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Cytomorphology and flow cytometry in monitoring patients treated for bladder cancer; preliminary results

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The sensitivity of voided urine and urine flow cytometry in detecting bladder cancer recurrence after combined modality treatement was studied in 106 patients. Two different techniques were used for preparation of the urine: conventional centrifugation which has been later replaced by milipore filter imprint technique. The cytologic diagnoses of patients without local recurrence were as follows: 1 (2%) positive, 6 (13 %) suspicious, 9 (19 %) atypical , 26 (56 %) negative , and 4 (9%) inadequate material for diagnosis. In 21 patients with local recurrence urinary cytology was positive or suspicious in 10 (47%), atypical in 6(28 %) and negative in 5 (23%) patients. DNA aneuploid stemline was seen in 2 patients with histologically confirmed recurrent in situ carcinoma and 6 patients without recurrent disease. Among them, there were 3 patients with positive cytology, 4 with suspicious and 1 patient with atypical cells in the urine.

Key words: bladder neoplasms-pathology; flow cytometry; urine-cytology

Introduction

The follow up of patients with bladder cancer is performed by cytology and cystoscopy with biopsy. Urinary cytology and bladder washing have distinct advantages over random or selected biopsies of bladder mucosa because they provide a wider sampling[1,2] and can identify flat carcinoma *in situ*, which is not recognized by cystoscopy. The sensitivity of cytologic examination partially depends on specimen collection and partially on the experience of the cytopathologists evaluating reactive cellular changes and changes after radio and chemo therapy. The accuracy of urinary cytology is lower in initial low-grade than in high grade tumors, therefore flow cytometry (FCM) analysis of exfoliated cells as a complementary test to urinary cytology was introduced.[3]

The aim of our study was to analyse results of urinary cytology and FCM measurements in monitoring the patients with bladder cancer treated with sequential transurethral surgery, multiple drug chemotherapy and radiation therapy.

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Material and methods

One thousand thirty seven urinary samples were collected from 106 patients, treated for bladder cancer with combined modality.[4] In 67 patients cytopathologic analysis of the urine was performed while in 39 patients DNA analysis was done as well. Two different techniques were used for urinary sample preparation: conventional centrifugation, and later miliporefilter imprint technique (filter device, Costar, Italy). All slides were immediately fixed in Delaunay and stained by Papanicolaou method. Cytologic reports were categorized as negative, atypical, suspicious, and positive.

The samples for DNA measurements were prepared by filtration of the urine through a miliporefilter, and by washing the filter in the mixture of 0.2 M citric acid and Tween 20-CA. The suspension was centrifuged at 400 g and the sediment fixed in 70% ethanol. Before staining, the samples

were centrifuged again, and the sediment was treated with 0.5% pepsin for 5 minutes. The nuclei were stained with 4-6-diamidino-2-phenilyndole (DAPI) and sulphorhodamine 101 (SR 101). Measurements were performed on a PARTEC PAS II (Munster, Germany) flow cytometer equipped with a 100W arc mercury high pressure lamp.

Classification of histograms

DNA histograms were classified into two categories: diploid and aneuploid. The histograms were classified as diploid if there was a single peak in the diploid channel position, while aneuploid histograms exhibited two or more G0 /G1 peaks. DNA index (DI) was calculated as the ratio of the modal chanel numbers of the aneuploid and diploid peaks.

Results

Cytopathologic analysis

Recurrent tumors were confirmed by histology in 21/67 patients; cytologic diagnoses in these patients were as follows: 10 patients (47%) had positive or suspicious cytopatologic diagnosis, 6 (28 %) atypical, and 5 patients (23 %) had negative diagnosis. The cytologic diagnoses in patients without local recurrence were as follows: 1 positive (2 %), 6 suspicious (13 %), 9 atypical (19 %), 26 negative (56 %), and 4 (9 %) material inadequate for diagnosis.

FCM analysis

In 39 patients there were 95 FCM measurements performed (1-8 measurements per patient). In 4 patients (13.6%) samples were inadequate for FCM analysis because of scarcity of the cells. From samples 8200-40000 signals were analysed at a flow rate of about 60/sec. CV ranged from 0.95-8, with the mean value of 2.6. DNA aneuploid stem lines were found in 8 patients, DI varied from 1.35-1.64.

Among aneuploid samples, there were 3 patients with positive cytology, 4 with suspicious and 1 patient with atypical cells in the urine. In only 2/8 patients recurrent carcinoma *in situ* was histologically confirmed. Aneuploidy antedated histologic diagnosis of recurrence by 11 and 20 months. Five patients were followed-up from 6 to 12 months, only one patient was followed-up for 3 years. The comparison of FCM data with cytopathologic diagnosis is shown in Table 1.

Table1. Comparison of FCM and cytopathologic analysis in 39 patients

	Cytologic diagnosis				
FCM analysis	neg	atyp	susp	pos	Total
diploid	14	7	3	3	27
aneuploid	–	1	4	3	8
inadequate	1	1	1	1	4
Total	15	9	8	7	39

neg = negative; atyp = atypical; susp = suspicious; pos = positive

Discussion

Since Papanicolaou and Marshal in 1945[5] described malignant cells in the sediment of the urine, cytology has become an important factor in the diagnosis and follow-up of patients with bladder carcinoma.Voided urine and irrigation specimen samples the entire bladder mucosa, and therefore the probability to identify recurrence is greater then in random biopsies. The sensitivity of the conventional urinary cytology is 50-75% and depends on several factors: on the number of specimens and the specimen collection technique, the histologic grade of the tumor, and on experience of the cytopathologist. The interpretation of cytopathologic findings in patients treated by chemo-, radio- and BCG therapy is difficult and demanding.

The sesitivity of urinary cytology in our study was 75 %, which corresponds to the data reported for Grade II and Grade III transitiocellular carcinoma. The comparison of cytologic diagnosis and ploidy analysis showed an excelent correlation: 7/8 patients with aneuploid stem lines had malignant or supicious cells in the urine. Originally, 7/8 patients had G3 transitiocellular carcinoma and 1 patient G2 tumor. Comparing cytopathologic diagnosis and DNA analysis with histology revealed some discrepancies. Of the 8 patients with aneuploid DNA histograms and suspicious or malignant cells in the urine, only 2 had recurrent cancer *in situ* documented histologically, while the remaining 6 patients had urothelial displasia. The duration of follow-up was 6 to 12 months in 5/6 patients, and 3 years in 1 patient. Now the question arises whether in these 6 patients endoscopically undetectable carcinoma *in situ* is present, or, perhaps, their cytologic and flow cytometric findings are false positive. We know that chemo- and radio-therapy may produce rather severe cellular changes[6] including enlargement of the cells, vacuolisation of the cytoplasm, enlarge-

ment of the nucleus, multinucleation of the cells, degeneration and necrosis of the superficial urothelial cells. These changes may be identified correctly with the knowledge of the patient's history.[7] Aneuploidy remains a strong marker of malignancy after treatement and is not influenced by prior intravesical chemotherapy or radition therapy.[8]

According to our experience and the data from literature, bladder washing is superior to voided urine technique regarding the preservation as well as the number of cells. In addition, the new milipore filter imprint technique collects a higher number of cells on slides. With new technique the procentage of inadequate samples was reduced to 2 % . Our experience with FCM analysis is still limited, but on the basis of this study, we consider FCM measurements very usefull and complementary to cytomorphology in monitoring patients with bladder carcinoma. To assess the definitive value of these two techniques a longer follow-up of at least two years or more is needed.[9]

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