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Nuclear morphometry lacks prognostic value in squamous cell carcinoma of the oesophagus

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Summary. In order to determine the possible influence in oesophageal squamous cell carcinoma of nuclear measurements on patients' postoperative survival and on various histological tumour features, we performed a nuclear morphometry study on 53 patients (50 males, 3 females) with a mean age of 57.4 years (37-79).

A statistical correlation was revealed between area, perimeter and diameter and the analysis was, therefore, performed only in terms of nuclear area.

No influence of nuclear area on postoperative survival was observed. Nor was a relationship found between mean nuclear area and either involvement of the oesophageal wall or degree of histological differentiation. The tumours showing expansive growth had a larger mean nuclear area than those of the infiltrative growth type, although differences did not reach statistical significance.

The nuclear area standard deviation (reflecting anisocytosis of the tumour) showed no correlation with survival.

In conclusion, our data do not support that measurement of nuclear parameters by static methods is of any prognostic value in surgically-treated squamous cell carcinoma of the oesophagus.

Key words: Carcinoma, Squamous cell, Oesophageal, Nuclear morphometry

Introduction

Squamous cell carcinoma of the oesophagus is a tumour with a high mortality rate. Aggressive therapy is required when the aim is curative (Skinner et al., 1986), whereas only palliative treatment is given when the prognosis is poor. Besides known factors of prognostic value such as transmural penetration of the oesophagus and lymph node metastases (Sugimachi et al., 1986), the ability to predict the biological behaviour of the tumour has also been studied by means of quantitative analysis of the DNA content of the tumour, by static cytophotometry (Matsuura et al., 1986; Stephens et al., 1989; Böttger et al., 1991) and by flow cytometry (Hasegawa, 1990; Ruol et al., 1990), producing results that are not in complete agreement. The nuclear characteristics of squamous cell carcinoma of the oesophagus and the possible relationship with postoperative prognosis have rarely been explored in the literature. The prognostic value of nuclear morphometry has been demonstrated in other types of tumour such as cancer of the ovary (Baak et al., 1988), breast (Van der Linden et al., 1986; Pesce, 1987), endometrium (Baak et al., 1981), kidney (Gutiérrez et al., 1992), and bladder (Portillo Martin et al., 1992) among others. For squamous cell carcinoma of the oesophagus, only one study has been published, that of Stephens et al. (1989) in which no conclusive results with regard to prognostic value were found. The aim of the present nuclear morphometry study was to determine more clearly the possible prognostic value of this technique in squamous cell carcinoma of the oesophagus.

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Materials and methods

A retrospective study was made of patients undergoing surgery for squamous cell carcinoma of the oesophagus (standard subtotal oesophagectomy with gastric replacement) at the University Hospital «Marqués de Valdecilla», Santander and at the Hospital «Nuestra Señora de Covadonga» Oviedo (Spain) between 1972 and 1990. Excluded from the study were those patients who had received preoperative radiotherapy and those who died from postoperative complications (that is, occurring before discharge from hospital). Thus the study included 53 patients (50 males and 3 females) whose ages ranged from 37 to 79 years (mean 57.4). The distribution by pathological staging and the clinical characteristics of the patients are shown in Table 1. Staging was determined using the UICC 1989 criteria.

The aims of the study were as follows:

1) To analyse the relationship between the post-

Table 1. Postoperative pathological stage.

TNM CLASSIFICATION	STAGE	NUMBER OF PATIENTS
T1NoMo	I	1
T1NxMo	I/IIB	1
T2NxMo	IIA/IIB	3
T2N1Mo	IIB	4
T2NoMo	IIA	3
T3NoMo	IIA	14
T3NxMo	IIA/III	5
T3N1Mo	III	22

operative survival of these patients and various nuclear measurements: a) perimeter; b) diameter; c) area. The standard deviation of the different parameters was used to reflect the anisocytosis of the tumour.

2) To study the possible influence of these parameters on a) involvement of the oesophageal wall; b) histological differentiation; c) growth type or type of infiltrative border.

The following anatomo-pathological criteria were used:

a) Wall involvement classified into mucosa, submucosa, muscular layer and adventitia.

b) Histological differentiation: well differentiated - manifest intercellular bridges and patent keratinization; poorly differentiated - cellular bridges and isolated keratinization; moderately differentiated - showing intermediate characteristics.

c) Growth type: expansive - characterized by a well-defined deep tumour border with a uniform growth front and displacement of healthy tissue; infiltrative - non-uniform depthwise growth of an infiltrative nature.

The clinical data of the patients were reviewed. New sections were taken of specimens preserved in paraffin blocks and stained with Haematoxylin-Eosin. The conventional factors were studied with an Axiomat Zeiss microscope with planapochromatic lenses.

Nuclear measurement was performed with a MOP-Videoplan analyzer (Kontron-Germany) with an analysis system (software, version 5.41, 1983). This consists of a BH-2 Olympus microscope, magnetic tablet, electronic pen, TV camera (Sony KX-14CP1) and a keyboard control unit. Selection of the nuclear area was made using the following criteria:

a) High cellularity;

b) Highest mitotic index;

c) Most evident atypias;

d) Avoidance of areas showing inflammation, necrosis or calcification.

Within the selected areas those cells situated at the periphery of the tumour ribbons were chosen. The outline of the nucleus was traced with the electronic pen, and automatic calculation was made for the perimeter, largest diameter and area (expressed in microns and square microns) and the corresponding standard deviations. All measurements were performed by the same operator. Reliability of the measurements was checked by estimating the coefficient of variation, defined as the standard deviation of a measurement, divided by the mean and multiplied by 100. For this the same structure was measured 120 times.

Table 2. Nuclear measurements and standard deviation. Area is expressed in μm^2; diameter and perimeter, in μm.

PATIENT	AREA	SD-A	DIAMETER	SD-D	PERIMETER	SD-P
1	57.41	14.89	9.09	1.65	28.83	3.53
2	116.36	52.75	14.34	4.22	41.68	10.00
3	46.07	10.47	8.54	1.39	27.79	3.03
4	48.06	19.24	9.22	2.25	27.00	4.95
5	85.67	45.48	12.53	4.03	36.87	9.87
6	80.61	32.14	11.29	3.11	33.97	7.95
7	58.55	26.00	9.66	2.44	28.68	6.23
8	49.07	18.37	9.21	1.92	27.52	4.96
9	71.22	31.33	11.42	3.48	31.86	7.61
10	78.51	25.24	11.02	2.38	33.53	5.77
11	97.01	26.30	11.93	2.09	36.54	4.96
12	100.08	30.86	12.10	2.21	38.90	5.97
13	70.47	23.59	10.30	2.36	36.67	5.40
14	73.74	28.52	11.02	2.50	32.98	6.63
15	29.31	9.88	7.59	1.98	21.78	3.90
16	50.82	23.53	9.68	2.52	28.25	6.52
17	90.31	37.49	12.41	3.46	36.93	8.36
18	68.29	24.77	11.51	2.61	33.09	5.84
19	94.47	50.54	11.40	3.09	34.97	8.46
20	64.03	32.24	10.47	3.27	30.78	8.10
21	144.29	60.43	14.75	4.11	47.06	10.80
22	41.40	13.09	8.40	2.08	25.42	4.27
23	111.04	42.66	13.14	2.87	38.93	7.13
24	74.32	18.00	10.30	1.59	31.51	3.79
25	52.18	26.96	9.07	2.79	27.76	7.05
26	110.64	73.28	13.86	5.29	40.07	12.67
27	62.19	24.17	10.97	2.83	31.28	5.78
28	86.46	28.47	11.93	2.55	34.92	5.62
29	57.21	21.49	10.46	2.56	30.90	5.66
30	59.78	21.26	9.17	1.83	28.53	4.87
31	87.01	65.47	11.83	3.75	34.61	11.01
32	74.97	29.86	10.82	3.00	32.21	7.58
33	57.47	26.15	10.87	2.78	30.06	7.05
34	77.09	25.69	10.48	2.08	32.12	5.14
35	77.83	40.85	11.55	3.63	34.10	8.28
36	73.24	21.11	11.45	2.81	33.49	5.24
37	103.20	43.00	13.60	3.83	39.80	9.11
38	93.18	25.10	12.15	2.22	35.98	4.82
39	62.85	26.82	10.89	2.75	31.41	6.29
40	73.39	34.24	10.49	3.39	32.13	8.05
41	92.81	35.89	12.08	2.75	36.66	6.98
42	43.74	31.74	8.00	2.85	24.58	7.92
43	63.47	21.23	9.42	2.12	29.45	5.34
44	72.46	25.82	10.88	2.79	31.75	5.99
45	67.22	23.58	10.04	2.33	30.99	6.44
46	71.92	29.71	10.85	2.76	31.65	6.91
47	50.02	21.60	9.37	2.17	27.36	5.65
48	52.73	19.57	9.40	2.20	27.71	4.99
49	47.75	18.38	9.11	2.31	26.84	5.14
50	88.49	36.19	11.23	2.60	35.12	6.73
51	63.18	22.85	10.27	1.91	30.12	4.84
52	68.32	32.90	9.96	2.49	30.28	7.03
53	58.17	22.32	9.64	2.16	28.95	5.39

Statistical analysis was performed with the Statistical Analysis System computer programme (S.A.S. Institute, Inc.).

Results

The coefficient of variation obtained was 1.09. The nuclear measurements are shown in Table 2. The mean values calculated were as follows:

a) Nuclear area: 72.45 μm^2 (29.31-144.29); sd: 21.60.

b) Nuclear diameter: 10.78 μm (7.59-14.75); sd: 1.55.
c) Nuclear perimeter: 32.22 μm (21.78-47.06); sd: 4.65.
Pearson's coefficient of correlation revealed a correlation between both area and perimeter (p = 0.0006) and between area and diameter (p=0.0001); the statistical analysis was, therefore, performed only in terms of nuclear area. A correlation was also observed between the values for the standard deviations of the three parameters (p=0.0001) so that the standard deviation for nuclear area was selected.

Histological findings are given in Table 3. In 2 cases, wall involvement was limited to the submucosa (3.8%); in 10 cases it reached the muscular layer (18.9%); and in 41 it extended to the oesophageal adventitia (77.4%). 17 cases were poorly differentiated histologically (32.1%); 31, moderately differentiated (58.5%); and 5, well differentiated (9.4%). Growth type was expansive in 28 tumours (52.8%) and infiltrative in 25 (47.2%).

We compared the mean nuclear area of patients surviving less than two years and more than two years. No influence of nuclear area on postoperative survival of the patients was observed (Fig. 1). Nor was a relationship found between mean nuclear area and either involvement of the oesophageal wall or degree of histological differentiation (Fig. 2). The tumours showing expansive growth had a larger mean nuclear area than those of the infiltrative growth type, although differences did not reach statistical significance (Fig. 2). The nuclear area standard deviation showed no correlation with survival.

Discussion

The well-known poor prognosis of oesophageal neoplasms has led to the study of numerous factors, both clinical and anatomo-pathological, with a view to the possible prediction of the outcome of each individual patient. Apart from information which could be provided to patients and their families, the main interest lies in being able to establish preoperatively a possible good or poor long-term prognosis that would permit the selection of the most appropriate therapy in each case, thus sparing patients aggressive measures when these offer them no benefit. To this end we studied the possible prognostic value of static nuclear morphometry - already demonstrated in other tumour types - in squamous cell carcinoma of the oesophagus so that, if it should prove of value, it might be applied to preoperative biopsy samples.

The main advantages of quantitative microscopy techniques - including nuclear morphometry - over conventional procedures are the greater objectivity, accuracy and reproducibility they offer compared with subjective observation. Moreover, there are some histological changes that are difficult for the human eye to observe and which morphometric methods can

Table 3. Histological characteristics of tumour and survival (expressed in months after surgery).

FACTOR	SURVIVAL	SIGNIFICANCE
Growth type		
Expansive	44	p=0.001*
Infiltrative	5	
Differentiation		
Well	25	
Moderate	29.6	N.S.
Poor	21.4	
Wall involvement		
Submucosa	100	p=0.04#
Muscular	33.3	p=0.6*
Adventitia	20.6	N.S.

*: Kaplan-Meier (Log-Rank); #: Chi-square test.

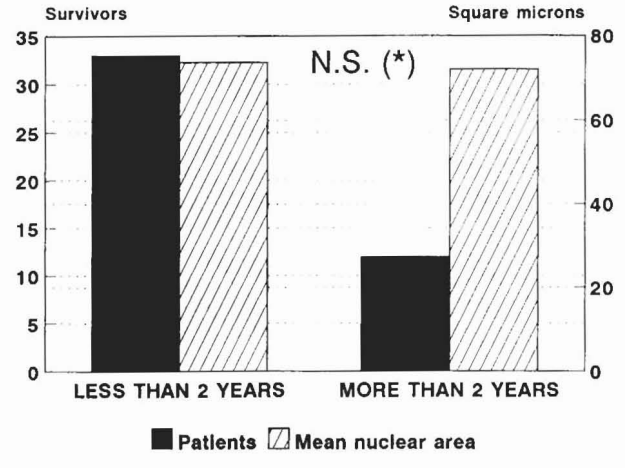

Fig. 1. Correlation between nuclear area and postoperative survival. Comparison of mean nuclear area of patients surviving less than 2 years and more than 2 years.

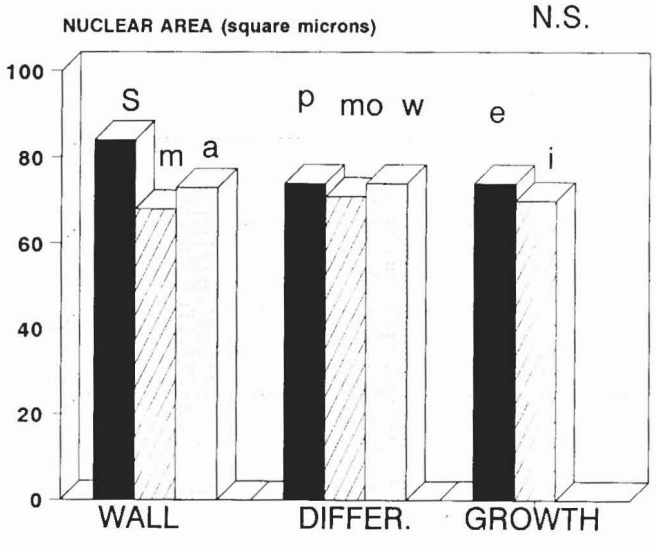

Fig. 2. Correlation between nuclear area and oesophageal layers affected by the tumour, degree of histological differentiation of the tumour and type of tumour growth. S: submucosa, M: muscular, A: adventitia, P: poor, Mo:moderate, W: good, E: expansive, I: infiltrating.

capture and evaluate by means of statistical analysis (Collan, 1984; Hall and Fu, 1985; Mariuzzi and Tosi, 1986). One factor that may affect the reliability of measurement is interobserver variation - which did not exist in our study since all measurement was performed by the same observer - and intraobserver variation. The latter is assessed by determining the coefficient of variation, which is considered acceptable when it does not exceed a value of 1.5 (Fleege et al., 1988).

In contrast to what is seen with other tumour types, no influence of nuclear area was found on the postoperative survival of patients undergoing surgery for squamous cell carcinoma of the oesophagus. Our data agree with those of Stepehens et al. (1989), who found no statistically significant relationship between nuclear area and survival. They did, on the other hand, find a clear correlation between nuclear area and wall involvement; thus tumours with areas larger than 70 μm^2 were more often associated with perioesophageal involvement than those with smaller areas. Nevertheless, in our much larger series no such correlation was observed; this might be because most of our cases were of tumours with complete invasion of the oesophageal wall whereas in the series of Stephens et al. (1989) there was a more even distribution of cases in terms of degree of involvement.

The failure of nuclear morphometry to correlate with survival in squamous cell carcinoma of the oesophagus is not surprising if one bears in mind the lack of correlation between degree of tumour differentiation and survival and between degree of differentiation and morphometry. Nor has it been possible to establish a correlation between nuclear area and prognosis in certain other tumour types such as papillary thyroid carcinoma (Ambros et al., 1989).

Another aspect studied was nuclear area standard deviation, which is an indicator of tumour cell anisocytosis. No correlation was found with survival although one might have been expected. Our results contrast with the findings of Ambros et al. (1989), who observed a significant influence of this parameter on the prognosis of papillary thyroid carcinoma, with a greater standard deviation being associated with a higher recurrence rate of the tumour. Also striking is the failure of a greater degree of anisocytosis to correlate with poorly differentiated tumours.

In conclusion, our data do not support that measurement of nuclear parameters by static methods is of any prognostic value in surgically-treated squamous cell carcinoma of the oesophagus.

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