Department of Biochemistry and Molecular Biology
Course: BMB- 309

An assignment on,

Challenges in Prostate Cancer Diagnosis

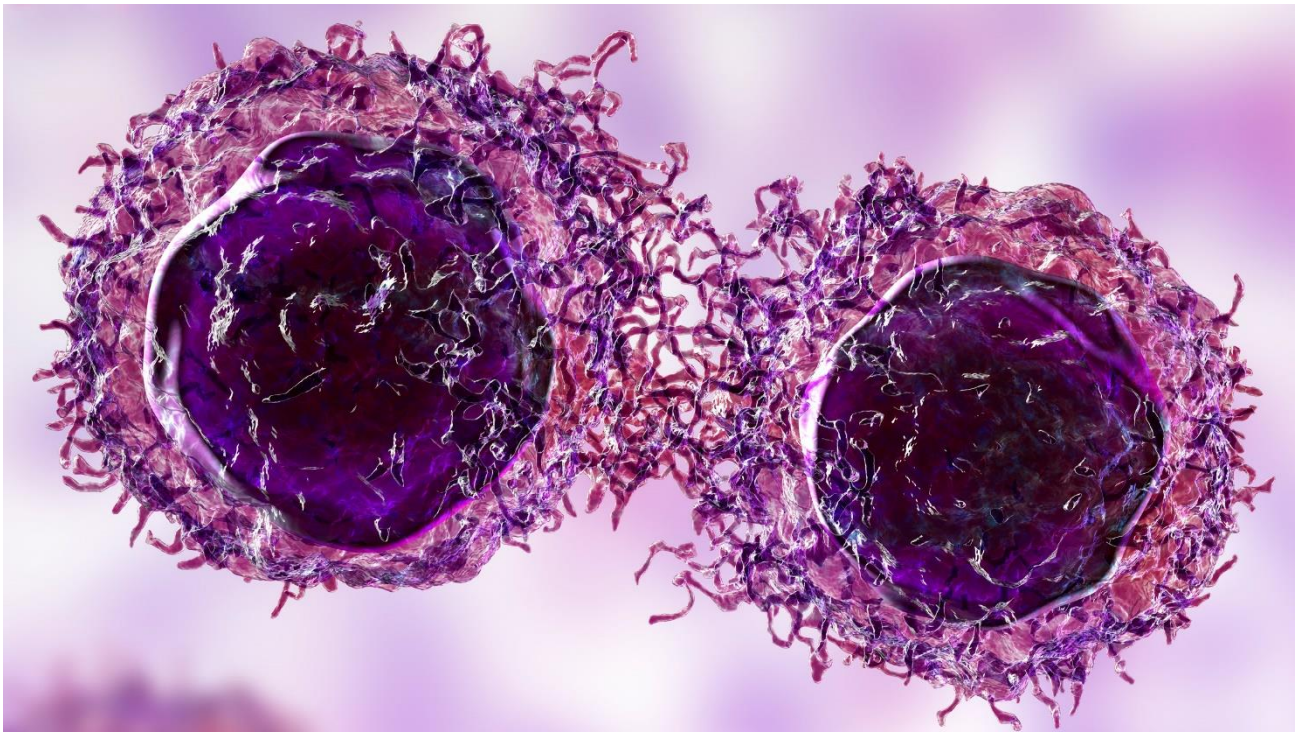
Submitted by,
Raina Islam
Roll-03
Class: 3rd year,B.S honors
Session: 2021-22

Submitted To,
Dr. Md. Zakir Hossain Howlader
Dr. A.H.M. Nurun Nabi
Dr. Md. Ismail Hossen
Dr. Rifat Ara Begum

Date of Submission:10.01.2023

Contents

List of Figures



Development of Cancer Cell

1.Background

Prostate cancer is the second most common cause of cancer death among man of United States.It is the most prevalent non-skin cancer in males that lowers quality of life and contributes to early mortality globally . Maximum males had greater incidence rates of prostate cancer than compared to the developing globe, resource regions like Oceania, North America, and Europe are present. Contrarily, death rates are greater in less developed portions of America, the Caribbean, and sub-Saharan Africa, where resources are scarcer. By 2030, there will be 1,700,000 additional prostate cancer diagnoses worldwide, with a projected rise of roughly 500,000 fatalities.

If prostate cancer is discovered as a consequence of screening, it will likely be in a more asymptomatic and curable stage than if screening had not been done. While this could give the impression that screening for prostate cancer is always a good idea, there are still screening-related problems that make it difficult to determine if the advantages of screening exceed the hazards for the majority of men. The development of serum **prostate-specific antigen (PSA)** and subsequent **U.S. Food and Drug Administration(FDA)** clearance of it for prostate cancer screening in the late 1990s,PSA testing has been a common procedure for detection around the globe. The prostate epithelial cells manufacture and release PSA, **a kallikrein-type serine protease(a family of proteases consisting of 15 closely related, secreted serine proteases with either trypsin-like or chymotrypsin-like specificity)** to maintain the liquefaction of seminal fluid. The limits of PSA testing as the optimum screening method for prostate cancer diagnosis, however, have been well documented in clinical trials over the years.

Poor specificity, which causes aggressive treatment due to overdiagnosis of slow-growing cancer that was not going to be fatal, is one of the drawbacks of PSA testing. 15% of instances of prostate cancer are found in men with extremely low blood PSA levels, and there is no definitive PSA cut-off that can ensure the existence or absence of a tumor.The majority of current diagnostic approaches, including immunoassays, are lab-based (e.g. ELISA). Immunoassays are extremely labor-intensive and time-consuming despite being highly sensitive and selective methods. Digital rectal examination and biopsy are two more highly invasive approaches for cancer detection. Prostate cancer molecular signatures have recently been created using novel genomic and proteomic approaches.These techniques provide a lot of data that could help us understand the condition better, but to use the data effectively and produce conclusions that are useful and practical, we may need to apply sophisticated algorithms.

Even while some of these novel marker-based tests have already been made available for purchase, the majority still require extensive clinical validation. This study discusses potential biomarkers, few of which have been developed into commercially available tests, and offers a critical assessment of the present state of prostate cancer detection. A variety of innovative tests based on the simultaneous detection of novel biomarkers are intended to improve prostate cancer screening, but more clinical research is necessary to confirm the advantages and efficacy of these methods for early and accurate diagnosis.(1)

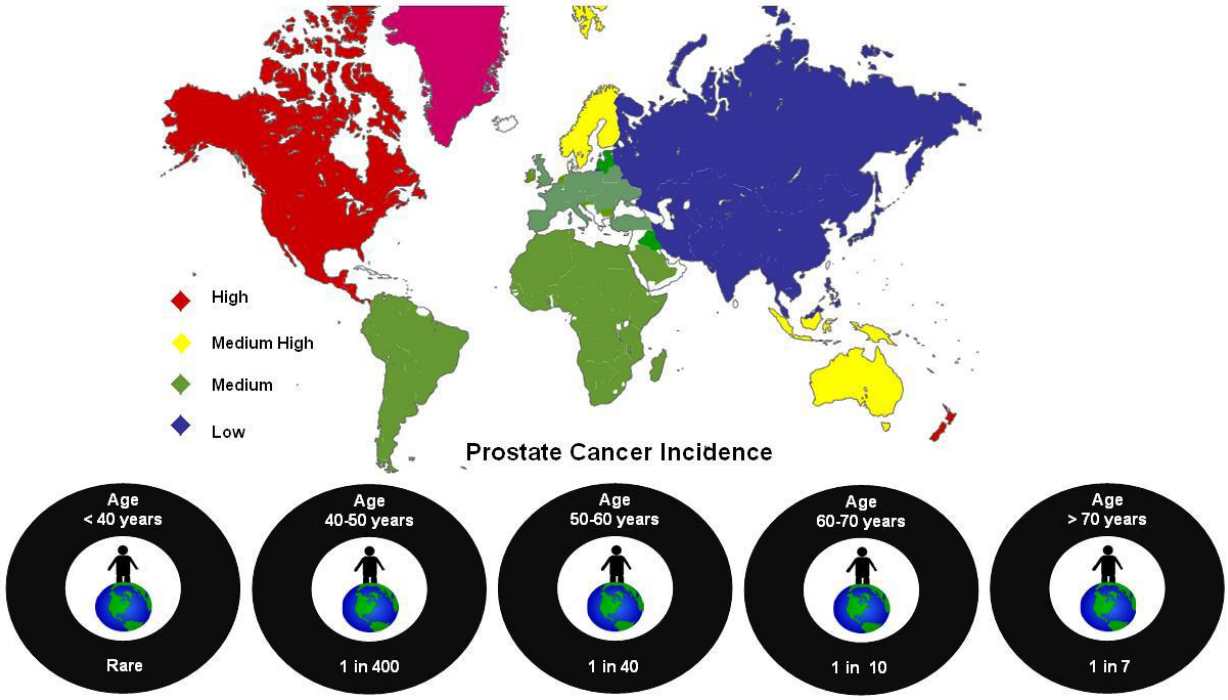


Figure 1: Map of the world showing the prevalence of prostate cancer and the estimated number of cancer cases by age(1)

2.History of Prostate Cancer Diagnosis

Herophilus, a Greek anatomist, discovered the prostate gland for the first time while dissecting cadavers. Antonio Ferri, a physician from Naples, was the first to explain how prostate cancers block the bladder path in 1530. The work of Vesalius, who created the first anatomical depiction of the prostate gland in 1538, came after this. In 1649, French physician Jean Riolan identified the link between insufficient urine flow and prostatic hypertrophy and first documented the disease's symptoms. All of these factors show that individuals in those times were aware of prostate tumors, yet George Langstaff did not report the first prostate cancer case in London until 1817. The first prostate cancer case was recorded in 1853 by J. Adams, a surgeon at The London Hospital who had detected it through histological analysis. This syndrome is "a very rare disease," Adams said in his paper. After that, 150 years later, prostate cancer has escalated to a serious public health issue.

Several males with primary and metastatic prostatic carcinomas were found to have increased blood prostatic acid phosphatase (PAP) values in 1938; these men tended to have bone metastases. PAP generated by the prostate gland and present in the semen of healthy men, controls the metabolism and cell development of the prostate glandular epithelium. Following Gutman's discovery, many studies investigated PAP activity and discovered a link between PAP enzymatic activity and prostate cancer. The first PAP colorimetric test was created as a result of this finding and was based on the enzymatic

activity of PAP. Low specificity, sensitivity, and poor stability of serum enzymatic activity—which is greatly influenced by reaction time, pH, and temperature—were these tests' main shortcomings. The creation of a radioimmunoassay for PAP in 1975 increased the sensitivity of the PAP activity assay. The sensitivity, however, remained insufficient to identify early-stage illness. A more sensitive and accurate assay that might enhance early diagnosis and result in more effective treatment of prostate cancer was urgently needed, according to the disadvantages of the PAP assay.(1)

2.1.The Prostate Specific Antigen era

In the 1960s and 1970s, a number of researchers worked on the identification of highly specific blood tumor markers that would be useful tools in the early diagnosis of prostate cancer. Flocks reported the characteristics of a particular prostate antigen in 1960. A new molecule called -seminoprotein was found in human serum 10 years later by Japanese forensic scientist Mitsuwo Hara and his colleagues. They suggested that this protein may be used as a forensic diagnostic marker in rape cases. Tien Shun Li and Carl G Beling revealed two proteins, E1 and E2, that they had isolated from human serum two years later, while doing research on male fertility. Later research revealed that PSA and E1 antigen were similar. In 1979, Ming C. Wang extracted an antigen from the prostate gland while working with an antiserum (p8) against prostate cancer cells. Initially known as PA (prostate antigen), it later became recognized as prostate-specific antigen (PSA). Further evidence from Wang's research team revealed the existence of PA in normal, benign, and cancerous prostate tissues as well as the chemical and immunological distinctions between PA and PAP. PSA was once thought to be extremely specific for the prostate gland, but later research showed that both males and females' periurethral and prostatic glands include dispersed cells that release PSA into the urine without changing serum levels.(1)

The PSA gene, which codes for a 261 amino acid pre-protein, is a member of the kallikrein gene family and is found on chromosome 19 in region 13q. ProPSA is a 237 amino acid protein that exhibits enzymatic activity, whereas PSA is an enzymatically inert 244 amino acid sequence with a leader sequence(Figure 2).

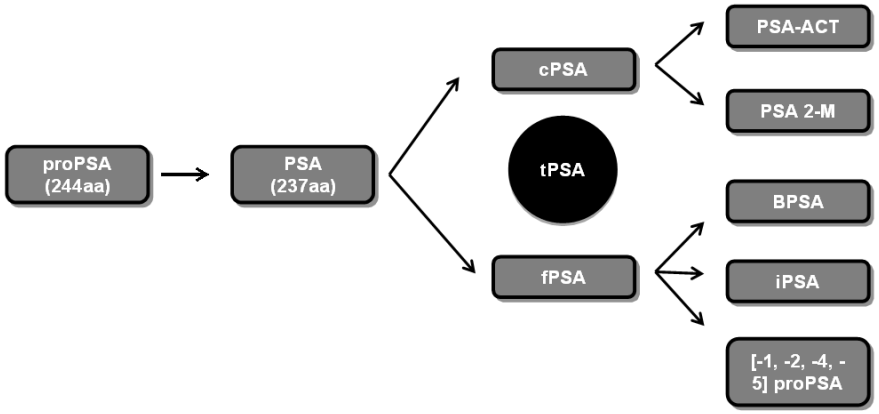


Figure 2: Schematic illustration of the PSA molecules that can be found in blood. The precursor of the enzymatically active PSA, known as ProPSA (244aa), is inactive (237aa). The majority of PSA that enters the bloodstream interacts with protease inhibitors, primarily 1-antichymotrypsin (ACT) and 2-macroglobulin. Blood can also include pro-PSA forms, which together with other PSA isoforms (such as benign PSA or BPSA and inactive PSA or iPSA) are referred to as "free PSA." Total PSA in circulation (tPSA) consists of both complexed and free PSA forms.(1)

Lilja and Stenman first identified the differences between the free (fPSA) and complex (cPSA) forms of PSA in serum in the early 1990s (Lilja, 1988). The majority of total PSA is composed of complex PSA, which is covalently bound to serum serine protease inhibitors like 1-antichymotrypsin and 2-macroglobulin. The remaining PSA is referred to as free pSA (fPSA), which also contains other PSA isoforms like (proPSA), benign PSA (BPSA), and inactive PSA (iPSA). Papsidero and colleagues carried out a quantitative assessment of PSA in blood in 1980. Following this research, Stamey and his associates conducted a ground-breaking study in which they examined 2,200 serum samples from both healthy individuals and cancer patients and determined the PSA's potential therapeutic usefulness as a prostate cancer diagnostic. Later, it was shown that glandular ducts act as effective barriers to prevent even a very little amount of PSA from entering the bloodstream. Hybritech Inc., San Diego, CA suggested that a blood PSA concentration exceeding 4ng/ml be taken into consideration as a marker for prostate illness in 1986. According to clinical research on pertinent populations, the ideal value for cancer detection should be between 2.8 and 4.0 ng/mL. The US Food and Drug Administration (FDA) authorized PSA testing as a monitoring tool to track the development of the illness in men who had previously received a prostate cancer diagnosis in 1986 after additional clinical research. Based on clinical evidence from a screening trial including 6,630 males aged 50 to 74, the FDA approved PSA, and 4.0 ng/ml was chosen as the "cut-off" in the 50 to 54 age range. Clinical trials were being conducted in the meanwhile to explore the PSA test's potential as a diagnostic tool. The FDA authorized the Hybritech Tandem PSA test in August 1994 to be used in conjunction with a digital rectal examination (DRE) to help in the early diagnosis of prostate cancer in men over 50. The FDA held the opinion that a person should not be diagnosed with prostate cancer solely only on the results of a PSA test. It should thus be used in combination with other diagnostic techniques, such as the digital rectal exam.However, the outcome of the biopsy would ultimately determine the diagnosis. To prevent pointless biopsies, the ratio of free PSA (fPSA) to total PSA (% fPSA) is employed in contemporary clinical practice. Urologists can utilize a cut-off of 25% of fPSA in males with total PSA values between 4 and 10 ng/mL to determine which individuals should have a biopsy.

2.2.PSA Associated Contoversies

The two key factors that revolutionized the diagnostic market for PSA testing were availability and FDA clearance. The rapid detection of prostate cancer made possible by PSA testing may reduce the disease's mortality rate, but it has also led to a rise in the number of needless prostate biopsies and treatments. The specificity and sensitivity of PSA testing have been put into question by a number of clinical investigations. Several males were diagnosed with cancer despite having PSA levels below the accepted "cut-off" level of 4.0 ng/mL. The PSA's sensitivity and specificity for men under the age of 60 were 0.18 and 0.98, respectively, at this cut-off value. Thus, just 2% of men would get an unneeded biopsy, while 82% of prostate malignancies would go undetected or missing. Since PSA levels are reported to be raised in many other prostate-related disorders such benign prostatic

hypertrophy (BPH) and prostatitis, another restriction is related to the specificity of PSA. False positives are a serious issue because of the biomarker's weak specificity, which leads to unnecessary biopsies and other therapies. One of the pioneering teams behind the development of PSA testing reported in a study by Stamey and colleagues that there is very little link between pre-operative serum PSA levels and cancer volume. Additionally, they stated that the "PSA era is over" in forecasting the risk of prostate cancer and that PSA is just a useful marker for benign prostatic hyperplasia.(2)

Prostate cancer seldom results in death because it normally spreads relatively slowly, unlike the majority of other malignancies. While the lifetime risk of dying from prostate cancer is only approximately 3.4%, the high PSA screening rate has increased the lifetime chance of being diagnosed with prostate cancer to 16%. Therefore, even without therapy, the majority of individuals with prostate cancer do not pass away from the condition.Numerous studies indicate that while at least 25% of men with PSA-detected prostate cancer would have an indolent condition, over 90% of them receive early treatment with surgery, radiation, or androgen deprivation therapy. After weighing the advantages and disadvantages of PSA-based screening for prostate cancer, the U.S. Preventive Services Task Force (USPSTF) advised against using PSA screening in men who have no symptoms in 2012. Cancer may be "overdiagnosed" in people with benign or slow-growing tumors who don't really need therapy, which can lead to problems including bowel, sexual, and urinary dysfunction from intensive treatments. Despite the fact that PSA falls short of the requirements for a standalone marker and all of the prior objections, it is still the most widely used and widely accessible clinical diagnostic for the diagnosis and follow-up of prostate cancer. After radical prostatectomy, radiation treatment, high intensity focused ultrasound, and cryotherapy, serum PSA should decline and stay at low levels; further elevations may be a sign of cancer recurrence. After androgen suppression therapy or treatment for localized prostate cancer, PSA kinetics may also offer prognostic data.

3.Prostate Cancer's Cellular and Molecular Progression

Men's prostates, which are walnut-sized glands, create seminal fluid that carries and nourishes sperm. It is divided into four zones: the periurethral transition zone, the central zone, the anterior fibromuscular stroma, and the peripheral zone. The bulkiest peripheral zone is where the majority of prostate cancers (60–75%) grow, but the transition zone may play a role in the onset of benign prostatic hyperplasia (BPH). According to histology, the human prostate is made up of a stromal compartment and a pseudostratified epithelium that are separated by a foundation membrane. Three terminally differentiated epithelial cells, the luminal, basal, and neuroendocrine cells, are present in the pseudostratified epithelium. New evidence suggests that either basal or luminal cell types are the primary source of most PCa. However, whether prostate cancers originating from a particular cell type are more aggressive than those originating from other cell types is up for contention.

The growth of prostate cancers that are malignant involves many steps (Figure 1). Premalignant lesions known as prostatic intraepithelial neoplasia emerge in the early stages (PIN). Localized invasive lesions then develop, and they eventually develop into advanced metastatic prostate adenocarcinomas. Only the high-grade PIN (HGPIN), also known as in situ carcinoma, is extensively recognized as a precursor to invasive prostate cancer. PIN are divided into low- and high-grade lesions. The HGPIN has significant genetic and molecular similarities with cancer cells and the PIN

is defined by the presence of luminal epithelial cells with prominent and expanded nucleoli. In contrast to the normal and hyperplastic epithelium, several proliferative abnormalities are also frequently observed in HGPIN. While proliferation happens in the basal cell compartment of the benign epithelium, it mostly happens on the luminal side of the ducts and acini in HGPIN. Premalignant lesions have been shown to overexpress a number of oncogenic proteins, including the -class glutathione S-transferase (GSTP1) and the anti-apoptotic BCL2. It is thought that increased BCL2 expression is what prevents cells from dying during the transition from normal to malignant tissue. While GSTP1 is found in more than 70% of high grade PINs, more than 90% of PCa patients lack it, probably as a result of promoter hypermethylation. High-grade PIN has also been linked to increased expression of the proliferation marker Ki-67 and reduced expression of the cyclin-dependent kinase inhibitor p27 (Kip1). In the high grade PIN, it has also been documented that tumor suppressor genes including PTEN and NKX3.1 lose their expression. High-grade PIN lesions have also been shown to have the TMPRSS2-ERG fusion gene, which is thought to be an early sign of prostate cancer. In a different investigation, it was discovered that adult mouse PCa cells overexpressing ERG will eventually produce localised PIN lesions and epithelial hyperplasia. It has also been proposed that downregulation of the miR-34 family, miR-23b, and miR-205 are significant occurrences in the formation of premalignant lesions. It has been proposed that activation of telomerase, PTEN deletion, and loss of RB1 are crucial during the transition from high grade PIN to carcinoma. A number of other factors have been linked to the progression of PCa from an early stage to a metastatic disease, including the loss of the miR-15A-miR-16-1 cluster on chromosome 13, mutation and amplification of the AR gene, overexpression of several oncogenes, including CXCR4, EZH2, and cMYB, activation of a number of oncogenic pathways, and a number of mutations, including FOXA1, BRACA1/2 and ATM.(2)

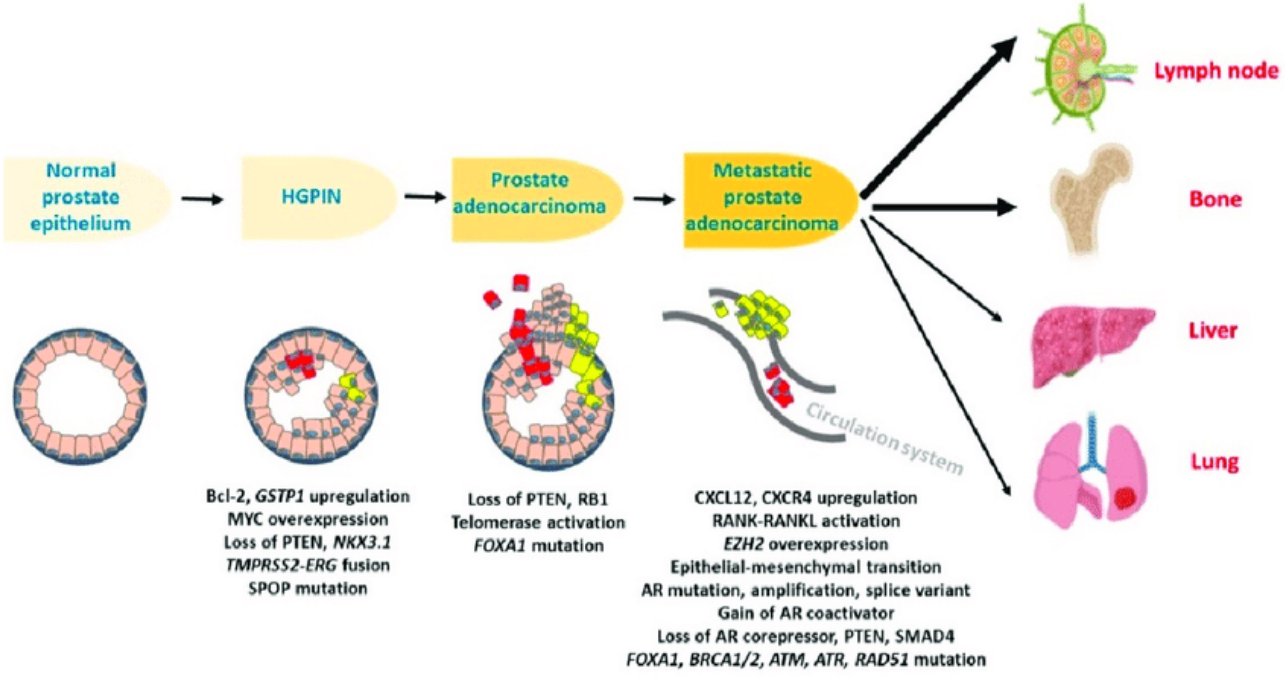


Figure 3: Prostate cancer cellular development. The luminal or basal cells of the healthy prostate epithelium are the source of prostate cancer. Prostatic intraepithelial neoplasia (PIN), a premalignant lesion, first appears. Only high-grade PIN (HGPIN) progresses to malignant invasive prostate

adenocarcinoma and eventually becomes a metastatic illness that spreads to the lymph nodes, bone, liver, and lung via the circulatory system. The development of prostate cancer has been associated with many molecular changes. BCL2, GSTP1, MYC, and PTEN are overexpressed in HGPIN, but NKX3.1, TMPRSS2-ERG fusion, and SPOP mutation are lost. Tumor suppressor genes like PTEN and RB1 are lost in early-stage prostate cancer, while some oncogenes with a high frequency of mutations, including FOXA1, are overexpressed.Multiple molecular changes, including overexpression and/or mutations in the tumor suppressor genes AR, ATM, ATR, RAD51, and CXCR4, as well as the loss of different tumor suppressors such SMAD4, have been observed during the progression to the metastatic stage.(2)

4.Diagnosis of Prostate Cancer

4.1.Prostate Specific Antigen(PSA) blood test

Nanograms per milliliter, or ng/mL, are the units used to test the PSA level in blood. There is no predetermined cutoff threshold that can definitively determine if a man has prostate cancer or not, although the likelihood of having prostate cancer increases as the PSA level rises. When determining whether a man would require additional testing, many doctors utilize a PSA cutoff number of 4 ng/mL or above. However, other physicians may advise that testing begin at a lower level, such as 2.5 or 3.

- The majority of men without prostate cancer have blood PSA values below 4 ng/mL. The PSA level often rises over 4 when prostate cancer manifests. A level under 4 does not, however, guarantee that a guy is cancer-free. If a biopsy is performed, 15% of men with a PSA under 4 will have prostate cancer.
- About 1 in 4 men who have a PSA score between 4 and 10 (commonly referred to as the "borderline range") may develop prostate cancer.
- Prostate cancer is more likely than 50% more likely if the PSA is more than 10.

You might require further tests to search for prostate cancer if your PSA level is high.(7)

4.1.1.Factors that increase PSA level

- **Enlargement of prostate:**PSA levels can be increased by conditions like benign prostatic hyperplasia (BPH), a non-cancerous prostate enlargement that affects many men as they age.
- **Older age:** Even in people without prostate abnormalities, PSA levels often increase gradually as they age.
- PSA levels may rise as a result of prostatitis, an infection or inflammation of the prostate gland.
- **Ejaculation:** This may temporarily increase the PSA. For this reason, some medical professionals advise men to hold off on ejaculating for a day or two before to testing.
- **Certain medications:** A spike in PSA may result from using male hormones like testosterone (or other drugs that increase testosterone levels).
- **Bicycling:** While not all studies have shown this, some have claimed that cycling may temporarily increase PSA levels (perhaps because the seat places pressure on the prostate).
- **Certain urologic procedures:** Prostate-related office procedures, such prostate biopsies or cystoscopies, have the potential to temporarily increase PSA levels. Digital rectal exams

(DREs) may marginally increase PSA levels, according to some research, although this hasn't been confirmed by others. However, if a PSA test and a DRE are being performed at the same time, some medical professionals suggest having the blood obtained for the PSA test before the DRE, just in case.(7)

4.1.2.Factors that lower PSA level

- **5-alpha reductase inhibitors:** Some medications, such as finasteride (Proscar or Propecia) or dutasteride (Avodart), which are used to treat BPH or urinary symptoms, can reduce PSA readings. These medications may influence the risk of prostate cancer.
- **Herbal concoctions:** Some concoctions marketed as dietary supplements may conceal a high PSA level. This is why it's crucial to inform your doctor if you're taking any supplements, even those that aren't specifically designed to support prostate health. PSA doesn't appear to be impacted by saw palmetto, a plant some men take to treat BPH.
- **Others medicines:** Several study has shown that long-term usage of some medications, including aspirin, statins (drugs used to decrease cholesterol), and thiazide diuretics (such hydrochlorothiazide), may lower PSA levels. These results need to be confirmed by other study. If you frequently use any of the medications, speak with your doctor before stopping for whatever reason.(7)

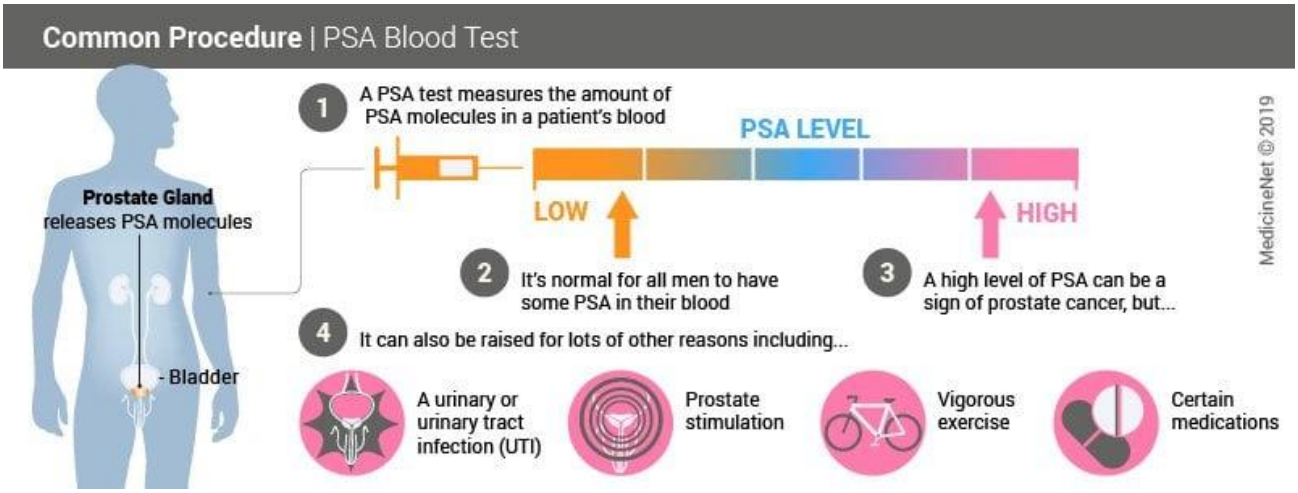


Figure 4: The various PSA levels and their implications for health are depicted in this figure.(8)

4.1.3.Special types of PSA tests

- **Percent-free PSA:** PSA of two main types is found in the blood. One version circulates free, whereas the other is bound to blood proteins (unattached). The ratio of the amount of PSA that circulates freely to the overall PSA level is known as the percent-free PSA (%fPSA). Those with prostate cancer have a lower proportion of free PSA than men without the disease.
- The percent-free PSA might be used to assist determine whether you should undergo a prostate biopsy if your PSA test result falls within the borderline range (between 4 and 10). With a lower percent-free PSA, you have a higher risk of developing prostate cancer and should likely get a biopsy.

- **Complexed PSA:** The quantity of PSA that is joined to other proteins is directly measured by this test. Although this test can provide the same amount of information as testing for total and free PSA, it is not commonly performed.
- **Tests that combine different types of PSA:** Some more recent tests combine the findings of many PSA tests to get an overall score that indicates the likelihood that a man has prostate cancer (particularly cancer that might need treatment). These tests consist of:

1. **The Prostate Health Index (PHI)**, which includes proPSA, free PSA, and total PSA readings,
2. **The 4Kscore test** combines the findings of human kallikrein 2 (hK2), total PSA, free PSA, intact PSA, and various additional variables.(7)

In order to decide whether men with slightly high PSA levels should undergo a prostate biopsy, these tests may be helpful. These tests might also be used to assist decide if a guy who has already undergone a prostate biopsy and it came out clear of cancer needs another one.

4.2.Digital Rectal Exam(DRE)

Prior to the 1990s, the (DRE) test was the most widely used method for detecting prostate cancer and other conditions in the lower rectum and pelvis. In order to find lumps or any hardening of the prostate gland, a regular screening test is utilized.

The doctor does a digital rectal exam (DRE) by inserting a finger into the rectum while wearing gloves and lubricant to feel for any lumps or hard spots on the prostate that might be cancer. The prostate is situated just in front of the rectum, as may be seen in the figure below **(Figure 5)**. Rectal examinations may reveal the presence of prostate tumors, which frequently start at the rear of the gland. Even though this inspection occasionally causes discomfort (particularly for males who have hemorrhoids), it often causes no pain and only lasts a short while.(15)

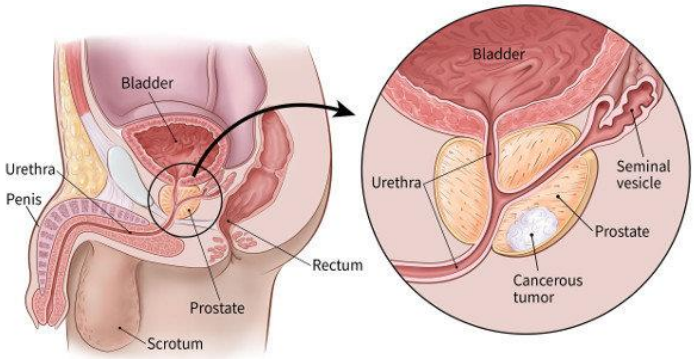


Figure 5: Position of prostate gland(15)

DRE is a helpful method for diagnosis when the volume of the prostate is around 0.2 mL or bigger since about 80% of prostate cancers are detected in the peripheral zone of the gland, mostly in a posterior location. Patients with a PSA level higher than 2 ng/mL are deemed to have prostate cancer if they have a worrisome DRE.

4.2.1.Gleason Grading System

Higher Gleason scores may be associated with a suspicious or abnormal DRE. The Gleason scale, created in the 1960s by doctor Donald Gleason, offers a score that aids in predicting the behavior of prostate cancer (Gleason, 1966). Based on how it appears under the microscope, prostate cancer is assigned a Gleason score.

The Gleason scale is based on the morphological structure of the tumor.It is frequently employed in the histological assessment of prostate cancer malignancy. We employ the following 5 histopathological diversity stages:

- **Gleason I:** Malignancy known as "pushing boundaries" when it predominates in glands that are equal in size and form and have a clear duct lumen.
- **Gleason II:** Greater size and form diversity of glandular ducts, greater abundance of interglandular connective tissue, and blurred borders.
- **Gleason III:** The limits of the infiltrated region are irregular but still stay within the defined, visible bounds. The shape and size of the gland ducts are much more varied, numerous, and single ducts are isolated from the others.
- **Gleason IV:** The glands are frequently confluent, the malignant epithelium develops cribriform and papillar structures, the margins are invaded by nest structures, and the glandular lumen is absent.
- **Gleason V:** A comedocarcinoma is a sort of band or nest of individual cells without the development of glandular structures, or a papillar or cribriform structure with severe necrosis in the core.(17)

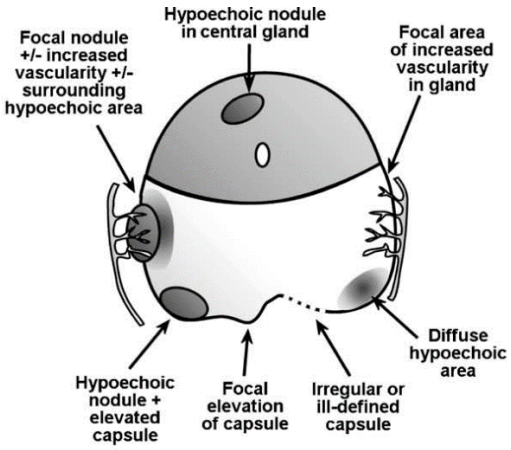
The Gleason score system's application is based on the Gleason sum from patterns of the two most prevalent fields with a certain level of differentiation. It can be used to predict the disease's likely future course and, consequently, the patient's future medical care.

The DRE is recognized as a helpful screening tool, but it is dependent on the abilities of the doctor performing and interpreting the test and is unable to identify prostate cancer on its own due to its lack of specificity and accuracy.

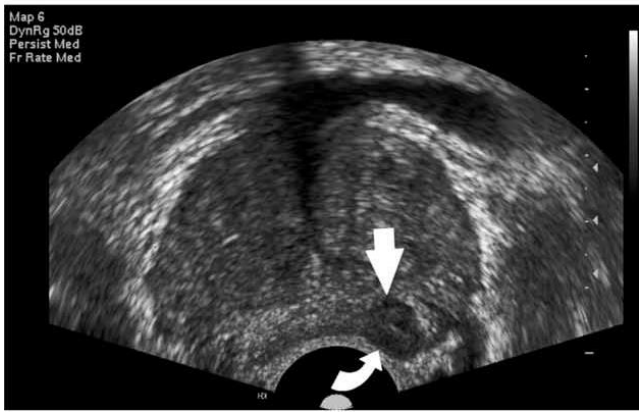
Prostate cancer is more difficult to detect with DRE than it is with the PSA blood test, although it can occasionally detect tumors in men with normal PSA levels. Because of this, it may be used into the screening process for prostate cancer.

4.3.Transrectal Ultrasound

It’s a procedure that makes use of ultrasonography and involves inserting a USG transducer into the rectus muscle. It enables a comparatively precise evaluation of the gland's size, echogenicity, and existence of localized lesions.(3)



(a)



(b)

Figure 6: Prostatic cancer indications on transrectal ultrasonography. (a) A line diagram showing the different sonographic appearances,(b) A 1 cm left peripheral zone nodule on a TRUS film with capsular elevation (curved arrow) is indicative of T2 prostatic cancer.(3)

When TRUS is insufficient, we can also employ Color-Doppler ultrasonography, which allows us to gauge the degree of vascularization in the targeted location.

One of the most common diagnostic techniques used nowadays is a targeted biopsy under the direction of TRUS or TRUS/MRI (Magnetic Resonance Imaging).

4.4.Positron Emission Tomography(PET)

It is **based on the use of radioactive samples**, which enables the assessment of the efficacy of the therapy as well as the detection of metastases in various body sections and the spread of the cancer.This radioactive sample targets **Prostate Specific Membrane Antigen(PSMA**) expressed in prostate cancer.

1.A radioactive material is injected into the patient's arm, and it travels through the body to places where PSMA is expressed.

2.The patient is seated into a system that combines a CT or MRI scanner with a PET scanner to obtain body images.

3.On the scan, the doctor may see body areas with elevated PSMA levels.

4.Making treatment decisions may be aided by information from the scan:

- Verify whether cancer has not spread to other organs.
- Detect a recurrence of prostate cancer following radiotherapy or surgery.
- Establish your eligibility for prostate cancer treatments that target PSMA.

5. Commercially marketed immunodiagnostic instruments based on PSA

There are two primary sectors that make up the PSA diagnostic market as a whole. It comprises of point-of-care (POC) devices, which include both lateral flow tests and handheld devices, and fully automated equipment, which are utilized by the bulk of clinical laboratories.

5.1.Centralised Lab Instruments

The widely utilized, big, automated, high-throughput equipment for PSA detection in centralized laboratories includes Thermo Scientific‘s BRAHMS Kryptor, Roche‘s Cobas and Elecsys assay, DiaSorin‘s LIAISON, Abbott‘s Architect and AxSym assays, BioMérieux‘s Vidas system, Access Hybritech from Beckman Coulter, Siemen‘s Advia Centaur, Immulite and Dimension assay, and Tosoh‘s AIA assay. These devices' benefits include consistent findings, low detection limits (to 0.008 ng/mL), high sample throughput, and a broad linear range (about 0.008 to 100 ng/mL). Despite being calibrated in accordance with WHO (World Health Organization) standards, it has been noted that PSA tests from various manufacturers exhibit inter-assay variability.

Despite the fact that the area of PSA automated devices is already developed, some of the most significant shortcomings in the existing technologies are the expense of the instruments, the unpredictability of the findings, and the requirement for skilled workers to oversee the systems. More individuals should survive cancer with a higher quality of life following diagnosis if aggressive illness and sensitivity are discovered early on. The adoption of quick near-patient devices placed in medical offices could make this easier.(13)

5.2.Point of Care Devices

Rapid near-patient diagnostic tests are mostly performed using lateral flow or dipstick devices, which were first developed in the middle of the 1980s. Double antibody sandwich assays and competitive assays are the two primary categories of lateral flow immunoassays.

Membranes or paper strips are used as the carrier surface on which antigens or antibodies are fixed in lateral flow diagnostic devices. Blood, serum, urine, and saliva are just a few of the many biological samples that may be examined. The sample flows automatically under capillary action after being placed on the membrane. The sample's antigen interacts with a labelled antibody at the sample pad and moves up the strip until it reaches the capture zone, where it binds to immobilized antibodies to produce a distinct visible band at a predetermined position. Despite improved PSA strip manufacture and marketing, its utility as a trustworthy instrument is still constrained by the absence of sensitive and precise analyte measurement. As was already mentioned, the diagnostic value of isolated individual PSA testing is relatively low.

Handheld or portable systems, frequently created by utilizing lateral flow technology on an automated detection platform, are other POC devices used for PSA detection. These innovative portable and user-friendly detection systems were developed in response to the requirement for point-of-care (POC) devices for quick and frequent monitoring of biomarkers generally, in order to improve the quality of patient treatment.(10)

These lateral flow-based devices have recently been commercialized by many businesses, which are listed in **Table 1**. The majority of the systems presented are based on sandwich-type immunoassays for quantitative measures, where the assay is timed following the addition of the sample, a sequence of reactions are started, and the result is then shown in a legible format. For the majority of POC devices, whole blood is recommended as the sample type since it requires the least amount of sample preparation and processing time.

Table-1: Performance characteristics of commercial point-of-care systems for prostate cancer diagnostics.(1)

Mfr	Device name	Principle of assay (Immunoassay)	PSA isoform	MR (ng/mL)	Sample volume (µL)	Assay time (mins)
Mediwatch UK	PSAwatch	Palette chromatographic membrane	T	0.5–25	35	10
Boditech	i-chroma	Lateral flow fluorescence	T	0.1-100 (S/P) or 0.5-100 (WB)	30	15
Qualigen	Fast Pack System	Paramagnetic particle chemiluminescent	T	0.04-50	100	11
VEDALAB	PSACHECK-1	Palette chromatographic membrane	T	1-100	25-50	10-15
Nano En Tek	FREND PSA PLUS	Lateral flow fluorescence	T	0.1-25	30	6
MH Medical Co., Ltd	Advanced Collaurum IA System PSA test reader	Collaurum Immunochromato graphic	T	NA	20	15
Infopia Co., ltd	SelexOn	Lateral flow AuNPs based optica	T	2-30	100	10

Mfr – Manufacturer; MR – Measurement Range; LOD – Lower Limit of Detection; F- Free; T- Total; NA – Not Available

6. Next Generation diagnostic tools for Prostate Cancer

Recent breakthroughs in proteomic and genomic technologies have led to the identification of a wide range of possible prostate cancer biomarkers. Numerous biomarkers have been proposed, including exosomes, prostasomes, circulating tumor cells, exosomes and protein markers, circulating miRNAs, autoantibodies, and genetic and protein markers (CTCs). Of all of these, protein biomakers have received the most attention because to their abundance and accessibility in bodily fluids. Additionally, changes in post-translational modifications and protein expression throughout time reflect cellular function. They are therefore excellent candidates for cancer diagnosis, staging, and treatment. **Table 3** lists a few suggested biomarkers from the literature that may be employed in addition to PSA to diagnose PCa.(12)

Table-2: Potential biomarkers and their relevance to prostate cancer are listed.(1)

Protein Biomarker	**Description**	**Function/ Role in prostate cancer**
Human kallikrein 2 (hK2)	Prostate-specific kallikrein (similar to PSA) with a potent protease activity	Synthesised by the prostate epithelium; hK2 regulates the activation of PSA. Hk2/PSA ratio could detect PCa with an improved specificity compared with tPSA and %fPSA tests in the PSA gray zone.
Prostate membranespecific antigen	Transmembrane glycoprotein	PSMA is overexpressed in PCa and this relates with disease progression and severity. Serum PSMA levels may be used to differentiate PCa from BPH in the PSA gray zone
α-Methylacyl-CoA racemase (AMACR) autoantibodies	AMACR is a peroxisomal and mitochondrial enzyme belonging to a family of isomerases.	AMACR is involved in the β-oxidation of branched-chain fatty acids. Detection of autoantibodies against AMACR in the PSA diagnostic range of 4-10ng/ml may help to discriminate PCa patients from healthy individuals.
Glutathione Stransferase pi (GSTP1) hypermethylation	Isoenzyme belonging to the glutathione-*S*transferases family.	Involved in DNA repair, this enzyme has a detoxification function. In PCa, GSTP1 promoter hypermethylation leads to GSTP1 silencing, which increases the susceptibility of

		cells to DNA damage. This occurs is >90% of PCa cases and up to 70% of high grade PIN (a precursor lesion for PCa)
Prostate secretory protein of 94 amino acids/β-microseminoprotein (PSP94/ MSP)	High density apolipoprotrein, synthetised in liver.	Influence 'serum-free' fatty acids levels by unknown mechanisms. Overexpression of ApoA-II is specific for PCa (when compared with colon, neck or lung cancer). Useful for discrimination of PCa from BPH when PSA levels < 4.0 ng.
Caveolin-1	Caveolae coat protein.	Performs molecular transport, cell adhesion and signalling functions. Overexpression in PCa promotes cancer survival, progression and metastasis. In patients with localised disease at diagnosis, Cav-1 could help to identify patients with clinical significant disease.

7.Overdiagnosis and Overtreatment of Prostate Cancer

Some men may receive a prostate cancer diagnosis through screening that they otherwise would not have received. They would never have died from it, nor would any signs have shown. Overdiagnosis is the discovery of a "disease" like this one that would never result in issues.

A difficulty with prostate cancer overdiagnosis is that many of these men may still have surgery or radiation therapy, either because the doctor is unsure of how rapidly the disease may grow and spread or because the patient feels uneasy knowing he has cancer but is not receiving treatment. Overtreatment is the treatment of a malignancy that would never have resulted in any issues. The main drawback of this is that, even if they weren't necessary, procedures like surgery and radiation can still have side effects that negatively impact a man's quality of life in terms of his bowel, urine, and/or sexual functions.(9)

8..Summary

Prostate cancer must be detected at an early stage with good sensitivity and specificity in order to allow for effective therapy and patient recovery. Unfortunately, there is currently no method that can reliably distinguish between PCa that is indolent and that is aggressive. As a result, many tumors that

are not expected to have a life-threatening natural history are overdiagnosed and treated. Numerous possible biomarkers have been investigated recently with the aim of enhancing diagnosis. A viable clinical technique for managing benign and malignant prostate illnesses is the identification of these biomarkers in conjunction with recognized PSA testing. However, further study and validation, such as retrospective and prospective clinical studies, are required to determine the overall clinical value of the tests before these biomarker tests are translated for general use. Immunoassay-based technologies for PCa biomarker identification are now very sensitive, portable, affordable, quick, and user-friendly thanks to recent advancements. Although technology has advanced, PSA-based gadgets are still unable to clear up the current uncertainties related to prostate cancer.

9.References

1. Sharma, S., Zapatero-Rodríguez, J. and O'Kennedy, R. (2017) "Prostate cancer diagnostics: Clinical challenges and the ongoing need for disruptive and effective diagnostic tools," *Biotechnology Advances*, 35(2), pp. 135–149. Available at: https://doi.org/10.1016/j.biotechadv.2016.11.009.

2. Saranyutanon, S. *et al.* (2019) "Therapies targeted to androgen receptor signaling axis in prostate cancer: Progress, challenges, and hope," *Cancers*, 12(1), p. 51. Available at: https://doi.org/10.3390/cancers12010051.

3. Raja, J. *et al.* (2006) "Current status of transrectal ultrasound-guided prostate biopsy in the diagnosis of prostate cancer," *Clinical Radiology*, 61(2), pp. 142–153. Available at: https://doi.org/10.1016/j.crad.2005.10.002.

4. Munteanu VC;Munteanu RA;Gulei D;Schitcu VH;Petrut B;Berindan Neagoe I;Achimas Cadariu P;Coman I; (no date) *PSA based biomarkers, imagistic techniques and combined tests for a better diagnostic of localized prostate cancer, Diagnostics (Basel, Switzerland)*. U.S. National Library of Medicine. Available at: https://pubmed.ncbi.nlm.nih.gov/33050493 (Accessed: January 10, 2023).

5. Morris, S.Y. (2017) *PCA3 test and prostate cancer: What to expect*, *Healthline*. Healthline Media. Available at: https://www.healthline.com/health/prostate-cancer/pca3-test?fbclid=IwAR1x9mr26aUFcrSlfcnwZiXPT8C9ikxxI4-SL3V144lNpMURsjLfo2ERzkg (Accessed: January 10, 2023).

6. Daniel H. Kwon, M.D. (2022) *Psma Pet Scan, JAMA Oncology*. JAMA Network. Available at: https://jamanetwork.com/journals/jamaoncology/fullarticle/2797264#:~:text=A%20prostate%2Dspecific%20membrane%20antigen,is%20expressed%20by%20prostate%20cancer. (Accessed: January 10, 2023).

7. *Can prostate cancer be found early?* (no date) *American Cancer Society*. Available at: https://www.cancer.org/cancer/prostate-cancer/detection-diagnosis-

staging/detection.html?fbclid=IwAR2cYa2ykyA8vdPuGKwAHtkDXvYgFPON4pJ1d-jb5YtuGaEi1d2XMlkLhc8 (Accessed: January 10, 2023).

8. Pamela I. Ellsworth, M.D. (2022) Prostate specific antigen (PSA) test, results, Levels & Ranges, MedicineNet. MedicineNet. Available at: https://www.medicinenet.com/prostate_specific_antigen/article.htm (Accessed: January 10, 2023)

9. Kelloff, G.J. et al. (2009) Challenges in clinical prostate cancer: Role of Imaging, AJR. American journal of roentgenology. U.S. National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2893141/ (Accessed: January 10, 2023).

10. Frame, I. and Cant, S. (2015) Current challenges in prostate cancer: An interview with prostate cancer UK - BMC medicine, BioMed Central. BioMed Central. Available at: https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-015-0411-7 (Accessed: January 10, 2023).

11. Purysko, A.S. et al. (2021) Invited commentary: Prostate cancer diagnosis-challenges and opportunities for artificial intelligence, RadioGraphics. Available at: https://pubs.rsna.org/doi/10.1148/rg.2021210187 (Accessed: January 10, 2023).

12. Cafasso, J. (2019) Prostate cancer complications: Overview and outlook, Healthline. Healthline Media. Available at: https://www.healthline.com/health/prostate-cancer-complications (Accessed: January 10, 2023).

13. Early detection of prostate cancer (no date) Cancer Council. Available at: https://www.cancer.org.au/cancer-information/causes-and-prevention/early-detection-and-screening/early-detection-of-prostate-cancer (Accessed: January 10, 2023).

14. Ablin, R.J., 2012. The United States Preventive Services Task Force Recommendation against prostate-specific antigen screening—point. Cancer Epidemiol. Biomarkers Prev. 21, 391-394.

15. Beltran, H., Yelensky, R., Frampton, G.M., Park, K., Downing, S.R., MacDonald, T.Y., Jarosz, M., Lipson, D., Tagawa, S.T., Nanus, D.M. and Stephens, P.J., 2013. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity.

16. Berger, M.F., Lawrence, M.S., Demichelis, F., Drier, Y., Cibulskis, K., Sivachenko, A.Y., Sboner, A., Esgueva, R., Pflueger, D., Sougnez, C. and Onofrio, R., 2011. The genomic complexity of primary human prostate cancer. Nature 470(7333), 214-220.

17. Pyda-Karwicka, M. *et al.* (2018) "Current status and new approaches in prostate cancer diagnosis," *Polish Journal of Public Health*, 128(4), pp. 166–169. Available at: https://doi.org/10.2478/pjph-2018-0033.