Prognosis of Operable Squamous Cell Carcinoma of the Esophagus

Relationship with Clinicopathologic Features and DNA Ploidy

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Background. **Reports on the influence of various prognostic factors in carcinoma of the esophagus are conflicting. The prognostic value of a set of clinicopathologic factors and DNA ploidy were examined in 74 patients with surgically resected squamous cell carcinoma of the lower and middle third of the esophagus.**

Methods. **All patients had surgery performed in a single thoracic surgical unit at the Tata Memorial Hospital between January, 1984 and December, 1987. The clinicopathologic factors studied were (1) gross residual disease at operation; (2) morphology of the tumor; (3) depth of microscopic invasion; (4) lymph node involvement; (5) histologic grade; (6) vascular and lymphatic embolism; and (7) sex. DNA ploidy and S-phase fraction (SpF) were determined by flow cytometry on archival tissues extracted from paraffin blocks. Ploidy status could be determined successfully in all 74 tumors, whereas SpF could be assessed only in 25.**

Results. **Of the various prognostic factors examined with the Cox stepwise regression model, residual disease (P = 0.000), depth of invasion (P = 0.047), and lymph node status (P = 0.077) were found to be correlated with overall survival.**

Conclusions. **DNA ploidy was not related to prognosis. The overall survival of this group of patients at 36 months was 28%, and median survival was 18 months. *Cancer* 1993; 72:20–4.**

Key words: esophageal neoplasm, squamous cell carcinoma, prognosis.

Results of treatment of symptomatic carcinoma of the esophagus remain unsatisfactory. The 5-year survival figures reported in the world literature are between 8% and 20%.[1,2] In Japan and in China, where cancer of the esophagus is endemic, early diagnosis in the presymptomatic stages by cytologic screening of high-risk populations has, however, resulted in cure rates between 70% and 90%.[3–5] At the Tata Memorial Hospital, Bombay, India, nearly 800 new cases of carcinoma of the esophagus are registered every year. Of these, 20% of patients are selected for surgery, 35% are subjected to radiation therapy, and the remainder (45%) who present with advanced disease are offered only symptomatic treatment.

Clinicopathologic factors related to prognosis of cancer of esophagus have been studied by several workers.[2,6–9] More recently, attempts have been made to investigate the relationship between certain biologic properties of the tumor, including DNA ploidy and S-phase fraction (SpF) and the clinical course of the disease.[10–17] The results of these studies have been conflicting. There is no clear consensus as to whether DNA ploidy and SpF are reliable prognostic factors in squamous cell carcinoma of the esophagus. We undertook the current study to evaluate the significance of certain established clinicopathologic prognostic factors, as well as flow cytometric assessment of ploidy status and SpF in relation to survival after surgery for squamous cell carcinoma of the lower and middle third of the esophagus. The underlying goal of the study was to identify a group of patients with operable tumors who potentially had such poor prognoses that they could be spared surgical intervention and treated by radiation therapy, chemotherapy, or other palliative measures.

Materials and Methods

This study is based on an analysis of 74 consecutive patients with squamous cell carcinoma of the lower and

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Table 1. Distribution of Prognostic Features

Morphology	
Stenotic	8 (11)
Ulceroproliferative	66 (89)
Residual disease	
Present	14 (19)
Absent	60 (81)
Depth of invasion	
T1T2	18 (25)
T3T4	56 (75)
Microscopic cut margins	
Positive	4 (5)
Negative	70 (95)
Lymph node status	
Positive	31 (42)
Negative	43 (58)
Grade	
I/II	42 (57)
III	32 (43)
Vascular and lymphatic emboli	
Present	6 (8)
Absent	68 (92)
Sex	
Male	39 (52)
Female	35 (58)
DNA ploidy	
Aneuploid	60 (81)
Diploid	14 (19)
SpF	
< 20%	12 (48)
> 20%	13 (52)

Values in parentheses are percentages.

middle third of the esophagus who underwent surgical resection by the Thoracic Service A at the Tata Memorial Hospital, Bombay, India, between January, 1984 and December, 1987. In keeping with the criteria laid down before the study was started, only patients who were residents of Bombay were included, and death (rather than relapse) was chosen as the end point of the study. This ensured that even if some patients failed to come for regular follow-up, their status ("dead or alive") could be ascertained from the well established Bombay Cancer Registry.[18] In the event, however, there were only four patients who were lost to follow-up and were not registered with the Cancer Registry as having died. These four cases were excluded from survival analysis.

The various clinicopathologic and biologic factors examined are listed in Table 1. The presence of residual disease was documented at surgery, as was the gross morphology of the tumor, which was classified as being either stenotic or ulceroproliferative in nature. The margin of surgical resection was kept at 5 cm from the edge of this tumor, and a limited mediastinal lymph node dissection was performed routinely. Lower neck nodes and recurrent laryngeal lymph nodes were not removed. Depth of invasion, lymph node status, vascular and lymphatic embolism, and the status of cut margins (at 5 cm) were evaluated at histologic analysis.

DNA ploidy and SpF were analyzed on tumor tissues embedded in paraffin blocks. Flow cytometry was performed using a laser-based, multiparameter flow cytometer (Epics Profile I. Coulter, Hialeah, FL) with 15-mW power and 488-nm excitation. Three to four 35-μm-thick sections were cut, and single-cell suspensions were prepared according to the method described by Hedley et al.[19] In brief, the 35-μm sections were dewaxed in xylene and rehydrated in descending grades of alcohol. The tissues were washed in distilled water, suspended in 0.5% pepsin in 0.9% sodium chloride at pH 1.5, and incubated in a water bath at 37°C with intermittent vortexing for 30 minutes. The suspension was washed with phosphate-buffered saline (PBS) at pH 7.2. The cells were filtered through a 35-μm nylon mesh and centrifuged. The pellet was treated with 0.1% Triton-X, incubated with RNAse, and stained with propidium iodide (50 μg/ml). Tumors were classified into diploid and aneuploid categories according to their DNA index (DI), which is the numerical ratio of the channel number of the G0/G1 peak of the experimental sample and that of the G0/G1 peak of the standard diploid sample. The latter consisted of cells from paraffin-embedded, normal esophageal lymph nodes. Normal diploid cells within the sections under study also were used as an internal standard. For diploid tumors, the DI ranged from 0.9 to 1.1, whereas for an aneuploid tumor, the DI was either less than 0.9 or more than 1.1 (Fig. 1). SpF was measured from the DNA histograms using a cell-cycle analysis computer software program (Cytologic, Coulter). Analysis was limited to samples in which the tumor cell population could be distinguished clearly from normal cells. In this study, an estimate of SpF was possible in 25 cases. This figure is considerably lower than that obtained for other solid tumors, such as breast cancer, in our hands and those of others.[19,20]

Statistical Analysis

The various clinicopathologic and biologic prognostic factors that were examined were dichotomous in nature (Table 1). Each prognostic factor was correlated individually with overall survival in a univariate analysis by the log rank test, and all together in a multivariate analysis using the stepwise Cox regression model.[21]

Results

Figure 2 shows the life table survival curve of the patients in the study. The overall 36-month survival of

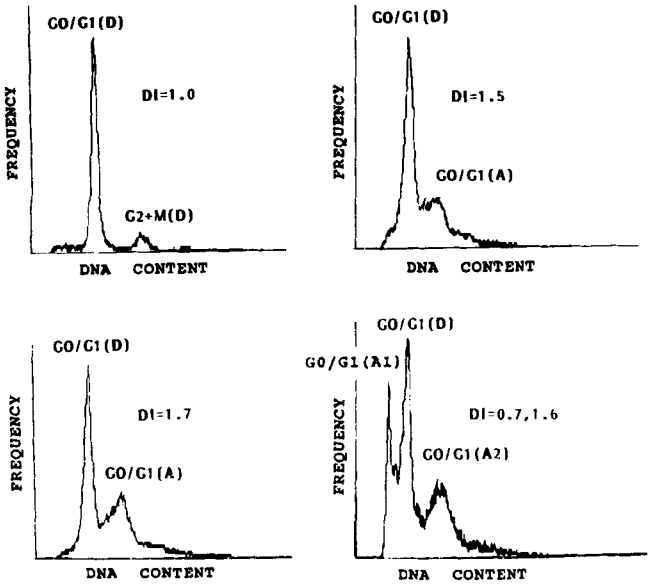

Figure 1. Representative DNA histograms of paraffin-embedded samples of squamous cell carcinoma of the esophagus. (Top left) Diploid (DI = 1.0); (top right) aneuploid (DI = 1.5); (bottom left) aneuploid (DI = 1.7); (bottom right) aneuploid (DI = 0.7, 1.6). (Top left) A diploid unimodal tumor showing a single cell population with one G0/G1 peak and a corresponding G2 + M peak with the S-phase fraction between the two peaks. (Top right, bottom left, bottom right) Aneuploid bimodal and multimodal tumors showing two and more than two cell populations that are in different phases of the cell cycle.

our patients was 28%, with a median figure of 18 months. Six patients who died in the postoperative period were excluded from survival analysis, as were the four who were lost to follow-up. Table 1, which lists the various clinicopathologic and biologic features, shows that these factors were dichotomous in distribution. Table 1 also shows that most of the tumors were ulceroproliferative in nature; only 11% were stenotic. Most patients in our study had advanced disease, and this is

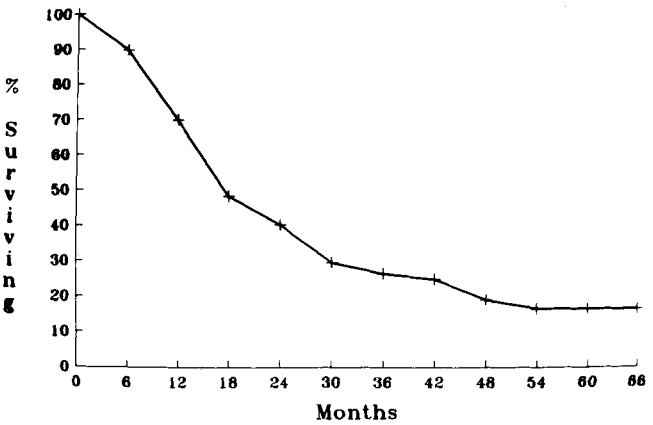

Figure 2. Overall survival of patients in the study.

Table 2. Univariate Analysis of the Relationship Between Various Prognostic Factors and Survival

Variable	Chi-square	*P* value
Residual disease	13.1	0.0003
Depth of invasion	6.95	0.084
Lymph node status	3.18	0.0746
Morphology	0.018	0.89
Tumor grade	0.615	0.433
Vascular and lymphatic embolism	0.59	0.44
Positive cut margin	0.305	0.58
Sex	0.5	0.4
DNA ploidy	0.005	0.94

reflected by the fact that 75% of the tumors belonged to T3 or T4 categories, and residual disease was present after resection in 19% of the cases. DNA ploidy was measurable in all 74 tumors. Most of the tumors (81%) were aneuploid, whereas 19% were diploid. SpF could be assessed in only 25 tumors.

Table 2 gives the relationship between the various prognostic factors and survival from esophageal cancer in a univariate analysis. The only prognostic factor that was found to be significantly correlated with survival was residual disease ($P = 0.0003$). Two other factors, depth of microscopic invasion and lymph node status, barely failed to reach statistical significance ($P = 0.084$ and $P = 0.074$, respectively). DNA ploidy did not correlate with survival ($P = 0.94$). Because SpF could be analyzed in only 25 of the 74 cases, this parameter was not included in survival analysis. Table 3 presents the results of a multivariate analysis using the Cox proportional hazard model. This analysis, which takes account of inherent interrelationships, if any, between the various prognostic factors, shows that the three prognostic factors that are independently related to survival are residual disease ($P = 0.000$), depth of invasion ($P = 0.47$), and lymph node metastasis ($P = 0.077$).

We analyzed separately the clinicopathologic features of 11 patients who survived 36 months or more. We found no difference in the distribution of various prognostic factors compared to that in the entire population, except that of this group of 11 patients none had residual disease after surgery.

Table 3. Multivariate Analysis of the Relationship Between Various Prognostic Factors and Survival*

Step no.	Variable entered	Chi-square	*P* value
1	Residual disease	16.83	0.000
2	Depth of invasion	3.936	0.047
3	Lymph node metastasis	3.137	0.077

* Cox proportional hazard model, stepwise results.

Discussion

Our result of a 28% overall survival after a mean follow-up of 36 months is a direct reflection of the relatively late stages at which cancer of the middle and lower third of the esophagus generally is diagnosed, and is comparable with figures reported in the Western literature.[1,2] We found residual disease, depth of microscopic invasion, and lymph node status to be independent indicators of prognosis. Patients with extraesophageal spread of the disease, postresection residual disease, and involved lymph nodes had a significantly poorer prognosis (Table 3). Of the three, residual disease had strongest prognostic influence ($P = 0.0003$). This was confirmed further by analysis of clinicopathologic features in those patients who survived for more than 3 years. None of them was found to have residual disease.

Skinner et al.[2] have shown that wall penetration and lymph node status are independent prognostic factors for squamous cell carcinoma of the esophagus. Lu et al.[6] have failed to identify depth of muscle invasion as a factor affecting long-term survival and have reported tumor length as a prognostic indicator. Akiyama and colleagues,[7] in a study of 1025 resected cases of squamous cell carcinoma of the esophagus, have found lymph node status to be the single most important prognostic factor. The 5-year survival in their node-negative patients was 53.8%, compared to only 15.3% in those in whom the lymph nodes were involved.

In our study, tumor morphology, tumor grade, vascular and lymphatic embolism, status of the microscopic cut margins, and the sex of the patient did not correlate with prognosis (Table 2). This is in agreement with the report of Skinner et al.,[2] who found tumor grade to be an insignificant prognostic factor, and that of Giuli and Sancho-Garnier,[8] who observed that the sex of the patient was unrelated to disease outcome. In a retrospective study of 2400 patients, however, Giuli and Gignoux[9] found that the 5-year prognosis was most favorable for patients with proliferative tumors (22%), intermediate for ulcerative tumors (19%), and worst for infiltrating lesions (12%). Our failure to observe a significant association between tumor morphology and survival may have been due to the relatively small number of patients included in our study.

The results of DNA analysis with regard to prognosis of cancer of the esophagus are conflicting.[10,11] In a retrospective study of 110 surgically resected cases of squamous cell carcinoma of the esophagus, Edwards et al.[10] reported a 70% incidence of aneuploidy, but the survival of these patients was similar to that in patients with diploid tumors. DNA ploidy did not correlate with other clinicopathologic factors. Matsuura et al.[11] measured DNA ploidy in 128 cases of esophageal carcinoma using a cytophotometric method. These workers reported that although 76.6% of patients with the aneuploid pattern had died by 2 years, only 34.4% with a diploid pattern died within this period. Our results agree with those of Edwards et al. in that we failed to observe an association between DNA ploidy status and survival in our group of patients.

In summary, our study revealed that residual disease left behind at surgery, the depth of microscopic invasion of tumor, and pathologic lymph node status are the three factors that determine prognosis for cancer of the esophagus. The study failed to establish that measurement of tumor DNA content by flow cytometry can be used to identify a subset of patients with symptomatic esophageal cancer that potentially has very poor prognosis, and which could be spared the cost and trauma of surgery and treated by conservative means.

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