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ARTICLE

The Clinical Usefulness of Nuclear Matrix Protein-22 in Patients with End-Stage Renal Disease and Microscopic Hematuria

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Abstract

Objectives: To evaluate the sensitivity, specificity, and false-positive rate of the nuclear matrix protein-22 (NMP22) test in patients with end-stage renal disease (ESRD) and microscopic hematuria in order to avoid unnecessary follow-up tests for patients with false-positive NMP22 test results. *Patients and Methods*: Patients with ESRD were screened for microscopic hematuria as part of the pre-transplant workup. Patients with documented microscopic hematuria underwent workup as recommended by the American Urological Association. *Results*: Between January 2006 and April 2012, 277 patients with ESRD were referred to the Department of Urology for pre-transplant evaluation. Fifty-seven (22.6%) patients were found to have microscopic hematuria and underwent further testing. Nineteen (33.3%) patients demonstrated a positive NMP22 test result and 38 (66.7%) had a negative NMP22 test result. The false-positive rate was 32.7%. The sensitivity and specificity of the NMP22 test in this patient population were 50% and 67%, respectively. The positive predictive value of the test was 52.6% and the negative predictive value 97.3%. Especially noteworthy, the two detected transitional cell cancers of the urinary bladder were both demonstrated during cystoscopy, independent of their NMP22 or urine cytology test result. *Conclusions*: Our study revealed a significantly increased NMP22 test false-positive rate, low sensitivity, and specificity in the setting of high prevalence of microscopic hematuria, proteinuria, and low glomerular filtration rate in patients with ESRD. Therefore, cystoscopy remains the gold standard for patients with ESRD and microscopic hematuria for pre-transplant evaluation.

Keywords: urinary bladder, urinary bladder neoplasms, kidney function tests, tumor markers, hematuria, false-positive reactions

INTRODUCTION

Renal transplantation is widely considered the best available therapy for patients with end-stage renal disease (ESRD) because of improved short- and long-term survival benefits over dialysis treatment.[1] According to the most recent statistical report of the United States Renal Data System (USRDS) from 2011, 17,736 kidney transplants were performed in the USA in 2009.[2] Most centers in the US use a multidisciplinary team approach to evaluate and select potential recipients.[3] Because of the involvement of the genitourinary tract in the renal transplantation process, urologists are often consulted regarding pre-transplant evaluation and treatment of potential renal transplant recipients. In order to avoid unforeseen problems during transplantation and in the post-transplant period, the urological pre-transplant evaluation intends to diagnose, treat, or optimize any underlying urological condition.[4] These conditions mainly include urinary tract infection, urolithiasis, upper and lower urinary tract obstruction, bladder dysfunction, and malignancies.[3,5] In recent years, several clinical studies have reported a higher incidence of malignancies in renal transplant recipients.[4] The incidence is not only higher compared to the general population, but also compared to similar patients on dialysis.[6] This higher incidence might be secondary to multiple factors, including the application of immunosuppressive agents that may cause DNA damage, interfere with normal DNA repair mechanisms, and alter immune surveillance mechanisms that ordinarily prevent the growth and development of malignancies and increased incidence

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of viral infections.[6] Kasiske et al. reported a significantly increased incidence of genitourinary malignancies with prostate cancer incidence (twofold increase), testicular and bladder cancer (approximately threefold increase), and kidney cancer (approximately 15-fold increase).[6] Therefore, cancer should continue to be a major focus of prevention in kidney transplantation. Part of the prevention is detection during the pre-transplant evaluation.

A considerable number of patients with ESRD present with microscopic hematuria, which by itself often triggers a urological workup to elucidate the underlying causes. According to the Guidelines of the American Urological Association, the workup should include imaging studies of the upper and lower urinary tract (computed tomography of the abdomen and pelvis, intravenous pyelogram/retrograde pyelogram, ultrasound), cystoscopy, and depending on the preferences of the urologist involved, urine cytology and/or other non-cytology based urine tests.[7]

Recent publications have reported an increasing false-positive rate of the nuclear matrix protein-22 (NMP22) test depending on renal function.[8] NMP22 is a protein involved in regulation of mitosis and is overexpressed in malignant urothelial cells. Apoptotic cells release NMP22 into the urine, where it can be detected and quantified.[9] As described previously, the NMP22 test demonstrates increased sensitivity but decreased specificity compared to urine cytology.[10] The performance of the NMP22 test can be influenced by several conditions such as urinary tract infection, previous instillation therapy, foreign bodies, benign prostate hyperplasia or stone disease, changes in urine composition, such as hematuria and pyuria.[9,11–14] Any of these conditions can cause false-positive test results. More recently, Todenhöfer et al. have reported that decreased glomerular filtration rates were associated with increased false-positive NMP22 results. The authors recommended that renal function should be considered when urine-based bladder cancer tests are interpreted.[8] Therefore, we report in a retrospective fashion our findings regarding the usefulness/accuracy of the NMP22 test in the context of a transplant workup in patients with ESRD and microscopic hematuria.

PATIENTS AND METHODS

Patients with ESRD referred to the Department of Urology were screened for microscopic hematuria as part of the pre-transplant workup. Patients with documented microscopic hematuria underwent workup as recommended by the American Urological Association.[7] The study received institutional review board approval (IRB# L12-096).

Urine samples obtained by catheterization or midstream urine collection were analyzed by dipstick analysis followed by confirmation urine microscopy using a Neubauer hemocytometer. In case of divergent results between dipstick analysis and microscopy, the urine microscopy results were used for further analysis. Microscopic hematuria was classified according to Todenhöfer et al. into grade 0 (no erythrocytes), grade I ($1 \leq$ erythrocytes/μl < 100), grade II ($100 \leq$ erythrocytes/μl < 250), and grade III ($\geq$250 erythrocytes/μl).[15] All patients with microscopic hematuria underwent white-light cystoscopy and imaging of the upper urinary tract (computed tomography of the abdomen and pelvis, retrograde pyelogram, ultrasound). Patients with suspicious cystoscopic findings were evaluated by transurethral biopsy and/or resection of suspicious lesions with histological assessment.

Urine Processing

NMP22 enzyme-linked immunosorbent assay was performed according to manufacturer recommendations (Alere NMP22® BladderChek® Test, Scarborough, ME, 04074 USA). The NMP22 test was used instantly after urine collection, allowing for test results and guidance for immediate clinical decision making to be obtained rapidly.

A concentration greater than 10 U/mL was used as the threshold for a positive test.[9] For urine cytology analysis, cytospin slides were stained after Papanicolaou and Marshall and microscopically assessed by a cytopathologist by light microscopy according to defined criteria.[16] For the urinalysis, the Iris system—AX-4280 and the automated microscopic IQ-200 were used.

Definition of Urinary Tract Infection

Urinary tract infections were determined by dipstick analysis, urine microscopy, and urine culture. Urinary tract infection was defined as at least 100 leukocytes per μl and more than 1 erythrocyte or at least 100 leukocytes per μl and the presence of urine nitrite.

Renal Function Parameters

Serum creatinine, glomerular filtration rate, and urine protein served as renal function parameters.[17] Creatinine was measured using the Cobas 6000 analyzer. The glomerular filtration rate was calculated using the modification of diet in renal disease formula.[18]

Exclusion Criteria

Since urinary tract infections, previous mechanical manipulation such as cystoscopies or catheterizations, urolithiasis, and foreign bodies are known to produce false-positive NMP22 test results, they were considered contraindications to the test.

Statistical Analysis

Statistical analysis was performed using the commercially available statistical program from XLSTAT (Addinsoft SARL, New York, NY, USA).

RESULTS

Between January 2006 and April 2012, 277 patients with ESRD were referred to the Department of Urology for pre-transplant evaluation. Fifty-seven (22.6%) patients were found to have microscopic hematuria, were compliant with the inclusion criteria, and underwent further testing. Two patients with microscopic hematuria were excluded according to the exclusion criteria. Dialysis was performed in 43 patients (75.4%), with peritoneal dialysis in 11 (19.3%) patients and hemodialysis in 32 (56.1%), whereas 14 (24.6%) were not on dialysis. The median patient age was 50 years with a range of 20–75 years. Male-to-female ratio was 34/23 patients (59.6%/40.4%). Median serum creatinine (range) and median glomerular filtration rate (range) were 6.55 mg/dL (2.1–21.1 mg/dL) and 8.1 mL/min/1.73 m^2 (2.53–31.03 mL/min/1.73 m^2), respectively. Median urine output in 24 h was 591.5 ml (range: 118.3–1656.2 ml). Proteinuria was common in this patient population and ranged from (0–750 mg/dL) with a median of 265.5 mg/dL. From the 57 (22.6%) patients, 19 (33.3%) demonstrated a positive NMP22 test result and 38 (66.7%) had a negative NMP22 test result. Further patient characteristics are summarized in Table 1 and the causes of end-stage renal disease (ESRD) in Table 2. The most common cause of end-stage renal disease in our patient population was diabetes mellitus in combination with arterial hypertension (53.5%), followed by arterial hypertension alone (14%), diabetes mellitus alone, and glomerulonephritis, both (7%).

Of the 19 (33.3%) patients with a positive NMP22 test, three also had positive urine cytology.

Table 2. Patient characteristics II—causes of end-stage renal disease.

Cause	No. of patients (%)
Diabetes mellitus	4 (7%)
Arterial hypertension	8 (14%)
Diabetes mellitus and arterial hypertension	30 (52.6%)
Prune-belly-syndrome	1 (1.75%)
Glomerulonephritis	4 (7 %)
Lithium-treatment-induced	1 (1.75%)
Lupus-nephritis	2 (3.5%)
Adult polycystic chronic kidney disease	3 (5.3%)
IgA-nephropathy	2 (3.5%)
Wegener's disease	1 (1.75%)
Chronic pyelonephritis	1 (1.75%)

Three patients had a negative NMP22 test but positive urine cytology. All patients with either positive NMP22 test or urine cytology underwent a random bladder biopsy. In the group of patients with positive NMP22 test, one patient was diagnosed with transitional cell cancer of the urinary bladder but none of the patients with positive urine cytology. Interestingly, none of the three patients with positive NMP22 test and positive urine cytology demonstrated a transitional cell cancer of the urinary bladder. Especially noteworthy, the 2 detected transitional cell cancers of the urinary bladder were both demonstrated during urethrocystoscopy, independent of their NMP22 or urine cytology test result. Detailed information is summarized in Table 3.

Based on our data collection, the false-positive rate was 32.7%. Sensitivity and specificity of the NMP22 test in

Table 1. Patient characteristics I—baseline demographics of evaluable patients ($n = 57$).

Characteristics	No. of patients (%) or test value (%)
Median age, year (range)	50 (20–75)
Male/female	34/23 (59.6%/40.4%)
Median body mass index (range)	28.3 (18.7–44.3)
Median serum creatinine, mg/dL (range)	6.55 (2.1–21.1)
Medium glomerular filtration rate (mL/min/1.73 m^2) (range)	8.1 (2.53–31.03)
Medium proteinuria, mg/dL (range)	256.5 (0–750)
NMP22 test results	
Positive	19 (33.3%)
Negative	38 (66.7%)
Positive NMP22 test results (M/F ratio)	
Positive	11/8 (19.3%/14.0%)
Negative	22/16 (38.6%/28.1%)
Urine cytology results (Patients with positive NMP 22 test)	
Normal	16 (84.2%)
Atypia	3 (15.8%)
Cancer cells present	0 (0 %)
Urine cytology results (patients with negative NMP 22 test)	
Normal	33 (86.8%)
Atypia	5 (13.2%)
Cancer cells present	0 (0%)
Median urine output in mL per 24 h (range)	591.5 (118.3–1656.2)
Type of dialysis performed	
None	14 (24.6%)
Peritoneal dialysis	11 (19.3%)
Hemodialysis	32 (56.1%)

Table 3. Urinary bladder biopsy results ($n = 21$).

Histopathological results	No. of patients (%)
Patients with positive NMP22 test ($n = 16$)	
Normal bladder urothelium	8 (50.0%)
Chronic cystitis	7 (43.7%)
Transitional cell cancer	1 (6.3%)
Patients with only positive urine cytology ($n = 3$)	
Normal bladder urothelium	2 (66.7%)
Chronic cystitis	1 (33.3%)
Patients with positive NMP22 test and positive urine cytology ($n = 3$)	
Normal bladder urothelium	1 (33.3%)
Chronic cystitis	2 (66.7%)
Patients with positive NMP22 test or urine cytology and abnormal urethrocystoscopy ($n = 6$)	
Normal bladder urothelium	1 (16.7%)
Chronic cystitis	4 (66.6%)
Transitional cell cancer	1 (16.7%)
Patients with negative NMP22 test/negative urine cytology but abnormal urethrocystoscopy ($n = 2$)	
Normal bladder urothelium	1 (50%)
Chronic cystitis	0 (0%)
Transitional cell cancer	1 (50%)

Table 4. NMP22 test sensitivity, specificity, and negative and positive predictive values.

	% Sensitivity	% Specificity	% Positive predictive value	% Negative predictive value
NMP22 test	50	67.2	52.6	97.3

this patient population were 50% and 67%, respectively. The positive predictive value of the test was 52.6% and the negative predictive value was 97.3% (see Table 4).

The study demonstrated two true-positive results, 37 true-negative results, 18 false-positive results and one false-negative result. No significant correlation between other potential modifying factors such as serum creatinine/glomerular filtration rate, degree of proteinuria, microhematuria, and leukocyturia could be demonstrated in this study.

DISCUSSION

Microscopic hematuria is a common finding in patients with ESRD. The causes can be manifold and include malignancies of the urinary tract, glomerular disease, urolithiasis, urinary tract infection, foreign bodies, bowel segment use, and instrumentation.[11,19] According to the AUA Guidelines, a urological workup for this condition should be initiated. The guidelines make no clear difference between patients with ESRD and those without.[7] Patients with ESRD have a higher incidence of certain types of malignancies including urological malignancies such as bladder cancer. Therefore, it is crucial to rule out these malignancies prior to renal transplantation and initiation of immunosuppressive treatment. Particularly, immunosuppressive medication can alter the natural course of these malignancies. Loss of immunological control can lead to more aggressive disease/phenotypes with higher stages at the time of diagnosis. In recent years, interest has shifted to the use of noninvasive methods to identify early stage bladder cancer in order to replace cystoscopy as an invasive test. Urine markers including the NMP22 test are gaining importance for primary diagnosis and in the tumor follow-up.[8] The NMP22 test is an accepted screening test/method for "general patient population" with acceptable sensitivity and specificity. However, as described by others, the NMP22 test has inherent limitations, which must be considered.[8,11,19]

In this study, the NMP22 test was used in a highly selected patient population with ESRD to screen for potential "malignancies of the urinary tract (transitional cell cancer)". Patients with ESRD are a subpopulation of patients with microscopic hematuria. We could show that the NMP22 test demonstrated a relatively low sensitivity and specificity, and a high false-positive rate. The 67.2% accuracy of the test shows that the test has limited value on how well the test can correctly identify or exclude transitional cancers in this particular patient population.

No significant correlation between other potential modifying factors such as serum creatinine/glomerular filtration rate,[8] degree of proteinuria,[20] microscopic hematuria,[11] and leukocyturia[11] could be demonstrated. The reasons for this are multiple overlapping conditions in this patient population. For example, patients with low-grade (grade I) microscopic hematuria can present with high proteinuria and vice versa.

A limitation of the present study is the fact that only two transitional cell cancers were present in the study

population. Therefore, the sensitivity results should be interpreted carefully. However, the specificity is low due to an extremely high false-positive rate. This can lead to unnecessary "worries or concerns" for the patient and may lead to unnecessary further invasive testing (anesthesia and bladder biopsies), which are cost intensive and put these usually high-risk patients at a higher risk for complications. Based on our data, NMP22 testing should be used wisely in this patient population. More importantly, in our study, all tumors were detected by cystoscopy. In our experience, patients with ESRD and microscopic hematuria should undergo only cystoscopy for evaluation of the lower urinary tract. Besides detecting lower urinary tract tumors, urethrocystoscopy is also useful for determining bladder capacity and lower urinary tract obstruction. Therefore, cystoscopy remains the mainstay of bladder cancer diagnosis, especially in patients with ESRD and microscopic hematuria undergoing pre-transplant evaluation. Our results also emphasize the need to strictly comply with exclusion criteria for the NMP22 urine tests, which should include patients with ESRD.[19]

A potential strategy to increase the predictive rate of the NMP22 test could be the use of phase-contrast microscopy. This allows a more reliable differentiation between glomerular and non-glomerular microscopic hematuria. Especially, non-glomerular microscopic hematuria is a concern for transitional cell cancers of the urinary tract. Therefore, introducing phase-contrast microscopy as a diagnostic step and the usage of the NMP22 test only for patients with non-glomerular microscopic hematuria could increase the predictive rate of the test.

CONCLUSION

To the best of our knowledge, this is the first study evaluating the condition of ESRD on the performance of the NMP22 test. Our study revealed a significantly increased NMP22 test false-positive rate, low sensitivity and specificity in the setting of high prevalence of microscopic hematuria, proteinuria and low glomerular filtration rate in patients with ESRD. Based on our data, the usage of NMP22 test in patients with ESRD gives no diagnostic advantage. Therefore, cystoscopy remains the gold standard for patients with ESRD and microscopic hematuria for pre-transplant evaluation.

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