Tumor Markers for Prostate Cancer: Update for 2012
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Prostate Cancer is a Major Health Threat for Men

- Worldwide: 670,000 new cases/year
- US: 240,000 new cases/year
- Europe: 225,000 new cases/year

http://info.cancerresearchuk.org/cancerstats/types/prostate/incidence/
Prostate Cancer - 2012
Dilemmas and Questions

1. Do we need to screen for early detection of prostate cancer?
2. Who needs to be screened?
3. Who needs to have a biopsy? And, if negative, is a second, third, or fourth biopsy warranted?
4. Who needs to be treated and when?
Limitations of Serum PSA For Early Detection

- Positive predictive value is only about 25% when PSA = 4-10 ng/ml and 2.5-4.0 ng/ml.
- The high false positive rate leads to many unnecessary biopsies.
- Cannot identify indolent tumors, which leads to unnecessary treatments for many subjects with damaging side effects.
- New biomarkers are needed!
Proposed Improvements

1. Serum Pro-PSA precursor forms
2. Other markers: Kallikreins (HK-2), EPCA, auto-antibodies and sarcosine
3. Gene detection in prostate cells released into urine
   ~ Panels of “selected” genes
   ~ Gene patterns defined by “supervised learning” mathematical analysis
Molecular Forms of PSA

Free PSA

PSA

proPSA

BPSA

intactPSA

AA
Complexed Disease

yes

237

239 - 244

no Cancer

237

no BPH

237

no benign

*active PSA not present in serum

Mikolajczyk et al, Urology 59, 797-802, 2002
Multiple Forms of proPSA

APLILSR-PSA (native proPSA)

[-7]Pro → ILSR-PSA

[-4]Pro → SR-PSA

[-2]Pro
Pro PSA and BPSA Immunoassays

Streptavidin Coated Microtiter Plate

DETECT
anti-PSA mAbs:

CAPTURE
Biotinylated anti-PSA mAb
Typical Proportions of proPSA Forms in Cancer Serum with PSA 4-10 ng/ml

intact non-native PSA 40%
proPSA 33%
BPSA 27%

[-4]pPSA 30%
[-2]pPSA 20%
[-5/-7]pPSA 50%
Early Clinical Studies with Prototype Research Assays

• 1091 serum samples from men enrolled in prostate cancer screening studies
  – Innsbruck PSA 2-4: 75 No Ca 71 Ca
  – PSA 4-10: 74 No Ca 77 Ca
  – St. Louis PSA 2.5–4: 245 No Ca 164 Ca
  – PSA 4-10: 241 No Ca 144 Ca

42% cancer. Extra-prostatic tumor in 12% with PSA 2.5-4 ng/ml and 25% with PSA 4-10 ng/ml
Sensitivity and Specificity of % Pro-PSA in PSA 4-10 ng/ml Range

• In the 4-10 ng/ml range, % pro-PSA detected 90% of cancers while avoiding 31% of unnecessary biopsies
• % Free PSA avoided 20% and complexed PSA avoided 19%
Sensitivity and Specificity of Pro-PSA: 2-4 ng/ml PSA Range

• Using a ratio of pro-PSA/free-PSA for recommending biopsy, 90% of cancers were detected while avoiding 19% of unnecessary biopsies

• % Free PSA avoided 10%, complexed PSA avoided 11%
NCI-EDRN Study of % -2 proPSA
Sokoll, L et al. Ca Epid Bio Prev 2010:19;1193

• N=566 subjects screened, 245 (43%) cancers detected

<table>
<thead>
<tr>
<th>Serum PSA</th>
<th>2.0-4.0</th>
<th>2.0-10.0 ng/ml</th>
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<tbody>
<tr>
<td>Sensitivity set at</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>Specificity</td>
<td>52%</td>
<td>45%</td>
</tr>
<tr>
<td>PPV</td>
<td>~50%</td>
<td></td>
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</table>
-2proPSA and Prostate Health Index

\[ \text{PHI} = (-2\text{proPSA}/\text{Free PSA}) \times (\sqrt{\text{T-PSA}}) \]

- Le, B et al. J Urol 2010: 183; 1355
- N=2034 subjects screened (PSA>2.5 or +DRE)
- 74 men biopsied, 30 (41%) cancers detected
- %proPSA: spec = 48% at sens = 89%; PPV=55%

- PHI = (-2proPSA/Free PSA) (sqrt T-PSA)
- PHI: spec = 65% at sens = 89%; PPV = 54%
Proposed Improvements

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Serum Kallikreins

• HK2 – A prognostic or diagnostic marker?
  Martin et al J Urol 175:104, 2006

• HK11 – A diagnostic marker?
  No significant differences were observed for HK 11, HK 11/PSA ratio and HK11 density in men with PCa (n =36) vs without cancer (n=78)

• So it seems there is no clinical role for serum kallikreins for prostate cancer
EPCA (Epitope 2.22 and 2.19)

- Early Prostate Cancer Antigen- is a nuclear matrix protein, originally described by Robert Getzenberg, reported to be a highly sensitive and specific test for prostate cancer.
- Onconome, Inc licensed the test, but has failed to validate the clinical claims for the test. So it seems the development of this test will be discontinued.
Sarcosine

• Sarcosine is an amino acid (glycine) metabolite, reported to be elevated in the urine of ~40% of PCa patients and not present in healthy controls.

• Early validation studies have not confirmed that claim!
  Jentmik et al. J Urol 2011: 185; 385
Auto-Antibody Tests for PCa

• Review article by Wang X, Lab Med 2008:39;165-171
  Gives basis for use of AABs and lists most promising antigens

• Panel test and autoantibody signatures for early detection of PCa
  No one marker has the desired sensitivity, panels must be used

• Koziol J et al. Clin Ca Res 2003:9;5120-6
  AB panel to antigens: c-myc,cyclinB1,IMP1,Koc,p53,p62,survivin

  Phage microarray defined 22 peptides for AAB test for PCa
Auto-Antibody Tests for PCa

- Oncimmune, LTD – 8 to 10 serum biomarker panel (TBD)
- Oxford Gene Technology – 15 serum biomarker panel
- Question of organ cancer specificity for each of these panel tests
- Assay technical issues - lot to lot consistency of recombinant antigens used for AAB capture, reference materials, standards and quality control preparations for routine clinical use
Proposed Improvements

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mRNA Based Detection of Prostate Cancer Cells in Urine

• Prostatic massage causes release of prostate cancer cells into the urine

• Detection of prostate specific genes in urine, such as DD3 (PCA3), has potential use for detection of prostate cancer.

Bussemakers, M, Ca Res 59:5975, 1999
PCA3 – Prostate Cancer Antigen

- Prostate-specific, non-coding mRNA\(^1\)
- Low expression level in normal prostate tissue
- Over-expressed in ~ 90% of prostate tumors (~ 60 to 100-fold), which allows for discrimination
- Feasibility of quantitative urine test first demonstrated in Schalken laboratory\(^2\)
- PCA3 Score: PCA3 mRNA levels normalized to prostate-specific housekeeping gene (PSA mRNA)

PCA3 Test Procedure – Gen Probe

- PCA3 and PSA mRNA concentrations measured in separate tubes

Quantitative ratio of PCA3/PSA mRNA = PCA3 Score

- PCA3 Score < cutoff → Lower risk of positive biopsy
- PCA3 Score > cutoff → Higher risk of positive biopsy

PCA3 Score Correlates with Probability of Positive Biopsy

- Overall 34% biopsy positive

Source: CE-marked European package insert
Subject group = 529 men scheduled for prostate biopsy
Summary for PCA 3 Test

- PCA 3 score improves the detection of prostate cancer. At a cutoff score of 35, the sensitivity is ~ 60% and specificity ~ 70%. The test predicts the probability of a positive biopsy, e.g. score > 50 has PPV of greater than 50% - but too many cancers are missed if it is used as a stand-alone test.

- The recent FDA approved use of PCA3 is selecting men for a second biopsy (high
A Four Gene Expression Signature for Prostate Cancer Cells Consisting of UAP1, PDLIM5, IMPDH2, and HSPD1

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* University of Texas, M.D. Anderson Cancer Center in Houston, TX, Clarient Inc, Aliso Viejo, CA

In collaboration with Thomas A. Stamey, MD
Department of Urology, Stanford University School of Medicine

Research effort to discover biomarkers for “aggressive” prostate cancer

1. Initiative of Dr. Thomas Stamey, Head Dept of Urology, Stanford University Medical Center
2. Radical Prostatectomy Specimens, n=87
3. Fresh frozen, laser microdissected, and carefully labeled cell types
4. Analyzed with Affymetrix U133A Microarray Chip (>20,000 genes)
Genes Overexpressed in PCa

Heat map of 19 genes (red means over-expressed, blue means unde-rexpressed). In color: the panel of complementary genes selected by SVM-RFE.
### Novel PCa biomarkers

<table>
<thead>
<tr>
<th>Name</th>
<th>UAP1</th>
<th>DKFZp564</th>
<th>IMPDH2</th>
<th>HSPD1</th>
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<td>Hs.632539</td>
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<td>4, 527.0 cR</td>
<td>3p21.2</td>
<td>2q33.1</td>
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<td>AL049969.1</td>
<td>J04208</td>
<td>BC002676.1</td>
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<tr>
<td>EC #</td>
<td>2.7.7.23</td>
<td>NA</td>
<td>1.1.1.205</td>
<td>NA</td>
</tr>
<tr>
<td>Pathway</td>
<td>Aminosugar metabolism</td>
<td>Not known</td>
<td>De novo guanine nucleotide biosynthesis</td>
<td>Mitochondrial Control of Apoptosis</td>
</tr>
<tr>
<td>Comment</td>
<td>Androgen response gene, assoc with PCa</td>
<td>Over-expressed in PCa. Similar to LIM domain protein.</td>
<td>Related to apoptosis</td>
<td>Chaperonin involved in mitochondrial protein import/folding</td>
</tr>
</tbody>
</table>
Summary of Studies to Establish the Urine Gene Profile Test

1. Optimized RT-PCR reaction conditions, prepared standard curves, selected positive (PC3) and negative (leukocyte) controls
2. Validated the assay using PC3 RNA spiked into urine
3. Established the sample collection conditions to ensure RNA stability in urine
4. Established a protocol for the separate assays of urine sediment and supernatant
5. Defined “S” score to report the normalized and standardized expression of the 4-target genes
Prospective, Blinded Validation Study

N= 46 men with PSA> 2.5 or +DRE

• The HDC urine four-gene profile test using two reference genes showed a sensitivity of 86% in 19/22 cancer cases (either or both sediment or supernatant was positive) and a specificity of 50% in 12/24 of the non-cancer subjects (both the supernatant and sediment were negative).

• The sensitivity of the HDC gene test was significantly better than the PCA3 test (64%/88%), while the specificity of the PCA3 test was better than the HDC test.

• A combined test –PCA3 with HDC genes – results in improved performance!
Other Gene Tests for PCA?

Gene test (AMACR, PCA3 and PSA ref gene) performed on urine sediment after prostatic massage in 43 Ca and 49 non Ca.
Sensitivity = 81% and specificity = 84%.

AMACR is alpha-methylacyl-CoA racemase
Other Gene Tests for PCA?

- ASCO Meeting 2009 Abstract Ross et al, Dana Farber. Blood test of six genes, 5 decreasing and one increasing in PCa. In 204 Ca, 110 BPH and 170 normals, sensitivity = 86% and specificity = 83%
- Test licensed to Source MDx, and clinical trial of 1000 men is reported to be underway.
Conclusion

I. Current PSA based cancer detection strategies must be enhanced to reduce the false positive rate and the number of unnecessary biopsies

II. Various “reflex test” opportunities include:
- Serum Pro-PSA variants (Beckman)
- Urine PCA3 (GenProbe)
- Urine 4-gene panel (Health Discovery Corp)

III. The next challenge in prostate cancer detection is to detect only the clinically significant cancers.