In human beings, 90% of urinary bladder neoplasms are transitional cell carcinoma (TCC). Among these tumors, about 70 to 80% are confined to mucosa or only invade to the lamina propria. These superficial tumors can be treated easily by transurethral resection or simple fulguration. However, 50 to 70% recurrence rate or second primary cancer is estimated within 5 years and, most lesions occur within the first year. Additionally, up to 10 to 20% of these superficial tumors will progress to invasive disease. Hence, regular follow-up and early detection are important key events of disease care.

In current clinical practice, cystoscopy and voided-urine cytology are routine diagnosed methods for survey of new or recurrent urothelial cancer. However, tumor surveillance by cystoscopy is invasive and difficult to perform in some circumstances, although flexible cystoscopes have made the procedure less uncomfortable. Voided-urine cytology is a non-invasive adjunct to cystoscopy for follow-up of patients with transitional cell carcinoma. Unfortunately, urine cytology is insensitive for low-grade tumor lesions. Only about 40% of well- to moderately differentiated tumors are diagnosed by urine cytology. More over, in terms of the tumor status by urine cytology is subjective, and the accuracy of...
of diagnosis of the disease depends on the experience of the cytopathologist.

Recently, several new tumor markers have been developed, such as bladder tumor antigen (BTA), fibrinogen degradative products, telomerase, hyaluronic acid, Lewis X, cytokeratin 20 and nuclear matrix protein (NMP).\(^5\)\(^-\)\(^11\) NMP consists of the insoluble structural framework of the nucleus, which includes the nuclear lamina and pore complex, internal ribonucleic protein network and residual nucleoli.\(^12\) These proteins play important roles in DNA topological organization, replication and transcription.\(^13\) Certain NMPs have been discovered as cancer-specific markers in colon, breast, bone, and prostate cancers.\(^14\) It has been shown that the concentration of the intracellular NMP22 in TCC is at least 25-fold greater than in mean level isolated from normal bladder urothelium.\(^15\) In our study, we compared the sensitivity and specificity of pathocytology and NMP22 to detect bladder TCC and assessed the correlation of NMP22 value with tumor grading and staging.

**METHODS**

Seventy-four patients were recruited between June, 2000 and May, 2001, as well as 18 healthy volunteers, totaling 92 subjects. There were 53 males and 39 females. These subjects were classified into 3 groups. Group 1 comprised 28 patients who had received transurethral resection of the urinary bladder TCC at least 3 months prior to enrollment. Group 2 consisted of 46 patients that suffered from either gross or microscopic hematuria. Group 3 was 18 volunteers who were not under physician care for any condition and had no symptoms. All subjects except the healthy volunteers had received intravenous urography to rule out upper urinary tract neoplasm. Urine analysis and urine pathocytology were obtained from all subjects. Those subjects that were disclosed with pyuria were excluded. Meanwhile, voided urine was collected separately in a NMP22 test kit (Matritech, Inc., New ton, Mas sa chu setts, USA.) containing a tainer containing urine stabilizers. The samples were frozen to -80°C immediately after collection. Subsequent cystoscopy was performed in Group 1 and Group 2. For a suspicious lesion, either simple biopsy or transurethral resection was undertaken. Staging of the bladder carcinoma was according to TNM criteria, and the World Health Organization grading system was used for tumor grading. Those patients that had no suspicious lesion found by cystoscopy or who had benign lesions revealed by pathology were considered true negative. Patients proved carcinomapathologically were considered true positive.

All collected urine samples were processed for NMP22 assay. Calibrators, controls and subjects urine samples were added to antibody-coated wells. After washing, a second antibody labeled with digoxigenin was added to react with the captured NMP antigen. Then the digoxigenin-labeled antibody was washed, reacted with an anti-digoxigenin antibody coupled to horseradish peroxidase and detected using o-phenyldiamine substrate. The concentration of NMP antigen in urine was proportional to the intensity of color development, and concentration of NMP22 in each sample was calculated from a standard curve.

A receiver operating characteristics (ROC) curve was constructed to determine NMP22 cutoff value for optimal sensitivity and specificity in detecting bladder tumor. Analyses to comparing the NMP22 values in 2 subgroups were done with Student-t test.

**RESULTS**

Twenty-four patients were disclosed to have TCC of the urinary bladder diagnosed by pathology and 68 were reported as negative. The mean ages of these patients were 69.1 years and 60.5 years, respectively. Of TCC cases, 9 patients were recurrent status and the other 15 patients were found for the first time. Pathologic staging revealed superficial stage (Ta and T1) in 19 cases and in invasive stage (above T2) in 5 cases. Ten of 24 TCC patients had low-grade (grade I and grade II) tumors and 14 patients had high-grade (grade III) carcinoma. Nine cases had single lesion and 15 cases had multiple lesions.

The ROC curve was created to determine the reference value of NMP22. Interpretation of the curve showed an optimal cutoff value of greater than 10 units per mL in differentiating positive from negative results (Fig. 1).
Using 10 units per mL as a cut off, the sensitivity was 91.7% (22/24) and the specificity was 72.1% (49/68). As shown in Table 1, the sensitivity and specificity of cytology were 37.5% (9/24) and 97.1% (66/68), respectively. The positive predictive value and false positive rate are also presented in Table 1.

The distribution of NMP22 levels according to various parameters of pathologically proven bladder cancer are summarized in Table 2. Both the median NMP22 values of invasive staging (82.0 U/mL) and high-grade (70.1 U/mL) tumors were greater than those of superficial staging (62.8 U/mL) and low-grade (62.8 U/mL) tumors. However, the significance test in staging, grading and multiplicity showed no statistical significance.

We compared the sensitivity of NMP22 to cytology in terms of different stage, grade and multiplicity of tumors (Table 3). Of 10 patients with low-grade tumor, NMP22 test had 8 cases with true positive. Among the high-grade cases, 13 of 14 patients had true positive NMP22 value. The sensitivities were 80% and 93%, respectively. On the other hand, the cytology detection rates were 25% and 50%, respectively, according to low-grade and high-grade. NMP22 had sensitivity of 84% (16/19) for superficial lesions and 100% (5/5) for invasive lesions, and the sensitivities of cytology

---

**Table 1. Comparison of NMP22 and urine cytology**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>False positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMP22</td>
<td>91.7</td>
<td>72.1</td>
<td>53.7</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td>(22/24)</td>
<td>(49/68)</td>
<td>(22/41)</td>
<td>(19/68)</td>
</tr>
<tr>
<td>Cytology</td>
<td>37.5</td>
<td>97.1</td>
<td>81.8</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>(9/24)</td>
<td>(66/68)</td>
<td>(9/11)</td>
<td>(2/68)</td>
</tr>
</tbody>
</table>

**Table 2. Correlation of NMP22 level to various parameters of TCC**

<table>
<thead>
<tr>
<th>Category</th>
<th>NMP22 levels, U/mL</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SD</td>
<td>median</td>
</tr>
<tr>
<td>Stageb</td>
<td>Superficial</td>
<td>149.97 ± 207.30</td>
</tr>
<tr>
<td></td>
<td>Invasive</td>
<td>100.44 ± 78.71</td>
</tr>
<tr>
<td>Gradec</td>
<td>Low grade</td>
<td>144.67 ± 204.34</td>
</tr>
<tr>
<td></td>
<td>High grade</td>
<td>136.08 ± 182.29</td>
</tr>
<tr>
<td>Multiplicityd</td>
<td>Single</td>
<td>167.70 ± 216.95</td>
</tr>
<tr>
<td></td>
<td>Multiple</td>
<td>122.85 ± 173.28</td>
</tr>
</tbody>
</table>

TCC = Transitional cell carcinoma.

b Tumors invading no more than the lamina propria were classified as superficial lesions and those invading beyond the lamina propria were classified as invasive.

c Low grade includes grade I and grade II, high grade means grade III.

d Multiplicity means grade I and grade II, high grade means grade III.

NS = No significance.
were 32% (6/19) and 60% (3/5), respectively. For multiplicity, 9 of 24 tumor patients were single-lesion and the remaining 15 cases were multiple-lesion. Similarly, the NMP22 had higher detection rate for multiple-lesion. Of 51 patients with low NMP22 level (<10 U/mL) and negative urine cytology, only one patient was found to have TCC.

**DISCUSSION**

In our study, we compared NMP22 with cytopathology in detection of the urinary bladder TCC, either recurrence or first episode. When using 10 U/mL as a cut off, the NMP22 sensitivity was 91.7% and the specificity was 72.1%. Comparing to cytology, which had sensitivity of 37.5% and specificity of 97.1%, the NMP22 test as say had higher sensitivity but much lower specificity. At the same time, lower positive predictive value (53.7% vs 81.8%) was observed in NMP22 test as say with high false positive rate (27.9% vs 2.9%). Soloway et al. reported in 1996 that the sensitivity, specificity and positive predictive value were the drawback of this new tumor marker as say. There is in ade quate evidence to consider total replacement of cytology with NMP22. But combination of NMP22 and cytology could be a proper way to avoid unnecessary cystoscopic surveillance.

**REFERENCES**


