Approval

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30/03/2016

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31/03/2016
INTRODUCTION
Prostate cancer (PCA) is the second most common cancer in men worldwide, with an estimated 1.1 million cases [wcrf.org] and 370,000 deaths in 2012 [globocan.iarc.fr]. The lowest disease incidence is observed in less developed countries, which also present the highest mortality rate. The highest disease incidence is found in North America, North-Western Europe, Australia and New Zealand, where also the lowest mortality rate is reported. Such data would correlate with a greater awareness of the disease through the increasing use of serum prostate specific antigen (PSA) as a screening tool, rather than to a true rise in the number of new cases. In Luxembourg roughly 300 patients are diagnosed with the disease every year [Registre Morphologique des Tumeurs, 2012].

Prostate cancer diagnosis
As of today, for the early diagnosis of prostate cancer (PCA) urology practice relies on three main diagnostic tools: total serum prostate specific antigen (PSA) and/or PSA-derived measures, digital rectal examination (DRE) and transrectal ultrasound (TRUS) - guided biopsy.

Each of these three measures has shortfalls that contribute to 75% of biopsies being done unnecessarily.

The Food and Drug Administration (FDA) firstly approved the use of PSA to monitor the progression of PCA in men who had been already diagnosed with the disease (1986) and, subsequently (1994), to test asymptomatic men for PCa, in conjunction with DRE. However, a growing body of clinical evidence has shown that PSA is not a particularly reliable biomarker for PCA screening and it suggests as well that PSA is also a poor indicator of tumor aggressiveness.

The usual PSA threshold used to recommend a biopsy has been 4.0 ng/mL. Despite being specific for prostate tissue, PSA is not PCA specific and has a very low predictive value estimated
at 25%-35% in the range of 2.6–10 ng/mL, often referred as the diagnostic grey zone. PSA concentration in blood is affected by prostate volume and hence, conditions such as lower urinary tract infections, prostatitis and benign prostatic hyperplasia (BPH) will cause high PSA concentrations. In case of PSA higher than 4 ng/ml (established diagnostic accuracy cut-off) but negative biopsy, it is not clear which follow-up strategy should be applied. Such scenario (PSA suggests PCa but biopsy is negative) can create a false sense of security in the patient and call for subsequent biopsies which, in turn, will induce anxiety and cause discomfort, pain, antibiotics-resistant inflammation and occasionally severe complications such as bleeding and septicemia.

New and non-invasive diagnostic tools are needed to discriminate men requiring surgical/chemical treatment for aggressive cancer, from men with indolent, non-life threatening tumors who could be enrolled in an active surveillance program and be spared from repeated biopsies and potential subsequent surgical intervention and related complications.

Prostate cancer gene 3

Several European and US studies have shown that Prostate Cancer Gene 3 (PCA3), a prostate-specific urine biomarker, helps identify men with a high likelihood of prostate cancer, independently from PSA levels, prostate volume and previous number of biopsies. Clinical evidence suggests as well that PCA3 can be used as a predictor of PCa significance.

A non-coding messenger RNA (mRNA) is expressed by the Prostate Cancer gene 3 (PCA3) in epithelial prostate cells. An over expression of PCA3 has been observed in up to 95% of PCA patients undergoing radical prostatectomy with levels 60-100 times higher in cancerous tissues than non-cancerous specimens. In contrast to serum PSA, PCA3 is not only prostate-specific but also PCa-specific, as it is undetectable in malignant tissue from other organs. Its levels are not affected by age or prostate volume, therefore prostatitis and BPH will not cause PCA3 elevation. PCA3 is also independent from PSA levels and the number of prior biopsies.
PCA3 test: assay cost in Luxembourg and clinical helpfulness

In Luxembourg, the hospital clinical laboratory applies a charge of 10.81 € for the PSA test (total PSA) versus a charge of 259 € for the PCA3 test (the test is outsourced to a laboratory in Gand, Belgium).

When is the PCA3 score helpful for the clinician?

PCA3 score can:

- increase the confidence when deciding whether a first biopsy, or a repeat biopsy, is needed especially when the patient presents with a PSA value in the diagnostic grey zone (2.5-10ng/ml)
- aid in making a decision whether to enroll a patient into an active surveillance program or to perform a surgical intervention
- be combined with demographic data, risk factors, family history, phi index, DRE and other biomarkers (such as PSA itself, including PSA forms and TMPRSS2:ERG fusion) thus providing more thorough information to the urologist to support his decision-making regarding biopsy execution and/or treatment avenues

AIM OF THE STUDY

In our study we sought to:

- evaluate the diagnostic performance of serum PSA versus urine PCA3 expressed as areas under each respective receiver operating characteristic (ROC) curve
- provide the Sensitivity and Specificity of PSA and PCA3
- provide PSA and PCA3 cut-offs for diagnostic accuracy for the analyzed sample of subjects

MATERIAL AND METHODS

PCA3 scores and PSA data

Two samples of post-DRE urine (20-30ml/sample), dated 6-months apart from each other (visit 1 or V1, and visit 2, or V2), were obtained from N=103 male patients attending one of the urology centers in
Luxembourg. For each subject, time-matched or closest to the visit date, serum PSA levels were determined by various Luxembourgish clinical biology laboratories. Patients' diagnoses were expressed as: no prostate pathology, prostate cancer, prostatitis, concurrent BPH and prostatitis.

In the IBBL laboratory, using transcription-mediated amplification (TMA) technology (PROGENSA™ PCA3 Assay, Gen-Probe), PCA3 mRNA molecules were amplified and the PCA3 score was calculated for each subject as \((\text{PCA3 mRNA}/\text{PSA mRNA}) \times 1\,000\). The greater diagnostic utility for this assay occurs at a cut-off of 35.

PSA data, received by IBBL via each urologist, were expressed as ng/ml of total PSA. The greater diagnostic utility for PSA occurs at a cut-off of 4ng/ml.

**Final diagnoses**

Final diagnoses were available for 90.3% study participants and stratified as follows: 30 BPHs; 14 prostatic cancers; 1 'other cancer'; 5 prostatitis; 9 BPH/prostatitis and, finally, 34 no prostate pathology.

**ROC curves, AUC, Sensitivity and Specificity**

In a ROC curve graph, the Sensitivity (true positive rate: ability of the test to correctly identify those with the disease) is plotted against the 1-Specificity (true negative rate: ability of the test to correctly identify those without the disease) thus generating a set of x-y coordinates corresponding to a particular decision threshold.

The chosen clinical decision threshold (greatest diagnostic accuracy of the assay), will be defined by the value corresponding to the best trade-off between Sensitivity and Specificity (red arrow in Fig.1 and Fig.2).

The area under the curve (AUC) defines the accuracy of a test. The perfect test has an area of 1 while an area of 0.5 represents a worthless test.

To evaluate PCA3 and PSA accuracy to discriminate prostate cancer from non-prostate cancer cases (i.e., non-cancer pathology, BPH, prostatitis, BPH and prostatitis), we plotted a ROC curve for each diagnostic tool and compared the correspondent AUCs.
RESULTS

PSA levels
PSA values were not consistently time-matched with PCA3 score calculation. In order to perform our analysis it was decided to pair, for each study participant, the highest PCA3 score (either obtained at V1 or V2), with the highest PSA concentration selected among all the values provided at different visit times.

PSA: ROC, AUC, Sensitivity and Specificity
Over N = 14 prostate cancer and N = 79 non-prostate cancer subjects, the PSA ROC curve (Fig.1) gave the following results:

AUC = 0.555 (95% CI = 0.349 to 0.762)

Sensitivity: 46.2%

Specificity: 82.4%

Cut-off diagnostic accuracy: 5.8 ng/ml (clinically accepted threshold is 4 ng/ml)

PCA3 scores
At visit 1 (V1), a PCA3 score was obtained for all the 103 study participants. At visit 2 (V2), a PCA3 score was obtained only for 72 of them. Missing values were due to sample over diluted that did not produce any result, or lack of urine sample for IBBL laboratory to analyze.

PCA3: ROC, AUC, Sensitivity and Specificity
Over N = 14 prostate cancer and N = 79 non-prostate cancer subjects, the PCA3 ROC curve (Fig.1) gave the following results:

AUC = 0.683 (95% CI = 0.539 to 0.826)

Sensitivity: 78.6%

Specificity: 63.3%
Cut-off diagnostic accuracy: 36 (clinically accepted threshold is 35)

**COMMENTS**

The PSA ROC curve depicted in Fig.1 gives and AUC of 0.555 so, in simple terms, the PSA assay is only marginally better at predicting PCa than flipping a coin which would give us an AUC=0.5. The PCA3 ROC curve depicted in Fig.2 gives and AUC of 0.683, showing that PCA3 assay has a better accuracy in discriminating subject with PCa from those presenting with inflammatory disease or disease-free.

**RECCOMENDATION**

Based on our results, showing that PCA3 has a better diagnostic performance than PSA, hence confirming clinical findings in US and other European countries, we recommend defining the use of PCA3 as standard of care with the necessary reimbursement of the assay by the Luxembourgish Ministry of Health.
Fig.1: PSA ROC Curve

PSA

AUC = 0.555 (95% CI = 0.349 to 0.762)

Sensitivity: 46.2%
Specificity: 82.4%

Cut-off diagnostic accuracy: 5.8 ng/ml (clinically accepted threshold is 4 ng/ml)
Fig. 2: PCA3 ROC Curve

PCA3

AUC = 0.683 (95% CI = 0.539 to 0.826)

Sensitivity: 78.6%

Specificity: 63.3%

Cut-off diagnostic accuracy: 36 (clinically accepted threshold is 35)