

CORRELATION OF HETEROGENEITY INDEX SCORE (HIS) OF DNA CONTENT IN BLADDER CANCER RECURRENCE*

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ABSTRACT—Automated flow cytometry (FCM) has been used to monitor the effects of therapy and progression of human bladder carcinoma. We have previously reported a computer-based model which has shown a correlation to relative mean DNA content in cell populations analyzed by FCM. Two patients with superficial transitional cell carcinoma of the bladder were followed for several months. Using FCM the patients' tissue samples were examined and heterogeneity index scores (HIS) were determined. Both cases had recurrence. The first patient has increased in grade with a respective increase in HIS within four months (grade I→II, HIS 27.2→80.8). The second patient also has recurrence in three months both times with a grade I tumor and a score of 106.2. High scores reflect large aneuploid populations which have shown to recur. Since such a correlation exists HIS may not only offer a more objective technique with quantitative results to monitor patients but possibly can distinguish the degree of tumor malignancy.

Flow cytometry (FCM) is a relatively new technique with an increasing range of applications in clinical medicine. However, after a review of the results of FCM, this method of cell cycle determination has been limited in describing the degree of diploidy and aneuploidy by its imprecise graphic interpretation.¹

In our previous study we attempted to establish a new methodology for a computerized determination of the relative mean DNA content in cell populations by FCM analysis (heterogeneity index score, HIS).² We also showed this index can provide a quantitative and objective description of aneuploidy. Tumor grades correlated well with heterogeneity index scores.

We present 2 cases of superficial bladder carcinoma. The HIS of the primary tumor is measured and clinical course described.

Material and Methods

Two cases of superficial bladder carcinoma are presented in which the patients' tissue samples were analyzed by FCM at the time of diagnosis. They were followed for several months without any other therapy.

Specimens were obtained by cold cup biopsies and relative mean DNA content was analyzed according to our previously described method.² Single-cell suspensions were obtained by mincing the tissue on a 125- μ m nylon mesh. Approximately $3-5 \times 10^5$ cells were stained by adding 25 μ g of propidium iodide, 500 mg of sodium citrate, and 5 mL of triton X-100.

An Ortho-System 50-H multiparameter flow cytometer equipped with an Ortho 2150 Computer (Ortho Diagnostic Inc., Westwood, MA) was used to determine DNA content and cell cycle determination.

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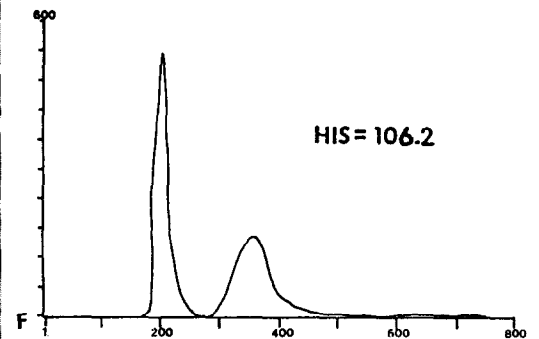
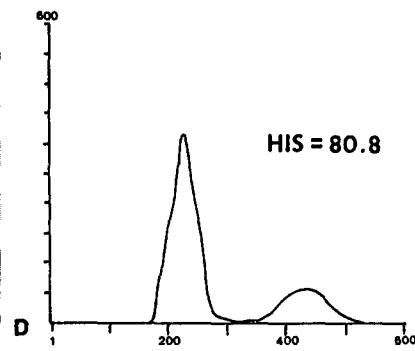
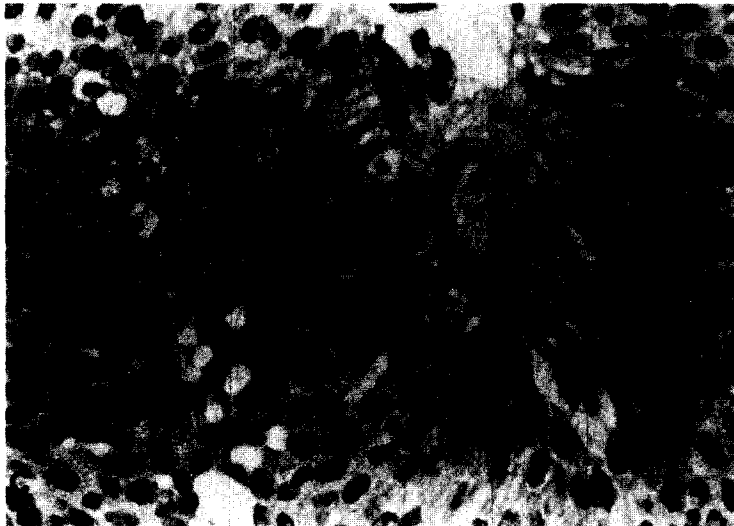
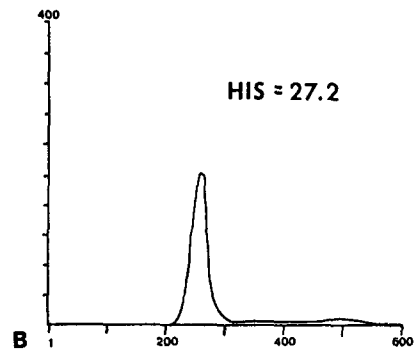
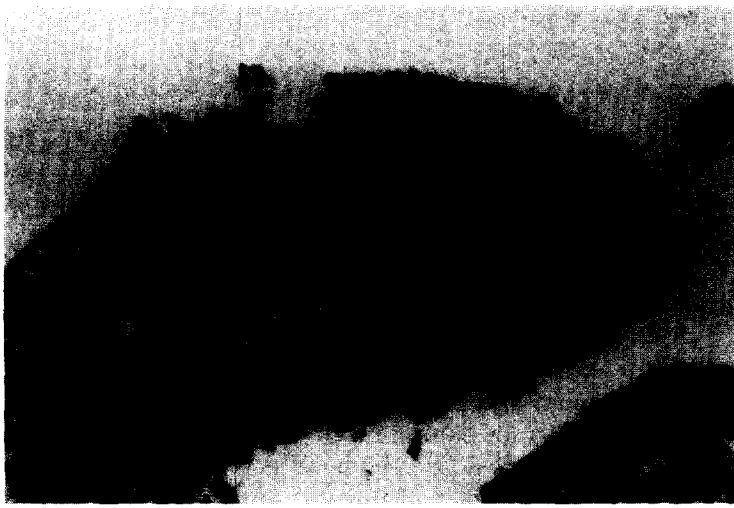


FIGURE 1. (A to D) Case 1. Pathologic slide (A) diagnosis transitional cell carcinoma grade 1. DNA histogram (B) diploid pattern with HIS of 27.7. Pathologic slide (C) showing recurrence with diagnosis of transitional cell carcinoma grade II. DNA histogram (C) aneuploid pattern with HIS of 80.8. (E and F) Case 2. Pathologic slide (E) diagnosis transitional cell carcinoma grade 1. DNA histogram (F) showing aneuploid pattern with HIS of 106.2.

Each DNA histogram was analyzed to establish distinct and objective graphic patterns. As previously reported the relative mean DNA content (heterogeneity index, HI) was calculated by the following formula:

$$\frac{\sum_{i=1}^{1000} 2X}{\text{Channel No. (Xi)} \times \text{Cell No./Channel (Yi)}} \div \text{Channel No. which has maximum Cell No. in First Peak}$$

To allow the results of the HI to be more facile in the clinical setting, HI was expanded into the heterogeneity index score (HIS): $HIS = (HI \times 100-200)$. In our previous study, HIS of transitional cell carcinoma grade I was 15.5 ± 11.7 and that of transitional cell carcinoma grade II was 25.9 ± 29.2 .

Results

Case 1

A sixty-seven-year-old Indian man was first diagnosed as having superficial bladder carcinoma in December, 1984. Cold cup punch biopsy and transurethral resection of bladder tumor (TURBT) was performed on a solitary tumor on the right posterolateral wall of the bladder. The pathologic diagnosis was reported as a grade I transitional cell carcinoma (TCC) (Fig. 1A). The patient received no adjuvant chemotherapy. Flow cytometric analysis was performed and HIS calculated. The DNA histogram performed on this tissue shows a diploid population of cells and HIS was calculated to be 27.2 (Fig. 1B). Four months later the patient was noted to have recurrent tumor superior and medial to the right ureteral orifice. Cold cup punch biopsy and TURBT revealed a grade II TCC with no muscle invasion (Fig. 1C). Follow-up DNA histogram revealed an aneuploid peak at 3.8 c and a HIS of 80.8 which reflects an increased DNA content (Fig. 1D).

Case 2

A seventy-four-year-old white woman with a three-year history of recurrent superficial bladder carcinoma had a cold-cup punch biopsy and TURBT in May, 1985. Pathologic diagnosis was reported as a TCC grade I tumor (Fig. 1E). FCM analysis demonstrated an aneuploid peak at 3.8 c and HIS was 106.2 (Fig. 1F). She received no adjuvant therapy but was found to have recurrent tumor at the dome three months later with a pathologic diagnosis of TCC grade I.

Comment

We report on 2 cases of recurrent superficial bladder carcinoma and calculated heterogeneity index scores (HIS). HIS is a concept originated from a new methodology for the accurate determination of the degree of aneuploidy and proliferative characteristics of tumor cells.² Our

previous study showed HIS correlated well with both proliferation and DNA indices. Since HIS and the histologic grading system are well correlated, this might suggest HIS could be used to clarify a tumor according to its differentiation.

In the first case the primary pathology was transitional cell carcinoma grade I with a HIS of 27.2 whose value is on the upper limit of the mean and SD (15.5 ± 11.7) for this class. The recurrent tumor progressed to a grade II, and HIS was 80.8 which reflects a high aneuploid population well beyond our mean and SD for grade II tumors. Therefore, this patient might be considered a high risk for possible recurrence. In our second case, the primary tumor was histologically a grade I but the DNA histogram reveals a large aneuploid population which reflects the high HIS. This might suggest to the clinician that there might be a high probability of tumor recurrence as shown by the follow-up cystoscopy.

Our observation of recurrence secondary to large aneuploid populations (and therefore elevated HIS) is in line with Gustafson, Tribukait, and Esposti³ who have reported a higher recurrence among aneuploid populations (94%). Since aneuploidy may be determined by its imprecise graphic representation, HIS offers the alternative of its basis on relative mean DNA content which could be indicative of tumor recurrence or prognosis.^{1,3}

In summary, high scores with low-grade tumors might alert the clinician to the possibility of recurrence. HIS can be assigned to such groups and can be followed more accurately and objectively. Such correlations also may provide a prognostic tool to the clinician. More studies are needed to define the benefits and deficiencies that HIS will provide.

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