# Tissue polypeptide antigen in the follow-up of patients with urinary bladder cancer compared with conventional urine cytology

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## Abstract

The incidence of bladder cancer has demonstrated a rapid increase during the last decades. *The aim* of this study is to determine the clinical value of serum tissue polypeptide antigen (TPA) as a tumour marker for urinary bladder cancer in comparison with conventional urine cytology. *Urine and blood samples* were obtained from a total of 108 patients (group A) with a known history of bladder cancer, who presented for their routine 3 month follow-up. These 108 patients included 45 patients with high grade and 63 patients with low grade bladder cancer, and 30 patients with lower urinary tract symptoms (LUTS) and no history of bladder cancer (group B). Urine and blood samples from fifty healthy adults (group C) were also tested; this group served as the control group for estimating the normal range of serum TPA values. In all group A patients cystoscopy and/or bladder biopsies were performed. All blood and urine samples were tested for TPA and conventional urine cytology respectively. *Results* showed that the upper normal range for TPA was 1.0ng/mL(0.9±0.04) in the control group. For the subgroups of patients with high and low grade bladder cancer elevated serum TPA levels were found in 52% and 40% of the patients respectively. The overall serum TPA sensitivity and specificity were 50% and 85% respectively for patients with known bladder cancer (group A). We found the sensitivity of cytology for high grade bladder (GIII) carcinomas to be 72%; however when urine cytology was combined with serum TPA the overall sensitivity reached 80%. *We conclude* that serum TPA combined with urine cytology may be used as a prognostic marker for bladder cancer.

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## Introduction

Bladder cancer represents the 4th most common malignancy in men and the 9th most common in women. In 2006, an estimated 61,420 of Americans were diagnosed with bladder cancer and 13,060 died as a result of this disease. [1] Bladder tumours are typically diagnosed by cystoscopy which is the gold standard for both detection and follow-up [2]. The proper follow-up for patients with bladder cancer managed with bladder preservation treatment consists of cystoscopy and urine cytology usually at 3 months intervals for the early detection of recurrences [3].

It is known that although conventional urine cytology is more specific for the detection of high grade bladder carcinomas (GIII) with high sensitivity its sensitivity is very low for the detection of superficial bladder tumours (low grade GI and GII) [4, 5]. In order to increase the sensitivity of urine cytology for the detection and monitoring of bladder cancer, we have studied tissue polypeptide antigen (TPA). This antigen is a protein with a molecular weight between 20,000-40,000 Daltons, a differentiation and proliferation tissue marker present in the proteolytic fragments of cytokeratins 8, 18 and 19 as a component of the cytoskeleton of non-squamous epithelia [6-8]. Increased levels of TPA have been reported in various malignant diseases as well as inflammatory disorders such as Crohn's disease and hepatitis. Tissue polypeptide antigen has also been detected in normal urothelium as well as in non-squamous neoplastic urothelium [10-13].

The aim of our study was to examine whether serum TPA either alone or combined with conventional cytology can improve the overall sensitivity for the detection of tumor recurrence in patients with low risk urinary bladder cancer.

## Subjects and methods

One hundred and eight patients (group A), mean patient age 68 years, were enrolled in this study. Forty five patients had a high grade and 63 patients a low grade bladder carcinoma following transurethral resection

of bladder tumours (TUR-BT). These patients were routinely followed-up every 3 months. Thirty patients (group B) presented with lower urinary tract symptoms (LUTS) and no evidence of bladder cancer were also enrolled. Fifty healthy individuals were tested as controls to estimate the normal values of serum TPA. None of them had a history or evidence of urinary bladder cancer, inflammatory disease of the urinary tract or renal failure. The clinicopathological data for all groups are displayed in Table 1.

**Table 1.** *The clinicopathological data for all groups studied.*

| | Patients' characteristics | No of patients |
|---|---|---|
| | Mean age (in years) | 68 ± 5 |
| | Female and Male | 35 and 73 |
| Group A TCC | Superficial tumour (pTa) | 63 |
| | Invasive tumour (pT1, pT2) | 45 |
| Group B F-TCC | Urolithiasis | 7 |
| | BPH | 15 |
| | Microhematuria | 8 |
| Group C | Control group (CG) | 50 |
| | TCC and F-TCC and CG | 188 |

Group A includes patients under follow-up with known transitional cell carcinoma and Group B patients free of TCC and without other malignancy. Healthy donors were also examined as control group C.

In groups A and B patients, cystoscopy or tumour biopsies were performed when necessary during the follow-up period. Urothelial tumour grade was defined according to the new World Health Organization/International Society of Urological Pathology consensus classification of urothelial neoplasms and stage was classified according to International Union Against Cancer (IUC) [14]. Freshly voided urine and blood samples were also obtained from all patients. Urine was cultured and the kind of the organisms grown was identified. Urine samples with significant growth of organisms were excluded from the study. Urine specimens were centrifuged and their sediment was used for standard PAP stain which revealed the existence of malignant cells (Fig. 1). With regard to cytology examination we described three groups as negative, suspicious and positive for malignancy.

Serum TPA was determined by an immunoradiometric assay (IRMA) using TPAcyk commercial kit, (DRG instruments® GmbH Germany). This is a one step in vitro diagnostic assay for the quantitative determination of cytokeratines 8 and 18 in the serum. The assay is a sensitive indicator of tumour

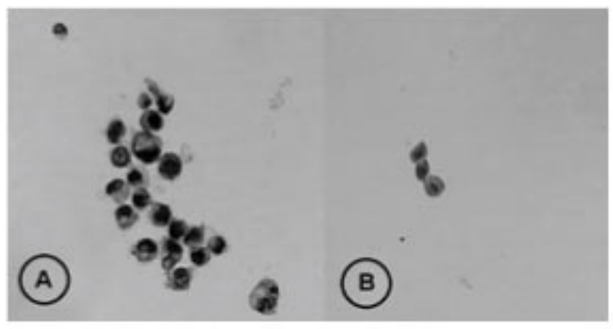


**Figure 1.** Urine smear: A: Sheets of urothelial carcinoma cells (Grade II) with Papanicolaou stain (X500). B: Benign urothelial cells (umbrella cells) (X500).

cells activity. It is a solid phase sandwich assay based on immunochemical technique. The assay of TPAcyk measures key epitopes of TPA fragments. The monoclonal 6D7 and 3F3 antibodies used in the test are specific for cytokeratin 8 and 18, with no detectable cross reactivity to other tumour associated antigens that may be present in patient samples. [14-15]. In this assay antiserum against TPA is incubated in excess with the sample, binding any TPA present. The TPA molecules were radiolabelled with iodine 125. The cut-off value was 30ng/mL.

The radioactivity in the precipitate was measured in a gamma counter (WLLAC WIZARD® 1470, Turku, Finland) and counts were converted to TPA concentration units by using a standard curve. In all groups studied, serum creatinine was examined. Cases with elevated creatinine were excluded from the study since elevated creatinine levels result in elevation in the serum TPA levels.

## Statistical analysis

Data analysis was performed using the statistical package SPSS version 11.00. The association of urine cytology and TPA with all prognostic parameters of bladder tumour (stage and grade) was assessed by a one way analysis of variance (ANOVA) test followed by tests of multiple comparisons, since urine cytology and TPA did not deviate from normality. The specificity and sensitivity of TPA and urine cytology were calculated using a receiver operating characteristic (ROC) curve analysis. A P value of less than 0.05 was considered to be statistically significant.

## Results

The cut-off value for TPA for the control group C was 1.0 ng/mL (0.9±0.04). During the three months of follow-up we found elevated serum TPA levels in 40% of the patients with superficial bladder cancer. Serum TPA correlated well with the stage of the disease as shown in Fig. 2. All patients had higher serum TPA levels when compared with the control group. The TPA serum levels are shown in Table 2. Elevated TPA levels were also found in 52% of patients with invasive bladder cancer.

During the three months follow-up, according to the pathology examination the distribution of patients with superficial urine bladder cancer was 45% with grade I, 30% with grade II and 25% with grade III cancer (Fig. 3). Regarding the stage of the disease, 58% of the patients were stage pTa, 23% were stage pT1 and 19% were stage pT2. Relevant to cytology, of the 63 patients with pTa and grade I to II, 60 patients were negative and 3 were suspicious for malignancy while of the 45 patients with T1 or T2 and grade I to III, there were 15 suspicious and 30 positive for malignancy.

One way analysis of variance (ANOVA) showed that

serum TPA was significantly higher (P=0.008) for patients with bladder cancer depending on the stage (pT) and grade of the cancer. There was no difference in TPA levels between superficial and invasive urine bladder cancer patients (Fig. 4).

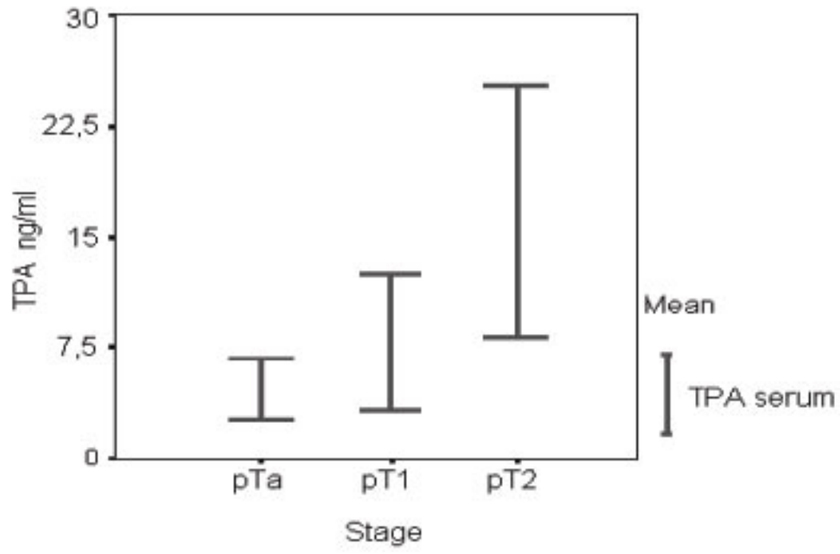


**Figure 2.** The distribution of urine and serum TPA according to histopathological stage.

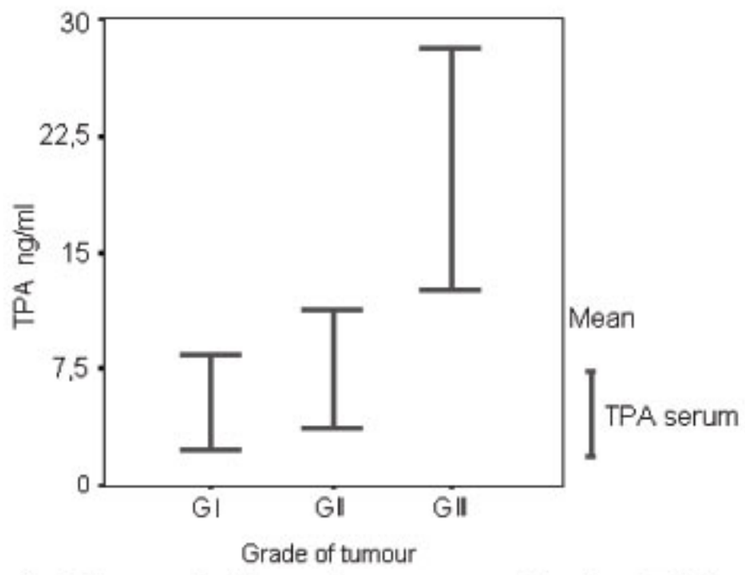


**Figure 3.** The variation of serum and urine TPA values according to tumour grading.

The variance of serum TPA levels and urine cytology are shown in Table 2. In this table it is clear that the positivity and higher levels of serum TPA correlate with more aggressive (advanced stage) of bladder cancers (pT1 and pT2).

**Table 2.** *Serum TPA levels in urinary bladder cancer in comparison to staging*.

| Stage | No | Serum TPA levels (ng/mL) | Urine cytology |
|---|---|---|---|
| PTa | 63 | 5.1 ± 1.4 | 60 (--)<br>3 (±) |
| PT1 | 25 | 8.04 ± 4.26 | 5 (±)<br>10(+) |
| PT2 | 20 | 17.33 ± 8.89 | 20(+) |
| Control | 50 | 0.9 ± 0.04 | |

pTa: papillary epithelial confined tumour, T1: tumours that invaded lamina propria, T2: superficial muscle invasive tumour. For urine cytology was used categories: negative, suspicious and positive for malignancy.

The sensitivity of serum TPA for group A and its subgroups of superficial pTa and invasive bladder tumour (pT1 and pT2) was 50% while the overall specificity was 85%. With regard to tumours grade, for low grade carcinomas, the sensitivity of urine cytology was 21% although it reached 52% when combined with serum TPA. For high grade (Grade III) bladder carcinomas the sensitivity of urine cytology was 72% and when combined with serum TPA reached 80% (Fig. 5). The individual sensitivities of conventional cytology, serum TPA values and the sensitivity of the combination of serum TPA and conventional cytology are shown in Table 3. This Table also shows the sensitivities as far as the stage and the grade of the disease are concerned.

**Table 3.** *Sensitivity of conventional cytology, tissue polypeptide antigen (TPA) measurement and the combination of TPA measurement and conventional cytology in comparison to the grade and stage of the disease.*

| | | Sensitivity | | |
|---|---|---|---|---|
| Grade | No | UC | TPA | UC and TPA |
| GI | 49 | 21 % | 50 % | 52 % |
| GII | 32 | 49 % | 57 % | 65 % |
| GIII | 27 | 72 % | 60 % | 80 % |
| Stage | | | | |
| pTa | 63 | 28 % | 49 % | 55 % |
| pT1 | 25 | 63 % | 52 % | 71 % |
| pT2 | 20 | 74 % | 65 % | 80 % |

Increased serum TPA (8.04-17.33ng/mL) levels were found in patients with pT1 and pT2 bladder cancer. However, serial measurements of TPA for monitoring disease activity were found to have limited value because of the low sensitivity of TPA, especially in patients with early stage urine bladder cancer.

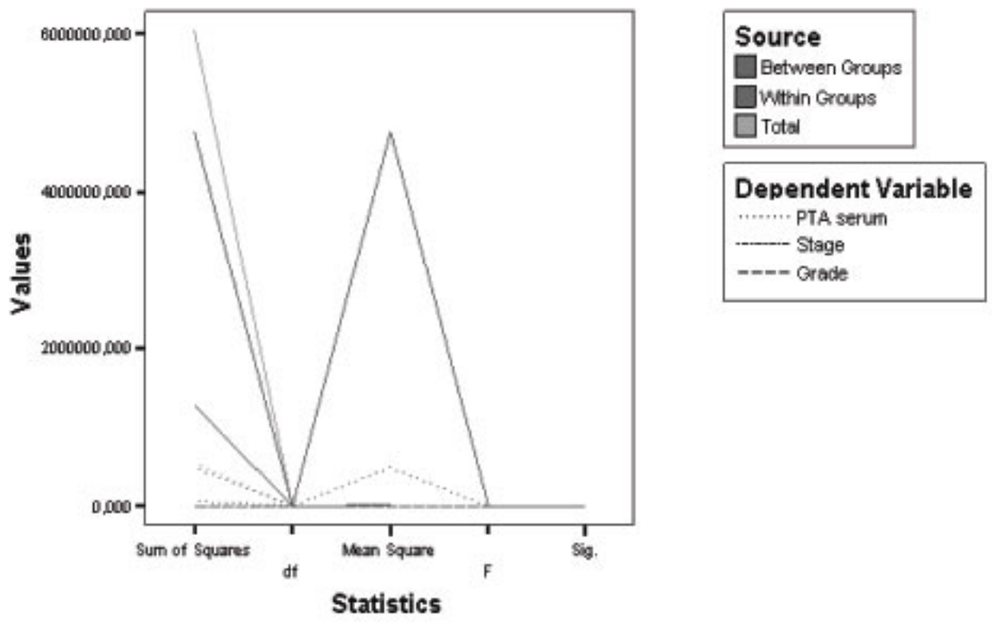


**Figure 4.** One way ANOVA analysis of variance. In this study serum TPA histopathological stage and tumour grading were the dependent variables.

## Discussion

Many cancer markers available are neither specific for malignancy nor allow early diagnosis [17]. The elucidation of the molecular events occurring during tumorigenesis may provide new markers that would be both specific for cancer and sensitive for early disease detection [18]. The marker TPA is a cytoplasmic constituent of normal non-epidermal human epithelia, maintained and increased in carcinomas of these epithelia. The proliferative activity of epithelial cells increases the release of TPA into the serum [9]. There can be a temporary elevation of TPA during infection or trauma. These levels are not as high as in patients with

known neoplasms and usually return to normal levels faster than other parameters such as the erythrocyte sedimentation rate [19].

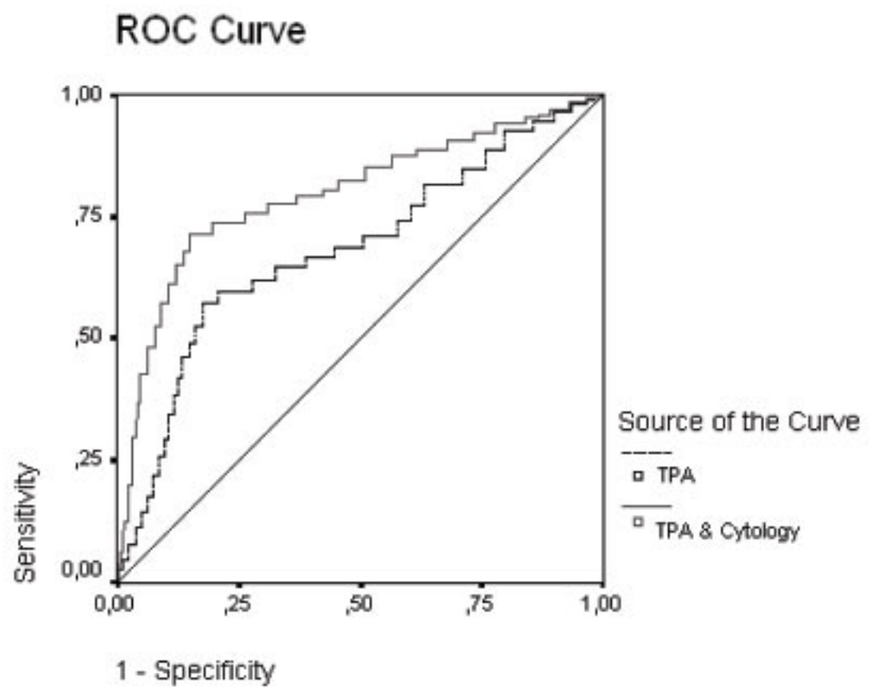


**Figure 5.** Percentages of sensitivity and specificity of serum TPA measurements and conventional cytology for all groups studied.

Numerous chemicals have been identified as bladder carcinogens in both animals and humans, including various aromatic amines, cyclophosphamide and related compounds [20]. Urinary tract carcinogens affect the urothelium via exposure through the urine rather than through blood. Animals exposed to carcinogens do not develop bladder tumours unless the urothelium is exposed to urine [21].

Superficial or low grade bladder cancer patients after the initial TURT usually have to adhere to a long-term follow-up protocol with repeated urine cytology and cystoscopic surveillance. Despite its low sensitivity, conventional urine cytology has been the standard non-invasive method for cancer detection and monitoring. Only very recently the methylation status of certain genes e.g. p16, p15, DAPK, RARβ etc. in bladder carcinoma was studied as diagnostic markers of the epigenetic changes in urine [22].

The elevated TPA levels found in invasive bladder cancer patients in our study is in agreement with the results from Maulard-Durdux et al (1997) who reported that in invasive urine bladder cancer, serum TPA levels correlated with initial tumor stage and represented a valuable parameter during the follow-up period [9], while others concluded that TPA serial measurements for monitoring disease activity has limited value because of the low sensitivity of TPA, especially for patients with low-stage disease, and also due to the occurrence of false positive results [23]. Increased levels of TPA were found in postoperative wound healing [24] and in 92.5% of dialysis patients without evidence of malignancy [25]. In another study although serum TPA levels correlated with initial tumour stage and grade, TPA was not as useful for detecting disease recurrence [6]. However the combination of both TPA measurements and conventional cytology seems to be a promising tool in detecting recurrence, for patients with bladder cancer.

Our study confirms that serum TPA levels are significantly higher in patients with bladder cancer in comparison with the control group, as has also been shown in other studies [24-26]. Two other studies confirmed that no significant serum TPA levels difference exists between superficial and invasive urine bladder cancer patients [27-29]. Moreover, TPA levels seem to correlate well with the stage and the grade of the disease [19, 29, 30].

According to other published studies, the sensitivity of serum TPA for the diagnosis of local bladder cancer ranges between 60%-70%, while for metastatic disease it ranges between 85%-100% [31-33]. Serum TPA tests can discriminate between local and disseminated disease, as they significantly correlate to the tumor burden and can be used either alone or combined with other conventional diagnostic techniques [34-36].

Clinical studies have also shown that serum TPA assays are valuable for the management of bladder cancer, since TPA as a single test can reliably differentiate between patients with and without metastatic disease [34-37], with 85% sensitivity and 79% specificity [36]. This differentiation is important for stages pT1GIII and pT2 patients with urine bladder cancer. An increased pre-therapeutic serum TPA value can predict disseminated disease for stages pT1GIII and pT2 patients with a sensitivity of 90% and 95% specificity [34-36].

*In conclusion*, according to our study, the sensitivity of TPA alone is low. The combination of TPA with urine cytology slightly increases its sensitivity which becomes fairly high (80%) only for high grade (Grade III) bladder cancers.

## Bibliography

1. Jemal A, Siegel R, Ward E et al. Cancer Statistics, 2006. *Cancer J Clin* 2006; 56: 106-30.
2. Madeb R, Golijanin D, Knopf J, Messing EM. Current state of screening for bladder cancer. *Expert Rev Anticancer Ther* 2007; 7: 981-7.
3. Burchardt M, Burchardt T, Shabsigh A et al. Current concepts in biomarker technology for bladder cancers. *Clin Chem* 2000; 46: 595-605.
4. Mian C, Pycha A, Wiener H et al. Immunocyt: a new tool for detecting transitional cell cancer of the urinary tract. *J Urol* 1999; 161: 1486-9.
5. Seripa D, Parrella P, Gallucci M et al. Sensitive detection of transitional cell carcinoma of the bladder by microsatellite analysis of cells exfoliated in urine. *Int J Cancer* 2001; 95: 364-9.
6. Menendez Lopez V, Galan JA, Fernández-Suárez A et al. Usefulness of tissue polypeptide antigen in the follow-up of bladder cancer. *Urology* 2003; 62: 243-8.
7. Sánchez-Carbayo M, Urrutia M, Silva JM et al. Urinary tissue polypeptide-specific antigen for the diagnosis of bladder cancer *Urology* 2000; 55: 526-32.
8. Hautmann S, Eggers J, Meyhoff H et al. [Tumor markers for bladder cancer: Up-to-date study by the Kiel Tumor Bank. *Urologe A* 2007; 46: 1508-13.
9. Maulard-Durdux C, Toubert ME, Hennequin C, Housset M. Serum tissue polypeptide antigen in bladder cancer as a tumor marker: a prospective study. *J Clin Oncol* 1997; 15: 3446-50.
10. Barak V, Goike H, Panaretakis KW, Einarsson R. Clinical utility of cytokeratins as tumor markers. *Clin Biochem* 2004; 37: 529-40.
11. Gaston KE, Grossman HB. Proteomic assays for the detection of urothelial cancer. *Methods Mol Biol* 2010; 641: 303-23.
12. Puoti C, Bellis L, Guarisco R et al. Quantitation of tissue polypeptide antigen (TPA) in hepatic and systemic circulation in patients with chronic liver diseases. *J Gastroenterol Hepatol* 2009; 12: 1847-51.
13. Tarantino G, Mazzarella C, Tarantino M et al. Could high levels of tissue polypeptide specific antigen, a marker of apoptosis detected in nonalcoholic steatohepatitis, improve after weight loss? *Dis Markers* 2009; 262: 55-63.
14. Montironi R, Lopez-Beltran A. The 2004 WHO classification of bladder tumors: a summary and commentary. *Int J Surg Pathol* 2005; 13: 143-53.

15. Wiklund B, Silen A, Nilsson S, Andersson E. Cytokeratin 8 and 18 fragments quantified in serum of healthy individuals and pancreatic cancer patients with a novel monoclonal assay. *J Tumor Marker Oncol* 1993; 8: 46.
16. Correale M, Tedone T, Abbate I. TPA-cyk assay in cytosol from primary breast cancer: preliminary results. *Int J Biol Markers* 1994; 9: 264-5.
17. Liou LS. Urothelial cancer biomarkers for detection and surveillance. *Urology* 2006; 67: 25-33; discussion 33-4.
18. Duffy MJ. Can molecular markers now be used for early diagnosis of malignancy? *Clin Chem* 1995; 41: 1410-3.
19. Costello CB, Kumar S. Prognostic value of tissue polypeptide antigen in urological neoplasia. *J R Soc Med* 1985; 78: 207-10.
20. Ross RK, Jones PA, Yu MC. Bladder cancer epidemiology and pathogenesis. *Semin Oncol* 1996; 23: 536-45.
21. Cohen SM, Masui T, Garland EM, Arnold LL. Effects of diet on urinary bladder carcinogenesis and cancer prevention. *J Nutr* 1997; 127: 826S-9S.
22. Chan MW, Chan LW, Tang NL et al. Hypermethylation of multiple genes in tumor tissues and voided urine in urinary bladder cancer patients. *Clin Cancer Res* 2002; 8: 464-70.
23. Van der Gaast A, Kirkels WJ, Blijenberg BG, Splinter TA. Evaluation of tissue polypeptide antigen serum levels for monitoring disease activity during chemotherapy in patients with transitional carcinoma of the urinary tract. *J Cancer Res Clin Oncol* 1992; 118: 626-8.
24. Putzki H, Student A, Breucker K, Jablonski M. Reaction of the tumor markers CEA and TPA to a surgical trauma. *J Surg Oncol* 1986; 33: 18-9.
25. Walz G, Kunzendorf U, Schwarz A et al. Elevated tumor markers in hemodialysis patients. *Am J Nephrol* 1988; 8: 187-9.
26.Moreno Sierra J, Maestro de las Casas ML, Ortega Heredia MD et al. [Serum and tissue quantification of tissue polypeptide antigen (TPA) in transitional cell carcinoma of the bladder]. *Arch Esp Urol* 1994; 47: 979-84.
27. Ecke TH, Schlechte HH, Schulze G et al. Four tumour markers for urinary bladder cancer--tissue polypeptide antigen (TPA), HER-2/neu (ERB B2), urokinase-type plasminogen activator receptor (uPAR) and TP53 mutation. *Anticancer Res* 2005; 25: 635-41.
28. Ecke TH, Lenk SV, Schlechte HH, Loening SA. Tissue polypeptide antigen (TPA) in comparison with mutations of tumour suppressor gene P53 (TP53) in patients with bladder cancer. *Anticancer Res* 2003; 23: 957-62.
29. Senatore S, Zizzi L, Blasi C et al. Tissue polypeptide antigen (TPA) in urinary bladder cancer cytology: a follow-up study. *Oncology* 1990; 47: 37-42.
30. Senatore S, Attolini A, Luccarelli S et al. Tissue polypeptide antigen in normal and neoplastic urinary bladder. Preliminary reports. *Oncology* 1987; 44: 118-23.
31. Weber K, Osborn M, Moll R et al. Tissue polypeptide antigen (TPA) is related to the non-epidermal keratins 8, 18 and 19 typical of simple and non-squamous epithelia: re-evaluation of a human tumor marker. *EMBO J* 1984; 3: 2707-14.
32. Adolphs HD, Oehr P. Significance of plasma tissue polypeptide antigen determination for diagnosis and follow-up of urothelial bladder cancer. *Urol Res* 1984; 12: 125-8.
33. Khanna OP, Wer B. Tissue polypeptide antigen (TPA) as a predictor for genito-urinary cancers and their metastases. *Urology* 1987; 30: 106-10.
34. Maulard C, Toubert ME, Chretien Y et al. Serum Tissue Polypeptide (S-TPA) in bladder cancer as a tumor marker. *Cancer* 1994; 73: 394-8.
35. Van Poppel H, Billen J, Goethuys H et al. Serum Tissue Polypeptide (TPA) as tumor marker for bladder cancer. *Anticancer Res* 1996; 16: 2205-8.
36. Lopez VM, Galan JA, Fernandez-Suarez A et al. Usefulness of tissue polypeptide antigen in the follow-up of bladder cancer. *Urology* 2003; 62: 243-8.
37. Pectasides D, Bafaloucos D, Antoniou F et al. TPA, TATI, CEA, b-HCG, PSA, SCC and CA 19-9 for monitoring transitional Cell carcinoma of the Bladder. *Am J Clin Oncol* 1996; 19: 271-7.
38. P.Markou. Serum Tissue Polypeptide Antigen. Radioimmunoassay in the Diagnosis and the Evaluation of Treatment of Breast, Lung and Bladder Cancer. *Hell J Nucl Med* 1998; 2: 40-7.