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Prognostic Significance of Ki67 Labelling in Resected Non Small Cell Lung Cancer

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One hundred and eleven tissue samples of primary non small cell lung cancer obtained from patients undergoing radical surgery for resectable disease were investigated for the presence and distribution of Ki67 related antigen using an immunohistochemical technique, as a marker of the proliferative activity of the tumour. No correlation was seen between Ki67 expression and clinico-pathological variables (sex, age, histology, grading and pTNM stage) but disease-free survival was significantly lower in patients with higher Ki67 score (> 25% positive cells) at diagnosis ($P < 0.03$). Growth fraction evaluated by Ki67 labelling may provide a complementary prognostic parameter in non small cell lung cancer.
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INTRODUCTION

PROLIFERATIVE ACTIVITY of a tumour can be evaluated by different morphological, immunohistochemical, cytofluorimetric and autoradiographic methods, which mostly label cells in S-phase of the cell cycle. Methods more representative of the proliferative status of a tumour as a whole are flow cytometry and Ki67 immunohistochemistry.

Ki67 is a murine monoclonal antibody, which detects a non-histone protein [1] expressed in all phases of cell cycle except G_0 and probably early G_1 [2]. In the promyelocytic leukaemia cell line HL60, the addition of affinity purified Ki67 inhibits DNA polymerase α activity to the same degree as special inhibitors (aphidicolin, *n*-ethylmaleimide) [3]. Ki67 labelling has not been routinely used but preliminary studies in different tumours show a good correlation with histopathological grade [4, 5] and prognostic behaviour [6, 7].

In this study tissue samples of non small cell lung cancer (NSCLC), obtained from patients undergoing radical surgery were analysed for the presence and distribution of Ki67 and related these with different clinical and pathological prognostic factors.

PATIENTS AND METHODS

A total of 111 patients (101 men, 10 women, age 38–74 years) with newly diagnosed, operable NSCLC were investigated. All patients received radical surgery. Immediately after excision, samples of the tumour and apparently normal bronchial mucosa were collected and kept at −70°C until processed.

Histological type and degree of differentation were assigned according to WHO criteria [8]. Post-surgical pathological stage (pTNM) was determined according to the TNM staging system for lung cancer [9]. After surgery, all patients were followed-up every 3 months. Immunohistochemical analyses were carried out according to a modification of the technique proposed by Hsu *et al.* [9] Frozen sections, 6 μm thick, were air-dried overnight and fixed in cold acetone immediately before staining. After washing, sections were incubated with Ki67 for 60 min. Afterwards, 100 μl of an anti-mouse biotinylated antibody (Vector Laboratories, Burlingame, California, U.S.A.) were added. Subsequently, sections were incubated for 45 min with a preformed avidin biotinylated horseradish peroxidase macromolecular complex (Vectastain, Vector Laboratories). Final staining was provided by means of 3,3′ diaminobenzydine tetrahydrochloride. Negative controls were obtained omitting Ki67 antibody and using anti-sheep IgG as primary antibody. Finally, sections were counterstained with haematoxylin. The proportion of Ki67 positive cells was determined by counting 1500 cancer cells randomly selected throughout each section using a 40× objective. The extent of the immunoreactivity was grouped into four as follows: 0–10% stained tumour cells = grade I; 11–25% = grade II; 26–50% = grade III; > 50% = grade IV. The χ^2 test was used to analyse correlations among immunoreactivities of Ki67 and factors of age, sex, histology, grading and pTNM. Survival curves were plotted using the Kaplan–Meier method with statistical significance calculated using the log-rank test.

RESULTS

Analysis of the results revealed no correlation among Ki67 expression and patient age, sex, histology, pTNM stage, extent of primary tumour and/or lymphonode involvement (Table 1). The immunohistochemical nuclear staining of neoplastic specimens showed a wide heterogeneity ranging from rare scattered cells to homogeneous pattern for nearly all cells examined suggesting that phenotypic heterogeneity is a major feature in NSCLC. Cytoplasmic staining was never detected. In 5 cases out of 111 Ki67 immunostaining was completely negative. No significant difference was observed between central and peripheral areas of the tumours. Thirty-six out of 111 NSCLC samples (32%) had more than one fourth of examined cells positive for Ki67; on the other side in 34 cases the labelling was confined to 10% or less of examined cells. Follow up study revealed that in the first years after radical surgery patients with higher Ki67 score (grade III–IV) at diagnosis had a significantly higher incidence of recurrence or relapse than those with a lower Ki67 score (grade I–II) ($P < 0.03$, log-rank test) (Fig. 1).

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Table 1. Clinical and pathological characteristics of patients

Characteristic	No. of patients	Ki67 labelling No. of positive cells (%) ≤ 25%	> 25%
Histology			
Squamous cell	54	38 (70)	16 (30)
Adenocarcinoma	33	22 (67)	11 (33)
Large cell	19	12 (63)	7 (37)
Mixed histology	5	3 (60)	2 (40)
T status			
T1	7	4 (57)	3 (43)
T2	63	42 (67)	21 (37)
T3	41	29 (71)	12 (29)
N status			
N0	53	37 (70)	16 (30)
N1	31	20 (65)	11 (35)
N2	27	18 (67)	9 (33)
pTNM			
I	32	20 (63)	12 (27)
II	23	15 (65)	8 (35)
III A	56	40 (71)	16 (29)
Grading* (n = 89)			
G_0	35	25 (71)	10 (29)
G_1	17	12 (71)	5 (29)
G_2	37	24 (65)	13 (35)

*Limited to squamous cell carcinoma and adenocarcinoma subtypes.

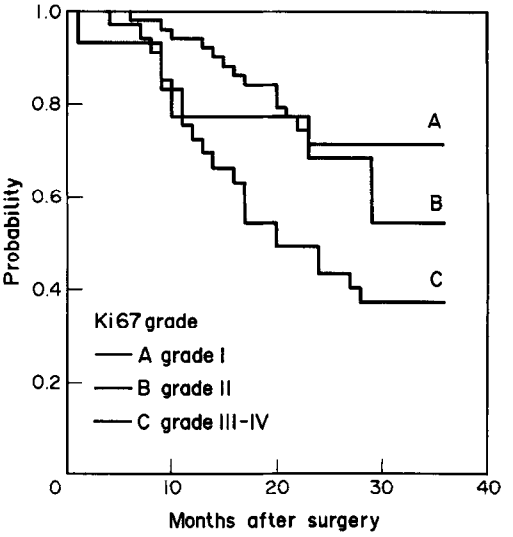

Fig. 1. Disease-free survival curves, showing Ki67 grades, for all tumour types. A = grade I, B = grade II, C = grades III and IV. ($P < 0.03$, log rank test).

DISCUSSION

In NSCLC several parameters have been established as relevant prognostic factors, extent of disease and pretreatment performance status being the key factors. Analysis of survival data within each TNM classification [9] results in a great variability of outcome and independent prognostic variables are needed. Cell kinetic data can be valuable for the assessment of new therapeutic approaches providing a theoretical framework for new treatment strategies, the more promising methods are flow cytometry and Ki67 or polymerase α/cyclin labelling. Studies in lung cancer had shown a possible relationship between DNA ploidy and biological behaviour in some of these tumours [11] while others failed to provide such evidence [12]. The evaluation of S-phase fraction by flow cytometry has been studied less exhaustively although it has been recognised as an independent prognostic factor [12]. Consequently flow cytometry may be of value but there are several restrictions in its widespread availability (i.e. requirement of a suspension of isolated cells, poor diagnostic reliability on bronchoscopic samples). Two different reports concluded that Ki67 labelling was a reliable method for assessing the proliferative status of lung tumours, if a sufficiently large number of fields were analysed to take into account intratumoral variability [13–14]. The proportion of labelled nuclei was significantly higher in small cell lung cancer [13] and in hypoploid or multiploid tumours [14].

A further study on 43 patients with radically resected NSCLC revealed that the 3-year disease-free survival rate was significantly lower in patients with a tumour positively determined for DNA polymerase α as detected by an experimental monoclonal antibody [15]. Our study failed to demonstrate any relationship between Ki67 expression and clinical or pathological variables. In the first years after surgery the analysis of the disease-free survival curves according to different Ki67 grades revealed an increased probability of relapse in patients with higher Ki67 scores. In conclusion, growth fraction evaluated by a semiquantitative Ki67 immunostaining may provide a complementary prognostic parameter in NSCLC.

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Thyroid Cancer in Children in Norway 1953–1987

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All cases of thyroid cancer in children aged 15 years or younger registered in Norway (1953–1987) are presented. 30 girls and 5 boys are included, the youngest being a 6-year-old boy. Half of the patients were in the age-group 14–15 years. As for adults, papillary thyroid cancer was most common. 70% of the patients presented with tumour growth outside the thyroid gland. In spite of this, only 2 children died of the disease during the follow-up time (maximum 37 years).
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INTRODUCTION

CANCER OF the thyroid gland is uncommon in most countries, accounting for about 1–2% of all registered new cancer cases in the general population. Among childhood malignancies it is even more rare, constituting 0.5% of all cancer in children in Norway [1]. It has, however, an interesting epidemiological profile with high risk in iodine-rich areas such as Iceland and northern Norway [2, 3]. The prognosis is considered to be good, depending on variables such as age, sex, stage and histological type. In a multivariate analyses published from the Norwegian Thyroid Cancer Project including 1026 differentiated tumours, only age and stage were of significant prognostic value[4].

Most articles on thyroid cancer in childhood are based on hospital records and thus are highly selected, focusing on clinical data [5, 6]. The present material includes all cases of thyroid cancer in children 15 years or younger reported to population based Cancer Registry of Norway.

MATERIALS AND METHODS

Since 1953 the Cancer Registry of Norway has received data on all cancer patients. Several types of data are stored based on clinical reports, histology and cytology reports as well as autopsy records. Death certificates on all cancer patients are received via The Central Bureau of Statistics of Norway. All available slides were reviewed (19 patients). The remaining histopathology-reports were re-read, recoded and all slides were classified according to the WHO [7]. Stage 1 includes tumours within the thyroid gland; no metastases found at the time of diagnoses. Stage 2 means regional metastases either in lymph nodes or in the adjacent soft tissue. Patients with distant metastases were classified as stage 3. Information on relapses and treatment were given on the clinical reports from the hospital responsible for treatment. The follow-up time varied from 2–37 years with a median of 17 years.

RESULTS

In the 35 year-period 1953–1987, 35 new cases of thyroid cancer in children 15 years or younger occurred in Norway, giving an average of 1 case per year in a population of about 1 million children in this age-group (Table 1). The figures are small, but there does not seem to be any increase in the past years. Girls dominated with 30 cases, giving a sex ratio of 6:1. The youngest patient was a 6-year-old boy, while 13 of the girls and 4 of the boys were in the age group 14–15 years (Table 2). All children were operated for primary diagnosed thyroid cancer; there were no occult carcinomas.

80% of cases were papillary and 2 girls had medullary tumours (Table 3). Anaplastic tumours did not occur in any of the cases. About half of the children had regional neck metastases, while 4 girls and 1 boy had distant metastases at the first hospital admission (Table 4). All patients were treated surgically, 26

Table 1. Number of cases in the period 1953–1987

	Period							
	53–59	60–64	65–69	70–74	75–79	80–84	85–87	Total
Boys	1	0	0	2	0	2	0	5
Girls	4	3	4	3	6	5	5	30
Total	5	3	4	5	6	7	5	35

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