Biological and Molecular Markers for Urological Tumours: where do we stand?

Frans M.J. Debruyne
Professor of Urology

Hong Kong November 6th, 2009

Markers for Kidney Cancer

?
Markers for Testis Cancer

HCG ß
LDH
A foetoproteine

Markers for Bladder Cancer
Diagnostic goals in bladder cancer

- Identification of primary or recurrent UCC
- Early detection of risk groups
- Improvement of Golden Standard

How do we diagnose bladder cancer?

- Symptoms: symptomatic or asymptomatic microscopic or macroscopic haematuria
- Key Investigation: Urethrocystoscopy and urine cytology
Golden Standards

1. Urethrocystoscopy (UCS) and 2. Urinary Cytology
Golden Standard 1: Urethrocystoscopy

- Invasive
- Bothersome for patient
- Expensive
- Sensitivity???

Sensitivity cystoscopy

- EORTC, 2410 pts, recurrence rate at first follow-up (Brausi et al, Eur Urol 2002):
  - Single tumours: Between 3.4 en 20.6%
  - Multiple tumours: Between 7.4 en 45.8%

**Conclusion:** During resection of TaT1 papillary tumours many lesions are missed
Can we improve the Golden standard 1?

Possible solution:

Photo Dynamic Diagnosis (PDD)
Golden Standard 2: Urinary Cytology

Non-invasive, but:

- Low sensitivity, mainly in low-grade lesions: 60-70%
- Useless in case of infection
- Useless during intravesical therapy
- Operator dependent
- High intra- and interobserver variation

What could be the answer to the shortcomings of both Urethrocystoscopy and Cytology?
The answer: urinary tests (markers)?

Requirements

• Non-invasive
• Highly sensitive and specific (low grade lesions?!)  
• Objective
• Simple technique with rapid results
• Low costs

Indication

• Diagnosis of primary UCC of the bladder

• Follow up:
  - Recurrence: to replace UCS or adapt follow up (preventing unnecessary cystoscopies)
  - Progression
Potential markers in bladder cancer

- E-cadherin (soluble form)
- Urinary urokinase-type plasminogen activator
- Fibrin/fibrinogen Degradation Products (FDP)
- Human complement factor H related proteins (BTA, TRAK, BTA stat)
- Nucleair Matrix Proteins (NMP22, BCLA-4)
- Cytokeratins (CK19=CYFRA 21-1, CK20, CK8/18=UBC)
- Tumor associated antigens (19A211/M344/LDQ10 = Immunocyt)
- Hyaluronic acid
- Telomerase
- Survivin
- Quanticyt

A practical marker overview of commercial available tests
### Summary of recent literature

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>sens (%)</th>
<th>spec (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>906</td>
<td>49.8</td>
<td>96.6</td>
</tr>
<tr>
<td>BTA</td>
<td>911</td>
<td>39.5</td>
<td>89.5</td>
</tr>
<tr>
<td>BTAsstat</td>
<td>1482</td>
<td>67.7</td>
<td>65.8</td>
</tr>
<tr>
<td>BTA TRAK</td>
<td>928</td>
<td>71.1</td>
<td>62.0</td>
</tr>
<tr>
<td>NMP22</td>
<td>1558</td>
<td>64.3</td>
<td>71.2</td>
</tr>
<tr>
<td>UBC</td>
<td>1580</td>
<td>60</td>
<td>84.1</td>
</tr>
</tbody>
</table>

### Marker review

- 10,000 pts
- All commonly used tests
- All tests: sensitivity > cytology (low grade / stage)
- All tests: specificity < cytology
- CIS sensitivity “surprisingly” low

Lotan et al. (Urology 2003)
Urinary markers: conclusions

- Improved sensitivity at the cost of some specificity
- No present marker can replace cystoscopy
- Disappointing performance:
  - Confounding factors (UTI, stones, intravesical therapy)
  - Quality and handling of urine
  - Early detection of subclinical disease (false-positives)
- Comparison of studies difficult (patients included, cutoff)

In all studies so called false positive tests are considered as an indication for early recurrence
Two ‘new’ ones

Immunocyt\textsuperscript{TM} / uCyt+

NMP22 dipstick (BladderChek\textsuperscript{TM})

Immunocyt\textsuperscript{TM} / uCyt+

- Immunocytologic fluorescence test
- 3 Antibodies
- Labour intensive
- Some very high specificities
- Sensitivities independent from grade
- Value has to be confirmed
BladderChek™

FDA approved for monitoring and diagnosis
30 minutes office test
Value has to be confirmed

Grossman et al. (JAMA '05): sens 56%, spec 86%
Akkad et al. (Abstract 628 EAU Istanbul): sens 55%
Hautmann et al. (Abstract 630 EAU Istanbul): sens 85%, spec 91%

Combination with cytology

Sensitivities of tumor markers increase if results are combined with cytology:

- Tetu et al. (Mod Pathol '05): Immunocynt sens 74% ↑ 84%
- Hakenberg et al. (Urology '04): UBC Elisa sens 71% ↑ 83%
- Perez Garcia et al. (Arch Esp Urol '02): NMP22 sens 32% ↑ 46%
- Gibanel et al. (Anticancer Res '02): BTA TRAK sens 52% ↑ 81%
Fluorescence in situ hybridization

Detection of chromosomal alterations

Vysis™ UroVysion Bladder Cancer Recurrence Kit:

- DNA probe based urine test
- Aneuploidy detection for chromosomes 3, 7, 17 and loss of 9p21 locus
- Placer et al. (Eur Urol ‘02): sens 80%, spec 85%
- Kipp et al. (J Urol ‘04): results unaffected by BCG treatment

Other new techniques

- Telomerase
  - TRAP or RT-PCR in urine or bladder washing
  - Large discrepancies between different studies

- Microarrays
  - High-throughput mean
  - Panel of tumor markers: transcription expression, DNA and protein

- Neural networking (input of several markers)
However

What does the patient want??
Patients opinion about urine tests versus UCS

Vriesema et al. (Urology 2000)

• Utility analysis in 102 “experienced” patients
  (87 men, 15 women; age 38-85 years, mean 67 years)
• Burden of cystoscopy is low
  - depending on number of cystoscopies
  - no sex and age difference
• 89% prefers UCS, if sensitivity of urine test < 90%

Take home messages!

• Flexible cystoscopy indeed “golden standard”;
  might become “platinum” with PDD (e.g.
  HAL), especially in case of CIS.

• Cytology not the best but easy and specific.

• At best use a marker together with cytology.

• Markers do not yet meet the demands of
  urologist nor the patient.
General Conclusion

Urinary markers can not (yet) replace cystoscopy in the diagnosis of primary and recurrent (superficial) bladder tumours

Potential progression markers for EORTC CIS study

• Ki-67 (proliferation, Anticancer res 21, 1495, 2001)
• CK-20 (easy, recurrence)
• pT1 sub classification
• p53 mutation analysis or IHC
• E-cadherin expression
• EGF-R over expression
Conclusion markers

• Many molecular markers are studied

• Many seem promising in initial reports

• However, clinical implication of molecular markers remains very limited and difficult

Markers for Prostate Cancer
Molecular diagnosis of Prostate Cancer


Prostate Cancer: Global Incidence and Mortality

<table>
<thead>
<tr>
<th>Country/Area</th>
<th>Incidence*</th>
<th>Mortality (Incidence*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Europe</td>
<td>80.4</td>
<td>36.3</td>
</tr>
<tr>
<td>Western Europe</td>
<td>94.5</td>
<td>34.3</td>
</tr>
<tr>
<td>World</td>
<td>17.8</td>
<td>6.7</td>
</tr>
</tbody>
</table>

*Incidence=rate of prostate cancer cases/deaths per 100,000 person-years of observation.
Prostate-Specific Antigen Has Revolutionized the Diagnosis of Prostate Cancer\textsuperscript{1,2}

- More than two decades after the introduction of PSA as a screening tool for prostate cancer:
  - No consensus regarding its use for broad population screening has been formulated
  - No common international standard for measurement of PSA has been adopted
  - No universally accepted lower cut-off value; no long-term data are available to determine the optimal PSA threshold value for detection of non-palpable but clinically significant prostate cancer
  - Use of PSA can lead to unwarranted biopsies and over-treatment

Prostate Cancer Detection

- PSA is a sensitive method to identify patients at risk of prostate cancer but
  - the lack of specificity leads to many unnecessary biopsies
  - significant cancers are found in patients with PSA < 4ng/ml

- Definite need for better diagnostic and prognostic tests
Unresolved Issues Surrounding Prostate Cancer and PSA

• While a useful screening tool:
  – Elevated PSA levels can be associated with unnecessary biopsies & over-treatment
  – PSA is influenced by several conditions/factors that can influence levels\(^2\)
    – **Does not distinguish between clinically significant and insignificant tumors**\(^3\)–\(^5\)

• Conclusion:

  **A better screening tool is needed to improve diagnosis and appropriate treatment of prostate cancer**\(^3\)

‘..Quotes from recent meetings..’

• WHO conference prognostic markers(Stockholm) sept 2004 & ICUD
  Paris June 2005

**We most urgently need a test that identifies clinically significant cancer**
New developments in the diagnosis of prostate cancer

Gene based diagnostics of prostate cancer
DD3...DD3PCA3...PCA3...uPM3

- PCA3DD3
  (Bussemakers et al., Cancer Res. 59: 5975-79, 1999)
- Overexpressed in >95% of PrCa
PCA3$^{DD3}$ is the most prostate-cancer-specific gene described to date

- Over expressed in >95% of Ca
- Expression restricted to the prostate

**PCA3 in situ Hybridization**
PCA3<sup>DD3</sup> QRT-PCR
Non malignant vs cancer

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>PCA3&lt;sup&gt;DD3&lt;/sup&gt; mRNA copies/ug tissue (X1 10&lt;sup&gt;5&lt;/sup&gt;)</th>
<th>Median PCA3&lt;sup&gt;DD3&lt;/sup&gt; mRNA copies/ug tissue (X1 10&lt;sup&gt;5&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign prostate tissue (n=12)</td>
<td>0.2 -10.1</td>
<td>2.4</td>
</tr>
<tr>
<td>&lt;10% PCa (n=13)</td>
<td>6.6 – 166.0</td>
<td>25.3</td>
</tr>
<tr>
<td>&gt;10% Pca (n=27)</td>
<td>7.0 – 994.0</td>
<td>158.4</td>
</tr>
</tbody>
</table>

PCA3<sup>DD3</sup>, a new marker for prostate cancer

- These data imply that a minority of cancer cells in a background of non malignant cells is sufficient to discriminate cancer from non cancer;
- rational basis for molecular PCA3<sup>DD3</sup> based analysis of urinary sediments after extended DRE
Cells in prostatic urethra

Digital Rectal Exam (DRE)
‘Molecular Uroscopy’
urinary PCA3 Marker test-uPM3
**uPM3 urine test**

- *uPM3* is performed on the first 20 to 30 ml of voided urine collected after careful digital rectal examination, prior to the biopsy.
- The urine sample is fixed with a buffer solution, refrigerated and processed within 3 days.
- Cell pellet lysis
- Extraction
- Amplification & Detection on Easy Q instrument

---

**Results first study Nijmegen**

- 114 urine samples collected of 114 men with PSA > 3 ng/ml.
- 26 men had PCa in their biopsies (Gold standard)
- 88 men were negative for prostate cancer
Results

8 men with prostate cancer had DD3 values less than $200 \times 10^{-3}$.

70 men without prostate cancer had DD3 values less than $200 \times 10^{-3}$.

70/78 men (90%) with negative DD3 values, were negative for having prostate cancer (based on biopsy results).

Negative predictive value of the test is 90%.

So, compared to serum PSA

Serum PSA: specificity of 20%

80 out of 100 men with negative biopsies have high PSA values.

DD3 reflex-testing on PSA: specificity of 84%

16 out of 100 men with negative biopsies have high DD3 values.

Reduction in the number of unnecessary biopsies!
Independent confirmatory studies uPM3

- Tinzl & Marberger, Vienna (European Urology, August, 2004-EAU, EU award 'best paper 2004')
- Fradet et al. (Urology, August 2004)

Patients and Samples (Fradet et al.)

- A urine sample post DRE was obtained before TRUS biopsies in 517 patients from 5 centers
- 443/517 (86%) samples were evaluable for which tPSA result was available
  - 292 (66%) negative biopsies
  - 151 (34%) positive biopsies
### uPM3 Sensitivity & Specificity

<table>
<thead>
<tr>
<th>tPSA</th>
<th>Nb</th>
<th>Se %</th>
<th>Sp %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4 ng/ml</td>
<td>94</td>
<td>74 (14/19)</td>
<td>91 (68/75)</td>
</tr>
<tr>
<td>4 – 10 ng/ml</td>
<td>243</td>
<td>59 (50/85)</td>
<td>91 (144/158)</td>
</tr>
<tr>
<td>&gt; 10 ng/ml</td>
<td>106</td>
<td>79 (37/47)</td>
<td>80 (47/59)</td>
</tr>
<tr>
<td><strong>Overall uPM3:</strong></td>
<td><strong>443</strong></td>
<td><strong>67 (101/151)</strong></td>
<td><strong>89 (259/292)</strong></td>
</tr>
</tbody>
</table>

---

### Second (UMCN) cohort-2004-2005

- 299 patients
- 108 cancers
- Sensitivity; 59 %
- Specificity; 80%
- ROC analysis; AUC, 0.725
Sensitivity=59% and Specificity= 80%

The test result variable(s): DD3 over PSA ratio per urine sediment has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

The test result variable(s): DD3 over PSA ratio per urine sediment has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

Under the nonparametric assumption
- Null hypothesis: true area = 0.5
- Under the nonparametric assumption

<table>
<thead>
<tr>
<th>Marker</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA3 (n = 299)</td>
<td>0.725</td>
</tr>
<tr>
<td>PSA (n = 299)</td>
<td>0.584</td>
</tr>
<tr>
<td>% fPSA (n = 68)</td>
<td>0.603</td>
</tr>
</tbody>
</table>
PCA3: 2004/05 study

- PCA3 is useful for diagnosis
- PCA3 can have prognostic value
  - long term follow up exploratory study (2006)
  - Long term follow up ‘this study’ (2007)

Conclusion

- PCA3/PSA gene based Dx of urinary sediment analysis after DRE may have prognostic value
- Molecular diagnostic test for PrCa is within scope
  - Special attention to sampling
  - ASR/IVD test launched in 2006 (GenProbe)
PCA3 Gene-Based Urine Test

- New paradigm in prostate cancer detection based on a molecular target markedly over-expressed in prostate cancer cells
- Concept of a molecular cytology sampling of the prostate
- Initial studies suggest significantly better diagnostic accuracy than serum PSA
- Commercial assay under development by Gene Probe of San Diego and expected mid 2006

The TMPRSS2-ERG fusion gene can be found in urinary sediments after DRE
Many groups are looking for new candidate markers for prostate cancer diagnosis

Upregulation of ETS family member genes ERG and ETV1 (Petrovics)


1. Confirm the findings of recurrent gene fusions in our PCa patient group

2. Test whether these insights can be used in a routine clinical setting
Sample collection (a):

- PCa tissue obtained after radical prostatectomy (n=29)
- Tumor rich areas microdissected
- RNA isolation (TRIzol)

Sample collection (b):

Urinary sediments (n=68)

Cells in prostatic urethra

Digital Rectal Exam (DRE)
RT-PCR:

To achieve a high specific and sensitive signal →

Southern blotting of the on agarose gel separated PCR fragments

Blots were hybridized with a (α)\(^{32}\)P-dATP labeled probe:

PCR-fragment (180 bp) cloned into pCR\(^{\circledR}\)-Blunt vector

 Autoradiography

 Bands analyzed and sequenced

TMPRSS2-ERG RT-PCR on prostate cancer tissue

TMPRSS2/ERG fusion product in 17/29 (59%) of PCas
**TMPRSS2–ERG gene fusions in urinary sediments:**

<table>
<thead>
<tr>
<th>Patient</th>
<th>PSA test</th>
<th>PCA3 test</th>
<th>Gleason score biopsies</th>
<th>Seq. T2-ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>73.4</td>
<td>+</td>
<td>3 + 4 = 7</td>
<td>1 -4</td>
</tr>
<tr>
<td>11</td>
<td>10.4</td>
<td>-</td>
<td>3 = 6</td>
<td>1 -4</td>
</tr>
<tr>
<td>15</td>
<td>7.7</td>
<td>+</td>
<td>3 + 3 = 6</td>
<td>1 -4</td>
</tr>
<tr>
<td>21</td>
<td>8.3</td>
<td>+</td>
<td>3 + 5 = 8</td>
<td>1 -4</td>
</tr>
<tr>
<td>22</td>
<td>8.2</td>
<td>+</td>
<td>3 + 5 = 8</td>
<td>1 -4</td>
</tr>
<tr>
<td>23</td>
<td>8.2</td>
<td>+</td>
<td>3 + 5 = 8</td>
<td>1 -4</td>
</tr>
<tr>
<td>24</td>
<td>8.2</td>
<td>+</td>
<td>3 + 5 = 8</td>
<td>1 -4</td>
</tr>
<tr>
<td>25</td>
<td>8.2</td>
<td>+</td>
<td>3 + 5 = 8</td>
<td>1 -4</td>
</tr>
</tbody>
</table>

**Introduction**

**Objectives**

**Methods**

**Results**

**Concl./Disc.**
This is the first report of the use of TMPRSS2/ERG fusion gene based PCa diagnosis in urine.