Abstract. The widespread use of the PSA test has led to increased detection of the disease at earlier stages and a reduction in the number of patients where metastatic disease is found at diagnosis. However, there are significant limitations to the PSA test such as its lack of specificity, elevation in benign disease and failure to detect a significant number of PSA-negative tumours. Therefore, PSA is now commonly regarded as an indicator of prostate volume and is not independently diagnostic or prognostic in prostate cancer. Due to these limitations, there is an urgent need for new prognostic biomarkers to enhance the clinical management of prostate cancer. There have been many recent advances in high-throughput technologies for measuring gene and protein expression in minimally invasive samples (e.g. blood, urine) that could more accurately predict disease progression. This review article gives a brief overview of biomarkers that are currently showing prognostic potential in prostate cancer research.

The Problems with PSA

Prostate cancer is the most common cancer and the second leading cause of cancer-related death affecting men in the UK: 10,000 men still die every year in the UK from prostate cancer (1). Currently, the only biomarker in broad clinical use for the diagnosis and prognosis of prostate cancer is the PSA test.

Prostate-specific antigen (PSA) is a serine protease secreted by the epithelial cells of the prostate and has a role in the liquefaction of seminal fluids. PSA can be detected in serum from a blood sample and is considered to be the most useful tumour marker currently in use (2, 3). Elevations of PSA are seen in prostate enlargements and the highest elevations are found with more advanced stages of the disease (4). The widespread use of the PSA test has led to an increased detection of the disease at earlier stages (5) and a reduction in the number of patients where metastatic disease is found at diagnosis (4); most men who are diagnosed with prostate cancer still have localized disease and are symptomless (6). As higher levels of PSA are found with more advanced stages of the disease and with worse outcomes, PSA has been used as a staging and prognostic tool (7). The PSA test is also extremely useful for monitoring the disease after treatment such as radical prostatectomy.

However, there are significant limitations of the PSA test as it is not cancer specific. PSA levels do not have a direct correlation with increasing grade and stage of prostate cancer (7). Although PSA is considered to be an effective predictor of pathological stage in patients with high levels, it shows a poor correlation with grade and progression in the 2-9 ng/ml PSA range (8); more men fall within this range than previously as a smaller proportion of prostate cancer patients are diagnosed with advanced stages of the disease. Elevations of PSA are seen in benign enlargements of the prostate such as benign prostatic hyperplasia (9), a condition that commonly affects older men, and also in prostatitis and after interventions such as biopsy (10). This lack of specificity of the test has led to the overdiagnosis of indolent disease (11).

Many unnecessary biopsies are performed based on abnormal PSA results. This invasive procedure carries a risk of infection and haemorrhage. In addition, difficulty with staging and grading the tumour at biopsy, due to the multifocal nature of the disease, has led to radical surgical procedures such as radical prostatectomy being carried out needlessly. Sardana et al. (12) state that 30% of tumours removed in radical prostatectomy procedures are found to be indolent and a “watchful waiting strategy” would have been more appropriate in these cases. There is no active treatment...
for the patient in the watchful waiting strategy but they are closely monitored with frequent PSA testing and examinations. Action is taken if required, such as biopsy. Significant survival benefits have not been found with early radical prostatectomy in comparison to watchful waiting in patients with an indolent form of the disease (13). Radical prostatectomy is associated with a 20% risk of incontinence, 70% of impotence, 3.5% risk of bowel injury, infection, thrombosis and haemorrhage (14). Conversely, the upper limit of normal of 4.0 ng/ml PSA has failed to detect a significant number of tumours (15, 16). Moreover, some aggressive prostate tumours can be PSA negative (17).

The prognosis after prostate cancer diagnosis is hugely variable and the molecular pathogenesis of the disease is not well understood (12). For a patient with organ-confined disease of a relatively indolent nature, there may be no significant impact on mortality for 15 years (18). An elderly patient diagnosed with a low-grade cancer will most likely die from other causes before they need treatment for prostate cancer and there may be no deleterious effect on quality of life (19). Unfortunately some patients present with an aggressive form of the disease where time to metastasis may be only 2 years (18). Metastatic spread is responsible for the lethality of cancer (14). These patients may present with late-stage invasive disease. At this stage, there are no curative options available for the patient as radical treatment is only successful in organ-confined disease. There is great need for new prognostic biomarkers to enhance the clinical management of prostate cancer patients as currently no marker exists that can distinguish between indolent and aggressive disease (14).

A simple non-invasive test is required that could differentiate between these two outcomes so that clinicians can offer the patient the most appropriate treatment strategy. Hopefully this would enable indolent forms of the disease to be treated with watchful waiting, thereby decreasing the number of negative biopsies and unnecessary surgical interventions and an active treatment pursued in more aggressive forms of the disease, where a watchful waiting strategy would lead to unchecked growth and cancer spread. This could ensure that treatment is restricted to those patients in whom it would be beneficial. It is very helpful to be able to predict the probable prognostic outcome preoperatively in order to avoid unnecessary procedures or plan optimal postoperative treatments to minimise recurrence (20). Currently, various factors are used to assess the patient with prostate cancer with regard to prognosis. Clinical prognostic factors are found by a physical examination of the patient. These could be evaluated by blood tests, medical imaging techniques and examination of biopsies by microscopy. Pathological prognostic factors can be assessed only by examination of the whole prostate, if and when the prostate is removed. Presently clinico-pathological factors that are used to predict failure of treatment include PSA (biological marker), Gleason score, pathological stage, surgical margin status, seminal vesicle invasion and tumour volume. The grading of biopsy material by the Gleason system is one of the most relevant predictors of patient outcome, as is strongly correlated to disease progression and survival (21-23). However, as previously mentioned, biopsy grading is difficult and when biopsy grading has been compared to grading of samples after radical prostatectomy, over half of the biopsies were found to be under or over-graded compared to the grading of radical prostatectomy samples (24).

PSA is called a biological marker (biomarker) because it is a molecule that can be objectively measured as an indicator of normal biological processes, pathogenic processes or pharmalogical responses to a therapeutic intervention (5). Biomarkers may be measured using biopsy tissue or from blood or urine samples. Biomarkers are most useful if they are able to be assayed from an easily obtained patient sample such as blood, without the need for an invasive procedure such as biopsy (4) and they must show high specificity to the particular organ.

Preoperative and postoperative nomograms have been devised that utilise PSA amongst other multiple clinical parameters for predicting stage of the disease, the likelihood of recurrence as measured by a rising PSA value and the chance of disease-free survival (25). Due to the limitations of PSA as a biomarker, there is an urgent need for new biomarkers that can be used as prognostic indicators in prostate cancer to effectively differentiate between indolent and aggressive disease. When assessing the effectiveness of a marker, often biochemical recurrence is used as an indication of failure of treatment. Biochemical recurrence is said to have occurred if there is a persistent increase in serum PSA value above the level detected immediately post-treatment. Some 30-40% of patients suffer a biochemical recurrence after radical prostatectomy (26). A rising PSA level after radical prostatectomy is extremely likely to indicate recurrence of prostate cancer (27) and progression of the disease to cancer spread and metastasis.

It may not be possible for one single biomarker to provide the necessary prognostic information about the patient to base treatment options on. Instead, panels of markers could be used to accurately predict the stage of disease and how it will progress (4). There have been many recent advances in high-throughput technologies for measuring gene and protein expression that have aided the search for new biomarkers (28).

**Genomics**

Gene expression analysis has been very useful for researching new biomarkers. Cancer is a genetic disease at the cellular level and complex changes in gene expression
are seen throughout its many stages. These changes in gene expression lead to alterations of protein levels and function which changes the cellular behaviour. This causes a normal cell to change into a cancerous cell, which will exhibit features of cancer such as uncontrolled proliferation and the potential to invade and spread around the body. There are multiple genetic changes that arise during metastasis. A gene that promotes cancer progression and metastasis will be up-regulated and a gene that inhibits cancer progression and metastasis will be down-regulated in a more aggressive cancer with a higher metastatic potential (14).

Various studies have used high-density DNA microarrays to examine gene expression in prostate cancer samples (29-31). Microarrays can analyse thousands of genes simultaneously to produce a gene expression profile of samples. Gene expression profiles can be found that are associated with different disease outcomes by using samples with known information about the progression of the disease. Molecular signatures for aggressive disease can be found that may be able to predict the progression of the disease (28). Low density arrays such as TLDA (Taqman Low Density Array) screen tissue samples against a small panel of genes.

There have been many advances recently in the study of proteomics which may in the future become more frequently used for biomarker discovery than gene expression studies. Proteins are the final products of gene expression and may give a more accurate representation of the functional molecules expressed by a cell, as only 48-64% of RNA transcripts may be translated into proteins (32).

**Proteomics**

Proteomic profiling may use mass spectrometry which can measure the mass-charge ratio of ionized molecules (peptides) (33). The most common mass spectrometry technique used for proteomic profiling is SELDI (surface-enhanced laser desorption/ionization) coupled to TOF (time of flight) analysers which can be used to produce a profile of the mass-charge ratio of all the proteins present in a serum sample and quantify the amount of each one (34). Some of the proteins may be identified by using data from sequence databases. However, many proteins are not identified, although this may not negate their usefulness as biomarkers (35).

**Novel Prognostic Biomarkers**

**PSA-derived Forms**

Various parameters of PSA have been used to try to improve its performance, increasing its diagnostic specificity and prognostic ability. PSA velocity (rate of PSA change over time) (12) has been found to be an independent predictor of recurrence of disease after radical prostatectomy (36). A PSA velocity of >2.0 ng/ml per year before diagnosis is correlated with a shorter disease-free survival time despite treatment by radical prostatectomy (37).

PSA is present in serum in various molecular forms falling into the two broad categories of free (unbound) and complexed (bound to protease inhibitors) forms (7). The free form constitutes 5-45% of the total PSA. The measurement of the percentage of free PSA (free PSA/total PSA x 100) has been found to address the problem of low specificity of the PSA test in patients whose PSA levels are in the 4-10 ng/ml range (38). Many unnecessary biopsies are performed on patients in this grey area as only 25% of patients biopsied after a result within this range will actually have prostate cancer (39, 40). It appears that with increasing percentage of fPSA, the risk of finding prostate cancer on biopsy is reduced and this would make the elevated PSA more likely due to benign prostatic disease instead of prostate cancer (38, 41). Catalona et al. (38) found that using a cut-off value of 25% fPSA, below which biopsy was initiated, they were able to detect prostate cancer with a 95% sensitivity and 20% of unnecessary biopsies could thus be avoided. This study (38) concurred with other studies in finding that % fPSA was significantly lower in those patients with aggressive disease (such as Gleason score 7 or greater, presence of metastases, positive surgical margins) than in those with nonaggressive disease (42, 43). Southwick et al. (44) found % fPSA to be a better predictor of postoperative pathological outcome than the Gleason grade, although other studies have not found % fPSA to be useful for staging of prostate cancer or to predict disease progression (45, 46). % fPSA does not improve the specificity of the tPSA test in patients with PSA levels above the 4-10 ng/ml range.

**Human Kallikrein-related Peptidase 2 (hk2)**

Another member of the same kallikrein gene family of secreted serine proteases as PSA is the human kallikrein-related peptidase 2 (hk2). Like PSA (hk3), hk2 shows greatest abundance in the prostate tissues (47); it is overexpressed in prostate cancer (48). hk2 levels can be measured in serum samples (49, 50). Some studies have found that hk2 may be useful in predicting, preoperatively, disease that is nonorgan-confined and the risk of biochemical recurrence (49, 51). However other studies (52) have not found hk2 to be more beneficial than PSA for prognostic prediction preoperatively. The ratio of hk2 to fPSA has been shown to be a useful tool in prostate cancer detection in patients within the 2-10 ng/ml PSA range (53, 54) and is helpful in determining biochemical recurrence risk in this group of patients (55). Other members of the kallikrein family may prove to have roles as prostate cancer biomarkers in the future.
α-Methylacyl-CoA Racemase (AMACR)

A promising biomarker is α-methylacyl-CoA racemase (AMACR). This enzyme is involved in oxidative metabolism and synthesis of branched chain fatty acids (56). Its gene is up-regulated in prostate cancer (57). mRNA of AMACR can be detected in serum and urine by reverse transcription PCR (58). Sreekumar et al. (59) measured autoantibodies to AMACR in serum as a highly sensitive and specific diagnostic test. In addition, AMACR may have a prognostic value as decreasing AMACR levels have been linked to increased risk of biochemical recurrence and worse prognosis in prostate cancer (60).

Insulin-like Growth Factors and Binding Proteins

There has been a considerable amount of literature citing an association between prostate cancer and the expression of insulin-like growth factors (IGFs), their receptors and binding proteins. Some studies have found an increased risk of prostate cancer development with an elevated level of IGF-1 (61) in serum, although the risk may only be marginally increased and could not outperform the PSA test (62). Of greater significance, with regard to prognosis are IGF-binding proteins (7). IGFBP-2 is the main IGF-binding protein in prostate epithelial cells. Substantially elevated circulating IGFBP-2 levels have been associated with patients with prostate cancer (63). The study by Shariat et al. (63) found an inverse correlation of IGFBP-2 levels with features of aggressive disease and progression to late stage disease such as higher Gleason score, extraprostatic extension and seminal vesicle involvement. It should be noted that these values are still higher than those of patients without cancer (63). IGFBP-3 (the main binding protein for IGF-1) levels have not been found to differ between patients with non-metastatic prostate cancer and healthy patients but are lowered and have an inverse correlation with development of metastases to the bone (63, 64). Preoperative measurement of serum levels of IGFBP-2 and IGFBP-3 may be useful in prostate cancer prognosis due to their association with the risk of disease progression and ease of measurement in the blood.

Urokinase Plasminogen Activator (uPA) and Receptor (uPAR)

The urokinase plasminogen activation cascade (uPA) is involved in degradation of the extracellular matrix and basement membrane, a process that is linked to carcinogenesis by enabling angiogenesis and metastasis (12). Central to this pathway is the urokinase plasminogen activator (uPA) which is activated by binding of its receptor (uPAR). This brings about the conversion of plasminogen to plasmin which is responsible for the degradation through activation of various proteases. uPA and uPAR have been detected at elevated levels in the serum of prostate cancer patients (65). Elevation of tissue levels of uPA and uPAR has been associated with invasion of the tumour, metastasis and an aggressive phenotype (66, 67). Interestingly, uPA and uPAR levels have been measured in blood and elevation of their levels associated with disease progression and metastasis (68, 69), particularly in bone metastasis (70). Preoperative uPA levels could be used to predict the likelihood of biochemical recurrence after surgery (4).

Early Prostate Cancer Antigen (EPCA)

Also concomitant with carcinogenesis are changes to the nuclear protein matrix and one of these proteins, early prostate cancer antigen (EPCA) has shown impressive results as a blood assay, with respect to specificity and sensitivity in identifying patients with prostate cancer from healthy patients (71), suggesting a putative role as a diagnostic marker for prostate cancer. Its role in prostate cancer prognosis is highlighted by Leman et al. (72) who have found EPCA-2 to be capable of distinguishing between disease that is confined to the prostate and that which is nonorgan-confined.

Transforming Growth Factor β1 (TGF-β1)

TGF-β1 plays a role in the regulation of cellular proliferation and differentiation as well as angiogenesis (12). A relationship has been found between elevation of the concentration of TGF-β1 in prostate cancer tissue samples and increased tumour grade and stage, presence of invasion and metastasis (73, 74). In addition, levels of TGF-β1 can be measured in plasma by immunoassay and studies have found a correlation between higher circulating levels of TGF-β1 and biochemical recurrence and features of aggressive disease such as extracapsular extension, seminal vesicle invasion, lymph node involvement and metastasis (75-78). Conversely other studies have not identified an association (79, 80).

Interleukin-6 Ligand and Receptor

Circulating levels of the cytokine IL-6 and its receptor have been found to be elevated in correlation with features of aggressive disease such as higher Gleason score, advanced stage, development of metastasis and decreased survival (81, 82). Kattan et al. (83) added preoperative plasma TGF-β1 and IL-6 soluble receptor levels before radical prostatectomy to a currently existing nomogram (84) which predicts risk of biochemical progression using pretreatment PSA levels, clinical stage and biopsy Gleason grade. They reported an enhanced prognostic ability of the nomogram with these
additional markers (83). The new nomogram was found to give a better prediction of disease recurrence at the 5-year point after radical prostatectomy.

**Enhancer of Zeste Homolog 2 (EZH2)**

EZH2 encodes a member of the polycomb group of proteins that has a function in transcriptional repression. EZH2 has been found to be overexpressed in metastatic prostate cancer compared to clinically localized disease by gene expression profiling of tissue. Higher concentrations of EZH2 in clinically localized disease are correlated with a poorer prognosis (85). The study by Varambally et al. (85) found that the measurement of EZH2 protein expression gave a better prediction of clinical outcome than surgical margin status, Gleason score or pre-operative PSA levels, making EZH2 a promising marker for distinguishing between indolent and aggressive prostate cancer. However a blood assay is not yet available for this protein (33). EZH2 expression has been used as a ratio with the important cell adhesion molecule, E-cadherin (28), to predict the risk of recurrence of the disease after radical prostatectomy.

**E-cadherin (ECAD)**

E-Cadherin expression is reduced in higher Gleason grade prostate tumours, with advanced stages of disease and with shortened survival (86, 87). In the study by Rhodes et al. (28), a high EZH2:ECAD finding was found to increase the chance of disease recurrence independently of the usual clinical and pathologic parameters, such as tumour stage, Gleason grade of tumour and pretreatment serum levels of PSA. The authors of the study (28) suggest that the addition of these two biomarkers to the nomogram developed by Kattan et al. (84) at diagnosis could aid in the identification of high-risk patients who have an increased risk of disease recurrence. The utility of E-cadherin as a prognostic marker would be greatly enhanced by the development of a serum test for this marker.

**Prostate Stem Cell Antigen (PSCA)**

Prostate stem cell antigen (PSCA) is a cell-surface glycoprotein and its presence is almost exclusive to the prostate (88). PSCA may play a role in tumorigenesis, possibly being involved with cell adhesion, signal transduction and also the prevention of apoptosis (88). Its expression is mainly confined to the basal cell population of the prostate (88), which is believed to be the focus of cancer transformation (89). Many studies have found PSCA to show increased expression in cancerous prostate tissue compared to normal prostate tissue (88, 90, 91). With regard to prostate cancer progression, such studies (90-93) also found a correlation between increased expression and increasing Gleason grade, stage and degree of metastasis. However immunohistochemical staining techniques were used in this research to analyse prostate tissue. A non-invasive assay to investigate expression of PSCA would require its detection in an easily obtained blood or urine sample. Measurement of PSCA RNA in peripheral blood has been carried out using reverse transcriptase polymerase chain reaction (RT-PCR) (94) with similar results to the studies on tissues, suggesting its potential as a blood-based biomarker for prostate cancer prognosis.

**Prostate-specific Membrane Antigen (PSMA)**

Prostate-specific membrane antigen (PSMA) is an integral membrane glycoprotein expressed on the surface of prostate epithelial cells. Its expression is very prostate-specific although weak expression is seen in other tissues (95, 96). Horoszewicz et al. (95) found the expression of PSMA to be upregulated in the epithelial cells of malignant prostate tissue compared to benign tissue by immunohistochemistry. Other studies have found its overexpression in the serum of prostate cancer patients (95, 97, 98). Serum concentrations of PSMA have been measured using ELISA (97) and Western blot assays (98). PSMA increases have been found to correlate with increasing Gleason score and stage progression of the disease especially in hormone-refractory disease (99-102), although several studies have concluded that its use in monitoring the disease is not significantly better than other prognosticators such as PSA (52, 103). The highly specific and sensitive immunoscintigraphic test called ProstaScint uses antibodies to PSMA to enable prostate cancer cells to be identified (104, 105), allowing the presence and location of recurrent disease in the prostate bed and lymph nodes to be determined after treatment (106), as well as being particularly useful prior to treatment in determining whether cancer is likely to recur (104, 106). RT-PCR has been used for analysis of PSMA in the buffy coat of blood. Although many studies using RT-PCR for PSMA analysis have not found a correlation between PSMA and grade and stage of disease or it to be particularly useful in the detection of hidden metastases, a combination of PSMA and PSA RT-PCR assays has found a correlation with stage and it was concluded that PSMA/PSA RT-PCR is a superior prognostic predictor of extracapsular extension of the tumour than serum PSA, clinical stage and biopsy Gleason score (107).

**Chromogranin A**

One of the most abundantly produced peptides of neuroendocrine cells of the prostate, chromogranin A (CgA) is used as a marker of neuroendocrine features (108). Neuroendocrine differentiation may be involved in the progression of prostate cancer (109). CgA can be
readily measured in serum samples and a wide array of studies have correlated increased levels of CgA with a higher grade and stage of disease and with decreased survival of patients, in particular in men with hormone-refractory disease (108, 110, 111). It may serve as a useful marker for showing progression to hormone-refractory disease and for the monitoring of patients undergoing endocrine treatment (110, 112). Although this marker shows promise as a prognostic marker, it would only be appropriate for prostate cancer where neuroendocrine differentiation is a feature.

**Hepsin**

Hepsin, a transmembrane serine protease that is found in large concentrations in prostate tissue, shows up-regulation of its gene in 90% prostate adenocarcinomas by expression profiling (113). Although some studies have shown that hepsin RNA expression increases with increasing stage and grade of the disease (113, 114), there is no test available yet for the detection of hepsin in urine or serum.

**TMPRSS2:ERG and TMPRSS2:ETV1**

**Gene Fusion**

Rearrangements of genes are frequently seen in cancer (12). In most cases of prostate cancer there are rearrangements of either of two genes of the ETS (E26 avian erythroblastosis virus) family of transcription factors (33, 115); ERG (ETS-related gene) or ETV1 (ETS variant gene 1) with a gene that encodes the membrane-anchored serine protease, TMPRSS2. The fusion gene TMPRSS2:ERG has been found to have a greater association with higher Gleason grade, metastasis and shortened survival than TMPRSS2:ETV1 (116).

**Ki-67**

A study by Miyake (20) investigated the prognostic potential of multiple molecular markers and several conventional prognostic markers to predict biochemical recurrence after radical prostatectomy. They found that the molecular marker Ki-67 and the conventional markers seminal vesicle invasion (SVI) and surgical margin status (SMS) could independently predict biochemical recurrence. They concluded that the use of these three markers together improved the ability to predict biochemical recurrence. Ki-67 has a role in regulation of the cell cycle and is a nuclear antigen. The fraction of nuclei that are positive for Ki-67 by immunohistochemical staining provides a Ki-67 index. A high Ki-67 index has been associated with earlier recurrence of disease and progression to metastatic disease (117, 118).

**p53**

Mutations of the p53 tumour suppressor protein which may lead to uncontrolled cellular proliferation have been associated with prostate cancer (119, 120). Presence of an abnoma p53 protein shows promise as a potential predictor of disease recurrence after radical prostatectomy (121, 122). Correlations have been found between this protein and features of aggressive disease such as presence of metastasis and decreased survival (123). These studies were conducted on prostate tissue as there is no currently available assay to measure this biomarker in blood. Other molecular markers which have a role in controlling the cell cycle have been explored. These include p27 which inhibits the cell cycle. Immunohistochemical studies on samples from radical prostatectomies have found an inverse correlation between p27 levels and worse prognosis (124).

**Conclusion**

Prostate cancer has a heterogeneous nature and the prognosis after a diagnosis of prostate cancer is extremely variable. It seems likely that future methodologies, in order to predict the course of the disease for an individual patient, will rely on novel prognostic information individual to the patient relating to the expression of multiple genetic or proteomic biomarkers. This would reflect the complex changes that take place during progression of the disease.

This review has given an overview of only a selection of molecular markers that may prove useful in providing both clinician and patient more information about the patient’s prognosis than is currently possible without invasive action. This could enable the most beneficial treatment strategy to be determined limiting mortality of the disease and its negative effects on quality of life.

**References**


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