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The Value of the ImmunoCyt/uCyt+ Test in the Detection and Follow-up of Carcinoma *In Situ* of the Urinary Bladder

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Abstract. *Background: Urine bound tests, which have been developed for the early detection of urothelial cancer (UC), do not seem to match cytology in the detection of carcinoma in situ (CIS) as their sensitivity in the case of CIS is poor. ImmunoCyt/uCyt+™ in CIS seems promising, but the number of analysed CIS is still small. The aim of the present study was to assess the value of this test in the detection and follow-up of carcinoma in situ of the urinary bladder. Patients and Methods: Thirty-five patients, with histologically verified CIS of the urinary bladder, were included in the study. At the first diagnosis, patients underwent cytology, cystoscopy, bioptical bladder mapping and ImmunoCyt/uCyt+™. All patients underwent BCG instillation therapy. The patients were followed with cytology, ImmunoCyt/uCyt+™, cystoscopy and bladder mapping after every BCG cycle and then every 3 months. Results: At the first CIS diagnosis, the sensitivity of cytology and ImmunoCyt/uCyt+™ was 100%. At the first control after therapy, cytology detected 81.8% of recurrences and ImmunoCyt/uCyt+™ detected 90.9%. At the second control, both tests each detected 50% of recurrences. At every control, the combination of both the tests together gave a sensitivity of 100%. The specificity of cytology after therapy improved from 88.2% at the first control up to 100% at the third control. The specificity of ImmunoCyt/uCyt+™ after BCG initially decreased from 70.6% to 55.5% and finally increased to 88.9%. Conclusion: ImmunoCyt/uCyt+™ is as sensitive as cytology in the first diagnosis of CIS. In the follow-up, even if it is less sensitive, its combination with cytology leads to detection of 100% of the recurrences. Despite decreasing specificity after therapy, the value remains acceptable and increases during maintenance therapy. The ImmunoCyt/uCyt+™ test could play an important role in controlling the response of patients to instillation therapies or in the modification of their application schedules.*

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Carcinoma *in situ* (CIS) of the bladder is a flat, high-grade non-invasive malignancy with a high tendency for progression. The diagnosis is based on a combination of cytology, cystoscopy and bioptical mapping (1). Although cytology has a low sensitivity in low-grade lesions and a high inter- and intra-observer variability, it is highly sensitive in the detection of high-grade lesions, especially of CIS (over 90%) (2), and is also highly specific.

At present, cystoscopy and histology are still considered the gold standard methods for the detection of primary or recurrent urethral cancer (UC) of the bladder. Patients with bladder tumour undergo 3-6 months cystoscopy and cytology follow-up. Despite technical improvements, cystoscopy is an invasive method causing the patient some discomfort. Furthermore, flat tumours or carcinoma *in situ* may be difficult to detect (3).

These limitations for both primary diagnosis and monitoring of patients after UC has been removed, led to the development of several urine bound tests for the early detection of UC. They do not, however, seem to match cytology since their sensitivity in the case of CIS is poor (1, 4).

ImmunoCyt/uCyt+™ in CIS seems promising, but the number of analysed CIS is still small (5). ImmunoCyt/uCyt+™ combines cytology with an immunofluorescence analysis and is highly sensitive for tumours of all grades. Its sensitivity, according to the grade of UCs, is much higher than for cytology in low-grade tumours and reaches comparable values in high-grade tumours (5-10). It detects cellular markers specific for bladder cancer in exfoliated cells of the urothelium using three fluorescent monoclonal antibodies (6). The antibody 19A211, labelled with Texas-Red, identifies a high-molecular-weight form of CEA, and M344 and LDQ10, labelled with Fluorescein,

Table I. *Sensitivities of cytology, ImmunoCyt/uCyt+™ and both the tests combined in 35 patients with CIS. Calculations of the sensitivity at the controls are based on the number of recurrences.*

	Diagnosis	Control	Control
Cytology	35/35 (100)	9/11 (81.8)	2/2 (50)
ImmunoCyt/uCyt+™	35/35 (100)	10/11 (90.9)	2/2 (50)
Both	35/35 (100)	11/11 (100)	4/4 (100)

Numbers in parenthesis are percentages.

Table II. *Specificities of cytology, ImmunoCyt/uCyt+™ and both the tests combined in patients after endovesical BCG therapy for CIS.*

	Control (17 pts)	Control (18 pts)	Control (9pts)
Cytology	2/17(88.2)	1/18(94.4)	0/9(100)
ImmunoCyt/uCyt+™	5/17(70.6)	8/18(55.5)	1/9(88.9)
Both	5/17(70.6)	8/18(55.5)	1/9(88.9)

Numbers in parenthesis are percentages.

are directed against mucins, which are expressed in most bladder cancers but not in normal cells of the transitional epithelium (6).

The aim of the study was to assess the value of the ImmunoCyt/uCyt+™ test in the detection and follow-up of CIS of the urinary bladder. Our preliminary data are presented.

Patients and Methods

Between July 2001 and July 2004, 35 patients with histologically CIS of the urinary bladder (mean age 73.3 years, range 52-93) were included in the study. At the first diagnosis all patients underwent cytology, ImmunoCyt/uCyt+™, cystoscopy and bioptical bladder mapping. All patients underwent BCG instillation therapy (weekly for 6 weeks in 10 cases, maintenance therapy in all other cases). The patients were followed with cytology, ImmunoCyt/uCyt+™, cystoscopy and bladder mapping after every BCG cycle and then every 3 months. The histopathological classification was performed according to McKenney *et al*. (11).

Cytology and ImmunoCyt/uCyt+™. Voided urine (40-80 ml) was collected from all patients and evaluated by liquid-based cytology (ThinPrep®) (Cytyc Corp., Boxborough, MA, USA) and ImmunoCyt/uCyt+™ (Diagnocure Inc., Quebec, Canada). The material was prepared as previously reported by Mian *et al*. (12) and slides stained according to Papanicolaou. The diagnostic results were categorized as previously reported (12). In short, specimens negative for malignancy or with atypia of any degree were categorized "negative", and specimens considered suspicious or positive for malignancy as "positive".

For the ImmunoCyt/uCyt+™ evaluation, slides were read under a fluorescence microscope (Provis AX 70 Olympus, Italy) using filters for Fluorescein and Texas Red emission light detection. Red fluorescence showed cells positive for high-molecular-weight glycosylated carcinoembryogenic antigen (CEA), and green fluorescence cells positive for bladder cancer mucins. Samples were considered positive when they showed at least one green or one red fluorescent cell.

Sensitivities and specificities were calculated using histology results as reference data.

Results

All patients were evaluated for cytology and ImmunoCyt/uCyt+™. At the first CIS diagnosis, cytology (35/35) and ImmunoCyt/uCyt+™ sensitivity was 100%. At the first control after weekly BCG therapy, cytology detected 9 out of 11 recurrences (81.8%), and ImmunoCyt/uCyt+™ detected 10 out of 11 (90.9%). The combined sensitivity of both tests together was 100%. At the second control after monthly BCG, cytology detected 2 out of 4 recurrences (50.0%), and ImmunoCyt/uCyt+™ detected 2 out of 4 (50.0%). The combination of both tests together gave a sensitivity of 100%. At the third control after 3-monthly BCG therapy, only one recurrence was observed and detected by cytology.

The specificity of cytology after the BCG therapy improved from 88.2% at the first control to 100% at the third control. The specificity of ImmunoCyt/uCyt+™ was 70.6% at the first control after weekly therapy, after which it decreased to 55.5% at the second control and then increased to 88.9% at the third control. Detailed data on sensitivity and specificity are given in Tables I and II.

Discussion

The untreated natural history of CIS shows a 5-year progression of more than 50% and an even higher recurrence rate (13, 14). Due to this high risk of progression and recurrence, patients with CIS undergo immuno- or chemotherapy and have to undergo multiple cystoscopies and biopsies in order to control the effectiveness of the intravesical therapy on tumour recurrence or progression. Moreover, cystitis after intravesical therapy decreases the diagnostic power of cytology and cystoscopy, so that any suspicious results need to be biopsied (1).

Urinary tumour markers have been evaluated as possible alternative non-invasive diagnostic methods to

cystoscopy (3, 15). The value of the recently developed new markers for detecting bladder cancer has already been described and proposed to replace follow-up cystoscopy in patients with UC (2). The sensitivities of most of these markers were disappointing in the diagnosis of CIS. This led to the conclusion that urinary cytology by an experienced and dedicated cytologist remains the gold standard in the diagnosis and follow-up of suspected or proven CIS (1).

Nevertheless, as reported by Pfister *et al*. (5), the incorporation of ImmunoCyt/uCyt+™ in the urinary cytology protocol resulted in a significant increase of up to 100% in sensitivity in the detection of CIS and grade 3 tumours with a high overall negative predictive value (NPV).

In our experience, both cytology and ImmunoCyt/uCyt+™ showed 100% sensitivity at the first CIS diagnosis. At the first control after BCG immunotherapy, sensitivities decreased to 81.8% for cytology and 90.9% for ImmunoCyt/uCyt+™. However, the 2 tests combined detected 100% of recurrences. At the second control, both methods failed to detect 50% of the tumours, but when combined they again detected 100%. Unfortunately, the number of recurrences at this control was very low and therefore warrants confirmation in further investigations.

The data of sensitivity for ImmunoCyt/uCyt+™ at first diagnosis are comparable with previously published studies on small CIS cohorts (5, 8) and for cytology higher than (1, 5) or comparable with previously reported studies (8, 16). The accuracy of cytology differs significantly between untreated and treated patients (17). Any kind of therapy reduces the diagnostic accuracy of cytological diagnosis. According to these previously published reports, the sensitivity of cytology in this CIS study decreased by 18.2% at the first control after therapy and became worse at the second control. The recurrence rate of the latter, however, was very low. In contrast, the sensitivity of ImmunoCyt/uCyt+™ decreased by only 8.1%, and, despite local instillation therapy, was higher than that of cytology. Both tests combined, however, offered 100% sensitivity. These data corroborate a previous study by Lodde *et al*. (18), who reported that overall sensitivity and specificity of ImmunoCyt/uCyt+™ in patients after BCG-instillation therapy were not significantly affected in statistical terms. The overall sensitivity of ImmunoCyt/ uCyt+™ in the BCG-group was reported to be about 85%, which was much higher than that of cytology and in accordance with most reports in large series (70-90%) (5-9). The specificity after instillation therapy in this study was 88.2% for cytology and 70.6% for ImmunoCyt/uCyt+™. At the second and third controls, cytology specificity increased constantly up to 100%. As reported by several authors, cytology excels, not only in its high accuracy, but also in its specificity of up to 100% in the diagnosis of CIS (1, 16, 17). Urinary markers used for the improvement of sensitivity in papillary tumours neither match the specificity, nor the sensitivity of cytology in CIS (1). ImmunoCyt/uCyt+™ seems to be promising in CIS patients. In fact, its specificity in this study decreased, at first from 70.6% to 55.5% at the first and second controls, respectively, but increased to 88.9% at the third control. The low specificity for ImmunoCyt/uCyt+™ at the first 2 controls could be explained by the fact that the BCG-instillation therapy might not have destroyed the whole tumour or CIS. A positive ImmunoCyt/uCyt+™ test could, therefore, reflect the presence of cells expressing antigens at a time when they can not be correctly interpreted morphologically due to the therapy effect.

The efficacy of a bladder tumour marker depends on its ability to provide early detection and insight into appropriate treatment decisions, as well as to monitor treatment response and tumour recurrence (2, 19). The presence of cells expressing the antigens after a weekly course of instillation with BCG could identify non-responders or a group of tumours which really benefit from longer cycles of instillation. Furthermore, as reported in other studies, "false-positive" ImmunoCyt/uCyt+™ became morphologically confirmed tumour recurrence within 3-6 months (2) in 28% of the patients, or a recurrence at 1 year in 47% of the cases *versus* 11.9% in a control group (9).

Both cytology and ImmunoCyt/uCyt+™ require experienced and trained cytologists for their evaluation. At our institution, procedures are standardised: using liquid-based cytology, slides are prepared and stained by the same technician for both methods with exact protocols and evaluated by the same cytologist. For urinary diagnosis and follow-up of patients with proven CIS by a dedicated cytologist, cytology remains the gold standard (1), but its sensitivity in the follow-up could be still improved by the use of the ImmunoCyt/uCyt+™ test.

Conclusion

ImmunoCyt/uCyt+™ is as sensitive as cytology in the first diagnosis of CIS. In the follow-up, even if it is less sensitive, in combination with cytology 100% of the recurrences seem to be detectable. However, due to the low number of recurrences in the present study, these findings need to be confirmed in further investigations. Despite decreasing specificity after therapy, the value remains acceptable and increases during maintenance therapy. The ImmunoCyt/uCyt+™ test could play an important role in controlling the response of patients to instillation therapies or in modifying their application schedules.

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