Prognostic Significance of Clinical Stage, Histologic Grade, and Nuclear DNA Content in Squamous-Cell Carcinoma of the Anus

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Specimens from 47 cases of anal squamous-cell carcinoma were examined in Stockholm county (1978 to 1981) with respect to clinical stage (43 cases), histologic grade (41 cases), and DNA content of the tumor cells (31 cases). Follow-up ranged from four to seven years (median, 5.5 years). The increased mortality in advanced stage and high-grade lesions was significant. Analysis of DNA content showed that most tumors were aneuploid. No statistically significant effect of DNA content on survival could be demonstrated. Thus, histologic grade and clinical stage seem to be the best predictors of patient outcome in squamous-cell carcinoma of the anus. [Key words: Anal carcinoma; Squamous epithelium; Clinical stage; Histologic grade; Ploidy; Survival.]

SQUAMOUS-CELL CARCINOMA of the anus is relatively uncommon. Together with cloacogenic carcinoma it comprises 1 to 4 percent of all colorectal malignancies. In Stockholm county the incidence of anal carcinoma is 0.9/100,000/year. At the time of biopsy diagnosis most tumors are advanced stage lesions associated with a high mortality rate, and most patients die within two years. Therapy usually centers around excision of the rectum, either radical or local, which may be followed by irradiation and cytostatic therapy.

Recent reports describe improved patient outcome following irradiation in combination with cytostatics as an alternative to primary surgical treatment.

Material and Methods

Patient Material: This report is based on a study of 47 consecutive anal tumors from 29 women and 18 men with invasive squamous-cell carcinoma diagnosed in Stockholm county. The average age was 68 years (range, 32 to 93 years). The material was selected from patients who were entered in the Swedish Cancer Register during 1978 to 1981.

Clinical staging according to UICC criteria (T1-T4) was possible in 43 cases. Tumor size was unknown in four cases. Histopathologic specimens from 42 cases were reevaluated by one experienced pathologist (KE) without
knowledge of the original classification or the clinical course.

The tumors were categorized histologically according to World Health Organization criteria as low (Grade I), intermediate (Grade II) and high (Grade III). Forty-one patients received either sphincter-preserving treatment (irradiation with or without cytostatics, local excision, 22 patients) or sphincter-sacrificing treatment (radical excision of the rectum with or without irradiation, 19 patients). No patient was treated prior to biopsy and histologic diagnosis.

Six severely ill patients with high-stage lesions received no treatment.

Material suitable for DNA analysis was available from 31 primary tumors. In 16, the amount of tissue available was too small for analysis.

**Histologic Material:** The original paraffin-embedded tumor specimens were used for this study. One representative block was selected from each tumor to provide two serial sections of 4 μ thickness. After deparaffining, one section was stained with hematoxylin and eosin and the other was postfixed in 10 percent neutral buffered formalin and stained according to the Feulgen technique (acid hydrolysis with 5N HCl at 22°C for 60 minutes).

Representative fields of the tumor in the Feulgen-stained preparation were selected for photography with guidance from the hematoxylin and eosin stained section. Usually ten fields were photographed in each case. The fields were chosen from different areas where the tumor tissue was well preserved histologically and where the architecture of the tumor tissue allowed DNA analysis. The goal was to select representative fields throughout the entire tissue section. A Leitz photomicroscope was used with 40 X 1.0 oil objective (refractive index 1.518) in monochromatic light (wave length 546 nm). The sensitivity of the film (Kodal Technical Pan 2415) was 29 DIN (ASA 540) and the developing time was 4 minutes at 22°C (Kodak D19).

**DNA Measurements:** After developing the film, the DNA content of the individual cells was measured. The photographic cytophotometric method used was a modification of that described by Adams in 1968. This method is based on light transmission measurements of the Feulgen-stained nuclei, the degree of blackness of which is taken as a measure of DNA content.

In the present study 100 tumor cells were measured in each tumor among the photographed microscopic fields. All tumor cells which could be analyzed (no overlapping of the Feulgen-stained nuclei) were measured. To define the normal diploid (2 c) value 50 control cells were measured. To facilitate comparison of the DNA values from various tumors, DNA content was expressed in c-units, 2 c being defined as the median value (Ps0) of the control cells in a given case. To distinguish nondiploid cells from diploid, an upper limit of 2.5 c was set for diploid values. This borderline was empirically selected as including most (> 90 percent) of the control cells. The percentage of tumor cells above the 2.5 c limit was taken as a measure of nondiploidy. Since this fraction of cells might include proliferating diploid cells, the percentages of tumor cells with DNA values above the 5 c level, i.e., the fraction of cells with values exceeding those of proliferating diploid cells, also were calculated. The percentage of tumor cells above the 5 c limit was taken as a measure of aneuploidy.

**Survival Data Analysis:** Patients were followed to December 31, 1985, resulting in follow-up from four to seven years (median, 5.5 years). Tumor-related causes of death were established from death certificates.

The significance of the relationship between a particular variable and patient survival was tested by the log rank method. Patients dying from causes other than squamous-cell carcinoma of the anus or still alive on January 1, 1986, were considered as censored observations.

**Results**

The correlation between clinical stage (UICC) and survival of the 43 patients for whom adequate clinical data were available is illustrated in Fig. 1. The five-year survival in clinical stage T1 through T4 was 91, 80, 16 percent, and zero, respectively. The increasing mortality with advancing stage was statistically significant ($P < 0.001$).