Abstract. Aim: To compare nuclear matrix protein 22 expression by BladderChek® and ELISA, as urine-based assays for bladder cancer (BC) detection. Patients and Methods: Urine samples of 100 BC patients and 100 controls were analyzed. Comparative statistical evaluations were based on sensitivity and specificity. Results: Seventy-one patients had primary and 29 recurrent BC. The sensitivity of BladderChek® was significantly higher compared to ELISA in the overall cancer cohort and in patients with primary BC (p<0.0001 and p=0.0001, respectively). Both tests demonstrated significant correlation of sensitivities and tumor stage/grade for the overall cancer cohort and for patients with primary BC. Both tests had specificity values of 100% in healthy individuals. Specificity was 93% for BladderChek® and 99% for ELISA in patients with benign diseases (p=0.048). Conclusion: BladderChek® may be clinically more useful for BC detection. Due to high specificity, BladderChek® could be used for high-risk screening. However, due to its low sensitivity, BladderChek® cannot replace but only complement cystoscopy for BC detection.

The gold standard for bladder cancer (BC) detection is cystoscopy, including fluorescence-guided techniques, in combination with cytology. Moreover, various urine-based tumor markers have been investigated as diagnostic tools (1-5).

Nuclear matrix proteins (NMP) are part of the structural frame of the nucleus (6). In urothelial cancer cells, protein expression of NMP22 is strongly increased. In addition, increased apoptotic and necrotic potential of urothelial tumor cells results in release of NMP22 into urine. Therefore, patients with BC display high NMP22 concentrations, creating a rationale for using NMP22 as a tumor marker (6).

Originally, NMP22 detection was achieved by a quantitative enzyme-linked immunosorbent assay (ELISA) in a laboratory, with obvious disadvantages and limitations, including: i) different cut-off values, ii) bench work required, iii) results not immediately available, and iii) results dependent on examiner’s experience (7). Consequently, a qualitative point-of-care version, the NMP22 BladderChek® was developed, which utilizes monoclonal antibodies to detect NMP22 that is elevated in BC cells. The test provides an absolute positive or negative result within 30 min.

The present study aimed to prospectively compare the diagnostic efficacy of the urine-based BladderChek® and ELISA for BC detection. A comparison of the diagnostic efficacy of these tests would help urologists to decide which test is most appropriate in their daily routine and whether the BladderChek® is sufficient for primary detection and follow-up of BC. Thus, our study provides additional evidence regarding the optimal use of NMP22 for BC detection.

Patients and Methods

Patients. From December 2003 to February 2006, patients with newly-diagnosed BC or with history of BC were included in this prospective study at the Department of Urology, Technische Universität München, Klinikum rechts der Isar, Munich, Germany. Urine samples of these pre-selected patients with histopathologically-confirmed transitional cell carcinoma (TCC) of the bladder were collected before transurethral resection. NMP22 ELISA and BladderChek® (both tests were kindly provided by Alere, Scarborough, Maine, USA) were employed according to the manufacturer’s guidelines in a parallel design. Tumor grade was classified using the 2004 World Health Organization/International Society of Urological Pathology consensus classification (8). Tumor stage was classified according to the 2002 TNM classification, approved by the Union for International Cancer Control (UICC) (9). A cohort of 30 healthy individuals and 70 patients with benign
urological diseases, such as hydrocele, spermatocele and urinary incontinence, served as controls. In lieu of a formal ethics committee, the principles of the Helsinki Declaration were followed. All participants received study information, and written informed consent was obtained from all patients and healthy controls. Regarding patients with BC, three subgroups were analyzed: (i) overall cancer patient group, (ii) patients with primary cancer and (iii) patients with recurrent BC.

**Exclusion criteria.** NMP22 is a non-specific protein that is released by high cell turnover. Various conditions can lead to increased NMP22 values in the absence of cancer and can cause false-positive results. Therefore, patients with urinary tract infections, foreign material in the urinary tract, urolithiasis, other carcinoma except TCC, previous intravesical instillation (chemotherapy, BCG) and urinary diversion were excluded.

**Urine examination.** Urine samples were obtained from all patients in the morning (midstream, second-void urine sample) and processed immediately. The samples for the ELISA were stored at −80°C and the morning (midstream, second-void urine sample) and processed.

**Statistical analysis.** Sensitivity and specificity values were monitored and classified in different patient groups and healthy individuals. The comparison of groups with respect to sensitivity and specificity was performed using the $\chi^2$-test, and comparisons between the ELISA and BladderChek were made using McNemar’s test. The comparison of quantitative markers was performed using the Kruskal Wallis test and Mann Whitney U-test, where appropriate. The level of significance was two-sided and set to 5% (p-value <0.05). A sample size of 94 pairs will have 80% power to detect a difference in proportions of 0.2 (i.e., a difference of 20% in sensitivity of the ELISA and BladderChek when the proportion of discordant pairs is expected to be 0.5 and the method of analysis is a McNemar’s test of equality of paired proportions with a 0.05 two-sided significance level. Statistical analyses and sample size calculation were carried out with IBM SPSS Statistics 19, StatXact version 5 (Cytel Software Corporation, Cambridge, Massachusetts, USA) and nQuery Advisor version 7 (Statistical Solutions, Saugus, Massachusetts, USA), respectively.

**Results**

One hundred Caucasian BC patients were included (71% male, 29% female). The patient and control subgroups showed no significant difference in age and sex. Table I lists characteristics of the studied cohort.

<table>
<thead>
<tr>
<th>NMP22 ELISA results (absolute values).</th>
<th>Cancer patients</th>
<th>Overall</th>
<th>Primary BC</th>
<th>Recurrent BC</th>
<th>Patients with benign urological diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pts. (n)</td>
<td>100</td>
<td>71</td>
<td>29</td>
<td>70</td>
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<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>67</td>
<td>65</td>
<td>68</td>
<td>55</td>
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</tr>
<tr>
<td>Range</td>
<td>61-73</td>
<td>60-71</td>
<td>63-75</td>
<td>49-71</td>
<td>48-69</td>
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<td>T-stage (n)</td>
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<td>pTis</td>
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<td>4</td>
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<td>Grade (n)</td>
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<tr>
<td>G1</td>
<td>37</td>
<td>22</td>
<td>15</td>
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<tr>
<td>G2</td>
<td>34</td>
<td>25</td>
<td>9</td>
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<tr>
<td>G3</td>
<td>29</td>
<td>14</td>
<td>15</td>
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<tr>
<td>Mean (U/ml)</td>
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<td>5.1</td>
<td>5.5</td>
<td>2.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Median (U/ml)</td>
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<td>51.5</td>
<td>22.7</td>
<td>3.3</td>
<td>2.1</td>
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<td>95% CI</td>
<td>19-68</td>
<td>18-85</td>
<td>4-42</td>
<td>2.7-3.8</td>
<td>1.4-2.7</td>
</tr>
</tbody>
</table>

**Sensitivity of NMP22 ELISA and NMP22 BladderChek.**

Employing the ELISA, we detected sensitivity values of 40%, 42% and 34% for the overall cancer cohort, primary and recurrent cancer groups, respectively. In the overall cancer patient group and in patients with primary BC, we observed a significant correlation between the ELISA sensitivity and tumor stage and grade. Increased sensitivity values were detected for cancer with advanced T-stage (pTis/pTa vs. pT1/pT2-4) and grade (G1 vs. G2/G3). BladderChek demonstrated sensitivity of 59% in the overall patient cohort. The respective values for patients with primary and recurrent BC were 63% and 48%. In line with ELISA results, BladderChek sensitivity values significantly increased with advanced T-stage and grade.

Sensitivity values of the BladderChek in the overall cancer cohort and in patients with primary BC were significantly higher than those obtained with the ELISA ($p<0.0001$ and
Sensitivities of the two tests in patients with recurrent BC were not significantly different ($p=0.059$).

**Specificity of NMP22 ELISA and NMP22 BladderChek®.** In healthy individuals, specificity values of 100% were determined with both tests. There was a borderline significant difference between the two NMP22 tests in the control group of patients with benign diseases. Specificity values of 93% for BladderChek® and 99% for ELISA were measured in this group ($p=0.048$).

**Discussion**

According to a recent review by Mowatt et al., NMP22 is the most applied FDA-approved urine-based tumor marker for BC detection and follow-up (1). In our study, we performed a head-to-head comparison of BladderChek® with the ELISA.

**NMP22 ELISA.** In literature, ELISA sensitivity in primary BC ranges from 44% to 100%, accompanied by specificity of between 60% and 95% (7). In our study, sensitivity of ELISA in the overall cancer cohort and primary BC group was 40% and 42%, respectively. Impressively, specificity values were 100% for healthy individuals and 99% for patients with benign diseases. Multiple reasons may explain differences between our results and those in the literature, such as different patient populations, pre-selected patients, pre-analytical steps, assay performance and use of different cut-off values. The cut-off recommended by the manufacturer is 10 U/ml. Literature results are based on cut-offs of between 6.4 and 20 U/ml, which affects sensitivity and specificity (10, 11). Although flexible cut-off values may allow for tailored use, results cannot be compared.

In our study, sensitivity of ELISA in patients with recurrent BC was 34%. In contrast, Soloway et al. found sensitivity of 70% for ELISA in patients with recurrent BC (11). Shariat et al. demonstrated sensitivity of ELISA of 54% in a cohort of 302 patients with recurrent BC (10). On this regard, the low number of patients with recurrent BC in our study might explain the low sensitivity values, fact which must not be overrated.

Various studies have included patient populations with multiple characteristics such as patients with or without history of BC, i.e. patients before transurethral resection and patients under surveillance (12, 13). The ability of NMP22 to detect BC is higher in patients with primary cancer. Recurrent BC is often found at lower stage and grade than primary cancer and therefore tumors release less NMP22 into the urine. To improve the study design and allow for multicenter comparisons, future studies should analyze equivalent subgroups of primary and recurrent BC.

**NMP22 BladderChek®.** Our results with BladderChek® are in line with the literature. Grossman et al. applied...
BladderChek® for the screening of 1,331 patients; sensitivity was 56% and specificity 86% (14). Moreover, Moonen et al. reported sensitivity of 57% and specificity of 89% with the BladderChek® in 73 patients with recurrent BC (12).

Only limited data are available for comparison of the two NMP22 tests (15-17). Hautmann et al. included 52 patients with BC and 23 controls; sensitivity values were 87% and 85% for ELISA and BladderChek®, respectively; specificity values were 83% and 91% for these tests, respectively (16). In contrast to our results, sensitivity and specificity values did not differ significantly between the two tests. Moreover, sensitivities were relatively high. These discrepancies might be attributed to the retrospective study design, small patient number and lack of exclusion criteria (kidney/testicular cancer). These malignancies can increase urinary NMP22, and therefore may cause false-positive results. Those patients must be excluded. In our study, specificity of the ELISA was higher compared to BladderChek®. However, this borderline significance must not be overrated.

**Relationship between NMP22 sensitivity and tumor stage/grade.** As in our study, many series have shown that sensitivity of urine-based NMP22 in patients with BC varies according to tumor stage and grade. Soloway et al. reported 100% sensitivity for invasive BC and 71% for superficial BC in the clinical use of combinations of the available diagnostic tools might be beneficial to determine the most accurate diagnosis of BC.

**Conflicts of Interest**

None.

**References**