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

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 86% 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.

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.

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

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 immunodiacetic assay (IRMA) using TPAck 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 cells activity. It is a solid phase sandwich assay based on immunochemical technique. The assay of TPAck 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 antisera against TPA is incubated in excess with the sample, binding any TPA present. The TPA molecules were radioiodinated 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 urinary 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 urinary 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.

![Histopathological Stage](image)

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

![Tumour Grading](image)

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.

<table>
<thead>
<tr>
<th>Stage</th>
<th>No</th>
<th>Serum TPA levels (ng/mL)</th>
<th>Urine cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTa</td>
<td>63</td>
<td>5.1 ± 1.4</td>
<td>3 (-)</td>
</tr>
<tr>
<td>PT1</td>
<td>25</td>
<td>8.04 ± 4.26</td>
<td>5 (+)</td>
</tr>
<tr>
<td>PT2</td>
<td>20</td>
<td>17.33 ± 8.89</td>
<td>20 (+)</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>0.9 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

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 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.

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Grade</th>
<th>No</th>
<th>UC</th>
<th>TPA</th>
<th>UC and TPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>49</td>
<td>21</td>
<td>50</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td>32</td>
<td>49</td>
<td>57</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>GIII</td>
<td>27</td>
<td>72</td>
<td>60</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

Stage
- pTa: 63 28% 49% 55%
- pT1: 25 63% 52% 71%
- pT2: 20 74% 65% 80%

Increased serum TPA (8.04-17.33 ng/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.

ANOVA

![ANOVA](image)

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].

![ROC Curve](image)

**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 TUR-T 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 [19] 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 serum 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 tumor 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 pT1G III 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**


