Urine Markers for Bladder Cancer

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University of British Columbia
# Disclosures

<table>
<thead>
<tr>
<th>Role</th>
<th>Company</th>
</tr>
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<tbody>
<tr>
<td>Consultant/Speaker</td>
<td>Abbvie, Astellas, Bayer, BioSyent, Ferring, Janssen, Lilly, Merck, Roche, Sanofi, Sitka, Spectrum</td>
</tr>
<tr>
<td>Grant Funding</td>
<td>GenomeDx, iProgen, New B Innovation</td>
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<tr>
<td>Clinical Trials</td>
<td>Genentech, Sitka, BioCancell, MDxHealth</td>
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</table>
The Gold Standard for Bladder Cancer Detection
In Search of the Ideal Bladder Cancer Biomarker

Can markers enhance performance of cystoscopy?

Can markers replace some use of cystoscopy?
Bladder Cancer Biomarkers - Clinical Utilities

Initial diagnosis of bladder cancer
- investigation of signs/symptoms (eg. hematuria)

Surveillance for bladder cancer recurrence/progression
- reduce the frequency of cystoscopies
- maintaining compliance to follow-up

Identifying non-visible (by cysto) tumors
- in the bladder (CIS vs atypia)
- upper tract monitoring

Predicting risk of progression

Screening for bladder cancer
Cytology

Pros:
- highly specific
- sensitive for high grade
- reveals the non-visible lesion
- non-invasive

Cons:
- very poor sensitivity for low grade
- dependent on expertise of cytopathologist
- frequent indeterminate reports
Ideal Marker: “Easier, better, faster, cheaper”

- adequate analytic performance/robustness
- result available in a timely fashion
- cost-effective
- improvement in diagnostic accuracy
- reduction of other diagnostic tests
### FDA “Cleared”

<table>
<thead>
<tr>
<th>Test</th>
<th>Company</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTA-Stat®</td>
<td>Polymedco</td>
<td>Diagnosis and surveillance</td>
</tr>
<tr>
<td>BTA-Track®</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMP22 (BladderCheck®)</td>
<td>Alere</td>
<td>Diagnosis and surveillance</td>
</tr>
<tr>
<td>uCyt+™ ImmunoCyt</td>
<td>Scimedx</td>
<td>Diagnosis and surveillance</td>
</tr>
<tr>
<td>UroVysion®</td>
<td>Vysis</td>
<td>Diagnosis and surveillance</td>
</tr>
</tbody>
</table>
NMP22
• Test detects NuMA (nuclear mitotic apparatus protein)
• Cut-offs 6 U/ml for recurrent tumors and 10 U/ml for incident tumors

BTA
• BTA Stat is office based test based on color change
• BTA TRAK is quantitative
• Identify a complement H related factor present in urine
ImmunoCyt™ (uCyt+™)

Cocktail of 3 monoclonal antibodies: LD Q10, M344 (mucin glycoproteins) and 19A211 (CEA)

UroVysion® identifies chromosome copy number alterations

- 12 cells loss of 9p21, and/or
- >4 cells with >2 copies 3, 7, 17
Why are we not using these tests more?

in setting of primary diagnosis:

• most studies have artificial “case-control” populations with high prevalence of disease
  – makes for overly optimistic report of test performance
    (especially positive predictive value = PPV)

• insufficient evaluation is a key reason for failure of tests to be implemented in clinical practice
Why are we not using these tests more?

In setting of surveillance for recurrence:

• higher prevalence bladder tumor, so much more likely to have acceptable PPV (but also higher risk of missing something)

• “anticipatory positive” is difficult to interpret
  – positive test before clinical evidence of recurrence

• main hurdle: lack of prospective studies defining consequence of positive or negative test
10. In a patient with a history of low-risk cancer and a normal cystoscopy, a clinician should not routinely use a urinary biomarker or cytology during surveillance. (Expert Opinion)

11. In a patient with NMIBC, a clinician may use biomarkers to assess response to intravesical BCG (UroVysion/C210 FISH) and adjudicate equivocal cytology (UroVysion/C210 FISH and ImmunoCyt/C212). (Expert Opinion)

Researchers have long attempted to identify and utilize urinary markers for bladder cancer detection. Five markers are currently approved by the FDA and/or commercially available in the U.S. (table 3).

At present, urinary biomarkers are insufficiently accurate to replace cystoscopy for diagnosis/surveillance, though some appear to have predictive utility for assessing response to intravesical BCG and may help interpret indeterminate cytology.

**TURBT/Repeat Resection: Timing, Technique, Goal, Indication.**

12. In a patient with non-muscle invasive disease who underwent an incomplete initial resection (not all visible tumor treated), a clinician should perform repeat transurethral resection or endoscopic treatment of all remaining tumor, if technically feasible. (Strong Recommendation; Evidence Strength: Grade B)

13. In a patient with high-risk, high-grade Ta tumors, a clinician should consider performing repeat transurethral resection of the primary tumor site within six weeks of the initial TURBT. (Moderate Recommendation; Evidence Strength: Grade C)

14. In a patient with T1 disease, a clinician should perform repeat transurethral resection of the primary tumor site to include muscularis propria within six weeks of the initial TURBT. (Strong Recommendation; Evidence Strength: Grade B)

Incomplete resection is likely a significant contributing factor to what have been described and diagnosed as early recurrences, as tumors have been noted at the first follow-up cystoscopic evaluation in up to 45% of patients.

**Intravesical Therapy, BCG/Maintenance, Chemotherapy/BCG Combinations.**

15. In a patient with suspected or known low- or intermediate-risk bladder cancer, a clinician should consider administration of a single postoperative instillation of intravesical chemotherapy (e.g., mitomycin C or epirubicin) within 24 hours of TURBT. In a patient with a suspected perforation or extensive resection, a clinician should not use postoperative chemotherapy. (Moderate Recommendation; Evidence Strength: Grade B)

16. In a low-risk patient, a clinician should not administer induction intravesical

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**Table 3. Performance characteristics of commonly used and FDA approved urinary markers**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Pos. Likelihood Ratio (95% CI)</th>
<th>Neg. Likelihood Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMP22® quantitative</td>
<td></td>
<td></td>
<td>3.05 (2.28-4.10)</td>
<td>0.40 (0.32-0.50)</td>
</tr>
<tr>
<td>Overall</td>
<td>69%</td>
<td>77%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>67%</td>
<td>84%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surveillance</td>
<td>61%</td>
<td>71%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMP22® qualitative</td>
<td></td>
<td></td>
<td>4.89 (3.23-7.40)</td>
<td>0.48 (0.33-0.71)</td>
</tr>
<tr>
<td>Overall</td>
<td>58%</td>
<td>88%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>47%</td>
<td>93%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surveillance</td>
<td>70%</td>
<td>83%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTA® quantitative</td>
<td></td>
<td></td>
<td>2.52 (1.86-3.41)</td>
<td>0.47 (0.37-0.61)</td>
</tr>
<tr>
<td>Overall</td>
<td>65%</td>
<td>74%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>76%</td>
<td>53%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surveillance</td>
<td>58%</td>
<td>79%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTA® qualitative</td>
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<td></td>
<td>2.80 (2.31-3.39)</td>
<td>0.47 (0.30-0.55)</td>
</tr>
<tr>
<td>Overall</td>
<td>64%</td>
<td>77%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>76%</td>
<td>78%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surveillance</td>
<td>60%</td>
<td>76%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UroVysion® FISH</td>
<td></td>
<td></td>
<td>5.02 (2.93-8.60)</td>
<td>0.42 (0.30-0.59)</td>
</tr>
<tr>
<td>Overall</td>
<td>63%</td>
<td>87%</td>
<td></td>
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<tr>
<td>Diagnosis</td>
<td>73%</td>
<td>95%</td>
<td></td>
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<tr>
<td>Surveillance</td>
<td>55%</td>
<td>80%</td>
<td></td>
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<tr>
<td>ImmunoCyt™</td>
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<td>3.49 (2.82-4.32)</td>
<td>0.29 (0.20-0.41)</td>
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<tr>
<td>Overall</td>
<td>78%</td>
<td>78%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>85%</td>
<td>83%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surveillance</td>
<td>75%</td>
<td>76%</td>
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</tr>
<tr>
<td>Cxbladder™</td>
<td></td>
<td></td>
<td>5.53 (4.28-7.15)</td>
<td>0.21 (0.13-0.36)</td>
</tr>
<tr>
<td>Overall</td>
<td>82%</td>
<td>85%</td>
<td></td>
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</tr>
</tbody>
</table>
How good is good enough? Patient Preferences

• n=200
• standard gamble method
• minimal accepted diagnostic accuracy at which urine test favored over cystoscopy
• 75% accept sensitivity >95%
• 21% accept sensitivity of 90-95%
• men who find cysto painful more likely to tolerate lower accuracy

Yossepowitch et al. J. Urol. 2007
NMP22 - Diagnosis

- prospective
- n=1331 @ 23 centres in US
- bladder cancer in 79
  - sensitivity = 55.7%
  - specificity = 85.7%
  - sensitivity of cytology = 15.8%
  - specificity of cytology = 99.7%
- NMP22 detected 4 cancers (3 T2, 1 Cis) missed by initial cystoscopy

Grossman et al. JAMA 2005
NMP22 - Surveillance

- prospective, multicentre
- n=668
- cancer recurrence in 103
  - cysto 91%
  - cysto + NMP22 99%
  - NMP22 detected 8/9 tumors not seen on cysto, including 7 high grade (1 by cytology only)
  - sensitivity = 49.5%
  - specificity = 87.3%

Grossman et al. JAMA 2006
Bladder Cancer Biomarkers - Clinical Utilities

Initial diagnosis of bladder cancer
- investigation of signs/symptoms (eg. hematuria)

Surveillance for bladder cancer recurrence/progression
- reduce the frequency of cystoscopies
- maintaining compliance to follow-up

Identifying non-visible (by cysto) tumors
- in the bladder (CIS vs atypia)
- upper tract monitoring

Predicting risk of progression

Screening for bladder cancer
Use of Fluorescence In Situ Hybridization to Predict Response to Bacillus Calmette-Guérin Therapy for Bladder Cancer: Results of a Prospective Trial

<table>
<thead>
<tr>
<th>Baseline FISH</th>
<th>FISH at 6 weeks</th>
<th>Patients at risk</th>
<th>Recurrence by 24 months (%)</th>
<th>Progression by 24 months (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>39</td>
<td>12.8</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>5</td>
<td>60.0*</td>
<td>40.0</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>35</td>
<td>22.9</td>
<td>8.6</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>55</td>
<td>48.9*</td>
<td>28.9</td>
</tr>
</tbody>
</table>
Recurrence

Baseline FISH

FISH at six weeks

FISH at three months

FISH at six months

Proportion without recurrence

Months

0 12 24 36 48 60

Months

0 12 24 36 48 60

Months

0 12 24 36 48 60

Months

0 12 24 36 48 60

p=0.141

p=0.008

p<0.001

p=0.001

Negative

Positive

Kamat et al J Urol 2012
Progression

Baseline FISH

FISH at six weeks

FISH at three months

FISH at six months

Proportion without progression

Months

Months

Months

Months

Kamat et al J Urol 2012
Cytokine Panel for Response to Intravesical BCG

- n=130 patients with intermediate- and high-risk NMIBC getting BCG with maintenance
- urine samples collected at baseline, before/after 6th induction dose BCG, and before/after 3rd maintenance BCG dose
- 12 cytokines measured; post-treatment levels compared to baseline

CyPRIT predicted the probability of recurrence with 85.5% accuracy.
Bladder Cancer Biomarkers - Clinical Utilities

Initial **diagnosis** of bladder cancer
- investigation of signs/symptoms (eg. hematuria)

**Surveillance** for bladder cancer recurrence/progression
- reduce the frequency of cystoscopies
- maintaining compliance to follow-up

Identifying **non-visible** (by cysto) tumors
- in the bladder (CIS vs atypia)
- upper tract monitoring

Predicting risk of **progression**

**Screening** for bladder cancer
UroScreen – Study Design

- Prospective surveillance cohort
- **Study group**: ~1700 chemical workers formerly exposed to aromatic amines
- Workers from two large chemical companies
- **Study period**: 2003 – 2011
- Assessment of bladder cancer risk factors
  Exposure to aromatic amines, tobacco smoking, and medical history
- **Annual examinations of spot-urine samples**
  - Microhematuria, urine cytology
  - Tumor marker tests (NMP22, UroVysion, survivin)
  - Urine creatinine, leucocytes

S Huber et al., BJU Int. 2012 Sep;110(5):699-708
1,772 subjects with at least one urinary examination

7,091 examinations of spot-urine samples

> 400 positive marker tests (cytology, NMP22, UroVysion)

Detection of 18 potential cases with bladder cancers
National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Bladder Cancer

Herbert A. Fritsche,¹ H. Barton Grossman,² Seth P Lerner³, Ihor Sawczuk⁴

¹Department of Laboratory Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; ²Department of Urology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; ³Department of Urology, Baylor College of Medicine, Houston, Texas, USA; ⁴Department of Urology, Hackensack University Medical Center, Hackensack, New Jersey, USA.
URINE TUMOR MARKERS IN BLADDER CANCER: NACB RECOMMENDATIONS

At this time, no tumor markers tests can be recommended for use in the diagnosis and clinical management of bladder cancer. This includes tests for making a differential diagnosis, assessing prognosis, staging of the disease or monitoring patients for the early detection of recurrent disease. There are no prospective clinical trial data that establish the utility of any of the FDA cleared markers or the proposed markers for increasing survival time, decreasing the cost of treatment or improving the quality of life of bladder cancer patients. In the following report, we describe the FDA cleared markers and the variety of newly proposed markers.

2006 & 2010
NMIBC AUA Guidelines 2016

1. In surveillance of NMIBC, a clinician **should not** use urinary biomarkers in place of cystoscopic evaluation. (Strong Recommendation; Evidence Strength: Grade B)

2. In a patient with a history of low-risk cancer and a normal cystoscopy, a clinician **should not** routinely use a urinary biomarker or cytology during surveillance. (Expert Opinion)

3. In a patient with NMIBC, a clinician **may use** biomarkers to assess response to intravesical BCG (UroVysion FISH) and adjudicate equivocal cytology (UroVysion FISH and ImmunoCyt). (Expert Opinion)

Chang SS et al, J Urol 2016
SO WHY ARE WE TALKING ABOUT URINE MARKERS?

On the horizon
AssureMDX – Discovery Study

DNA methylation of three genes:
- TWIST1, ONECUT2 and OTX1

Mutation analysis of three genes:
- FGFR3, TERT and HRAS

- n=154 hematuria patients (n=74 with bladder cancer)
- AUC 93%
- sensitivity: 97% specificity: 83%
- PPV: 23% NPV: 99.9%

(assuming 5% prevalence on BCa in hematuria population)

van Kessel et al; J Uro 2016
AssureMDX – Prospective Validation

- prospective study at three centers
- n=200 hematuria patients (n=97 with bladder cancer)
- AUC 96%
- sensitivity: 93% specificity: 86%
- PPV: 25.7% NPV: 99.6%
  (assuming 5% prevalence of BCa in hematuria population)
- 81.7% reduction in diagnostic cystoscopy
- North American multicenter prospective validation in 700 patients launching in 2017

van Kessel et al; J Uro 2016
European Multicenter Prospective Study

- DNA methylation of OTX1
- Mutation analysis of FGFR3 and TERT
- n=977 patients – primary detection and surveillance
- AUC, NPV, PPV not reported

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td><strong>Diagnosis - Primary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- low grade</td>
<td>81%</td>
<td>-</td>
</tr>
<tr>
<td>- high grade</td>
<td>94%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Surveillance - Recurrence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- low grade</td>
<td>57%</td>
<td>59%</td>
</tr>
<tr>
<td>- high grade</td>
<td>72%</td>
<td>55%</td>
</tr>
<tr>
<td><strong>OVERALL</strong></td>
<td>69%</td>
<td>-</td>
</tr>
</tbody>
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Beukers et al; J Uro Dec 31, 2016
• quantitative PCR to measure 5 mRNA:
  • MDK, HOXA13, CDC2, IGFBP5, CXCR2

Triage
incorporate
patient risk
profile
(age, sex, smoking,
exposures,
characteristics of
hematuria)

Detect
qPCR panel
alone in high
risk patients:
gross hemat +
risk factors

Monitor
surveillance of
patients with
prior NMIBC
Cx-Bladder Triage

Biomarker Gene Descriptions
- MDK: Blood vessel growth and cell migration
- HOXA13: Cell differentiation
- CDC2 (CDK1): Cell division
- IGFBP5: Programmed cell death
- CXCR2: Inflammation

Phenotypic Clinical Characteristics
- Age: Approximately 90% of people with bladder cancer are over 55
- Gender: Men are up to 4 times more likely to develop bladder cancer than women
- Smoking History: Current or ex-smokers are 3 times more likely to get bladder cancer than non-smokers
- Haematuria History: The occurrence, frequency, and duration of haematuria is a classic warning sign of possible bladder cancer
Cx-Bladder Triage

red – high grade
green – low grade
clear – no tumor

## Cx-Bladder Detect

- n=485 patients with gross hematuria

<table>
<thead>
<tr>
<th>Tumor Characteristic</th>
<th>Cxbladder</th>
<th>NMP22 BladderChek</th>
<th>NMP22 ELISA</th>
<th>Cytology</th>
</tr>
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<tbody>
<tr>
<td>Overall Sensitivity</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>82%</td>
<td>38%</td>
<td>50%</td>
<td>56%</td>
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<td>Stage Sensitivity</td>
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<tr>
<td>Tis</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
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<td>Ta</td>
<td>68%</td>
<td>38%</td>
<td>35%</td>
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<tr>
<td>Specificity</td>
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</tr>
<tr>
<td>85%</td>
<td>96%</td>
<td>88%</td>
<td>96%</td>
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</tbody>
</table>

O’Sullivan J Urol 2012
• prospective study at 11 US centers
• 803 patients with prior NMIBC, pre-cystoscopy
  • training (n=354) and validation (n=449) cohorts
• also tested UA, NMP22, cytology
• algorithm includes previous tumor occurrence information
Cx-Bladder Monitor

- 11% detection rate of UC (64% Ta, 14% T1, 22% Tis)
- 50% low grade/PUMLMP and 50% high grade
- Sensitivity 93%
  - Outperformed all other tests across all stages and grades
- 95% for HG and 86% for LG
- NPV of 97%
- Could this be used to reduce number of cystoscopies?
Next Generation Sequencing

20 non-cancer, 120 primary cancer and 89 cancer-free after TURBT

6 frequently mutated genes

70% sensitivity and 97% specificity
1. DEFINING genetic signatures

- Tumour biopsy → Tumour DNA
- Blood draw → Germline DNA
- DNA sequencing
- Tumour-specific genomic variants
  - deletion
  - translocation (tumour)
  - insertion
  - reference

2. DEVELOPING clinically applicable assays

- Personalized assays
- Digital droplet PCR analysis
- Breakpoint identification
- PCR validation

3. MONITORING clinical outcomes by liquid biopsies

- Plasma → Cell-free DNA
- Urine → Cell-free DNA

Birkenkamp-Demtröder et al Eur Urol 2016
The evolution of urine markers for bladder cancer