

The clinical utility of atypical cytology is significantly increased in both screening and monitoring for bladder cancer when indexed with nuclear matrix protein-22

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OBJECTIVES

To assess atypical cytology as a positive bladder tumour marker and to determine if indexing atypical cytology to nuclear matrix protein-22 (NMP22) can decrease the false-positive results or increase the positive predictive value (PPV).

PATIENTS AND METHODS

In all, 197 patients at risk of bladder cancer were identified as having atypical urine cytology; 126 were incident (screening)

cases and 71 were prevalent (monitoring) cases of bladder cancer. All patients with atypical cytology were evaluated using office cystoscopy. All cancers were confirmed histologically and patients had a negative upper tract study within a 1-year interval. The atypical cytology was then indexed with NMP22 values in an effort to decrease the false-positive results.

RESULTS

Atypical cytology detected 17 cancers in the 126 patients who were screened, giving a PPV of 13% (17/126). When stratified by NMP22, using a threshold of >10 U/mL, the PPV increased to 71% (15/21). In the 71 patients who were being monitored, atypical

cytology detected 43 cancers, for a PPV of 61% (43/71). When stratified by NMP22 using a threshold of >6 U/mL, the PPV increased to 92% (35/38).

CONCLUSIONS

The clinical utility of atypical cytology was significantly increased in both screening and monitoring for bladder cancer when indexed with NMP22 levels.

KEYWORDS

bladder cancer, nuclear matrix protein, urinary cytology, haematuria and cystoscopy

INTRODUCTION

The early detection of bladder cancer allows for effective local treatment and optimises the success of surgical therapy. In most patients superficial bladder cancer can be treated successfully with minimally invasive procedures, e.g. transurethral resection or fulguration, which precludes the need for more aggressive surgical therapies. Survival rates reflect the importance of an early diagnosis; when detected at superficial clinical stages Ta and T1, the 5-year survival rate of bladder cancer is 82–95%, whereas corresponding survival rates for muscle-invasive and metastatic disease are 50% and 6%, respectively [1,2].

Currently there is no standard method for the noninvasive early identification of bladder cancer. Patients who present with symptoms

of microscopic or gross haematuria or other irritative voiding symptoms are often screened with an upper tract study, urine analysis, urinary cytology, and office cystoscopy. However, urine cytology lacks sensitivity and office cystoscopy lacks specificity. The sensitivity of voided urine cytology is 40–50% for high-grade disease but is reported to be as low as 20–30% for low-grade low-stage disease. Conversely, the specificity of office cystoscopy for cancer detection is <10% when evaluating patients with microscopic haematuria. Although urinary cytology has been the reference standard for noninvasive testing, its low sensitivity misses a significant number of cancers and might result in a delayed diagnosis. While cystoscopy remains the reference standard for invasive testing, the primary indication for cystoscopy, which is haematuria, has a low specificity (many false-

positive results). Therefore it is an inefficient tool for screening for bladder cancer. The development of a highly sensitive urinary test for detecting TCC of the bladder could greatly affect the ability to effectively screen symptomatic patients at risk of bladder cancer.

Many researchers have tried to evaluate noninvasive methods to accurately and easily identify the presence of bladder cancer [3–6]. Several diagnostic urinary tumour markers developed from new molecular technologies, e.g. nuclear matrix protein-22 (NMP22), bladder tumour antigen, telomerase) are being tested for screening and monitoring in high-risk populations [5,6].

The recent introduction of urinary tumour markers potentially challenges the efficacy of the current diagnostic evaluation. In a series of recent studies, researchers evaluated the

efficacy of urinary tumour markers for detecting recurrent bladder cancers. These studies show that these new urinary markers have excellent sensitivity, particularly in their ability to detect low-grade, low-stage tumours. The sensitivity of these tumour markers was reported to be two to three times greater than that of cytology, which translates into improved cancer detection. Despite excellent sensitivities, the low specificities and more importantly low positive predictive values (PPVs) limit these tumour markers. Specificity is frequently cited as an measure of efficacy for screening test [7,8].

Despite the low sensitivity, voided cytology is a widely accepted adjunctive test for the diagnosis and monitoring of patients with bladder cancer, as it is not invasive. Several investigators suggested increasing its sensitivity by considering all atypical cytology as positive, a valuable aid in identifying bladder cancer [9,10]. In a comparative study between the sensitivity of positive cytology and the atypical cytology, we found that atypical cytology was twice as sensitive as a positive cytology for detecting bladder cancers, but the false-positive rate was also increased. Compared with the NMP22 test, atypical cytology is less sensitive for identifying bladder cancers, and has a lower PPV for both screening and monitoring [11]. Thus, treating an atypical cytology as positive significantly improves the cancer detection rate, but at the cost of an increased false-positive rate.

The purpose of the present study was to determine whether indexing atypical cytology with NMP22 could enhance the clinical utility of atypical cytology, by increasing the PPV in groups of patients being screened or being monitored.

PATIENTS AND METHODS

In all, 197 patients with atypical urine cytology and at risk of bladder cancer (January 1997 to October 2000) were identified; these patients were being either initially evaluated, or followed up, in our urology clinic. Of the 197 with positive urine cytology, 126 were incident (screening) and 71 were prevalent (known/monitoring) cases of bladder cancer. All patients were evaluated by outpatient cystoscopy, the incident cases presenting with microhaematuria, gross

Variable, %	Overall	Incident	Prevalent	<i>TABLE 1</i> <i>The sensitivity, specificity, PPV and NPV for patients with atypical cytology when indexed to NMP22, in the incident and prevalent groups, and the cancer detection rate</i>
Sensitivity	83.3	88.2	81.4	
Specificity	93.4	94.5	89.3	
PPV	84.7	71.4	92.1	
NPV	92.8	98.1	75.8	
Cancer-detection rate, PPV (95% CI)				
Atypical	30.5 (24–37)	60.6 (48–72)	13.5 (8–21)	
Atypical/NMP+ve	84.7 (73–92)	92.1 (78–98)	71.4 (48–88)	

Group	NMP22, n/N (%)		<i>TABLE 2</i> <i>The cancer detection rate of atypical cytology indexed with NMP22 in the screening and monitoring groups</i>
	Positive	Negative	
Screening (atypical cytology, 17/126, 13%)			
	21/126 (16.7)	105/126 (83.3)	
Atypical cytology	15/21 (71)	2/17 (12)	
Monitoring (atypical cytology 43/71, 61%)			
	38/71 (54)	33/71 (47)	
Atypical cytology	35/38 (92)	8/43 (19)	

haematuria, and/or irritative voiding symptoms, or as part of monitoring in the prevalent cases. If a tumour was identified, biopsies were taken and subsequent transurethral resection of bladder tumour (TURBT) performed. If no obvious tumours were present, the suspicious areas were sampled by cold-cup biopsy. All cancers were histologically confirmed, and an experienced urologist performed all surgical procedures. The urinary tests were conducted at our institution and all patients had a negative upper tract study within a 1-year interval. Atypical cytology was retrospectively indexed to NMP22 values, assessed at the same time as the initial cytology. The NMP22 assay used was a double-monoclonal antibody immunoassay for measuring the nuclear mitotic apparatus protein designated NMP22, and used stabilized urine samples. The assay is approved by the USA Food and Drug Administration for detecting occult recurrent TCC after TURBT [11,12]. An NMP22 value of >10 U/mL was considered positive for potential urothelial malignancy in the present screened high-risk patients who presented with haematuria or chronic irritative voiding symptoms. The threshold value of 10 U/mL was determined to be the optimum using receiver operator characteristic analysis in previous studies by our group [7,8]; for monitored patients with a history of bladder cancer the threshold was >6 U/mL.

The sensitivity, specificity, PPV and negative PV (NPV) were calculated for the overall incident and prevalent subgroups using

McNemar's test. The sensitivity of NMP22 to detect specific stages and grades was assessed in patients with atypical urine cytology; 95% CI were also calculated for the PPV.

RESULTS

Of the 197 patients with atypical cytology who were evaluated, 60 (30%) had histologically confirmed bladder cancer. When stratified using NMP22 levels, cancer was detected in 50 of the 60 (83%) patients. Gross haematuria was the presenting symptom in 24% (47/197) of the patients, and microscopic haematuria in 25% (49/197). When all atypical cytology was considered as positive and indexed with NMP22, the overall specificity improved to 93.4% and the cancer detection rate (PPV) improved to 84.7% (from 30.5%). The overall sensitivity and specificity, PPV and NPV of NMP22 when indexed with atypical cytology, both in the screening and monitoring subgroups, are shown in Table 1.

In the screening (incident) group of 126 patients, atypical cytology detected all 17 cancers (100% sensitivity by design), with a PPV of 13.5% (or a false-positive rate of 87%; 17/(17 + 109)). When stratified by NMP22 with a threshold of >10 U/mL, the PPV increased to 71% ((15/(12 + 6)); Table 1) but the sensitivity decreased to 88% (15/17). In the remaining 105 patients with a NMP22 level of <10 U/mL, two cancers were detected (12%), giving a NPV of 98% (Table 2).

Bladder cancer stage	Overall	Incident	Prevalent	<i>TABLE 3</i> <i>The sensitivity, as n/N (%), of atypical cytology indexed with NMP22 to detect specific stages and grades</i>
CIS	60	33	100	
Ta	65	100	63	
T1	75	0	75	
T2	100	100	100	
T3	100	100	100	
Grade				
I, low	36	0	36	
II, moderate	91	80	94	
III, high	96	83	100	

In the monitoring (prevalent) group of 71, atypical cytology detected 43 cancers (100% sensitivity by design), with a PPV of 61% (or a false-positive rate of 39%) (43/(43 + 28)). When stratified using the NMP22 threshold of >6 U/mL, the PPV increased to 92% (35/(35 + 3)) (Table 2) but the sensitivity decreased to 81% (35/43). In the remaining 33 patients with an NMP22 level of <6 U/mL, only eight cancers were detected (19%), giving a NPV of 76% (Table 2).

When NMP22 was combined with atypical cytology, 60% of carcinoma *in situ* (CIS) and all invasive cancers were identified. In the incident group, the sensitivity of NMP22 for detecting CIS when indexed with atypical cytology was 33%, but it was 100% in the prevalent group. In both the incident and prevalent groups, the NMP22 test alone was able to detect all invasive cancers. When stratified by the grade of the tumour, NMP22 combined with atypical cytology detected 36% of grade 1, 91% of grade 2 and 96% of grade 3 tumours (Table 3).

DISCUSSION

The early detection of bladder cancer in patients at risk of newly diagnosed or recurrent cancer might significantly affect the options and effectiveness of local therapies. The greatest challenge in the management of superficial bladder cancer is to diagnose cancer before progression to invasive disease. The sensitivity of urinary cytology in screening a symptomatic population is too low (30–50%) to optimally detect bladder cancer at its earliest presentation. Conversely, a positive urine analysis with microscopic or gross haematuria lacks the specificity to screen patients at risk, and predisposes to too many unnecessary cystoscopies. While cystoscopy remains the reference standard for the early detection of bladder cancer, with a sensitivity of >90%, its limitation is the low

specificity and PPV when used to evaluate microscopic haematuria or chronic irritative voiding symptoms. While recent reports of new urinary tumour markers show markedly better sensitivity than urinary cytology, the limitation of these markers (like microscopic haematuria) is the low specificity, which clinically manifests as a high false-positive rate [13,14].

Despite the potential error from subjective interpretation and low overall sensitivity, voided urinary cytology is a widely accepted adjunctive test for the diagnosis and monitoring of patients with bladder tumours. The efficacy of urinary cytology for detecting bladder tumour is now being reassessed because of the recent availability of rapid tests reported to be more sensitive and specific. The limitations of urine cytology are suboptimal sensitivity and professional costs.

In the present study we retrospectively evaluated the cancer detection rate and false-positive rate generated by indexing atypical cytology results with the NMP22 tumour marker assay for patients being screened or monitored for bladder cancer. The results support the view that considering all atypical cytology as positive, and indexing the result with NMP22, the specificity and PPV could be increased to 93% and 85%, respectively. In the screening group, atypical cytology alone had a low PPV, limiting its usefulness. When indexing atypical cytology with a positive NMP22, the PPV in the screening population increased from 13% to 71% and diagnosed all early and invasive bladder carcinomas. In the monitoring group, atypical cytology alone had a cancer detection rate of 61%; indexing atypical cytology with a positive NMP22 increased the cancer detection rate from 61% to 92%, enhancing its clinical utility in monitoring patients with a history of bladder cancer. The sensitivity of NMP22 when indexed with atypical cytology to diagnose

early bladder cancer was only 33%, vs 100% in the incident group, but its utility in detecting invasive bladder cancers was 100%.

This refinement in the clinical application of atypical cytology indexed with NMP22 could significantly influence the early detection of patients with predisposing factors for bladder cancer. Although cystoscopy is the reference standard for diagnosis, its sensitivity is variable, especially in lesions difficult to visualize, such as CIS and low-grade papillary lesions. When atypical cytology is indexed with NMP22 the evaluation before cystoscopy alerts the physician to the likelihood of a neoplasm and can therefore enhance the disease detection rate of cystoscopy (92% in the prevalent group; 71% in the incident group).

It is impractical to screen all people at increased risk of bladder cancer by cystoscopy, and therefore a cost-effective, noninvasive method is necessary. The predicted advantage of indexing atypical cytology with the NMP22 test is that it is equally effective in diagnosing superficial tumours and muscle-invasive cancers. Evaluating those patients with haematuria using the NMP22 test and urinary cytology could enhance this protocol by reducing the number of cystoscopies. This also allows for the identification of all invasive disease, and the ability to detect more cancers than with cytology alone, with minimal added expense.

In conclusion, when screening using atypical cytology the PPV or cancer-detection rate is only 13%, limiting its role as a screening method because of the many false-positive results. However, when atypical cytology is stratified by NMP22 level, the cancer-detection rate increases to 71%. In monitoring patients with atypical cytology the PPV or cancer-detection rate is 61%; when atypical cytology is stratified by NMP22, the cancer detection rate increases to 92%. The combination might be useful in adjusting surveillance cystoscopy.

CONFLICT OF INTEREST

None declared.

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Abbreviations: P(N)PV, positive (negative) predictive value; NMP, nuclear matrix protein; TURBT, transurethral resection of bladder tumour; CIS, carcinoma *in situ*.