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Eur J Cancer, Vol. 26, No. 5, pp. 622–625, 1990.
Printed in Great Britain

0277-5379/90$3.00 + 0.00
Pergamon Press plc

Thymidine Labelling Index as Prognostic Factor in Resected Non-Small Cell Lung Cancer

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To assess the prognostic value of tumor proliferative activity, 89 patients with operable non-small cell lung cancer were studied. Tumor samples were obtained during surgery and cell kinetics were analyzed by the *in vitro* thymidine labelling index (TLI). The overall median TLI (2.9) was used to identify two subsets of patients with high and low proliferating tumors. In univariate analysis survival was significantly longer in patients with lower TLI ($P = 0.047$) and with stage I–II ($P = 0.003$) and T1–T2 tumors ($P = 0.043$). In multivariate analysis, stage was the most important prognostic parameter ($P = 0.004$). The risk of death for patients with TLI higher than 2.9 was increased (hazard ratio = 2.01, CI = 0.96–4.27).
Eur J Cancer, Vol. 26, No. 5, pp. 622—625, 1990.

INTRODUCTION

PROGNOSIS in patients with non-small cell lung cancer (NSCLC) is generally poor, even when the disease is resectable at diagnosis. Among operated patients, 5 year survival ranges from 27% to 37% and no factor, except stage, has been consistently shown to influence survival [1, 2]. The thymidine labelling index (TLI) is the percentage of cells in DNA synthesis in a tumor population, which reflects proliferative activity [3, 4]. Tumor proliferative activity correlates with clinical factors, including receptor status, grading, histology, tumor size, and with prognosis in tumors such as breast cancer, non-Hodgkin's lymphoma, myeloma and leukemia [5–9]. In contrast, little is known about NSCLC growth rate and its importance in prognosis [10, 11]. We have investigated the relation between clinical and kinetic indices and have assessed the prognostic importance of TLI in patients operated on for NSCLC.

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PATIENTS AND METHODS

We studied 89 consecutive patients undergoing radical surgery in the Department of Thoracic Surgery, S. Martino Hospital, between November 1984 and February 1988. No patient had been previously treated with chemotherapy or radiotherapy. Eighty-two patients had a pneumonectomy or lobectomy and 7 patients a bilobectomy or atypical lung resection. Two patients died within 24 h of surgery, for causes not related to the tumor, and were excluded from survival analyses. Moreover, 12 patients with T3 or N2 tumor received radiotherapy to the chest after surgery. Clinical and histological variables, including age, sex, type of surgery, histology, tumor size, nodal status and stage of disease were analyzed for their relation to TLI and survival.

TLI

At least 1 cm^3 from the cut surface of the tumor was transported to the laboratory in RPMI 1640 (Flow) in a sterile vial on ice. Kinetic analyses were done on single cell suspensions [3, 12]. Cells (2×10^6 to 3×10^6/ml) were suspended in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and incubated with 10 μCi/ml [^{3}H]thymidine (Amersham) for 30 min at 37°C in a Dubnoff shaker. Radiolabelling was stopped by adding cold phosphate buffered saline (PBS) and, after two washings in PBS, cells were cytocentrifuged onto slides and fixed in methanol/acetic acid (3:1) for 15 min at room temperature. Slides were then dipped in NTB-2 nuclear track emulsion (East Kodak) exposed for 24 h at 4°C and stained with hematoxylin/eosin after gold-activated autoradiography [12].

Tumor cells containing more than 5 nuclear grains over background were scored as labelled and the percentage of labelled cells over all tumor population represents the fraction of cells in DNA synthesis. At least 1000 cells in consecutive fields were scored for TLI by two independent observers.

Statistics

TLI distributions in the subgroups of each variable were compared by the median test. Actuarial survival was estimated according to Kaplan–Meier and survival curves in different subgroups were compared by the log-rank test. For ordinal variables, such as stage and nodal status, the association with survival was tested by Mantel's extension of the Mantel–Haenszel test (chi-square test for trend). To assess the prognostic value of TLI, while taking into account the confounding effect of other prognostic factors, a multivariate proportional hazard model was fitted to the data. Age, TLI and stage were included in the model, and histological type was used as a stratification variable. Variables were removed from the model by a stepdown procedure, based on a partial likelihood ratio test. The proportionality assumption was confirmed by visual inspection of the residuals.

*Table 1. Patients' characteristics and median TLI**

Variables	No.	Median TLI
Overall	89	2.9 (range 0.1–18.4)
Age		
<60	42	3.2
≥60	47	2.8
M/F	83/6	2.9/1.9
Surgery		
Lobectomy	46	3.2
Pneumonectomy	36	2.7
Other	7	2.7
Histology		
ADK	30	2.9
Squamous	45	2.8
Large cell	6	3.3
Mixed ADSQ	8	2.8
Tumor size		
T1	8	2.0
T2	49	2.8
T3	32	2.9
Nodal status		
N–	35	2.9
N1	29	2.7
N2	25	2.9
Stage		
Ia–Ib	27	3.2
II	15	2.2
III	47	2.9

*No significant differences between TLI within subgroups (median test).
ADK = adenocarcinoma; ADSQ = adenocarcinoma (squamous).

RESULTS

Clinical characteristics and median TLI of patients are shown in Table 1. Median age was 60 (range 36–77). TLI was not

Table 2. Survival analysis

Variables	No.	Obs/Exp	*P* (log-rank)	Median survival (days)
Age				
<60	41	1		650
≥60	46	0.92	0.803	691
Histology				
ADK	28	1		731
Squamous	45	1.23	0.431	650
Other	14	1.88		381
Tumor size				
T1–T2	55	1		731
T3	32	1.95	0.043	384
Nodal status				
N0	35	1		689
N1	27	1.07	0.098*	†
N2	25	2.01		301
TLI				
<2.9	41	1		*
≥2.9	46	2.03	0.047	449
Stage				
Ia–Ib	26	1		691
II	14	0.86		†
III	47	3.24	0.003*	381

*Test for trend.
†Proportion of survivors higher than 50%.

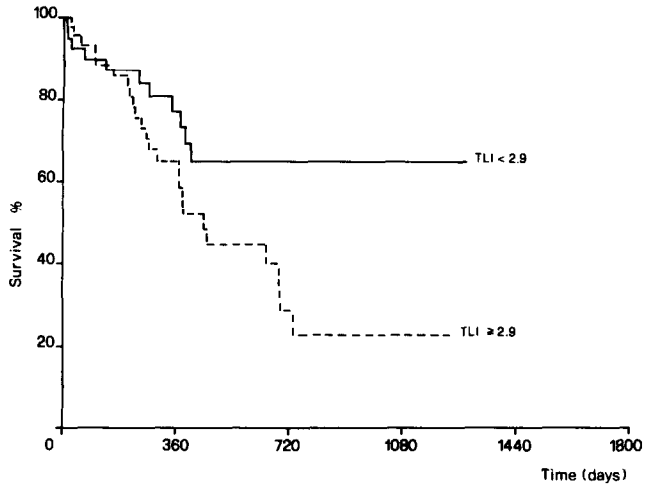

Fig. 1. Relation between TLI and survival in patients with resected non-small lung cancer.

significantly associated with any of the variables under study. Non-significant increases of TLI values were observed in men, in patients younger than 60, in tumors resected by lobectomy and in large cell histology. Median follow-up was 340 days (range 13–732). Actuarial median survival in the whole group was 689 days with 60% of the patients alive at the time of the analysis. The overall media TLI (2.9; range 0.1–18.4) and median age were used as cut-off values to identify two subgroups of patients for survival analyses. Standard criteria were used to subgroup the other variables. Moreover, since less than 10% of the patients had T1 tumors, T1 and T2 tumors were considered together.

Univariate survival analyses are shown in Table 2. Patients with a higher TLI had a shorter overall survival than those with a lower ($P = 0.047$) (Fig. 1). Moreover, patients with smaller (T1–T2) tumors or stage I and II had a better prognosis than patients with T3 or stage III tumors ($P = 0.043$ and $P = 0.003$, respectively). None of the other clinical variables was significantly related to survival, although N2 patients had almost twice the mortality observed in N0–N1 patients.

In multivariate analysis (Table 3), stage and TLI were the only factors affecting survival; stage was the most important predictor of survival. A two-fold increase in the risk of death was observed in the group of patients with a TLI greater than 2.9, which was of borderline statistical significance.

DISCUSSION

Few studies have investigated the association between TLI and survival in lung cancer, and the results have been inconsistent.

*Table 3. Multivariate analysis by Cox's model**

	Relative risk (95% CI)	*P*
Age (<60 vs ≥60)	1.09 (0.52–2.26)	0.813
Stage (I vs II vs III)	1.89 (1.17–3.06)	0.004
TLI (<2.9 vs ≥2.9)	2.02 (0.96–4.27)	0.057

*Histology was used as stratification variable.

Moran and Strauss [10] studied *in vivo* [^{3}H]thymidine labelling in 28 patients with lung cancer. Among 17 previously untreated patients, median survival times of patients with TLIs below and above the mean of 11 were 32 and 26 weeks, respectively (not significant). However, 10 of the 17 patients had undifferentiated carcinomas and 6 had small-cell carcinomas. In 42 patients with NSCLC, Volm *et al.* [13] reported a significant difference in survival between patients with tumors with a low (under 4.2%) and a high fraction of labelled S-phase cells ($n = 13$, $P = 0.04$). The selection of the cut off value of 4.2% was not explained. In 38 of their patients, Kerr and Lamb [14] reported no association between T1 and survival. Indeed, if squamous and large cell carcinomas were considered alone, a trend of borderline significance ($P = 0.05$) was observed, suggesting that survival was longer in those patients who had tumors with higher TLIs. However, the statistical analysis used does not appear fully appropriate. Our Kaplan–Meier and Cox reanalysis of their data shows no association between TLI and survival ($P = 0.24$).

Our study showed, in a larger and more homogeneous series of consecutive patients than those previously reported, that TLI is significantly associated with survival in univariate analysis. Furthermore TLI retains its prognostic value when stage, histology and age were adjusted for in multivariate analyses, thus making TLI an independent predictive factor. Despite being of borderline statistical significance, apparently due to the small sample size, the association between TLI and survival in multivariate analysis was strong. The rate of death two-fold among patients whose tumors showed a TLI above the median compared with the other patients.

The prognostic value of DNA content in lung cancer has been investigated [13, 19–17]. However, TLI was not related to ploidy. Previous studies have reported contrasting results for histological subtype and cell kinetics [3, 10, 14, 18, 19]. For instance, among the 28 cases described by Moran and Straus [10], only 6 were of the squamous type, and all were poorly differentiated. Hainau *et al.* [18] showed that undifferentiated forms of squamous cell carcinomas have a higher rate of cell proliferation that differentiated forms of the same cell type. In our series of consecutive patients, selected only on the basis of presurgical resectability, no difference in the distribution of TLIs between squamous cell cancer and adenocarcinomas was observed. These two tumor types are known to have a different natural history and metastatic pattern. Nevertheless, in most clinical trials they behave similarly in terms of response to therapy and survival. The similar distribution of TLI values in these two tumor types may account for their clinical behaviors. The high median TLI observed in the 6 large cell carcinomas in our series confirms previous observations and is reflected in the poor prognosis commonly experienced by these patients.

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Acknowledgements—We thank Miss M. Pratolongo and Miss P. Baracchini for typing the manuscript.

Eur J Cancer, Vol. 26, No. 5, pp. 625–628, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Modification of 5-Fluorouracil Activity by High-dose Methotrexate or Leucovorin in Advanced Colorectal Carcinoma

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21 patients with advanced colorectal carcinoma were entered into a phase II study to evaluate efficacy and toxicity of methotrexate (MTX), 1500 mg/m² rapid infusion on day 1, combined with continuous infusion of 5-fluorouracil (5-FU), 600 mg/m² per 24 h on days 1–4. 12 patients who had progressive disease during this regimen subsequently received high-dose leucovorin, 200 mg/m² bolus injection on days 1–4, combined with 4 days' continuous infusion of 5-FU. In the MTX/5-FU group 1 pathologically proven complete remission and 3 partial remissions were seen (response rate 20%). The median progression-free interval was 30 weeks. In 12 patients with progressive disease leucovorin/5-FU stabilized disease in 2 (17%). Toxicity in both regimens was tolerable, gastro-intestinal side-effects being most frequent. There were no treatment-related deaths. Median survival time was 10 months. Serum levels of carcinoembryonic antigen before treatment or doubling-time during progression did not correlate with survival.
Eur J Cancer, Vol. 26, No. 5, pp. 625—628, 1990.

INTRODUCTION

FOR advanced colorectal carcinoma there are few therapeutic options. Only 5-fluorouracil (5-FU) has a constant but low activity, inducing remission in 8–25% of patients. These remissions are usually partial and not lasting (average duration 7–8 months). Effects on survival, if any, are marginal [1–3]. Several modifications enhance the effectiveness of 5-FU [3]. Among these is continuous infusion, which augments response and decreases toxicity but does not improve survival [4, 5].

Biochemical modulation of 5-FU activity involves the pharmacological manipulation of the intracellular metabolic pathway.

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