ORIGINAL ARTICLE – CLINICAL ONCOLOGY

CrossMark

No influence of smoking status on the performance of urine markers for the detection of bladder cancer

Susanne Deininger[1] · J. Hennenlotter[1] · S. Rausch[1] · K. Docktor[1] · E. Neumann[1] · I. A. da Costa[1] · J. Bedke[1] ·
A. Stenzl[1] · T. Todenhöfer[1]

Abstract

Purpose The performance of urinary markers for detecting bladder cancer (BC) is influenced by various factors. The aim of the present study was to evaluate the influence of smoking habits on the performance of four commonly used urine markers.

Methods Urine samples of 723 patients with suspected BC were analysed using urine cytology, fluorescence in situ hybridization (FISH), immunocytology (uCyt+ test), and quantitative nuclear matrix protein 22 (NMP22) immunoassay. The smoking habits of all patients were recorded and a cystoscopy performed within 2 weeks after urinary marker testing. Rates of false negative and false positive results were compared between non-smokers, former smokers, and current smokers by contingency analyses.

Results We included 723 patients in this study, 431 (59.6%) of which were non-smokers, 215 former smokers (29.7%), and 77 (10.7%) current smokers. 148 patients (20.5%) had a tumour at the time of urinary marker testing. Respective rates of false positive test results among non-smokers, former smokers, and current smokers were: 16.3, 19.1, and 11.5% ($p = 0.81$) for urine cytology; 36.8, 42.0, and 32.7% for the uCyt+ test ($p = 0.88$); 18.0, 19.1, and 13.5% for FISH ($p = 0.66$); and 69.5, 71.6, and 71.2% for NMP22 ($p = 0.67$). Respective rates of false negatives among non-smokers, former smokers, and current smokers were: 31.4, 15.1, and 28.0% for cytology ($p = 0.34$); 21.4, 22.6, and 16.0% for uCyt+ test ($p = 0.67$); 24.3, 13.2, and 28.0% for FISH ($p = 0.88$); and 10.0, 18.9, and 8.0% for NMP22 ($p = 0.80$).

Conclusions Our results strongly suggest that smoking habits do not affect performance characteristics of urinary markers in the diagnostics of BC.

Introduction

While cystoscopy remains the gold standard for the diagnosis and surveillance of bladder cancer (BC) (Babjuk et al. 2011), less invasive and discomforting alternatives such as urine marker tests continue to be studied. From among the established urine marker tests, urine cytology is currently the only urinary marker being recommended by the European Association of Urology (EAU) guidelines, to be used in combination with cystoscopy for the detection of high grade tumours and carcinoma in situ (Babjuk et al. 2017). The test consists of the microscopic analysis of urothelial cells in urine for criteria of malignancy and while it has been shown to have a high specificity, its sensitivity, in particular for the detection of low-grade tumours, is rather low (Schroeder et al. 2004). To remedy this sensitivity issue, additional molecular tests such as immunocytology (uCyt+ test) have been introduced. The uCyt+ test is based on the immunocytical staining of two antigens that are typically expressed by urothelial cancer cells (mucin glycoprotein and carcinoembryonic antigen) (Comploj et al. 2013). Another well-established urine marker test is UroVysion®, where a marker detects specific, frequently appearing chromosomal aberrations associated with the presence of urothelial carcinoma by fluorescence in situ hybridization (FISH) (Bubendorf 2011). The Nuclear Matrix Protein 22 (NMP22) test

✉ Susanne Deininger
   susanne.deininger@mail.de

[1] Department of Urology, Eberhard-Karls-University,
    Tübingen, Germany

is an Enzyme-linked immune sorbet assay, marking the NUMA-1-protein (nuclear mitotic apparatus protein 1) in the patient's urine.

Several studies described the interaction between certain influencing factors with urine marker test results, including hematuria, which can increase false positive results of molecular tests such as NMP22 (Atsu et al. 2002; Oge et al. 2002; Todenhofer et al. 2013). Other interfering factors are urinary tract infections, inflammation (Atsu et al. 2002), instrumented urinary sampling (Sharma et al. 1999; Todenhofer et al. 2012), and age (Horstmann et al. 2013).

One of the main risk factors for the development of BC is smoking, which is associated with 50% of bladder cancer cases (Burger et al. 2013; Freedman et al. 2011). This association is thought to be triggered by dimethylnitrosamine (DMN), formaldehyde, benzo(a)pyrene, and other contents defined in the list of hazardous tobacco smoke components (Talhout et al. 2011).

Despite the strong influence of smoking on the development of BC, the influence of this risk factor on the performance of cellular and protein-based urinary markers in BC diagnostics has not yet been examined. The aim of our study was thus to evaluate the effect of smoking habits on the accuracy of the four commonly used urinary markers mentioned above: cytology, fluorescence in situ hybridization (FISH), immunocytology (uCyt+ test), and quantitative nuclear matrix protein 22 (NMP22) immunoassays.

Materials and methods

Patient cohort

The study is a retrospective data analysis of medical and pathological reports of 723 patients presenting to our outpatient clinic on suspicion of bladder cancer. During the initial presentation, all patients were routinely interviewed for their smoking habits and classified into three categories: non-smoker, current smoker, former smoker. Four commonly used urinary markers for the detection of bladder cancer (BC) were evaluated: urine cytology, uCyt+ test, FISH, and NMP22. The patients underwent cystoscopy by an experienced investigator within 2 weeks after urine marker testing. Patients presenting suspicious cystoscopy results were evaluated by transurethral resection and subsequent histological work-up. The study was approved by the ethics committee of the University hospital Tübingen (number 400/2009 A).

Urine marker tests

For urine cytology, specimens were cytospinned, stained (Papanicolaou and Marshall 1945), and evaluated by a cytopathologist according to the WHO/ISUP classification system 2004 (Epstein et al. 2005) and the recommendations by the Papanicolaou Society of Cytopathology (Layfield et al. 2004). The following characteristics of urothelial cancer cells were considered: papillary clusters comprised of cells with eccentric nuclei, single cells with eccentric nuclei, increased nuclear-to-cytoplasmic ratio, cells with irregular nuclear borders, and cells with coarse chromatin. The FISH test (with cytospins) was performed as described previously (Riesz et al. 2007). The test was considered to have a positive outcome if either of the following two conditions occurred: (1) the presence of at least three signals from a minimum of two different chromosomes (from among chromosomes 3, 7, 17) per cell in at least four nuclei out of 25 morphological suspicious cells, or (2) the absence of a signal of 9p21 in at least 12 of 25 nuclei (Kipp et al. 2008). The uCyt+ test was performed according to the manufacturer's recommendations (Mian et al. 1999) and the test outcome was considered to be positive if at least one definitely positive red or green cell was present (Greene et al. 2006). Also the NMP22 Enzyme-Linked Immunosorbent Assay (ELISA) was performed according to the manufacturer's recommendations and a threshold of 10 IU/ml was used to separate the positive (greater than threshold) from the negative results (Shariat et al. 2006).

Statistical analysis

Commercially available software (JMP®, version 10.0, SAS Institute Inc., Cary, NC, USA) was used to calculate the overall sensitivities and specificities, as well as positive and negative predictive values (PPV and NPV) for non-smokers, former smokers, and current smokers. We also performed Cochran–Armitage tests for trend to compare the rates of false positives and false negatives between the three smoking categories. A p value ≤ 0.05 was considered as significant.

Results

A total number of 723 patients [636 males (88.0%) and 87 females (12.0%)] with a median age of 68 years (range 11–92) were retrospectively enrolled in the study. The smoking status was available for all patients yielding 431 (59.6%) non-smokers, 215 (29.7%) former smokers, and 77 (10.7%) current smokers. Cystoscopy and transurethral resection with subsequent histological evaluation revealed the presence of BC in 148 patients. Pathological staging classified 81 (54.7%) tumours as pTa, 33 (22.3%) as pT1, 19 (12.8%) patients as pT2, 4 patients (2.7%) as pT3a, and 7 patients (4.7%) as pure pTis. Thirty-one patients (20.9%) had concomitant pTis. The tumour grading was G1 in 59 patients (39.9%), G2 in 49 (33.1%), and G3 in 40 (27.0%) (Table 1).

Table 1 Patients' characteristics

Number of patients	723
Smoker status non/former/current	431 (59.6%)/215 (29.7%)/77 (10.7%)
Median age in years (range)	68 (11–92)
Gender, male/female	636 (88.0%)/87 (12.0%)
Histologically confirmed tumour	148 (20.5%)
Pathological staging	
pTa	81 (54.7%)
pT1	33 (22.3%)
pT2a	19 (12.8%)
pT2b	4 (2.7%)
pT3a	4 (2.7%)
Only pTis	7 (4.7%)
Concomitant pTis	31 (20.9%)
Grading	
G1	59 (39.9%)
G2	49 (33.1%)
G3	40 (27.0%)

Sensitivities, specificities, PPV and NPV according to smoking status are shown in Table 2.

There was no statistically significant difference between the rates of false positive urine tests between non-smokers, former smokers, and current smokers. Urine cytology yielded false positives for 16.3% of non-smokers, 19.1% of former smokers, and 11.5% of current smokers ($p = 0.81$), while for the uCyt+ test the corresponding percentages are 36.8, 42.0 and 32.7%, respectively ($p = 0.88$). False positives in the FISH test were 18.0% of non-smokers, 19.1%

of former smokers and 13.5% of current smokers ($p = 0.66$), while for NMP22 presented the highest overall percentages with 69.5, 71.6, and 71.2%, respectively ($p = 0.67$). With regard to false negatives, no significant difference was detected among the three smoker categories with any of the methods used. Specifically, in non-smokers, former smokers, and current smokers, the false negative rates of urine cytology, uCyt+ test, FISH and NMP22 were 31.4 vs. 15.1 vs. 28.0% ($p = 0.34$), 21.4 vs. 22.6 vs. 16.0% ($p = 0.67$), 24.3 vs. 13.2 vs. 28.0% ($p = 0.88$) and 10.0 vs. 18.9 vs. 8.0% ($p = 0.80$), respectively (Fig. 1).

Discussion

Smoking seems to cause a number of changes in the human metabolism: smokers typically exhibit increased levels of inflammatory markers like C-reactive protein, white blood cell count, and fibrinogen (Bakhru and Erlinger 2005; de Maat et al. 1996) and several lipid metabolites have also been shown to be associated with smoking (Wang-Sattler et al. 2008). Such alterations are not only present at a metabolic, but also a genetic level: allelic losses on chromosomes 3p, 9p, 17p, 5q, and 13q can be detected both in current and former smokers. In a study performed by Mao et al. (1997), the loss of heterozygosity at chromosome 3p14 appeared in 88% of bronchial biopsies of smokers and in 45% of former smokers. Wistuba et al. (1997) demonstrated that the percentage of allelic losses and microsatellite alterations in bronchial biopsies of smokers and former smokers showed no statistical difference between the two groups, but

Table 2 Sensitivities, specificities, PPV, NPV according to smoking status

Urine marker test	Smoking status	Sensitivity (%)	Specificity (%)	PPV	NPV
Urine cytology	OA	75.0	83.0	53.6	92.8
	NS	68.6	83.7	44.9	93.2
	FS	84.9	80.9	59.2	94.2
	CS	72.0	88.5	75.0	86.8
uCyt+/ImmunoCyt	OA	79.1	62.1	34.9	92.0
	NS	78.6	63.2	29.3	93.8
	FS	77.4	58.0	37.6	88.7
	CS	84.0	67.3	55.3	89.7
FISH	OA	79.1	82.1	53.2	93.8
	NS	75.7	82.0	44.9	94.6
	FS	86.8	80.9	59.7	94.9
	CS	72.0	86.5	72.0	86.5
NMP22	OA	87.2	29.7	24.2	90.0
	NS	90.0	30.5	20.1	94.0
	FS	81.1	28.4	27.0	82.1
	CS	92.0	28.9	38.3	88.2

OA overall, *NS* non-smoker, *FS* former smoker, *CS* current smoker, *FISH* fluorescent in situ hybridization, *NMP22* nuclear matrix protein 22
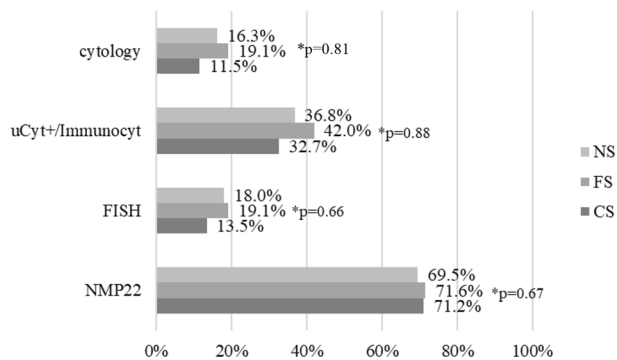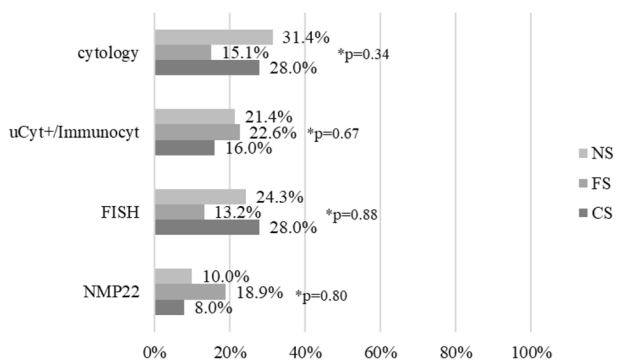
a rate of false positive test results

b rate of false negative test results

Fig. 1 Rate of false positive and false negative test results (*NS* non-smoker, *FS* former smoker, *CS* current smoker, *FISH* fluorescent in situ hybridization, *NMP22* nuclear matrix protein 22)

were considerably different compared to non-smokers. In a genetic analysis on bronchial brushing biopsies, Spira et al. (2004) found that smokers showed an increased gene expression to regulate inflammation (CYP1B1, DBDD and others) and a decreased expression of tumour suppressor genes (TU3A, SLIT1 and -2, and GAS6). While former smokers who had quit smoking less than 2 years ago showed genetic analysis results that were similar to current smokers, former smokers who had quit more than 2 years ago showed results that were more akin to those of non-smokers (Spira et al. 2004). These data indicate that former smokers might still suffer from smoking-related genetic alterations during a transitional time period of up to 2 years after quitting. To date, it is unclear whether currently available urine markers, either cellular or protein based, are able to detect premalignant alterations caused by smoking. Moreover, a potential influence of smoking on the performance of these markers is also unknown. The present study therefore aimed to compare the performance of four commonly available urine markers in a large cohort of patients with suspicion of BC. Our study showed that neither conventional urine cytology nor the uCyt+ test are influenced by smoking habits. Moreover,

smoking appeared to have no effect on levels of NMP22 or the amount of genetic alterations detected by the FISH test.

The detection of microscopic malignant features in urothelial cells by urine cytology shows a sensitivies of 16–84% depending on grading of the tumour (Yafi et al. 2015). In our study, the sensitivity among both, the entire study group (75.0%) and the group of former smokers (84.9%), was relatively high. Previous studies reported cytology specificities of 95–98.3% (Kehinde et al. 2011; Planz et al. 2005), which are superior compared to the present study. Our overall specificity was 83.0% with a maximum of 88.5% for current smokers. Blessing et al. (2016) showed that urine of smokers contained statistically increased concentrations of necrotic cells and clusters of cells showing dysplastic changes (enlargement in nuclear cytoplasm ratio and irregular nuclear border), as well as leucocytes and erythrocytes. This finding may lead to the assumption that the rate of false positive urine cytologies would be increased in smokers. Our results do not support this assumption as we found no statistically significant differences between the rates of false positives in non-smokers, former smokers, and current smokers.

uCyt+ is a microscopic test based on fluorescent staining of two antigens that are typically expressed by urothelial cancer cells (mucin glycoprotein in green and carcinoembryonic antigen in red) (Comploj et al. 2013). Literature reports of the sensitivity of the uCyt+ test vary from 74 to 85% (Tetu et al. 2005; Vergara-Lluri et al. 2014), which seems in good agreement with both our overall sensitivity of 79.1%, and also the maximum of 84.0% which we observed for current smokers. While the literature values on the specificity of the uCyt+ test are in the 31–62% range (Tetu et al. 2005; Vergara-Lluri et al. 2014), our study showed an overall specificity of 62.1% (with a maximum among current smokers of 67.3%). Sajid et al. (2007) reported a significant increase in blood levels of carcinoembryonic antigen (CEA) in smokers compared to non-smokers. An influence of smoking on the detection of CEA in urothelial cells could not be shown in our study as there were no statistically significant differences between the rates of false positive uCyt+ tests in non-smokers, former smokers, and current smokers.

The FISH test detects numeric chromosomal aberrations and gene translocations, deletions, and duplications via fluorescent DNA probes. It is generally used for genetic analyses, prenatal diagnostics, and for the detection and follow-up of BC (UroVysion®), where it has shown sensitivities of 69–84% and specificities of 84–91% (Sokolova et al. 2000; Zeng and Zhou 2010). In our study, we observed a similar performance with an overall sensitivity of 79.1% and a maximum of 86.8% for the group of former smokers. The overall specificity was 82.1% with the maximum of 86.6% occurring in the group of current smokers. The FISH test identifies aneuploidy in

chromosomes 3, 7, and 17 and the loss of the 9p21 locus (Sokolova et al. 2000). Studies have shown how smoking can cause allelic losses of chromosomes 3p, 9p, 17p, 5q and 13q (Mao et al. 1997; Zhang et al. 1997). Souto et al. (2010) showed that the oral epithelium cells of smokers had a statistically higher percentage of aneuploidy compared to non-smokers ($p < 0.05$). Furthermore, smoking can interfere with spermatogenesis: Pereira et al. (2014) showed that unlike non-smokers, smokers suffered from increased chromosomal segregation anomalies in spermatogenesis and had elevated rates of disomies of chromosome 3 in sperm. Other studies reported aneuploidy of chromosomes X, Y, 8 (Rubes et al. 1998) and 18 (Robbins et al. 1997) in the sperm of smokers. These data indicate that smoking could interfere with the results of the FISH test. However, our study showed no such interference as there was no statistical difference in the rate of false positive test results in FISH between the study groups.

Data from Kipp et al. (2009) seem to indicate the possibility of anticipatory positive FISH tests in patients with recurrent urothelial cancer: in patients without macroscopic tumour at time of urine marker testing the percentage of suspicious cells correlated negatively with the time to tumour recurrence and to progression to muscle invasive tumour ($p < 0.001$). Since our study was a retrospective data analysis with no standardized follow-up program for the included patients, we have no information about the medical history following the urine marker test. We therefore cannot make any statements as to whether some of the patients with positive FISH test, but without macroscopic tumour at the time of urine maker testing, developed BC at a later date.

The NMP22 test is an enzyme immunoassay marking the NUMA-1- protein in patient's urine. NUMA-1 seems to be essential for the organization and stabilization of spindle poles in cell division (Zeng 2000) and is excreted via urine. The test has shown sensitivities of 26–56% and specificities of 33–86% in the diagnosis of BC (Grossman 2005; Lotan et al. 2017). Our study showed an overall sensitivity of 87.2% and a maximum sensitivity 92.0% for the group of current smokers, whereas overall specificity was 29.7% (with a maximum specificity of 30.5% for the group of non-smokers). Lotan et al. (2008) showed that among men, smoking as a risk factor was correlated to a 35.4% increase of the PPV value of NMP22. In our study, the PPV of NMP22 was 20.1% in the group of non-smokers, compared to 38.3% among smokers, which represents a relative increase of 91.1% in PPV. Nuclear matrix protein as indicator for apoptosis can also be found in the saliva. Imirzalioglu et al. (2005) demonstrated a statistically significant difference between the levels of NMP in the saliva of smokers and non-smokers which could lead to the assumption that the excretion of nuclear matrix protein in smokers can also be increased in urine and therefore lead to increased rates of false positives for the NMP22 test. This was not the case in our study.

The present study is limited by its retrospective character. No information was available with regard to the duration of active, past or non-smoking for any of the patients. Furthermore, no standardized follow-up analysis of the patients was conducted after the urine marker testing which would be required to examine the potential role of anticipatory positive test results.

Conclusions

The results of our study indicate that smoking status has no effect on the accuracy of four commonly used urine marker tests and has therefore no bearing on test interpretation. Nevertheless, smoking, as one of the main risk factors for the development of BC, needs to be considered when estimating the individual BC risk for patients with BC-related findings such as hematuria.

Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. In the study a retrospective chart review but no specific study-related investigations were performed in the cohort. Therefore, in accordance with the ethics committee no informed consent was obtained in the framework of the study.

Conflict of interest No conflict of interest.

References

Atsu N, Ekici S, Oge OO, Ergen A, Hascelik G, Ozen H (2002) False-positive results of the NMP22 test due to hematuria. J Urol 167(2 Pt 1):555–558

Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Bohle A, Palou-Redorta J, Roupret M (2011) EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder, the 2011 update. Eur Urol. https://doi.org/10.1016/j.eururo.2011.03.017

Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Zigeuner R (2017) EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol 71(3):447–461. https://doi.org/10.1016/j.eururo.2016.05.041

Bakhru A, Erlinger TP (2005) Smoking cessation and cardiovascular disease risk factors: results from the Third National Health and Nutrition Examination Survey. PLoS Med 2(6):e160. https://doi.org/10.1371/journal.pmed.0020160

Bubendorf L (2011) Multiprobe fluorescence in situ hybridization (UroVysion) for the detection of urothelial carcinoma—FISHing for the right catch. Acta Cytol 55(2):113–119. https://doi.org/10.1159/000323652

Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Lotan Y (2013) Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 63(2):234–241. https://doi.org/10.1016/j.eururo.2012.07.033

Comploj E, Mian C, Ambrosini-Spaltro A, Dechet C, Palermo S, Trenti E, Pycha A (2013) uCyt+/ImmunoCyt and cytology in the detection of urothelial carcinoma: an update on 7422 analyses. Cancer Cytopathol 121(7):392–397. https://doi.org/10.1002/cncy.21287

Blessing A, Ikechi E, Ayobami F (2016) Cytology analysis of urine among cigarette smokers. Am J Biomed Sci 8(1):56–67

de Maat MP, Pietersma A, Kofflard M, Sluiter W, Kluft C (1996) Association of plasma fibrinogen levels with coronary artery disease, smoking and inflammatory markers. Atherosclerosis 121(2):185–191

Epstein JI, Allsbrook WC Jr, Amin MB, Egevad LL (2005) The 2005 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma. Am J Surg Pathol 29(9):1228–1242

Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC (2011) Association between smoking and risk of bladder cancer among men and women. JAMA 306(7):737–745. https://doi.org/10.1001/jama.2011.1142

Greene KL, Berry A, Konety BR (2006) Diagnostic utility of the ImmunoCyt/uCyt + test in bladder cancer. Rev Urol 8(4):190–197

Grossman HB (2005) Detection of bladder cancer using a point-of-care proteomic assay. JAMA 16(293)(7):810–816

Horstmann M, Todenhofer T, Hennenlotter J, Aufderklamm S, Mischinger J, Kuehs U, Schwentner C (2013) Influence of age on false positive rates of urine-based tumor markers. World J Urol 31(4):935–940. https://doi.org/10.1007/s00345-012-0906-1

Imirzalioglu P, Uckan S, Alaaddinoglu EE, Haberal A, Uckan D (2005) Cigarette smoking and apoptosis. J Periodontol 76(5):737–739. https://doi.org/10.1902/jop.2005.76.5.737

Kehinde EO, Al-Mulla F, Kapila K, Anim JT (2011) Comparison of the sensitivity and specificity of urine cytology, urinary nuclear matrix protein-22 and multitarget fluorescence in situ hybridization assay in the detection of bladder cancer. Scand J Urol Nephrol 45(2):113–121. https://doi.org/10.3109/00365599.2010.533694

Kipp BR, Tanasescu M, Else TA, Bryant SC, Karnes RJ, Sebo TJ, Halling KC (2009) Quantitative fluorescence in situ hybridization and its ability to predict bladder cancer recurrence and progression to muscle-invasive bladder cancer. J Mol Diagn 11(2):148–154. https://doi.org/10.2353/jmoldx.2009.080096

Kipp BR, Tyner HL, Campion MB, Voss JS, Karnes RJ, Sebo TJ, Zhang J (2008) Chromosomal alterations detected by fluorescence in situ hybridization in urothelial carcinoma and rarer histologic variants of bladder cancer. Am J Clin Pathol 130(4):552–559. https://doi.org/10.1309/DFJUHY3WPC9GUU2W

Layfield LJ, Elsheikh TM, Fili A, Nayar R, Shidham V (2004) Review of the state of the art and recommendations of the Papanicolaou Society of Cytopathology for urinary cytology procedures and reporting: the Papanicolaou Society of Cytopathology Practice Guidelines Task Force. Diagn Cytopathol 30(1):24–30. https://doi.org/10.1002/dc.10401

Lotan Y, O'Sullivan P, Raman JD, Shariat SF, Kavalieris L, Frampton C, Darling D (2017) Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. Urol Oncol. https://doi.org/10.1016/j.urolonc.2017.03.008

Lotan Y, Shariat SF, the NMP22 Study Group (2008) Impact of risk factors on the performance of the nuclear matrix protein 22 point-of-care test for bladder cancer detection. BJU Int 101(11):1362–1367. https://doi.org/10.1111/j.1464-410X.2008.07473.x

Mao L, Lee JS, Kurie JM, Fan YH, Lippman SM, Lee JJ, Hong WK (1997) Clonal genetic alterations in the lungs of current and former smokers. J Natl Cancer Inst 89(12):857–862

Mian C, Pycha A, Wiener H, Haitel A, Lodde M, Marberger M (1999) Immunocyt1: a new tool for detecting transitional cell cancer of the urinary tract. J Urol 161(5):1486–1489

Oge O, Kozaci D, Gemalmaz H (2002) The BTA stat test is nonspecific for hematuria: an experimental hematuria model. J Urol 167(3):1318–1319

Papanicolaou GN, Marshall VF (1945) Urine sediment smears as a diagnostic procedure in cancers of the urinary tract. Science 101(2629):519–520. https://doi.org/10.1126/science.101.2629.519

Pereira CS, Juchniuk de Vozzi MS, Dos Santos SA, Vasconcelos MA, de Paz CC, Squire JA, Martelli L (2014) Smoking-induced chromosomal segregation anomalies identified by FISH analysis of sperm. Mol Cytogenet 7(1):58. https://doi.org/10.1186/s13039-014-0058-7

Planz B, Jochims E, Deix T, Caspers HP, Jakse G, Boecking A (2005) The role of urinary cytology for detection of bladder cancer. Eur J Surg Oncol 31(3):304–308. https://doi.org/10.1016/j.ejso.2004.12.008

Riesz P, Lotz G, Paska C, Szendroi A, Majoros A, Nemeth Z, Kiss A (2007) Detection of bladder cancer from the urine using fluorescence in situ hybridization technique. Pathol Oncol Res 13(3):187–194. https://doi.org/10.1007/BF02893498

Robbins WA, Vine MF, Truong KY, Everson RB (1997) Use of fluorescence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men. Environ Mol Mutagen 30(2):175–183

Rubes J, Lowe X, Moore D 2nd, Perreault S, Slott V, Evenson D, Wyrobek AJ (1998) Smoking cigarettes is associated with increased sperm disomy in teenage men. Fertil Steril 70(4):715–723

Sajid KM, Parveen R, Durr-e S, Chaouachi K, Naeem A, Mahmood R, Shamim R (2007) Carcinoembryonic antigen (CEA) levels in hookah smokers, cigarette smokers and non-smokers. J Pak Med Assoc 57(12):595–599

Schroeder GL, Lorenzo-Gomez MF, Hautmann SH, Friedrich MG, Ekici S, Huland H, Lokeshwar V (2004) A side by side comparison of cytology and biomarkers for bladder cancer detection. J Urol 172(3):1123–1126. https://doi.org/10.1097/01.ju.0000134347.14643.ab

Shariat SF, Marberger MJ, Lotan Y, Sanchez-Carbayo M, Zippe C, Ludecke G, Karakiewicz PI (2006) Variability in the performance of nuclear matrix protein 22 for the detection of bladder cancer. J Urol 176(3):919–926. https://doi.org/10.1016/j.juro.2006.04.017

Sharma S, Zippe CD, Pandrangi L, Nelson D, Agarwal A (1999) Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. J Urol 162(1):53–57

Sokolova IA, Halling KC, Jenkins RB, Burkhardt HM, Meyer RG, Seelig SA, King W (2000) The development of a multitarget, multicolor fluorescence in situ hybridization assay for the detection of urothelial carcinoma in urine. J Mol Diagn 2(3):116–123. https://doi.org/10.1016/S1525-1578(10)60625-3

Souto GR, Caliari MV, Lins CE, de Aguiar MC, de Abreu MH, Mesquita RA (2010) Tobacco use increase the number of aneuploid nuclei in the clinically healthy oral epithelium. J Oral Pathol Med 39(8):605–610. https://doi.org/10.1111/j.1600-0714.2010.00907.x

Spira A, Beane J, Shah V, Liu G, Schembri F, Yang X, Brody JS (2004) Effects of cigarette smoke on the human airway epithelial cell transcriptome. Proc Natl Acad Sci USA 101(27):10143–10148. https://doi.org/10.1073/pnas.0401422101

Talhout R, Schulz T, Florek E, van Benthem J, Wester P, Opperhuizen A (2011) Hazardous compounds in tobacco smoke. Int J Environ Res Public Health 8(2):613–628. https://doi.org/10.3390/ijerph8020613

Tetu B, Tiguert R, Harel F, Fradet Y (2005) ImmunoCyt/uCyt+ improves the sensitivity of urine cytology in patients followed

for urothelial carcinoma. Mod Pathol 18(1):83–89. https://doi.org/10.1038/modpathol.3800262

Todenhofer T, Hennenlotter J, Kuhs U, Tews V, Gakis G, Aufderklamm S, Schwentner C (2012) Influence of urinary tract instrumentation and inflammation on the performance of urine markers for the detection of bladder cancer. Urology 79(3):620–624. https://doi.org/10.1016/j.urology.2011.10.067

Todenhofer T, Hennenlotter J, Tews V, Gakis G, Aufderklamm S, Kuehs U, Schwentner C (2013) Impact of different grades of microscopic hematuria on the performance of urine-based markers for the detection of urothelial carcinoma. Urol Oncol 31(7):1148–1154. https://doi.org/10.1016/j.urolonc.2011.10.011

Vergara-Lluri ME, Hu E, Rao JY, Levin M, Apple SK, Moatamed NA (2014) Comparative evaluation of ProEx C and ImmunoCyt/uCyt assays in atypical urine cytology. Arch Pathol Lab Med 138(9):1215–1222. https://doi.org/10.5858/arpa.2013-0433-OA

Wang-Sattler R, Yu Y, Mittelstrass K, Lattka E, Altmaier E, Gieger C, Illig T (2008) Metabolic profiling reveals distinct variations linked to nicotine consumption in humans—first results from the KORA study. PLoS One 3(12):e3863. https://doi.org/10.1371/journal.pone.0003863

Wistuba II, Lam S, Behrens C, Virmani AK, Fong KM, LeRiche J, Samet JM, Srivastava S, Minna JD, Gazdar AF (1997) Molecular damage in the bronchial epithelium of current and former smokers. J Natl Cancer Inst 89(18):1366–1373

Yafi FA, Brimo F, Steinberg J, Aprikian AG, Tanguay S, Kassouf W (2015) Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. Urol Oncol 33(2):66 e25–66 e31. https://doi.org/10.1016/j.urolonc.2014.06.008

Zeng C (2000). NuMA: a nuclear protein involved in mitotic centrosome function. Microsc Res Tech 49(5):467–477. https://doi.org/10.1002/(SICI)1097-0029(20000601)49:5<467::AID-JEMT9>3.0.CO;2-V

Zeng Z, Zhou XJ (2010) Detection of bladder cancer by FISH test: a meta-analysis. Zhonghua Bing Li Xue Za Zhi 39(2):75–78

Zhang ZF, Shu XM, Cordon-Cardo C, Orlow I, Lu ML, Millon TV, Scher H (1997) Cigarette smoking and chromosome 9 alterations in bladder cancer. Cancer Epidemiol Biomark Prev 6(5):321–326