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Epidermoid carcinoma of the lung in stage I: Factors of prognostic interest

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Summary. Factors affecting stage I epidermoid cancer of the lung were studied in a series of 29 patients treated only by surgery and followed up for ten years. A set of 13 variables with a possible influence on prognosis were investigated. The application of the Cox Univariate Analysis to the different variables showed the grade of cell differentiation and the mitotic index to be predictors. In the Cox Multivariate Analysis, the proportional regression equation revealed two independently significant variables ($p<0.01$), which were the Mitotic Index and Nuclear Area. Grouping patients on the basis of the prognostic variables indicated allows a better prediction for survival to be made for this series of patients.

Key words: Epidermoid carcinoma, Stage I, Morphometry, Mitotic index, Nuclear area

Introduction

The prognosis of lung cancer seems to depend on a large number of factors, some specifically related to the tumour and others to the host in whom the tumour is growing (Fraser and Pare, 1978), but it also depends on the interaction between the two (Feinstein and Wells, 1982).

Recognising factors of prognostic value is essential both to plan the treatment of patients and as a basis to improve therapeutic practice (Slack et al., 1972; Sather, 1986).

The prognosis of lung cancer is basically related to tumour stage, which is expressed by means of the TNM classification (Mountain, 1986).

The TNM classification is of great prognostic significance when T and N are taken both individually and as a whole. However, patients with the same histological type and with the same TNM stage are often seen to evolve differently, which indicates that there must be other factors affecting prognosis. A homogeneous series of surgically-treated epidermoid carcinoma of the lung in stage I of the new TNM classification (T1 or T2 and NO) were reviewed in order to assess what factors are of value in predicting survival in lung cancer.

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Materials and methods

The clinical records of patients admitted to the «Marqués de Valdecilla» National Hospital, Santander (Spain) with lung cancer and operated on prior to December, 1983, were reviewed. Those patients with epidermoid morphology and classified as T1 NO MO or T2 NO MO who underwent complete surgical resection surviving postoperatively were selected. The series was thus reduced to 29 patients.

In all these cases the parameters which might be of prognostic significance were collected and the following assessments were made.

Clinical Assessment

The following variables were evaluated: a) age at the time of diagnosis; b) tumour location - which took in two possibilities, central, when the tumour was observed by bronchoscopy, and peripheral, when the tumor was not visible at the preoperative bronchoscopy; c) initial ligature of the pulmonary veins - with two possibilities (+ve and -ve) depending on whether the ligature of the pulmonary veins had been performed as a first step in the resection of the lung or as a second or third step; d) tumour size - maximum diameter of the tumour was taken into account and was expressed in centimetres; e) T status in TNM classification - the criteria of the new 1986 classification (Mountain, 1986) were followed for T1 and T2; f) survival time - the date of the surgical operation was taken as zero and survival time to be the number of months from surgery to death or to review for

this study, which was at ten years; and g) state - the three possibilities considered were alive, dead or lost.

Pathological Assessment

A pathologist who did not know the outcome of the cases reviewed prospectively all the histological preparations to establish the following parameters: a) grade of cell differentiation - the criteria of the WHO were followed and the three grades considered were well, moderately and poorly differentiated; b) pleural infiltration - expressed as either present or absent, infiltration of the visceral pleura was considered positive when tumour cells were observed in the mesothelial layer or the fibroelastic layer of the pleura; c) tumoral permeation of pulmonary lymphatic vessels - this was labelled positive when clumps of tumour cells were detected in the pulmonary parenchyma adjacent to the tumour inside structures that appeared to be lympatic vessels, but only when capillary endothelium was clearly identified and no erythrocytes were present; d) tumoral permeation of blood vessels - this was positive when microscopy showed that the tumour infiltrated all layers of the vessel and protruded into the lumen, or that there were tumoral thrombi in contact with the vessel wall; and e) tumoral necrosis - this was labelled positive when a homogeneous magma of cell debris surrounded by neoplastic cells was observed.

Morphometric Assessment

A kontron MOP-VIDEOPLAN Analyzer, which uses the September 1983 5.41 version of Kontron Standard Copyright Software, was used. Preparations of tumour tissue embedded in paraffin were sectioned at 5 μm and stained with haematoxylin and eosin. Each preparation was viewed under a x 100 oil-immersion objective and the camera lucida was used to measure 100 nuclei by means of a cursor. The mean nuclea area (NA) was then obtained. Two groups of patients were established, one with NA <55 μm^2 and the other with Na >55 μm^2. The same preparations were used to obtain the mitotic index (MI), for which a x40 lens with a total magnification of 400 was used to count the number of mitoses existing in ten adjacent fields; for a mitosis to be considered as such the criteria of Baak and Oort (Baak and Oot, 1983) were applied. Patients were divided into three groups: <35 mitoses, 35-45 mitoses, and >45 mitoses. Epithelium/Stroma ratio was also assessed, for which a x10 lens was used. The outline of the epithelium was traced with the cursor. When the epithelium had been separated from the stroma, measurement was made by tracing the outline of the stroma since the separations mainly corresponded to the retraction of the epithelium. To avoid duplications in measuring, the areas already measured were displayed on the monitor. It was thus possible to know which areas had been measured and which still had to be measured. From three to ten fields were measured depending on their homogeneity and a computer carried out the calculation of the percentages of epithelium and stroma.

Statistical Assessment

With the above-mentioned variables actuarial survival tables were made using the Kaplan-Meier method (Kaplan and Meier, 1958) and the different possibilities presented by the variables in terms of survival were compared by means of the Long-Rank Test (Peto et al., 1977). The Cox multiple regression analysis was also performed (Cox, 1972). This analysis identifies the variable which provides most independent information and the result is expressed as a hazard function in which the factors contributing to the model appear in descending order of importance. The Cox multivariate analysis was performed with the BMDP programme (Dixon, 1981).

Results

The ages of the patients in this series ranged from 45 to 73 years with a mean age of 59. The tumour was located centrally in 17 patients (58.6%) and peripherally in 12 (41.4%). Ligature of the pulmonary veins was

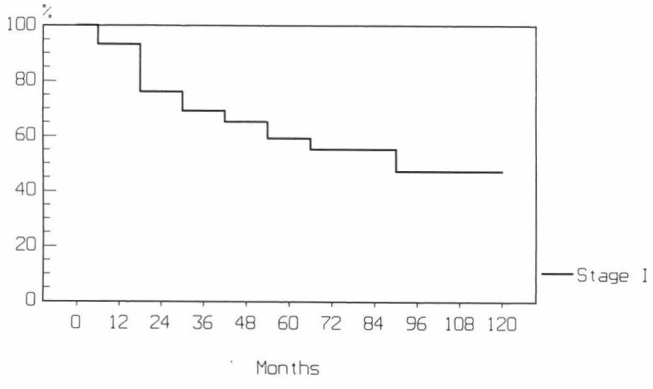

Fig 1. Survival in stage I epidermoid carcinoma of the lung.

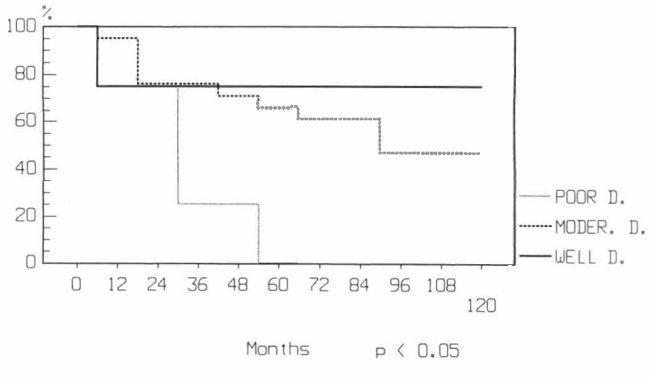

Fig. 2. Survival in stage I epidermoid carcinoma of the lung in relation to grade of cell differentiation.

performed as a first step in surgery in 7 cases (24%) and as a second or third step in 22 (76%). Tumour size ranged from 2 cm to 11 cm with an average of 5.4 cm. In 21 (72.4%) cases the tumours showed moderate cell differentiation against 4 (13.8%) with good and 4 (13.8%) with poor differentiation. The visceral pleura was infiltrated in 8 patients (27.6%) compared with 21 (72.4%) in which it was tumour-free. Tumoral permeation of the lympatic vessels was detected in 14 (48.3%) patients while 15 patients (51.7%) showed none. Tumoral permeation of the blood vessels was present in 6 (20.7%) but absent in 23 (79.3%) of the patients. Tumoral necrosis was observed in 14 (48.3%) of the cases but was not detected in the other 15 (51.7%). Most of the tumours (86.2%) were classified as T2, the remaining 13.8% being T1. The measured Nuclear Area ranged from 34.07 μm^2 to 105.22 μm^2, with an average of 62.20 μm^2. The MI ranged from 19 to 74 mitoses, with a mean of 42.17. The mean Epithelium/Stroma ratio was 60.47%, ranging from 82.72% to 28.47%.

The accumulated survival of the patients in our series for Stage I Epidermoid Carcinoma was 65% at five years and 47% at ten years (Fig. 1). 14 patients died during follow-up: 4 due to the local recurrence; 8 due to metastases; and 2 due to the development of another tumour.

Of all the variables included in this study those which on the survival curves, showed prognostic significance after 10 years' follow-up were Grade of Cell Differentiation (Fig. 2) and MI (Fig. 3). NA was on the significance borderline (Fig. 4). Besides these variables, significant differences in survival after 5 years were obtained between patients with and without tumoral permeation of the peritumoral lymphatic vessels (Fig. 5) and between those with and without tumoral necrosis (Fig. 6). No correlation with prognosis was found for tumour location, initial ligature of the pulmonary veins, tumour size, pleural infiltration, tumoral permeation of the blood vessels, T Status nor Epithelium/Stroma ratio.

When the Cox Regression Analysis was performed, the different variables were introduced independently into the regression and Grade of Cell Differentiation and MI were seen to be predictive (Table 1). When each independent variable was introduced stepwise into the proportional regression, a group of two independently significant variables was found ($p<0.01$): these were MI and NA (Table 2).

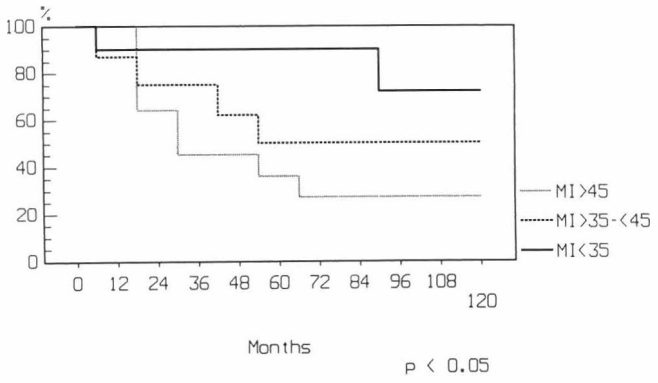

Fig. 3. Survival in stage I epidermoid carcinoma of the lung in relation to the mitotic index.

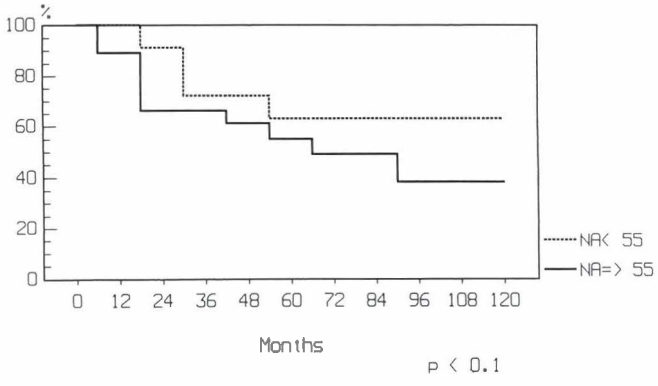

Fig. 4. Survival in stage I epidermoid carcinoma of the lung in relation to nuclear area.

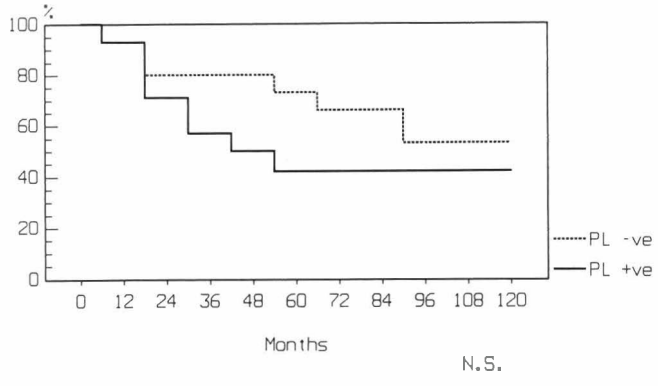

Fig. 5. Survival in stage I epidermoid carcinoma of the lung in relation to the permeation of the peritumoral lymphatic vessels (PL).

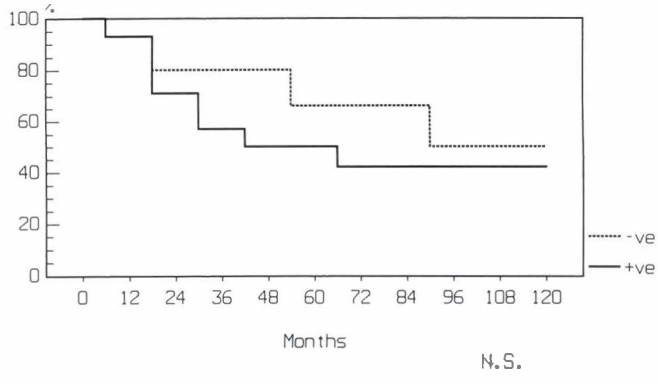

Fig. 6. Survival in stage I epidermoid carcinoma of the lung in relation to tumoral necrosis.

Table 1. Contribution of each variable to the Cox Univariate Regression Model.

VARIABLE	chi^2	p
Age	0.0471	0.8228
Location	0.4895	0.5087
Ligature of veins	0.6044	0.5571
Tumour size	0.0216	0.8781
Grade of cell differentiation	4.4954	0.032*
Visceral pleura infiltration	0.0037	0.9487
Tumoral permeation of lymphatic vessels	1.149	0.2837
Tumoral permeation of blood vessels	0.8832	0.6502
Tumoral necrosis	0.7978	0.6246
T status	0.0094	0.9195
Nuclear area	1.9910	0.1541
Mitotic index	6.3843	0.011*
Epithelium/Stroma ratio	0.1049	0.74492

*: statistically significant ($p<0.05$).

When Grade of Cell Differentiation was introduced with MI, the model was worse, which means that the two variables are correlated and the former does not contribute more information than that contributed by MI. On the other hand, when NA, which was not significant in the univariate analysis, was introduced together with MI, the model improved significantly. This means the two variables are independent.

Discussion

As is well known, in 1986 the system of lung cancer staging was modified (Mountain, 1986) with tumours classified as T1, N1 being excluded from Stage I thus leaving T1 NO and T2 NO tumours, both of which have a similar survival rate (Williams et al., 1981).

In attempt to establish the factors affecting the prognosis of Stage I Epidermoid Cancer in particular, in our series different clinical, pathological and morphometric factors were assessed.

There are few studies on the relationship of different morphometric variables with the prognosis of lung cancer. However, morphometry has been used in tumour pathology to distinguish between benign and malignant tumours (Kempson, 1976; Norris, 1976; Ranaldi et al., 1986), to classify malignant tumours (Barry and Sharkey, 1986; Nomori et al., 1986) and to establish a prognosis for the latter (James and Davey, 1979; Crissman et al., 1984; Baak et al., 1985).

In our series, the variables which showed prognostic significance in the univariate analysis were Grade of Cell Differentiation and MI. As was to be expected, the two variables are correlated; the poorer the differentiation observed, the more mitoses and vice versa. When the multivariate analysis was performed, however, the Grade of Cell Differentiation did not contribute more information than MI. Although there are more sophisticated methods to establish the degree of cell proliferation, the MI method is the oldest, the easiest, the quickest, and the cheapest (Baak, 1990). Takise et al. (1988) found that MI was capable of establishing prognostic differences for adenocarcinomas of the peripheral lung of less than 2 cm in diameter. The importance of the MI in the prognosis of lung cancer has also been established by James et al. (1979). On the other hand, Lee et al. (1989) found no relation between the MI and prognosis; their definition, however, was the number of mitoses per thousand neoplastic cells.

Table 2. Cox regression - Final set of predictors.

VARIABLE	EXP(Beta)	chi^2	p
Mitotic index	1.05996	8.38938	0.0039
Nuclear area	1.03893	4.39910	0.0339

chi^2 for the model 10.52095: p= 0.0053.

Tumoral necrosis, which was considered as present or absent, only established significant prognostic significance after five years' follow-up, but this significance was lost at ten years. Elson et al. (1988) reported the significance of tumoral necrosis in the prognosis of lung cancer, but their classification was absent, focal or extensive. On the other hand, Lipford et al. (1984) found no prognostic significance. Nor did Lee et al. (1989) correlate tumoral necrosis with prognosis in a series of Stage III non-small cell lung cancers; in their study only the peritumour lymphoid index correlated with survival and their results do not support the hypothesis that tumoral necrosis is responsible for lymphoid infiltration.

As for Nuclear Area, though it did not establish significant differences for survival at ten years, this was seen to be higher in patients who had a smaller NA (Fig. 4). Takise et al. (1988) obtained similar results for NA. However, they were able to observe the correlation between prognosis and the standard deviation of the mean of the measured nuclear areas, which indicates the degree of atypia for each tumour; the greater the standard deviation, the greater the degree of atypia and the poorer the prognosis. However, in our series, NA was the second most important independent factor contributing information to the multivariate regression model; this is in agreement with the reports of Tosi et al. (1982) for breast cancer and Lee et al. (1987) for thyroid carcinoma.

The prognosis for Stage I Epidermoid Carcinoma of the Lung is influenced in our series by the MI and to a lesser extent by NA. It is, therefore, necessary to take these factors into account when establishing a prognosis and planning treatment.

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