RESEARCH ARTICLE

Diagnostic Role of Survivin in Urinary Bladder Cancer

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Abstract

Background: Early diagnosis of carcinoma of bladder remains a challenge. Survivin, a member of the inhibitor of apoptosis (IAP) protein family, is frequently activated in bladder carcinoma. The objective of this study was to investigate urinary survivin as a marker for diagnosis of urinary bladder. Materials and Methods: We examined urinary survivin concentration in 28 healthy individuals, 46 positive controls and 117 cases of histologically proven TCC prior to transurethral resection, using ELISA, and compared values with findings for urinary cytology. Results: Survivin was found to be significantly higher in the cancer group (P<0.05). A cut off value of 17.7 pg/ml was proposed, with an approximate sensitivity of 82.9% and specificity of 81.1% (P<0.0001), whereas urine cytology had a sensitivity of 66.7% and a specificity of 96.0%. Conclusions: Urinary survivin can be used as a non-invasive diagnostic biomarker for TCC bladder, both for primary and recurrent disease.

Keywords: Survivin - urinary bladder cancer - urinary biomarker - diagnosis

Asian Pacific J Cancer Prev, **14 (1)**, 81-85

Introduction

The American Cancer Society reported that about 69,250 new cases of urinary bladder cancer (52,020 men and 17,230 women) were diagnosed whereas 14,990 cases died (10,670 men and 4,320 women) in 2011 (Seigel, 2011). Although bladder cancer ranks lower in total number of cancer-related deaths than it does in incidence, it has the eminence of being the malignancy with the fastest rate of recurrence which results in a very high prevalence of tumors all over the world (Agarwal, 2008).Numerous institutions routinely use screening cystoscopy, urinary cytology and random bladder biopsies in an attempt for initial diagnosis of transitional cell carcinoma (TCC). It is well known that, painless hematuria, the most prevalent symptom, is found in only 4-10% of cases of bladder cancer (Grossfeld and Carroll, 1998). However, microhematuria is undetectable by simple observation and is more often caused by cystitis, which may cloud the clinical assessment if occurring concurrently (Davido and Getzenberg, 2002). Most of the marker studies for bladder cancer are cross-sectional, observational case control studies. Some of the studies also used heterogeneous patient populations and those being evaluated for hematuria and those with a history of bladder cancer (Konety and Getzenberg, 2001). So, there is a strong need for new markers for screening, initial diagnosis, surveillance for recurrent lesions, detection of early progression and prediction of the biological potential of a particular tumor with the ultimate aim to alter clinical management of patients (Goebell, 2008).

Some programs have been unable to prevent bladder cancer despite aggressive screening with cystoscopy and others have claimed that they have found lower stage tumors as a result. It is axiomatic that screening tests, to be effective, must be safe, rapid and inexpensive. They should also have adequate sensitivity, specificity and predictive value. These screening programs, however, have not met these universal tenets and, therefore, none of the protocol has been clinically useful for screening patients with the preclinical symptoms of bladder cancer.

The application of biomarkers, as an adjunct or to supplant cystoscopy, as a screening test for diagnosis of bladder cancer in patients has not been extensively investigated and is the need of the hour.

In last few years, abundant tumor markers including nuclear matrix protein 22 (NMP22), bladder tumour antigen (BTA), telomerase, fibrinogen degradation products, lewis X antigen, cytokeratins, survivin, and BLCA-4 (Kausch and Bohle, 2001; Lokeshwar and Soloway, 2001; Boman, 2002; Davido and Getzenberg, 2002) have been studied for bladder cancer, which few of them have been evaluated in well-defined cohorts of patients to determine their independent diagnostic and

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prognostic value with known clinicopathological criteria. In the present study, we have evaluated the urinary survivin performed by ELISA (enzyme-linked immunosorbent assay) for TCC of urinary bladder and compared the results with the conventional urinary cytology.

Survivin, a 16.5-kDa protein, is one of the family members of Inhibitor of Apoptosis Protein (IAP) and has unique role in apoptosis (Altieri, 2003) and control of cell division (Uren et al., 2000; Giodini et al., 2002). Although exclusively expressed in embryonic tissues (Ambrosini, 1997), survivin was reported to be expressed in bladder cancer for the first time by Swana et al. (1999) by using immunohistochemistry (Swana et al., 1999). Detection of survivin in the urine sample of bladder cancer patients was done by Smith et al. (2001) for the first time (Smith et al., 2001). Such studies found a positive correlation between survivin expression and prognosis of disease in several types of other carcinomas like neuroblastoma, (Azuhata et al., 2001) colorectal cancer, (Ikeguchi and Kaibara, 2002; Rodel et al., 2002) breast cancer (Ryan et al., 2006) and esophageal squamous cell carcinoma (Wang, 2007). A multivariate statistical analysis revealed that survivin expression is an independent prognostic factor for disease progression in bladder cancer. Shariat et al. (2000) reported that urine survivin is a strong, self-regulating predictor of the presence of bladder cancer in high grade tumor (Shariat et al., 2004). In another study, Weikert et al. (2005) were able to detect survivin mRNA in urine 68% patients with bladder cancer. None of the healthy patients had detectable urinary survivin mRNA in their study. Overall, a sensitivity of 68.6% and a specificity of 100% for urinary survivin mRNA, compared to sensitivity of 31.4% and a specificity of 97.1% for voided cytology for detection of TCC of the urinary was found (Weikert et al., 2005).

Taking the hypothesis that survivin could be used as a functional marker of early diagnosis in both new-onset and a recurrent bladder tumor, the current study was designed to assess the clinical utility of survivin as a diagnostic biomarker with the help of ELISA in detection of bladder cancer and it was compared with the cytology as the conventional marker, in TCC from a clinically mixed population of patients. Our study was prospective case series that tracks patients with known disease.

Materials and Methods

Experimental design and characteristics

In this prospective case series study, healthy controls and patients with urinary tract benign disease and TCC of urinary bladder were included after signing voluntary informed consent. The confirmation of bladder cancer, the clinicopathologic status and detailed history of each patient was assessed with the standard procedures including biopsy. A total of 191 (163 males and 28 females) subjects were enrolled in this study. Subjects of the study were as follows: healthy control participants (Group I, n=28); patients diagnosed with noncancerous urinary tract conditions such as, benign prostate hyperplasia (BPH), urinary tract infection (UTI), urethral stricture or urolithiasis (Group II, n=46) and TCC bladder patients {(ICD-O code: 8120/0, 2 and 3) (Fritz et al., 2000) (Group III, n=117)}. Out of 117 TCC cases, 103 were males and 14 were females, with the median age of 57 years (range 34-82 years). Group III had four sub groups which included patients with a) primary superficial, b) muscle invasive, c) recurrent cases and d) treated patients with superficial disease (patients after transurethral resection (TUR) or any adjuvant treatment or immunotherapy) and who were without bladder tumors.Tables 1 and 2 summarize demographics and tumor characteristics. All patients were biopsy confirmed cases. The post-surgical pathological stage was classified according to the revised tumor-node-metastasis (TNM) staging system (UICC, 2002) (Sobin and Wittekind, 2002). The urinary bladder were graded according to the World Health Organization grading system (1998) (Epstein et al., 1998).

Patients with the history of bowel interposition surgery and other malignancies like squamous and adenomatous carcinoma of urinary bladder and patients with any concurrent malignancy or disease like Tuberculosis, Diabetes mellitus, Hepatitis B or C infection or HIV infection were excluded from the study. Fifty patients were determined as superficial (28 primary, 22 recurrent cases). Among 67 invasive tumors, we observed 14 tumors as low grade and 53 tumors as high grade.

Sample collection and processing

A single and naturally voided midstream urine sample was obtained prospectively from all subjects. Approximately 50 cc. of sample was collected and immediately after collection, urine were put on ice and centrifuged as soon as possible (not later than an hour interval) at 3,000 rpm, 4ºC, for 7 minutes. Supernatant was then applied on urine concentrator (Amicon® Ultra-4 Centrifugal Filter Unit, Millipore, USA), and stored at -80ºC. All samples were brought to room temperature before use. ELISA was done with Quantikine® Human Survivin Immunoassay kits (RnD systems, MN, USA) with the minimum detectable dose (MDD) from 1.58-9.96 pg/mL.

Cytology

The urine cytology was analyzed from fresh urine. All the urine cytology results were interpreted by a single observer. The cytopathologist was not aware of the patient's disease status.

Statistical summary

Data was summarized as mean±SE. Groups were compared by student's t-test and one way analysis of variance (ANOVA) followed by Bonferroni post hoc test. Discrete (categorical) variables were compared by chi square (χ^2) test. Diagnostic significance of variables assessed by receiver operating characteristic (ROC) curve analysis. A two tailed (α=2) probability $p<0.05$ was considered statistically significant.

Results

Basic characteristics

The basic characteristics viz. age, sex, smoking

habit and survivin concentration of three groups are summarized in Table 1. Age, sex and smoking habit did not differ significantly (p>0.05). However, the survivin concentration differed significantly between the three groups. In cancer patients (n=117) the survivin concentration was significantly different and higher (p<0.001) as compared to both healthy controls (n=28) and non malignant patients (n=46) while it did not differ (p=0.367) between healthy controls and non malignant patients as measured by ELISA.

Association between clinicopathological parameters and survivin concentration

The association between clinicopathological parameters and survivin concentration in cancer patients are summarized in Table 2. The survivin concentration showed direct and significant (p<0.001) correlation with stage, nodal status and cytology while it did not show any association (p=0.349) with grade. A significant association (p<0.001) was found in urinary survivin levels in patients with early stage disease (Ta-T2) versus advanced stage disease (T3-T4) and for all study sub-groups Vs control (p<0.001). The differences among the sub-groups were significant (p<0.05) except in treated superficial cases Vs primary superficial cases (p=1.0). Urinary survivin levels were analyzed with reference to nodal status patients with TCC. Out of 117 bladder cancer patients, 15 were node positive (N1) and rest of 102 node negative (N0) case. The difference between the two groups was statistically

Table 1. Basic Characteristics and Survivin Concentration (pg/ml) of Three Groups

Characteristics		Controls (n=28)	Non malignant urological disease (n=46)	TCC cases (n=117)	p value
Age in years (Mean, Range)		52.71 (28-70)	58.57 (25-77)	57.76 (34-82)	0.085
Sex	Male	24 (85.7%)	36 (78.3%)	103 (88.0%)	0.286
	Female	4 (14.2%)	10 (21.7%)	14 (12.0%)	
Smoker	Yes	25 (89.3%)	39 (84.7%)	95 (81.1%)	0.563
	No	3 (10.7%)	7 (15.2%)	22 (18.8%)	
Survivin (Mean±SE)		7.77±0.44	14.59±0.76	39.97±2.17	p<0.001

Table 2. Association of Clinicopathological Parameters and Survivin Concentration in Cancer Patients

Characteristics		Bladder cancer patients n=117 (100%)	Survivin (pg/ml) Mean±SE	p value
Stage	Early stage (I+II)	71 (60.7)	29.35 ±1.94	p<0.001
	Advance stage (III+IV)	46 (39.3)	56.35±3.44	
Nodal status	Negative	102 (87.2)	37.33±2.12	p=0.001
	Positive	15 (12.8)	57.87±7.51	
Grade	Low	29 (24.8)	36.41±4.09	
	High	88 (75.2)	41.14±2.55	
Primary		69 (58.9)	45.52±3.03	0.573
Recurrent		27 (23.1)	42.56±3.14	
Cytology	Negative	39 (33.3)	27.71±2.71	p<0.001
	Positive	78 (66.7)	46.10±2.70	
Study Sub-groups				
	Primary superficial	28 (23.9)	23.74±1.79	
	Muscle invasive	41 (35.0)	60.40±3.35	
	Recurrent cases	27 (23.1)	42.56±3.14	
	Treated superficial	21 (17.9)	18.39±1.92*	

*Primary superficial treated superficial (p>0.05)

Table 3. Comparison of Sensitivity for Survivin and Cytology in Urinary Bladder Cancer

Groups	Characteristics	Urinary cytology Sensitivity (%)	p value	Urinary survivin Sensitivity (%)	p value
Stage	Early stage	47.89	p<0.001	77.46	p<0.001
	Advance stage	95.65	p<0.001	91.30	p<0.001
Node status	Node positive	80.00	p<0.001	93.33	p<0.001
	Node negative	64.71	p<0.001	81.37	p<0.001
Grade	Low grade	41.38	0.003	79.31	p<0.001
	High grade	75.00	p<0.001	84.09	p<0.001
Overall	TCC cases	66.67	p<0.001	82.91	p<0.001
	Non	6.52*	0.693	17.47	p<0.001
History	Primary	73.91	p<0.001	86.96	p<0.001
	Recurrent	81.48	p<0.001	92.59	p<0.001

*Patients with the symptom of chronic inflammation with catheterization may lead to false diagnosis of bladder cancer

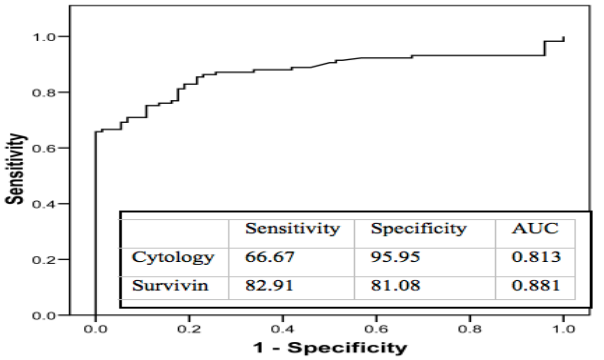

Figure 1. ROC Curve of Urinary Survivin, which Predicts the Presence of Bladder Cancer in Terms of Sensitivity and Specificity. AUC: area under the curve

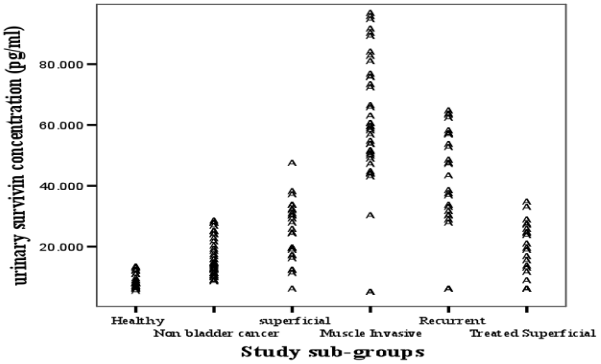

Figure 2. Analysis of Survivin Concentration. Scatter plot shows ELISA absorbance values in healthy individuals, non cancer patients bladder cancer

significant (p=0.001). Out of 15 node positive bladder cancer cases, 14 had higher urinary survivin levels, whereas 12 were urinary cytology positive. A significant difference was not found in survivin expression between primary (n=69) and recurrent (n=27) cases (p=0.573).

Receiver operating characteristic curve analysis and survivin specificity

Elevated levels of survivin detected in urine obtained from cancer vs. controls, a receiver operator characteristics (ROC) curve was constructed (Figure 1), by plotting sensitivity versus 1-specificity and was typically used to determine an optimal cut-off value. The area under the ROC curve was 0.881 with an optimal cut-off value 17.74 pg/ml was proposed, corresponding to a sensitivity of 82.91% (95%CI: 74.84-89.23%) and specificity of 81.08%

(95%CI: 70.30-89.25%) for TCC cases.

Comparison of survivin and cytology

Out of 117 cancer cases, 78 (66.7%) were found cytology positive and 96 (82.1%) were found survivin positive. Overall sensitivity of survivin was 82.91% and specificity was 81.08%, whereas urine cytology had sensitivity of 66.67% (95%CI: 57.36-75.11%) and specificity of 95.95% (95%CI: 88.61-99.16%). Table: 3 shows the comparison of voided urine cytology and survivin in different groups.The diagnostic accuracy of survivin and cytology was compared at different levels of patient features. The urinary survivin concentration showed more sensitivity than cytology (grade wise, stage wise and wise), but cytology was considerably more sensitive for high-grade tumors. Our results clearly demonstrated that both survivin expression pattern and cytology give almost equal sensitivity for higher grade, whereas in the patients of lower grade, the sensitivity of survivin was 79.31% in comparison to the 41.38% sensitivity cytology (Table 3). Survivin expression levels also detected both primary and recurrent cases more accurately.

Discussion

At the time of initial diagnosis of TCC, 80% of the patients present with superficial papillary tumors (stage pTa or pT1) (Walsh et al., 1997), but there are no well-recognized clinical techniques available for its early and accurate diagnosis. Hence it is important to diagnose bladder cancer accurately with the help of a simple and cost effective method. Urine cytology is another popular method for early detection having variable sensitivity (21-40%) (Fuessel et al., 2004), this drawback explains the most part of poor criteria of identifying well-differentiated, low-grade TCC (Brown, 2000). Our results show the sensitivity and specificity of urinary cytology as 67.67% and 95.95% respectively. Given the emerging data (Figure 2) suggesting an important role survivin, we attempted to judge the sensitivity and specificity of urinary survivin over urine cytology. We found that urinary survivin had better sensitivity in patients of lower grade /early stage as compared to cytology. However both survivin and cytology showed almost equal sensitivity for higher grade/advance stage patients. It was also found that the difference between sensitivity of survivin and cytology for both primary and recurrent cases good (Table 3).

It is well known that urine cytology is not suitable for the diagnosis of lower grade carcinoma of urinary bladder. Also, the urine sample for cytology needs to be processed which is sometimes not possible. Overall, our results indicate that survivin gives higher positive results in comparison to cytology, particularly in low grade, early stage disease. Interestingly, these findings are in concurrence with many researches in last few years. Sun et al. (2006) found a sensitivity of 36.4% and specificity of 100% for urine cytology compared with survivin (sensitivity of 70.2%, specificity of 85.0%) in the diagnosis of TCC (Sun et al., 2006). It has been documented that survivin has a high expression in exfoliated cells in urine of the patients with TCC and only slightly expressed in healthy individuals (Ambrosini, 1997). The sensitivity of the test in our patients with TCC is 82.91%. This is in harmony with the results of Sharp et al. (2002) who found the survivin expression in urine samples of bladder cancer patients, and its sensitivity was 100% and specificity was 95% and Ohsawa et al. (2004) who used ELISA system with sensitivity of 42.4% and specificity of 88.9% in 40 cases of TCC (Sharp et al., 2002; Ohsawa et al., 2004). Our results also demonstrated that using the best cut-off point determined by the ROC curve, survivin can act as a better biomarker for diagnosis and surveillance of urinary bladder cancer vs. urinary cytology.

With the encouraging results of urinary survivin by ELISA system over conventional urinary cytology, the diagnosis of urinary bladder malignancy by examination of urinary survivin can become more reliable and least invasive. Based on the results of our study, we may recommend urinary survivin as a suitable diagnostic marker for the early diagnosis and monitoring the bladder cancer. Further evaluation of urinary survivin in a larger patient population is warranted before the use of urinary survivin for routine clinical use and early detection of urinary bladder cancer.

Acknowledgements

We declare that we have no conflict of interest. first author (Anupam Srivastava) is thankful to Indian Council of Medial Research, New Delhi Government India for providing Senior Research Fellowship (IRIS ID: 2010-03160) under the guidance of corresponding author. All authors are also thankful to Prof. M K Mishra, Dr. Seema Nayak and Dr. Nidhi Pradhan for their invaluable contribution. We thank all the patients for their participation in the study.

References

Agarwal PK, Black PC, Kamat AM (2008). Considerations on the use of diagnostic markers in management of patients with bladder cancer. *World J Urol*, **26**, 39-44.

Ambrosini G, Adida C, Altieri D (1997). A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med*, **3**, 917-21.

Altieri DC (2003). Survivin in apoptosis control and cell cycle regulation in cancer. *Progress in Cell Cycle Res*, **5**, 447-52.

Azuhata T, Scott D, Takamizawa S, et al (2001). The inhibitor of apoptosis protein survivin is associated with high-risk behavior of neuroblastoma. *J Pediatr Surg*, **36**, 1785-91.

Boman H, Hedelin H, Holmang S, (2002). Four bladder tumor markers have a disappointingly low sensitivity for small size and low grade recurrence. *J Urol*, **16**, 780-3.

Brown FM (2000). Urine cytology. It is still the gold standard for screening? *Urol Clin North Am*, **27**, 25-37.

Davido HT, Getzenberg RH (2002). Utilization of a urine-based assay for BLCA-4 in the detection of bladder cancer. *J Clinical Ligand Assay*, **25**, 83-6.

Epstein JI, Amin MB, Reuter VR, et al (1998). The world health organization/international society of urological pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. bladder consensus conference committee. *Am J Surg Pathol*, **22**, 1435-48.

Fritz A, Percy C, Jack A, et al (2000). International Classification of Diseases for Oncology, 3rd edition, World Health Organization, Geneva.

Fuessel S, Kueppers B, Ning S, et al (2004). Systematic in vitro evaluation of survivin directed antisense oligodeoxynucleotides in bladder cancer cells. *J Urol*, **171**, 2471-6.

Grossfeld GD, Carroll PR (1998). Evaluation of asymptomatic microscopic hematuria. *Urol Clin N Am*, **25**, 661-76.

Goebell PJ, Groshen SL, Schmitz-Drager BJ (2008). Guidelines for development of diagnostic markers in bladder cancer. *World J Urol*, **26**, 5-11.

Giodini A, Kallio MJ, Wall NR, et al (2002). Regulation of microtubule stability and mitotic progression by survivin. *Cancer Res*, **62**, 2462-7.

Ikeguchi M, Kaibara N (2002). Survivin, messenger RNA expression is a good prognostic biomarker for oesophageal carcinoma. *Bri J Cancer*, **87**, 883-7.

Kausch I, Bohle A (2001). Bladder cancer. II. Molecular aspects and diagnosis. *Eur Urol*, **39**, 498-506.

Konety BR, Getzenberg RH (2001). Urine based markers of urological malignancy. *J Urol*, **165**, 600-11.

Lokeshwar VB, Soloway MS (2001). Current bladder tumor tests: does their projected utility fulfill clinical necessity? *J Urol*, **165**, 1067-77.

Ohsawa I, Nishimura T, kondo Y, et al (2004). Detection of urine survivin in 40 patients with bladder cancer. *J Nippon Med Sch*, **71**, 379-83.

Rodel F, Hoffmann J, Grabenbauer GG, et al (2002). High survivin expression is associated with reduced apoptosis in rectal cancer and may predict disease-free survival after preoperative radiochemotherapy and surgical resection. *Strahlenther Onkol*, **178**, 426-35.

Ryan BM, Konecny GE, Kahlert S, et al (2006). Survivin expression in breast cancer predicts clinical outcome and is associated with HER2, VEGF, urokinase plasminogen activator and PAI-1. *Ann Oncol*, **17**, 597-04.

Siegel R, Ward E, Brawley O, Jemal A (2011). Cancer statistics, 2011. the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*, **61**, 212-36.

Swana HS, Grossman D, Anthony JN, et al (1999). Tumor content of the antiapoptotic molecule SURVIVIN and recurrence of bladder cancer. *N Engl J Med*, **34**, 1452-53.

Smith SD, Wheeler MA, Plescia J, et al (2001). Urine detection of survivin and diagnosis of bladder cancer. *JAMA*, **285**, 324-8.

Shariat SF, Casella R, Khoddami SM, et al (2004). Urine detection of survivin is a sensitive marker for the noninvasive diagnosis of bladder cancer. *Urol*, **171**, 626-30.

Sobin LH, Wittekind CL (2002). TNM classification of malignant tumors, 6th edition. John Wiley & Sons, New York.

SUN Yi, HE Da-lin, MA Qiang, et al (2006). Comparison of seven screening methods in the diagnosis of bladder cancer. *Chinese Med J*, **119**, 1763-71.

Sharp JD, Hausladen DA, Maher MG, et al (2002). Bladder cancer detection with urinary survivin, an inhibitor of apoptosis. *Front Biosci*, **7**, 36-41.

Uren AG, Wong L, Pakusch M, et al (2000). Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype. *Curr Biol*, **10**, 1319-28.

Viv Bewick, Liz Cheek, Jonathan Ball (2004). Statistics review 13: Receiver operating characteristic curves. *Critical Care*, **8**, 508-12.

Wang T, Qian X, Liu B (2007). Survivin: potential role in diagnosis, prognosis and targeted therapy of gastric cancer. *World J Gastroenterol*, **13**, 2784-90.

Weikert S, Christoph F, Schrader M, et al (2005) Quantitative analysis of survivin mRNA expression in urine and tumor tissue of bladder cancer patients and its potential relevance for disease detection and prognosis. *Int J Cancer*, **116**, 100-04.

Walsh PC, Retik Ab, Vaughn ED, et al (1997) Campbell's Urology. Philadelphia, WB Saunders, Co., 2354-57.