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REVIEW

The emerging landscape of tumor marker panels for the identification of aggressive prostate cancer: the perspective through bibliometric analysis of an Italian translational working group in uro-oncology

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ABSTRACT

Molecular heterogeneity and availability of different therapeutic strategies are relevant clinical features of prostate cancer. On this basis, there is an urgent need to identify prognostic and predictive biomarkers for an individualized therapeutic approach. In this context, researchers focused their attention on biomarkers able to discriminate potential life-threatening from organ-confined disease. Such biomarker could provide aid in clinical decision making, helping to choose the treatment which ensures the best results in terms of patient survival and quality of life. To address this need, many new laboratory tests have been proposed, with a clear tendency to use panels of combined biomarkers. In this review we evaluate current data on the application in clinical practice of the most promising laboratory tests: Phi, 4K score and Stockholm 3 as circulating biomarkers, Mi-prostate score, Exo DX Prostate and Select MD-X as urinary biomarkers, Confirm MDx, Oncotype Dx, Prolaris and Decipher as tissue biomarkers. In particular, the ability of these tests in the identification of clinically significant PCa and their potential use for precision medicine have been explored in this review.

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KEY WORDS: Prostatic neoplasms; Tumor biomarkers; Diagnosis.

Prostate cancer (PCa) is the most frequently diagnosed cancer in men and represents the second major cause of death for cancer in this population.[1]

The first case of PCa was described by Adams in the mid-nineteenth century who defined the pathology as very rare.[2] After 150 years, PCa has become a significant public health problem.

Recent estimates have calculated that in 2020, 191,930 new cases will be diagnosed and 33,330 patients will die of PCa in the USA.[3]

In the late 1980's, PSA was introduced in screening procedures and the incidence of PCa doubled.[4] In turn PCa-specific mortality rates was reduced.[5]

Widespread use of PSA for screening led to overdiagnosis and already in 2001 Stamey invited to weigh the consequences of the use of PSA for about twenty years in screening procedures.[6] In 2012 the USA Preventive Services Task Force (USPSTF) issued a recommendation against the use of PSA in screening procedures.[7] Richard Ablin (who discovered the PSA in 1970) emphasized the two main limits of PSA: 1) it has low specificity, 2) it does not distinguish between indolent and clinically significant tumors.[8]

Routine use of diagnostic biopsies, an invasive procedure associated with several side-effects such as pain and bleeding, paved the way to the identification of clinically insignificant PCa. Consequently, a high rate of overdiagnosis and overtreatment were observed for PCa.[9]

After biopsy, a patient diagnosed with PCa is stratified in risk category according to clinical and pathological findings, such as Gleason score, tumor stage and PSA levels.[10] Unfortunately, the prognostic performance of these systems is unsatisfactory in distinguishing aggressive tumors. In addition, therapeutic strategies other than prostatectomy and far less invasive are currently available, such as active surveillance (AS), however personalized therapy guided by predictive biomarkers still lack in the clinical management of PCa patient.

Hence there is a strong need of new biomarkers information not only for early identification of the disease, but also for the discrimination of clinically significant tumors. Many new tests[11] have been proposed, based on panels of circulating, urinary and tissue biomarkers. In this review, we assess the latest evidence on the impact of these tests on clinical decision-making and future perspectives.

Search strategy

A narrative search was conducted on PubMed for all publications with relation to panel of biomarkers of PCa, using the following combinations of MeSH terms: prostate cancer, biomarker or tumor marker. Initially, titles and abstracts of these studies were screened and reviewed on the basis of the established selection criteria.

Selection criteria

The following inclusion criteria were applied: identification of human PCa biomarkers, indication on PCa prognosis or aggressiveness; evidence for the clinical benefit of the biomarkers. All publications (except reviews, editorials, studies made on animal models of PCa or on cell model systems and abstracts) were considered. Table I summarizes the included studies for each biomarker.

The main eligibility criterion was the presence of data on the ability of the test in the identification of aggressive PCa. Publications were excluded when the authors report studies evaluating PCa with very low or very high PSA levels. Studies were selected after reading the abstract. The selection was shared with the working group.

This project was realized by an Italian working group that includes molecular biologists, oncologists and urologists with expertise in the field of PCa biomarker testing.

For each marker the available data were used to evaluate: 1) area under the receiver operatic characteristic curve (AUC); 2) decision curve analysis (DCA). For each publication, the working group analyzed methodological quality, and clinical impact.

TABLE I.—*Summary of the studies for each biomarker included in the bibliometric analysis.*

Biomarker	N. included studies
Phi	10
4K score	3
Stockholm3	3
Proclarix	3
Select MDx	2
MiProstate score	1
ExoDx prostate	1
PCA3	9
Confirm MDx	7
Oncotype Dx	1
Prolaris	1
Decipher	5

Test based on circulating biomarkers

Phi, 4K score and Stockolm 3 are multiplex test including different molecular forms of PSA. Free PSA was distinguished from complexed PSA firstly in 1990.[12] In 2000 Mikolajczyk[13, 14] described different components of free and complexed PSA. PSA is synthesized via a complex enzymatic pathway, which includes different molecular forms and the isoform [-2]pro-PSA is preferentially synthesized in malignant cells.[15]

In PCa, damaged tissue architecture causes reduced enzymatic activity in the lumen of the gland eliciting an increase of circulating levels of complexed PSA and pro-PSA and a decrease of free PSA.[16] Literature data indicated that a free/total PSA ratio of less than 10% is associated with malignancy in only about 56% of cases.[17]

To overcome free/total PSA ratio inadequacy phi (prostate health index) was developed. Phi combines total PSA, free PSA and -2proPSA producing a risk index of positive biopsy.[18]

Following FDA approval of Phi in 2010, a lot of studies[19-21] aimed to compare the diagnostic performance of phi with free/total PSA ratio. A meta-analysis in 2014 demonstrated that the probability to identify a positive biopsy is significantly increased using the phi in place of the free/total PSA ratio in subjects with PSA ranging from 2 to 10 ng/mL.[22] Moreover, phi is significantly associated to aggressiveness of the tumor defined at radical prostatectomy,[23] as confirmed also by Tosoian on a large cohort of patients (N.=1663).[24] In addition, it has been recently demonstrated that p2PSA seems to be more sensitive than tPSA in predicting earlier BCR within 3-year follow-up.[25]

A review of literature data suggested that, if used routinely, phi could reduce the number of unnecessary biopsies without losing clinically significant tumors, so it could be a useful tool for treatment decision making.[26-28] The 4K score test combines the value of 4 kallicreins (PSA, free PSA, iPSA and hk2) with clinical variables such as age, DRE and previous biopsy outcome, producing a risk index related to the presence of clinically advanced PCa. Some authors showed that 4K score is significantly increased in metastatic PCa.[29, 30] Compared to phi, 4K score showed a performance comparable both for the prediction of positive biopsies and of high-grade tumors.[31]

More recently, Stockholm 3 (S3M) test was developed at the Karolinska Institute in Stockholm.[32] S3M combines several clinical variables (age, family history, previous biopsies, and DRE) with 232 single nucleotide polymorphisms and six protein markers (total PSA, free PSA, intact PSA, hK2, MSMB, and MIC1).[33] In a recently published study on about 60,000 subjects, S3M showed an AUC significantly higher than PSA for the identification of high-grade tumors and the use of this test reduced the number of unnecessary biopsies without losing high-grade tumors.[32, 34]

Collectively, phi, 4K score and S3M showed comparable diagnostic performance for the identification of clinically significant tumors, even if a direct comparison of the three test is still lacking. At present, the cost of Phi is lower and this biomarker is the only FDA approved and with CE mark. Conversely, S3M presents a relevant limitation: the test has been validated on a large population, including mainly north-European men and the algorithm contains also 232 SNPs. Thus, this test is currently available for clinical use only in Sweden.

More recently, a new test named Proclarix has been developed,[35] combining thrombospondin-1 and cathepsin D serum level with patient age, PSA and fPSA.[36] Proclarix showed a better ability compared to PSA in predicting positive biopsy in men with PSA in grey zone, negative DRE and prostate volume ≥35 mL.[36] Macagno *et al*. recently demonstrated the good analytical performance of thrombospondin-1 and cathepsin D immunoassays as components of a CE-IVD marked test.[37]

Tests based on urinary biomarkers

In last decade, several urinary tests have also been developed PCA3 (prostate cancer antigen 3) and the T2:ERG fusion gene are urinary tests for two prostate cancer–associated RNA markers.[38, 39]

PCA3 score is significantly associated with positive biopsy In a head-to-head comparison with phi PCA3 avoids a lower number of unnecessary biopsies without lose cases of cancer compared to phi.[40, 41] In addition PCA3 correlation

with aggressiveness was controversial. Auprich *et al.* demonstrated that PCA3 was significantly correlated with tumor volume, Gleason score and pathological stage, but not with the presence of extracapsular extension or the invasion of the seminal vesicles.[42] Shalken *et al.* Previously found no significant correlation with tumor volume and with Gleason score and pathological stage.[43] At present, PCA3 is recommended only for patients undergoing to repeated biopsy[44-46] in international guidelines. Similarly, T2: ERG was significantly associated with the presence of cancer, but not with poor prognosis.[47, 48] In addition, both T2: ERG and PCA3 had scarce clinical benefit in African-American men, indicating that ethnicity could be an important bias in the diagnostic performance of these two tests.[49]

The multiplex tests Mi-prostate score (MiPS) and ExoDx Prostate Intelliscore included PCA3 and T2: ERG. MiPS combines PCA3 score, T2: ERG and serum PSA, providing a risk index significantly associated with the detection of clinically significant PCa.[48, 50] ExoDx Prostate score is based on the evaluation of PCA3 and T2:ERG RNA content in the exosomes isolated from urine of men with suspected PCa.[38, 51] ExoDX Prostate score combined with clinical variables such as PSa, age, race and family history ameliorated the identification of clinically significant PCa.[38, 51, 52]

Recently, Select MDx has been developed. This test was designed to weigh the expression of three genes HOXC6, DLX1 and KLK3 in post-DRE urine. The combination of these values with tPSA, PSA density, DRE, age and family history produced an index significantly associated with the presence of high-grade cancers.[38] The test showed a negative predictive value of 98% for aggressive cancers.[53]

EAU guidelines recommended PCA3 for patients undergoing repeated biopsy and SelectMdx to identify the risk of high-grade cancer (https://www.scribd.com/document/376891488/2018-edition-of-the-european-association-of-urology-eau-guidelines).

Test based on tissue biomarkers

A direct evolution of the former Select MDx test was the Confirm MDx (Confirm MDx for Prostate Cancer; MDx Health, Inc, Irvine, CA) which was developed to aid urologists in the diagnostic algorithm of those patients with previously negative biopsies. Previous studies examining patients who have had multiple biopsies have shown indeed a 20-30% false negative rate.[54, 55] This false negative rate may potentially lead to further biopsies simply because of the concern in high-risk patients that a negative biopsy is not sufficient.[56] Confirm MDx is a commercially available test that uses multiplex methylation-specific polymerase chain reaction (PCR) to determine the methylation status of prostate cancer–associated genetic biomarkers (APC, GSTP1 and RASSF1) in prostate biopsy cores that were negative. This tests for methylation patterns in normal tissue that surrounds tumor suggesting the normal core was in close proximity to a missed cancer.[57] A positive test was found to be a strong independent predictor of finding prostate cancer in a subsequent biopsy while a negative result provides a higher negative predictive value than standard histopathology alone.[55, 58] This assay has the potential to help guide urologists on decisions regarding the need for repeat biopsy for patients with a previous negative one but who are still considered to be at risk for prostate cancer. In 2014, Wojno *et al*.[59] demonstrated a low repeat biopsy rate for men with a history of negative biopsy who also had a negative Confirm MDx result on testing of the residual prostate tissue (<5% rate of repeat prostate biopsies, indicating a potential 10-fold reduction from previous rates). The test achieved a 90% NPV within 30 months of the initial biopsy. In another subsequent validation trial, an 88% NPV was reported, and the test was the most significant predictor of biopsy results.[60] One of the most promising applications of Confirm MDx testing might be in aiding the selection of men who undergo AS instead of treatments with curative intent. To offer AS safely, the risk of underestimating the metastatic and local invasive potential of the individual tumor has indeed to be minimized. In this critical decision-making scenario, the adoption of new potentially predictive models incorporating imaging (*e.g.* mpMRI) and epigenetic testing as well as the commonly used clinical variable

such as history, PSA values etc. might therefore in future open the doors for a better selection and safe management of the increasing number of patient reporting a previous negative biopsy or enrolled in AS programs.

Oncotype Dx Genomic Prostate Score (Oncotype Dx Genomic Prostate Score (GPS; Genomic Health Inc., Redwood City, CA, USA) is a quantitative real-time PCR assay performed on samples obtained by needle biopsy that tests for 12 cancer-related genes. These include from pathways involved in androgen production, cell proliferation, cellular organization, and stromal response as well as housekeeping genes. The test includes quantitative 17-gene RT PCR assay on manually micro-dissected tumor tissue. The test was optimized for use with very small tissue input (six 5-micron sections of singe needle biopsy block with as little as 1 mm tumor length). In one of the first experience analytically validating the Oncotype DX prostate cancer assay, Knezevic *et al*.[61] found that out of 412 prostatectomy patients the GPS of their prostate biopsies successfully met all prespecified assay quality metrics in 395 (96%) cases. Gene assays were shown to precisely account for molecular expression over different inputs varying from a minimum of 0.005 ng to 320 ng. The overall mean biases at pPCR inputs were indeed less than the 10% of the whole assays measured guaranteeing optimal analytical accuracy.

In one of the most recent clinical application of the assay, Klein *et al*.[62] tested the reliability of Oncotype Dx to test the association between the GPS and pathologic stage and grade at RP. Combining the 17 genes in the Oncotype Dx with the GPS algorithm allowed for the prediction of high grade and high stage disease with an OR of 2.1 (95% CI: 1.4-3.2) when adjusting for Cancer of the Prostate Risk Assessment score (CAPRA). In this experience, out of the 732 candidate genes analyzed for correlation with clinical outcomes after biopsy, 288 (39%) were found to predict clinical recurrence despite heterogeneity and multifocality while 198 (27%) were predictive of aggressive disease after adjustment for prostate-specific antigen, GS and cT stage. From these initial validations, emerges the possibility for the Oncotype Dx to enable improved treatment decisions for men diagnosed with early-stage PCa. If on one hand the possible implications of the present tool may offer interesting application in the field of men selected for AS programs, on the other hand, we have to acknowledge how main results above summarized include in their cohort population many patients with low-volume intermediate risk PCa (GS 3+4) for whom many centers would currently consider primary treatments strategies (*i.e*. surgery or RT) rather than AS programs limiting thus the possibility to reproduce the aforementioned experiences and the reliability of the Oncotype Dx.

Prolaris is a commercially available 46-gene panel (31 cell-cycle progression [CCP] genes and 15 housekeeping genes) developed by Myriad Genetics (Salt Lake City, UT, USA). This assay is performed on RP specimens as well as prostate biopsies and is aimed predicting adverse features that might benefit from adjuvant therapy and is useful in counseling patients deciding between active surveillance and treatment in low risk PCa.[63] To date, the available series which rely on clinical application of this biomarker were overall concordant in confirming the role of CCP score as an independent predictor of PCa death, BCR, and metastasis after RP.[64, 65] Furthermore, Cooperberg *et al*.[66] confirmed the ability of the CCP score to predict BCR after RP and found that a combined model incorporating the CCP with the CAPRA score had a better prognostic value for both the overall cohort and a low-risk subset.

The additional benefit of this specific tool could be early identification of those patients who after primary treatment of csPCa may benefit from upfront adjuvant modalities of therapy (both RT or ADT) in order to contrast the likely of later cancer specific recurrence or progression.

Decipher is a tissue based genomic classifier (GC) developed by GenomeDx Biosciences (Vancouver, BC, Canada) and Mayo Clinic (Rochester, MN, USA). Decipher predicts the risk clinical metastasis after surgery and is based on 22 RNA biomarkers.[67] Data available from a systematic review and metanalysis of five studies have cumulatively assigned to the GC tool an overall increase in the predic-

tive accuracy. This improvement in the 10-year distant metastasis predictive accuracy of clinical parameters jumped from an AUC of 0.76 to 0.81.[68] When the cancer specific risk of death from PCa was compared with clinicopathological variables expressed as Cancer of the Prostate Risk Assessment (CAPRA) score, Cooperberg *et al*.[69] demonstrated an even higher discrimination ability in predicting the unfavorable outcome (AUC: 0.75 *vs*. AUC: 0.78, respectively). Similar to the Prolaris test, a Decipher score ≥0.4 or ≥0.6 according to two different experiences was found to be, together with the classical clinical and pathologic variables, a predictive covariate to guide treatment decisions after RP. In the first study Den *et al*.[70] demonstrated in a cohort of high-risk PCa patients who subsequently underwent adjuvant vs. salvage RT that there was a significant difference in terms of cumulative incidence of metastasis at 5 years (6% *vs*. 23%, respectively; P=0.008). Dalela *et al*.[71] tested the combination of Decipher and other PCa adverse prognostic features such as high GS, pathology report and lymph-node involvement for the assessment of immediate adjuvant RT *vs*. observation. In patients presenting with a preoperative Decipher score ≥2, adjuvant RT was able to reduce the 10-year recurrence rate. Men with a higher Decipher score and unfavorable pathology could thus potentially benefit from preoperative testing in order to be considered for further adjuvant regimens following RP.

Critical analysis and evaluation of cost-effectiveness

Even though the growing body of evidence suggesting potential utility and benefits from the routine clinical implementation of such novel genetic biomarkers, their adoption into clinical practice has been limited so far and seem mainly limited by their costs. Based on the available evidence, some of the aforementioned biomarkers could help in discriminating between aggressive and non-aggressive tumors with an additional value compared to the prognostic parameters currently used by clinicians, as well as could represent guiding tools in influencing different clinical scenarios ranging from the algorithm of the re-biopsy patients in the diagnostic setting to the use of adjuvant therapies after primary interventions such as RP or RT.[72, 73] While cost-effectiveness data on genomic biomarkers is limited, several analyses suggests that that overdiagnosis and overtreatment should be reduced by testing with the appropriate biomarker and the subsequent limitation in unnecessary medical costs. Although from our analysis we can conclude the existence of a potential usefulness of these biomarkers in clinical practice, specifically, the use of Oncotype DX, and Confirm MDx demonstrated a positive economic impact on the healthcare costs, whereas the incorporation of other markers may be uncertain (Decipher) or negative (Prolaris). Prospective trials with long-term follow up will be crucial in determining the impact of commercially available genomic biomarkers on oncologic and quality of life outcomes in prostate cancer.

Conclusions

The need to have laboratory test able to discriminate PCa patient with indolent cancers is still not satisfied. However, search to identify prognostic biomarkers that would reduce overdiagnosis and overtreatment of organ-confined disease became more intensive.

Fortunately, panels of biomarkers combined together and/or with clinical parameters, provide risk estimation models (Figure 1, Table II) with improved ability to stratify patients and allow individualized therapeutic approach.[74]

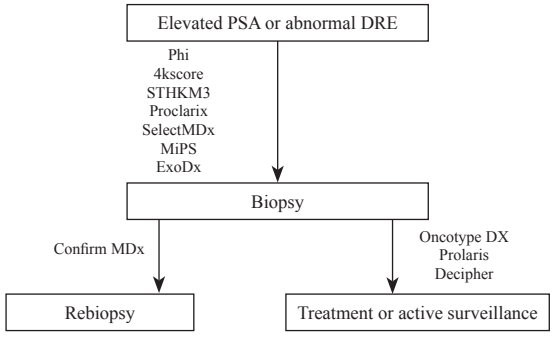

Figure 1.—Biomarker panels in the diagnostic pathway of prostate cancer.

TABLE II.—*Comparison between each biomarker.*

Test	Specimen type	Variables	Use in clinical practice	Indication (biopsy setting)	Commercial availability
Phi	Blood	(-2)proPSA, / fPSAx√tPSA	Increases the power to detect PCa, especially high grade (Gleason ≥7) PCa, in men ≥50 years, negative DRE, PSA between 2 and 10 ng/mL	Initial + repeat	Yes
4K score	Blood	tPSA, fPSA, iPSA, hK2, clinical variables	Better accuracy in the detection of metastatic PCa	Initial + repeat	Yes
Stockholm 3	Blood	tPSA, fPSA, iPSA, hK2, MSMB, MIC-1, clinical variables, 232 SNPs	To distinguish benign and high-grade malignant prostatic diseases	Initial	No
MiPS	Post-DRE Urine	TMPRSS2-ERG, PCA3, tPSA	To determine the probability of detecting aggressive disease at biopsy	Initial + repeat	No
SelectMDX	Post-DRE Urine	HOXC6, DLX1, tPSA, clinical variables	High negative predictive values to reduce unnecessary prostate biopsies	Initial + repeat	Yes
ExoDx	Post-DRE Urine	Exosomal PCA3 and ERG	To determine the probability of detecting aggressive disease at biopsy	Initial + repeat	No
Confirm MDX	Biopsy-prostate cores	GSTP1, APC, RASSF1	High negative predictive values to reduce unnecessary repeat prostate biopsies	Repeat	Yes
Oncotype Dx	Biopsy-prostate cores	17-gene expression panel involved in multiple pathways	Improves risk discrimination of PCa into very low, low and modified intermediate risk	Initial	Yes
Decipher	Biopsy-prostate cores	22-gene multi-pathway expression panel	A tool to stratify biopsy positive PCa patients in treatment planning	Initial	Yes
Prolaris	Prostate tissue	48 gene-expression panel involved in cell-cycle progression	Assessment of disease aggressiveness	Post-prostatectomy	Yes

PSA: prostate-specific antigen; fPSA: free PSA; PHI: prostate health index; tPSA: total PSA; PCa: prostate cancer; DRE: digital rectal examination; 4K: four kalikrein; MiPS: Mi Prostate Score.

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