# PSA AND OTHER BIOMARKERS FOR EARLY DETECTION, DIAGNOSIS AND MONITORING OF PROSTATE CANCER

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Prostate cancer (PCa) is a leading cause of illness and death among men in Europe and the United States. With widespread screening for prostate-specific antigen (PSA) and digital rectal examination (DRE), as well as early treatment of localized prostate cancer, however, the mortality due to PCa decreases. For a Caucasian male, the lifetime risk of developing PCa is 16%, but the risk of dying of PCa is only 3%. Many more cases of PCa do not become clinically evident, as indicated in autopsy series, where PCa is detected in one-third of men under the age of 80, and in two-thirds of older men. These data suggest that PCa, while easily detectable, often grows so slowly that most men die of other causes before the disease becomes clinically advanced.

## 10.1 Risk factors for prostate cancer

Age, ethnicity, genetic factors, dietary factors, lifestyle-related factors, and androgens are the most important contributors to the risk of PCa.

### 10.1.1 Age

PCa rarely occurs before the age of 45, but the incidence rises rapidly thereafter. With the aging of our population, the incidence of PCa has increased sharply, to the extent that 50% of men over the age of 80 exhibit underlying PCa.

### 10.1.2 Race / ethnicity

The risk of PCa is dramatically higher among blacks, intermediate among white and Hispanic men, and lowest among Asians. Population differences in androgen levels, dietary factors, socioeconomic factors or genetic factors

are possible explanations. The risk of PCa among Asians increases when they immigrate to North America - implicating the environment and lifestyle-related factors in causing PCa.

### 10.1.3 Genetic factors

The risk of PCa is approximately two-fold elevated in men with an affected first degree relative (brother, father), compared to those without an affected relative, and increases with a greater number of affected family members; men with 2 or 3 affected first-degree relatives had a 5- and 11-fold increased risk of PCa, respectively. Early age of onset in a family member also increases the risk. Among twins, 42% of cases of PCa were attributed to inheritance, with the remainder most likely attributable to environmental factors.

Inherited prostate cancer-susceptibility genes have been identified on multiple chromosomes. The RNASEL gene, which encodes an endoribonuclease that degrades viral and cellular RNA, has been linked to the hereditary prostate cancer (HPC1) gene on chromosome 1q. An increased risk of PCa is associated with mutant RNASEL alleles that encode a less active enzyme.

Another candidate prostate-cancer-susceptibility gene, the macrophage-scavenger receptor 1 (MSR1) gene, located at 8p22, encodes subunits of the macrophage-scavenger receptor that is capable of binding a variety of ligands, including bacterial lipopolysaccharide and serum oxidized low-density lipoprotein.

The presence of BRCA1/2 mutations may increase the risk of developing PCa at least 2 to 5-fold.

### 10.1.4 Androgens

Androgens and the androgen receptor (AR) regulate the early embryological differentiation and later growth cycles of the prostate. The prostate is a walnut sized secretory organ of the genitourinary tract system, which produces most of the fluids in semen that provide nutrients for sperm. It is formed initially from the urogenital sinus and undergoes two significant growth cycles in life. The first occurs from puberty to approximately age 25 and the second at ages 40–60. The organ consists of both luminal and basal epithelial components.

Differentiated luminal glandular epithelial cells express the AR.

The AR plays a critical role in the prostate. Its primary function is to provide responsive gene products for differentiation and growth, but under abnormal conditions it contributes to the development of PCa. Males who

are castrated at early ages do not develop PCa, implying that androgens are risk factors for PCa development. High levels of plasma androgens are associated with a high incidence of PCa.

In genital tissue including the prostate, testosterone is converted by 5a-reductase type II to the more active androgen, dihydrotestosterone (DHT). DHT binds to the AR, thereby initiating downstream effects, including proliferation, differentiation, and prevention of apoptotic cell death in the prostate. Androgen binding induces the release of heat shock proteins, hyperphosphorylation, conformational changes, and dimerization of the AR. The ligand-receptor complex then translocates to the cell nucleus where it binds to androgen responsive elements located within the promoters of androgen-responsive genes.

Polymorphic variants of several genes involved in androgen action, including the androgen-receptor (AR) gene and the steroid 5a-reductase type II (SRD5A2) gene, have been proposed as possible contributors to the risk of PCa. In the case of AR, polymorphic polyglutamine (CAG) repeats have been described. That affects transcription and activation of this gene. Blacks, who have a relatively high risk of PCa, have shorter CAG repeats, whereas Asians, who have a relatively low risk of PCa, have longer CAG repeats. Genetic epidemiologic studies have shown a correlation between an increased risk of PCa and the presence of short androgen-receptor CAG repeats.

Polymorphic variant SRD5A2 alleles that encode 5a-reductase enzymes with increased activity have been associated with an increased risk of PCa and with a poor prognosis for men with PCa.

Metastatic prostate cancers are lethal because they are heterogeneously composed of both androgen-dependent and androgen-independent malignant cells. For those cells that are androgen-dependent, androgens activate the AR so that transcription of death-signaling (apoptotic) genes is repressed. Hormone therapies (androgen ablation) allow these genes to be expressed, triggering the biochemical cascade that results in apoptotic cell death, resulting in the eradication of the large fraction of androgen-dependent cancer cells. For this insightful work, the Nobel Prize in Physiology or Medicine was awarded to Dr. Charles Huggins in 1966. Hormone ablation in metastatic PCa is usually achieved by androgen suppression, antiandrogens, or a combination of the two. Initially, many tumours (~80%) respond favorably to this treatment by regression in size but progression is inevitable, because of the emergence of androgen-independent prostate cancer cells. Many AR mutations have been detected in prostate cancers, especially in those that progress despite hormonal treatment. AR amplification, accompanied by overexpression of androgen receptors, may promote the growth of androgen-independent prostate-cancer cells by increasing the sensitivity of prostate-cancer cells to low levels of circulating androgens.

### 10.1.5 Diet

Increased total fat intake, animal fat intake, and consumption of red meat, coffee, and zinc supplements have been associated with an increased risk of PCa although data are inconsistent. A link between high calcium intake, vitamin D deficiency and PCa risk has been suggested. Other reports suggest a protective effect of fish consumption, particularly those varieties containing high amounts of omega-3 fatty acids. Prospective studies are consistent with a protective role for lycopene (from tomatoes), vitamin E and selenium. Intake of vegetables, fruits, milk, carotenoids, and the vitamins A and C is not consistently related to reduced PCa risk.

### 10.1.6 Vasectomy and ejaculatory frequency

Vasectomy may increase the risk of PCa, an effect that appears to increase with time after the procedure, but data are not consistent. An association between ejaculatory frequency and a lower risk of PCa has been suggested, but there is no protective effect from being married or having more sexual partners.

### 10.1.7 Prostatitis

Chronic or recurrent prostate infection or inflammation probably initiates carcinogenesis in symptomatic or asymptomatic prostatitis. Inflammatory cells produce microbicidal oxidants that might cause cellular or genomic damage in the prostate. Two inherited susceptibility genes, RNASEL and MSR1, and the decreased risk of PCa associated with the intake of antioxidants or nonsteroidal antiinflammatory drugs (NSAIDs) support this hypothesis. Several studies suggest an increased risk of PCa in men with prostatitis and in those with a history of syphilis or gonorrhea. However, PSA values can be elevated with prostatitis, leading to more prostate biopsies and a greater likelihood of making the diagnosis of cancer.

### 10.1.8 Insulin-like growth factor

Insulin-like growth factor (IGF)-1 and its binding protein, IGFBP-3, modulate cell growth and survival, and are important in tumour development. High IGF-1 concentrations are associated with increased risk of PCa, and serum IGFBP-3 suppresses the mitogenic action of IGF-1.

### 10.1.9 Obesity and physical activity

Some studies suggest a positive relationship of serum insulin, obesity, body mass index, waist-hip circumference and other anthropometric measures with PCa risk. Other proposed risk factors (such as lack of exercise) are currently being studied.

## 10.2 Molecular biology of prostate cancer

Prostate cancer, once generally diagnosed at an advanced stage in older men, is now more often detected at an early stage in younger men as a consequence of more widespread PSA screening. This trend has changed the definition of a “case” of cancer, since many men who would have qualified as controls in previous genetic and epidemiologic studies are now known to have PCa. Control groups also include a large, often unknown proportion of subjects with benign prostate hypertrophy (BPH). BPH may also be androgen dependent and affected by the same genetic polymorphisms. Despite these limitations, genetic studies have provided remarkable clues to the causes of PCa.

Although prostate cancer typically presents in men over the age of 65, prostatic carcinogenesis is probably initiated much earlier. Lesions of prostatic intraepithelial neoplasia (PIN), which are thought to represent a precursor of adenocarcinoma, do not always progress to invasive disease. PIN represents a spectrum of dysplastic changes that are limited to prostatic acini and do not invade the basement membrane. PIN appears to represent an intermediate stage between normally differentiated prostatic tissue and prostatic adenocarcinoma. PIN precedes cancer by about ten years. Basal cell layer disruption occurs with progressive loss of differentiation and increased proliferative activity that is accompanied by genetic alterations that affect the AR and other molecules involved in the regulation of cell survival and apoptosis. Progressive accumulation of these genetic alterations facilitates cellular transformation from normal prostate epithelium to PIN, invasive neoplasia, and the state of androgen independence.

At the time of diagnosis, prostate-cancer cells contain many mutations, gene deletions, gene amplifications, chromosomal rearrangements, and changes in DNA methylation that are associated with genetic predisposition (eg, 1q deletions), that result in amplification of oncogenes (eg, c-myc, beta catenin, HER-2/neu, Ras, MKP-1, EZH2, Bcl 2, telomerase) or that result in the loss of function of tumour-suppressor genes (eg, GSTP1, NKX3.1, PTEN, p27, p53). These alterations accumulate over several decades with the progression of prostate cancer. Many chromosomal aberrations and candidate genes or their protein products are under study for their value in clinical staging with the goal of more closely tailoring the selection of treatment options, and, perhaps more importantly, might reveal additional targets for therapy.

Over-expression of the Bcl-2 oncogene by prostate cancers decreases apoptosis, and upregulation of Bcl-2 (particularly in combination with p53 mutations) is a frequent and important step in the progression to advanced or hormone independent disease. The Bcl-2 protein is a potential target for clinical intervention.

Telomerase compensates for telomere shortening during cell division by synthesizing telomeric DNA, thereby maintaining telomere length. Upregulation of telomerase and amplification of telomeric DNA is detected in up to 90% of prostate cancers, and in high-grade PIN. Telomerase has been exploited both as a diagnostic tool and a therapeutic strategy in PCa. A urinary assay for telomerase has been proposed as a noninvasive means of detecting PCa.

Hypermethylation of the tumour suppressor GSTP1 gene, encoding glutathione S-transferase (GSTP1), prevents the transcription of GSTP1. GSTP1 is absent in more than 90% of prostate-cancers and also in PIN. GSTP1 serves as a “caretaker” gene, defending prostate cells against genomic damage mediated by carcinogens and various oxidants at sites of inflammation. Epithelial cells in proliferative inflammatory atrophic lesions, which are thought to be a precursor to PIN and PCa, show many molecular signs of stress caused by inflammatory oxidants, such as high levels of GSTP1 and cyclo-oxygenase-2 (COX-2). Loss of the GSTP1 caretaker function, as cells of proliferative inflammatory atrophy give rise to cells of PIN and to prostate-cancer cells, increases the prostate's vulnerability to genomic damage caused by oxidants and dietary carcinogens.

Somatic allelic losses in the tumour-suppressor phosphatase and tensin homologue gene (PTEN, also termed MMAC1) located on chromosome 10q are common in prostate cancers and may promote abnormal proliferation of prostate cells. PTEN is present in normal epithelial cells and in cells in PIN, but is frequently reduced in PCa cells, particularly in cancers of a high grade or stage. PTEN act as a tumour suppressor by inhibiting the phosphatidylinositol 3'-kinase–protein kinase B (PI3K–Akt) signalling pathway that controls the cell cycle and apoptosis.

### 10.3 Prostate specific antigen

Prostate specific antigen (PSA) is a glycoprotein that is expressed by both normal and neoplastic prostate tissue. It is a member of the human kallikrein (hK) gene family, located on chromosome 9, encoding serine proteases that have many structural similarities and significant homologies. PSA (hK3) is produced by the prostatic epithelial cells and the periurethral glands, but its physiological function is still not well understood. It is secreted into the seminal fluid, where it is involved in liquefaction of the seminal coagulum. Investigators have reported that PSA may act as a tumour suppressor, a cell growth inhibitor, an anti-angiogenic molecule, or as an apoptotic molecule whereas other suggest that PSA may, through its proteolytic activity, promote tumour progression and metastasis. PSA may

cleave IGFBP-3, thus liberating IGF-1 that is a mitogen to prostatic stromal and epithelial cells.

PSA's relative tissue specificity makes it valuable as a tumour marker for prostatic cancer, although recent publications have reported that PSA is widely expressed, at lower concentrations than in prostate, in many tissues including the female breast. PSA is efficacious as a screening and diagnostic analyte and, together with a digital rectal examination (DRE), it has become the standard test for detecting PCa. The absolute value of serum PSA is useful for determining the extent of PCa, and assessing the response to PCa treatment particularly surgical prostatectomy, because complete removal of the prostate gland should result in PSA being undetectable. Measurable PSA after radical prostatectomy indicates residual prostatic tissue or metastasis, and increasing PSA concentrations indicate recurrent disease.

### 10.3.1 PSA expression and processing

PSA is regulated at the transcriptional level by the AR through androgen response elements in the promoter region of the gene. PSA is produced as a prohormone (proPSA) by the secretory cells that line the prostate glands (acini), and secreted into the lumen, where the first seven amino acids of the propeptide (244 residues) are removed by hK2 to generate enzymatically active PSA (237 residues). This molecule undergoes proteolysis to generate inactive PSA, which enters the bloodstream and circulates in an unbound state (free PSA). Active PSA, that diffuses into the circulation, is rapidly bound by protease inhibitors. Of the total PSA (**tPSA**) in serum, the majority is complexed (**cPSA**) with serum proteins $a_1$-*antichymotrypsin* (ACT) (70-90%), $a_1$-antitrypsin and protein C. An additional proportion that is complexed with $a_2$-macroglobulin has low or no immunoreactivity in most commercial PSA immunoassays. Free (noncomplexed) PSA (**fPSA**) accounts for 10-30% of tPSA.

In prostate cancer, PSA expression per cell is lower than in normal prostate epithelium. However, the increased serum PSA concentration in PCa is attributable to increased cell numbers and destruction of the basement membrane, basal cells, and normal tissue architecture. As a result, higher amounts of the secreted proPSA have direct access to the circulation, and a larger fraction of the PSA produced by malignant tissue escapes proteolytic processing (ie, activation of proPSA to active PSA, and degradation of active PSA to inactive PSA).

In men with a normal prostate, the majority of fPSA in the serum reflects the mature protein that has been inactivated by internal proteolytic cleavage. In contrast, this cleaved fraction is relatively decreased in PCa. Thus, the percentage of fPSA is lower in the serum of men with PCa (and conversely, the %cPSA is higher) compared to those who have a normal prostate or BPH. This finding has been exploited in the use of the fPSA/tPSA ratio and cPSA to distinguish between PCa and BPH as a cause of an elevated PSA.

### 10.3.2 Reference ranges

A serum PSA concentration above 4 ng/mL is considered abnormal in most available immunoassays, with a diagnostic grey zone between 4 and 10 ng/mL. Total PSA distribution in men aged 55-70 years shows that

- Most men (81%) have PSA values in the range of 0-2 ng/mL
- More than 50% of men with PSA > 10 ng/mL have prostate cancer
- 5% of all men are found in the grey zone, of which ~20% have prostate cancer
- Men with low PSA values (2-4 ng/mL) have a ~15% likelihood of having PCa

*Age-specific reference ranges*

The PSA concentration increases yearly in men over the age of 40; it increases at a faster rate in elderly men. Age-specific reference ranges may improve specificity and positive predictive value of the serum PSA in screening for PCa. However, it should be recognized that the use of a higher upper range of normal for older men reduces the sensitivity of serum PSA testing for the detection of early PCa, potentially missing an unacceptable number of clinically significant cancers in older men.

*Race-specific normal ranges*

Specific ethnic and racial groups may require different definitions of a “normal” PSA value. Serum PSA concentrations are significantly higher in black compared with white men. However, the utility of race-specific normal reference ranges remains unclear.

### 10.3.3 Causes of an elevated serum PSA

The major causes of an elevated serum PSA include benign prostatic hyperplasia (BPH), prostate cancer, prostatic inflammation and perineal trauma.

*Benign prostatic hyperplasia*

The most common explanation for an elevated serum PSA is BPH because of the very high prevalence this condition in men over the age of 50. BPH produces more PSA per gram than normal prostate tissue. Serum PSA levels overlap considerably in men with BPH and those with PCa.

Treatment for BPH with *finasteride*, an inhibitor of 5-a-reductase, can reduce serum PSA concentrations. Finasteride decreases serum PSA by 50% due to direct interference with the prostatic intracellular androgen response mechanism. Thus, the appropriate serum PSA reference range for men receiving finasteride is one-half that of men not receiving the drug.

*Prostate cancer*

When the conventional cutoff of 4 µg/L is used, tPSA is clearly more sensitive than DRE for the detection of PCa, but is has low specificity. False positive tests (due to BPH or prostatitis) occur primarily in men age 50 or older. In this age group, 15 of every 100 men will have elevated PSA levels (> 4 ng/mL). Of these 15 men, 12 will be false positives and only 3 will turn out to have cancer.

Furthermore, although the majority of prostate cancers express PSA, 20 to 50% of men with prostate cancers have serum PSA values < 4.0 ng/mL. It is difficult to recognize false-negative test results, because prostate biopsies are generally not performed if the PSA is normal. Most prostate cancers are slow-growing and may exist for decades before they are large enough to cause symptoms. Those cancers that are detected at a time when the serum PSA is < 4.0 ng/mL have a higher likelihood of being organ confined than cancers detected at a time when the PSA level is > 4.0 ng/mL. The absolute level of serum PSA can predict local disease extent:

- A serum PSA of 4.1 to 10.0 ng/mL at the time of diagnosis increases the likelihood of finding a tumour larger than 0.5 mL (a volume considered clinically significant by many investigators) which is either confined to the prostate capsule, and therefore most amenable to curative therapy, but also increases the odds of finding extracapsular extension by 5.1-fold.
- A serum PSA > 10.0 ng/mL increases the likelihood of extraprostatic extension by 24 to 50-fold.

*Prostatic inflammation*

Prostatitis is an important cause of an elevated PSA. Many physicians will initially treat a man with an elevated serum PSA for prostatitis, and then obtain a repeat serum PSA; a return of the PSA to normal is expected if prostatitis was solely responsible. The fPSA/tPSA ratio is unable to distinguish chronic inflammation from PCa, as both conditions lower the percentage of free PSA.

*Perineal trauma*

Any perineal trauma can increase the serum PSA. DRE may cause minor transient elevations that are clinically insignificant. Mechanical manipulation of the prostate by cystoscopy, prostate biopsy, or transuretheral resection (TUR) can more significantly affect the serum PSA. A serum PSA determination after cystoscopy is reliable, but a serum PSA determination should not be obtained for at least six weeks after either a prostate biopsy or TUR. Bicycle riding does not significantly affect serum PSA. In addition, sexual activity can minimally elevate the PSA (usually in the 0.4 to 0.5 ng/mL range) for approximately 48 to 72 hours after ejaculation.

### 1.3.4 General limitations of tPSA assays.

- 80% of all patients with total PSA in the diagnostic grey zone (4-10 ng/mL) have negative biopsies.
- Low specificity in the grey zone: approx. 20% of all patients with PSA between 4-10 ng/mL have prostate carcinoma.
- For the cut-off of 4,0 ng/mL sensitivity for total PSA is in the range of 68-80% and has a specificity of ~25-30%.
- PSA is also released in men with BPH and serum levels are proportional to prostate size.
- Asymptomatic and DRE negative men with PSA concentrations = 4 ng/mL are usually not biopsied in clinical routine. Approx. 20% of prostate cancers are found in this group.
- tPSA is unable to differentiate between aggressive and non-aggressive prostate cancers.

### 10.3.5 Advances in PSA testing

Various diagnostic approaches have been proposed using tPSA, fPSA or cPSA combined with DRE or transrectal ultrasonography (TRUS) to improve the differentiation between prostatic cancer and BPH. These modifications would presumably be most useful for PCa screening when the total PSA is 4.0 to 10.0 ng/mL, the range in which decisions regarding further diagnostic testing are most difficult. However, in clinical practice, the use of these techniques has not resulted in superior patient outcomes compared to simple PSA testing.

*PSA density*

PSA density considers the relationship of the PSA level to the size and weight of the prostate. In other words, an elevated PSA might not arouse suspicion in a man with a very enlarged prostate. TRUS is used to measure prostate volume. Serum PSA is then normalized by prostate volume to give a prostate density, with higher PSA density values (greater than 0.15) being more suggestive of PCa than BPH. However, there are inherent difficulties to measuring PSA density, which include errors of prostatic volume measurement with TRUS and an intrapatient variation of up to 15% in PSA density with repeated measurements. In addition, there is considerable overlap between patients with PCa and those with BPH.

*PSA velocity*

Another approach has been to assess the rate of PSA change over time (the PSA velocity). An elevated serum PSA that continues to rise over time is more likely to reflect PCa than one that is consistently stable. A PSA velocity cutoff of 0.75 ng/mL per year can distinguish patients with PCa from those with either BPH or no prostate disease with a specificity of 90 and 100%, respectively. However, men with PCa often have a PSA velocity of less than 0.75 ng/mL per year, especially those with lower PSA levels.

Multivariate and ROC analyses suggest that calculation of PSA velocity and PSA doubling time are of limited value in screening.

*Serum fPSA*

As noted previously, PCa is associated with a lower serum fPSA as compared to benign conditions. The percentage of free PSA (fPSA/tPSA) has been used to improve the sensitivity of cancer detection when total PSA is in the normal range (< 4 ng/mL), and, most often, to increase the specificity of cancer detection when total PSA is in the "gray zone" (4.1 to 10.0 ng/mL). In this latter group, the lower the value of fPSA/tPSA, the greater the likelihood that an elevated PSA represents cancer and not BPH. As an example, using a cutoff of < 10% fPSA/tPSA, the probability of cancer is 56%, compared to only 8% of men with a ratio >25%. Use of %fPSA in the grey zone 4.1 to 10.0 ng/mL can eliminate 20-25% of unnecessary biopsies. Furthermore, use of %fPSA in the tPSA range of 2.0 to 3.9 ng/mL can predict tumour aggressiveness and thus can be used for risk stratification to select treatment options. Equimolar recognition of free and complexed PSA in immunoassays is crucial for correct measurement.

*Serum cPSA*

Immunoassays for ACT-complexed PSA (cPSA) would theoretically provide a similar enhanced degree of specificity as fPSA/tPSA but require only the measurement of a single analyte. In addition to the obvious economic advantage, the variability associated with nonequimolarity of different manufacturers' assays for total or free PSA could be avoided. cPSA, cPSA/tPSA or fPSA/cPSA perform better than tPSA alone, and are similarly effective as fPSA/tPSA in reducing the rate of unnecessary biopsies. Although determination of ACT-PSA would have the analytical advantage of measuring the major and not the minimal fraction and the clinical advantage of measuring the fraction of serum PSA directly related to PCa, over-recovery due to interferences with the ACT-cathepsin G complex hampers accurate ACT-PSA assays. In men with febrile urinary tract infection (UTI), sustained elevations of cPSA and tPSA for up to 6 months after UTI could be falsely interpreted as a sign of PCa.

*New promising markers*

*Human glandular kallikrein* (hK2) and the inactive fPSA forms *proPSA* (precursor PSA, associated with PCa) and BPSA (“benign PSA”, an internally cleaved PSA isoform associated with BPH) have been proposed as potential discriminatory tools and may improve the early detection of PCa in men with tPSA < 4 ng/mL. Although early results are encouraging, further evaluation is anticipated.

## 10.4 Screening for prostate cancer

Survival in men with prostate cancer is related to many factors, one of the most important being extension of the tumour beyond the prostate capsule at the time of diagnosis. The ten-year survival among men with "early stage" cancer confined to the prostate is 75%, compared with 55 and 15%, respectively, among those with regional extension and distant metastases. Thus, a screening program should identify those men with early stage tumours that have not spread beyond the prostate capsule.

The combination of DRE plus serum PSA testing, followed by TRUS-guided prostate biopsy if either test is positive, is the screening strategy that is now most commonly recommended. New refinements in serum PSA assays which may add discriminatory value to the test including tPSA density, tPSA velocity, fPSA/tPSA, cPSA, cPSA/tPSA, and fPSA/cPSA could substantially reduce the number of unnecessary prostate biopsies that are done because of false-positive test results. Additional serum measurements of BPH-associated BPSA, cancer-associated proPSA, and hK2 are promising developments to improve the specificity of PSA screening, but not yet used in clinical practice.

### 10.4.1 Recommendations

Major medical associations and societies have not developed a clear consensus regarding recommendations for serum PSA-based screening for the early detection of PCa. Evidence is insufficient to determine whether the benefits outweigh the harms (including frequent false-positive results and unnecessary anxiety, biopsies, and potential complications of treatment) for a screened population. The diagnostic procedure may cause significant side effects, including bleeding and infection. PCa treatment often causes incontinence and impotence.

Although PSA-based screening in asymptomatic men may lead to diagnosis at an early stage, it is not known whether it actually saves lives. Given uncertainties about the effectiveness of screening and the balance of benefits and harms, the cost-effectiveness of screening for PCa is impossible to determine. PSA screening may be cost-effective for men aged 50 to 69 years. Men older than 70 to 75 years are unlikely to benefit substantially from screening because of their shorter life-expectancy and higher false-positive rates. For men who have a very low PSA concentration (< 0.5 to 1.0 ng/mL), it may be reasonable to stop screening after the age of 65, or at least to reduce the frequency of screening, since very few go on to develop PCa.

In 2002, the European Group of Tumour Markers (EGTM) and the National Academy of Clinical Biochemistry (NACB) proposed the following guidelines for PSA as tumour marker:

- PSA must not be used alone but should be evaluated in conjunction with DRE
- Biopsies for DRE negative patients with PSA < 4 ng/mL are not recommended
- The EGTM does not recommend age- and race-specific reference values in contrast to the NACB
- Both recommend %fPSA in the range 4-10 ng/mL, if DRE is negative
- Blood should be drawn before any manipulation of the prostate and several weeks after resolution of prostatitis
- The EGTM does not recommend PSA screening but it is recommended by the NACB.

If the serum PSA concentration is abnormal, the test should be repeated within two to four weeks for confirmation. Men should be provided with adequate information regarding the risks and benefits of screening so that they can make informed decisions.

### 10.5 Diagnosis of prostate cancer

Early prostate cancer usually causes no symptoms and is found by a PSA test and/or DRE. When symptomatic, PCa can cause urological problems such as inability to urinate or difficulty starting or stopping the urine flow, urinary urgency, nocturia, the need to urinate more frequently, weak or interrupted urine flow, pain or burning during urination; these symptoms are also present in men with BPH, and are more likely to be caused by BPH than cancer.

Haematuria, haematospermia and erectile dysfunction are signs of advanced PCa. A small percentage of men present with non-specific symptoms due to metastatic disease such as bone pain or, rarely, spinal cord compression.

#### 10.5.1 Serum PSA elevation

Malignant prostate tissue generates more PSA than normal or hyperplastic tissue, probably because of increased cellularity and disruption of the prostate-blood barrier. Serum PSA should be obtained prior to biopsy, both for diagnostic and prognostic purposes. Prostate biopsy is advised if serum PSA is > 4 ng/mL, even in the presence of a normal DRE.

#### 10.5.2 Abnormal DRE

All men with induration, asymmetry, or palpable nodularity of the prostate gland require further diagnostic studies to rule out PCa, particularly if they are over the age of 45 or have other risk factors for the disease. Even if the

serum PSA is in the normal range (ie, < 4 ng/mL), prostate biopsy may be indicated in men with a DRE examination that is suspicious for cancer.

### 10.5.3 Prostate biopsy

Prostate biopsy is the gold standard for PCa diagnosis. If symptoms or test results suggest PCa, a needle biopsy is performed guided by TRUS. Several biopsy samples are often taken from different areas of the prostate. If PCa is strongly suspected (e.g. due to a very high PSA level) a repeat biopsy may be needed to rule out false negative biopsy results.

### 10.5.4 Molecular detection in urine

Molecular assays for urinary detection of PCa are beginning to be explored. Initial studies suggest that these tests have very high specificity, thereby differentiating PCa from BPH. Promoter hypermethylation of the GSTP1 gene is one of the earliest molecular changes in PCa, and a test has been developed for urinary detection after prostatic massage. Others have evaluated a urinary assay for telomerase as a molecular marker of PCa.

## 10.6 Grading the Prostate Cancer

The Gleason histological scoring system assigns a grade from 1 to 5 to each area of cancer, based upon the degree of glandular differentiation and structural architecture. Grade one represents the most well-differentiated appearance, and grade five represents the most poorly differentiated. Because prostate cancers often have areas with different grades, a primary and secondary grade score are reported, and combined to yield the Gleason score between 2 and 10. The higher the Gleason score, the more likely it is that the cancer will grow and spread rapidly. Scores of 2 through 4 are often grouped together as low, 5 and 6 are intermediate, and scores of 7 to 10 are considered high.

If the prostate histology is reported as either atypical or high-grade PIN, there is a 30% to 50% chance of finding cancerous tissue somewhere else in the prostate gland. For this reason, repeat prostate biopsies are often recommended in these cases.

## 10.7 Staging the prostate cancer

If a prostate biopsy specimen is interpreted as containing carcinoma, staging is performed for choosing treatment options and predicting a patient's survival. Overall survival at ten years after radical prostatectomy

or radiation therapy is high, but the likelihood of remaining disease free (defined as an undetectable serum PSA) at ten years following treatment is related to both the biology of the cancer, which is approximated by its grade, stage, and volume, as well as whether the surgical margins of excision are positive. Thus, using various tests, the primary goal of staging is to rule out the presence of disease outside of the prostate gland, and to assess the likelihood of finding potentially resectable, organ-confined disease. DRE results, PSA level, and Gleason score are used to decide which other tests (if any) to perform. Men with a normal DRE result, a low PSA, and a low Gleason score may not need any other tests, because the chance that the cancer has spread is low.

The tumour-node-metastasis (TNM) system is widely used for staging prostate cancer. Once the T, N, and M categories have been determined, this information is combined, along with the Gleason score, in a process called *stage grouping*. Men are assigned an overall stage from I (the least advanced) to IV (the most advanced).

Imaging tests, including TRUS, *computed tomography* (CT scan), *magnetic resonance imaging* (MRI), and the *radionuclide bone scan*, are most widely used.

Serum tPSA alone is of limited value for staging, but may help to predict disease extent in men with PCa:

- There is a higher likelihood of finding organ-confined disease when serum PSA is < 4.0 ng/mL.
- A serum PSA concentration of 4.1 to 10.0 ng/mL at the time of diagnosis of PCa increases the likelihood of finding an organ-confined tumour larger than 0.5 mL, but also increases the odds of finding extracapsular extension by 5.1-fold.
- A serum PSA concentration > 10.0 ng/mL increases the likelihood of finding extraprostatic extension by 24 to 50-fold. CT scan of the abdomen and pelvis and bone scan should always be performed in these patients.

Volume-adjusted (density) parameters of tPSA, cPSA, and fPSA significantly enhance the prediction of extraprostatic disease extension in men with nonpalpable PCa.

### 10.7.1 Molecular staging

RT-PCR testing of blood for prostate cancer-specific gene expression, or “molecular staging”, is a promising technique for choosing treatment options. PSA-producing cells can be detected in the systemic circulation of men with newly diagnosed clinically localized prostate cancer using RT-PCR for PSA mRNA. The clinical utility of these refinements is not clear. This molecular marker showed no additional benefit for predicting tumour stage

or volume in men with clinically localized disease, while others suggest prognostic utility in those with advanced hormone-refractory disease.

## 10.8 Follow-up of men with prostate cancer after initial therapy

A consequence of diagnosing PCa at an earlier stage in younger men is a significant increase in the utilization of locally aggressive but potentially curative therapies, *radical prostatectomy* (RP) and *external beam radiation therapy* (EBRT). Other therapeutic options include *brachytherapy, cryoablation, hormonal therapy* (androgen ablation), and *watchful waiting*. Advances in the therapeutic modalities have reduced the incidence of side effects and now offer patients a choice of treatments depending on many factors such as tumour characteristics, age, and co-morbidity.

### 10.8.1 Localized prostate cancer

Serum PSA is the mainstay of surveillance testing in men who have undergone therapy for localized PCa. Many of these men desire close follow-up, with early intervention if a recurrence is detected, typically by a progressively rising serum PSA level. While the optimal frequency of PSA testing has not been established, every 6 to 12 months is reasonable. Most experts also recommend annual DRE.

The majority of recurrences following RP or RT are asymptomatic. One consequence of routine monitoring of serum PSA following local therapy is the identification of men with a *"PSA-only" (biochemical) disease recurrence*, in which post-treatment increases in serum PSA over baseline are not accompanied by other symptoms or signs of progressive disease.

*Surgery*

RP is a common treatment for early stage PCa. Recent surgical innovations are *laparoscopic prostatectomy and cryosurgery*. All prostate tissue is removed following successful RP. Thus, any detectable PSA in the serum using a standard immunoassay theoretically indicates remaining prostate tissue, and presumably represents persistent/recurrent disease. By European consensus, PSA relapse after RP has been defined as a value of 0.2 ng/mL with one subsequent rise.

*Radiation therapy*

EBRT may be the primary treatment (instead of surgery) in early stage PCa. It may also be used after surgery to destroy remaining cancer cells in the area, or in palliative care. *Conformal radiation therapy* more precisely targets the cancer and spares normal tissue. This may permit the use of higher radiation doses without increasing side effects. *Brachytherapy* may also be used.

The definition of a “PSA-only” or biochemical recurrence following RT is more complicated. It is unreasonable to expect PSA levels to fall to undetectable levels since there is benign tissue remaining after RT. Furthermore, PSA levels tend to fluctuate or “bounce” after RT. PSA recurrence is defined as three consecutive increases in PSA after radiation therapy.

### 10.8.2 “PSA-only” recurrence following local therapy

Salvage RT is a treatment option for localized recurrence after RP. It is most successful when it is administered at a time when disease burden is low (ideally, when the serum PSA is below 2 ng/mL), and should be initiated when PSA levels reach 1.0-1.5 ng/mL.

Other options for men with a PSA-only recurrence include salvage RP, cryotherapy or brachytherapy for radiation patients, traditional or non-traditional hormonal therapy, and observation.

The *PSA doubling time* can predict both clinical metastasis-free survival and prostate cancer-specific mortality for men with a PSA-only recurrence. A PSA doubling time of <3 months identified men who were 19.6-fold more likely to die of PCa than those with a PSA doubling time of = 3 months.

### 10.8.3 Metastatic prostate cancer

For men who present with nodal involvement or distant metastatic disease, or who develop a systemic recurrence after initial local therapy, *traditional hormone therapy* (androgen ablation) is the standard treatment, effectively palliating symptoms in 80 to 90 percent of men, and possibly prolonging survival.

Blocking of androgen signaling results in a decrease in tumour volume as well as a decline in serum PSA in the majority of patients. Endocrine therapy involves androgen depletion by *orchiectomy* or by treatment with *luteinizing hormone releasing hormone (LHRH) agonists* as well as blockade of the AR with *anti-androgens*. These therapies can be applied singly or in combination. The combination of an LHRH agonist or orchiectomy and an antiandrogen is commonly referred to as *"complete androgen blockade"* (CAB), since it removes the influence of both testicular and adrenal androgens. However, therapeutic responses are typically limited; almost all tumours progress to androgen independence within two to five years.

Overt metastatic disease may not become evident for many years in men with a biochemical failure following local therapy. As a result, in asymptomatic men, it is unclear what constitutes the appropriate serum PSA level at which hormonal therapy should be instituted. Practices vary widely from starting treatment when the serum PSA is barely detectable (ie,

0.4 ng/mL), to waiting until various higher levels are achieved (ie, 10, 20, or even 50 ng/mL), or until clinical or symptomatic progression of disease. Many advocate initiating treatment early in the course of a PSA recurrence in the hope of delaying disease progression and possibly prolonging survival. Others have argued that there is no evidence for a significant survival benefit with any form of androgen deprivation, and that treatment is best deferred until clinical metastases or symptoms develop. Androgen deprivation is not curative, and it is associated with side effects that can alter quality of life (hot flashes, loss of libido, decreased muscle mass, mild anemia, osteoporosis).

New evidence suggests that there may be a role of *intermittent androgen deprivation* (IAD) rather than continuous suppression. The benefits of IAD include a possible delay in the emergence of androgen-independent tumour growth, and the potential for regaining potency and libido during the time when androgen deprivation is withheld. Reinitiation in IAD is based upon predefined threshold levels of serum PSA, which vary from 4 to 20 ng/mL, or upon serum testosterone levels rising out of the castrate range (typically >50 ng/mL).

Non-traditional, oral hormone therapy using *finasteride* (a 5-a-reductase inhibitor) and *flutamide* (a nonsteroidal antiandrogen) has been explored in the setting of PSA-only progression. These agents block the intraprostatic conversion of testosterone to DHT, and block the cytoplasmic DHT receptor, respectively. Since testosterone conversion is blocked selectively within the prostate, serum testosterone levels are maintained during administration of finasteride and flutamide. As a result, most men retain their pretreatment libido, potency, muscle mass, and erythropoietic capacity.

*Guidelines for follow-up*

A PSA level < 0.4 ng/mL after hormonal therapy can be considered as an indicator of a positive response. Although serial PSA testing in men with metastatic disease has never been shown to prolong life expectancy, rising PSA is an indication of treatment failure, signaling the need to consider alternative therapies. It is reasonable to measure PSA in men with metastatic disease every six months, more frequently if the serum PSA begins to rise or the patient complains of symptoms.

### 1.8.4 Watchful waiting

Watchful waiting is advised when the risks and possible side effects of surgery, radiation therapy, or hormonal therapy may outweigh the possible benefits. If watchful waiting is recommended, the patient will be monitored closely and will be treated if symptoms occur or get worse. This option is most appropriate for men ages 70 to 75 and older, and those with substantial co-morbid disease that may severely limit their life expectancy (< 10-15 years).

Watchful waiting may also be advised for some men with early stage prostate cancer and those who have a low predicted likelihood of aggressive disease (ie, a normal to minimally abnormal DRE, a low Gleason score (2-4), and a slowly increasing serum PSA concentration (< 1 ng/mL per year). In these men, initial observation is employed as a means of distinguishing rapidly progressive disease from slower growing cancers, with the plan to initiate definitive potentially curative therapy if a significant change in the serum PSA concentration, DRE, or biopsy Gleason score is detected. The use of genetic markers may in the future distinguish between patients most likely to benefit from radical therapy and those in who either palliation or “watch and wait” is more appropriate.

*Guidelines for follow-up*

DRE and serum PSA measurements should be performed every three to six months, depending on the clinical situation. Repeat prostate biopsy may be warranted in the face of rising PSA values. A re-biopsy may also be warranted in patients on watchful waiting one year after initial diagnosis to assess for disease progression or unrecognized higher grade disease.

## 10.9 Follow-up of chemotherapy in hormone resistant prostate cancer

The median survival of men with hormone-resistant prostate cancer (HRPC) is approximately 12 months. However, newer docetaxel-based chemotherapy regimens are associated with higher rates of both objective and biochemical (PSA) response, and median survival that approaches two years.

For men undergoing chemotherapy for HRPC, changes in serum PSA correlate with objective disease progression, treatment response, and survival. A minimum PSA decline ("PSA response") of at least 50% from baseline PSA is a common treatment endpoint for clinical trials conducted in men with HRPC. However, some men with aggressive metastatic disease have low serum PSA values (HRPC with low PSA production). Histologically, these patients show neuroendocrine (ie, small cell) features or poorly differentiated prostate adenocarcinomas.

## 10.10 Chemoprevention strategies in prostate cancer

Identification of specific cancer risk factors permits the selection of high-risk individuals. These “risk biomarkers”, when they can be measured quantitatively and are modifiable by an intervention (eg, a drug or micronutrient), may become an intermediate endpoint for chemoprevention trials. Primary chemoprevention of PCa has become an appealing alternative strategy to early detection. In secondary chemoprevention, efforts are

directed toward the detection of disease at an early stage when effective treatment may provide the best opportunity for a cure.

Possible ways by which chemopreventive agents may influence a risk biomarker include carcinogen blocking, antioxidant or anti-inflammatory activity, anti-proliferative or cytostatic activity. There are currently no definitively proven effective chemopreventive strategies for PCa. However, a number of agents have been and continue to be studied including selenium, vitamin E, vitamin D, 5-a-reductase inhibitors, cyclooxygenase-2 inhibitors, retinoids, lycopenes, soy, and green tea.

### 10.10.1 Finasteride

Men who are deficient in 5-a-reductase do not develop PCa. Finasteride is an inhibitor of 5-a-reductase type II whose safety and tolerability has been demonstrated in large long-term trials for the treatment of BPH. Blocking the conversion of testosterone to DHT results in a significant reduction of DHT with normal or increased testosterone levels, an effect that may limit side effects. In 2003, the Prostate Cancer Prevention Trial demonstrated that finasteride is associated with a 25% reduction in the 7-year period prevalence of PCa in men over age 55 years with normal DRE and initial PSA <3.0 ng/mL. Other data suggest that 5-a-reductase inhibitors reduce serum PSA in men with localized or advanced, primary or recurrent PCa.

### 10.10.2 Vitamin E and selenium

Experimental observations suggest that oxidative damage is associated with PCa. *In vitro* studies demonstrate potent inhibitory activity of the antioxidant vitamin E and selenium compounds on cell proliferation consistent with apoptosis in PCa cell lines. A large prospective study in healthy men, the *Selenium and Vitamin E Cancer Prevention Trial* (SELECT), comparing selenium, vitamin E, and the combination of both agents compared to placebo was initiated by the National Cancer Institute in July 2001 and is still in progress.

### 10.10.3 Vitamin D analogs

A link between vitamin D deficiency and PCa risk has been suggested. Preclinical studies support an antiproliferative, antimetastatic, and differentiating effect of 1,25-dihydroxyvitamin $D_3$ ($1,25(OH)_2D_3$) and its analogs in PCa, providing a rationale for the consideration of these compounds as potential chemopreventive agents. Circulating 1,25(OH)2D3 binds to *vitamin D receptors* (VDR) in prostatic epithelial cells. VDR functions as a ligand-dependent transciption factor that binds to vitamin D responsive elements (VDRE) on target genes. Several molecular

epidemiological studies link VDR polymorphisms with PCa risk and/or progression. Cross-talk between the androgen- and vitamin D signaling pathways has been reported for PCa cells in which VDR and AR are upregulated. 1,25(OH)2D3 exerts antiproliferative activity predominantly by cell cycle arrest, but also induces apoptosis by downregulation of Bcl-2. Growth arrest is mediated by induction of IGFBP-3. A major limitation to the clinical application of 1,25(OH)2D3 (calcitriol) is the induction of hypercalcemia; as a result less calcemic analogs of 1,25(OH)2D3 with more antiproliferative activity are being developed and will be more useful clinically.

### 10.10.4 Nonsteroidal antiinflammatory compounds

Regular use of NSAIDs, which inhibit COX-2, may be associated with a lower risk of PCa. Elevated prostaglandin levels, and upregulation of cyclooxygenase-2 (COX-2), a key enzyme in the conversion of arachidonic acid to prostaglandins, are found in PCa cell lines, and apoptosis follows the withdrawal of arachidonic acid and/or its metabolites. COX-2 inhibitors have an apoptotic effect on PCa cell lines. One COX-2 inhibitor, Exisulund, is in phase II/III trials for the treatment of PCa.

### 10.10.5 Micronutrients

Retinoids, substances in tomatoes (lycopenes), soya beans (isoflavones), and polyphenolic constituents of green tea also seem to be preventive. These cancer-chemopreventive micronutrients can induce apoptosis, inhibit cell growth or arrest the progression of the cell cycle, or inhibit the invasiness of PCa cells by decreasing expression of genes related to angiogenesis and metastasis.

## 10.11 New developments in prostate cancer research and treatment

Recent insights in the molecular basis of prostate cancer have provided potential advances in treatment options. These include novel uses of chemotherapeutics, complete and intermittent androgen ablation, vaccines, immunotherapeutics, angiogenesis inhibitors, AR-pathway-specific therapy and gene therapy as well as genetic screening tests.

*Apoptosis* is an important target for PCa treatment, since impaired ability to undergo apoptosis plays an important role in the evolution from androgen-dependent to androgen-independent PCa. Expression of the PTEN protein, an inhibitory regulator of the PI3K/Akt pathway, is frequently lost in advanced PCa. Rapamycin (Sirolimus), an immunosuppressive drug, inhibits

a downstream component of the PI3K pathway and forces PCa cells to enter apoptosis.

*Gene therapy* may reverse the malignant phenotype of PCa cells by replacing a missing tumour suppressor gene (eg, p53, PTEN) or down-regulating gene expression of oncogenes (eg, Bcl-2).

Several prostate tissue-specific antigens and their epitope peptides that are recognized by cytotoxic T lymphocytes can be target molecules in specific *immunotherapy* for prostate cancer. Induction of the host immune system by vaccine therapy with prostate antigen mRNA-transfected dendritic cells is being explored. Several clinical trials are underway.

### 10.11.1 Molecular biomarkers

Recent advantages in proteomic and genomic technologies such as DNA microarrays have been used to characterize gene expression profiles of prostate cancer tissue, which could help to identify men at high risk who would benefit from more intensive screening or from chemoprevention trials. Gene expression of *a-methylacyl-coenzyme A racemase* (AMACR) may provide a more sensitive screening test for PCa than the PSA blood test currently in use. EZH2 (*enhancer of zeste homolog 2*), a transcriptional repressor, is overexpressed in advanced (metastatic) prostate cancers and may indicate aggressive cancer. This could eventually help distinguish men who need treatment from those who might be better served by watchful waiting. In the future, gene expression profiling of prostate biopsy material may revolutionize the management of PCa patients.

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