Metabolomic diagnosis of prostate cancer

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2nd Urology Forum, 15-16th May 2015, Athens
Current screening and diagnosis

• PSA, fPSA, PSAV, PSADT
• DRE
• Biopsy
• Prediction tools
• MRI
Current screening and diagnosis

- PSA
  - Low Sp
  - No cut-off – exclusion of PCa diagnosis
  - Screening – modest reduction in mortality

<table>
<thead>
<tr>
<th>PSA (ng/ml)</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>2,8-5%</td>
</tr>
<tr>
<td>1-2,5</td>
<td>10,5-14%</td>
</tr>
<tr>
<td>2,5-4</td>
<td>22-30%</td>
</tr>
<tr>
<td>4-10</td>
<td>41%</td>
</tr>
<tr>
<td>&gt;10</td>
<td>69%</td>
</tr>
</tbody>
</table>


Mortality Results from a Randomized Prostate-Cancer Screening Trial

Gerald L. Andriole, M.D., E. David Crawford, M.D., Robert L. Grubb III, M.D., Saundra S. Buys, M.D., David Chia, Ph.D., Timothy R. Church, Ph.D., Mona N. Feud, M.D., Edward P. Gelmann, M.D., Paul A. Kvale, M.D., Douglas J. Reding, M.D., Joel L. Weissfeld, M.D., Lance A. Yokochi, M.D., Barbara O’Brien, M.P.H., Jonathan D. Clapp, B.S., Joshua M. Rathmell, M.S., Thomas L. Bleyer, B.S., Richard N. Hayes, Ph.D., Barnett S. Kramer, M.D., Grant Izmirlian, Ph.D., Anthony B. Miller, M.B., Paul F. Pinsky, Ph.D., Philip C. Prorok, Ph.D., John K. Gahagan, Ph.D., and Christine D. Berg, M.D., for the PLCO Project Team®.
Current screening and diagnosis

- Ultrasound-guided prostate biopsy
  - > 60-70% of initial biopsies = negative
    - 20% false negative
  - 20-30% confirmation rate for the 2nd US-guided prostate biopsy
  - 45% of diagnosed PCa = low-risk

Panel NGR. NCCN Clinical Practice Guidelines in Oncology – Prostate Cancer Treatment.2012; version 3.0
Current screening and diagnosis

• Prediction tools
  
  o significant variability - low/high end of the risk spectrum
  
  o at least 30–50% of men assessed as very low risk under AS will require treatment
  
  o among high risk men with Gleason 8–10 tumors, 15 year PCa mortality may be lower than 40%

Current screening and diagnosis

- mpMRI
  - PZ PCa
    - T2WI+DWI+DCE-MRI => Se 83%
    - T2WI+DWI+DCE-MRI + spectroscopy => Se 86% Sp 100%
  - PZ+TZ PCa
    - T2WI+DCE-MRI+spectroscopy => Se 73% Sp 89%

Tamada T, Sone T, Higashi H, Jo Y, Yamamoto A, Kanki A, et al. Prostate cancer detection in patients with total serum prostate-specific antigen levels of 4-10 ng/mL: diagnostic efficacy of diffusion-weighted imaging, dynamic contrastenhanced MRI, and T2-weighted imaging. AJR Am J Roentgenol 2011;197:664e70
Current screening and diagnosis

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HIGH VOLUME CENTERS
3T MRI

Tamada T, Sone T, Higashi H, Jo Y, Yamamoto A, Kanki A, et al. Prostate cancer detection in patients with total serum prostate-specific antigen levels of 4-10 ng/mL: diagnostic efficacy of diffusion-weighted imaging, dynamic contrastenhanced MRI, and T2-weighted imaging. AJR Am J Roentgenol 2011;197:664e70
Current screening and diagnosis

• Increased screening + early detection of indolent PCa => large disparity: incidence vs lethality

• Overdiagnosis/overtreatment
Current screening and diagnosis

Screening 781 pts
Treat 27 pts
Avoid one death due to PCa

Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up

Fritz H Schröder, Jonas Hugesson, Monique J Roobol, Teuvo T. Tammela, Marco Zappa, Vere Nelen, Maciej Kwiatkowski, Marcos Lujan, Liisa Mäkitinen

Lancet 2014; 384: 2027–35
Current screening and diagnosis

- need for new biomarkers
  - better screening strategies
  - indolent vs aggressive

- ultimate goal of current research:
  - to minimize false positives
  - to treat only those men who are at the greatest risk for aggressive forms of PCa
  - to reduce testing and treatment for those at low risk for the disease

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Recent Advances in Metabolic Profiling And Imaging of Prostate Cancer
Roopa Thapa and Mark A Titus
New biomarkers

Timeline for Development Of Clinical Biomarkers For Prostate Cancer Progression

- **1930s**: Prostatic acid Phosphatase (PAP)
- **1985**: Prostate-specific antigen (PSA) gene kallikrein 3 (KLK3)
- **1999-present**: Biomarker discovery from “omics” platforms:
  - Proteomics
  - Genomics
  - Metabolomics
- **2000-present**: Long noncoding RNA prostate cancer antigen 3 (PCA3)
- **2007-present**: TMPRSS2-ERG gene fusions
Metabolomics?!
New biomarkers

- **Metabolomics** = evaluation of the patterns and concentration of low molecular weight metabolites over broad classes of compounds in a tissue or organ

- intermediate and end products of the *intracellular* biochemical reactions

- mass typically in the range of 80–1000 Daltons
New biomarkers

• the known metabolome - smaller than the number of genes, transcripts, or proteins

• downstream of changes in genes and proteins

=> metabolomics may more clearly characterize altered cellular networks

• metabolic alterations occur early -> targets for intervention

• Blood, urine and tissue
Advantages?
New biomarkers

• several circulating glycerophospholipid, fatty acid, energy and related metabolites - inversely associated with aggressive prostate cancer up to 20 years prior to diagnosis
## Characteristics of prostatic tissue

<table>
<thead>
<tr>
<th>Normal prostate/ BPH</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>- High levels of <strong>zinc</strong> in prostatic fluid (500–1000 times that in blood)</td>
<td>- Inhibit the mitochondrial (m-)aconitase activity</td>
</tr>
<tr>
<td>- Seminal fluid – high concentration of <strong>citrate</strong> (8000–15000 nmol/g of tissue)</td>
<td>- Due to the inhibition of m-aconitase, which converts citrate to isocitrate in the TCA cycle</td>
</tr>
</tbody>
</table>

### Characteristics of prostatic tissue

<table>
<thead>
<tr>
<th>Malignant prostate</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>- citrate levels decrease to 1000–2000 nmol/g</td>
<td>- malignant prostate cells oxidize citrate in the TCA cycle</td>
</tr>
<tr>
<td>- low levels of zinc</td>
<td>- decreased expression of zinc transporters</td>
</tr>
<tr>
<td>- spermine, phosphocholine and total <strong>choline</strong>-containing compounds are increased</td>
<td>- increased demand for lipid synthesis</td>
</tr>
<tr>
<td>- increased uptake of choline</td>
<td>- increased expression of choline transporters</td>
</tr>
</tbody>
</table>

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Prostate metabolome

- **Lipids**
  - Inositol-1-phosphate
  - Glycerol-3-phosphate

- **Amino-acids**
  - Leucine
  - Proline
  - Sarcosine

- **Xenobiotics**

- **Nucleotides**
  - Uracil

- **Peptides**

- **Energy molecules**
  - Citrate
Methods in metabolomic profiling

- Mass spectrometry
  - Gas chromatography
  - Liquid chromatography
- Nuclear magnetic resonance spectroscopy
- Magnetic resonance spectroscopic imaging
Disadvantages?
<table>
<thead>
<tr>
<th>Prostate cancer association</th>
<th>Metabolite association</th>
<th>Specimen types</th>
<th>Analytical methods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased in PCa vs. matched benign tissue</td>
<td>total choline, phosphocholine, glycerophosphocholine, phosphoethanolamine glycerophosphoethanolamine, lactate, alanine, sarcosine, uracil, kyurenine, glycerol-3-phosphate, leucine, proline</td>
<td>Prostatectomy, biopsy</td>
<td>HR-MAS, LC/GC-MS, GC-MS</td>
<td>6, 19, 20, 24, 25, 37</td>
</tr>
<tr>
<td>Increased in PCa vs. non-cancer specimen (unmatched)</td>
<td>total choline, free choline, sum of glycerophosphocholine +phosphocholine, lactate:alanine ratio, sarcosine, xylionic acid, dihydroxybutanoic acid</td>
<td>Biopsy, urine sediment or supernatant</td>
<td>HR-MAS, ID GC-MS, LC-MS/MS</td>
<td>6, 21, 3, 34, 35</td>
</tr>
<tr>
<td>Increased in metastases vs. primary tumor (unmatched)</td>
<td>sarcosine, uracil, kyurenine, glycerol-3-phosphate, leucine, proline</td>
<td>Biopsy of metastatic site, prostatectomy</td>
<td>LC/GC-MS</td>
<td>6</td>
</tr>
<tr>
<td>Increased in bone metastases vs. normal bone (matched)</td>
<td>sarcosine, cholesterol, myo-inositol-1-phosphate, citric acid, fumarate, glycerol-3-phosphate</td>
<td>Bone biopsy</td>
<td>GC-TOFMS</td>
<td>38</td>
</tr>
<tr>
<td>Increased in primary tumor from metastatic vs. non-metastatic disease</td>
<td>aspartagine, threonine, fumaric acid linoleic acid</td>
<td>Biopsy</td>
<td>GC-TOFMS</td>
<td>38</td>
</tr>
<tr>
<td>Association with plasma levels from metastatic vs. non-metastatic disease</td>
<td>Increased glutamic acid, taurine, phenylalanine. Decreased stearic acid.</td>
<td>plasma</td>
<td>GC-TOFMS</td>
<td>38</td>
</tr>
<tr>
<td>Decreased in PCa vs. matched benign tissue</td>
<td>ethanolamine, citrate, spermine, spermidine, putrescine</td>
<td>Prostatectomy, biopsy</td>
<td>HR-MAS</td>
<td>19, 20, 24, 25</td>
</tr>
<tr>
<td>Decreased in PCa vs. non-cancer specimen (unmatched)</td>
<td>citrate, myo-inositol, spermine, valine-leucine, hydroxybutyrate, glutamine, pyrimidine, ribofuranoside, xylopyranose</td>
<td>Biopsy, EPF, urine supernatant</td>
<td>HR-MAS, 1H-NMR, ID GC-MS</td>
<td>21, 22, 23, 34</td>
</tr>
<tr>
<td>No association with prostate cancer vs. non-cancer specimen (unmatched)</td>
<td>sarcosine</td>
<td>Urine supernatant, serum</td>
<td>ID GC-MS, LC-MS/MS</td>
<td>31, 33, 34, 36</td>
</tr>
<tr>
<td>Association with metastases vs. primary tumor (unmatched)</td>
<td>sarcosine</td>
<td>Serum</td>
<td>LC-MS/MS</td>
<td>36</td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
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</tr>
<tr>
<td>Association with increasing PSA</td>
<td>Decreased citrate, polyamines. Increased choline, phosphocholine.</td>
<td>Prostatectomy</td>
<td>HR-MAS</td>
<td>26</td>
</tr>
<tr>
<td>Association with increasing Gleason score</td>
<td>Increased total choline, free choline, phosphocholine, sum of glycerophosphocholine +phosphocholine. Decreased citrate, polyamines</td>
<td>Prostatectomy, biopsy</td>
<td>HR-MAS</td>
<td>19, 21, 26</td>
</tr>
<tr>
<td>Association with increasing stage</td>
<td>Decreased citrate, polyamines Increased choline, phosphocholine.</td>
<td>prostatectomy</td>
<td>HR-MAS</td>
<td>26</td>
</tr>
<tr>
<td>Association with increased probability of biochemical recurrence</td>
<td>spermine, glutamine, glutamate, myo-inositol, phosphoryl choline, scyloinositol</td>
<td>Biopsy</td>
<td>HR-MAS</td>
<td>27</td>
</tr>
<tr>
<td>No association with PSA, Gleason score, stage</td>
<td>sarcosine</td>
<td>Urine supernatant and sediment, serum, prostatectomy</td>
<td>ID GC-MS, LC-MS/MS, GC-MS</td>
<td>31, 33, 35, 36, 37</td>
</tr>
</tbody>
</table>
Biases

• variability in the physiological and pathological state of the samples
• specimen collection procedure
• size and selection of the study cohorts
• methodologic differences
• lack of standardization
Our experience
Our experience

• Total 134 patients
  o 85 before biopsy (39 BPH, 46 PCa)
  o 37 confirmed PCa, before RP
  o 12 controls

• LC-QTOF-(ESI+)-MS - Bruker Daltonics MaXis Impact
  • Aminoacid
  • Metabolomics
  • Lipidomics
Our experience

- Seric aminoacid quantitative analysis

  lysine
  phenylalanine
  alanine
  glycine

PCa vs BPH and control (p<0.05)

Putluri et al, 2011
Teahan et al, 2011
Kami et al, 2013

arginine

Vissers et al, 2005

PCa vs BPH and control (p<0.05)
Our experience

- Urine aminoacid quantitative analysis
  - glutamine
  - alanine
  - leucine
  - hystidine
  - phenylalanine
  - tryptophane
  - tyrosin

PCa vs BPH

PCa vs BPH
Conclusion

- No single metabolite, BUT the metabolic flux of metabolites in a particular pathway may be more useful for screening and prediction of PCa aggressiveness.
Conclusion

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FUTURE

- PCa fluxomics
- Pharmacometabolomics
- Personalised medicine
Thank you!
Mass spectrometry

- **Sreekumar et al, 2009** – 1126 metabolites (52 significant)
  - Diff benign vs localized PCa vs metastatic PCa
  - Sarcosine

- **McDunn et al, 2013**
  - glycerol-3-phosphate, sarcosine, kynurenine, proline, threonine, and uracil - mPCa
  - Sarcosine levels - elevated only in tissue biopsies with a Gleason pattern of 8 or worse

New biomarkers

- Prostate health index
- TMPRSS2-ERG gene fusion
- α-methyl-coenzyme A racemase (AMACR)
- circulating tumor cells (CTCs) in the blood stream
- prostate derived exosomes

- PCA3
  - most promising
  - non-invasive
  - detected in urine of patients with metastatic disease
  - can be used in conjunction with the PSA test for prostate cancer screening

15,16,17 REFERINTEEEEE
Mass spectrometry

- initial separation of metabolites by GC or LC, followed by ionization of metabolites and resolution according to mass-to-charge ratio

- advantage – high Se and detection of metabolites at very low concentrations

- extensive sample preparation (particularly for GC-MS), and metabolite detection can be complicated by differences in ionization efficiency, stability, extraction efficiency, and fragmentation behavior
Nuclear magnetic resonance spectroscopy

- exploits the behaviour of molecules when placed in a magnetic field, allowing the identification of different nuclei based on their resonant frequency

- for a mixture of metabolites in a biological sample the different patterns of energy release are represented as peaks in a chromatogram, and the area of the peaks is indicative of the relative concentration of each type of metabolite

- liquids or tissue extracts

- advantages: low cost, minimal sample preparation requirements, high reproducibility, ability to quantify metabolites, and identification of unknown metabolites
Magnetic resonance spectroscopic imaging

- elevated choline + reduced citrate