Contemporary review of prognostic markers of prostate cancer from a pathologist perspective

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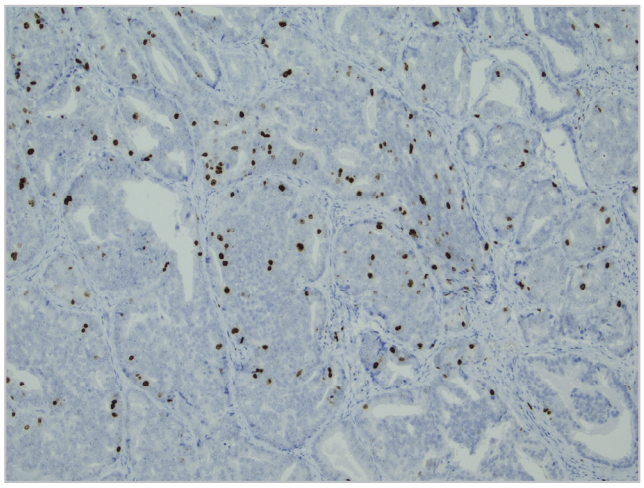
Prostate cancer is the most frequently diagnosed malignant tumour in men worldwide. To treat this condition, prognostic markers to distinguish indolent from aggressive disease, and biomarkers for metastatic forms are needed. From a pathologist's perspective, despite the plethora of emerging biomarkers, none to date has made its way into clinical practice. The need for prognostic and predictive markers following histological evaluation remains. This overview of some putative immunohistochemical and genetic markers reveals the pitfalls of biomarker research, notably verifiability, validity and interlaboratory comparison. Meta-analyses and extensive cooperation between pathology departments are a sine qua non. Codes of Best Practice such as the REMARK guidelines have been advocated as a path forward. Currently, the most widely used and validated prognostic marker remains the Gleason score. Ki67 along with PTEN are the most promising prognostic markers.

PROGNOSTIC MARKERS OF PROSTATE CANCER

- **There's a need to better stratify patients' prognosis following the histological evaluation, for adequate therapy choice.**
- **This study presents an overview of current and recently discussed immunohistochemical and genetic markers.**
- **The immunohistochemical stains discussed are PSA, PSMA, Ki67, PD-L1, CDK19, PTPN12, EZH2 and E-cadherin.**
- **The genetic markers discussed are TMPRSS2:ERG, SPOP, IDH-1, PTEN, TP53, RB1, CHD1 and SPINK1, androgen receptor, BRCA1 and BRCA2, ATM, MYC and CCND1.**
- **Gleason score remains the most widely used and validated prognostic marker.**
- **Ki67 and PTEN are the most promising potential prognostic markers.**
- **The problem of potential molecular markers is their difficult interlaboratory comparability.**



The researchers in prognostic pathology should adhere to the REMARK guidelines to achieve consensus, enable valid comparisons of published data, and validate new markers.

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Graphical Abstract

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INTRODUCTION

Prostate cancer (PCa) is the most frequently diagnosed malignant tumour in men worldwide, with a steadily increasing incidence in the Czech Republic, 149.8 new cases per 100,000 men in 2021 (ref.[1]), accounting for 1/4 of all newly diagnosed malignancies in men. The median age at diagnosis was 69 years[1]. With a rate of 27.6 deaths per 100,000 men, most often aged 77 years (median), prostate cancer is third in the ranking of malignant tumour deaths albeit 95.8% of treated patients survive for more than 5 years, and if the tumour is diagnosed at clinical stage I or II, the 5-year survival rate in the last 10 years is as high as 100% (ref.[1]). This was achieved in approximately 65% patients[1]. The survival rate of patients diagnosed in stage III of the disease is higher than 95%, but there is a significant difference in patients with stage IV, of whom less than 50% survive for more than 5 years[1]. At this clinical stage, however, the condition is detected in only 11% of patients[1]. For this reason, there is a need to identify possible risk factors and importantly, prognostic and predictive indicators of the risk of progression which can be used in routine clinical and pathological practice[1,2].

Radical prostatectomy (RP) or radiotherapy (RT) are offered as treatment options, depending on the clinical stage of the disease, patient age, existing health conditions, and in localized and locally advanced disease. When metastatic disease is found, systemic treatment is indicated. However, given the increasing number of low-risk cancers (T1 stage, PSA < 10 ng/mL, Gleason score 3+3) (ref.[3]), active surveillance is gaining more importance. This includes a strategy where radical treatment is only indicated on confirmation of more aggressive forms of the disease (e.g. an increase of the number of positive cancer cores in prostate rebiopsy, evidence of a Gleason score ≥ 3+4 or higher PSA dynamics[3].

In the monitoring of already diagnosed and treated patients, PSA (ref.[4]) (discussed below) is used as a sign of the biochemical progression, or increase in serum PSA levels. This may indicate the presence of viable tumour tissue, e.g. in the bed after prostatectomy, in the lymph nodes or in metastatic lesions. The definition of biochemical recurrence (BCR)/persistence differs after RP (any increase or progression of PSA after initially zero values) and after radical radiotherapy of the prostate (increase in PSA by more than 2 ng/mL (ref.[4]) above the lowest achieved value, called the nadir[4].

For the biochemical progression status of prostate cancer in an environment of very low serum testosterone concentrations, we use the term castration-resistant prostate cancer (CRPC). This was originally proposed by the Prostate Cancer Working Group 2 in 2008 to define the clinical or biochemical progression status of PCa in an environment of very low serum testosterone concentrations. The castration environment is defined as a sustained serum testosterone level below 50 ng/dL or 1.7 nmol/L (ref.[5]).

GLEASON SCORE

An integral part of histological examination is the determination of the Gleason score (GS), which was proposed in 1966 by Professor Gleason and has undergone a number of modifications since then. Histologically, prostate cancer is formed by small glands which differ from non-neoplastic glands mainly by the absence of basal cells. While in normal glands there are two layers of cells – the outer layer of basal cells and the inner layer of luminal cells, carcinomas consist of only one layer of luminal tumour cells. By far the most common histological type is acinar adenocarcinoma which is essential for determining the Gleason score[6-8].

According to the original scoring system, the architecture of the tumour cells is evaluated and classified from 1 to 5, where type 5 corresponds to the least differentiated type of carcinoma. In practice, however, types 1 and 2 do not occur, which is why the pathologist should not describe a category lower than 3. In the case of prostate biopsy, both the most common type of tumour and the least differentiated, i.e. the most aggressive type (e.g. 3+5) are reported. When evaluating material acquired by RP, the most common and the second most common type of tumour are reported, and if a minor component with the worst type is present, it is stated in brackets – e.g. 3+4+(5) (ref.[6-8]).

The resulting score is the sum of both numbers and can reach values of 6–10, based on which they are classified into so-called Gleason grade groups, which have prognostic significance for the patient. The Gleason score 3+3=6 falls into grade group 1 and represents the least aggressive tumour variant, which means a 97.5% (ref.[7]) chance of 5-year survival without biochemical progression of the tumour (see below)[6-8].

What is the percentage chance of 5-year survival without biochemical progression and to which grade group the appropriate Gleason score belongs is summarized in Table 1 (ref.[7]).

Table 1. Relation between Gleason score and biochemical progression.

Grade group	Included Gleason score	Chance of 5-year survival without biochemical progression
Grade group 1	Gleason score 3+3=6	97.5%
Grade group 2	Gleason score 3+4=7	93.1%
Grade group 3	Gleason score 4+3=7	78.1%
Grade group 4	Gleason score 4+4 =8; 3+5=8; 5+3=8	63.6%
Grade group 5	Gleason score 4+5=9; 5+4=9; 5+5=10	48.9%

Adapted from Shah and Zhou, 2016 (ref.[7]).

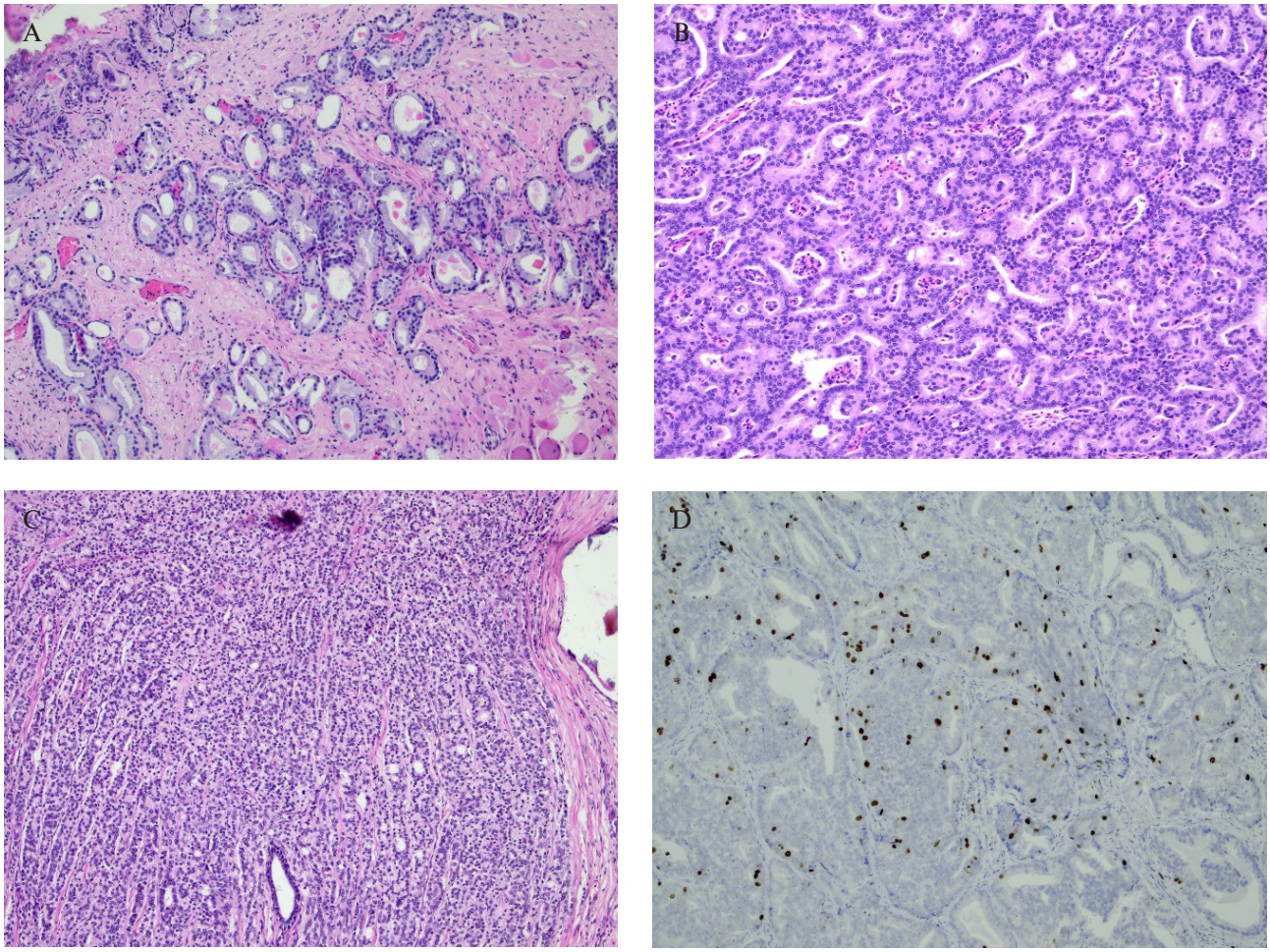

Fig. 1A. PCa rated as Gleason score 3+3 (magnification 100x). **1B.** PCa rated as Gleason score 4+4 (magnification 100x). **1C.** PCa rated as Gleason score 4+5 (magnification 40x). **1D.** IHC staining showing Ki67 expressed in approximately 17% of nuclei in PCa Gleason score 4+3 (magnification 100x). All figures were obtained from the authors own collection and were captured at the Department of Clinical and Molecular Pathology.

IMMUNOHISTOCHEMICAL MARKERS

PSA

Prostate-specific antigen, also called kallikrein-3, is a protein produced by the prostate glands that is of great importance for the diagnosis and clinical management of patients with prostate cancer. It is secreted almost exclusively into the ejaculate and its physiological function is to facilitate the movement of sperm. Normally, it is only found in minimal concentrations in blood serum, but in prostate disease, the PSA (ref.[9]) level can be elevated due to the prostate glands' disruption. This is used in the diagnosis and monitoring of the course of PCa treatment, as this protein is highly specific for the prostate and is not commonly produced in other tissues. However, a higher PSA (ref.[9]) level alone may not be indicative of PCa, since it may also be elevated in other physiological or pathological circumstances (urinary tract infection, endoscopic examination of the urinary tract or benign prostatic hyperplasia)[9].

Common clinical practice shows that the extent of the disease and the aggressiveness of the tumour are not always associated with PSA, as locally advanced or metastatic prostate cancers are often present with practically normal or only slightly elevated PSA values[9].

In the practice of a pathologist, PSA (ref.[9]) has mainly diagnostic significance. High specificity for prostate tissue can be utilised in the immunohistochemical staining, which can be helpful in differentiating possible metastatic prostate carcinoma in the case of poorly differentiated metastatic tumours (usually an advanced – castration-resistant prostatic carcinoma, CRPC). Even under these circumstances, tumours retain PSA expression in more than 80% of the cases[10].

It is also possible to use the PSA prognostically. In the normal epithelium of the prostate, immunohistochemical staining of PSA (ref.[9]) is noticeably more expressed at the apical pole of the cells, and the cells generally stain with strong intensity. Weaker staining intensity and loss of apical staining predominance were found in tumours bearing the TMPRSS2:ERG (ref.[11]) fusion gene or in PTEN (ref.[11]) deleted tumours. Those findings were associated with a significantly worse prognosis and more aggressive tumour behaviour, which also applies to carcinomas with the genetic changes mentioned (discussed further in text). Statistically, the prognostic value of

the intensity of immunohistochemical staining of PSA (ref.[9]) is so significant that it can stand as an independent prognostic marker[10,11].

PSMA

Prostate-specific membrane antigen is known by many names, most notably glutamate carboxypeptidase II. It is a transmembrane glycoprotein with enzymatic activity. In prostate cancer, its role is possibly to regulate angiogenesis which is crucial for tumour growth and metastasis. PSMA itself can be regarded, *inter alia*, as a prognostic factor, since high PSMA expression has been found in patients with aggressive forms of PCa (ref.[12]).

In recent years, there has been significantly more interest in this molecule, in the diagnosis and treatment of prostate cancer, as PSMA is present in up to 95% of these tumours[12]. Until recently, CT/MRI (ref.[13]) of the abdomen and skeletal scintigraphy were used as the basis for diagnosing PCa persistence or progression. According to the guidelines of the European Association of Urology and the European Society of Medical Oncology, using ^{68}Ga-PSMA PET/CT for restaging is preferred, as it has higher sensitivity and specificity[13].

PSMA also has a unique role in theranostics, i.e. the use of radioligand in the treatment of carcinoma (radioligand therapy, RLT). The radioligand ^{177}Lu-PSMA-617 is used to destroy tumour cells, where this ligand is purposefully taken up in tumour-affected foci[14]. Studies have shown at least 50% or greater decrease in serum PSA levels in 43% of patients with metastatic castration-resistant cancer (mCRPC). The response to this therapy was better in patients with mCRPC, which also showed higher PSMA expression. At the same time, better results of therapy were achieved in lymph node metastases than in metastatic bone disease. Thus, PSMA imaging is also predictive in nature[14]. Our department has also contributed to the development of new anti-PSMA radioligands with potentially improved efficiency[15].

Ki67

Ki67 is a well-known marker of cell proliferation, as it is expressed in cells throughout the cell cycle outside the G0 phase. As uncontrolled proliferation is one of the main characteristics of malignant tumours, Ki67 is considered a promising prognostic marker, but it is not yet used for this purpose in routine practice[16]. A higher Ki67 positivity rate in radical prostatectomies correlates with a higher Gleason score, a higher stage, more frequent invasion into the seminal vesicles and growth outside the prostate capsule. There was also a correlation with a shorter progression-free period and with the overall patient survival[16,17].

Mathieu et al.[18] conducted a multi-institutional study in which the authors also confirmed Ki67 status as an independent predictor of BCR. Interestingly, Ki67 labelling does not add any further prognostic value in patients with evident signs of aggressive disease such as lymph node involvement, positive surgical margins, extracapsular extension, seminal vesicles invasion or Gleason score 8 and higher. Therefore, its main benefit is limited to low-risk disease, meaning PCa with GS 7 and absence of these aggressive features.

However, the dilemma of comparing heterogenous studies on Ki67 remains. Published articles do not always provide sufficient information on the patient cohort and, they use different ways of data processing among other things. Thus, it is impossible to compare the final evaluation of a given immunohistochemical examination[19]. The techniques for processing the biological material are also different, and even though automated methods are now introduced, there are still variations between laboratories. In addition, the pathologist's assessment also comes into question as in this case it is never completely objective. Therefore, there is not yet a comprehensive scheme for the evaluation of Ki67 (ref.[19]) as a prognostic marker and the current WHO (ref.[8]) classification of urogenital tumours (2022) does not recommend its routine use in diagnostic practice so far[8,19].

PD-L1

The evaluation of PD-L1 expression and the possibilities of application of anti-PD-1 therapy have been a widely researched and discussed recently[20]. It is an established therapy in the case of some tumour lesions, e.g. non-small cell lung cancer. Various phases of clinical trials are still underway for other tumours, and prostate cancer is no exception[20].

Increased PD-L1 expression was found in prostate cancer that correlated with a higher Gleason score, higher stage, as well as with increased androgen receptor expression. Positive resection margins were also more frequent, and higher expression correlated with obesity. On the other hand, there was no correlation with age, PSA or the presence of lymph node metastases[21,22].

Despite the correlations of increased PD-L1 expression mentioned above, anti-PD-1 therapy is usually ineffective as a single therapeutic modality in cases of prostate cancer. The task of the PD-L1 molecule in a tumour is to escape the immune surveillance, and it is now clear that prostate cancer has other specific mechanisms, about which very little is known so far[23].

That aside, increased PD-L1 expression is also found in tumours with high microsatellite instability, mismatch repair disorders and higher mutational burden (e.g. with concomitant mutations of the Rb, BRCA2 or TP53 genes). Simultaneously, a higher proportion of tumour-infiltrating lymphocytes was found in the latter group. In these patients, anti-PD-1 therapy is more effective. Trials combining anti-PD-1 therapy with androgen receptor blockers in men with mCRPC are currently underway, where this combination was found to achieve a longer progression-free and overall survival[23,24]. Shim et al.[25] also confirmed a positive correlation between higher Gleason score and PD-L1 expression in prostate needle biopsy of carcinoma samples and suggested the administration of PD-1 inhibitors as a possible part of early neoadjuvant therapy.

Although the applicability of anti-PD-1 therapy in prostate cancer is still very limited, increased expression of PD-L1 alone may serve as an unfavourable prognostic factor[20-25].

CDK19

Cyclin-dependent kinase 19 is part of the Mediator complex, which binds to transcription factors and RNA polymerase II, thus regulating transcription. High expression of CDK19 correlates with higher Gleason score, higher stage of disease (higher pT category within TNM), higher Ki67 expression, nuclear expression of androgen receptor and ERG positivity[26].

From a group of cases with zero expression of CDK19, 5-year progression-free survival was recorded in 73.7% of patients. In the moderate-intensity staining group, 56.9% of patients survived 5-years without progression, and among the strong expressors only 30.4%. CDK19 had the ability, independent of other markers, to predict the disease progression[26].

PTPN12

This is a non-receptor protein tyrosine phosphatase with tumour suppressor effects. It is used, for example, in the dephosphorylation of HER2 and c-ABL, thus regulating signalling pathways involved in communication with the extracellular matrix, especially the response to mechanical stress and cell adhesion. It is only expressed weakly or not at all in normal prostate tissue, while it is variably positive in most cancers. Strong PTPN12 positivity is indicative of high genomic instability of the carcinoma and indicates an unfavourable prognosis and a more aggressive tumour phenotype. It is also associated with more advanced stage of the disease (higher pT category within TNM classification), higher Gleason score, metastatic lymph node involvement, positive resection margins, higher Ki67 expression and early biochemical progression[27]. Positive immunohistochemical staining was observed in 86.4% of tumours with the presence of the TMPRSS2:ERG fusion gene and in 58.4% of tumours that lacked this fusion gene[28]. In ERG-negative tumours, the prognostic value of PTPN12 is more pronounced, but it is important in both subgroups. PTPN12 can potentially serve as a stand-alone prognostic marker, but also in combination with others[27,28].

EZH2

The Enhancer of zeste homolog-2 (EZH2) is a catalytic subunit of the Polycomb Repressive Complex 2 (PRC2), which takes part in histone methylation, thus regulating genetic expression, for example by silencing tumour suppressor genes and leading to oncogenesis. This protein also participates in stem cell renewal, maintenance and their differentiation. It is overexpressed in aggressive solid tumours, including PCa and promotes epithelial-mesenchymal transition (EMT) by inhibiting E-cadherin expression[29]. Similarly, EZH2 promotes progression into CRPC and neuroendocrine differentiation, as well as aggressive tumour behaviour such as angiogenesis and vascular invasion. Moreover, EZH2 promotes AR expression but alters its signalling pathway, resulting in CRPC resistance even to potent AR signalling inhibitors (ARSi) such as enzalutamide. According to Abdelrahman et al.[30] strong nuclear EZH2 staining positively correlated with higher initial PSA serum levels, Gleason score ≥ 7, higher stage, lymph nodes involvement, frequent metastases and early biochemical progression[29-31].

E-cadherin

E-cadherin is a transmembrane protein, which in normal epithelial tissues binds to β-catenin in the cell cytoplasm. Its role is pivotal in cancer progression, since normal E-cadherin expression prevents EMT, where E-cadherin along with other proteins such as desmoplakin and cytokeratins is down-regulated. During the EMT E-cadherin is cleaved into smaller subunits, one of them being sE-cad fragment, which is capable of EGFR (ref.[30]) pathway activation, resulting in cancer cell invasion and proliferation as well as loss of cell-to-cell adherence. Those finding suggest, that changes during EMT happen on the molecular level before the EMT is morphologically assessable, thus immunostaining can serve as a prognostic method. Normal E-cadherin expression is regarded as moderate or strong membranous staining along with weak to negative cytoplasmic staining in > 70% cells[30]. Other staining patterns are considered aberrant. In their study Abdelrahman et al.[30] observed aberrant E-cadherin expression in 89.3% cases of advanced PCa, positively correlating with higher initial PSA serum levels. Loss of membranous expression was strongly associated with lymph nodes involvement and presence of distant metastases. E-cadherin evaluation thus may serve as a prognostic marker. Its future employment however remains debatable, since various studies have not confirmed the above-mentioned findings[30,32].

Huber et al.[33] analysed 28 markers with prognostic potential according to the available recent literature. Multivariate analysis confirmed the prognostic value of PSMA, stromal AR and EZH2 at the significance level of 0.01. For patients with GS 7 or less, the prognosis may also be made by E-cadherin. Huber et al.[33] point out the considerable limitations of studies aimed at finding new prognostic markers: for example, poor comparability of laboratories, unsuitable design of studies where tissues were obtained by radical prostatectomy, which can itself be curative, and confusion of the terms prognostic and predictive. Definitions of biochemical progression have also varied in studies. Therefore, the authors suggest and recommend adherence to the REMARK guidelines which set out the criteria for studies of prognostic biomarkers[33,34].

GENETIC CHANGES

To date, several important molecular subtypes of prostate cancer have been described. Some of them involve the fusion of the TMPRSS2 transcript with exons of genes from the ETS family[35]. It is a group of genes whose protein products serve as transcription factors regulating cell proliferation and differentiation, cell migration, apoptosis, angiogenesis, as well as the expression of oncogenes and tumour suppressor genes, which affect tumour progression and facilitate their metastasizing. Fusion with ERG (46%) is the most frequent, followed by ETV1 (8%),

ETV4 (4%) and FLI1 (1%) (ref.[35]). Presence of one of the fusion variants precludes fusion with other genes of the ETS (ref.[35]) family at the same time[35,36].

Other subtypes are characterized by point mutations in the following genes: 11% are SPOP mutated, 3% are FOXA1 mutated and 1% harbours IDH-1 mutation[35]. FOXA1 (Forkhead box A1) (ref.[35]) regulates androgen receptor-mediated transcription and promotes proliferation in PCa. However, apart from the relatively high frequency of occurrence of the mutated FOXA1, the prognostic or predictive significance of this gene has not yet been established and therefore is not further mentioned[35].

Other frequent changes are, for example, mutations in PTEN, TP53 or RB1 genes as well as epigenetic changes, e.g. silencing of the STAT6 gene[35-39].

TMPRSS2:ERG

This fusion gene is present in almost half of prostate cancer cases. TMPRSS2 is an androgen-regulated serine protease expressed mainly in the epithelial cells of the prostate. Due to the fusion of TMPRSS2 and ERG, there is a significant upregulation of the ERG protein. ERG expression itself does not have prognostic potential, but it affects the expression of approximately 1600 genes in the prostate epithelial cells, some of which are mentioned above[40]. In the past, the presence of TMPRSS2:ERG (ref.[40]) was considered an unfavourable prognostic factor, however, this has been later disproved and there is currently no consensus on whether the presence of this fusion gene is of any prognostic significance. Nevertheless, Chalmers et al.[40] argue in their large study of 10 189 patients, that PCa bearing the TMPRSS2:ERG fusion gene is a separate subunit characterized by development in younger patients and a more aggressive biological nature.

Furthermore, the role of this gene in the pathogenesis is significant, as it has already been detected in precursor lesions. Park et al.[41] reported that TMPRSS2:ERG fusion gene is present in 15% cases of HG PIN. Van Leenders et al.[42] even stated, that in their cohort of 21 HG PIN samples, 52% harboured this fusion gene. Shresta et al.[43] found the presence of TMPRSS2:ERG in proliferative inflammatory atrophy (PIA), which is also considered a precursor lesion of prostate cancer, either by direct transformation of PIA into PCa or though the HG PIN. The authors of the study believe that bacterial prostatitis should be considered a legitimate risk factor for cancer. Interestingly, Propionibacterium acnes has clearly been associated with prostate cancer risk[41-44].

SPOP

In prostate cancer, point mutations most commonly occur in this gene. The functions of SPOP include ubiquitination and protein degradation, but it has also been involved in maintaining genome stability by modulating the repair of double-stranded DNA breaks[45]. At the same time, the presence of the SPOP (ref.[45]) mutation is excluded with TMPRSS2:ETS (ref.[45]) fusions[35,36]. In prostate cancer, the SPOP gene increases the activity of the AR by preventing its ubiquitination and preventing degradation of AR co-activators[45]. Moreover, mutated SPOP leads to 17β-hydroxysteroid dehydrogenase (17βHSD) enzyme degradation, which causes increased intracellular testosterone levels[45]. Mutations in SPOP (ref.[45]) have been detected in HG-PIN (ref.[45]), but it cannot be used as a prognostic factor, as there has been no correlation with stage or Gleason score, or with the risk of biochemical progression or mortality. It is, however, a fairly common mutation associated with the expression of the SPINK1 (ref.[46]) gene (discussed further)[35,46]. Moreover, men with SPOP mutated mCRPC had shown better therapeutic response to ADT (ref.[35,36,45-47]).

IDH1

Isocitrate dehydrogenase-1 is a cytoplasmic enzyme required for the conversion of isocitrate to 2-oxoglutarate. Mutations in IDH1 are common in gliomas and acute myeloid leukaemia. In prostate cancer though they only occur in 1% of cases, but at younger age. Mutant IDH1 has the ability to convert alpha-ketoglutarate to 2-hydroxyglutarate, which leads to stabilization of HIF1A and higher angiogenesis. It is considered that in the future, angiogenesis inhibitors and IDH1 inhibitors could be useful for this group of patients[35].

In addition to those described above, other gene alterations are found in PCa, but they occur along with the subtypes discussed. PTEN, TP53 and RB1 mutations or deletions occur more frequently in the ETS-fusion subtype, while CHD1 and SPINK1 alterations are more common in the group of ETS-negative tumours (i.e. SPOP and IDH1) (ref.[35]).

PTEN

It is a tumour suppressor gene that is able to stop proliferation by inhibiting the G1 phase of the cell cycle. Loss of PTEN (ref.[48]) function correlates with ERG (ref.[48]) overexpression and its deletion correlates with higher tumour aggressiveness, higher Gleason score and higher stage[35]. While PTEN loss was already detected in approximately 20% of primary tumours, in metastatic PCa was PTEN loss detected in up to 40% of tumours[48]. It has also been found that the PTEN deletion occurs more frequently in intraductal cribriform prostate cancer (IDCP), which is considered a sign of poor prognosis. Nevertheless, Spieker et al.[49] argue in their study that the very presence of cribriform morphology is a marker of a worse prognosis independent of the PTEN loss. On the other hand, PTEN deletion in carcinomas with glomeruloid morphology was a predictor of earlier biochemical recurrence[49,50]. Jamaspishvili et al.[48] also found that PTEN loss corelated with shorter biochemical recurrence-free survival (BRFS) and also identified a subset of patients within low risk PCa group with shorter BFRS. The loss of PTEN (ref.[48]) in PCa also leads to higher involvement of the so-called Warburg effect, which may offer new therapeutic possibilities in the future at the level of intervention in glycolysis[51,52]. PTEN expression status can also be assessed by immunohistochemical staining[48-53].

TP53

Loss of function of the tumour suppressor gene TP53 also leads to a more aggressive PCa phenotype, alterations of the gene occur in 40–60% cases. Rather than in the primary tumour, the mutated TP53 is more frequently present in metastatic carcinomas and castration-resistant cancers[35]. The presence of TP53 mutation or deletion is also common in carcinomas with neuroendocrine differentiation[54].

TP53 product p53 protein can also be detected by in clinical practice well-established IHC stain, which can identify either overexpression or loss of expression, both indicating a TP53 status[35,55].

RB1

The function of this gene's eponymous protein is to bind transcription factors E2F and control entry into S-phase. It is a tumour suppressor gene capable of inhibiting proliferation[56]. According to Armenia et al.[57] RB1 mutations occur both in primary PCa and in metastatic PCa. While the mutated status is not very common in primary PCa (up to 3%) (ref.[35]), it is significantly higher in metastatic PCa (28%) (ref.[35,57]). In both cases however it is a sign of unfavourable prognosis. Like TP53, RB1 mutations are often found in carcinomas with neuroendocrine differentiation[35,54,58].

Alterations in PTEN, TP53 and RB1 genes can occur in the same tumour simultaneously. They are more common in advanced tumours, but presence of any of them in the primary tumour can be quite unfavourable. The loss of these genes leads to the development of aggressive carcinoma, which is no longer stimulated through the AR and has a great metastatic potential. If the loss of PTEN, TP53 or RB1 is already present in the primary lesion, this tumour poses a risk of early development of the castration-resistant carcinoma. If routine screening of these alterations in tumour suppressor genes were available, patients could be better stratified into low- and high-risk groups[59].

CHD1 and SPINK1

Mutations of CHD1 (ref.[35]) occur in various tumours, while deletion is less common. That however does not apply to PCa, where deletion is the most frequent type of CHD1 (ref.[35]) gene mutation[47]. CHD1 loss can be detected in approximately 5–10% of PCa cases, of which 80% is in the SPOP mutated group. Such event occurs already in localised PCa and serves as a metastatic driver[35]. The function of CHD1 (ref.[35]) is to "unpack" chromatin and elongate during transcription. According to clinical trials, men with mCRPC harbouring SPOP mutation and CHD1 loss proved a higher response to abiraterone therapy[47].

Tumours with mutated CHD1 and SPOP are characterized by overexpression of **SPINK1**, which is associated with aggressive tumour behaviour and correlates with disease progression even after prostatectomy[35,47,59].

SPINK1 is an inhibitor of serine proteases, commonly found in pancreatic tissue, where its function is to inhibit prematurely activated trypsin[60]. In case of prostate cancer SPINK1 outlier expression occurs in almost 10% (ref.[61]) of cases. Supposedly, SPINK1 (ref.[61]) is able to activate EGFR (ref.[61]) and induce EMT, which promotes aggressive tumour behaviour also through increased vascular invasion[35,62]. Patients with SPINK1 overexpression were characterized by a shorter survival time and higher risk of biochemical recurrence after RP, therefore its prognostic significance is possible. Its therapeutic use is also not excluded in the future[35,37,61,62].

Androgen receptor

As prostate cancer is a hormone-sensitive tumour, androgen receptor (AR) plays a key role in its development and therapy. It is the main transcription factor in normal prostate tissue as well as in carcinoma. Some of the genes described above (TMPRSS2:ETS, SPOP, FOXA1) are related to AR (ref.[35]) activity, and are involved in the pathogenesis of PCa, while changes in AR itself also affect prognosis and treatment. There are several structural variants of AR, as well as point mutations in the AR gene that lead to activation of the receptor independently of androgen stimulation. AR alterations are present in more than 60% of castration resistant PCa (CRPC) cases. Point mutations of AR (ref.[63]) are present in 15–30% CRPC, leading to the resistance against the AR targeted agents (ARTA; e.g. enzalutamide and abiraterone acetate) (ref.[63]). AR amplification is detected in up to 50% CRPC, leading to disease progression even with androgen-deprivation therapy (ADT) and ARTA (ref.[63]). Therefore, the detection of such aberrations could serve as an important predictive marker, as there is no point in treating these patients with AR signalling inhibitors[35,63].

Recently, attention has also been paid to variant forms of the androgen receptor (AR-Vs), which arise mainly due to changes in the primary protein structure of the receptor and by the alternative splicing. AR-Vs are characterized by the absence of a ligand-binding domain, i.e. they are not dependent on androgen signalling. However, the DNA-binding domain is retained, making these AR variants still capable of regulating the transcription of other genes. This creates an active form of AR that is resistant to drugs affecting androgen signalling. AR-Vs can be activated by homodimer formation within one variant or heterodimer with another AR-V, which explains the mechanism of progression to CRPC in ADT. AR-Vs have also been detected in non-cancerous prostate tissue and in early low-grade carcinomas, but at much lower levels than in CRPC. They are probably intermediates of the ongoing alternative splicing that do not affect tumour progression and were detected only due to the high sensitivity of the method used (RT-PCR) (ref.[63-64]).

The most explored is **AR-V7** or SE splice variant-7, which is the most common in CRPC. In these tumours, a poorer therapeutic response to abiraterone and enzalutamide was noted. Another AR-Vs studied include AR-V1, AR-V3, AR-V9 and ARv567es (ref.[64]). It is hypothesized that in the future, the presence of AR-Vs could serve as a marker of resistance to AR inhibitors. At the same time, it is a possible therapeutic target, especially in patients with CRPC. Potential drugs are currently being devel-

oped, which are able to block the androgen signalling by binding to AR-Vs outside the ligand-binding domain[64].

BRCA1/2

BRCA proteins 1 and 2 are well known in connection with mammary and ovarian cancer in women. However, their importance is also considerable in prostate cancer. In the general European population, men over 65 (ref.[65]) years of age have a 7.14% (ref.[69]) risk of developing PCa in BRCA2 mutation carriers and for BRCA1 carriers, the risk is 1.78% (ref.[69]). These proteins are involved in DNA damage repair (DDR), specifically in repair of double strand breaks (DSB) by homologous recombination. DDR disorders are particularly prevalent in patients with mCRPC and account for up to 25% genetic alterations. The most common DDR disorder in PCa is the BRCA2 mutation. The presence of the mutated BRCA gene in PCa is associated with a more aggressive biological nature of the primary tumour and a worse prognosis in patients with mCRPC. These men are diagnosed at younger age, Gleason score is higher and metastases to lymph nodes are more frequent[66]. On the other hand, the BRCA mutation means a higher chance for a good therapeutic response of the tumour when PARP inhibitors are administered[66]. At the same time, a higher therapeutic response to RLT can be assumed[65-69].

ATM

ATM acts as a DNA damage checkpoint and indirectly may activate DNA repair through homologous recombination. ATM alterations are present in approximately 5–10% (ref.[70]) of advanced PCa. Alterations in the group of DDR genes (BRCA1/2, ATM) can lead to more aggressive behaviour of tumours. However, in their work on a sample of 631 patients, Neeb et al.[70] did not find a correlation between a worse prognosis and negative ATM immunohistochemical staining. ATM negativity was however associated with higher genome instability. Loss of ATM predicts the possible response of PCa to combined treatment with ATR and PARP inhibitors or with ATR inhibitors only. Compared to PCa with the BRCA2 (ref.[72]) mutation, PARP inhibitors alone are significantly less effective against tumours with the ATM mutation, however these tumours tend to be more sensitive to ATR inhibitors. Similarly to BRCA2, ATM mutated PCa is expected to have a higher RLT success rate[68]. ATM mutations also predict the possible efficacy of carboplatin chemotherapy[68,70-74].

MYC

The c-Myc signalling pathway is currently being studied as a potential therapeutic target in the treatment of malignancies. Activation of c-Myc promotes tumour growth and invasion by inhibiting apoptosis and stimulating proliferation. In PCa, overexpression of MYC causes resistance to chemotherapy (paclitaxel and docetaxel) and radiotherapy. Simultaneously, overexpression of MYC can serve as an independent prognostic factor of PCa and is associated with shorter survival, worse clinical stage and more frequent presence of lymph node metastases. In their study of 106 patients, Dong et al.[75] also observed a higher Gleason score and a higher Ki67 in association with MYC overexpression[76].

Labbé et al.[77] conducted a study where they demonstrated in the PCa model that a diet containing high amounts of saturated fats leads to alterations of metabolism that further affect (increase) the expression of MYC, leading to increased cell proliferation and higher tumour load. In a mouse model, it was found that switching from a high fat to a low-fat diet leads to a decrease in MYC expression. These findings are consistent with the fact that obesity and increased dietary intake of saturated fats are associated with a higher risk of PCa development and progression and higher mortality[77].

CCND1

In their work, Armenia et al.[57] found that 2% of localised PCa and 9% of metastatic PCa harbour CCDN1 amplification. Product of this gene is cyclin D1 protein, which is a key part of cell cycle regulation. Cyclin D1 inhibits the activity of pRB protein, which allows the cells to enter the G1 phase of the cell cycle[78]. CCDN1 (ref.[57]) amplification is a known prominent oncogenic feature in several human malignancies, for example in mantle cell lymphoma or breast carcinoma and prostate carcinoma seems to be no exception. Nakamura et al.[79] states, that CCDN1 amplification corelates with higher incidence of perineural invasion. There was, however, no connection between CCDN1 amplification and other PCa attributes such as serum PSA level, Gleason score, stage, lymph node involvement, Ki67 proliferation index or patients' age. According to Nakamura et al.[79] there is also significant connection between CCDN1 and ER-beta expression, while there was no correlation with AR expression status, which implies, that PCa oncogenesis might also be driven by oestrogens[57,78,79].

CONCLUSION

Despite the wide range of molecular markers known and investigated today, only a few of them enter clinical-pathological practice and do not become a common part of the diagnostic algorithm of histological examination. The Gleason score remains widely used prognostic marker, which has undergone many modifications since its introduction. However, its disadvantage is the inter-observer variability among pathologists. It is necessary then to find other markers that will help us to better stratify the risks. Logically, attention turns to immunohistochemical markers, both new and long known. The Ki67 (ref.[16]) proliferation index is one of them, which has been found to be strongly correlated with the clinical course of the disease in individual studies, but the design of the studies allows only a very limited comparison, and therefore Ki67 (ref.[16]) has not yet been validated. A similar problem applies to the other new IHC markers. To find a consensus and a valid comparison of published data, the REMARK guidelines[34] have been published, which propose a uniform design of studies. Despite those facts,

Ki67 (ref.[16]) along with PTEN (ref.[48]) remain the most promising IHC stain so far[53], also thanks to the fact, that immunohistochemistry is widely available and easy to use method.

Genetic or cytogenetic methods can also be used to identify alterations of some genes that are important in the pathogenesis, but also have prognostic and predictive value. The promising genetic markers are AR (ref.[35]) aberrations, for prediction of therapy response to ARTA/ARPI (ref.[63]) and BRCA (ref.[65]) status for treatment with PARP (ref.[66]) inhibitors. However, similarly to IHC, most mutations are not routinely screened.

The latest possibilities in imaging methods can diagnose the clinical progression of tumours with high accuracy and there are also new possibilities of biochemical analysis. PET/CT using radionuclide Ga-68-PSMA-11 and whole-body MRI are now replacing the previously used CT and bone scintigraphy using technetium 99-m (ref.[13]). A more sensitive liquid chromatography with tandem mass spectrometry (LC-MSMS) should be used for biochemical examination, as opposed to the more obsolete chemiluminescence. However, recent data suggest that serum luteinizing hormone levels are even more indicative of the castration environment than testosterone levels, even as measured by LC-MSMS. Therefore, the current definition of CRPC now appears to be outdated and should be redefined[5].

To put new methods into practice, it will be necessary to perform large prospective studies and meta-analyses to verify the marker reliability and justification. Thanks to the increasing utilization of the Next Generation Sequencing (NGS) in detection of both diagnostic and predictive genetic mutations, its financial demands are decreasing, thus making the NGS increasingly available for wider cohort of patients in the near future. This promise establishing of the prognostic and predictive prostate cancer NGS panel, which might include the above-mentioned genes: TMPRSS2 fusions, SPOP, IDH-1, PTEN, TP53, RB1, CHD1, SPINK1, AR variants, BRCA1 and BRCA2, ATM, MYC and more.

Search strategy and selection criteria

The articles cited were found via PubMed search engine. Key words prostate cancer and prognostic markers were used. The immunohistochemical stains and genes examined were selected based on the knowledge of contemporary research convergence and authors' experience. Articles published within recent 5 years were preferred.

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