Bladder Cancer

Validation of the Diagnostic Value of NMP22® BladderChek® Test as a Marker for Bladder Cancer by Photodynamic Diagnosis

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Abstract

Objectives: The aim of the present study was to validate the sensitivity and specificity of the new “point-of-care” NMP22® BladderChek® test compared to photodynamic diagnosis (PDD).

Methods: Voided urine samples from 100 patients with suspicion of bladder cancer were collected to perform the NMP22® BladderChek® test and voided urinary cytology. The nuclear matrix protein 22 (NMP22) levels were measured by a lateral flow immunochromatographic qualitative assay, using 10 U/ml as the cut-off value. Subsequently patients underwent PDD, using 5-aminolevulinic acid or hexyl-aminolevulinate; previous bladder washings for cytology were collected. Sensitivity and specificity of the NMP22® BladderChek® test were compared with cytology and PDD.

Results: Forty of the 100 patients had urothelial malignancies (22 pTa, 4 pT1, 3 pT2, 9 carcinoma in situ, 2 pTx; 16 G1, 6 G2, 18 G3). The sensitivity was 65% for the NMP22® BladderChek® test, 44% for voided cytology, 75% for washing cytology, and 93% for PDD. Specificity rates were 40%, 78%, 62%, and 43%, respectively. Positive predictive values were 0.42, 0.58, 0.53, and 0.52 and the negative predictive values 0.63, 0.68, 0.82, and 0.9, respectively.

Conclusions: The results demonstrate that the NMP22® BladderChek® is an easily applied test, giving diagnostic findings within 30 min. However, validated by the highly sensitive PDD, the NMP22® BladderChek® test demonstrates poor specificity and sensitivity and, therefore, cannot be recommended for screening or surveillance in daily clinical routine use. Further studies with careful patient selection are necessary to identify the patient population that might benefit from the NMP22® BladderChek® test.

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1. Introduction

Bladder cancer is the sixth most frequent malignant disease in the world (American Cancer Society 2005, www.cancer.org). On first diagnosis 75–85% of the cases show non–muscle-invasive stages. Fifty to 70% of these patients with superficial tumours have one or more recurrences after the initial treatment and in about one third a progression is observed. Therefore, a frequent and long-term follow-up screen is essential.

For many decades, cystoscopy and cytology have been the main diagnostic tools and can be considered as the current “gold standard” for initial diagnosis and surveillance. However, cystoscopy is costly, invasive, and uncomfortable. In addition, urine cytology is of limited value due to dependency on cytopathologists and a low sensitivity, especially for low-grade lesions. Hence, many noninvasive marker tests have been investigated extensively in recent years, for example, BTAstat, BTAttrak, fibrin/fibrinogen degradation product (FDP), ImmunoCyt, fluorescence in situ hybridisation (FISH; UroVysion), and nuclear matrix protein (NMP22), which is approved by the US Food and Drug Administration. Many studies have investigated the potential of the quantitative enzyme immunoassay NMP22 test, reporting sensitivities of 44–100% and specificities of 60–95% [1]. Nevertheless, this test has to be done in the laboratory and does not provide the urologist of the patient with an immediate result, which is preferred to initiate instant management.

The present study investigated the efficacy of the new “point-of-care” (POC) version of the NMP22® bladder cancer test—the NMP22® BladderChek® test—compared with photodynamic diagnosis (PDD), which has proven to be the most sensitive endoscopic detection method to date [2], and compared its diagnostic value against urine and bladder washing cytology.

2. Materials and methods

2.1. Patients

From September 2004 to April 2005, 100 patients (29 women, 71 men, mean age, 67.9 yr) were included in the study. Seventy patients had a history of recurrent bladder cancer (Table 1) and 30 patients were included due to suspicion for bladder cancer (haematuria, urgency, suspicious cytology). For exclusion of upper urinary tract tumours all patients underwent ultrasound. Intravenous pyelography, computed tomography scanning, or retrograde ureteropyelography were performed in patients with primary tumour suspicion or unclear findings in the ultrasound.

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTa</td>
<td>46</td>
</tr>
<tr>
<td>pTis</td>
<td>18</td>
</tr>
<tr>
<td>PT1</td>
<td>8</td>
</tr>
<tr>
<td>PT2</td>
<td>4</td>
</tr>
</tbody>
</table>

2.2. NMP22® BladderChek® test

The NMP22® BladderChek® test is an immunochromatographic assay using monoclonal antibodies in a lateral flow strip, detecting the nuclear matrix protein NMP22 with a cut-off value of 10 U/ml (Matritech, Germany). Two different antibodies are used in the POC assay, one as a capture antibody and one as a reporter. Four drops of freshly voided urine (previously maintained at least 2 h in the bladder) are added to the sample well to perform the test. If NMP22 is present in the urine at a concentration of >10 U/ml, it will interact with the monoclonal antibodies and can subsequently be visualised by binding to the secondary antibodies.

2.3. Procedure

Patients were asked to maintain the urine in the bladder for about 2 h. Prior to the endoscopic procedure, freshly voided urine was sampled for urine cytology, NMP22® BladderChek® bladder tumour test, and sediment analysis. Subsequently, 1.5 g 5-aminolevulinic acid (5-ALA; Medac, Wedel, Germany) or hexyl-aminolevulinate (Hexvix®, Photocure ASA, Oslo, Norway) dissolved in 50 ml 5.7% sodium monohydrogenphosphate was instilled intravesically. Bladder washing for cytology was collected before PDD and white light endoscopy (WLE) was performed as described before [3].

Cytopathologic analysis was considered positive when malignant cells were present in the Papanicolaou-stained samples. Histologic diagnoses of biopsies were carried out according to the TNM classification (World Health Organization 1997) by two independent pathologists who were blinded for the results of the NMP22® BladderChek® and PDD.

Exclusion criteria were bowel interposition, invasive procedures within the last 14 d, other urogenital tumours, foreign bodies (e.g., stones, stents, nephrostomies, etc), and urinary tract infection. In case of pathologic urine sediment, significant bacteriuria was excluded by bacteriologic culture. Furthermore, patients had not been subject to intravesical installation for at least 4 wk prior to the examination.

2.4. Statistics

The values for sensitivity and specificity and positive and negative predictive values of the different tests were determined based on the results of the histologic examination. Different groups of patients were formed: patients presenting with primary suspicion for bladder cancer and patients for follow-up.
3. Results

One hundred freshly voided urine samples were collected. Forty patients (40%) showed a histologically confirmed urothelial malignancy. Table 2 summarises the histologic findings according to the TNM classification and grading. In Table 3 the sensitivity and specificity of the different tests are summarised. WLE, PDD, NMP22® test, washing cytology, and urine cytology detected these tumours with a sensitivity of 88%, 93%, 65%, 76%, and 44%, respectively, whereas specificity was 45%, 43%, 40%, 62%, and 78%, respectively. The positive and negative predictive values of WLE, PDD, NMP22, washing cytology, and urine cytology were 0.51, 0.42, 0.53, and 0.58 and 0.84, 0.90, 0.63, 0.82, and 0.68, respectively. Twenty-one of the 100 patients had microhaematuria in the sediment, and using microhaematuria as a marker for bladder cancer, sensitivity was 38% and specificity 83%.

Table 2 – Patient characteristics: bladder tumour stage and grade

<table>
<thead>
<tr>
<th>Bladder tumour stage</th>
<th>Stage</th>
<th>pTa</th>
<th>pT1</th>
<th>≥pT2</th>
<th>pTis</th>
<th>pTx</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>No bladder cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td></td>
<td>16</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Grade 2</td>
<td></td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Grade 3</td>
<td></td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>22</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>2</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 3 – Sensitivity and specificity of the different diagnostic devices

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WLE</td>
<td>88 (35/40)</td>
<td>45 (27/60)</td>
<td>51</td>
<td>84</td>
</tr>
<tr>
<td>PDD</td>
<td>93 (37/40)</td>
<td>43 (26/60)</td>
<td>52</td>
<td>90</td>
</tr>
<tr>
<td>NMP22</td>
<td>65 (26/40)</td>
<td>40 (24/60)</td>
<td>42</td>
<td>63</td>
</tr>
<tr>
<td>Washing cytology</td>
<td>76 (26/34)</td>
<td>62 (37/60)</td>
<td>53</td>
<td>82</td>
</tr>
<tr>
<td>Voided cytology</td>
<td>44 (15/34)</td>
<td>78 (40/51)</td>
<td>58</td>
<td>68</td>
</tr>
</tbody>
</table>

PPV = positive predictive value; NPV = negative predictive value; WLE = white light endoscopy; PDD = photodynamic diagnosis; NMP22 = nuclear matrix protein 22.

The necessity of a new noninvasive marker for the diagnosis and surveillance of bladder cancer is evident. Cystoscopy, which is considered to be the gold standard, is invasive, morbidity producing, and costly. Cytology lacks diagnostic efficacy due to its observer variability and to its low sensitivity, especially in low-risk tumours [4]. Initial studies with markers like NMP22 are for the most part promising; however, successive reports often fail to show comparable results. In a recently published meta-analysis, van Rhijn et al. reported a sensitivity of the NMP22® BladderChek® in different subgroups of patients. Moreover, an additional group was formed with patients who had no instillation therapy within the prior 2 yr. Table 5 shows the sensitivities of NMP22 compared with the invasive methods for the different tumour stages and grades.

Regarding the histologic findings of the patients in whom the NMP22 was false positive (n = 36), only 7 showed regular urothelium. The remaining showed chronic inflammatory lesions (chronic erosive cystitis, n = 6; chronic scarred cystitis n = 6; chronic follicular cystitis n = 4; and chronic unspecific cystitis n = 2), scar (n = 3), hyperplasia (n = 3), metaplasia (n = 2), or papillomas (n = 3).

4. Discussion

The necessity of a new noninvasive marker for the diagnosis and surveillance of bladder cancer is evident. Cystoscopy, which is considered to be the gold standard, is invasive, morbidity producing, and costly. Cytology lacks diagnostic efficacy due to its observer variability and to its low sensitivity, especially in low-risk tumours [4]. Initial studies with markers like NMP22 are for the most part promising; however, successive reports often fail to show comparable results. In a recently published meta-analysis, van Rhijn et al. reported a sensitivity of the NMP22 ranging between 47% and 100% and specificity between 55% and 98% [5]. The divergence in the reported sensitivity and specificity could be...
attributed to patient selection and to varying cut-off values between 7.5 and 12.5 U/ml. Considering the sensitivity of the test, the authors concluded that the NMP22 cannot reduce the number of follow-up cystoscopies.

Until recently, the NMP22 test was available as a quantitative enzyme-linked immunosorbent assay that had to be performed in the laboratory. The newly developed, easy to use NMP22 BladderChek test is a lateral flow immunochromatographic qualitative assay with a cut-off value of 10 U/ml, presenting as a rapid device (providing a result within 30 min).

Very few studies using this test have been published at this time. Grossmann et al. [6] used the NMP22 BladderChek as a screening device in 1331 patients without history of bladder cancer but with an increased risk (smokers and patients with dysuria and haematuria). They found a sensitivity and specificity of 55.7% and 85.7%, respectively, and considered the test as a useful tool supplementary to cystoscopy. Similarly, Tomera et al. [7] reported an improved performance of cystoscopy in combination with the NMP22 test and also reported a higher sensitivity at every disease stage compared to cytology.

Recently Moonen et al. [8] reported an improved sensitivity compared to cytology in 73 patients during follow-up of recurrent bladder cancer (57.1% vs. 42.9%). Nevertheless, in this study negative test results in patients with Ta, grade 1 tumours were not scored as false, thus improving the overall sensitivity. The NMP22 BladderChek showed a specificity of 89.8%.

However, the assessment of a noninvasive test depends on the sensitivity of the endoscopic detection device in use. The limitation of standard cystoscopy became evident in studies comparing standard white light cystoscopy with fluorescence endoscopy, often referred to as PDD. PDD had increased detection and decreased recurrence rates after fluorescence-guided tumour resection [2]. In a previous study, Lipinsky et al. [9] had shown that the effectiveness of the BTAnstat test seems to be reduced in comparison with PDD; 2 of 14 high-grade flat lesions had been missed by the BTAnstat test. In the present study, 8 of 16 G1 tumours and 4 of the 18 high-risk (G3) lesions were not detected by NMP22, resulting in a sensitivity of 65%, which is similar to that recently observed by Grossmann et al. and by Moonen et al.

Specificity of PDD is known to be low (43%) and leads to numerous unnecessary biopsies. Its high sensitivity, however, ensures reliable removal of all tumour lesions, which has been extensively studied in the past [10–12]. In this study, PDD led to additional diagnosis of two tumours, both of which were high-grade carcinoma in situ. In comparison to former studies, the benefit of PDD is rather low, which may be attributed to the fact that in the present population only a few patients showed carcinoma in situ. Previous studies proving the benefit of PDD dealt with many more patients with carcinoma in situ [3,10]. So the positive effect of PDD may statistically be impaired by the absolute number of patients and tumours. NMP22 was initially reported to be a promising noninvasive marker with high specificity, which would avoid unnecessary invasive procedures. However, its specificity of 40% in the present study is very low, compared to the specificity reported by Grossmann (85.7%). One reason for these discrepancies could be that NMP22 was used in the study of Grossman for screening in a population of patients with voiding symptoms or risk factors for developing bladder cancer, but no patient had a history of bladder cancer. In contrast, 70% of the patients had recurrent tumour in the present study. In a similar population, Moonen reported a specificity of 89.8% in the patient subgroup with recurrent disease; unfortunately, no information was provided on former intravesical therapies. Thirty percent of our patients, who did not show malignancy histologically, had chronic inflammatory lesions (n = 18). Therefore, the low specificity could be referred to former manipulation (transurethral resection, instillation), although formal exclusion criteria were considered. An impairment of specificity of NMP22 by benign inflammatory conditions was described previously. Chang et al. [13] reported a positive predictive value of 21.2% using a cut-off value of 7.5 U/ml with elevated NMP22 levels in patients with

| Table 5 – Sensitivity of NMP22 and the invasive methods according to the tumour grading and stage |
|----------------------------------|----------------|----------------|----------------|----------------|----------------|
|                                  | G3             | G1-2           | Superficial (Ta) | Superficial (Tis) | Invasive (T1) | Invasive (≥T2) |
| NMP22                           | 78             | 55             | 55             | 78             | 50             | 100            |
| WLE                             | 78             | 95             | 95             | 56             | 100            | 100            |
| PDD                             | 89             | 95             | 95             | 78             | 100            | 100            |
| Washing cytology                | 83             | 77             | 82             | 78             | 75             | 67             |

NMP22 = nuclear matrix protein 22; WLE = while light endoscopy; PDD = photodynamic diagnosis.
benign urothelial conditions. They concluded that the NMP22 does not provide a good diagnostic tool for screening because of the high rate of false-positive results. This is underlined by the histologic findings of patients without tumour but positive NMP22 in the present study. The low specificity of cytology in the present study (washing cytology, 62%) might also be contributed to the high frequency of patients with earlier resections and instillations.

However, specificity of NMP22 could not be improved even by exclusion of all patients with history of bladder cancer (47%, Table 4), and also specificity remained poor after exclusion of all patients who had received instillations (mitomycin or bacille Calmette-Guérin) in the last 2 yr (36%, Table 4).

The high rate of false-positive results with the NMP22® BladderChek® may indicate small tumours that can be visualised neither by WLE nor by PDD. But most patients with a false-positive result underwent follow-up cystoscopy after 3–10 mo (n = 27; 3 patients missed follow-up because of death or severe non-urologic disease, 6 were lost to follow-up). Only three of these patients had developed bladder cancer at the follow-up examination.

5. Conclusion

The NMP22® BladderChek® is a noninvasive and rapid test for the diagnosis of bladder cancer. However, the value of the test is limited by its low specificity, presumably due to frequent positive reaction in benign conditions. Therefore, exact selection of patients is essential to avoid unnecessary further invasive procedures. Additional studies are warranted to determine the most advantageous patient cohort for the use of this noninvasive marker.

Conflict of interest

There is no commercial association that might pose a conflict in connection with this study.

References


Editorial Comment

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The management of superficial bladder cancer remains a perpetual dilemma between excessive surveillance for indolent disease and sometimes delayed care in aggressive tumours. This study matches NMP22® BladderChek®, a new “Point of Care” version of the NMP22 Bladder Cancer test to fluorescence cystoscopy (PDD), considered by the authors as the standard management for detecting any urothelial malignancy. White light cystoscopy,
native and bladder washing cytology were proposed as standard references.

The idea to oppose a simple office, urinary marker test such as NMP22BC to the most efficient endoscopic technique such as PDD is valid, nevertheless the use of both techniques aims at different objectives.

The first one is a screening test in competition with other urinary markers, whereas PDD is an additional tool to standard cystoscopy not only improving detection of flat urothelial malignancies but optimising the whole resection process of bladder tumours. Besides, the close sensitivities of both WLE and PDD, found in this study, debates the validity of PDD as a standard reference.

The authors conclude that NMP22 cannot be recommended for screening or surveillance in a daily clinical use. But, in the group of 30 patients without any history of bladder cancer, the sensitivity of NMP22 was estimated at 60%, a value similar to the one reported by Grossman et al. [1]. Is it so bad? What was the specificity in this group of patients?

Moreover, the sensitivity of NMP22 for high grade disease was 78%; PDD only detected two more patients (2/18 ~11%) with high grade disease. Is it so bad again?

For a better understanding of the potential use of a test such as NMP22, we probably should include it in nomograms for specific groups of patients, as advocated by Shariat et al. [2].

A “new” NMP BladderChek test, with an adaptive cut-off value modulating sensitivity and specificity according to different situations such as screening versus surveillance, could be investigated.

References