# Biologic markers of urothelial cell cancer of the bladder

*Jakub Dobruch[1], Theo M. de Reijke[2], Andrzej Borówka[1]*

[1]Postgraduate Medical Education Center, Department of Urology, Department of Urology, Interforest Specialist Hospital, Warsaw, Poland
[2]Department of Urology, Academic Medical Center, University of Amsterdam, The Netherlands

KEY WORDS

bladder ◗ bladder cancer ◗ markers

ABSTRACT

Cancer of the urinary bladder (BCa) is a major cause of morbidity and mortality all over the world. Clinical and pathological features have limited capacity to detect bladder cancer patients at high risk of recurrence and mortality. Several tissue and urine markers were introduced to improve the prediction of oncological end points. The aim of the study is to evaluate the role of biologic markers of the urothelial cell cancer of the bladder. To this aim a PUB-MED search was performed and studies on urothelial cell cancer markers were collected. Several urine and tissue urothelial cell cancer markers were presented. None have gained wide-spread clinical use, their accuracy needs further assessment. Only few prospective trials were published so far. There is growing need to initiate prospective trails that would evaluate the role of urothelial cancer markers. A studied panel should be constructed based upon the most promising markers.

## INTRODUCTION

Cancer of the urinary bladder (BCa) is estimated to be the seventh most prevalent type of cancer worldwide, accounting for approximately 3.2% of the international cancer burden [http://www.who.int/whr/2004/en/]. With an estimated 68,810 new cases and 14,100 deaths attributable to the disease in the US during 2008, BCa is a major cause of mortality and morbidity [1]. The most frequent histological type of bladder cancer is urothelial cell carcinoma of the bladder. This type of BCa accounts for more than 90% of all bladder cancer cases. At initial diagnosis, bladder cancer patients may be classified into two major groups according to the pathological stage. Up to 75% of these are diagnosed with non-muscle-invasive BCa (NMIBC), which is confined to the urothelium or the suburothelial connective tissue. Most of these patients can be successfully managed with transurethral resection (TUR) and intravesical therapy. However, this group of patients is heterogeneous and includes well differentiated, noninvasive papillary tumors as well as high-grade malignant lesions. The high grade lesions are frequently associated with a risk for future invasive growth and subsequent metastatic potential. These patients are usually treated with adjuvant bacillus Calmette-Guérin (BCG) immunotherapy. Despite a complete TUR up to 50-70% of NMIBC recurs and almost 15% of them progress into muscle invasive disease [2]. The remaining 25% of patients present with muscle-invasive disease (MIBC) and when clinically localized surgery involving cystectomy and pelvic lymphadenectomy is the mainstay of treatment for this disease.

## Biomarkers – rationale

### *Invasive bladder cancer*

Although the relative 5-year survival rate for all stages of bladder cancer amounts to 82%, the 5-year survival rates for mucosa confined, however invasive cancers and distant metastasis differ substantially. They range from 94% to 6%, respectively [3]. The current prognostication of invasive BCa is traditionally based on criteria such as tumor stage and grade, because these have found to be of significant prognostic value [4]. However, these prognostic parameters cannot predict the long-term outcome of bladder cancer with sufficient certainty and tend to be affected by intra- and inter-observer variability. Despite the increasingly widespread use of prognostic nomograms, current predictive accuracy in this setting remains below 80% [5]. Clinicopathological criteria do not reveal the response to a particular therapy, which may cause a certain level of over- or undertreatment. The selection of appropriate candidates for systemic neoadjuvant or adjuvant chemotherapy might also be improved; inadequate patient selection, based on TNM criteria, might be the reason for the significant variations in outcomes of published chemotherapy trials for BCa. The use of biomarkers might be particularly beneficial in elderly patients with BCa, as significant co-morbid conditions represent important competing risks.

### *Non-muscle-invasive bladder cancer*

Bladder cancer is one of the most expensive malignancies per patient to treat, considering the time from diagnosis to death. Due to the high risk of recurrence, the patient has to submit to lifelong surveillance with cystoscopy and urine cytology [6]. The identification of molecular biomarkers might offer better risk prediction. For BCa, these 'risks' are recurrence (local or systemic), disease progression, and disease-specific mortality. In patients with NMIBCa similar to conventional urine cytology, biomarkers that are present in urine (soluble) may be used as an adjunct to cystoscopy [7]. They may also serve to decrease the number of invasive procedures such as cystoscopy, provided that adequate cancer control is maintained. Supplanting follow-up cystoscopies with a soluble biomarker may yield a considerable decrease in health care costs [8]. Identifying 'high-risk' patients is crucial, as they might benefit from early radical intervention (e.g. immediate radical cystectomy in a patient with non-muscle-invasive but aggressive BCa). In summary, the use of biomarkers might allow for more-individualized and less-radical management in selected patients.

## Biomarkers – identification

According to Biomarkers Definitions Working Group a biological marker (biomarker) is a characteristic that is objectively

measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention [9]. Investigation of the utility of molecular biomarkers is divided into four stages: first, identification of potential markers; second, small or single-center retrospective studies; third, larger multicenter retrospective studies; fourth, prospective validation studies. Currently, many biomarkers have been evaluated retrospectively, but few prospective validation studies have been performed. While a biomarker might have prognostic capability, it does not necessarily add to the predictive accuracy of known pathologic features [10]. Without evaluating the gain in predictive accuracy, one might inappropriately assume that a marker with prognostic capability on univariate analysis will be clinically useful.

In order to identify potential biomarkers in BCa, it is important to first understand the biology of the tumor. BCa develops through a multistep process. Evidence suggests that, in urothelial tumorigenesis, distinct pathways lead to the development of non-muscle-invasive tumors and to more-aggressive muscle-invasive neoplasms. Low-grade papillary tumors frequently show a constitutive activation of the receptor tyrosine kinase–Ras pathway, exhibiting activating mutations in the HRAS and fibroblast growth factor receptor 3 (FGFR3) genes. Seventy percent of low-grade noninvasive papillary tumors have been shown to harbor FGFR3 mutations compared with 10-20% of invasive tumors, which strongly suggests the role of the activated FGFR3 gene in the genesis of low-grade papillary tumors [11-13]. In contrast, carcinoma *in situ* (CIS) and invasive tumors frequently show alterations in the TP53 and RB genes.

## Non-muscle invasive bladder cancer

Low grade non-muscle invasive tumors demonstrate constitutive activation of cellular growth factor-signaling pathways specifically the receptor tyrosine kinase among the four is FGFR3. It was shown that expression of an activating mutant FGFR3 gene correlates with a non-invasive clinical course [12]. FGFR3 plays a role in cell growth, differentiation, proliferation, and angiogenesis. A large study in NMIBC found that mutations in *FGFR3* were associated with increased rates of recurrence, but only in patients with pTa tumours of low grade; no association was found with disease progression or survival [14]. HRAS is a human oncogene and in the normal state is a key signaling intermediate of receptor tyrosine kinases. HRAS mutations constitutively activate HRAS protein and enable growth factor-independent signal propagation [15]. Over-expression of activated HRAS is sufficient to induce urothelial tumorigenesis contributing to the low-grade noninvasive papillary tumors [16]. Ki-67 is a molecule detected in actively growing cells and represents a direct measurement of cell proliferation. Ki-67 labeling index in NMIBC is an independent predictor for recurrence [17].

Markers of urothelial origin might be found in urine, these are named soluble and can be assessed with urine tests or evaluated in the specimen taken during transurethral resection or radical surgery, these are named tissue markers. Currently many soluble ones are tested in NMIBCa patients whereas the majority of tissue markers are used in patients with invasive disease. Some have been implemented in different BCa settings.

## Biomarkers in non-muscle-invasive BCa

### *Soluble biomarkers*

Definitely the recurrence of NMIBC can be recognized during cystoscopic evaluation of the bladder. Its presence might be suggested by a number of urine tests that evaluate soluble or cell-associated biomarkers. Most of these tests have a better sensitivity but lower specificity than voided urinary cytology (VUC) used routinely in clinical practice.

**Table 1.** Sensitivity and specificity of BTA-stat in studies with at least 100 patients.

| Author (year) | Sensitivity | Specificity |
|---|---|---|
| Sarosdy (1997) [32] | 67% | 72% |
| Pode (1999) [26] | 82.8% | 68.9% |
| Leyh (1999) [34] | 65% | 64% |
| Heicappell (2000) [38] | 62.9% | 93% |
| Giannopoulos (2001) [30] | 72.9% | 64.6% |
| Raitanen (2001) [27] | 56% | 85.7% |
| Boman (2001) [31] | 73.6% | 83.3% |
| Mean | 68.6% | 75.9% |

**Table 2.** Sensitivity and specificity of NMP22 in studies with at least 50 patients.

| Author (year) | | Sensitivity | Specificity | Cut-off NMP22 in urine (U/ml) |
|---|---|---|---|---|
| Wiener (1989) [22] | | 48% | 69% | 10.0 |
| Ramakumar (1999) [25] | | 53% | 60% | 3.6 |
| Sanchez-Carbayo (1999) [48] | | 75.7% | 95% | 14.6 |
| Mian (2000) [50] | | 55.5% | 79% | 10 |
| Giannopoulos (2001) [30] | | 63.5% | 75% | 8 |
| Boman (2002) [31] | Primary tumors | 65% | 75% | 4 |
| | Recurrent tumors | 45% | 64% | |
| Mean | 57.9% | 73.8% | | 3.6 – 14.6 |

VUC is based on microscopic evaluation of the urine sediment to look for cancer cells that are exfoliated from the tumor surface. Sensitivity of the test ranges from 31% to 90% [18-20], however, it is different in patients with G1, G2, G3 tumors and in patients with CIS in whom it ranges from 3% to 22%, from 10% to 61%, from 32% to 90%, and from 67% to 73% respectively [21-26]. Specificity of VUC is very high, it amounts to 100% [21-27].

ImmunoCyt is the test that combines VUC with the immunofluorescence technique. Labeled antibodies directed against carcino-embryonic antigen (CEA) and mucin secreted by cancer cells after adhesion at the surface of cells filtered from urine are easily visible in the microscope. Sensitivity in cases of G1, G2 and G3 tumors is 84%, 88%, and 96.5% respectively; specificity is almost 80% [28, 29]. Unfortunately, there is substantial inter-observer variability and the cost of the test is high.

BTA-Stat/BTA-TRAK detects bladder tumor antigen now identified as human complement factor H and complement factor H-related protein. BTA-stat is available in routine ambulatory practice. Its sensitivity is, however, tumor grade dependent and specificity ranges from 53 to 83% and from 54 to 93%, respectively (table 1) [22, 25, 26, 30-41]. The FinnBladder group study revealed overall sensitivity and specificity to be lower than for cytology being 56-19.2% *vs* 85.7-98.3%, respectively [42].

NMP-22 is a nuclear matrix protein involved in the cell cycle. The test uses monoclonal antibodies and an immunoenzymatic technique. Sensitivity and specificity ranges a lot from 60-85% and 42-100%, respectively (Table 2) [22, 25, 30, 43-51]. Many authors have reported that sensitivity decreases markedly during detection of bladder cancer recurrence. In the setting with other urologic pathology the utility of the test is limited [46].

Hyaluronic acid (HA) is a glycosaminoglycane (GAG) that was shown to promote progression and metastases. It is efficacious in detection of low grade/low stage disease but further studies with large cohorts of patients are needed.

Cytokeratins are cytoskeletal proteins.

Among them, cytokeratins 8, 18, 19, and 20 were found to be over-expressed in BCa. Promising results of early studies were not replicated by later ones. Soluble Fas (sFas) inhibits apoptosis. As predictor of BCa recurrence the test outperformed NMP22. However, further studies are needed [52].

Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs are involved in degradation of the extracellular matrix. MMP-2 and MMP-9 correlate with invasiveness of Bca and allow prediction of future progression [53]. Telomerase (T) regenerates telomeres, which are repeated sequences of nucleotides found at the end of chromosomes. Sensitivity of the test that evaluates T in detection of BCa presence varies from 9-100%. The variability is speculated to result from poor stability of T in urine. The test could have a place in cases with inconclusive results of cytology [54-56].

Fluorescence in situ hybridization (FISH) detects chromosomal aberrations (Uro Vysion). The test is expensive and the criteria of the test positivity are not well established.

Microsatellite DNA are 2-6 base pair repeats throughout the human genome. Its analysis (MSA) has shown variations in BCa cases within 4p, 8p, 9p & q, and 11p. Little consensus on which marker should be used has been presented so far [57].

The sensitivity and specificity of before mentioned tests are summarized in Table 3.

**Table 3.** Sensitivity and specificity of urine tests.

| Test | Sensitivity | | Specificity | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| VUC | 48% | 16-89% | 96% | 81-100% |
| Urine test – dipstick | 68% | 40-93% | 68% | 51-91% |
| NMP22 | 75% | 32-92% | 75% | 51-94% |
| BTA stat | 68% | 53-89% | 74% | 54-93% |
| ImmunoCyt | 74% | 39-100% | 80% | 73-84% |
| UroVysion | 77% | 73-81% | 98% | 96-100% |

## Muscle invasive bladder cancer

Regarding patients with advanced disease, the Southwestern Oncology Group (SWOG) 8710 trial (neoadjuvant chemotherapy plus radical cystectomy vs. radical cystectomy alone) has evaluated the prognostic significance of Ki-67, p53 and angiogenic changes. Patients whose tumors had elevated Ki-67 expression had increased progression-free survival (median 66 months vs. 12 months; P = 0.063) and a non-significant increase in overall survival [58].

The most intensively studied molecular biomarker for muscle invasive BCa is the p53 protein, which inhibits cell-cycle progression at G1-S transition. Many studies have suggested that increased p53 expression (i.e. nuclear accumulation) predicts poor outcome in BCa, both in patients treated with TUR for NMIBC and those who undergo radical cystectomy for muscle-invasive disease [59, 60]. In patients with MIBCa, p53 gene and protein statuses were significantly associated with stage and clinical outcome [61]. However, according to the first to prospectively examine the role of a p53 in homogeneous patient population with a primary diagnosis of T1 bladder cancer with no additional therapy, failed to show any significant findings [62]. For muscle invasive bladder cancer in SWOG 8710 trial increased p53 expression was not associated with decreased overall p (hazard ratio 1.48; P = 0.15) or progression-free survival [63]. Authors of a recently published meta-analysis suggest that after 10 years of research, evidence is not sufficient to conclude whether changes in P53 act as marker for outcome in patients with bladder cancer [64]. There is now, an ongoing adjuvant chemotherapy study at the University of Southern California, randomizing patients with nuclear p53 accumulation (p53+) to receive adjuvant chemotherapy or observation within 10 weeks following radical cystectomy.

Besides p53 protein status, other important cell-cycle regulatory molecules have been studied as potential biomarkers in both NMIBC and muscle invasive BCa. These include the pRb, the p21 and p27 proteins, and the cyclins D1, D3 and E. In immunohistochemistry studies of patients who underwent radical cystectomy, decreased expression of both p21 and p27 were predictors of BCa recurrence and disease-specific survival in both NMIBC and MIBC. Cyclins play a crucial role in malignant urothelial transformation during tumorigenesis. Also, the elevated expression of cyclins D1 and D3 have been reported to be independent predictors of progression-free survival in patients with NMIBC. A different study, however, found that tissue expression of cyclin D1 or E1 did not add independent prognostic value in patients with NMIBC [65].

Apoptosis is programmed cell death that results from a cascade of intrinsic and extrinsic signals. Among the apoptotic markers assessed in BCa specimens of all stages, the absence of caspase-3, increased expression of Bcl-2 and increased survivin expression (>10% of BCa cells expressing survivin) are associated with increased risk of recurrence and decreased BCa-specific survival in patients treated with radical cystectomy and bilateral pelvic lymphadenectomy [66]. Other characteristics predictive for at least one relevant oncologic endpoint in patients with BCa include: thrombospondin-1, microvessel density, E-cadherin, VEGF, RhoGD12. Their clinical utility needs to be further evaluated.

Considering the multistep tumorigenesis and complexity of molecular alterations in BCa, the past few years have shown a strong trend towards simultaneous assessment of multiple molecular biomarkers. Shariat et al. reported that altered expression of p53, pRB, p21, and p27 was independently associated with an increased risk of BCa progression in patients with NMIBC [67]. A marker panel approach also showed superiority to assessment of single markers in terms of prognostic value in patients with muscle-invasive BCa.

## Biomarkers – validation

The most important challenge for researchers investigating molecular biomarkers in BCa remains validation, beyond identification of independent predictive value. Only a few studies have advanced to the important biomarker validation step of adding information to established predictive parameters or nomograms. In one retrospective study that included 191 patients with pTa–T3N0M0 BCa treated with radical cystectomy, the addition of a number of altered biomarkers (cyclin E1, p53, pRB, p21 and p27) to a nomogram based on TNM staging significantly increased the predictive accuracy for BCa-specific mortality to 86.9% [68]. The ultimate goal for marker validation is a prospective study. Such studies in BCa are noticeably scarce. Prospective studies in NMIBC have evaluated the utility of p53 in predicting recurrence and progression, but showed no benefit of assessing p53 status [62]. A large study in NMIBC found that mutations in FGFR3 were associated with increased rates of recurrence, but only in patients with pTa tumors of low grade; no association was found with disease progression or survival. Patients whose tumors had elevated Ki-67

expression had increased progression-free survival. Currently, at the University of Texas Southwestern Medical Center, there is a prospective study investigating a marker panel (p53, p21, p27, cyclin E and Ki-67) in all patients with high-grade or invasive disease (≥pT1).

### Biomarkers – response to therapy

While identifying patients at risk for recurrence or progression after radical cystectomy is an important goal, molecular biomarkers also have the potential to identify which therapies might be most beneficial for an individual patient. For example, in breast cancer, hormone receptor status has been guiding therapy for years. In BCa, potential biomarkers predicting response to chemotherapy protocols include growth factors and their receptors (EGFR [epidermal growth factor receptor], bFGF [basic fibroblast growth factor] and VEGF [vascular endothelial growth factor]), apoptotic markers (Bcl-2, EMMPRIN and survivin), the nucleotide excision repair system, and p53. Gefitinib, which targets EGFR, has yielded promising results in preclinical *in vitro* and subcutaneous *in vivo* models of BCa.

Patients with advanced disease were screened for erbB-2 overexpression, and combination therapy, consisting of trastuzumab, paclitaxel, carboplatin, and gemcitabine, was applied to 44 of 57 erbB-2-positive patients [69].

A great improvement in the treatment of advanced renal cell carcinoma has been achieved by using the multi-targeted agents sorafenib and sunitinib. After demonstration of efficacy in preclinical investigations these tyrosine kinase inhibitors are currently being investigated in therapeutic clinical trials of BCa.

## CONCLUSIONS

The main challenge for researchers investigating molecular biomarkers in BCa is to translate acquired knowledge into clinical practice as quickly as possible. Standardization of methods, both technological and statistical, is required to enable assessment of the accuracy of prognostic marker panels. Such standardization should include producing a universal definition of test positivity and a clear definition of clinical endpoints. BCa is a heterogeneous disease, reflecting multiple and complex molecular alterations during tumorigenesis and progression. Current staging systems have a limited capacity to predict BCa recurrence, progression and disease-specific survival. An individualized approach to treatment, taking into account molecular information, is likely to improve patient outcomes. After almost two decades of intensive research the field of molecular biology, no molecular biomarker has successfully entered the arena of clinical BCa management. An encouraging number of candidate tissue biomarkers for BCa are available, however, including cell-cycle regulators, apoptosis markers, extracellular-matrix modulating molecules, and factors promoting tumor cell growth and angiogenesis. Many of these molecules have been demonstrated to be predictive of oncologic endpoints in patients with BCa after treatment with curative intent. Now, attention should focus on the few candidate biomarkers that have shown a gain in predictive accuracy when used alongside conventional clinical and pathological parameters. Prospective trials that evaluate neoadjuvant and adjuvant chemotherapy, as well as targeted therapy protocols in advanced cases and surveillance of NMIBC patients must be designed to validate the promising data on molecular biomarkers for BCa. Inadequate sensitivity may result in undiagnosed tumors and impaired cancer control, particularly in patients at high risk for progression. Poor specificity may translate into unnecessary anxiety in patients and physicians, and it may prompt unwarranted assessment.

Apart from oncological endpoints, patients' related concerns in the trials evaluating biomarkers should also be analyzed. According to recently published data, 200 consecutive patients previously diagnosed with NMIBC who were undergoing outpatient flexible cystoscopy as follow-up were interviewed. Of them, 75% would accept the results of a urine test as a replacement for cystoscopy only if it was capable of detecting more than 95% of recurrent bladder tumors and an additional 21% would accept it if the urine test was at least 90-95% accurate [70]. The majority of patients undergoing routine cystoscopic surveillance would not compromise the diagnostic accuracy afforded by cystoscopy for the benefit of using a noninvasive assay and the choice was mainly driven by concern related to false-negative results. Understanding that flexible cystoscopy is well tolerated by the majority of patients, future clinical trials on biomarkers should focus not only on oncological and budgetary considerations, but also on patient related concerns.

## REFERENCES


1. Jemal A, Siegel R, Ward E et al: *Cancer statistics, 2008.* CA Cancer J Clin 2008; 58: 71-96.
2. Sylvester RJ, van der Meijden AP, Oosterlinck W et al: *Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials.* Eur Urol 2006; 49: 466-475.
3. Mitra AP, Datar RH, Cote RJ: *Molecular Pathways in Invasive Bladder Cancer: New Insights Into Mechanisms, Progression, and Target Identification.* JCO 2006; 10: 5552-5564.
4. Shariat SF, Karakiewicz PI, Palapattu GS et al: *Outcomes of radical cystectomy for transitional cell carcinoma of the bladder: a contemporary series from the Bladder Cancer Research Consortium.* J Urol 2006; 176: 2414-2422.
5. Bochner BH, Kattan MW, Vora KC: *Postoperative nomogram predicting risk of recurrence after radical cystectomy for bladder cancer.* J Clin Oncol 2006; 24: 3967-3972.
6. Botteman MF, Pashos CL, Redaelli A et al: *The health economics of bladder cancer: a comprehensive review of the published literature.* Pharmacoeconomics 2003; 21: 1315-1330.
7. Grossman HB, Soloway M, Messing E et al: *Surveillance for recurrent bladder cancer using a point-of-care proteomic assay.* JAMA 2006; 295: 299-305.
8. Nam RK, Redelmeier DA, Spiess PE et al: *Comparison of molecular and conventional strategies for followup of superficial bladder cancer using decision analysis.* J Urol 2000; 163: 752-757.
9. *Biomarkers Definitions Working Group: Biomarkers and surrogate endpoints: preferred definitions and conceptual framework.* Clin Pharmacol Ther 2001; 69: 89-95.
10. Bolenz C, Lotan Y: *Molecular biomarkers for urothelial carcinoma of the bladder: challenges in clinical use.* Nat Clin Pract Urol 2008; 5: 676-685.
11. Rieger-Christ KM, Mourtzinos A, Lee PJ et al: *Identification of fibroblast growth factor receptor3 mutations in urine sediment DNA samples complements cytology in bladder tumor detection.* Cancer 2003; 98: 737-744.
12. van Rhijn BW, van der Kwast TH, Vis AN et al: *FGFR3 and P53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma.* Cancer Res 2004; 64: 1911-1914.
13. Bakkar AA, Wallerand H, Radvanyi F et al: *FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder.* Cancer Res 2003; 63: 8108-8112.
14. Hernández S, López-Knowles E, Lloreta J et al: *FGFR3 and Tp53 mutations in T1G3 transitional bladder carcinomas: independent distribution and lack of association with prognosis.* Clin Cancer Res 2005; 11: 5444-5450.

15. Ehdaie B, Theodorescu D: *Predicting tumor outcomes in urothelial bladder carcinoma: turning pathways into clinical biomarkers of prognosis*. Expert Rev Anticancer Ther 2008; 8: 1103-1110.
16. Zhang ZT, Pak J, Huang HY et al: *Role of Ha-ras activation in superficial papillary pathway of urothelial tumor formation*. Oncogene 2001; 20: 1973-1980.
17. Yurakh AO, Ramos D, Calabuig-Fariñas S et al: *Molecular and immunohistochemical analysis of the prognostic value of cell-cycle regulators in urothelial neoplasms of the bladder*. Eur Urol 2006; 50: 506-515.
18. Lambl VD: *Über Harnbalsenkrebs. Ein Beitrag zur mikroskopischen Diagnostik am Krankenbe*tte. Vierteljahrschr Prakt Heilk 1856; 49: 1-32.
19. Papanicolau GN, Marshall VP: *Urine sediment smears as a distinct diagnostic procedure in cancers of the urinary trac*t. Science 1945; 101: 519-520.
20. Halachmi S, Linn JF, Amiel GE et al: *Urine cytology, tumour markers and bladder cancer*. Br J Urol 1998; 82: 647-654.
21. Landman J, Chang Y, Kavaler E et al: *Sensitivity and specificity of NMP-22, telomerase and BTA in the detection of human bladder cancer*. Urology 1998; 52: 398-402.
22. Wiener HG, Mian C, Haitel A et al: *Can urine bound diagnostic tests replace cystoscopy in the management of bladder cancer?* J Urol 1998; 159: 1876-1880.
23. Johnston B, Morales A, Emerson L et al: *Rapid detection of bladder cancer. A comparative study of point of care tests*. J Urol 1997; 158: 2098-2101.
24. Leyh H, Hall R, Mazeman E: *Wertigkeit von BTA-Test und Urincytologie bei der Diagnostik und Nachsorge des Harnblasenkarcinoms*. Akt Urol 1998; 29 (S): 57-61.
25. Ramakumar S, Bhutyan J, Besse C et al: *Comparison of screening methods in the detection of bladder cancer*. J Urol 1999; 161: 388-394.
26. Pode D, Shapiro A, Wald M et al: *Noninvasive detection of bladder cancer with the BTA stat test*. J Urol 1999; 161: 443-446.
27. Raitanen M-P, Aine R, Rintala E et al: *Differences between local and review urinary cytology and diagnosis of bladder cancer. An interobserver multicenter ana*lysis. Eur Urol 2002; 41: 284-289.
28. Mian C, Pycha A, Wiener H et al: *ImmunoCyt: a new tool for detecting transitional cell cancer of the urinary trac*t. J Urol 1999; 181: 1486-1489.
29. Pfister C, Chautard D, Devonec M et al: *Immunocyt test improves the diagnostic accuracy of urinary cytology. Results of French multicenter study*. J Urol 2003; 169: 921-924.
30. Giannopoulos A, Manousakas T, Gounari A et al: *Comparative evaluation of the diagnostic performance of the BTA stat test, NMP22 and urinary bladder cancer antigen for primary and recurrent bladder tumors*. J Urol 2001; 166: 470-475.
31. Boman H, Hedelin H, Holmang S: *For bladder tumor markers have a disappointingly low sensitivity for small size and low grade recurrence*. J Urol 2002; 167: 80-83.
32. Sarosdy MF, Hudson MA, Ellis WJ et al: *Improved detection of recurrent bladder cancer using the Bard BTA stat test*. Urology 1997; 50: 349-353.
33. Sharma S, Zippe CD, Pandrangi L et al: *Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat*. J Urol 1999; 162: 53-57.
34. Leyh H, Marberger M, Conort P et al: *Comparison of the BTA stat test with voided urine cytology and bladder wash cytology in the diagnosis and monitoring of bladder cancer*. Eur Urol 1999; 35: 52-56.
35. Irani J, Desgrandchamps F, Millet C et al: *BTA stat and BTA TRAK: A comparative evaluation of urine testing for the diagnosis of transitional cell carcinoma of the bladder*. Eur Urol 1999; 35: 89-92.
36. Mian C, Lodde M, Haitel A et al: *Comparison of quantitative assays the UBC Rapid test and the BTA stat test in the diagnosis of urothelial cell carcinoma of the bladder*. Urology 2000; 38: 228-231.
37. Serreta V, Pomara G, Rizzo I et al: *Urinary BTA-stat, BTA-Trak and NMP22 in surveillance after TUR of recurrent superficial transitional cell carcinoma of the bladder*. Eur Urol 2000; 38: 419-425.
38. Heicapell R, MÜller M, Fimmers R et al: *Quantitative determination of urinary human complement factor H-related protein (hcfHrp) in patients with bladder cancer, healthy controls, and patients with benign urologic disease*. Urol Int 2000; 27: 181-184.
39. Taitanen M-P, Marttila M, Nurmi M et al: *Human complement factor H related protein test for monitoring bladder cancer*. J Urol 2001; 165: 374-377.
40. Banos JLG, Rodrogo MHR, Juarez FMA et al: *Usefulness of the BTA stat test for the diagnisis of bladder cancer*. Urology 2001; 57: 685-689.
41. Babjuk M, Koštiurova M, Mudra K et al: *Qualitative and quantitative detection of urinary human complement factor H-related protein (BTA stat and BTA TRAK) and fragments of cytokeratins 8, 18 (UBC Rapid and UBC IRMA) as markers for transitional cell carcinoma of the bladder*. Eur Urol 2002; 41: 34-39.
42. Raitanen MP; FinnBladder Group: *The role of BTA stat Test in follow-up of patients with bladder cancer: results from FinnBladder studies. Raitanen MP; FinnBladder Group*. World J Urol 2008; 26: 45-50.
43. Soloway MS, Bringgman JV, Carpinto GA et al: *Use of a new tumor marker, urinary NMP 22, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment*. J Urol 1996; 156: 3643-367.
44. Miyanga N, Akaza H, Ishikawa S et al: *Clinical evaluation of nuclear matrix protein 22 (NMP 22) in urine as a novel marker for urothelial can*cer. Eur Urol 1997; 31: 163-168.
45. Landman J, Chang Y, Kavaler E et al: *Sensitivity and specificity of NMP-22, telomerase and BTA in the detection of human bladder cancer*. Urology 1998; 52: 398-402.
46. Serretta V, Lo Presti D, Vasile P et al: *Urinary NMP22 for the detection of recurrence often transurethral resection of transitional cell carcinoma of the bladder. Experience on 137 patients*. Urology 1998; 52: 793-796.
47. Witjes JA, van der Poel HG, Balken MR et al: *Urinary NMP-22 and karyometry in the diagnosis and follow-up of patients with superficial bladder cancer*. Eur Urol 1998; 33: 387-391.
48. Sanchez-Carbayo M, Herrero E, Megias J et al: *Comparative sensitivity of urinary Cyfra 21-1, urinary bladder cancer antigen, tissue polypeptide antigen and NMP22 to detect bladder cancer*. J Urol 1999; 162: 1951-1956.
49. Zippe C, Pandrangi L, Agarwal A: *NMP22 is a sensitive, cost effective test in patients at risk for bladder cancer*. J Urol 1999; 161: 62-65.
50. Mian C, Lodde M, Haitel A et al: *Comparison of the monoclonal UBC-ELISA test and the NMP22 ELISA test for the detection of urothelial carcinoma of the bladder*. Urology 2000; 55: 223-226.
51. Ghahal R, Darshane A, Browning AJ et al: *Evaluation of the clinical value of urinary NMP22 as a marker in the screening and surveillance of transitional cell carcinoma of the urinary bl*adder. Eur Urol 2001; 40: 415-421.
52. Svatek RS, Herman MP, Lotan Y et al: *Soluble Fas--a promising novel urinary marker for the detection of recurrent superficial bladder cancer*. Cancer 2006; 106: 1701-1707.
53. Eissa S, Ali-Labib R, Swellam M et al: *Noninvasive diagnosis of bladder cancer by detection of matrix metalloproteinases (MMP-2 and MMP-9) and their inhibitor (TIMP-2) in urine*. Eur Urol 2007; 52: 1388-1396.
54. Eissa S, Swellam M, Ali-Labib R et al: *Detection of telomerase in urine by 3 methods: evaluation of diagnostic accuracy for bladder cancer*. J Urol 2007; 178: 1068-1072.
55. Sanchini MA, Gunelli R, Nanni O et al: *Relevance of urine telomerase in the diagnosis of bladder cancer*. JAMA 2005; 294: 2052-2056.
56. Bravaccini S, Sanchini MA, Granato AM et al: *Urine telomerase activity for the detection of bladder cancer in females*. J Urol 2007; 178: 57-61.
57. von Knobloch R, Hegele A, Brandt H et al: *Serum DNA and urine DNA alterations of urinary transitional cell bladder carcinoma detected by fluorescent microsatellite analysis*. Int J Cancer 2001; 94: 67-72.
58. Margulis V, Shariat SF, Ashfaq R et al: *Ki-67 is an independent predictor of bladder cancer outcome in patients treated with radical cystectomy for organ-confined disease*. Clin Cancer Res 2006; 15: 7369-7373.
59. Shariat SF, Tokunaga H, Zhou J et al: *p53, p21, pRB, and p16 expression predict clinical outcome in cystectomy with bladder cancer*. J Clin Oncol 2004; 22: 1014-1024.

60. Chatterjee SJ, Datar R, Youssefzadeh D et al: *Combined effects of p53, p21, and pRb expression in the progression of bladder transitional cell carcinoma*. J Clin Oncol 2004; 22: 1007-1013.
61. George B, Datar RH, Wu L et al: *p53 Gene and Protein Status: The Role of p53 Alterations in Predicting Outcome in Patients With Bladder Cancer*. J Clin Oncol 2007; 1: 5352-5358.
62. Dalbagni G, Parekh DJ, Ben-Porat L et al: *Prospective evaluation of p53 as a prognostic marker in T1 transitional cell carcinoma of the bladder*. BJU Int 2007; 99: 281-285.
63. Grossman HB, Tangen CM, Cordon-Cardo C et al: *Evaluation of Ki67, p53 and angiogenesis in patients enrolled in a randomized study of neoadjuvant chemotherapy with or without cystectomy: a Southwest Oncology Group Study*. Oncol Rep 2006: 16: 807-810.
64. Malats N, Bustos A, Nascimento CM et al: *P53 as a prognostic marker for bladder cancer: a meta-analysis and review*. Lancet Oncol 2005; 6: 678-686.
65. Shariat SF et al: *Association of cyclin D1 and E1 expression with disease progression and biomarkers in patients with non-muscle-invasive urothelial cell carcinoma of the bladder*. Urol Oncol 2007; 25: 468-475.
66. Karam JA et al: *Use of combined apoptosis biomarkers for prediction of bladder cancer recurrence and mortality after radical cystectomy*. Lancet Oncol 2007; 8: 128-136.
67. Shariat SF et al: *Predictive value of cell cycle biomarkers in non-muscle invasive bladder transitional cell carcinoma*. J Urol 2007; 177: 481-487.
68. Shariat SF, Karakiewicz PI, Ashfaq R et al: *Multiple biomarkers improve prediction of bladder cancer recurrence and mortality in patients undergoing cystectomy*. Cancer 2008; 112: 315-325.
69. Hussain MH, MacVicar GR, Petrylak DP et al: *Trastuzumab, paclitaxel, carboplatin, and gemcitabine in advanced human epidermal growth factor receptor-2/neu-positive urothelial carcinoma: results of a multicenter phase II National Cancer Institute trial*. J Clin Oncol 2007; 25: 2218-2224.
70. Yossepowitch O, Herr HW, Donat SM: *Use of urinary biomarkers for bladder cancer surveillance: patient perspectives*. J Urol 2007; 17: 1277-1282.

**Correspondence**

Jakub Dobruch
Interforest Specialist Hospital
Department of Urology
2, Bursztynowa Street
04-749 Warsaw
phone: +48 503 072 230
kubadobr@wp.pl