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Active Surveillance in Prostate Cancer: Current and Potentially Emerging Biomarkers for Patient Selection Criteria

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Abstract

Background: Prostate cancer (PCa) represents one of the most frequent malignancies and the fifth leading cause of cancer death in adult men worldwide. PCa mortality rates have been declining in several Western countries; one of the possible reasons may be related to the application of prostate-specific antigen early detection policies. These early detection protocols increase PCa-specific patient survival; however, a high percentage of these cases corresponds to low-risk PCa that grows very slowly and is unlikely to metastasize to threaten survival. Many low-risk PCa patients receive aggressive therapies, such as radical prostatectomy and radiotherapy, that are costly for patients and/or health systems and generate side effects that affect the quality of life. An alternative to surgery and radiotherapy treatments for low-risk PCa is active surveillance (AS), a strategy based on close disease monitoring and intervention only if the disease progresses. However, proper identification of low-risk PCa patients at the time of diagnosis is essential for the effectiveness AS. The selection of AS candidates remains challenging; thus, effective prognostic biomarkers are needed. ***Summary:*** This review article addresses the characteristics of the current and emerging PCa prognostic biomarkers, including tests available for tissue, blood, and urine analyses, for the appropriate selection of PCa patients for AS. In addition, and based on published literature, we performed a selection of potential new biomarkers that can distinguish low-risk PCa. ***Key Messages:*** The literature search yielded four tissue-based tests, two blood-based tests, and six urine-based tests that can be used to determine PCa risk classification. However, most available tests are expensive; thus, cost-effective analyses are needed in order to obtain the approval of government agencies and to be financed by the health systems. Available prognostic urine tests have shown great progress over the last years, and they have the advantage of being minimally invasive; therefore, they may become a routine disease progression test for patients under AS. In addition, new research conducted in the last decade has shown promising biomarkers, including mRNA, miRNA, long non-coding RNA, and metabolites, that could improve existing tests or allow the development of new tools for AS patient selection.

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Introduction

Prostate cancer (PCa) represents one of the most frequent neoplasms and is the fifth leading cause of death from cancer in adult men worldwide [1]. Due to the high incidence and mortality rate, several developed countries have applied early screening policies using serum prostate-specific antigen (PSA) measurements [2]. Unfortunately, this strategy has induced a dramatic increase in the PCa incidence rate, where a high percentage of these cases represents low-risk PCa [3]. These patients are characterized by having a disease that does not often produce symptoms and does not cause death. Therefore, standard therapies (i.e., radical prostatectomy [RP] and radiotherapy) for these patients are unnecessary and generate side effects that adversely affect the quality of life. In addition, these therapies are expensive for patients and health care systems [4]. An alternative therapeutic option for patients with low-risk PCa is active surveillance (AS), which reserves treatment for only those patients in which disease progression can be demonstrated. AS strictly depends on the accurate identification of patients with low-risk PCa at the time of diagnosis that will have the greatest benefit from AS. Currently, it is widely accepted that the indication for AS is for patients with low-risk PCa (Gleason 3 + 3, International Society of Urological Pathology grade, ISUP 1) and favorable intermediate risk (Gleason 3 + 4: ISUP 2). In addition, there are other certain criteria that are generally part of the inclusion criteria, but depending on each center they are used such as family history of PCa, PSA density <0.15, PSA <10 ng/mL. Regarding the criteria for progression or reclassification, the most important is a change in the histological pattern on the re-biopsy, for example, from Gleason ISUP 1 to 2 or from ISUP 2 to 3 or 4. The current way to follow a patient in this modality of treatment is with PSA every 6 months, prostate MRI during the first year, and mainly the first repeat biopsy or confirmatory biopsy during the first year. Then, both the MRI and subsequent biopsies can be spaced out and done every 2–3 years.

Biomarkers are molecules that can provide information about the diagnosis, progression, prognosis, and prediction of pharmacological response. These include the presence of specific cell types, proteins, metabolites, RNA, DNA mutation, polymorphism, or epigenetic modifications [5]. Different types of biomarkers have been studied in PCa as tools for analysis of risk of progression, with potential application in the accurate identification of candidates for AS. In addition, multiparametric magnetic resonance imaging (mpMRI) has improved to represent an important tool in diagnosis, staging, and monitoring of PCa patients.

The correct selection of candidates for AS, and the best approach for monitoring, remains challenging for clinicians. One of the main concerns in the AS selection criteria is the fact that about 36% of patients with low-grade PCa, based on diagnostic prostate biopsy analysis, were found to have high-grade disease upon prostatectomy [6]. Therefore, there is a substantial interest in identifying new biomarkers that can be utilized to improve selection for AS and to monitor patients on AS. In this review, we summarized current and emerging PCa biomarkers and commercially available tests that help determine which patients are most appropriate for AS.

Tissue Biomarkers

Prostate tissues are obtained during needle biopsy from patients who are suspected to have PCa. These tissue specimens are used to evaluate the presence of cancer and to determine the Gleason grade. Gleason grade is informative about the biology of the tumor but often is insufficient to establish the most suitable treatment for each patient. Prostate biopsy specimens are limited in number and size and, thus, represent only a sampling of the prostate or the PCa. Therefore, further information regarding cancer aggressiveness and progression risk must be obtained through PCa tissue-specific biomarkers. There are several potential biomarkers for prognosis and commercially available tests that use these biomarkers to augment prognostic capability and to guide selection of a specific treatment, such as AS.

Long Noncoding RNAs

In addition to transcripts of protein-coding genes, the human genome is a template for a large amount of noncoding RNAs that include long noncoding RNAs (lncRNAs) with >200 nucleotides. Dysregulation of these lncRNAs plays an important role in tumor progression. Alterations in their expression, their primary and secondary structure, and their binding proteins are associated with metastasis, invasion, and patient survival [7]. lncRNAs for which a correlation has been shown between expression level and disease aggressiveness are described below.

Colon Cancer-Associated Transcript 2

Colon cancer-associated transcript 2 (CCAT2) is located at locus 8q24, a chromosomal region where multiple loci have been associated with PCa susceptibility. In a

study using PCa tissues from 96 patients, Kaplan-Meier survival analysis showed a poorer overall survival and progression-free survival in those patients with higher CCAT2 expression levels [7, 8]. Moreover, multivariate analyses indicated that CCAT2 status was an independent prognostic indicator or biomarker for PCa.

PCa-Associated Transcript 14

PCa-associated transcript 14 is transcriptionally regulated by androgen receptor and is differentially expressed depending on PCa aggressiveness [9]. Two studies, one using Affymetrix gene expression analyses in 131 primary and 19 metastatic prostate tumor tissues, and the second using microarray data from 355 prostate tissues, showed that PCa-associated transcript 14 was highly expressed in low-grade disease and loss of its expression predicted disease aggressiveness and recurrence [9, 10].

PCa-Associated Transcript 29

PCa-associated transcript 29 is an androgen-regulated tumor suppressor that exhibits a suppressive phenotype that includes inhibition of cell proliferation, migration, tumor growth, and metastases [11]. Kaplan-Meier analysis of data collected from RP tissue from 51 PCa patients showed that patients with lower levels of PCa-associated transcript 29 expression had significantly higher rates of biochemical recurrence [11].

Downregulated RNA in Cancer, Inhibitor of Cell Invasion and Migration

Downregulated RNA in cancer, inhibitor of cell invasion and migration (DRAIC) is a hormone-regulated lncRNA that inhibits cancer cell migration and invasion. Sakurai et al. [12], utilizing information from 80 PCa patients, showed that lower expression of DRAIC predicted a shorter disease-free survival in patients. Therefore, higher levels of DRAIC represented a predictor of a good prognosis.

Long Intergenic Nonprotein Coding RNA 1296

Long intergenic nonprotein coding RNA 1296 is located at chromosome 14q11.2. In 2017, a study of lncRNA expression levels in PCa tissues from 73 patients that underwent surgical resection showed a positive correlation between long intergenic nonprotein coding RNA 1296 expression and Gleason score, tumor stage, and shorter biochemical recurrence-free survival [13].

SWI/SNF Complex Antagonist Associated with PCa

SWI/SNF complex antagonist associated with PCa (SChLAP1) is located at chromosome 2q31.3. High levels of SChLAP1 expression have been associated with a more aggressive PCa phenotype [7]. In a study, overexpression of SChLAP1 was identified as the highest ranked gene that correlated with prognostic value and metastatic progression [14]. In addition, in a multivariate modeling analysis, SChLAP1 expression was an independent predictor of biochemical recurrence within 5 years, metastasis within 10 years, and death within 10 years [15].

Urothelial Carcinoma Associated 1

Urothelial carcinoma associated 1 (UCA1) is overexpressed in various human cancers [16]. Moreover, in a study performed on 40 PCa tissue specimens, high UCA1 expression was observed in patients with Gleason score ≥8 compared to Gleason score <8 [16]. Further investigations must be performed in order to determine whether UCA1 expression status might predict differences in Gleason score <8.

Proteins

Phosphatase and Tensin Homolog

Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene, and its inactivation in PCa has been shown to be associated with higher Gleason score, more aggressive disease features, worse prognosis, and PCa recurrence [17, 18]. PTEN inactivation, by deletion or mutation, is identified in ~20% of primary PCa from RP samples and in ~50% of castration-resistant tumors [17].

Ki-67

Ki-67 is determined via immunohistochemistry, and results are reported as a proliferation index. In a multiinstitutional tissue microarray study including over 192 RP specimens, a high Ki-67 proliferation index was strongly associated with a higher Gleason score, seminal vesicle invasion, extracapsular extension, and probability of recurrence [19].

Commercially Available Prostate Tissue Tests

Several biomarker tests have been shown to predict PCa progression and are recommended to inform decision-making for AS; these include Decipher, Prolaris, Oncotype DX Prostate, and ProMark [20, 21] (shown in Table 1).

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Table 1. Commercialized tissue-based biomarker tests for PCa risk stratification

Test	Company	Available, year	Biomarkers	Substrate	Clinical endpoints	Target patient	Technique	Certification	References
Decipher	GenomDX (Vancouver, BC Canada)	2015 in USA	22 coding and noncoding RNAs (*LASP1*, *IQGAP3*, *NFIB*, *S1PR4*, *THBS2*, *ANO7*, *PCDH7*, *MYBPC1*, *EPPK1*, *TSBP*, *PBX1*, *NUSAP1*, *ZWILCH*, *UBE2C*, *CAMK2N1*, *RABGAP1*, *PCAT-32*, *GLYATL1P4/ PCAT-80*, and *TNFRSF19)*	FFPE tissue from prostate tumor biopsies	Scores from 0 to 1 classify patients as high risk 0.61–1.0 (1 in 5 risk of metastasis), average risk 0.46–0.6, or low risk 0–0.45 (1 in 42 risk of metastasis)	Patients with localized disease on biopsy	RNA microarray	CLIA-certified. Test is not approved by the FDA. NCCN guidelines state that the test can be considered to be used in patients with localized tumors, but studies to compare effectiveness are necessary to prove clinical utility and improve risk stratification. The test is not cited by the EAU guidelines. Limited Medicare coverage	Alford et al. (2017) [20] Eggener et al. (2020) [21] Glaser et al. (2017) [47] Kim et al. (2019) [6] Nevo et al. (2020) [27]
Prolaris	Myriad Genetics, Salt Lake City, UT, USA	2012 in USA	*31* CCP genes*: CDKN3, RRM2, RAD54L, RAD51, CDC20, CDC2, BUB1B, PLK1, TOP2A, PTTG1, FOXM1, KIF11, KIAA0101, NUSAP1, CENPF, ASPM, DLGAP5, BIRC5, KIF20A, TK1, PBK, ASF1B, C18orf24, CDCA3, MCM10, PRC1, DTL, CEP55, CENPM, CDCA8, and ORC6L*	(1) Prostate biopsy or a (2) RP specimen	Score from 0 to 10, with each 1 unit increase reflecting a doubling of risk of disease progression	Patients with AS	mRNA expression level	Prolaris is validated by the FDA for use in men with NCCN with low-risk PCa and for post-prostatectomy patients who are at high risk for PCa recurrence. It has also been incorporated as an option in the NCCN and European Association of Urology guidelines for PCa	Alfrod et al. (2017) [20] Cuzick et al. (2011) [24] Eggener et al. (2020) [21] Nevo et al. (2020) [27]
Oncotype Dx	Genomic Health, Redwood City, CA, USA	2013 in USA	Use 5 reference genes (*ARF, ATP5E, CLTC, GPS1, and PGK1*) and 12 genes representing 4 biologic pathways with known roles in PCa tumorigenesis: the androgen pathway (*AZGP1, KLK2, SRD5A2,* and *FAM13C*), cellular organization (*FLNC, GSN, TPM2,* and *GSTM2*), stromal response (*BGN, COL1A1,* and *SFRP4*), and proliferation (*TPX2*)	Paraffin-embedded prostate needle biopsy tissue	Score from 0 to 100, where a value 0 is lowest risk and 100 is highest risk	Patients with AS	Real-time RT-PCR	CLIA-certified	Cullen et al. (2015) [29] Cullen et al. (2021) [33] Eggener et al. (2019) [26] Klein et al. (2014) [28] Lin et al. (2020) [34] Nevo et al. (2020) [27]
ProMark	Metamark, Waltham, MA, USA	2014	Use 8-protein signature: DERL1, CUL2, SMAD4, PDSS2, HSPA9, FUS, pS6, and YBOX1	Biopsy tissue	Score from 1 to 100 indicating the aggressiveness of PCa	Patients with AS	Quantitative multiplex proteomic	CLIA-certified laboratory and accreditation from the College of American Pathologists	Alfrod et al. (2017) [20] Eggener et al. (2020) [21] Blume-Jensen et al. (2015) [36]

FFPE, formalin-fixed paraffin-embedded; FDA, US Food and Drug Administration; NCCN, National Comprehensive Cancer Network; CLIA, Clinical Laboratory Improvement Amendments.

Decipher® PCa Test

Decipher is a gene expression assay developed to predict PCa aggressiveness [22]. The expression of 22 genes involved in cell proliferation, migration, tumor motility, androgen signaling, and immune system evasion is evaluated from formalin-fixed, paraffin-embedded tissue specimens. The results obtained from biopsy samples are used to determine a Decipher score (from 0 to 1) and categorize patients as high, intermediate, or low risk to decide between AS and traditional treatment [21]. Kim et al. [6] and Herlemann et al. [23] performed retrospective studies in biopsy samples and RP tissue from PCa patients and found that Decipher was an independent predictor of unfavorable disease and might improve the selection of candidates for AS.

Prolaris

Prolaris is a gene expression-based assay that evaluates genes associated with growth rate and potential tumor aggressiveness. The test can be performed on either a prostate biopsy or RP specimens [20, 21]. The test reports a score based on the average expression of a total 46 genes, including 31 cell cycle progression (CCP) genes known to be upregulated in aggressive PCa, with expression levels compared with 15 reference genes [24]. According to the National Comprehensive Cancer Network (NCCN) guidelines, it is recommended for patients with very low and low-risk PCa with at least 10 years of life expectancy at diagnosis [22]. Prolaris Biopsy reports a CCP score that ranges from 0 to 10; a higher score indicates a more aggressive cancer, and each unit increase reflects a doubling of risk of disease progression. Prolaris CCP score shows a direct correlation with tumor aggressiveness and recurrence and provides information on the risk of PCa death. Prolaris has shown clinical utility to guide AS decision-making after PCa diagnosis [25].

Oncotype Dx

Oncotype is an RT-PCR-based test that analyzes the expression of 17 genes, 5 reference genes, and 12 cancer-related genes of different neoplastic pathways involved in 4 biological processes that include androgen signaling, cellular organization, stromal response, and cellular proliferation [26]. The test uses prostate needle biopsy tissue specimens from PCa patients diagnosed with Gleason score 3 + 3 or 3 + 4 to predict aggressiveness. Oncotype reports a "Genomic Prostate Score" (GPS) that ranges from 0 to 100, where a value 0 represents the lowest risk and a value of 100 the highest risk [27]. It also reports three important endpoints: (i) the risk for PCa death; (ii) the risk for metastasis within 10 years; and (iii) the risk for subsequent upgrading to adverse pathology on RP [22, 25]. The test is used most often to help in the selection of patients for AS. Klein et al. [28] analyzed retrospectively 395 needle biopsies from patients with low to intermediate clinical risk who were candidates for AS and showed that GPS predicted the presence or absence of adverse pathology. In addition, two studies analyzed the effectiveness of the Oncotype test among 431 and 134 patients with very low-, low- and intermediate-risk PCa, who underwent needle biopsy and demonstrated that GPS predicted the PCa aggressiveness [29, 30]. Two retrospective studies by Kornberg et al. showed that GPS at baseline is an independent predictor of adverse pathology and is associated with an upgrade reclassification [31, 32]. Moreover, GPS predictive value has been validated in African American and Caucasian American PCa patients [33]. However, two additional studies [34, 35] did not find association of GPS with grade reclassification in surveillance biopsy and in patients with NCCN very low- and low-risk PCa, GPS did not demonstrate significant differences by disease volume at prostate biopsy.

ProMark

ProMark uses immunofluorescent staining and quantitative multiplex proteomic analyses to measure the expression of 8 proteins involved in cell proliferation, stress response, and signaling pathways [36]. ProMark reports a score between 1 and 100, with a low ProMark score reflecting a less aggressive PCa. The test predicts cancer aggressiveness and PCa-specific mortality in patients with Gleason score 3 + 3 or 3 + 4 to guide treatment decision-making [20, 21].

Blood Biomarkers

Tumor markers found in blood are a main diagnostic tool used for clinical diagnosis and prognosis in numerous types of cancers. Tumor markers in blood are molecules produced by malignant cells or by benign cells from the tumor microenvironment in response to malignant cells that are released into the bloodstream. These kinds of biomarkers allow early detection of cancer and evaluation of treatment response and may serve as indicators of disease progression. Several blood biomarkers have been identified that can assist with PCa patient selection for AS.

Table 2. Commercialized blood-based biomarker tests for PCa risk stratification

Test	Company	Available, year	Biomarkers	Substrate	Clinical endpoints	Target patient	Technique	Certification	References
4Kscore® test	OPKO Health, Miami, USA	Information not found	Kallikrein proteins as pro-PSA and pro-hK2	Noninvasive blood test marketed (plasma)	The test result itself is a personalized predictive value that calculates the risk (probability) of finding a Gleason score 7 or higher PCa	Men with prior negative biopsy results and presently elevated PSA levels	Level expressions	Was approved by the FDA in 2021	Alford et al. (2017) [20] Borque-Fernando et al. (2019) [53] Duffy (2020) [5] Glaser et al. (2017) [47] Punnen et al. (2018) [52]
PHI	Beckman Coulter, Brea, CA, USA	2012	Formula that combines all three forms: total PSA, fPSA, and p2PSA	Noninvasive blood test marketed (serum)	PHI scores of 0–26.9, 27.0–35.9, 36.0–54.9, and ≥55.0 correlate with probabilities of Gleason ≥7 cancer on biopsy of 9.8%, 16.8%, 33.3%, and 50.1%, respectively	Reports risk of aggressive cancer as a four-tiered probability based on various score cutoffs for use in men aged ≥50 years with serum PSA 4–10 ng/mL and negative DRE findings	Using the equation {(p2PSA/fPSA) × tPSA1/2} to predict risk of Gleason ≥7 disease on biopsy	Was approved by the FDA in 2012	Catalona et al. (2011) [54] Duffy (2020) [5] White et al. (2017) [9]

DRE, digital rectal examination; FDA, US Food and Drug Administration.

Proteins

Prostate-Specific Antigen

PSA was identified originally as a blood marker for monitoring treatment response [3]. PSA can bind to normal plasma proteins; however, the majority of PSA in blood is free or unbound (free PSA [fPSA]). PSA levels do not differentiate between low and intermediate PCa risks; however, the ratio of fPSA and total PSA, in addition to the presence of p2PSA or pro-PSA is useful in the determination of PCa risk and aggressiveness [37] and currently is used as biomarkers in commercially available blood tests (shown in Table 2). Other studies have determined that PSA density is an independent predictor of progression in men with low-risk PCa during AS; however, the best PSA cut-off value remains under investigation [38].

Caveolin-1

Caveolin-1 (Cav-1) is a scaffolding protein and a major structural component of caveolae, and it is secreted by PCa cells. Cav-1 is reportedly overexpressed in PCa cells and is associated with disease progression through multiple mechanisms and signaling pathways [39–41]. Moreover, high levels of Cav-1 in serum and plasma of PCa patients are associated with adverse clinicopathological features [40]. A recent retrospective study showed that Cav-1 plasma levels improve the risk stratification for AS patients (OR 1.82, 95% CI, 1.24–2.65, $p = 0.002$) [39].

Hormones

Testosterone

Low serum levels of total testosterone (<3 ng/mL) have been associated with high-grade PCa and high PCa stage at diagnosis [42]. Another study reported that men with free testosterone levels ≥1.5 ng/dL are more likely to have low-risk PCa [43]. However, more studies are required before levels of total or free testosterone can be utilized for AS decision-making.

miRNAs

Several miRNAs have shown differential expression between low- and high-risk PCa patients [44, 45]. Shen et al. [45] determined that the expression of four miRNAs, miR-20a, miR-21, miR-145, and miR-221, could distinguish high- from low-risk PCa patients by D'Amico score with an area under the curve of 0.824. In addition, circulating miRNA-375 and miRNA-141 levels were correlated with high Gleason score or lymph node-positive status [44]. A prospective study, using serum samples from 2 independent AS cohorts of 196 and 133 patients, showed

that a 3-miR (miRNA-223, miRNA-24, and miRNA-375) score predicted patient reclassification (validation OR 3.70, 95% CI, 1.29e10.6) [46].

Circulating Tumor Cells

Detection of circulating prostate cells or circulating tumor cells (CTCs), depending on the study and the identification markers utilized, has gained much attention as potential markers to predict clinically significant PCa. Several studies have reported that an increased number of these circulating cells (circulating prostate cell and CTC) correlate with more rapid progression of the disease [47]. Murray et al. [48] showed, in a prospective study that included 1,123 PCa patients, that negative CTC correlated with low-grade, small volume tumors, and most often would comply with the criteria for AS. In addition, more recent work by Murray et al. [49]confirmed that presence of CTCs in PCa patients fulfilling AS criteria represents a high risk of disease upgrade; thus, these men may not be ideal candidates for AS.

Stockholm3 Test

The Stockholm3 test is a PCa risk model based in a multivariate algorithm that combines analyses of plasma protein biomarkers (total PSA, fPSA, human glandular kallikrein 2 [hK2], macrophage inhibitory cytokine-1, microseminoprotein-beta, 101 germline genetic markers, and 5 clinical variables (age, first-degree family history of PCa, a previous biopsy, digital rectal examination [DRE], and prostate volume assessed by transrectal ultrasound at PCa diagnosis) [50, 51]. In a recent study, Olsson et al. evaluated Stockholm3 test in AS prospective cohort (n = 280) and showed that adding the Stockholm3 test as a selection tool before mpMRI increased sensitivity by 27% to detect GS >6 and by 53% to detect clinically significant PCa compared with systematic biopsies [4, 40]. At present, Stockholm3 is available in Sweden, Finland, Norway, and Denmark, and it has been validated in several European countries.

Commercially Available Blood Tests

So far, two commercially available blood tests have been developed to predict high-risk PCa and to provide valuable information to guide decision-making when selecting patients for AS, 4Kscore® test, and Prostate Health Index (PHI) (shown in Table 2).

The 4Kscore® Test

This test measures four biomarkers: total PSA (human kallikrein 3); fPSA; intact PSA; and hK2. The levels of both kallikreins increase in circulation as the tumor becomes poorly differentiated, perhaps due to loss of tissue architecture [52]. This test assesses the patient's risk for aggressive PCa after an abnormal PSA result or DRE [27] and is especially useful when considering whether to perform a prostate biopsy [52]. The 4Kscore® test provides a percent risk ranging from 1 to 95% to evaluate PCa aggressiveness. A study that analyzed the benefit of the 4Kscore® test in 137 patients concluded that this test was a useful tool in the decision-making process as to whether to perform a confirmatory biopsy as the first step in the AS program [53].

Prostate Health Index

PHI was approved by the US Food and Drug Administration (FDA) in 2012 based on the analysis of three different isoforms of PSA: fPSA; total PSA; and (−2)pro-PSA (p2PSA) using the mathematical formula: phi = ([−2]pro-PSA/fPSA) × √PSA to predict risk of Gleason sum ≥7 diseases in prostate biopsies [54]. p2PSA refers to a pro-protein isoform of PSA with a 2-amino acid leader peptide sequence (normally pro-PSA has a 7-amino acid leader). p2PSA has been identified as the most PCa-specific isoform of PSA and is associated with more aggressive disease [54].

Urine Biomarkers

The use of urine samples has emerged as a noninvasive diagnostic and prognostic method for PCa diagnosis and determination of PCa aggressiveness [55]. Different biomarkers obtained from urine samples have been studied; some are commercially available, and several others are currently under preclinical investigation or in clinical trials.

mRNAs

Few mRNAs have been detected in PCa patient urine with prognostic value. Increased mRNA levels of homeobox C6 (*HOXC6*), distal-less homeobox 1 (*DLX1*), and Tudor domain containing 1 (*TDRD1*) in urine have been correlated with clinically significant cancer at biopsy or prostatectomy, high Gleason score, upgrading of Gleason grade at prostatectomy, and tumor size [55]. TMPRSS2:ERG fusion transcript is already in use in several commercially available tests to detect aggressive PCA [56] (shown in Table 3).

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Table 3. Commercialized urine-based biomarker tests for PCa risk stratification

Test	Company	Available, year	Biomarkers	Substrate	Clinical endpoints	Target patient	Technique	Certification	References
ExoDx Prostate (IntelliScore)	Exosome Diagnostics, Inc., Waltham, MA	Information not found	Exosomal RNA or expression of PCA3, TMPRSS2-ERG, and SPDEF.	Noninvasive urine test (urine after DRE)	The result in men with <15.6 has low risk or benign, potentially monitoring patient and >15.6 higher risk, and proceeds to biopsy	Men at risk of PCa (50-year-old or older men, PSA level between 2 and 10 mg/mL	RNA-Seq data from extracellular vesicles	Not currently FDA-cleared, but has CLIA accreditation	Nevo et al. (2020) [27] Tutrone et al. (2020) [63]
SelectMDx	MDxHealth, Irvine, CA	Information not found	Expression of DLX1, HOXC6, and TDRD1	Noninvasive urine test (urine after DRE)	The test identifies aggressive PCa (Gleason score ≥7)	The test has an accuracy of 98% for detecting significant (Gleason 3+4 and higher) PCa	Reverse transcription PCR	Not currently FDA-cleared, but has CAP accreditation, included in the 2020 NCCN Guidelines for Prostate Cancer Early Detection CLIA-certified	Alford et al. (2017) [20] Duffy et al. (2020) [5] Haese et al. (2019) [64] Nevo et al. (2020) [27] Van Neste et al. (2016) [55]
Michigan Prostate Score	The University of Michigan Comprehensive Cancer Center, USA	2013	Expression of PSA, T2MPRSS:ERG, and PCA3 in combination	Noninvasive urine test (urine after DRE)	The test has a very high predictive accuracy (AUC 0.85) for high-grade PCa. A score of 1–100 reflects the percent chance of finding any PCa on biopsy, and the score report also provides a risk estimate for detecting cancer of Gleason score ≥7	Predicted the likelihood of cancer and the likelihood of high-risk cancer (clinically significant Gleason 7 or higher)	Serum PSA level, urine PCA3 mRNA, and urine TMPRSS2:ERG mRNA	CLIA-certified	Alford et al. (2017) [20] Duffy et al. (2020) [5] Lin et al. (2013) [59] Nevo et al. (2020) [27] Tomlins et al. (2016) [56]
Prostarix risk score	Metabolon, Durham, NC, USA	2013	Four metabolites, sarcosine, glycine, alanine and glutamate	Noninvasive urine test (urine after DRE)	Used in a proprietary algorithm to generate a risk score, ranging from 0 to 100 that predicts the likelihood of cancer on a prostate biopsy result	Can improve the sensitivity and specificity of PCa risk stratification in men with negative DREs and moderately elevated PSA levels (4–10 ng/mL)	This quantitative liquid chromatography-mass spectroscopy-based test measures the concentrations of four amino acids in the cell pellet of urine spun down post-DRE	Not currently FDA-cleared	Lee et al. (2020) [61] Malik et al. (2019) [65]
PTEN/ TMPRSS2:ERG	Metamark, Waltham, MA, USA	Information not found	Fusion gene TMPRSS2:ERG and the tumor suppressor gene PTEN	Noninvasive urine test (urine after DRE)	Use in men with Gleason 3+3 or 3+4 disease on biopsy, as well as those with an atypical/HGPIN	May be of use in both prognosis and diagnosis when combined with other clinical parameters	The presence of *TMPRSS2:ERG* and/or the absence of *PTEN* indicates a more aggressive cancer	CLIA-certified	Alford et al. (2017) [20] Trock et al. (2016) [18]
PROGENSA PCA3	Metamark, Waltham, MA, USA	2012	PCA3 and PSA RNA	Noninvasive urine test (urine after DRE)	A PCA3 score is determined using PCA3/PSA RNA ratio	May be of use in both prognosis and diagnosis when combined with other clinical parameters	The assay combines transcription-mediated amplification and hybridization protection assay, to amplify target RNA and detect amplicon, respectively	Was approved by the FDA in 2012	Alford et al. (2017) [20] Duffy et al. (2020) [5] Malik et al. (2019) [65] Nevo et al. (2020) [27] Pastor-Navarro et al. (2021) [66]

AUC, area under the curve; DRE, digital rectal examination; FDA, US Food and Drug Administration; NCCN, National Comprehensive Cancer Network; CLIA, Clinical Laboratory Improvement Amendments; CAP, College of American Pathologists.

Long Noncoding RNAs

Prostate Cancer Antigen 3

Prostate cancer antigen 3 (PCA3) is a proven urine biomarker that is utilized by several commercially available tests, such as PROGENSA PCA3, Michigan prostate score (MiPS), and ExoDx Prostate(IntelliScore). PCA3 levels have been shown to be useful for early detection of PCa. Tosoian et al. [57] showed that PCA3 score obtained during AS was higher in men who underwent Gleason grade reclassification GR, suggesting that PCA3 provides incremental prognostic information in the AS setting. In addition, Wei et al. [58] reported that PCA3 had a positive predictive value (PPV) of 80% for an initial biopsy. In those with a PCA3 score> 60 units, the specificity for PCa was 0.91 (95% CI, 0.87–0.94) and the sensitivity was 0.42 (0.36–0.48). However, PCA3 levels in urine as an individual biomarker for AS decision-making, such as in the PROGENSA PCA3 test, remain controversial [59].

SCHLAP1

SCHLAP1 is found at high levels in urine sediment samples after prostate exam in PCa patients, and its level positively correlates with Gleason score [7]. Moreover, SChLAP1 expression levels independently predict poor outcomes, including metastasis within 10 years, suggesting that SChLAP1 represents a promising biomarker for aggressive PCa [60].

Metabolites

Several metabolites in urine have been analyzed as markers to improve diagnosis and identify PCa progression, including alanine, aspartate, and glutamate, among others [61]. In addition, several studies found decreased levels of glycine, glutamine, tyrosine, and citrate; and elevated levels of valine, taurine, and leucine in PCa patient urine [62].

Commercially Available Urine Tests

Several urine-based tests have been developed to predict high-risk PCa that might help in the selection of patients for AS (shown in Table 3).

ExoDx Prostate(IntelliScore)

This is an exosomal RNA (lncRNA, mRNA) test designed for detection of PCa based on a 3-gene expression assay that includes PCA3, ERG (including TMPRSS2-ERG fusion gen), and SPDEF (sterile alpha motif-pointed domain-containing ETS transcription factor). ExoDx uses a reverse transcription-based quantitative polymerase chain reaction to measure the levels of the three mRNAs [27]. The test has been validated to discriminate between Gleason 6 and Gleason ≥7 PC [63].

SelectMDx

SelectMDx is a test that measures the mRNA levels of two genes, DLX1 and HOXC6. The test is used to stratify patients with clinically significant PCa disease and to select patients for AS [55, 64].

Michigan Prostate Score

MiPS is a test that combines serum PSA levels with two urine mRNA and lncRNA biomarkers, transmembrane protease serine 2 (*TMPRSS2):ERG* and PCA3, respectively. The test reports a score ranging from 1 to 100, which reflects the likelihood of finding PCa on prostate biopsy and the risk of developing a clinically significant disease (Gleason sum ≥7) [5, 27, 56].

Prostarix Risk Score

This is a test that determines the concentration of 4 metabolites (sarcosine, glycine, alanine, and glutamate) in urine to generate a risk score between 0 and 100 that predicts the chances of finding PCa on prostate biopsy [55, 65].

PTEN/*TMPRSS2:ERG*

This test is a molecular assay for the use on prostate biopsies with Gleason score 3 + 3 or 3 + 4, atypia or high-grade prostatic intraepithelial neoplasia (HGPIN). In men with atypia or HGPIN, the use of PTEN/*TMPRSS2:ERG* test might lead to an earlier diagnosis of potentially aggressive PCa. The test predicts PCa aggressiveness by measuring the presence or absence of PTEN and the *TMPRSS2:ERG* translocation/gene fusion [18]. The fusion gene *TMPRSS2:ERG* involves the *TMPRSS2* and v-ets erythroblastosis virus E26 oncogene homolog (*ERG*).

PROGENSA PCA3

PROGENSA PCA3 is the first FDA-approved urine-based molecular test that detects the overexpression of the PCA3 gene [66]. In conjunction with clinical information, this test could help in the decision to repeat a biopsy. The benefit of this test for AS patient selection or monitoring of disease progression has been suggested; however, results have been controversial and more studies are needed to resolve its potential contribution on these processes [59].

Multiparametric Prostate Magnetic Resonance Imaging and High-Resolution Micro-Ultrasound

Beyond the available prognostic tests based on biomarkers, the introduction of mpMRI has changed the strategy to detect PCa because it significantly reduces the detection of insignificant PCa when used as a triage tool to select men for prostate biopsy [21]. Prostate Imaging Information and Reporting System (PI-RADS) score helps locate clinically significant PCa (Gleason sum ≥7 and/or volume ≥0.5 cc and/or extraprostatic extension) [47]. Overall, prostate mpMRI performed according to PI-RADS v2 demonstrated high sensitivity and specificity for the detection of clinically significant PCa. A systematic review of the diagnostic performance of PI-RADS v2 for detection of PCa showed 89% sensitivity for clinically significant PCa, with a specificity of 64% [67].

In men suitable for AS, mpMRI of the prostate is often obtained to ensure that high-grade disease has not been missed [47]. However, there is limited prospective evidence regarding the diagnostic accuracy of serial mpMRI for monitoring patients on AS. The role mpMRI in AS monitoring continues to evolve. mpMRI currently cannot replace prostate biopsy nor has its use been compared to or combined with tissue, blood, or urine-based biomarkers.

High-resolution micro-ultrasound (micro-u/s) has the capability of imaging PCa based on detecting alterations in ductal anatomy. Micro-u/s is a low-cost, single-session option for prostate screening and targeted biopsy [68]. Therefore, it has a role in the diagnosis of PCa and, more recently, a role in AS patient selection criteria has also been demonstrated. In a multicenter prospective study that included 1,040 men who had previous mpMRI and underwent ExactVu micro-u/s, biopsies were taken from both mpMRI targets and micro-u/s targets, and systematic samples [68]. Overall, 39.5% were positive for clinically significant PCa. Micro-u/s had better sensitivity and negative predictive value than mpMRI (sensitivity was 94 vs. 90%, respectively (p = 0.03), and negative predictive value was 85% versus 77%, respectively). Specificities of micro-u/s and MRI were both 22%, with similar PPV (44 vs. 43%). In a systematic review recently published by Parker et al. [69], the authors reviewed the utility of different modern ultrasounds in AS for PCa. Twelve studies, utilizing the range of ultrasound parameters of B-mode, micro-u/s, color Doppler, contrast ultrasound, and elastography, were included. The review concluded that micro-u/s is a comparable tool to mpMRI for AS patient selection.

Therefore, micro-u/s is a relatively new tool for diagnosis and follow-up in a patient on AS. Micro-u/s is less expensive than mpMRI with better sensitivity and negative predictive value and, at least, same specificities and PPV; however, it is still not universally available.

Discussion

Overtreatment has long been a dilemma in PCa that has been somewhat attenuated with the adoption of AS. However, the ability to properly select patients with low-risk PCa who will not progress during AS remains a major challenge for clinicians. Misclassifying PCa risk is an evident concern and is one of the main reasons for patient anxiety and underutilization of AS [70]. Consequently, substantial efforts have been invested to identify new biomarkers that can distinguish intermediate and high-risk PCa from low-risk PCa [71]. Additionally, biomarkers with high sensitivity and specificity are needed to monitor disease progress during AS.

Many prostate tissue-based tests have been developed to identify PCa and recognize high-risk disease [20]. Commercial tests able to differentiate clinically relevant diseases have been evaluated for their potential to properly select patient candidates for AS. Oncotype DX, ProMark, PTEN/TMPRSS2:ERG, Prolaris, and Decipher provide valuable information for AS decision-making. However, more validation studies are required to solve some disparate data results. The advantages of tissue-based tests are that analyses are done in the tissue affected; however, cancer heterogeneity might affect the test results. In addition, these tests are in general expensive; thus, cost-effectiveness research is needed [72]. Moreover, its use in the disease follow-up is limited, since serial biopsies entail procedure complications, which increases patient anxiety and reduces patient compliance.

Further studies are required regarding the best test for each patient and how they should be used in conjunction with mpMRI. The use of both mpMRI and tissue-based biomarkers provides valuable information; however, both are costly [21]. In terms of imaging, micro-u/s has emerged as a comparable and less expensive tool for AS patient selection than mpMRI; however, it is still not widespread available [73].

Liquid biopsy in PCa using blood or urine samples offers the advantage that they can be obtained easily to provide continuous information during AS management. Therefore, these types of non- or minimally invasive tests have been under extensive research. Moreover, these biomarkers are not influenced by tumor sampling which suggests greater stability in the assays with a global dis-

ease assessment [40]. Currently, two blood-based tests, PHI and 4Kscore, are FDA-approved and both tests predict the risk value of PCa with Gleason score ≥7. Specifically, PHI is a noninvasive and economically affordable test that has been shown to improve PCa diagnosis and to provide valuable information to manage PCa patients [21]. The effectiveness of PHI and other biomarker tests must be analyzed in conjunction with each other, and with mpMRI, to improve PCa detection, treatment, planning, and prognosis [71]. Regarding the urine-based tests commercially available, one has been FDA-approved, Progensa. Other tests based on urine samples have been developed, such as SelectMDx, Exosome Dx, and MiPS, which have provided evidence of the clinical benefits of their use. However, validation information is necessary for their approval as predictive markers. Urinary biomarkers are the ideal sample to reduce morbidity for AS selection and to facilitate compliance in men on AS. However, it must be taken into account that the urinary markers used should be minimally modified by external effects such as diet, microbiota, and other diseases which can affect urine metabolome and biomarker levels [62].

From the point of view of the clinical utility of biomarkers, there is a consensus that they are really necessary; however, they are undoubtedly underused. They are already indicated and recommended in clinical guidelines such as the NCCN in the low-risk and intermediate-risk group. They highlight the role of tissue markers such as Prolaris and Oncotype DX, to which decipher has been added in AS. Biomarkers, also, have an emerging role, assuming a dynamic profile of the cancer and, therefore, replacing follow-up biopsies. Micro-u/s is complementary to resonance. In general, in patients with PSA between 4 ng/mL and 10 with ng/mL and a normal micro-u/s, biopsy would not be indicated. In AS, micro-u/s and resonance can be combined, since the latter is more validated than micro-u/s. There is currently agreement that low-risk and intermediate-risk patients are candidates for AS. In the intermediate-risk group with <5% pattern 4, there is a significant percentage of patients who ultimately have only pattern 3 in the surgical specimen, so these patients should be treated with AS. However, in patients with intermediate risk, with >5% pattern 4, biomarkers, especially tissue ones, have a crucial role in the adequate selection for AS, since up to 30% of metastatic disease has been found in the pre-MRI era at 15-year follow-up. These patients could be candidates for focal therapy, without the use of biomarkers, which is why biomarkers can play a significant role in defining best candidates for AS, especially in intermediate-risk group.

Conclusion

Even though some improvements have occurred in selection criteria for identification of patients suitable for AS, the role for each commercially available test remains unclear. New and better biomarkers for PCa that detect reliably clinically relevant disease remain an unrealized need. The scenario in which biomarkers play a critical role, especially tissue biomarkers, is in the group of patients with intermediate risk (Gleason 3 + 4, ISUP 2) with more than 5% of grade 4. Imaging also plays a critical role with an emerging tool as micro-u/s which is less expensive than mpMRI. This is a key point, because financial toxicity plays a major role in deciding whether or not to use a biomarker. Thus, more studies, especially with collection of long-term outcomes and cost-effectiveness analysis, are needed to define the appropriate test for each clinical case to further improve patient selection for AS and to promote personalized medicine.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Paula C. Sotomayor and Ignacio F. San Francisco contributed to the review conception and design, to the acquisition of data, to the drafting of the manuscript, and to the critical revision of the manuscript for important intellectual content. Juan C. Aguilar contributed to the drafting of the manuscript. Karen Mujica contributed to the acquisition of data and to the drafting of the manuscript. Alvaro Zuñiga contributed to the acquisition of data. Alejandro S. Godoy contributed to review conception and design, to the drafting of the manuscript, and to the critical revision of the manuscript for important intellectual content. Gary J. Smith contributed to the drafting of the manuscript and to the critical revision of the manuscript for important intellectual content. James L. Mohler contributed to the review conception, design, and drafting of the manuscript and to the critical revision of the manuscript for important intellectual content. Gonzalo Vitagliano contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the manuscript.

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