Modern urology perspectives on prostate cancer biomarkers

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Introduction Prostate cancer (PCa) is the most common type of cancer among men in Europe. Current recommendations for screening and diagnosis are based on prostate-specific antigen (PSA) measurements and the digital rectal examination (DRE). Both of them are triggers for prostate biopsy. Limited specificity of the PSA test brings, however, a need to develop new, better diagnostic tools. Several commercially available variations of the PSA test including: prostate health index (PHI), 4Kscore as well as molecular PCA3 score, have already revealed its value, lowering the number of unnecessary biopsies.
Material and methods This review summarizes published results of the current most promising, clinically proven and experimentally evaluated PCa biomarkers which have potential for creation of new diagnostic tests.
Results In the last few years new approaches for providing significantly better biomarkers, an alternative to PSA, have been introduced. Modern biomarkers show improvement in being used as not only a diagnosis procedure, but also for staging, evaluating aggressiveness and managing the therapeutic process. The most promising group are molecular markers, among them microRNAs(miRNAs) and long noncoding RNAs (lncRNAs) are most frequent. Their superiority, over standard PSA, in predicting tumor formation in early stages, and clinically non-symptomatic metastases has been noticed. Extracellular vesicles presence in biofluids have brought focus of many research groups, indicating their potential significance. This group of nanoparticles has potential not only in diagnostic and therapy management process, but also as a potential therapeutic target.
Conclusions Finding better PCa biomarkers, replacing the current PSA measurement, is firmly needed in modern urology practice.

INTRODUCTION

Prostate cancer (PCa) is the most frequent type of cancer among males in Europe. Over the last few years, the stabilization of incidence rates in Western and Northern regions of Europe has been observed. As for the Eastern and Southern regions, the continuous rise of incidence was determined, reaching similar levels as the Northern and Western parts [1]. Predicted mortality in Europe for 2018 is around 77,000 deaths caused by PCa which is higher than observed in 2012: 71,840 deaths [2]. High PCa occurrence is a global problem, as a recent estimation of PCa incidence in USA for 2018 is around 164,690 of new PCa cases with estimated deaths related to PCa of 29,430 patients [3].

Current screening and diagnosis procedures of PCa recommended by EAU-ESTRO-SIOG are based on measurements of prostate-specific antigen (PSA) levels and detecting abnormalities through the digital rectal examination (DRE). PSA is considered to be a better predictor of cancer than the DRE or transrectal ultrasound (TRUS). Only after the DRE/TRUS and PSA test, biopsy should be considered to confirm suppositions. Twelve-core biopsy is recommended for the best diagnostic value with additional cores from suspected areas after DRE/TRUS. In the last decade due to many favorable

results reuse ofmultiparametric prostate magnetic resonance imaging (mpMRI) in DRE/TRUS place is considered. For pathological analysis, the Gleason Score is recommended to determine PCa grade. The Gleason Score is a resultant of the Gleason grade of the most extensive pattern and the highest pattern, regardless of its extent [4].

Despite the fact, there are many predicting models, considering not only age groups but also different risk aspects, usage of PSA tests in screening procedures is the main reason of overdiagnosis and overtreatment [5]. In such a situation searching for better tools to diagnose PCa is needed to prevent its overdiagnosis and overtreatment, which is actually observed with insufficient benefits in both overall and cancer-specific survival [6].

Why prostate-specific antigen is not a good marker for prostate cancer diagnosis?

Elevated levels of PSA is correlated with higher PCa risk. Since PSA screening has been introduced, the number of diagnosed PCa cases has increased and the mortality rates have decreased. Despite this fact, PSA remains a controversial biomarker [7]. U.S. Preventive Services Task Force Recommendation in 2012 was against the usage of PSA for PCa screening. Afterwards, observations showed better diagnosis rates of higher risk diseases, but also a reduction in the diagnosis of intermediate risk PCa [8]. Despite the high correlation between elevated PSA and PCa, there are several other factors causing elevated PSA level. Prostatitis, benign prostate hyperplasia (BPH) or any prostate trauma can result in the rise of PSA level. In contrast, numerous drugs including aspirin, are proven to decrease PSA level in the blood [9, 10]. Moreover, sensitivity and specificity of PSA is highly dependent of the cut off value, as for the most common 4 μg/ml it achieves 20% and 65%, respectively [11]. Anadditional fact that should be considered is the presence of the different forms of PSA in serum and their diagnostic importance. In the present reports, evaluation of their presence and ratios reveals higher specificity and sensitivity than total PSA level.

Prostate-specific antigen based tests as a possible better diagnostic tools

Prostate Health Index

Prostate Health Index (PHI) is a mathematical formula consisting of total PSA, free PSA (fPSA) which is observed to rise in PCa, as well as [−2] proPSA serum isoform (p2PSA), isoform of PSA most closely related to PCa. The equation for PHI, containing all of these forms of PSA is: (p2PSA/fPSA) × √PSA. Multicenter studies indicated that PHI reveals higher specificity than any of its components, where at 95% sensitivity PHI specificity was at 36.0% vs. 17.2% and 19.4% for total and percent fPSA, respectively [12]. Additionally, in the biopsy naïve population it has been validated that PHI shows better detection rates than PSA/fPSA, and reduces up to 40% of unnecessary biopsies. Moreover, the results of this study showed that use of 24 PHI cut off instead of PSA, could lead to avoiding even 21% of unnecessary invasive treatment of clinically insignificant cancers [13].

4Kscore

Four-kallikrein panel, also known as 4Kscore, is a test composed of a panel of four kallikrein proteins (total PSA, free PSA, intact PSA, and human kallikrein-related peptidase 2), age, DRE, and the history of prior biopsies, that allows to calculate the individual patient's risk of high-grade PCa. One of the most important strengths of this test is its personalization, as it includes patients' individual clinical history. A multi-institutional trial, confirmed that 4Kscore has significant potential to lower the number of unnecessary biopsies even up to 58% with delaying diagnosis of only 4.7% of Gleason ≥7 with a cut off ≥15% [14]. There are several studies confirming that the 4KScore can predict metastases even up to 20 years ahead with high accuracy with the area under the receiver operator curve (AUC) around 0.8–0.9 [15].

Prostate-specific antigen glycosylation

Oncogenic process is often connected with significant alterations of the cellular glycosylation patterns. Connecting this phenomenon with PSAs high specificity, results in rising of its predictive value. Analysis of 5 different lectin immunosorbant assays created to detect sialylation of total and free PSA revealed that α2,6-linked sialylation of total PSA has the potential to rise to its predictive value [16]. More complex studies on detection of PSA glycosylation specific changes in PCa indicated that not only sialylation, but also fucosylation changes between PCa and healthy/BPH patients. Establishing two methodologies of analysis of the core fucosylation and the sialic acid linkage of PSA N-glycans allowed to describe potential of α2,3-sialic acid percentage and the core fucosylation ratio in separating high-risk PCa from low-risk PCa or BPH patients. Use of the 30% cut off for percentage of α2,3-sialic acid results in 85.7% sensitivity and 95.5% specificity. Moreover,

correlation between percentage of α2,3-sialic acid in PSA with the Gleason Score of the tumor have been observed with AUC = 0.97 [17]. According to the obtained results analysis of PSA, glycosylation patterns can become additional diagnostic tools for patients with suspicious DRE/TRUS, lowering the number of unnecessary biopsies.

Other biomarkers with confirmed clinical significance

Prostate-specific membrane antigen

Prostate-specific membrane antigen (PSMA) is a transmembrane protein expressed in all prostatic tissue types, as well as, in carcinoma tissue. In opposite to PSA, this protein is an integral part of the cellular membrane and is not secreted by epithelial prostate cells. Attempts to assess PSMA level in serum or urine and correlate it with presence of clinically significant carcinoma have ended with unsatisfactory results [18]. Histopathological analyses revealed that within carcinoma tissue, highly homogenous elevated expression of PSMA was observed, indicating PSMA as a potential therapeutic target [19].

However, PSMA has presented very promising results in diagnosis when combined with positron emission tomography-computed tomography (PET/CT) within 68Ga-labelled PSMA PET/CT gaining the most interest. Many studies focused on staging and restaging with results indicating polled 65.5% sensitivity and 95% specificity [20]. The lesion-based analyses after 68Ga-labelled PSMA PET/CT revealed 76.6% sensitivity and 100% specificity. But what is also important, this method allows depicting not only localized carcinoma, but also metastatic sites [21]. Nevertheless, none of the adverse effects of 68Ga-labelled PSMA PET/CT have been reported in these studies [20].

PCA3

DD3, also known as PCA3, is a non-coding, prostate specific messenger RNA(mRNA). This mRNA is not only highly prostate specific, but is also found to be highly overexpressed, from 10 up to 100 times, in tumor area compared to non-neoplastic tissue. PCA3 assay is performed from urine samples collected after prostate massage, for example post DRE, with use of time-resolved fluorescence-based quantitativereverse transcription polymerase chain reaction (RT-PCR). The sensitivity of testing PCA3 with such methods reveals AUC = 0.72, in which with a cut off level of 0.2 achieved specificity and sensitivity which was at 83% and 67%, respectively [22].

More recent studies evaluated commercially available PROGENSA® PCA3 assay, and its clinical usefulness. This test relies on a different method of assay as it is based on transcription-mediated amplification (TMA™) which allows standardization and automation of many steps reducing pre-analytical and analytical mistakes [23]. Results of the study have showed that with a PCA3 score of 25 achieved sensitivity was 77.5% and specificity was 57.1%. Achieved AUC for predicting high grade, significant and any cancer was 0.638, 0.689 and 0.707, respectively. Additionally, combining PCA3 with models including SOC factors, such as age, DRE result, family history of PCa, race, tPSA and number of previous negative biopsies, allows to significantly improve PCa prediction, meaningfully rising AUC, specificity as well as negative and positive predicting values [24].

Circulating tumor cells and liquid biopsy

Circulating tumor cells (CTC) are defined as tumor cells circulating in peripheral blood whose origin is primary tumor or metastatic sites. CTC are also acknowledged as a mechanism of cancer metastases. One of the commercially available methods of isolation and quantification of CTC from blood (Cell-SearchTM) is based on expression of EpCAM (epithelial cell adhesion molecule) on their surface and immunoseparation of such cells with automated fluorescent staining for cytokeratin and nucleic acid. Study results focused on correlation of CTC level with staging and PCa diagnosis, showed low reliability of this biomarker in non-metastatic PCa. On the other hand significant increase in metastatic PCa was observed with highest values for osseous and visceral metastatic patients [25].

Liquid biopsy is a more complex method than CTC enumeration alone, as it also includes CTC nucleic material and cell-free circulating tumor DNAs and RNAs in blood. One of the methods of analysis is use of the Next Generation Sequencing. Advantage of such approach is inclusion in analysis CTC population that does not present EpCAM, after epithelial-to-mesenchymal transition (EMT). EpCAM is a transmembrane glycoprotein involved in cell to cell adhesion, migration and signalization of epithelial cells. EMT is one of the mechanisms of PCa metastases. Epithelial carcinoma cells through EMT lose their adhesion and gain migratory properties, which allow them to leave the primary tumor and enter the bloodstream as a CTC [26]. Results of liquid biopsy brings not only diagnostic and prognostic value, but also enables prediction of response to certain treatment. Androgen receptor (AR) and

its splice variants analysis reveal occurrence and mechanisms of development and progression of castrate resistant PCa (CRPC). Especially investigated is the AR-V7 variant, which is believed to be associated with the resistance to abiraterone and enzalutamide treatment [27].

Experimental biomarkers with potential clinical implication

microRNAs

MicroRNAs (miRNAs) are small endogenous single-stranded non-protein coding RNAs. Their mechanism of downregulation is based on imperfect complementary binding to target mRNAs 3'UTR region. Differences in expression of miRNAs in oncogenic processes, including PCa, have been multiply reported, suggesting their potential as biomarkers. Analysis is performed from serum samples byquantitative reverse transcription PCR (RT-qPCR), microarrays, or small RNA-sequencing. Upregulation of serum level of miR-9-3p, miR-330-3p-3p, and miR-345-5p in PCa patients compared to non-cancer individuals have been confirmed. Additionally, miR-345-5p has been suspected to play oncogenic role in PCa promoting CRPC cell growth and migration [28]. Network vulnerability analysis of differentially expressed or deregulated microRNAs is a novel bioinformatics model for biomarker discovery. Analysis of primary and metastatic PCa have identified two previously reported (miR-101-3p and miR-145-5p) and three new (miR-204-5p, miR-198 and miR-152) potential miRNA biomarkers for differentiating primary PCa from metastatic [29].

Recent studies are focusing on obtaining quality-assured results and on the standardization of the measurement which is fundamental in the diagnosing process. A plasma panel of four miRNAs including miR-4289, miR-326, miR-152-3p and miR-98-5p, evaluating their ability to predict prostate cancer has been checked. Results indicated that their combined predictive value is higher (AUC = 0.88) than individual [30]. Studies on miRNA isolation form urine exfoliated cells from first-catch of urine after prostate massage revealed that urine can also be a sufficient source for such analysis. Moreover, significant downregulation of let-7 family miRNAs among PCa patients sample was observed, suggesting their high diagnostic value and potential as a non-invasive biomarker for PCa [31]. Considering these results, a new panel of miRNAs can bring many information of not only diagnostic but also stratifying and predictive value, depending on the chosen set of miRNAs.

ZNF154

Epigenetic modifications, such as CpG islands methylation, which often leads to gene expression inhibition and function loss, are important for tumor diagnosis and treatment strategies. Analysis of hypermethylation patterns of genes in PCa indicated the highest significance for ZNF154 gene. In PCa, ZNF154 showed 3 methylation sites compared to BPH, and downregulation of its expression have been noticed. Analysis of these sites have shown high predictive value in distinguishing between the BPH and PCa with AUC=0.9003, confirming that ZNF154 can be used as a specific marker for the diagnosis of PCa. Additional relations between ZNF154 methylation level in advanced tumor stages and AR activity indicates it as a potential therapeutic target [32].

LAMC1

Mass spectrometry-based proteomic analysis of the conditioned media from PC-3 and DU-145 cell lines, which originates from different metastatic sites of human prostate cancer, revealed 128 up-regulated, 83 down-regulated and 6 mutated peptides. Overexpression and high secretion of LAMC1 (laminin gamma 1), which is confirmed to play a role also in other types of cancer, preferably by DU-145 cells, indicate that it can be used as a metastases location biomarker. Detected six mutated peptides needs to be evaluated on PCa patients samples to confirm their potential in diagnosis and prognosis of PCa [33].

RNCR3

Important role of long non-coding RNAs (lncRNAs) in development and progression of different types of cancer have been suggested in many studies, as well as their stability in body fluids. RNCR3 is reported to effect cell proliferation and differentiation, but the exact role in cancer is not yet clear. Recent study showed that RNCR3 expression is increased in malignant prostatic tissue. Moreover, correlation between RNCR3 level and tumor size, Gleason Score, clinical stage and survival rates was observed, indicating its potential role as an important biomarker for diagnostic and therapeutic reasons [34].

CCAT1

Another example of potentially useful lncRNA in diagnosis and treatment of PCa is CCAT1. qRT-PCR analysis of CCAT1 expression revealed its elevated level in PCa specimens. Although surprisingly it was

not related to age, PSA and Gleason score, but significantly involved in lymph node metastasis. Suppression of CCAT1 in prostate cancer cell line PC-3 with small interfering RNA resulted in reduction of their proliferation and migration ability, suggesting its role in these processes. The exact molecule mechanism of CCAT1 is yet to be discovered, but wields possibilities of providing new molecular markers and treatment targets for PCa [35].

Alternative approaches to PCa diagnosis

Sarcosine/Creatinine urinary ratio

Metabolomic analyses of PCa patients' urine have uncovered its potential as an alternative method of detection of carcinoma. Several compounds have shown significance in correlation with presence of PCa, among them sarcosine revealed in highest association. Unfortunately, subsequent study findings were questionable, thus attempts to improve its diagnostic value by combining it with creatinine level has been made. Comparison results between sarcosine/creatinine ratio (Sar/Cr), PSA and free/total PSAs predictive values revealed superiority of Sar/Cr. In group of any PSA level the AUC of Sar/Cr was 0.841 compared to 0.728 of PSA and 0.797 of free/total PSA, moreover with use of 0.062 cut off its sensitivity and specificity were at 81.3% and 75.9%, respectively. Additionally, significant differences between the urinary Sar/Cr ratio in patients with Gleason Score ≤6, 7, or ≥8 have been noticed, therefore showing its high potential to replace the PSA test as a diagnosing tool for PCa [36]. However, considering

Table 1. *Currently used and experimentally evaluated prostate cancer biomarkers*

	Marker	Sensitivity	Specificity	Advantages	Disadvantages
1	PSA (PHI, 4Kscore, PSA glycoforms)	Up to 85.7%	Up to 95.5%	Simple and fast detection methods	In many cases is leading to over-diagnosis and over-treatment
2	PSMA	76.6%	100%	More specific than PSA	Useful only in pathomorphological analysis and in vivo assessment with 68Ga-PSMA PET/CT
3	PCA3	83%	67%	Possible non-invasive evaluation	The most appropriate cutoff for PCA3 score remains controversial
4	Circulating Tumor Cells	n.a.	n.a.	Change in number correlates with response to treatment	Mainly associated with overall survival rate only
5	miRNA-345-5p miRNA-101-3p miRNA-145-5p miRNA-204-5p miRNA-198 miRNA-152 miRNA-4289 miRNA-326 miRNA-152-3p miRNA-98-5p miRNA-210	n.a.	n.a.	Allows non-invasive personalized management of therapy	Depends directly on the ability to obtain quality-assured results and on the standardization of the measurement
6	ZNF154	n.a.	n.a.	Hypermethylation correlates with development and recurrence	Exact function of ZNF154 in PCa still is not uncovered
7	LAMC1	n.a.	n.a.	Overexpression and extracellular secretion can suggest metastases	Depends directly on the ability to obtain quality-assured results and on the standardization of the measurement
8	RNCR3	n.a.	n.a.	Increased expression is correlated with tumor size, Gleason score, and clinical stage	Depends directly on the ability to obtain quality-assured results and on the standardization of the measurement
9	CCAT1	n.a.	n.a.	Increased expression is correlated with cancer progression, also is potential treating target	Depends directly on the ability to obtain quality-assured results and on the standardization of the measurement
10	Urinary Sarcosine/Creatinine ratio	Up to 81.3%	Up to 75.9%	Might be used as a potential indicator of metastatic prostate cancer	Specificity of the urinary sarcosine/creatinine ratio in the diagnosis of patients with low PSA levels is not well described yet
11	Extracellular vesicles	Up to 83%	Up to 92%	Exosomes can be source of many different markers and enables personalized treatment	Difficult isolation methods and variability of exosomes limits their present diagnostic value

PCa – prostate cancer, PHI – prostate health index, PSA – prostate-specific antigen, PSMA – prostate-specific membrane antigen, n.a. – not available

the poor clinical translation of the previously evaluated Prostarix assay, metabolomic profiling is rather unlikely to become a clinically useful test.

Extracellular vesicles

Extracellular vesicles (EV) are heterogeneous group of nanoscale membranous vesicles present in biological fluids. EV are actively released by cells and their contribution in cell-to-cell signaling has been reported, gathering curiosity for their potential diagnostic value and as a therapeutic target. There is growing amount of evidence of elevated amount of released EV during oncogenic process, but their role is strictly dependent on their cargo. Two classes of EV are gathering particular interest: exosomes and large oncosomes. Large oncosomes are EVs released at quantifiable levels only by tumor cells, containing specific, oncogenic material including signaling factors involved in: cell metabolism, mRNA processing and cell growth. Their amount is significantly related with cancers aggressiveness [37]. Exosomes are defined as EV of endosomal origin with a diameter between 30 and 100 nm. They are composed of lipids, proteins, mRNAs, miRNAs and even genomic DNA. Their presence in both: blood and urine have been proven by many studies, as well as their potential in PCa diagnosis. Several studies investigated exosomal proteins among which one of the best results were obtained with PSMA for exosomes isolated from plasma. Results revealed a risen amount of PSMA in PCa compared to BPH patients with an AUC = 0.943, specificity and sensibility at 92% and 83%, respectively. Additionally, significant correlation with Gleason Score and risk of recurrence was proven [38]. Exosomes can be a source of different molecular markers, among which miRNAs show most promising results. Analysis of urine isolated exosomes revealed miR2909, miR19b, miRNA21, and miR375 as a potential PCa diagnosis markers [39].

CONCLUSIONS

Currently recommended diagnostic tools for prostate cancer (PCa) provide many false positive results causing not only costly, but more importantly potentially harmful management of clinically non-significant prostate changes. Developing new, more specific tools is thus firmly needed. Among emerged new biomarkers, many still need large studies proving their diagnostic potential on larger groups (Table 1).

PCa is a malignancy causing alternations in the presence and level of many proteins, as well as many changes at molecular level, giving possibilities for different detection methods. Diagnostic tools used nowadays, especially prostate-specific antigen (PSA) level analysis, results in a high rate of overdiagnosis and overscreening. From the urologists point of view, differentiation of clinically significant PCa from not significant benign prostatic hyperplasia (BPH) is crucial. Novel tools need to enable better diagnostic resolution. Several different PSA test modifications, such as the Prostate Health Index (PHI) or 4Kscore have elevated its predictive value not only by including different forms of PSA present in body, but also considering individual risk based on clinical history. Another interesting branch is a group of molecular markers, with microRNAs (miRNAs) at the forefront. Their potential to reveal clinically significant changes at the very early stage is encouraging. Rising number of more advanced studies, as well as standardization of the analysis methods, yields promise of gaining tools to predict PCa before full tumor development.

On the other hand alternative approaches with use of metabolomics, and extracellular vesicles raise a number of possible sources of new, yet to be discovered, biomarkers and treatment targets.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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