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Cytological Changes Induced by Intravesical Bacillus Calmette-Guérin Therapy for Superficial Bladder Cancer

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Key Words
Superficial bladder cancer · Bacillus Calmette-Guérin · Instillation therapy · Urinary cytology

Abstract
To evaluate cytological changes of urothelial cells with intravesical instillation therapy of the bacillus Calmette-Guérin (BCG), cytological specimens of voided urine from patients with superficial bladder cancer (pTa and pT1) treated with intravesical BCG therapy were examined. The following three groups of patients who had no evidence of recurrence more than 2 years after the treatment were studied: groups 1 and 2, patients who were treated with BCG (n = 22) and epirubicin, a derivative of doxorubicin (n = 22), respectively, for prophylaxis of intravesical recurrence after transurethral resection (TUR); and group 3, patients receiving no intravesical therapy after TUR (n = 12). Sixteen cytological characteristics were studied before and after the treatment in each group. In group 1 patients translucent nuclei and prominent nucleoli, vacuolization of cytoplasm, and eosinophilic cytoplasmic inclusions were frequently observed in urothelial cells as well as an increase in granulocytes, especially within 3 months after BCG instillation therapy. In group 2 patients an increased nuclear/cytoplasmic ratio, hyperchromatic nuclei and prominent nucleoli of urothelial cells were transiently found within 1–2 months after intravesical epirubicin therapy. In group 3, translucent nuclei and prominent nucleoli of urothelial cells were found within 1–2 months after TUR. In conclusion, cytological changes induced by BCG therapy are nonspecific and reactive in nature, different from those due to chemotherapeutic agents and distinguishable from malignant changes of urothelial cells.

Introduction

Superficial tumors (stages Ta and T1), which account for 70–80% of all bladder cancers, are usually managed by conservative treatment including transurethral resection (TUR) and intravesical chemotherapy or immunotherapy. However, among the affected patients, 50–70% demonstrate intravesical recurrence and 10–20% feature progression, defined as the development of muscle invasion or distant metastasis [1, 2]. Disease progression is significantly associated with unfavorable patient survival.

Chemotherapeutic agents including thiotepa (triethylene thiophosphoramide), mitomycin C, and doxorubicin have been used for eradicating tumor cells and preventing intravesical recurrence. In recent years epirubicin (4′-epidoxorubicin), a derivative of doxorubicin, has been ap-

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plied for intravesical instillation therapy [3–5]. This chemotherapeutic agent was synthesized with the aim of finding anthracycline analogues with an improved spectrum of antitumor activity and lower toxicity. Since the introduction of intravesical bacillus Calmette-Guérin (BCG) therapy by Morales et al. [6], many studies have demonstrated that this approach is effective for preventing recurrence and progression [7–9] and it has now become widely used for this purpose. The remarkable efficacy of BCG in the treatment of superficial bladder cancers has resulted in significant changes in the management of these lesions.

It has generally been known that intravesically applied chemotherapeutic agents induce various significant morphological changes of normal and neoplastic urothelial cells [10–14]. However, only a few studies have concentrated on urinary cytology after intravesical BCG instillation therapy, and the findings were not consistent [15, 16]. We therefore evaluated patients who received intravesical BCG therapy for prevention of intravesical recurrence after TUR of superficial bladder cancer, comparing them with individuals receiving intravesical epirubicin therapy.

Materials and Methods

Patients

The following three groups of patients with superficial bladder cancer (stages pTa or pT1) were treated at Nagoya University Hospital and three affiliated hospitals from September 1992 to December 1996: groups 1 and 2, patients who were treated with BCG (n = 22) and epirubicin (n = 22) instillation, respectively, for prophylaxis of intravesical recurrence after TUR; and group 3, control subjects, patients who received no intravesical therapy after TUR (n = 12). For all of the patients, endoscopically visible tumors were resected and no concomitant carcinoma in situ (CIS) was histologically demonstrated. No cases had recurrence more than 2 years after the treatments.

Patients in each group were followed up by cystoscopic examination and urinary cytology, biopsies being performed when abnormal lesions were found endoscopically or cytological studies suggested the presence of a tumor.

Table 1 shows details for the total of 56 patients in the three groups. The study group comprised 43 males and 13 females with a male-to-female ratio of 3.3:1. Their ages at diagnosis ranged from 23 to 87 years with a mean of 66.0 ± (SD) 13.5 years. They were followed up for a median of 47 months with a range from 24 to 75 months.

Intravesical BCG and Epirubicin Therapies

For prophylaxis of recurrence intravesical BCG therapy was initiated 2–4 weeks after TUR. Either 40- or 80-mg doses of Tokyo 172 strain BCG (BCG Co. Ltd., Tokyo, Japan) were instilled into the bladder with 40 ml of normal saline, and retained there for 2 h [17]. BCG was weekly administered for 6–10 weeks and no maintenance therapy was thereafter performed. Because we demonstrated that a 40-mg BCG low-dose (Tokyo 172 strain) regimen was useful for preventing recurrence, with low frequency of toxicity [17], most of the recent patients received the low-dose regimen on the basis of the responsible urologist's decision. In group 2 patients epirubicin instillation therapy was initiated 1–2 weeks after TUR. A dose of 40 mg epirubicin (Farmitalia Carlo-Erba, Milan, Italy) was instilled into the bladder with 40 ml of normal saline and retained there for 2 h [5]. This chemotherapeutic agent was administered weekly for 6–10 weeks. Indications for either BCG immunotherapy or chemotherapy at our hospitals include multiple, recurrent, and large tumors.

Cytological Examinations

For urinary cytology voided urine from the patients was centrifuged and sediments were smeared on glass slides for staining with the Papanicolaou technique [18]. For the present study a total of 5 specimens were examined for each patient in groups 1 and 2 at the following 5 time points: before treatment, 1 or 2 months after the end of the instillation therapy, and 3, 6 and 12 months after the instillation treatment. In group 3 patients, 5 specimens were similarly examined before treatment, 1 or 2 months after TUR, and 3, 6 and 12 months after TUR. Sixteen cytological parameters were investigated as listed on the left side of tables 2–4. Numbers of inflammatory cells and exfoliated urothelial cells were described semiquantitatively as follows: – = absent or infrequent; + = identified easily, and ++ = prominent or of marked degree. Increase in nuclear/cytoplasmic ratio was defined as an increase when the ratios were found to be more than 50 and 70% in urothelia of superficial and deep layers, respectively.

The following five diagnostic categories were used for final diagnosis as previously reported [19]: unsatisfactory for cytological diagnosis, negative, repeat test suggested, suspicious of cancer, and positive for cancer. Non-assessible specimens belong to the category of 'unsatisfactory for cytological diagnosis'. For the purpose of the present study all cytological specimens were reevaluated for use of diagnostic categories without knowledge of clinical and pathological information.

Histological Classification and Statistical Analysis

All tumors were histologically diagnosed at the time of TUR. Tumors were staged according to the UICC system [20]. Histologically, they were graded using the criteria of the Armed Forces Institute of Pathology [21]. Several tumors were histologically found to be of mixed grade and thus were arbitrarily classified from the predominant grade.

Differences in frequencies were evaluated by the Fisher's exact probability test or Mann-Whitney U test when indicated. The multiple test among three groups was considered by Bonferroni's adjustment of the significance level, that is multiplying the obtained p values by 3.

Results

Patient Characteristics

Table 1 shows details of the patients in the three groups. Those treated with intravesical BCG therapy (group 1) or epirubicin therapy (group 2) had a signifi-

cantly higher incidence of multiple tumors than the control subjects (group 3). Patients in group 1 had a significantly higher incidence of high-grade tumors than those in group 2. Follow-up intervals were significantly longer in group 1 than in group 2.

In group 1 doses of 40 mg BCG were given to 20 of 22 patients (91%) and 80 mg to the remaining 2 (9%). The mean number of times BCG was given was 7.5 ± (SD) 1.2 with a median value of 8 times. In all 22 patients in group 2 doses of 40 mg epirubicin were instilled. The mean number of times given was 6.5 ± (SD) 1.1 with a median value of 6 times.

Cytological Findings before and after Treatments

Tables 2–4 summarize cytological findings for the three groups. In group 1 patients translucent nuclei and prominent nucleoli of urothelial cells as well as remarkable increase in granulocytes were more frequently observed than in group 3 ($p < 0.05$, table 2, fig. 1). These changes were remarkable within 3 months after TUR. The incidence of both vacuolization of cytoplasm and eosinophilic cytoplasmic inclusions, the latter indicating degenerative change [16], was significantly higher in group 1 than in group 3. Increased nuclear/cytoplasmic ratios were found in 2 patients (9%) 1–2 months after BCG therapy and in 1 patient (5%) 3 months after BCG therapy. Appearance of macrophages and epitheloid cells

Table 1. Details of patients studied

Clinical and pathological categories	Group 1: BCG (n = 22)	Group 2: epirubicin (n = 22)	Group 3: no treatment (n = 12)
Male/female	17/5	16/6	10/2
Age, years			
Mean ± SD	65.7 ± 13.9	68.1 ± 14.6	62.4 ± 10.5
Range	43–84	23–87	44–79
Treatment history of bladder cancer			
Yes	8 (36%)	7 (32%)	1 (8%)
No	14 (64%)	15 (68%)	11 (92%)
Prior instillation therapy			
Yes	1 (5%)	6 (27%)	1 (8%)
No	21 (95%)	16 (73%)	11 (92%)
Number of tumors			
Solitary	5 (23%)	10 (45%)	12 (100%)
Multiple	17 (77%)***	12 (55%)**	0 (0%)
Tumor size			
≤ 1 cm	14 (64%)	11 (50%)	8 (67%)
1–3 cm	8 (36%)	7 (32%)	3 (25%)
> 3 cm	0 (0%)	4 (18%)	1 (8%)
Histological grade			
Grade 1	1 (5%)	9 (41%)	4 (33%)
Grade 2	18 (81%)	13 (59%)	8 (67%)
Grade 3	3 (14%)*	0 (0%)	0 (0%)
Histological stage			
pTa	12 (55%)	16 (73%)	11 (92%)
pT1	10 (45%)	6 (27%)	1 (8%)
Months of follow-up after treatment			
Median	61*	43	44
Range	25–75	24–72	25–74

* Significantly different from the value for group 2 (Mann-Whitney U test, $p < 0.05$).

** Significantly different from the value for group 3 (Fisher's exact probability test, $p < 0.01$).

*** Significantly different from the value for group 3 (Fisher's exact probability test, $p < 0.001$).

was found after the BCG therapy in 1 of the 22 patients (5%). Anisonucleosis was found in 1 patient (5%) within 1–2 months after BCG therapy. Neither multiple nuclei in cells other than superficial layer urothelia nor irregular shaped nuclei were apparent.

In group 2 patients the following cytological changes of urothelial cells were noteworthy 1–2 months after the epirubicin therapy (table 3, fig. 2): increased nuclear/cytoplasmic ratios in 4 (18%), hyperchromatic nuclei in 4 (18%), and prominent nucleoli in 5 (23%), although the differences in incidence between groups 2 and 3 did not reach statistical significance. Irregularly shaped nuclei and anisonucleosis were each found in 1 case (5%). The remarkable incidence of the above cytological changes decreased 3 months after the therapy. Increase in granulocytes was also noted within 3 months.

In group 3 the background of the specimens showed a moderate increase in granulocytes within 3 months after

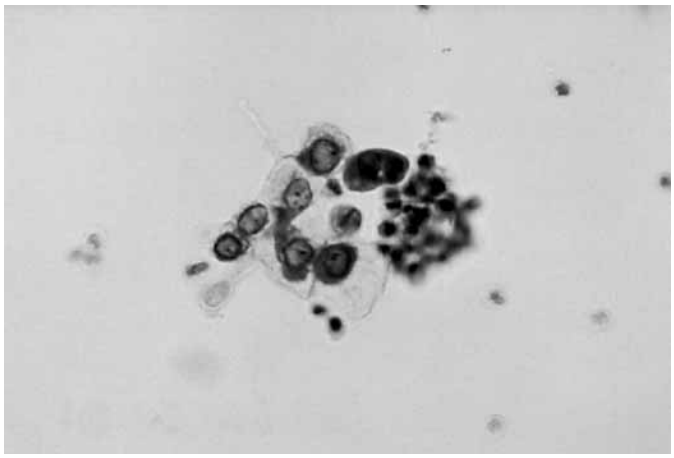

Fig. 1. Cytological findings for a patient treated with intravesical BCG therapy. Note translucent nuclei and prominent nucleoli of urothelial cells and increase in granulocytes. Papanicolaou staining. × 400.

Table 2. Cytological findings for 22 patients treated with intravesical BCG therapy (group 1)

Cytological findings	Before TUR	After instillation therapy			
		1–2 months	3 months	6 months	12 months
Inflammatory cells					
Increase in granulocytes					
+	5 (23%)	11 (50%)	3 (14%)	1 (5%)	1 (5%)
++	2 (9%)	7 (32%)*	3 (14%)	1 (5%)	0 (0%)
Increase in lymphocytes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Appearance of macrophages	0 (0%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)
Appearance of epitheloid cells	0 (0%)	0 (0%)	1 (5%)	0 (0%)	0 (0%)
Urothelial cells					
Increase of superficial cells					
+	1 (5%)	0 (0%)	1 (5%)	1 (5%)	1 (5%)
++	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Increase of deep layer cells					
+	4 (18%)	2 (9%)	3 (14%)	1 (5%)	1 (5%)
++	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Increased nuclear/cytoplasmic ratio	14 (64%)*	2 (9%)	1 (5%)	0 (0%)	0 (0%)
Multiple nuclei	5 (23%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Irregular shape of nucleus	12 (55%)*	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hyperchromatic nucleus	11 (50%)*	2 (9%)	0 (0%)	0 (0%)	0 (0%)
Translucent nucleus	2 (9%)	8 (36%)	7 (32%)*	2 (9%)	1 (5%)
Anisonucleosis	7 (32%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)
Pyknosis	0 (0%)	2 (9%)	3 (14%)	2 (9%)	0 (0%)
Prominent nucleoli	12 (55%)	12 (55%)	7 (32%)*	2 (9%)	0 (0%)
Vacuolization of cytoplasm	3 (14%)	7 (32%)*	5 (23%)	2 (9%)	1 (5%)
Eosinophilic cytoplasmic inclusion	1 (5%)	7 (32%)*	3 (14%)	0 (0%)	1 (5%)

+ = Feature identified easily; ++ = feature prominent or of marked degree.
* Significantly different from the values for group 3 (Fisher's exact probability test, $p < 0.05$).

TUR in 5 of the 12 patients (42%, table 4). Regarding urothelial cells, translucent nuclei were observed in 3 patients (25%) and prominent nucleoli in 4 (33%) within 1–2 months after TUR. No significant changes in urothelial cells were subsequently found during the clinical course.

Diagnostic Categories before and after Treatments

Table 5 shows the frequencies of diagnostic categories before and at four time points after the treatments. Before treatments the incidence of positive findings was significantly higher in group 1 than in group 3 ($p < 0.05$).

In group 1 no positive findings in cytology were seen after the BCG treatment, but suspicious findings were seen for 2 patients (9%) 1–2 months after the instillation therapy and for 1 patient (5%) 3 months after the therapy. In group 2 positive and suspicious findings in diagnostic categories were each found for 2 patients (9%) 1–2 months after the epirubicin instillation therapy. There-

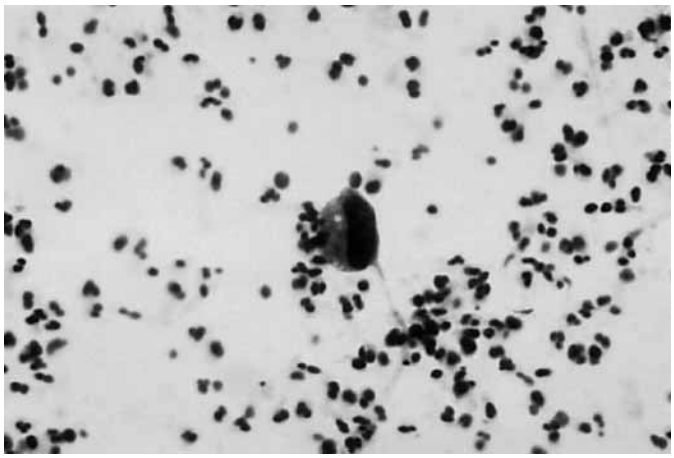

Fig. 2. Cytological findings for a patient treated with intravesical epirubicin therapy. Note increased nuclear/cytoplasmic ratio and hyperchromatic, irregular-shaped nuclei. Papanicolaou staining. × 400.

Table 3. Cytological findings for 22 patients treated with intravesical epirubicin therapy (group 2)

Cytological findings	Before TUR	After instillation therapy			
		1–2 months	3 months	6 months	12 months
Inflammatory cells					
Increase in granulocytes					
+	6 (27%)	8 (36%)	4 (18%)	1 (5%)	2 (9%)
++	1 (5%)	6 (27%)	0 (0%)	0 (0%)	0 (0%)
Increase in lymphocytes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Appearance of macrophages	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Appearance of epitheloid cells	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Urothelial cells					
Increase in superficial cells					
+	2 (9%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)
++	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Increase in deep layer cells					
+	11 (50%)*	2 (9%)	1 (5%)	0 (0%)	0 (0%)
++	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Increased nuclear/cytoplasmic ratio	10 (45%)	4 (18%)	0 (0%)	0 (0%)	0 (0%)
Multiple nuclei	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Irregular shape of nucleus	6 (27%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)
Hyperchromatic nucleus	10 (45%)	4 (18%)	1 (5%)	0 (0%)	0 (0%)
Translucent nucleus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Anisonucleosis	5 (23%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)
Pyknosis	0 (0%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)
Prominent nucleoli	7 (32%)	5 (23%)	0 (0%)	0 (0%)	0 (0%)
Vacuolization of cytoplasm	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Eosinophilic cytoplasmic inclusion	0 (0%)	1 (5%)	1 (5%)	0 (0%)	1 (5%)

+ = Feature identified easily; ++ = feature prominent or of marked degree.

* Significantly different from the value for group 3 (Fisher's exact probability test, $p < 0.05$).

after 1 patient (5%) in group 2 had suspicious findings 3 months after the treatment. In group 3 no positive or suspicious cytological categories were found during the entire clinical course after TUR.

Discussion

In general, patients with superficial bladder cancer need to be periodically followed up by cytology and cystoscopic examinations after TUR [22]. Cytological findings after surgical treatment and intravesical instillation therapy play an important role in the management of those patients. Intravesical BCG therapy is widely used for preventing recurrence in patients with multiple or recurrent superficial tumors as well as eradication of tumor cells in cases of CIS of the bladder [7–9, 17, 23]. Although it is well known that BCG induces inflammation within the bladder wall resulting in sloughing of the epithelium and probable destruction of cancer cells, the precise mechanisms of action remain unclear, and several immunological effects are also speculated to be involved. In the era of intravesical BCG therapy, it is important to evaluate cytological changes induced by the therapy. Positive findings of postoperative cytology are regarded as a significant prognostic indicator of tumor recurrence after intravesical BCG treatment [24].

Only a few investigators have reported urinary cytology findings after intravesical BCG instillation therapy and the findings were not consistent [15, 16]. Mack and Frick [15] reported cytological changes in patients with recurrent bladder cancer and CIS after TUR or biopsy to include enlarged, hyperchromatic nuclei, increased prominent nucleoli, anisonucleosis, distorted nuclear/cytoplasmic ratio of urothelial cells besides significant increase in inflammatory cells. They stressed that cytological changes

Table 4. Cytological findings for 12 patients without intravesical instillation therapy (group 3)

Cytological findings	Before TUR	After instillation therapy			
		1–2 months	3 months	6 months	12 months
Inflammatory cells					
Increase of granulocytes					
+	4 (33%)	5 (42%)	5 (42%)	1 (8%)	0 (0%)
++	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Increase in lymphocytes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Appearance of macrophages	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Appearance of epitheloid cells	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Urothelial cells					
Increase in superficial cells					
+	2 (17%)	2 (17%)	0 (0%)	1 (8%)	0 (0%)
++	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Increase in deep layer cells					
+	1 (8%)	3 (25%)	1 (8%)	0 (0%)	0 (0%)
++	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Increased nuclear/cytoplasmic ratio	3 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Multiple nuclei	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Irregular shape of nucleus	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hyperchromatic nucleus	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Translucent nucleus	0 (0%)	3 (25%)	0 (0%)	0 (0%)	0 (0%)
Anisonucleosis	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pyknosis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Prominent nucleoli	4 (33%)	4 (33%)	0 (0%)	0 (0%)	0 (0%)
Vacuolization of cytoplasm	1 (8%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)
Eosinophilic cytoplasmic inclusion	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

+ = Feature identified easily; ++ = feature prominent or of marked degree.

Table 5. Cytological diagnostic categories before and after treatment

Cytological categories	Group 1: BCG (n = 22)	Group 2: epirubicin (n = 22)	Group 3: no treatment (n = 12)
Before treatment			
Unsatisfactory sample	0 (0%)	0 (0%)	0 (0%)
Negative	4 (18%)	9 (41%)	7 (58%)
Repeat examination	2 (9%)	1 (5%)	2 (17%)
Suspicious	2 (9%)	4 (18%)	1 (8%)
Positive	14 (63%)*	8 (36%)	2 (17%)
1–2 months after treatment			
Unsatisfactory sample	0 (0%)	0 (0%)	0 (0%)
Negative	20 (91%)	16 (73%)	12 (100%)
Repeat examination	0 (0%)	2 (9%)	0 (0%)
Suspicious	2 (9%)	2 (9%)	0 (0%)
Positive	0 (0%)	2 (9%)	0 (0%)
3 months after treatment			
Unsatisfactory sample	0 (0%)	1 (5%)	0 (0%)
Negative	19 (86%)	20 (90%)	12 (100%)
Repeat examination	2 (9%)	0 (0%)	0 (0%)
Suspicious	1 (5%)	1 (5%)	0 (0%)
Positive	0 (0%)	0 (0%)	0 (0%)
6 months after treatment			
Unsatisfactory sample	0 (0%)	0 (0%)	0 (0%)
Negative	22 (100%)	22 (100%)	12 (100%)
Repeat examination	0 (0%)	0 (0%)	0 (0%)
Suspicious	0 (0%)	0 (0%)	0 (0%)
Positive	0 (0%)	0 (0%)	0 (0%)
12 months after treatment			
Unsatisfactory sample	0 (0%)	0 (0%)	0 (0%)
Negative	21 (95%)	22 (100%)	12 (100%)
Repeat examination	1 (5%)	0 (0%)	0 (0%)
Suspicious	0 (0%)	0 (0%)	0 (0%)
Positive	0 (0%)	0 (0%)	0 (0%)

* Significantly different from the value for group 3 (Fisher's exact probability test, $p < 0.05$; Mann-Whitney U test, $p < 0.05$).

such as hyperchromasia and distorted nuclear/cytoplasmic ratio, which usually suggest the presence of tumors, impair the interpretation of cytology in patients treated with topical BCG therapy. Piscioli et al. [25] reported morphological changes of urothelial cells in patients with tuberculosis of the bladder and suggested that atypical cells may be found in the urine with this condition. The present study demonstrated that intravesical BCG therapy induces nonspecific, reactive morphological changes of the urothelia accompanied by inflammation, consistent with the report by Koss [16, 26] of easy differentiation from neoplastic changes. Most cytological changes in urothelial cells were observed within 3 months after the end of intravesical BCG therapy. Our finding that translucent nuclei and prominent nucleoli in urothelial cells appear within 1 or 2 months after TUR in group 3 is in line with the description by Koss [26] that the influence of this surgery is mainly apparent within 6 weeks. These findings suggest that cytological examinations can be most safely performed 3 months after the end of instillation therapy.

In patients treated with intravesical BCG instillation therapy the urinary sediment usually shows inflammatory cells and may contain clusters of elongated, carrot-shaped epitheloid cells sometimes accompanied by multinucleated giant cells of Langhans type [16] as in the case of tuberculosis of the bladder [25]. In the present study, however, epitheloid cells were identified in only 1 of 22 patients (5%) treated with the BCG therapy and no multinucleated giant cells were observed in any cytological specimens.

BCG doses and the number of instillations have been empirically determined according to BCG strains including Pasteur, Armand-Frappier, Connaught, Tice, and Tokyo strains [27]. Pagano et al. [28] reported that a low-dose (75 mg of Pasteur strain) BCG regimen was effective with a low frequency of treatment-related toxicity, as compared with the results of the 150-mg standard regimen. Usually, a dose of 80 mg BCG (Tokyo strain) has been used in Japan [27, 29]. We previously demonstrated that a 40-mg BCG low-dose (Tokyo strain) regimen maintains the efficacy of preventing recurrence, with low frequency of toxicity [17]. In the present study no significant differences in cytological findings were observed between the 40- and 80-mg dose groups.

Chemotherapeutic agents administered intravesically, including thiotepa, mitomycin C, and doxorubicin, induce significant morphological changes in urothelial cells and their derivatives [10–14]. We previously reported cytological changes caused by mitomycin C, including enlargement and increased transparency of nuclei, and the appearance of prominent and multiple nucleoli [14]. In the present study, treatment with epirubicin was found to be associated with an increased nuclear/cytoplasmic ratio, hyperchromatic nuclei, and prominent nucleoli of urothelial cells in a limited number of cases. These cytological findings resulted in positive and suspicious findings in diagnostic categories each for 9% patients (shown in table 5), leading to confusion with neoplastic changes of urothelia. It is not clear whether nuclear hyperchromasia found in the cases treated with epirubicin is attribut-

able to the drug itself or due to persistent tumor cells after TUR. Clearly, urologists must provide cytopathologists with precise information on intravesical instillation therapy because any kind of therapy influences the accuracy of cytological diagnosis [30].

In conclusion, cytological changes induced by BCG therapy represent nonspecific, reactive changes, different from those caused by chemotherapeutic agents, and are distinguishable from malignant change of urothelial cells.

References

1 Soloway MS: Overview of treatment of superficial bladder cancer. Urology 1985;26 (suppl): 18–26.
2 Heney NM, Nocks BN, Daly JJ, Prout GR Jr, Newall JB, Griffin PP, Perrone TL, Szyfelbein WA: Ta and T1 bladder cancer: Location, recurrence and progression. Br J Urol 1982;54: 152–157.
3 Whelan P, Cumming JA, Garvie WHH, Hargreave TB, Kirk D, Newling DWW, Robinson MRG, Smith PH: Multi-centre phase II study of low dose intravesical epirubicin in the treatment of superficial bladder cancer. Br J Urol 1991;67:600–602.
4 Melekos MD, Chionis HS, Paranychianakis GS, Dauaher HH: Intravesical 4′-epi-doxorubicin (epirubicin) versus bacillus Calmette-Guérin: A controlled prospective study on the prophylaxis of superficial bladder cancer. Cancer 1993;72:1749–1755.
5 Okamura K, Murase T, Obata K, Ohshima S, Ono Y, Sakata T, Hasegawa Y, Shimoji T, Miyake K: A randomized trial of early intravesical instillation of epirubicin in superficial bladder cancer. Cancer Chemother Pharmacol 1994;35 (suppl):31–35.
6 Morales A, Eidinger D, Bruce AW: Intracavitary bacillus Calmette-Guérin in the treatment of superficial bladder tumors. J Urol 1976;116: 180–183.
7 Brosman SA: BCG in the management of superficial bladder cancer. Urology 1984;23 (suppl):82–87.
8 deKernion JB, Huang M, Lindner A, Smith RB, Kaufman JJ: The management of superficial bladder tumors and carcinoma in situ with intravesical bacillus Calmette-Guérin. J Urol 1985;133:598–601.
9 Herr HW, Laudone VP, Badalament RA, Oettgen HF, Sogani PC, Freedman BD, Melamed MR, Whitmore WF Jr: Bacillus Calmette-Guérin therapy alters the progression of superficial bladder cancer. J Clin Oncol 1988; 6:1450–1455.
10 de Voogt HJ, Rathert P, Beyer-Boon ME: Praxis der Urinzytologie. Berlin, Springer, 1979.
11 Rasmussen K, Peterson BL, Jacobo E, Penick GD, Sall J: Cytologic effects of thiotepa and Adriamycin on normal canine urothelium. Acta Cytol 1980;24:237–243.
12 Murphy WM, Soloway MS, Finebaum PJ: Pathological changes associated with topical chemotherapy for superficial bladder cancer. J Urol 1981;126:461–464.
13 Pust R, Ebner H, Kraushaar J, Rothauge CF: Diagnostische Bewertung von Zellatypien nach Gabe von Mitomycin C (Mitomycin medac®) und Adriamycin (Adriblastin®). Therapiewoche 1982;32:507–514.
14 Koshikawa T, Leyh H, Schenck U: Dignostische Probleme der Urinzytologie nach lokaler Instillationstherapie mit Mitomycin beim oberflächlichen Harnblasenkarzinom. Aktuelle Urol 1991;22:209–212.
15 Mack D, Frick J: Diagnostic problems of urine cytology on initial follow-up after intravesical immunotherapy with Calmette-Guérin bacillus for superficial bladder cancer. Urol Int 1994; 52:204–207.
16 Koss LG: Diagnostic Cytology of the Urinary Tract with Histopathologic and Clinical Correlations. Philadelphia, Lippincott-Raven, 1996.
17 Takashi M, Wakai K, Ohno Y, Murase T, Miyake K: Evaluation of a low-dose intravesical bacillus Calmette-Guérin (Tokyo strain) therapy for superficial bladder cancer. Int Urol Nephrol 1995;27:723–733.
18 Papanicolaou GN, Marshall VF: Urine sediment smears as a diagnostic procedure in cancers of the urinary tract. Science 1945;101: 519–520.
19 Takashi M, Schenck U, Leyh H, Treiber U: Use of diagnostic categories in urinary cytology. Acta Cytol 1998;42 (suppl):577.
20 Hermanek P, Sobin LH: UICC-International Union against Cancer TNM Classification of Malignant Tumours, ed 4. Heidelberg, Springer, 1987.
21 Koss LG: Tumors of the Urinary Bladder. Atlas of Tumor Pathology, 2nd ser, fasc 11. Washington, Armed Forces Institute of Pathology, 1975.
22 Schwalb MD, Herr HW, Sogani PC, Russo P, Sheinfeld J, Fair WR: Positive urinary cytology following a complete response to intravesical bacillus Calmette-Guérin therapy: Pattern of recurrence. J Urol 1994;152:382–387.
23 Takashi M, Shimoji T, Murase T, Sakata T, Sobajima T, Suzuki Y: Intravesical bacillus Calmette-Guérin (Tokyo 172 strain) therapy for carcinoma in situ of the bladder. Int Urol Nephrol 1997;29:557–563.
24 Badalament RA, Gay H, Cibas ES, Herr HW, Whitmore WF Jr, Fair WR, Melamed MR: Monitoring intravesical bacillus Calmette-Guérin treatment of superficial bladder carcinoma by postoperative urinary cytology. J Urol 1987;138:763–765.
25 Piscioli F, Pusiol T, Polla E, Failoni G, Luciani L: Urinary cytology of tuberculosis of the bladder. Acta Cytol 1985;29:125–131.
26 Koss LG: The Urinary Tract in the Absence of Cancer: Diagnostic Cytology and Its Histopathologic Bases. Philadelphia, Lippincott, 1992, pp 890–993.
27 Herr HW: Use of bacillus Calmette-Guérin vaccine: Indications and results; in Soloway MS, Paulson DF (eds): Problems in Urology: Transitional Cell Malignancy. Philadelphia, Lippincott, 1992, vol 6, pp 484–492.
28 Pagano F, Bassi P, Milani C, Meneghini A, Maruzzi D, Garbeglio A: A low dose bacillus Calmette-Guérin regimen in superficial bladder cancer therapy: Is it effective? J Urol 1991; 146:32–35.
29 Akaza H, Hinotsu S, Aso Y, Kakizoe T, Koiso K: Bacillus Calmette-Guérin treatment of existing papillary bladder cancer and carcinoma in situ of the bladder: Four-year results. Cancer 1995;75:552–559.
30 Wiener HG, Vooijs GP, van't Hof-Grootenboer B: Accuracy of urinary cytology in the diagnosis of primary and recurrent bladder cancer. Acta Cytol 1993;37:163–169.