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Predicting prognosis in lung cancer: Use of proliferation marker, Ki67 monoclonal antibody

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Predicting Prognosis in Lung Cancer: Use of Proliferation Marker, Ki67 Monoclonal Antibody

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Abstract

An investigation was carried out to assess the prognostic significance of proliferation marker Ki67 in a group of tung cancer patients treated by surgery (limited disease). Tissue was not available for Ki67 immunostaining in inoperable group. The diagnosis is established by bronchial biopsy which does not carry enough tissue for frozen section and counting. This study is supplemented by estimating the prognostic significance of histological sub-types in the operable group and in a group of inoperable patients with extensive disease. These are usually treated by radiotherapy and/or chemotherapy. In all, 267 patients were studied including 105 treated by surgery. These patients attended King's College and Brompton Hospital, UK, between 1986 and 1989. With regard to proliferation marker Ki67 done for the surgical group, only patients with Ki67 scores of less than 5% did survive significantly longer than the rest. Histology did not make any significant contribution in determining prognosis in both operable and inoperable groups. Although follow-up is limited (mean 20 months), Ki67 antibody seems promising in identifying low and high grade disease in the initial stage of lung cancer. It may prove useful for category of patients with high scores to be placed on chemotherapy/radiotherapy. Results suggest that in the case of lung tumour, proliferative activity is a better prognostic indicator than histological type (JPMA 48:66, 1998).

Introduction

For patients with lung cancer, the choice of therapy consists of surgery, radiotherapy and chemotherapy. In general, the choice depends on applying the criteria of operability and histological diagnosis. Non-small cell carcinomas, i.e. squamous, adenocarcinoma and large cell anaplastic, are thought to have a better prognosis than small cell carcinomas, but the role of histology in assessing prognosis in lung cancer has been the subject of much controversy. Although some histological studies have found it useful in predicting survival[1-3], in other studies, its limitations inpredictingprognosis have been noted[8-7]. A study carried out the study on a series of 267 patients with lung cancer[8], confinned Stanley's6 findings that survival was significantly belier with limited disease than with extensive disease in both non-surgical, ($P<0.0001$) and surgical groups ($P<0.01$); that Feinstein staging and Karnofsky performance status generally showed the survival differences expected, but there were no differences associated with the age or sex of the patients. Most of these prognostic determinants with the exception of histology, have only been measurements of the state of advancement of the disease and physical condition of the patient Significant difference was found in survival between patients treated by surgery and those treated by radiotherapy and/or chemotherapy ($P<0.000l$). Surgery cannot be said to have improved prognosis, because surgical patients were selected on the basis of spread of the disease. Also, it may be that treatment cannot be regarded as a prognostic factor. An improved way of assessing the outlook for patients with lung cancer and of identifying subgroups with better prognosis or better responsiveness to different forms of therapy would therefore, be a good step forward. Many methods

have been used in the past to estimate dividing cells in different lung tumours. Most of these studies have concentrated on autoradiotherapy with tritiated thymidine. For example, Hàinau et al[9] showed higher growth fraction in large and small cell carcinomas, Kerr et al[10] found little difference between small cell and non-small cell carcinomas on the basis of their tritiated thymidinc data.
This study explored the possibility of estimating proportion of actively proliferating tumour cells with the help of the monoclonal antibody .Ki[6,7], which detects a nuclear protein expressed by cells in active phases of the cell cycle[11]. The anligen is a non-Histone protein constituting part of the nuclear matrix during interphase and of the chromosome scaffold during mitosis. It is a timer not an initiator of ccli division. The antigen recognized by Ki[6,7] is required for DNA synthesis in vitro and a gene (s) on human chromosome 10 is associated with its expressions. This method has been used for this purpose in other malignant tumours[12], but in lung cancers data is scanty[13].

Materials and Methods

Patients: The non-surgical group consisted of all new patients attending the King's lung cancerclinic between September01, 1286 and August 31, 1989 who did not undergo surgery. The surgical group consisted of patients operated at Brompton and King's College Hospital within the same period and for whom, a tumour specimen was available. The criteria which determined selection for surgery included disease staging based on various diagnostic procedures, but only patients with disease limited to one lung without lymph node involvement were considered suitable. Pulmonary, cardiac and liver function tests played a significant role in the decision. In patients with poor physical status and advanced age, the above selection criteria were more strictly scrutinized. Histology played little role, since even patients with samil cell carcinomas had resections if the disease was apparently confined to one lung.
Tissue: Fresh frozen tissue is required for the estimation of growth fraction by the Ki67 immuno method. Tissue from 105 of this series of 267 patients who attended King's College and Brompton Hospital between 1986 and 1989 was available for this purpose. The alkaline phosphatase anti-alkaline phosphatase (APAAP) method of immunostaining was applied to visualize Ki67 labelled cells. Sections from the frozen tumour were cut at 6 cm and immunostained after fixation in acetone for 30 min. Alkaline phosphatase anti alkaline phosphatase (APAAP) method was selected because background staining due to endogenous peroxidase could be avoided. Sections were incubated with primary antibody to Ki67 (Dakopatts M722) for 1 hour in a wet chamber at dilutions varying from 1:25 to 1:100 depending onthe batch of antibody, followed by secondary rabbit antimouse antibody at a dilution of 1:30, then by the APAAP complex (Dakopatts D65 1) at a dilution of 1:25 each for 30 minutes. For negative control primaiy antibody was replaced by serum. Section were washed with Tris-buffered saline between incubations. Alkaline phosphatase activity was demonstrated by a simultaneous capture method using a substituted naphthol as the substrate and a diazonium salt as the capture reagent. Section were counter stained with Meyer's haematoxylin[14].. In each specimen, over 1,000 cells were counted in random fields (except in 5 tumours where counts ranged from 500 to 900 cells), using a x40 objective with an eye piece graticule. All nuclei with red staining, irrespective of intensity were counted as positive, The total number of tumour cells counted were divided by the nuniberofKi67 positive tumour cells, provided aKi67 index for each tumour.
Follow UI): Details from each of the 267 patients were entered on a protocol prepared with the help of Department of Community Medicine. Follow up of patients was carried out in the out- patient clinic, through medical records and by correspondence with general practitioners. The mean follow-up time from entry to the series was 20 months but the appearance of first symptom related to lung cancer was taken as the starting point for survival curves rather than the date of diagnosis or date of first attendance. This being the earliest indicator of the disease process, could easily be distinguished by the

patient from ordinary smoking related problems.

Histological type: Four main histologic groups, squamous cell carcinoma (119 patients), adenocarcinoma (54 patients), large cell anaplastic (29 patients) and small cell carcinoma (37 patients) were entered in the protocol. The squamous and adenocarcinoma designation encompass all degrees of differentiation. Histological type was not known for 28 patients.

Statistics: For analysis of the data, a life table was constructed for each group of patients and survival probabilities determined[15] so that survival curves could be drawn. A chi- squared approximation to the conditional log rank test for group heterogeneity[16] was used to test differences between survival data for the various groups.

Results

One hundred and forty two of these patients have died during the study, including twenty patients for whom Ki67 scores were available.

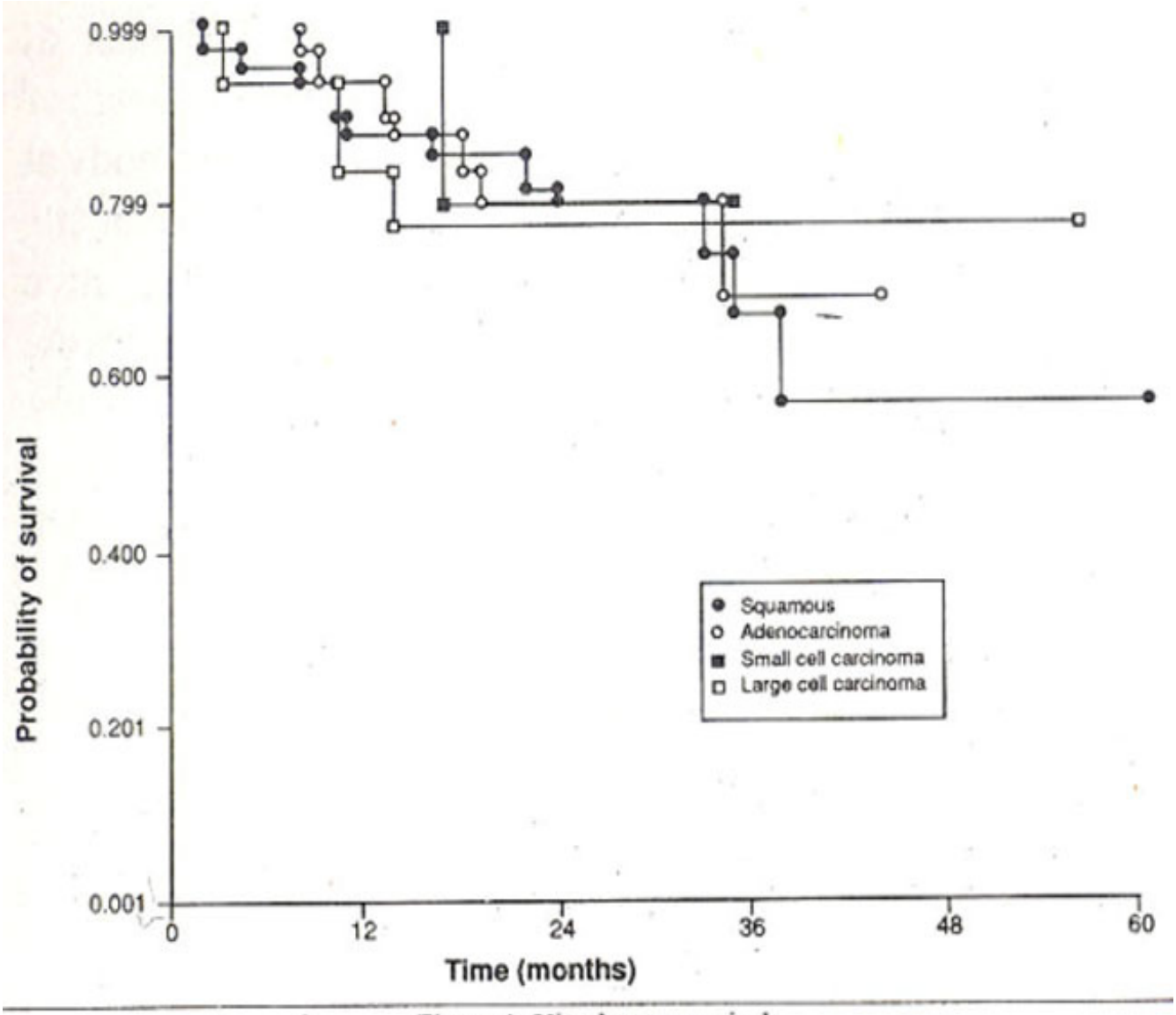

Figure 1. Histology - surgical.

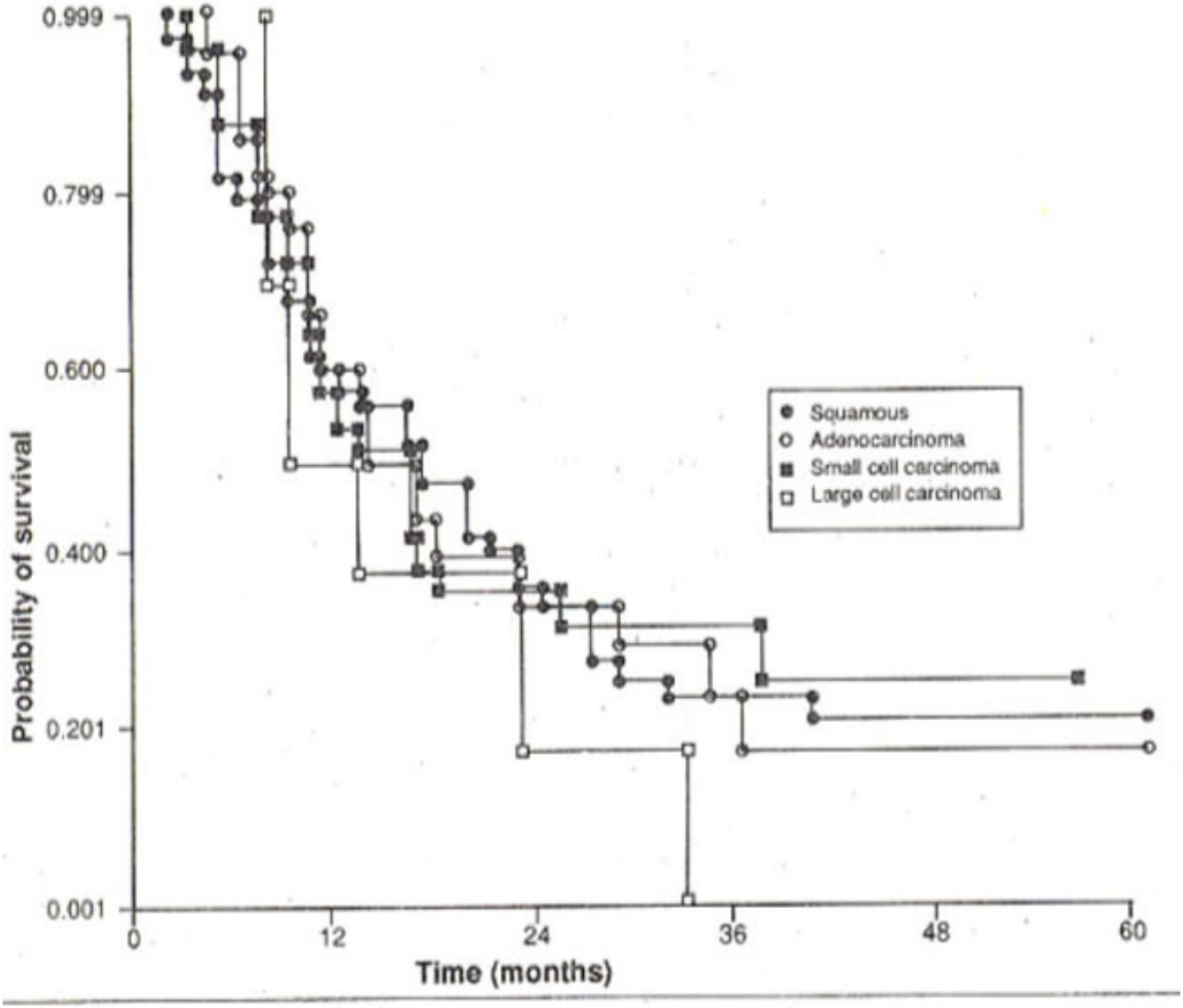

Figure 2. Histology - non-surgical.

Figures, 1 and 2 show that histological subtype did not affect survival, as no significant differences were found within the surgical or non-surgical groups. (overall P>0.9 and >0.8 respectively).

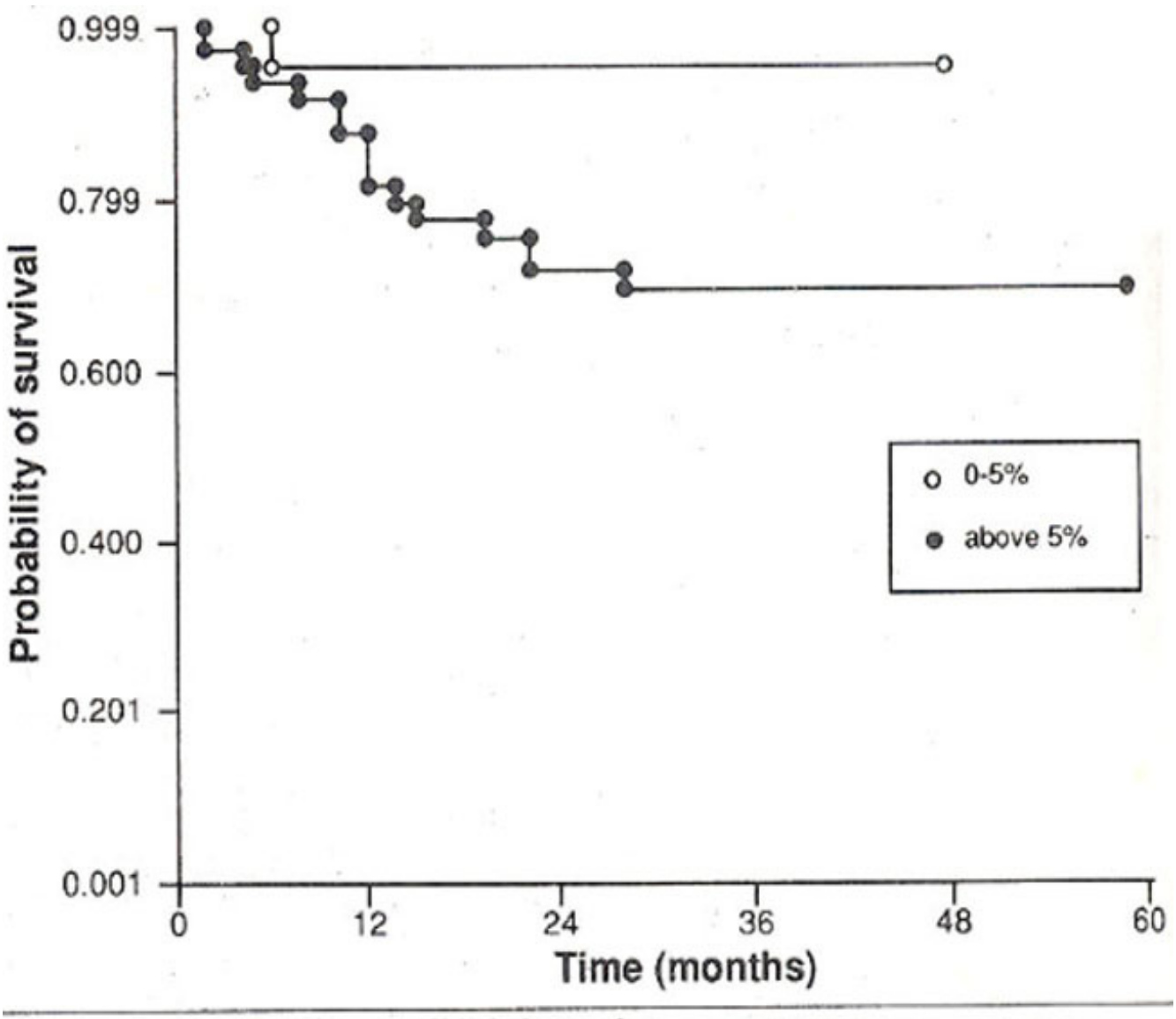

Figure 3. Ki67 in human lung cancers.

Figure 3 shows that patients with Ki67 scores of less than 5% did survive significantly longer than the rest, P<0,02. To make sure that the cut-off was really at 5%, the Ki67 results wre further analyzed. It was shown that survival with scores of 0-5% was significantly betterthanwiththose of>5- 10%, P<0.02. Also, the difference between scores of 0-10% and those over 10% was not significant P>O.2. Thus, 5% would indeed appear to be the appropriate cut-off for survival benefit. The mean Ki67 score for squamous cell carcinomas was 15.71, adenocarcinoma 10.99, large cell anaplastic 20.76 and small cell carcinomas 23,75.

Discussion

This study was started with the hypothesis that estimation of proliferative activity would be more relevant than histological type to assess lung cancer prognosis. In their publication, Watkin et al[7] identified histological type together with age at diagnosis as important prognostic factor in lung cancer. The overall distribution of cell types showed a predominance for squamous cell carcinoma. The five year survival for adenocarcinoma, squamous cell, undifferentiated and small cell carcinoma (aftertreatment) being 22.5, 18.5, 10 and 3.5% respectively. Nevertheless, survival curves for all

histological types showed a rapid initial fall within 12 months. It is this initial period where most of the patients with lung cancer succumb to their disease and hence, methods are required which can categorize low and high grade disease, so that aggressive chemotherapy could be prescribed. With the invention of various immunological methods, new markers have emerged, of which Ki67 monoclonal antibody is one and is being extensively used for estimating proliferating cells. Various markers of cell proliferation have been used in the past, but follow-up of the patients h9s given disappointing results for lung tumours. Kerr et al[17] followed 46 primary bronchogenic carcinoma cases for which thymidine labeling index was measured for 5 years, and found no correlation between reduced survival and higher tumour thymidine labeling index. Five year survivors in their series had small tumours, had T_1 status and stage I disease. Similarly, Weiss[18] determined mitotic index in 37 lung cancer patients and found no correlation with the survival, Ki67 score, as evidenced by our results, is more promising. Although Ki67 counting was confined to the surgical group of patients and therefore, for the most part to limited disease cases, there would appear to be clear distinction between survival rates in patients with low (<5%) and high (>5%) scores. Estimation of growth fraction by Ki67 has an edge over other methods, since it stains cells in all phases of the cell cycle including late Gi, S, G2 and M as compared to tritiurn labeling or uptake ofbromodeoxyuridine which label cells in S-phase only. Very little information is available on follow-up data of various tumours stained by Ki67 antibody. Hall et al[19] analyzed 91 patients with non-Hodgkin's lymphomas and found a vety strong correlation between low Ki67 index and low grade histology, high Ki67 index and high grade histology. They found worse survival for low grade lymphoma with relatively high Ki67 index (>5%) than those with an index of <5%. which is similar to our results. In their follow up data of lung cancer patients, Tungekar et al[13s] have shown almost similar results, whereas patients with low scores survived longer than those having highscores. This differencebeing significant only inearly part. Onthe basis of our results which are presently shortterm, itniay be concluded that histology on its own is of little value in assessing pmgnosis. With regard to otherfactors consisted, type of treatment is significant but this is probably due to selection of patients with limited disease for surgely. Extent of the disease is the important prognostic indicator, other significant factots being simply indicators of this extent Ki67 score would seem to be potential piognostic indicator, pmvided only broad categorization is attempted i.e. higher or lower than 5%. Complete follow-up of these patients is being undertaken and will be published as soon as possible. Immunohistochemical staining of bronchial biopsy material with Ki67 could help distinguish prognostically meaningful groups in inoperable cases as well.

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