CA19.9 and CEA in Transitional Cell Carcinoma of the Bladder: Serological and Immunohistochemical Findings

AXEL HEGELE1*, VERENA MECKLENBURG1*, ZOLTAN VARGA3, PETER OLBERT1, RAINER HOFMANN1 and PETER BARTH2

1Department of Urology and Pediatric Urology and 2Institute of Pathology, University Medical Center Marburg, Marburg, Germany; 3Department of Urology, Kreisklinikum Sigmaringen, Germany

Abstract. Background: Reliable blood and urine markers for transitional cell carcinoma of the bladder (TCC) do not currently exist. The aim of this prospective study was the serological and immunohistochemical evaluation of CA19.9 and CEA in TCC and to assess the correlation with different TCC stages. Patients and Methods: CA19.9 and CEA levels were prospectively determined in samples from 231 patients with TCC suspected bladder lesions, before transurethral tumor resection. Additionally, these serum parameters were determined in 11 patients with initial metastatic TCC. Immunohistochemical analysis on CA19.9 and CEA was performed in 83 patients. Results: Neither CA19.9 nor CEA levels were significantly elevated in TCC patients. Patients with muscle-invasive TCC showed significantly higher levels of CEA (p=0.008) and CA19.9 (p<0.001) compared to those with superficial TCC. Significantly higher levels were also evident with increasing grade of malignancy. Metastatic TCC showed significantly elevated CA19.9 levels compared to muscle invasive TCC as well as locally advanced (pT3/pT4/pN+) compared to localized TCC (pT2/pN0). Immunohistochemical staining revealed a strong correlation between CA19.9 serum levels and staining intensity.

Conclusion: CEA and CA19.9 are not useful markers in primary diagnosis of TCC. However, in instances of elevated CEA and CA19.9 levels where gastrointestinal malignancy has been excluded, evidence of TCC should be sought. If elevated CEA and CA19.9 are present in TCC, serum levels correlate with tumor invasion and grade of malignancy.

*These Authors contributed equally to this work

Correspondence to: PD Dr. Axel Hegele, Department of Urology and Pediatric Urology, University Medical Center, Baldingerstrasse, 35033 Marburg, Germany. E-mail: hegele@med.uni-marburg.de

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Following prostate cancer, bladder cancer represents the second most frequent malignancy of the genitourinary tract and the ninth most common cause of cancer worldwide, with rising incidence (1). In 2008, 69,000 cases of bladder cancer were newly diagnosed and 14,000 cancer-related deaths were attributed to it (2-4). More than 90% of bladder tumors are transitional cell carcinomas (TCC); squamous cell carcinomas (5%) and adenocarcinomas (2%) are found only rarely (5). Of all newly diagnosed cases, approximately 75% present at the superficial stages (Ta, T1, Tis), 20% at the muscle-invasive stages (≥T2) and around 5% as metastatic disease. Additionally, superficial TCC will recur in 70% of cases and will progress to muscle-invasive stages in about 20% (6, 7). Early detection is required for effective treatment and various urinary, serological and molecular markers have been described for TCC (8-12). Due to low screening sensitivity/specificity and immense screening costs, none of these markers are routinely used in the clinic. The Food and Drug Administration of the USA has only accredited superficial TCC urinary tests such as the BTA and NMP22 tests (13, 14). Since no reliable markers predicting TCC presence, TCC muscle invasiveness or metastatic TCC have been established to date, cystoscopy, which causes some discomfort for the patient, still represents the gold standard in the diagnosis of TCC. Thus, it is of great interest to identify optimal, reliable, cost-effective markers for early detection of TCC and for its adequate monitoring after primary therapy.

Carcinoembryonic antigen (CEA) is an established and routinely used marker for staging and follow-up of colorectal carcinomas (15, 16). Carbohydrate-antigen 19-9 (CA19.9) has shown effectiveness as a marker of pancreatic cancer with prognostic and therapeutic significance (17, 18). In some cases, elevated levels of CEA and CA19.9 have been demonstrated in TCC of the bladder and the upper urinary tract (19, 20). Additionally these markers may be helpful predicting extravesical disease and presence of lymph node metastases in TCC (21, 22). The aim of the present study was to

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evaluate the clinical value of CEA and CA19.9 as tumor markers for the diagnosis of TCC and to determine the relationship between marker levels and histopathological results.

Patients and Methods

The study included 231 patients with TCC suspected bladder lesions (173 male, 58 female; mean age 70, range 21-93 years), enrolled between June 2008 and February 2010, after approval by the local Institutional Review Board (No.176/08).

Serum levels of CEA and CA19.9 were determined before transurethral resection (TUR-B) of a suspected bladder tumor. Patients with a medical history of colorectal and/or pancreatic cancer were excluded from the study. After tumor resection, serum levels are correlated with the histopathological findings.

Another 11 patients (9 male, 2 female; mean age 66, range 44-74 years) presented with initial metastatic TCC. Blood samples were collected in 5 ml tubes (Sarstedt, Germany) and processed for plasma within 30 min of collection. After centrifugation (2500 rpm, 10 min, 20°C) and removal of supernatant, CEA and CA19.9 were measured using a Cobas e601® analyzer (Roche AG, Germany) according to the manufacturer’s recommendations.

The pathohistological diagnosis of the resected main tumor specimen was performed by the Department of Pathology at the Medical School of Philippus University Marburg (P.B.) according to the UICC classification of 2002 (23).

CEA and CA19.9 were also detected in samples from 83 patients by standard ABC-immunohistochemistry using DAB as the chromogen. The extent of immunohistochemical staining was graded using a three tiered scale. Positivity in fewer than 25% of the tumor cells was defined to be low and cases with more than 25% and fewer than 50% of the tumor being stained were defined as intermediate expression. Those tumors with more than 50% of the tumor stained were defined to be in the high expression category.

Figure 1. Box plot showing CA19.9 and CEA serum levels in patients with TCC and in controls.

Figure 2. Box plot showing CA19.9 and CEA serum levels in patients with TCC in relation to grade of malignancy.
Statistical analysis was performed using the nonparametric Mann-Whitney U-test to compare the results of TCC patients with controls. The Kruskal-Wallis ANOVA test was used to analyze the differences between the different groups and subgroups (SPSS® for Windows, Version 17). Statistically significance was accepted to have been reached for \( p \)-values <0.05.

Results

The intra-assay variance was 1.53%-3.3% and 1.39%-1.45% for CEA and CA19.9 respectively. The inter-assay variance was 1.39%-4.08% and 1.38%-2.39% for CEA and CA19.9 respectively.

In 71 patients (51 male, 20 female; mean age 67, range 21-93 years) TUR-B and subsequent investigation of the resected tissues showed benign findings. These patients served as the control group.

Fourteen patients with malignant bladder tumors but histologically excluded TCC (squamous cell carcinoma (n=6), breast cancer metastases (n=2), prostate cancer (n=3), endocrine carcinoma (n=3)) were excluded from the analysis.

In 146 patients (115 male, 31 female; mean age 70, range 41-89 years), histological examination revealed TCC: 74% (n=108) with superficial, non-muscle invasive (NMIBC) and 26% (n=38) with muscle-invasive (MIBC) bladder cancer.

Compared to controls, patients with TCC showed neither significantly different CEA (\( p=0.234 \)) nor CA19.9 (\( p=0.061 \)) levels. Using the manufacturer’s recommended cut-off levels (CEA 5 μg/l, CA19.9 37 kU/l), a sensitivity of 15.5%/5.5% and specificity of 94.4%/98.6% was found for CA19.9 and CEA respectively.

Patients with MIBC (n=38) showed significantly elevated CEA (\( p=0.008 \)) and CA19.9 serum levels (\( p<0.001 \)) compared to NMIBC (n=108, Figure 1). No difference was found between pTa (n=70) and pT1 (n=38) in the NMIBC group. Concerning TCC grading, significantly higher CEA (\( p=0.031 \)) and CA19.9 (\( p=0.001 \)) serum levels were found with increasing grade of malignancy (Figure 2).

In locally advanced MIBC and/or in presence of lymph node metastases (≥pT3/N+, n=19), CA19.9 was significantly elevated (\( p=0.04 \)) compared to MIBC confined to the bladder muscle (pT2N0, n=19), whereas CEA was not. A total of 11 (57.9%) cases of locally advanced MIBC presented with elevated CA19.9 levels. There were no significant differences in CEA levels between these two MIBC groups (\( p=0.7 \)) and only one patient (5.3%) had an elevated serum level.

Samples from patients with metastatic TCC displayed the highest serum levels of CA19.9 (\( p=0.003 \)) but not for CEA (\( p=0.105 \)). A total of 72.2% (n=8) presented elevated CA19.9.

<table>
<thead>
<tr>
<th>Clinico-pathological parameters</th>
<th>No. of patients</th>
<th>%</th>
<th>CA19.9 (kU/l)*</th>
<th>CEA (μg/l)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>71</td>
<td>100</td>
<td>10.5 (± 12.6)</td>
<td>2 (± 1.2)</td>
</tr>
<tr>
<td>Age, years (mean ±SD)</td>
<td>67.2 (± 12.4)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>51</td>
<td>71.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>28.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCC</td>
<td>146</td>
<td>100</td>
<td>12 (± 49.2)</td>
<td>2 (± 1.6)</td>
</tr>
<tr>
<td>Age, years (mean ±SD)</td>
<td>70.3 (± 10.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>115</td>
<td>78.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>31</td>
<td>21.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMIBC</td>
<td>108</td>
<td>74</td>
<td>10 (± 19.6)</td>
<td>2 (± 1.3)</td>
</tr>
<tr>
<td>pTa</td>
<td>70</td>
<td>46.6</td>
<td>9 (± 11.7)</td>
<td>2 (± 1.1)</td>
</tr>
<tr>
<td>pT1</td>
<td>38</td>
<td>25.4</td>
<td>11 (± 29.2)</td>
<td>3 (± 1.5)</td>
</tr>
<tr>
<td>MIBC</td>
<td>38</td>
<td>26</td>
<td>24.5 (± 82.9)</td>
<td>2 (± 2.1)</td>
</tr>
<tr>
<td>pT2</td>
<td>19</td>
<td>13</td>
<td>14 (± 16)</td>
<td>2.5 (± 1.9)</td>
</tr>
<tr>
<td>pT3/pT4/N+</td>
<td>19</td>
<td>13</td>
<td>46 (± 103)</td>
<td>2 (± 1.9)</td>
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<tr>
<td>Grading</td>
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<td></td>
<td></td>
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<tr>
<td>G1</td>
<td>41</td>
<td>28.1</td>
<td>7 (± 10)</td>
<td>2 (± 1)</td>
</tr>
<tr>
<td>G2</td>
<td>68</td>
<td>46.6</td>
<td>12 (± 13.3)</td>
<td>2 (± 1.2)</td>
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<tr>
<td>G3</td>
<td>37</td>
<td>25.3</td>
<td>21.5 (± 85.3)</td>
<td>3 (± 2.3)</td>
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<tr>
<td>Met. TCC (M+)</td>
<td>11</td>
<td>100</td>
<td>63 (± 532)</td>
<td>2 (± 64.1)</td>
</tr>
<tr>
<td>Age, years (mean ±SD)</td>
<td>66 (± 10.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>81.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>18.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are given ± standard deviation. NMIBC: Non muscle-invasive bladder cancer; MIBC: muscle-invasive bladder cancer; met.TCC: metastatic transitional cell carcinoma.
and 27.3% (n=3) elevated CEA levels. Patient characteristics and CEA/CA19.9 levels are summarized in Table I.

Immunohistochemical staining in 83 patients revealed a strong correlation between staining intensity and serum levels for CA19.9 ($p=0.004$) but not for CEA ($p=0.478$). Different staining intensities are shown in Figure 3 and Table II.

Discussion

An ideal tumor marker should offer a high sensitivity and specificity, correlation with tumor mass and at the same time should indicate response to therapy. To date, no such optimal tumor marker is available for TCC. However, CEA and CA19.9 are validated and routinely used markers for colorectal and pancreatic cancer respectively (15-18). Data concerning these markers in TCC are limited. Some cases of elevated levels of these markers in TCC of the bladder and the upper urinary tract have been reported (19, 20). The current prospective study of 231 patients demonstrated that neither CEA nor CA19.9 serum levels are useful in the primary diagnosis of TCC, due to their inadequate sensitivity and specificity.

In the face of elevated CEA and CA19.9 serum levels in TCC, these prospective data clearly demonstrate a correlation with muscle-invasive disease and higher tumor grading. Significant elevated CEA and CA19.9 serum levels were found in muscle-invasive TCC compared to superficial disease. Additionally patients with locally advanced disease or lymph node metastases (≥T3/N+) presented significantly higher CA19.9 levels. These findings are in agreement with the data of Margel et al., who described significantly elevated CA19.9 levels in locally advanced TCC (n=45) and lymph node metastases (n=17) compared to muscle-invasive disease. In multivariate analysis they also postulated that CA19.9 serum level is a predictor of lymph node decay (21, 24). Similar results were presented in a study by Pectasides et al., which determined CEA and CA19.9 levels in 76 patients with different TCC stages (22). In 28 patients with locally advanced disease, 39.2% had elevated CEA and 35.7% elevated CA19.9 levels. Higher CEA and CA19.9 levels were found in samples from patients with metastases due to TCC (n=48), 41.6% and 39.5%, respectively. In the current cohort of 157 patients with TCC, 19 presented with
locally advanced disease. Only one patient (5.3%) had an elevated CEA level in contrast to 11 patients (57.9%) with elevated CA19.9 levels. In patients with metastatic disease, CEA was elevated in three (27.3%) cases and CA19.9 in eight (72.2%) cases. These different findings maybe explained by the different patient numbers included in the studies and by the distinct extent of CEA and CA19.9 levels in TCC. Additionally, Pectasides et al. described significant changes of CA19.9 levels depending on different responses to therapy (22). The correlation of CA19.9 level and in addition of CEA level, with response to therapy was confirmed by Cook et al. in 74 patients (25).

Chuang et al. tested the expression of CA19.9 in TCC cell lines and 42 TCC tissue samples (26). Using immunohistochemistry they found negative CA19.9 staining in 70% of high-grade and 71% of invasive TCC cases. These data are in contrast to the immunohistochemical and serological findings of the current study. This study is the first to present data showing a positive correlation of CA19.9 serum levels with staining intensity, verifying TCC as the source of CA19.9 production. Additionally, CA19.9 was significantly elevated in serum of patients with high-grade TCC. Chuang et al. did not correlate immunohistochemical findings with serological parameters. Kurokawa et al. did not find a correlation of serum CA19.9 levels to either grade and stage or to staining property (27). These differences may be explained by the low number of examined TCC patients (n=43) in their study compared to the current patient cohort and the improved technical ability of laboratory equipment since previous studies were published. Moreover, discrepancies between various studies concerning this topic are likely to be due to methodological aspects such as staining technique and definition of thresholds for the assessment of immunohistochemical reactions.

In conclusion, the current prospective data obtained from a large number of patients clearly showed that neither CEA nor CA19.9 serum levels are useful tools in the primary diagnosis of TCC. When CEA and CA19.9 serum levels are elevated during routine examination and a gastrointestinal malignancy can be excluded, one should be mindful of the existence of CEA/CA19.9-producing TCC and thus examine the urologic tract. These data show a correlation of CEA and CA19.9 serum levels with stage and grade of TCC disease confirmed by immunohistochemical findings. CA19.9 is more promising than CEA. The utility of CA19.9 serum levels monitoring response to therapy should be checked in further studies as well as role of CA19.9 as a prognostic factor in TCC.

References


Table II. CEA and CA19.9 serum levels categorized by staining intensity group.

<table>
<thead>
<tr>
<th>Staining Intensity</th>
<th>CA19.9 (μg/l)</th>
<th>CEA (kU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (n=45)</td>
<td>9 (± 20.6)</td>
<td>3 (± 2.5)</td>
</tr>
<tr>
<td>Intermediate (n=20)</td>
<td>22 (± 37.4)</td>
<td>2 (± 1.7)</td>
</tr>
<tr>
<td>Strong (n=17)</td>
<td>63 (±108.7)</td>
<td>3 (± 14.9)</td>
</tr>
</tbody>
</table>

Data are given ± standard deviation.

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