The post PSA era: new developments in biomarkers, imaging and biopsy techniques in prostate cancer detection

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New technologies for the detection of prostate cancer are emerging regularly. The authors summarise recent advances in prostate cancer diagnostics, including serum, urine and tissue markers, and outline improvements in imaging and biopsy techniques.

Box 1. Serum biomarkers for prostate cancer

- Prostate health index
  - the prostate health index score is the ratio of a PSA precursor (proPSA) to free PSA
  - may help diagnose men with a PSA of 2–10ng/ml by making PSA more specific
- Circulating tumour cells
  - men with hormone-refractory prostate cancer are said to have high levels of circulating tumour cells, which may determine treatment response after hormonal treatment and chemotherapy
- Circulating tumour DNA
  - tracking tumour-specific mutations, for example in the androgen receptor, over courses of treatments may ultimately help predict recurrence and channel appropriate treatment

In the era of PSA testing, are we doing more harm than good? 40 000 men are diagnosed with prostate cancer annually in the UK (Cancer Research Statistics). The lifetime risk of a prostate cancer diagnosis is reported to be one in every nine men; however, the risk of death may be as low as 2%. Curative treatment is possible for localised prostate cancer, but carries with it the potential for significant side-effects impacting on quality of life.

Approximately 60% of patients will experience erectile dysfunction following radical therapy and, although the rates of severe urinary incontinence are low, up to 30% continue to report some symptoms at long-term follow-up.[1] Unfortunately, even detecting prostate cancer early may not reduce the risk of dying from the disease. It is because of this discordance that screening for prostate cancer is controversial.

The PSA test combined with digital rectal examination (DRE) is the standard to screen a man with a suspicion of prostate cancer,[2] but there are caveats to the test. PSA is produced by all epithelial tissue and although specific to the prostate, it is not specific to cancer. PSA may be elevated in benign conditions, including prostatitis and benign prostatic hyperplasia. It has also been suggested that higher-grade prostate tumours may actually produce less PSA as the tumour volume increases.[3]

Although the traditional PSA value of >4ng/ml has a 94% specificity for detecting prostate cancer, this has a sensitivity of only 20%.[4] This low sensitivity means the test fails the primary goal of a screening test – detecting cancer. Coupled with this, only one in four men with an elevated PSA will be found to have prostate cancer and a man with a PSA <1.0ng/ml still carries a 10% risk of having the disease.[5] As a result, patients

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with PSA-detected prostate cancer are largely overdiagnosed and overtreated.

Recent studies reporting on outcomes from screening found no survival benefit in the USA, but a reduction in prostate cancer mortality in Europe.[6] However, any benefit from screening may take up to 10 years and to save one death, 1055 men need to be screened and 37 cancers treated.[7] This compares unfavourably to breast cancer, where three women need to undergo treatment to save one life. There is an urgent need to diagnose prostate cancer confidently and to stratify men with the disease into those who have aggressive or indolent disease for optimal management.

SERUM, URINE AND TISSUE BIOMARKERS

Unfortunately, we do not have a reliable alternative to PSA. Transrectal biopsy is invasive and MRI expensive for mass screening; only a simple blood or urine test could satisfy all the screening test criteria set by Wilson.[8] Thankfully, research on 'biomarkers' of prostate cancer, whether from serum, urine or tissue, has accelerated recently to try to tackle this problem. Genomic profiling may allow characterisation of high-risk patients early on. This, coupled with advances in imaging and biopsy techniques, should help advance our diagnostic strategy.

An alternative to mass screening is selective screening of high-risk populations. The IMPACT study targets men with BRCA mutations and is expected to report in 2018, and the Profile study has just started recruiting men with a strong family history of prostate cancer for screening.

A biomarker is a molecule or molecules (such as genes, proteins or DNA) that can be detected in a body substance (blood, urine or tissue) and represents a biological process or disease. Boxes 1–3 highlight advances in serum, urine and tissue biomarkers, while Box 4 showcases recent advances in genomic markers of prostate cancer, which may guide treatment.

Box 2. Urine biomarkers for prostate cancer

- PCA3 (Progensa)
  - messenger RNA found highly expressed in prostate cancer tissue but not in benign prostatic hyperplasia, able to be detected in urine and has role in predicting need for further biopsy after an initial negative one
  - licensed by the FDA in this setting as 'Progensa'
- TMPRSS-ERG fusion
  - this fusion may be associated with a more aggressive tumour more likely to metastasise and higher mortality
  - may also be found in urine
  - may be useful when combining it with markers such as PCA3 and PSA, *eg* Mi-Prostate Score (University of Michigan Health System)
- Prostarix
  - a test designed to help decide whether to biopsy men who have a normal digital rectal examination and a moderately raised PSA
  - urine is run on a liquid chromatography mass spectrometer
  - levels of metabolites between benign and cancerous tissues are different and may predict stage and 5-year recurrence risk

Box 3. Tissue biomarkers for prostate cancer

- PTEN
  - loss of PTEN is common in prostate cancer and associates with poorer prognosis
  - the PTEN fluorescence *in-situ* hybridisation test may be used on biopsy specimens to determine potential for progression when low or intermediate Gleason score tumours are detected
- Ki-67
  - Ki-67 is a tissue marker of proliferation, and has been shown to be a marker of PSA relapse after surgery
- E-cadherin
  - E-cadherin is a protein responsible for cellular adhesion, and loss leads to carcinogenesis and correlates with increasing tumour grade and may prognosticate survival
- EZH2
  - EZH2 overexpression is reported to be the best predictor of biochemical recurrence or metastases and combined with E-cadherin is a strong predictor of recurrence
- ProMark
  - ProMark is a new tissue-based assay to differentiate indolent from aggressive disease using a panel of protein markers on high-throughput immunohistochemistry

Box 4. Recent advances in genomic markers for prostate cancer

- Confirm MDx
  - DNA methylation is able to switch off genes at the DNA level, found in normal tissue around cancer – the 'field effect'
  - Confirm MDx assays methylation in tissue to determine risk of tumour in 'normal' tissue
  - tested in a large cohort of men with negative initial biopsy: negative predictive value of >90%
- Prostate core mitomic test
  - deletion of mitochondrial DNA (mtDNA) characterises prostate cancer
  - levels of mtDNA in negative prostate biopsies have a negative predictive value of 91% and can predict the presence of missed tumour in 17 out of 20 men 1 year before diagnosis
- Oncotype Dx
  - a collated gene list tested on biopsy tissue, can reclassify prostate cancer into very low, low and modified intermediate-risk disease
  - the aim is to confidently stratify treatment groups including active surveillance
- Prolaris
  - another gene panel tracking prostate cancer proliferation, which can be used on biopsy or prostatectomy cancer tissue
  - the higher the 'score', the higher the risk of disease progression, therefore warranting closer monitoring or additional treatment

RECENT ADVANCES AND CURRENT PRACTICE

With the increased utilisation of high-throughput genomic analyses on prostate cancer tissues, there is frenzied interest in being able to use the most highly cancer-specific genes or pathways ('signatures') in disease recognition and prognostication. Currently only the PCA3 test is performed in specialist centres in the UK. It is best placed before a second biopsy in determining risk of cancer[9] rather than as a screening tool like PSA. The value cut-off is controversial but adding PCA3 to conventional nomograms for decision making comprising DRE, PSA prostate volume and age increases diagnostic accuracy by 7.1%.[10] Another limitation of PCA3 is that it may not predict aggressive disease.[11]

In terms of their use within the NHS, PCA3 and newer tests such as Oncotype Dx and Prolaris are highly restricted because of financial constraints and availability of backhouse technical support, as well as limited robust clinical data supporting the genomic tests. As further data from clinical studies of the newer assays with mature follow-up emerge, as well as cost decreases in terms of the genomic strategies employed, it is hoped that these tests will become increasingly useful clinically.

IMAGING

Imaging traditionally had a limited role in the staging of higher-risk tumours, with MRI utilised for local staging, and CT or bone scintigraphy for nodal or bone metastases. However, multiparametric MRI, combining anatomical and functional tumour imaging, has been validated as an accurate means of detecting prostate tumours.[12] Functional imaging of the prostate offers a way of improving risk stratification by characterising tumour grade and aggressiveness, with options including diffusion-weighted MR imaging, dynamic contrast-enhanced MRI, MR spectroscopy and positron emission tomography (PET) imaging.

MR has been shown to have high sensitivity for predominantly Gleason 3 lesions with a volume ≥0.5cm^3 (approximately a 10mm sphere) and Gleason ≥4+3 lesions with a volume of ≥0.2cm^3 (7mm sphere).[13] Although MR may lack sensitivity for low-grade, low-volume lesions, it can be argued that these are the very low risk tumours that tend to be overtreated. A negative MRI also shows potential for excluding the presence of significant cancer, with negative predictive values approaching 90%.[14] As such, a number of authors have advocated moving MRI earlier in the diagnostic pathway, replacing transrectal ultrasound (TRUS) biopsy as the initial diagnostic test for prostate cancer (Figure 1).[15]

^{18}Fluorine-labelled fluorodeoxyglucose PET-CT has become part of the clinical work-up for a number of cancers; however, it is limited in prostate cancer by the reduced glycolytic activity of lower-grade tumours and artefact due to activity in the bladder.[16] Choline and acetate are other PET tracers available for use in prostate cancer, and can be radiolabelled with either ^{18}Fluorine or ^{11}Carbon. Carbon has the advantage of better maintaining the metabolic structure of the tracer but has a greatly reduced half-life of 20 minutes (versus 120 minutes for ^{18}F), necessitating on-site cyclotron production.

Acetate is incorporated into membrane fatty acid, and prostate cancer cells preferentially use fatty acid metabolism for energy production.[17] Choline is a marker of malignant tissue as a result of increased cell membrane metabolism, a feature also exploited by conventional MR spectroscopy.

Current PET imaging remains inferior to MRI for the detection and local staging

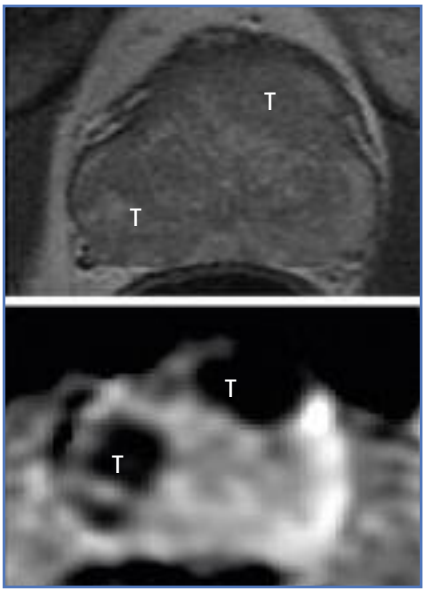

Figure 1. Prostate tumour (T) seen on MRI T2-W imaging followed by subsequent diffusion-weighted imaging for confirmation

of prostate cancer, but may play a role in staging more advanced disease. Both choline and acetate tracers have shown promise for the restaging of patients with biochemical failure after radical therapy, but a recent meta-analysis concluded that there is limited evidence for their routine use in staging patients with proven, but untreated, high-risk prostate cancer.[18]

BIOPSIES

Current practice for biopsy is a 10- or 12-core TRUS-guided prostatic biopsy. However, TRUS biopsy may miss up to half of cancers, and consistently undergrades around one third of tumours.[19] Patients who are deemed suitable to have active surveillance may not choose it because of the risk of missing higher-grade disease elsewhere in the prostate.

Biopsies performed using the transperineal route are gaining recognition. These offer certain advantages over a standard transrectal approach, and are considered after an initial negative TRUS biopsy. Transperineal ultrasound-guided biopsy utilises a sterile technique, reducing the risk of biopsy-associated infection, and has an increased ability to detect cancers located in the anterior and apical regions of the prostate, two areas frequently undersampled transrectally.[20]

MRI for secondary biopsy guidance following an initial negative TRUS biopsy has also been shown to improve tumour detection and the accuracy of grading.[21] Such targeting can be cognitive, under direct MRI guidance, or using fusion software for ultrasound guidance. The latter combines the diagnostic advantages of MRI with the real-time targeting of ultrasound. Future refinements to the transperineal technique are aimed at reducing operative time and allowing performance under local anaesthesia (Figure 2).

SUMMARY

Prostate cancer diagnosis is undergoing a revolution and necessarily so (Box 5). Although commonly diagnosed, the death rate from prostate cancer is low. The surgical treatment is so radical that we may expose men who may not die from their disease to life-limiting sequelae such as impotence and urinary incontinence. Prostate cancer screening may save lives, but needs years to manifest a benefit and involves treating 37 men to save one life.

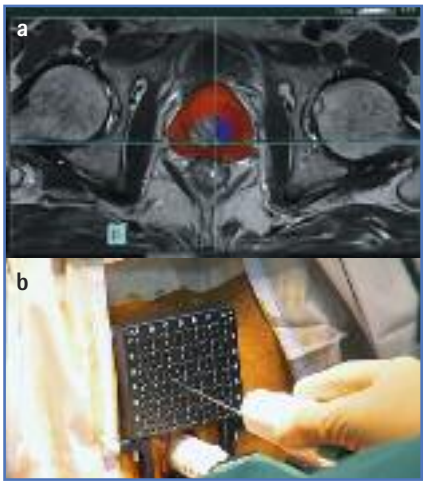

Figure 2. a) 3D mapping of the prostate using an MRI–transrectal ultrasound fusion device. b) The grid and biopsy technique used for transperineal biopsies, which is becoming increasingly used alongside the 3D visualisation of the prostate during biopsies

On the other hand, men with seemingly indolent disease are often upstaged and upgraded after prostatectomy. We need to better detect who to biopsy and if we diagnose cancer, prognosticate better. We have described here a plethora of recent advances in the diagnostic pathway, including serum, urine and tissue markers of prostate cancer, and improvements in imaging and biopsy techniques. Given the heterogeneity of prostate cancer, it is unlikely

Box 5. The future for prostate cancer diagnostics

- **Prostate cancer diagnostics will be based on initial PSA and digital rectal examination followed by multiparametric MRI and serum or urine testing**
- **The presence of a lesion on MRI and biological characteristics, for example high PCA3 and TMPRSS-ERG fusion score, will determine decision to biopsy, also taking into account surgeon and patient factors**
- **If the biopsy is negative, tests like the Confirm MDx or mtDNA assay will confirm true negativity**
- **In the presence of a positive biopsy, the Prolaris, Oncotype Dx or ProMark tests may be applied to decide whether active surveillance is appropriate or treatment with a curative intent is needed**
- **Ultimately, the high initial cost of these tests (Oncotype Dx is currently $4175) will be tempered by a reduced number needing surgery (a robotic prostatectomy costs in the region of £20 000), reduced days off work, higher quality of life in terms of potency and continence, and marked reduction in overtreatment, which is the goal for patient-centred healthcare**

that a one-size-fits-all approach will suffice, and a combination of investigations will likely be tailored to individuals to determine the optimal management.

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