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Long-term Prognostic Significance of Thymidine Labelling Index in Primary Breast Cancer

T. G. Cooke, P. D. Stanton, J. Winstanley, G. D. Murray, R. Croton, S. Holt and W. D. George

Tumour growth rates, as measured by incorporation of tritiated thymidine, have been reported as being of prognostic importance in breast cancer. We have measured the thymidine labelling index (TLI) of 185 early breast cancers, followed-up for a minimum of 8 years. Above median TLI was associated with higher tumour grade, but not with other prognostic factors. TLI was not predictive of survival in either univariate or multivariate analysis. The inter- and intra-observer reproducibilities of TLI measurements were poor, which may be a factor limiting its usefulness as a prognostic indicator in breast cancer.
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INTRODUCTION

THERE HAVE been a number of publications evaluating the usefulness of thymidine labelling in breast cancer since it was first applied by Johnson and Bond [1]. However, little attention has been paid to the reproducibility of the measurement itself. We present here a cohort of patients whose primary treatment was by surgery alone, followed up for a minimum of 8 years, in whom thymidine labelling index (TLI) of the primary tumour was measured at initial presentation. We also report the inter- and intra-observation variability in the assessment of TLI.

PATIENTS AND METHODS

Patients

185 tumours were studied between 1978 and 1982. All had stage I and II breast cancer, treated by mastectomy and axillary dissection. No patients were given adjuvant therapy. They have been prospectively followed up, and flagged in the Regional Cancer Registry to ensure accuracy of mortality data. The close of the study was taken as 1 January 1990, when the minimum follow-up of surviving patients was 93 months.

Methods

(*a*) *Thymidine labelling.* The TLI was measured by the method of Meyer and Bauer [2]. Fresh tumour was divided into five 2 mm cubes, which were added to tubes containing 5 ml of RPMI and 0.2 ml of ^{3}H-thymidine (925 μBq/ml; specific activity 1.6 Tbq/mmol). Tubes were incubated in a shaking water bath for 2 h at 37°C, at 3 atm produced by addition of 10 ml of a 95% O_2/5% CO_2 mixture. Tissue was then fixed in formalin and wax embedded. Five micron sections were mounted on histological slides and Kodak AR stripping film applied. Autoradiographs were exposed at 4°C for 28 days, and counterstained with haematoxylin and eosin.

Labelling was assessed in 2000 nuclei per tumour, four separate areas of 100 nuclei on each of five slides. Nuclei were considered to be positive if there were more than 10 reduced silver grains over them, although negative nuclei never demonstrated more than three grains. The TLI was taken as the proportion of positive nuclei. Inter- and intra-observer variability were assessed in 20 tumours.

(*b*) *Other data.* Histological grade was assigned by a single pathologist according to the criteria of Bloom and Richardson. ER assays were performed using the dextran coated charcoal

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Table 1. Distribution of prognostic variables, and comparison of TLI between groups within each variable

Variable		*n*	%	TLI % Mean	TLI % Median	*P* value
Tumour Size	T1	8	5	3.4	2.4	
	T2	119	73	4.5	3.2	0.529 (KWA)
	T3	37	23	4.7	4.2	
Nodal Status	N0	57	43	4.4	2.6	0.407 (MWU)
	N1	76	57	5.0	3.4	
Hist. Grade	I	37	33	3.4	3.0	
	II	46	41	4.5	3.1	0.032 (KWA)
	III	30	26	6.2	5.1	
ER Status	ER+	93	58	3.9	2.7	0.123 (MWU)
	ER−	68	42	5.5	3.3	
Menopausal	Pre	55	38	4.9	3.8	0.168 (MWU)
	Post	91	62	4.1	2.1	
*erb*B-2 Status	Neg	129	78	4.0	2.8	0.266 (MWU)
	Pos	37	22	4.8	3.1	

KWA = Kruskal–Wallis One Way Analysis of Variance; MWU = Mann–Whitney U Test.

technique, with a cut off of 5 fmol oestradiol/mg cytosol protein. c-*erb*B-2 staining was performed with the 21N polyclonal antibody (a gift from Dr W. Gullick).

(*c*) *Statistical analysis.* TLI was related to other variables by the Mann-Whitney U test and the Kruskall–Wallis one-way analysis of variance. Univariate survival analysis was performed using the life-table method, expressed as the log rank probability. Patients alive at the close of the study, or dead of unrelated causes, were treated as censored observations. Multivariate survival analyses were performed with the Cox proportional hazards model, using both forward and backward selection of variables. Reproducibility was analysed by the method of Bland and Altmann [3].

RESULTS

Values of TLI ranged from 0.3 to 19.1% (median 3.2%). The relationship between TLI and other data is indicated in Table 1. There is a tendency for higher values of TLI to be associated with indicators of poor prognosis, but this is statistically significant only in respect of tumour grade.

Inter- and intra-observer variability are expressed as the ratio between the two estimates for each of the 20 cases studied. The mean of these ratios for the inter-observer study was 1.01, standard deviation 0.55. For the intra-observer study the values are: mean 1.09, S.D. 0.49. In neither case is there a mean difference between the two sets of estimates, but the standard deviations are high for data of this type. In 17% of cases the two observations varied by more than a factor of 2.

Univariate survival analysis based on TLI values above or below the median shows a trend to improved survival in the latter group, but this is not statistically significant (Fig. 1). Analysis dividing the patients into 4 groups based on the quartiles of the TLI distribution was also carried out. Although this demonstrates a difference in the outcome for the extreme groups, there is no statistically significant trend.

Multivariate analysis of survival was carried out in a model containing node status, size, *erb*B-2 staining and TLI. Data

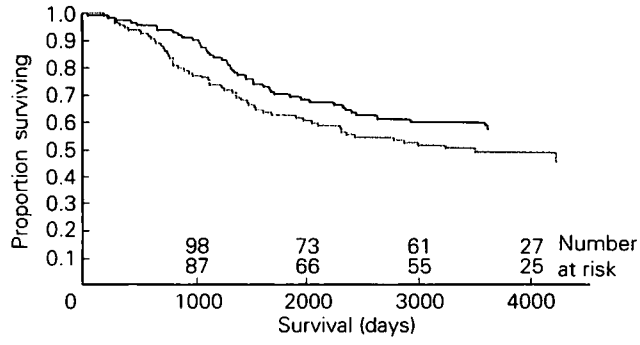

Fig. 1. Disease-related survival curves for 185 breast cancers, stratified by TLI above or below median value.—TLI <7.25; . . . TLI >7.25; *P* = 0.11 (logrank).

were available for 164 patients. Within this group, nodal status is by far the strongest variable, and tumour size is also independently prognostic. Neither *erb*B-2 status nor TLI show a statistically significant influence on survival.

DISCUSSION

In this series the TLI is not statistically predictive of long-term survival in stage I and II breast cancer. This is in contrast to the results of Silvestrini *et al.* [4], Tubiana *et al.* [5] and Meyer *et al.* [6]. However, the observed survival difference at 6 years in our population is 13% in favour of tumours with below median TLI. This accords with Silvestrini *et al.* figures of 12% for node negative and 14% for node positive patients. Meyer's group have presented their results dividing TLI into three groups. The survival advantage for tumours in the lowest TLI band over those in the highest is 23% at 5 years. Our own observed difference at that stage between the uppermost and lowest quartiles of TLI is 24%. Although we have not reproduced the statistical significance of these studies, our data are very similar.

The determination of TLI shows poor inter- and intra-observer reproducibility. Only Meyer's group have presented any such data before [7]. They reported the inter-observer variability on 5 tumours, expressed as the coefficient of variation of three estimates. The mean coefficient of variation for the 5 tumours was 21%. Analysing our own data this way, the mean coefficient of variation is 24%. It would seem that our results are in keeping with the only other available figures, and that TLI is not a reproducible measurement in our hands or those of Meyer.

In summary, this series does not show a significant prognostic survival effect of TLI, but is compatible with previous data showing that low TLI predicts improved outcome. The clinical usefulness of this is reduced by the poor reproducibility of the measurement itself, and the time consuming methodology involved.

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The Value of Follow-up in Patients Treated for Squamous Cell Carcinoma of the Head and Neck

Morten Boysen, Oscar Lövdal, Johan Tausjö and Finn Winther

Clinical findings, treatment and results have been recorded prospectively in 661 patients with carcinoma of the head and neck. With an average follow-up of 3 years 7813 follow-up consultations revealed 220 recurrences. The overall "recurrence pick-up rate" and subsequent "cure rate" was 1:36 and 1:113 consultations, respectively. Laryngeal carcinomas treated with radiotherapy and oral carcinomas receiving radiotherapy and limited resections showed recurrence "cure-rates" of 1:89 and 1:110, respectively. For other tumour sites the average "cure-rate" was 1:238. Only 39% of the recurrences were detected through physical examination. Follow-up consultations revealed 9.1% of second primaries. More time should be spent on training patients to recognise symptoms and signs of recurrence. Routine follow-up is rarely indicated beyond the third year after completion of treatment, or in patients for whom we have little to offer in terms of curative treatment.
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INTRODUCTION

ONE OF the main objectives of follow-up of patients treated for malignant disease is to discover and treat early recurrences. The benefit of prolonged routine follow-up in terms of increased survival has been questioned for several types of carcinomas [1–4]. In a previous retrospective study on squamous cell carcinoma of the head and neck we found that successful treatment of recurrences was almost exclusively achieved in patients with laryngeal carcinoma initially treated with irradiation alone [5].

Since 1983 we have prospectively recorded relevant clinical data and the outcome of all follow-up consultations of patients treated for squamous cell carcinoma of the head and neck. In this study we have evaluated to what extent our follow-up regimen has been effective in detecting early recurrences and, once a recurrence was detected, whether further treatment resulted in cure. Furthermore, we have recorded how the recurrences were detected—through symptoms or signs that the patients reported or by physical examination at scheduled follow-up consultations.

PATIENTS AND METHODS

From May 1983 to May 1988, 807 consecutive and previously untreated patients with confirmed squamous cell carcinoma of the head and neck were admitted to our department. The clinical findings, treatment, results and outcome of all follow-up consultations have been recorded prospectively. Excluded from the present study were 50 patients with disseminated disease, other serious illnesses or poor general condition which made treatment with curative intent impossible. In addition we excluded 96 patients in whom initial control of the primary tumour and/or neck metastases was not achieved (i.e. where the operative findings or histological examination of the surgical specimens showed an incomplete resection and patients who at the first appointment 6 weeks after completion of the treatment had residual tumour locally, regionally or at distant sites). Our study focusses on the remaining 661 patients who were considered "free of disease" 6 weeks after the completion of the therapy.

Of the 661 patients 77% were male and 23% female, ranging in age from 27 to 88 years (mean 63 years). Table 1 presents the material according to site and the UICC classification of 1982 [6]. In general, the smaller tumours (T1-2) received radiotherapy with surgery for suspected residual tumour and the larger combined radiotherapy and surgery. In cases where resection of part of the mandible was considered necessary surgery had preceded radiotherapy. Radiotherapy was given in once-a-day fractions of 2 Gy 5 days a week from an external megavolt source and directed towards the primary site and usually both sides of the entire neck. The primary site received 66–80 Gy. Most cases of oral carcinomas, not involving the mandible, received external irradiation of 47–50 Gy and an additional 20–30 Gy as brachytherapy for the primary tumour. The clinically negative neck received 47–50 Gy and the positive neck 50 Gy with an

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