding the need for repeat biopsy based on the patient's specific probability of low vs high risk disease on subsequent repeat biopsy.

PCA3

Another test taking advantage of advancements in molecular biology is the PCA3 test. Prostate Cancer Gene 3 (PCA3), formerly referred to as DD3 after its discovery in the late 1990's (29), is a gene that is highly overexpressed in prostate cancer cells

Table 2. Markers to Aid in Deciding Whom to Rebiopsy

TEST	INDICATION	SCIENCE	PERFORMANCE	RESULTS	COST
ConfirmMDx® MDxHealth	Biopsy tissue based test for patients who are repeat biopsy candidates • Provides risk stratification on decision for repeat biopsy • Eligibility: Patients with a prior negative or HGPIN biopsy result in past 24 months	Three-gene methylation assay to detect an epigenetic field effect associated with the cancerization process at the DNA level.	NPV 96%	• Negative ConfirmMDx result: Avoid repeat biopsy and monitor with routine screening. • Positive ConfirmMDx result: Suspicious areas marked as positive providing repeat biopsy guidance on a prostate map.	• $3,300 • Mdxhealth.com
Progensa® PCA3 Assay Hologic, Inc.	Urine-based test, post DRE, which adds useful info when PSA or DRE is inconclusive • For patients who are considering first or repeat biopsy • FDA approved for use in conjunction with other patient info to aid in the decision for repeat biopsy in men ≥ 50 years	Test detects PCA3 gene that is highly specific for prostate cancer. Measures concentration of prostate cancer gene3(PCA3) and prostate specific antigen (PSA) RNA in post-DRE urine and calculates ratio of PCA3 molecules to PSA molecules to produce the PCA3 score.	NPV 90%	• As the PCA3 score increases, the likelihood for positive biopsy increases. As the PCA3 score decreases, the likelihood for a positive biopsy decreases • The greatest diagnostic utility occurs at a cutoff score of 35	• $300-500 • Pca3.org
4Kscore® OPKO	Indicated for men with an abnormal PSA or DRE as a reflex blood test combined with clinical information to determine an individual patient's risk for high-grade prostate cancer on biopsy • For patients who are considering a first biopsy or who have had a prior negative biopsy vs HGPIN on first biopsy	Laboratory test measuring 4 kallikreins in the blood (Total PSA, Free PSA, Intact PSA and hK2) and combined with the patient's clinical information in an algorithm to predict the individual patient's risk of having high-grade prostate cancer versus indolent or no cancer.	AUC 0.82	• 4K results provide percent probability (positive predictive value) of an individual patient's risk of having high-grade prostate cancer on biopsy.	• $595 • Clinical.opko.com • Patient support program available through OPKO
PTEN/ TMPRSS2-ERG (PTEN/ ERG) Metamark	Bopsy tissue based test. Useful in men with G6, G3+4=7, HGPIN or Atypia on initial biopsy to help determine need for rebiopsy based on risk of aggressiveness of CaP (based on risk of biochemical recurrence).	PTEN tumor suppressor gene deletion associated with aggressive CaP. T2-ERG fusion protein found in 40-80% of prostate cancers, with method of fusion (i.e. deletion vs insertion of intervening genes) determining aggressiveness prediction. FISH used to detect T2-ERG fusion, as well as PTEN presence	HR of 2.49 for biochemical failure if + for PTEN deletion and T2:ERG fusion present.	• 1) PTEN deletion negative, T2:ERG fusion negative • 2) PTEN deletion positive, T2:ERG fusion negative • 3) PTEN deletion negative, T2:ERG fusion positive • 4) PTEN deletion positive, T2:ERG fusion positive • Progressively worse biochemical recurrence rates going 1 → 4. As such, PTEN:ERG +/+ associated with aggressive CaP disease. Consider re-biopsy.	• Variable cost • Metamarkgenetics.com

compared to normal prostate tissue (30). PCA3 is the first gene based test specific to prostate cancer, offered by Hologic, Inc., and marketed as Progensa PCA3 assay in the USA. Using a post digital rectal exam urine sample, the specimen is analyzed for both PCA3 mRNA, as well as PSA mRNA to generate a PCA3 score (PCA3 Score=PCA3 mRNA/PSA mRNA x 10-3). With an increasing PCA3 score, there is a greater probability of positive biopsy on needle biopsy of the prostate, with a PCA3 score of 35 being used as the recommended diagnostic cutoff. Unlike PSA, PCA3 is not affected by prostate volume (31). It has regulatory approval for use in patients who are suspected of having CaP based off of PSA alone or in addition to a suspicious DRE, who have had a previous negative needle biopsy of the prostate. Specifically, those who have had previous negative biopsies in the past were found to have a 58% sensitivity and 72% specificity for positive 2nd biopsy when using a PCA3 score cutoff of 35 (32). Modern use of the PCA3 includes its incorporation with other testing modalities. For example, when coupled with MRI, the PCA3 score had a positive predictive value of 91.66%, with a negative predictive value of 96% in one series (33). Overall, the PCA3 score is useful in determining need for repeat biopsy in the setting of a negative first time biopsy, and in conjunction with other tests and/or nomograms. Still, the PCA3 is not itself capable of detecting GS ≥ 7, unlike other tests that are available such as SelectMDx or the 4k score.

ConfirmMDx

ConfirmMDx is also available through MDxHealth, and provides a "second look" for patients with a negative previous biopsy. ConfirmMDx applies advancements in epigenetics to interrogate possible abnormal DNA methylation changes associated with "field effects" around prostate cancer tumors. The "field effect," first described in the 1950's by Slaughter er al (34), is DNA methylation change that occurs secondary to the cancer process itself in histologically normal appearing tissues adjacent to cancer foci. Importantly, identifying field effect can help identify falsely negative pathologic results. It is thought that less than <1% of all prostate tissue is biopsied on standard sextant TRUS biopsy of the prostate, lending to a 20-30% false negative rate (35). Given this, ConfirmMDx was designed in part to evaluate tissues that are seemingly histologically normal under the microscope for abnormal changes at the DNA level (i.e., methylation). In a sense, the test helps to "expand the circumference" of the original biopsy specimen. The test uses a quantitative PCR assay to identify abnormally methylated genes from tissue that has undergone epigenetic changes due to its proximity to CaP cells. Specifically, the test evaluates GSTP1, APC, and RASSF1 gene array, with GSTP1 the most widely studied epigenetic biomarker currently (36–39). The ConfirmMDx test has been validated through numerous studies. The Europe-based MATLOC study highlighted the test's ability to confirm the absence of cancer in histopathologically negative biopsy cores, demonstrating a NPV of 90% for all risk cancers. This is in comparison to histopathology alone, which has a NPV of 65-75% (40). Additionally, the multi-institutional USA-based DOCUMENT study revealed that ConfirmMDx was the most significant predictor of prostate cancer detection on repeat biopsy when compared to age, PSA, and HGPIN vs Atypia on initial biopsy as independent predictors of prostate cancer of repeat biopsy (41). Recent refinements

of the analytics used in ConfirmMDx have led to the creation of the EpiScore, which analyzes the intensity of DNA methylation to further stratify methylation-positive men into high vs low risk, with an associated 96% NPV for high risk disease (42). Ongoing studies (the PASCUAL study, Neal Shore, expected 2017) are currently being performed in an effort to verify previous data suggesting that ConfirmMDx actually changes physician ordering patterns in clinical practice and reduces repeat biopsies (43). In short, the test is useful in patients with a negative biopsy, being considered for repeat biopsy based on clinical factors.

TMPRSS2-ERG

The TMPRSS2-ERG protein variant is found in 40-80% of prostate cancer, making it the dominant genetic variant. TMPRSS2-ERG is a fusion protein of the transmembrane protease serine 2 gene and the v-ets erythoblastosis virus E26 oncogene homolog gene. Analysis is done on post DRE urine sediment, and the quantitative results are taken into account along with PSA density, Gleason score, tumor density, and number of positive cores. Together, these are known as the Epstein criteria, and can be predictive of clinically significant prostate cancer (44). The results of this test can also be combined with the PSA and PCA3, and is available through the University of Michigan as the MiPS test. The MiPS test has a sensitivity of 80% and specificity of 90% for detection of prostate cancer (GS >6) on subsequent diagnostic needle biopsy (45), helping to reduce unnecessary biopsies.

PTEN

PTEN (Phosphatase and Tensin) is a tumor suppressor gene, that when deleted, portends a poor prognosis in prostate cancer patients. The test is performed via a FISH assay on prostate biopsy tissue, and can determine hemizygous vs homozygous gene deletion. There is a large body of literature showing deletion of the PTEN gene can lead to all of the following: higher Gleason grade, cancer progression, post treatment recurrence, advanced local disease as well as metastatic disease, and death (46). It is useful in determining risk of cancer progression is patients initially deemed low risk, and whether or not treatment vs observation may be appropriate. It can also help in determining need for rebiopsy, especially in the setting of atypia or HGPIN (47). Interestingly, PTEN/TMPRSS2:ERG combination tests have been commercialized, with improved determination of the risk of disease progression. This can help in determining the need for immediate re-biopsy vs monitoring (48).

MARKERS TO AID IN PREDICTING DISEASE AGGRESSIVENESS AND TREATMENT DISCUSSIONS (TABLE 3)

Know Error

Prior to treating a patient, the provenance of the patient's biopsy sample must be assured. The Know Error system was created to address the issue of biopsy or biomarker contamination, and avoid providing an incorrect diagnosis to the patient. Occult specimen swapping or contamination with another patient's specimen is collectively known as Specimen Provenance Complications (SPCs), and occur at rates as high as 3-5%, leading to possible patient and physician angst and harm (49). Specimen provenance testing assays confirm the tissue being analyzed is actually the index patient's tissue. Know Error uses a bar coding and a comparative buccal swab-to-biopsy DNA specimen provenance assay to ensure that biopsy and/or biomarker tests are free of contamination. Specimen provenance complications of prostate biopsies were found to occur on average in 1 of every 200 prostate biopsies in a recent series, with an estimated $880 million spent on extraneous healthcare and medical-legal costs (50). Utilizing this type of testing can be useful if the clinical picture does not correlate with the biomarker or biopsy data. Although it is not found in every laboratory, it has potential to become standard of care.

Utilizing this type of testing can be useful if the clinical picture does not correlate with the biomarker or biopsy data.

Oncotype Dx

Oncotype Dx Prostate Cancer Assay, available through Genomic Health Inc., utilizes advancements in RT-PCR assay development to better understand a patient's tumor biology. Oncotype Dx is available also for other cancers, such as colon and breast, with much the analytical validation for the prostate cancer assay based off of these previous assays (51). Oncotype Dx for prostate cancer is able to analyze genes from paraffin embedded prostate biopsy needle specimens, and requires only 1mm of prostate tissue to perform the analysis. The test targets four distinct cellular pathways to analyze 12 target genes thought to be related to prostate cancerization. When combined with 5 reference genes, an algorithm is used to ultimately generate a GPS, or Genomic Prostate Score, ranging from 0-100. Higher scores suggest more aggressive disease (51). The power of this test comes in its optimization and validation, which has shown it to be a more significant predictor of cancer aggressiveness (high grade or pT3 disease) vs standard clinical predictors found in current prostate cancer nomograms, and relies on very small quantities of tissue to do this (51–53). Development and gene selection studies directly addressed the issues of tumor heterogeneity and multi-focality as well as tumor under-sampling. The validation studies were performed through multiple institutions and cohorts of racially diverse men with varying degrees of low to intermediate risk prostate cancer, with both prostate biopsies and RP specimens used in design and validation of the test. This test is ideal for men newly diagnosed with early stage clinically low risk prostate cancer who, during shared decision making discussions, are attempting to decide between early intervention vs active surveillance (51). The GPS score combined with NCCN risk provides a likelihood of favorable disease within the prostate to help the patient make a more informed decision. Recently, the GPS gene profile has been used evaluate histologically normal appearing tissue adjacent to CaP foci from pathologic stage T1/T2 RP specimens from patients found to have metastatic or locally recurrent disease, and has shown promising results in predicting prostate cancer outcomes (54). The authors attribute these findings to the previously

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Table 3. Markers to Aid in Determining Disease Aggressiveness

TEST	INDICATION	SCIENCE	RESULTS	COST
Oncotype DX® Genomic Health	Biopsy tissued based test to help determine how aggressive cancer is by providing a liklihood of favorable patholgy. • For patients that are NCCN Very Low, Low & Intermediate Risk • Provides personalized Risk Assessment, aids in the decision for active surveillance or immediate treatment	Assay looks at 17 genes within 4 pathways (androgen signaling, stromal response, cellular organization, proliferation) to assess tumor aggressiveness.	• Genomic Prostate Score (GPS) from 0 to 100. • Likelihood of freedom from high grade and/or non- organ-confined disease. • GPS is reflective of the biology of the tumor at the time of biopsy.	• $4,180 • Oncotypedx.com • Medicare covered for NCCN Very Low and Low risk disease
ProMark™ Metamark Genetics	Biopsy tissue based prognostic assay for patients with biopsy Gleason Scores 3+3 and 3+4 • For patients who are deciding between active surveillance and treatment • Provides a personalized risk score • Can be used as stand-alone risk score or combined with NCCN risk categories	Eight-protein signature predicts cancer aggressiveness (>4+3 and/or non-organ confined). Selected markers eliminate sampling variability, provides a direct analysis of cancerous regions of interest. Test requires 4 tissue sections.	• ProMark Score gives a personalized % probability of aggressive cancer. • Interpretation as stand-alone result and in combination with NCCN risk categories. • Results delivered within an easy-to-interpret, personalized report.	• Cost: $3,900 • Metamarkgenetics.com
Prolaris® Myriad Genetics	Biopsy tissue based test for patients who are Active Surveillance candidates. • Available post-prostatectomy available to determine relative risk of BCR	46-gene expression signature includes cell cycle progression genes selected based upon correlation with prostate tumor cell proliferation	• Prolaris score • Biopsy is < or = or > than AUA risk group and estimates. 10year mortality risk. • Post-surgical results are similar but provide 10 year risk for BCR.	• $3,400 • Prolaris.com
Decipher® GenomeDx Biosciences	Tissue based test for patients with adverse pathology post-surgery (radical prostatectomy) • Provides metastatic risk stratification that can help guide post-operative treatment decisions • For patients with pT3 or positive surgical margin or rising PSA • Helps determine the need & optimal timing of radiation	22 RNA biomarkers across multiple biological pathways associated with metastatic progression including cell cycle progression, immune system modulation and androgen signaling. Measures each patient tumor's metastatic risk. Based on the whole transcriptome analysis platform.	• Decipher provides probability of metastasis at 5 years after surgery, and 3 years after detectable PSA • Decipher high risk men may benefit from adjuvant radiation • Decipher low risk men can be safely observed with PSA monitoring	• Cost: $3,400 cash pay price • Genomedx.com

discussed "field effect" phenomenon, and may prove useful in further improvements of the Oncotype Dx prostate cancer assay. This may also have implications in men being considered for focal therapy.

ProMark

ProMark uses an immunofluorescent automated proteomics imaging platform on paraffin-embedded prostate biopsy specimens to quantify 8 specific protein biomarkers. Using protein biomarkers, rather than mRNA for example, makes the test more resistant to sampling variability and pathologist discordance. This test can identify low vs high risk disease, is predictive of prostate cancer lethality, and has a tumor detection specificity of 99.9% (55–57). Overall, ProMark provides another tool to determine need for biopsy with reliable risk stratification based on initial biopsy tissues.

Prolaris

Prolaris, available from Myriad Genetic Laboratories, Inc. provides information on 10 year CaP specific mortality utilizing personalized risk assessment through analysis of a patient's tumor cell growth characteristics (CCP-cell cycle progression score). It is used in a similar set of patients as the OncotypeDx test. By evaluating a 46-gene expression profile of genes specific to cell cycle regulation and progression (31 CCP, 15 housekeeper genes), risk stratification can be obtained for low vs high risk of disease progression. Cell proliferation is known to be the underlying mechanism by which cancer thrives. Prolaris takes advantage of this knowledge, and by using genes associated with cell cycle progression, can predict the aggressiveness of an individual's specific prostate cancer (58). Low expression of the CCP gene profile is associated with low risk of disease progression, with high expression being associated with higher risk of progression (59). Prolaris can be used on a biopsy sample or on post-prostatectomy specimens (59). In its validation testing, Prolaris was found to be an independent predictor of biochemical recurrence and death in two individual treatment cohorts (TURP vs RP) (59). Previous literature has examined physician decision making pre- vs post-Prolaris testing regarding recommendations for treatment for CaP, and found an overall 65% change in the initial physician recommendation, concordant with the Prolaris results (60). The Prolaris test provides three pieces of clinical information for use during patient visits: the Prolaris score, the US distribution, and a mortality risk assessment (61). The Prolaris score itself provides a metric of aggressiveness based on the individuals CCP score and individual tumor biology. The US distribution percentile provides the patient's relative risk based on the Prolaris score compared to others in the same AUA risk group. Finally, a risk mortality assessment is provided, which combines the patient's clinical-pathologic information with the Prolaris score and determines a 10 year prostate cancer-specific mortality risk. Given that the Prolaris test can be used on both biopsy tissue as well as RP specimens, it has value in multiple settings. When counseling a patient post-biopsy, it provides information that can help in deciding to pursue definitive treatment vs surveillance. When used post operatively, it can provide valuable risk stratification and help in determining the need for post-surgical interventions (58).

Decipher

The Decipher Prostate cancer classifier test (GenomeDx Biosciences) predicts the probability of metastatic disease following RP, and also provides analysis of the predicted aggressiveness of the tumor (62). Decipher specifically analyzes 22 RNA biomarkers associated with aggressive disease via whole-transcriptome microarray assay from high risk RP specimens. Following expression of these biomarkers, a 5 year post-surgical probability of metastasis is calculated. The initial validation studies of the Decipher methodology have shown utility especially in the post-operative phase of treatment, with an AUC of 0.79 for predicting 5 year post prostatectomy metastasis (62–64). For example, the Decipher methodology lead to a reclassification of 60% of men to lower risk categories post-operatively, with 98.5% of these men metastasis free at 5 years post-

operatively (62). Moreover, physicians' recommendation for post-operative radiation therapy decreased by 50% after receiving Decipher results showing reclassification to lower risk (63,64). In patients determined to be high risk based on Decipher results, studies have shown an 80% reduction metastasis risk in those who received adjuvant radiation vs salvage radiation (65,66). Another important analysis can be performed on those with biochemical recurrence following prostatectomy. They benefit from Decipher testing, as Decipher metastasis risk stratification can help determine need for salvage radiation vs more intense post-operative intervention including hormonal or other therapies (45). Recently, the test was expanded to be able to additionally utilize prostate biopsy samples for analysis. In a small study of 57 men status-post radical prostatectomy, their preoperative needle biopsy specimens were reviewed. At a median follow up of eight years, eight patients had metastasized and three died of prostate cancer. Decipher was found, through multivariable analysis, to be a significant predictor of metastasis when controlling for standard preoperative clinical findings using the preoperative biopsy tissue. In this way, Decipher can be used clinically in determining preoperatively the likelihood for multi-modal therapy, as well as to set patient expectations early (65). Importantly, Karnes and colleagues suggest that in using a "genomic classifier" such as Decipher, men traditionally labeled as "intermediate risk" may be actually identified as high risk for metastasis. In the same vein, those with pathologic high risk disease may actually be at lower risk of metastasis, thus changing the paradigm and again resetting expectations (62).

NOVEL MARKERS

AR-V7

AR-V7 is a marker found in circulating tumor cells ("CTCs"-used to monitor therapeutic response in CaP patients), and has recently become commercially available through Johns Hopkins' Molecular Diagnostics Lab. Notably, Epic biosciences has received FDA approval for their AR-V7 test, and has partnered with Genomic Health to commercially launch their test in 2017. AR-V7 itself is a splice variant of the androgen receptor, and can be found in approximately 30% of men with CRPC. AR-V7 positivity is suspected in men treated with long term hormonal therapies for their prostate cancer. Previous reports correlate the presence of AR-V7 in CTCs that may predict resistance to enzalutamide and abiraterone (45). Specifically, having a negative AR-V7 was associated with a better PSA response to abiraterone and/or enzalutamide, as well as a better progression-free survival and overall survival when taking abiraterone and/or enzalutamide (67). More recent literature has provided validation for the test. Scher and colleagues found that CTC expression of AR-V7 protein in mCRPC patients, as a treatment-specific biomarker, was associated with superior survival on taxane therapy over ARS-directed therapy in a prospective clinical practice setting (68). Exciting new data has also shown that AR-V7 variants can be targeted with PIP5K1α inhibitors (ISA-2011B) to overcome enzalutamide resistance of CaP cells (69). The ultimate goal of this marker is to aid in the treatment of men with mCRPC in selecting appropriate chemotherapies (i.e., those that target the AR vs those that do not) (45).

HSD3B1

A recently released paper from Hearn et al details the association of a novel genetic marker: HSD3B1 (1245A>C). HSD3B1 (1245A>C) is an allele associated with CRPC, as it encodes an altered enzyme that increases the amount of DHT from extra-gonadal sources. The authors reviewed 443 patients with CaP, and found an association with this allele and resistance to ADT. They postulate that HSD3B1 can prove useful in the future to help determine which CaP patients will do better on ADT vs those that that may need an escalation of therapy sooner due to an increased likelihood of ADT failure (70).

CONCLUSIONS

While there are numerous markers available to aid in risk stratification and decision making regarding prostate cancer, selecting the right test for the right patient in the right setting remains challenging. Patient factors as well as cost considerations make selecting the test of choice that much more difficult. A useful "model of organization," as well as treatment algorithm help in determining which is the appropriate next best step in the work up of CaP patients. All providers who treat CaP patients benefit from a better understanding of these biomarkers. As referenced above, the field of biomarker design is rapidly evolving, and exciting new genomic testing is on the forefront. Future tasks should be aimed at consolidation of markers, to take advantage of individual markers strengths, and increase clinical utility.

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PSA <1.5 is Predictive of Negative SelectMDx Result

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INTRODUCTION

As the prevailing cause of cancer-associated death in men, prostate cancer (PCa) remains an important topic in the field of urology[1]. This year, 161,000 men will be diagnosed with PCa and 26,000 are expected to die from this disease. Autopsy studies have confirmed histologically apparent PCa in approximately 42% of men over 50 who died of other causes[2]. Consequently, most men die with PCa rather than because of it. Hence, screening patients more efficiently for PCa is also becoming important to minimize over-diagnosis and over-treatment[3,4]. However, PSA, which serves as the initial screening tool for PCa, has poor specificity for this disease. Thus, decisions to biopsy patients need to be supplemented by additional biomarker tests.

New studies have identified a PSA of 1.5 as a more appropriate screening cut-off to identify men at risk for PCa[5,6]. In addition to utilizing the new PSA cut-off for screening, our institution also uses a relatively new urinary biomarker test called SelectMDx to monitor our patient population on active surveillance (AS) as well as prior to prostate biopsy. The SelectMDx urine test provides the likelihood of detecting PCa and high grade prostate cancer (HG PCa) with Gleason pattern 4 and 5 upon subsequent biopsy[7]. The test measures the mRNA levels of the homeobox C6 (HOXC6) and distal-less homeobox 1 (DLX1) biomarkers in urine samples post-DRE. Higher expression levels of HOXC6 and DLX1 are associated with an increased probability for HG PCa.

Our institution provides an IRB approved annual screening for the members of our community during Prostate Cancer Awareness Week (PCAW). Last year, we offered the SelectMDx test as part of the screening protocol during PCAW. We investigated whether SelectMDx could be predictive of PCa and HG PCa in patients using PSA of 1.5 as a screening cut-off. We aimed to determine if any correlation could be found in the degree of positivity and/or negativity of the SelectMDx test after looking at a screening population split between those above and below a PSA of 1.5.

METHODS

Our institution advertised PCAW to the members of our community with mail-out materials. The population that arrived for screening was generally a heavily screened population over many years. Specifically, the majority of this population appears each year for this screening event. During the screening event, we collected serum samples and post-DRE, first-void urine specimens from each participant. A large, de-identified database of results with PSA, testosterone, cholesterol, SelectMDx, and DRE results were stored and surveyed after labs were completed. We compared PSA results against SelectMDx test results for probability of PCa and HG PCa at biopsy. Likelihood of diagnosing PCa and HG PCa at biopsy is reported as a probability (p) between 0-100%.

New studies have identified a PSA of 1.5 as a more appropriate screening cut-off to identify men at risk for PCa[5,6]

RESULTS

We received 199 participants and successfully collected post-DRE first-void urine samples from all but 13 during our PCAW screening event. Average age of the 186 patients was 66 and average PSA was 2.17. There were 81 patients with a PSA <1.5, and none of these men were predicted to

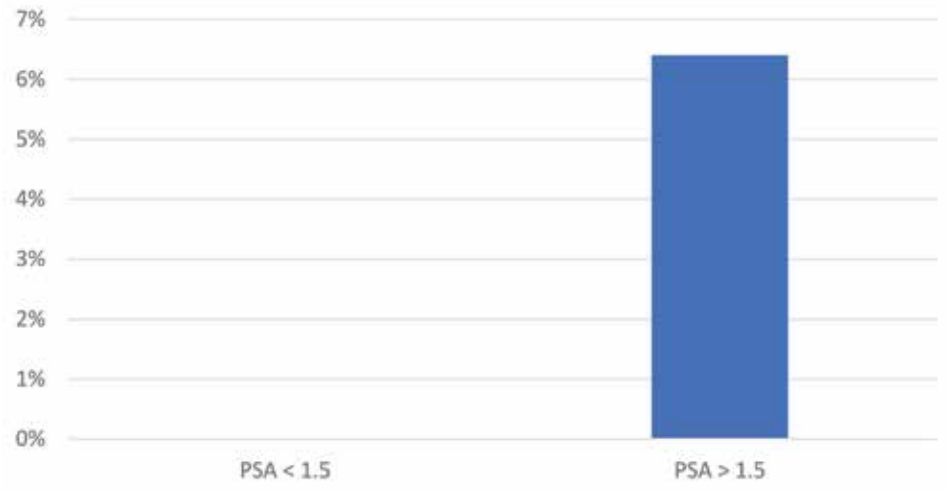

Figure 1. **Percentage of Positive SelectMDx Results**

have PCa by SelectMDx (Figure 1). There were no positive SelectMDx tests in this cohort. In the other cohort, there were 109 patients with a PSA >1.5, and seven of these patients received a positive SelectMDx test result. The average risk of these seven patients predicted by SelectMDx was 43% for PCa and 17% for HG PCa.

CONCLUSION

The SelectMDx test reinforced the original data that a PSA <1.5 is a more appropriate screening cut-off that represents a low-risk subset of patients. In our cohort of patients, no one with a PSA <1.5 had a positive SelectMDx test result. The only positive SelectMDx test results were produced from the cohort of screening patients with a PSA >1.5. Additionally, our data also supports referring patients with a PSA >1.5 to further urologic intervention. Although the SelectMDx test has sufficient sensitivity and NPV to indicate patients with high-grade prostate cancer, the test may also produce false positives3. We plan to follow up with our seven positive SelectMDx patients to determine whether they have taken steps for further urologic intervention and investigate their cancer status with a TRUS or transperineal mapping biopsy. Nonetheless, our data reinforce the proposition of setting a new PSA cut-off at 1.5 and the need to supplement decisions to biopsy with one or more biomarkers.

DISCUSSION

With prostate cancer still the leading cause of cancer mortality in men, it remains a very important topic in men's health as well as urology[1]. Additionally, screening patients appropriately for the likelihood of PCa is equally important. New studies have proposed setting a PSA standard of 1.5[2,3]. PSA values above 1.5 are now proposed to be high risk and those less than 1.5 would constitute a low risk group of patients. In an effort to validate and test this new PSA cut-off, we sought to determine how it would fair when supplemented with the SelectMDx test, which is a post-void DRE urinary biomarker that determines a patient's risk of having PCa as well as HG PCa. During our institution's annual screening event during PCAW, we hosted almost 200 patients and received final lab results on just over 180 patients. After analyzing each patient's SelectMDx test and DRE results, we divided the patient population to those above and below a PSA of 1.5. Approximately 44% of our screening population had a PSA <1.5, and, interestingly, none of these patients received a positive SelectMDx result. The only positive results were produced in the screening population with a PSA >1.5.

These results tend to affirm that patients with a PSA <1.5 are a low risk population and >1.5 is a higher risk of having PCa. However, the only way to confirm and diagnose PCa is with a biopsy. We might propose a future study in which we follow-up with these patients who received a positive SelectMDx result. As these patients are in a high-risk subset with a PSA >1.5 and they have a positive SelectMDx test result, we believe it will be beneficial for these patients to follow-up with a urologist for further intervention. As the SelectMDx test is not 100% accurate, the only way to determine the PCa status of these patients is with a highly sensitive and specific prostate biopsy, such as a transperineal mapping biopsy[8].

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AGE

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INTACT PSA

DRE

TOTAL PSA

hK2

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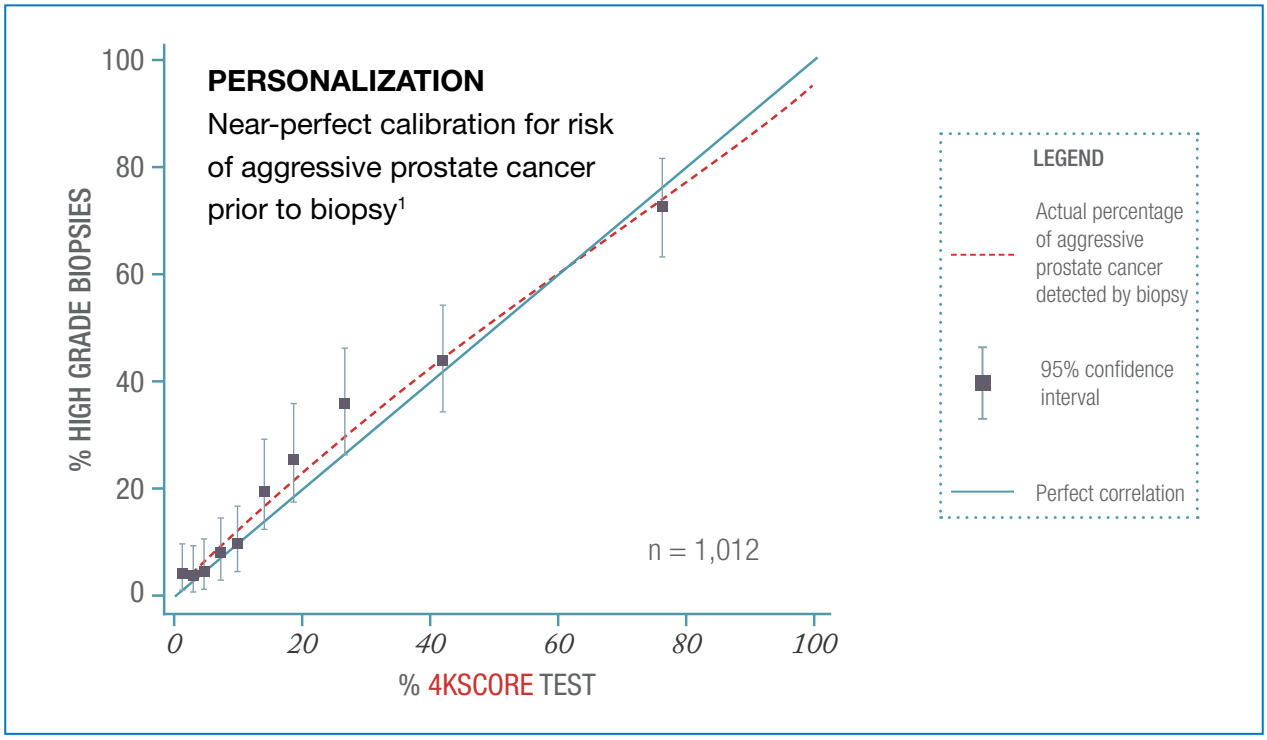

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