IMMUNOCYT*: A NEW TOOL FOR DETECTING TRANSITIONAL CELL CANCER OF THE URINARY TRACT

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ABSTRACT

Purpose: The limitations of cytology and the invasiveness of cystoscopy for detecting bladder cancer generate increasing interest in noninvasive, urine bound diagnostic tools. We assessed the diagnostic value of the newly developed immunocytochemical test, Immunocyt, which detects cellular markers specific for transitional cell cancer in the voided urine of patients with bladder cancer.

Materials and Methods: Participating in our prospective study were 264 consecutive patients with a mean age of 65.9 years, including 114 in whom symptoms were suggestive of bladder cancer and 150 who were being followed after complete transurethral resection of superficial transitional cell carcinoma. Voided urine specimens were evaluated by standard cytology and the Immunocyt test, which traces the monoclonal antibodies M344, LDQ10 and 19A211 against transitional cell carcinoma in exfoliated urothelial cells. In all cases cystoscopy was subsequently performed and any suspicious lesion was evaluated by biopsy.

Results: Histologically proved transitional cell carcinoma was found in 79 patients. Immunocyt with cytology had 89.9% sensitivity overall (84, 88 and 96.5% in grades 1 to 3 disease, respectively). A total of 34 (43%), 3 (3.8%) and 34 (43%) cases were positive on Immunocyt only, cytology only and both evaluations, respectively. In 8 cases (10.1%) both tests were negative. Overall Immunocyt only was 86.1% sensitive (84, 84 and 89.6% in grades 1 to 3 disease, respectively) and 79.4% specific. Overall cytology only was 46.8% sensitive (4, 52 and 79.3% in grades 1 to 3 disease, respectively) and 98.2% specific.

Conclusions: Immunocyt is a noninvasive, highly sensitive test for detecting transitional cell carcinoma of all grades and stages. When combined with conventional urinary cytology, it may replace cystoscopy in select patients, especially in followup protocols of low grade transitional cell carcinoma.

KEY WORDS: urinary tract; cancer, transitional cell; immunofluorescence; monoclonal antibody; cytology ucyt product clinical trial bladder cancer

Although cystoscopy is the most efficient method currently available for detecting primary or recurrent transitional cell carcinoma of the bladder, it is invasive and causes significant patient discomfort. Furthermore, flat tumors or carcinoma in situ may be difficult to detect.[1] Urinary cytology is noninvasive and effective for diagnosing high grade lesions but it has only 11 to 17% sensitivity in grade 1 disease, which is the most common type of transitional cell carcinoma.[1-4]

The limitations of cytology and cystoscopy for making the primary diagnosis and monitoring patients after transitional cell carcinoma removal led to the development of new urine bound tests for the early detection of transitional cell carcinoma.[1,3,4] Methods based on the immunological detection of soluble antibodies in voided urine are of particular interest. Kavaler et al reported that the detection of telomerase activity in the voided urine of patients with bladder cancer is 91% sensitive.[5] Klein et al detected CK-20 in the voided urine of patients with bladder cancer with 82.8% sensitivity and 100% specificity.[6] However, these assays are technically complicated, require highly sophisticated laboratory expertise and equipment, and are not suitable for routine cytology laboratories to perform. Recently simpler methods for detecting bladder tumor antigen in the urine have become commercially available, such as the BTA Statt and BTA Trakt assays,[7-9] and the NMP22 assay$ for nuclear matrix protein.[4] These tests are more sensitive than cytology in low grade tumors but their specificity is so low that cystoscopy is still always essential. Moreover, in grade 3 tumors the sensitivity of these assays is approximately 25% lower than that of routine cytology, and so cytology also remains necessary.[1]

In an attempt to overcome these problems Fradet and Lockhart developed the Immunocyt test, a new approach combining cytology and an immunofluorescence assay.[10] Immunocyt detects cellular markers specific for bladder cancer in exfoliated cells of the transitional epithelium using 3 fluorescent monoclonal antibodies.[10] Antibody 19A211 labeled with Texas red identifies a high molecular weight form of carcinoembryonic antigen.[11] Antibodies MO.344 and **LDQ10** labeled with fluorescein are directed against mucins,[12] which are expressed in most bladder cancer but not in normal transitional epithelium cells. We assess the diagnostic value of the Immunocyt assay for detecting bladder cancer in comparison to and combination with conventional cytology.

PATIENTS AND METHODS

From November 1997 to March 1998 we prospectively obtained voided urine specimens from 264 consecutive patients, including 60 women and 204 men 21 to 93 years **old** (mean age 65.9) who were undergoing cystoscopy. Of the **265** pa-

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tients 114 had symptoms suggestive of bladder cancer and 150 were being followed after complete transurethral resection of transitional cell carcinoma at least 3 months previously. For any lesion suspicious on cystoscopy biopsy or transurethral resection was done. Histopathological classification was performed according to International Union Against Cancer criteria.[13]

We collected 50 to 100 ml. specimens of voided urine from each patient and divided them into 2 aliquots. One aliquot was used for standard Papanicolaou and Giemsa staining, and cytological evaluation. Diagnostic results were categorized as previously described by Koss et al.[14] Briefly specimens negative for malignancy or with atypia of any degree were categorized as negative and those considered suspicious or positive for malignancy were categorized as positive.

We used 20 to 40 ml. of the sample for evaluation by the Immunocyt assay. Samples were immediately fixed with an equal volume of 50% ethanol and 1 ml. of a special fixative solution, and then incubated for 1 hour. Cells were collected by filtration through a 25 mm. polycarbonate membrane filter of 8 μm. porosity and connected to a vacuum pump. Filters were then rinsed with 3 ml. of Saccomanno solution and cells were blotted on 2 consecutive silanized slides. Cells were fixed using Merckofix* spray. Before proceeding to Immunocyt staining slides were controlled for cell content with the number of cells on a slide serving as a quality control measure. Slides containing less than 500 cells were excluded from study. A positive slide and a negative control slide guaranteed a correct staining procedure.

For the Immunocyt procedure slides were initially stained by a modified Papanicolaou method using consecutive incubation with Harris hematoxylin differentiator (70% ethanol-ammonium hydroxide), and OG-6 and EA-65 solutions. After rehydration in distilled water cells were incubated with 150 μl. of a blocking solution for 20 minutes at room temperature in a closed humid chamber. The blocking solution was drained from the slides, which were incubated with the Immunocyt antibody cocktail for 1 hour at room temperature. Slides were then rinsed twice in phosphate buffered saline containing 0.5% Tween 20 and in pure phosphate buffered saline, and mounted with a coverglass. Fixative solution, negative and positive controls, blocking solution and the antibody cocktail are provided in the Immunocyt kit.

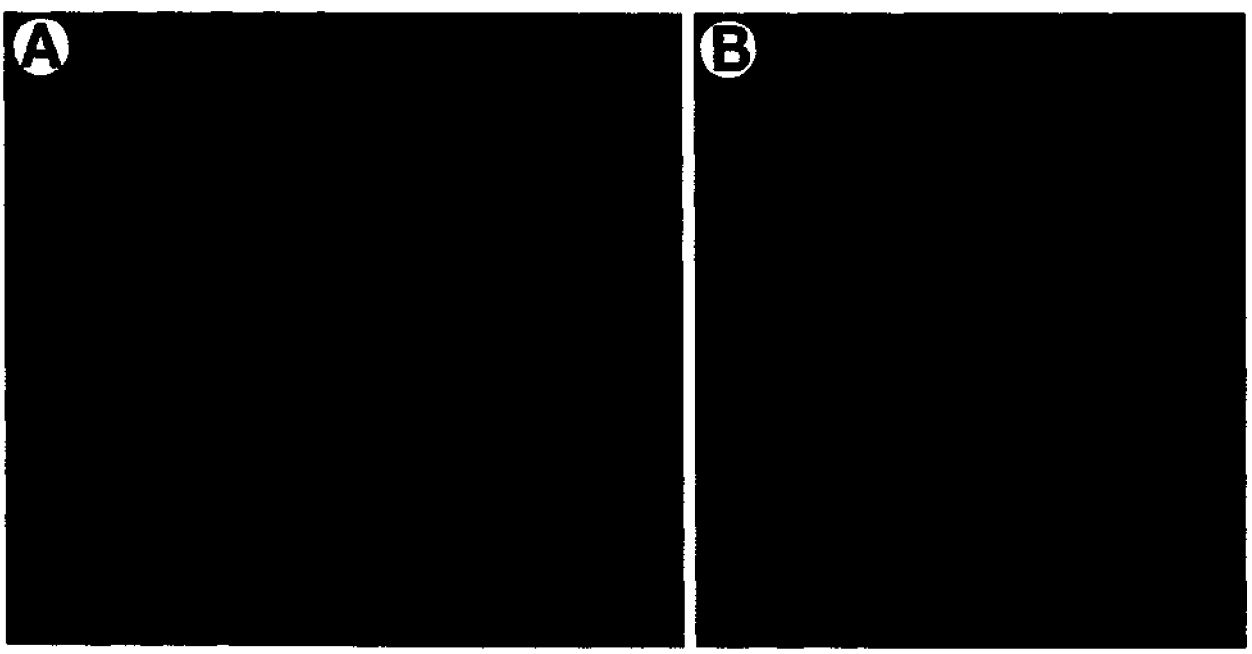
Slides were read under a fluorescence microscope using filters for fluorescein and Texas red emission light detection. Red fluorescence indicated cells positive for high molecular weight glycosylated carcinoembryonic antigen and green fluorescence indicated cells positive for bladder cancer mucins. Samples were considered positive when there was at least 1 green or 1 red fluorescent cell. Sensitivity, specificity, and the negative and positive predictive values of cytology and Immunocyt were calculated with cystoscopy and histological evaluation considered the gold standard.

* Merck, Darmstadt, Germany.

RESULTS

Of the 264 cases 249 were evaluable. We rejected 15 specimens because there were fewer than 500 cells per slide. Later cystoscopy revealed that all 15 cases were negative for transitional cell carcinoma. Of the remaining 249 evaluable cases histological testing verified transitional cell carcinoma of the urinary tract in 79, including 23 of 107 (21.5%) suspicious for transitional cell carcinoma and 56 of 142 (39.4%) during followup (table 1). Since the prevalence of transitional cell carcinoma in the 2 groups was different, predictive values were calculated separately. In 170 patients cystoscopy and cytology were negative. Table 2 shows the false-positive results of Immunocyt and cytology in these patients.

Of the 79 cases of transitional cell carcinoma 34 (42.8%) were positive by Immunocyt only, whereas only 3 (3.8%) were positive by cytology only. Table 3 shows the sensitivity of cytology and the Immunocyt assay correlated with disease grade and stage. The sensitivity of voided urine cytology increased from 4 to 79.3% in grades 1 to 3 disease, whereas the sensitivity of Immunocyt was 84, 84 and 89.6% in grades 1 to 3 disease, respectively. However, when cytology and Immunocyt were combined, sensitivity was 84% (21 of 25 cases), 88% (22 of 25) and 96.5% (28 of 29) in grades 1 to 3 transitional cell carcinoma, respectively. For stages pTa, pT1 and pT2 or greater transitional cell carcinoma the sensitivity of both tests was 88.3% (38 of 43 cases), 90% (18 of 20) and 91.6% (11 of 12), respectively. Cytology had a false-positive



A, red fluorescence shows cells positive for high molecular weight glycosylated carcinoembryonic antigen. *B*, green fluorescence shows cells positive for bladder cancer mucin.

TABLE 1. *Patient data*

	No. Pts.
Total	264
Beingfollowed	150
Suspicion of transitional cell Ca	114
Nonevaluable (less than 500 cells/slide)	15
Transitional cell Ca bladder	77
Transitional cell Ca ureter	2
Free of transitional cell Ca	170
Followup after transurethral resection	86
Cystitis	10
Upper tract urolithiasis	24
Benign lesions of lower urinary tract (benign prostatic hyperplasia, nephrogenic adenoma or inverted papilloma)	16
Renal cell, prostatic or cervical Ca	7
Microhematuria	27

TABLE 2. *Final findings in 170 patients free of transitional cell carcinoma on cystoscopy with false-positive results on Immunocyt and cytology*

	No. Pts. False-Pos./Total No. (%)		
	Immunocyt	Cytology	Immunocyt + Cytology
Followup after transurethral resection	17/86 (20)	2/86 (2)	17/86 (20)
Cystitis	4/10 (40)	0/10	4/10 (40)
Upper tract urolithiasis	2/24 (8)	0/24	2/24 (8)
Benign lesions of lower urinary tract (benign prostatic hyperplasia, nephrogenic adenoma or inverted papilloma)	8/16 (50)	0/16	8/16 (50)
Microhematuria	4/27 (15)	1/27 (4)	4/27 (15)
Renal cell, prostatic or cervical Ca	0/7	0/7	0/7
Totals	35/170 (21)	3/170 (2)	35/170 (21)

TABLE 3. *Sensitivity of the 2 methods according to grade and stage in 79 patients with transitional cell carcinoma*

	No. Pts.	% Sensitivity (No. pts./ total No.)		Immunocyt + Cytology
		Cytology	Immunocyt	
Grade:				
1	25	4 (1/25)	84 (21/25)	84 (21/25)
2	25	52 (13/25)	84 (21/25)	88 (22/25)
3	29	79 (23/29)	90 (26/29)	97 (28/29)
Stage:				
pTa	43	21 (9/43)	86 (37/43)	88 (38/43)
pT1	20	70 (14/20)	85 (17/20)	90(18/20)
pT2 or Greater	12	83 (10/12)	83 (10/12)	92 (11/12)
pTis (Ca in situ)	4	100 (4/4)	100 (4/4)	100 (4/4)

rate of 2% (3 of 170 cases) and a false-negative rate of 53% (42 of 79), while Immunocyt had a false-positive rate of 21% (35 of 170) and a false-negative rate of 14% (11 of 79). Both tests had a false-positive rate of 21% (35 of 170 cases) and a false-negative rate of 10% (8 of 79). Tables 4 and 5 show the sensitivity, specificity, and positive and negative predictive values of the 2 tests.

TABLE 4. *Sensitivity and specificity of Immunocyt and cytology in 249 evaluable patients*

	% Sensitivity (No. pos./79 with Ca)	*9c* Specificity (No. pos./170 with Ca)
Cytology	46.8(37)	98.2 (167)
Immunocyt	86.1 (68)	79.4 (135)
Immunocyt and cytology	89.9 (71)	79.4(135)

TABLE 5. *Negative and positive predictive values of Immunocyt and cytology evaluated separately in 107 patients with suspected transitional cell carcinoma and 142 being followed*

	9c Pos. (No. false-pos ./total No. Ca-free*)	% Neg. (No. false-neg./total No. with Ca*)
Cytology:		
Diagnostic (suspicious for Ca)	93 (1/84)	90 (9/23)
Followup	92 (2/86)	72 (33/56)
Immunocyt:		
Diagnostic (suspicious for Ca)	54 (18/84)	97 (2/23)
Followup	73(17/86)	89 (9/56)
Immunocyt + cytology:		
Diagnostic (suspicious for Ca)	55(18/84)	99 (1/23)
Followup	75(17/86)	90 (7/56)

* Cystoscopy results.

DISCUSSION

Monoclonal antibodies may be used for detecting transitional cell carcinoma cells exfoliated in voided urine.[2] Immunocytochemical methods using voided urine specimens have the advantage of being performed noninvasively. The determinations of urine carcinoembryonic antigen,[15] bladder tumor antigen using the BTA Stat and BTA Trak assays,[7-9] and nuclear matrix protein using the NMP22 test[4,16] have been investigated as new diagnostic methods to substitute for voided urine cytology. Sarosdy et al reported 67% sensitivity and 72% specificity for the BTA Stat assay,[7] whereas Soloway et al reported 70% sensitivity and 79% specificity for the NMP22 test.[4] Wiener et al compared the BTA Stat and NMP22 tests to urinary cytology, and noted 48 and 57% sensitivity, respectively, and approximately 70% specificity for both tests.[1] Without doubt these methods facilitate the detection of low grade tumors but specificity is not high enough to render cystoscopy unnecessary. Furthermore, these assays have lower sensitivity in high grade tumors, and so cytology is still needed as well.[1,7,10,15]

The Immunocyt test is highly sensitive in all grades of disease. Our study confirms the findings of Fradet and Lockhart[10] that overall sensitivity is approximately 2-fold higher than that of cytology. According to transitional cell carcinoma grade sensitivity was much higher for Immunocyt than for cytology in low grade disease, and it reached comparable values in high grade disease. Similar results were obtained in correlation with tumor stage. Immunocyt specificity was lower than that of cytology but comparable to that reported by Fradet and Lockhart. Of the false-positive results 50% involved patients being followed after transurethral resection of transitional cell carcinoma (table 2). These findings may ultimately signal tumor recurrence. Combining cytology with the Immunocyt assay improved sensitivity even further, particularly in grade 1 disease, yet specificity remained high. When cytology and Immunocyt are negative, cystoscopy may be avoided in select patients, particularly after transurethral resection of low grade, low stage transitional cell carcinoma that has a lower risk of recurrence. The frequency of followup would be decreased to 6 instead of 3 months.

The negative predictive value of Immunocyt in suspicious and followup cases is comparable to that of the BTA Stat and NMP22 assays,[1,4,7] and higher than that of cytology. The positive predictive value of these tests is inferior to that of cytology. Although the positive predictive value of cytology with Immunocyt is low (55 and 75% in suspicious and followup cases, respectively), the high positive predictive value of cytology and the high negative predictive value of Immunocyt may lead to overall improvement in diagnostic yield. Combining the tests provides the higher sensitivity of Immunocyt than conventional cytology and other commercially available diagnostic tests, while preserving the advantage of the high specificity of cytology. As reported by Fradet and Lockhart, the presence of 1 green or 1 red cell appears to be the best cutoff point at which to obtain a high negative predictive value, thus, avoiding false-negative results.[10]

The clinical usefulness of a diagnostic test also depends on procedure duration and technical expenditure. Immunocyt may be performed within 2 hours in specimens previously stained according to the Papanicolaou procedure for standard cytology.[10] Cytology and the Immunocyt assay are done by the same technician using the same urine specimen. However, to evaluate the specimen trained personnel with cytological knowledge are needed. Therefore, it is advisable to perform the test at institutions where a trained cytologist and all technical equipment are available.

CONCLUSIONS

Immunocyt is a noninvasive, highly sensitive test for detecting transitional cell carcinoma of all grades and stages. When combined with conventional urinary cytology, it may replace cystoscopy in select patients, especially in followup protocols of low grade transitional cell carcinoma.

Diagnocure, Inc., Saint-Foy, Quebec, Canada, provided the Immunocyt test kits.

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