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Clinical outcome and bromodeoxyuridine derived proliferation indices in 100 colonic and rectal carcinomas

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Aim: *In vivo* labelling of human colonic and rectal tumours with bromodeoxyuridine (BrdUrd) and analysis by flow cytometry (FCM) allows the labelling index (LI), S phase duration (Ts) and the potential doubling time (Tpot) of the tumour to be estimated *in vivo*.
Methods: The data for a series of 100 tumour specimens from 97 patients with colonic and rectal carcinoma was reported in 1991, and correlated with Dukes' classification and histological differentiation.
Results: This study reports the eventual outcome of the 97 patients after 12 years. There were no significant associations between proliferation data of the index tumours and patient outcome. No adverse events were identified which could be attributed to the use of the halogenated pyrimidine label *in vivo*.
Conclusion: Dynamic cell proliferation indices provide detailed information on the cell kinetics of colorectal tumours but these do not correlate with clinical prognostic markers or outcome.

Key words: colonic cancer; rectal cancer; bromodeoxyuridine; cell proliferation; outcome.

INTRODUCTION

Flow cytometry (FCM) and *in vivo* pulse labelling with the halogenated pyrimidines bromodeoxyuridine (BrdUrd) or iododeoxyuridine (IodUrd) allow the DNA index, the total labelling index (LI) and aneuploid labelling indices, and two time dependent indices, the S phase duration (Ts) and the potential doubling time (Tpot), to be calculated from single biopsy samples of human tumour specimens labelled *in vivo*.[1,2] We reported the dynamic indices in a large cohort of colonic and rectal carcinomas in 1991.[3] No significant associations were found between Dukes' stage or histological classification and kinetic parameters or in a preliminary survival analysis in 1993.[4] Subsequently Wilson[5] reported on a series of 125 tumours labelled with IodUrd, and Terry[6] reported on 101 rectal tumours labelled with BrdUrd. Measurements were broadly similar. Other studies have suggested that the proliferation rate might be an independent marker of prognosis in colorectal cancer,[7] and some that rapidly growing cancers (high LI and/or low Tpot) were associated with longer patient survival.[8] In this short paper we report the long term follow up data in our cohort of patients in respect of the multiple proliferation indices including parameters with a time component.

PATIENTS, MATERIALS AND METHODS

Ninety-seven patients (54 men and 43 women, age range 42–90 years, mean age 74 years) with 100 adenocarcinomas of the colon and rectum were studied between 1988 and 1991. There were 49 colonic tumours and 51 rectal tumours. Twenty-three colonic tumours were located in the caecum and ascending colon, four in the transverse colon, nine in the left colon and 13 in the sigmoid colon. Three female patients had two synchronous colonic tumours. Cell proliferation data from the index tumour was used in each case for the survival analysis. Hospital ethical committee approval was given for the study. Informed consent was obtained from all patients prior to administration of a single intravenous dose of 250 mg bromodeoxyuridine (BrdUrd), a thymidine analogue which labels DNA in the S phase of the

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Table 1 The surgical treatment of patients within the cohort

Operation	Colonic tumours	Rectal tumours
Subtotal/total colectomy	4	
Right hemicolectomy/ extended right hemicolectomy	22	
Transverse colectomy	2	
Left hemicolectomy	4	
Sigmoid colectomy	10	
Hartmans procedure	3	7
Anterior resection	1	27
Abdomino-perineal excision		12
Per-anal excision		3
Palliative defunctioning colostomy		2
Total	46	51

cycle of proliferating cells. The injection was given between 2.4 and 16 h (mean 6.7 h) prior to surgery. Surgical Treatment is summarised in Table 1. Eight patients underwent postoperative radiotherapy, and one patient a course of oral 5FU.

Tumour samples from the time of surgical excision were studied by multiparameter flow cytometric analysis of DNA and BrdUrd uptake. The technical details and findings have previously been reported,[3] and reviewed.[1] Where multiple samples were studied from any one tumour, the mean values for the flow cytometric data for each parameter were used in the survival analyses. Follow up data, using death as the endpoint, was collected for all patients. In 92% of cases follow up was either to death or for over 10 years; follow up was available for a lesser period of time in only the remaining 8% of cases.

STATISTICS

Survival analysis was performed using SPSS for windows version 10 (SPSS inc., Chicago, Ill, USA). Kaplan–Meier plots were used to demonstrate survival over the period of the study. The period of follow-up ranged from 18 to 151 months.

The effect on survival of the cell proliferation indices, and other known prognostic factors was assessed using Cox proportional hazards regression. Each prognostic variable was initially analysed independently. Single variable analysis was performed on each cell proliferation variable using three different cut off values to discriminate between groups. The cut off values analysed were those for the lower quartile, median, and upper quartile (for LI: 6.1, 9.0 and 11.9; for Ts: 10.8, 13.7 and 16.9; and for Tpot: 3.1, 4.5 and 7.4 respectively). The Wald test was used to assess the significance of any findings. The additional effect of adding both LI and Ts, or Tpot alone (which is derived from LI and Ts) independently to models controlled for Dukes' stage, histological differentiation, tumour ploidy, and patient age and sex, was also investigated using Cox proportional hazards regression model.

Table 2 The aggregate data for Dukes' stage, tumour differentiation, ploidy, and the dynamic proliferation parameters in the 97 colonic and rectal carcinomas used for survival analysis

	Number of tumours	Value (range)
Dukes' stage		
A	6	
B	37	
C	51	
Endoanal surgery	3	
Tumour differentiation		
Well	5	
Moderate	63	
Poor	29	
Ploidy		
Diploid	46	
Aneuploid	51	
Median		
LI (%)	97	9.0 (0.7–22.2)
Ts (h)	96	13.7 (4.0–28.6)
Tpot (d)	95	4.51 (1.7–21.4)

RESULTS

Two primary tumours (2.0%) failed to yield usable data measurement of the Ts and Tpot. Ploidy and BrdU LI was measured satisfactorily from at least one block from every tumour. The tumour characteristics including Dukes' stage, histological differentiation, ploidy and proliferation parameters are summarised in Table 2. At last follow up, after up to 12 years, 76 patients had died and 21 were still alive. The current median and mean survival are 24 and 55 months respectively (range: 0–151 months).

Dukes' stage, and patient gender were statistically significant predictors for death in this cohort in both single and multiple variable models (for multiple variable models: Hazard ratio: 2.44; 95% CI: 1.45–4.08, and 0.51; 0.31–0.85 respectively). This is demonstrated in Table 3. Patient age predicted death in multiple variable models (Hazard ratio: 1.47; 95% CI: 1.01–2.15). Single predictor variable Cox regression models demonstrated the median cut off value to be the best discriminator between groups for the cell proliferation indices. For analysis with the median value used to discriminate between groups for the cell proliferation indices Histological Differentiation, DNA ploidy, LI, Ts and Tpot failed to predict outcome in single or multi-variable models (Table 3). Figures 1 and 2 are the Kaplan–Meier

Table 3 Single and multi-variable analysis of predictors of survival using median values (Table 2) as the cut off value to discriminate between groups. In addition to the data presented the effect of adding Ts and LI instead of Tpot to an additional multivariate model was examined. LI and Ts were not significant predictors of survival in this model (for LI: $P = 0.75$, Hazard ratio = 0.92, 95% CI = 0.56–1.53; for Ts: $P = 0.89$, Hazard ratio = 1.04, 95% CI = 0.65–1.67)

Explanatory variable	Single variable analysis					Multivariate analysis	
	Group	Number	*P*-value	Mean survival (months)	95% CI of mean survival (months)	*P*-value	Hazard ratio (95% CI)
Dukes' stage	A/B (Non-metastatic)	43	<0.001	79.2	61.8–97.0	0.001	1
	C/D (Metastatic)	51		31.6	19.8–43.4		2.44 (1.45–4.08)
Gender	Male	54	0.023	44.2	30.6–57.7	0.009	1
	Female	43		68.5	49.5–87.4		0.51 (0.31–0.85)
Age	40–59	16	0.062	76.4	48.3–104.5	0.047	1
	60–79	59		54.3	39.4–69.2		1.47 (1.01–2.15)
	79–99	22		40.0	18.5–61.4		1.47 (1.01–2.15)
Potential doubling time (Tpot)	Below median	52	0.075	63.5	46.7–80.3	0.082	1
	Above median	45		45.2	30.2–60.1		1.55 (0.95–2.53)
Histological differentiation	Well	5	0.281	71.9	19.0–124.8	0.257	1
	Moderate	63		56.2	42.7–69.7		1.35 (0.81–2.26)
	Poor	29		47.2	47.2–68.7		1.35 (0.81–2.26)
Labelling index (LI)	Below median	50	0.513	49.9	42.1–75.1		
	Above median	47		59.4	34.7–66.2		
S phase duration (Ts)	Below median	49	0.892	54.8	39.2–68.8		
	Above median	48		55.1	38.0–74.1		
Ploidy	Diploid	46	0.525	58.1	41.1–75.1	0.635	1
	Aneuploid	51		51.3	36.0–67.0		1.127 (0.69–1.86)

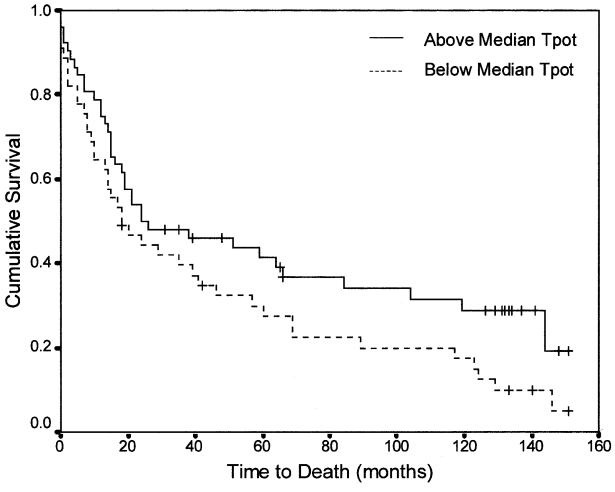

Figure 1 Kaplan–Meier plot of survival of 97 patients by Tpot. The solid line denotes patients with above median Tpot and the dotted line patients with below median Tpot. The crosses (+) denote patients censored from the analysis.

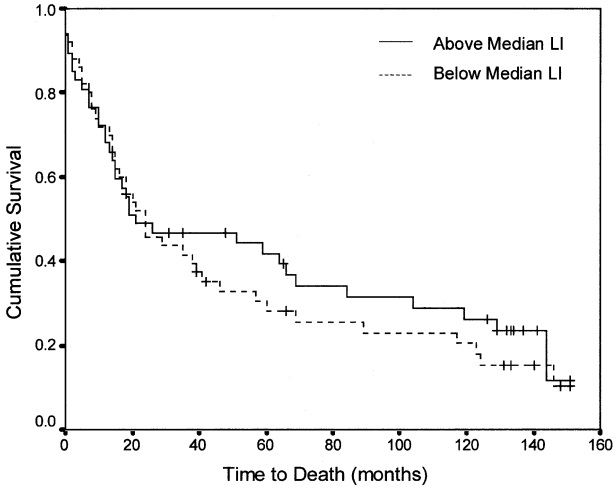

Figure 2 Kaplan–Meier plot of survival of 97 patients by LI. The solid line denotes patients with above median LI and the dotted line patients with below median LI. The crosses (+) denote patients censored from the analysis.

curves for time to death by Dukes' Stage and Tpot. There was no evidence of any short or long term toxicity or therapeutic benefit from the dose of the BrdUrd used for tumour labelling.

DISCUSSION

This is a small study with a pragmatic selection bias for patients with larger tumours originally undertaken to describe the time variable proliferation indices of a large

cohort of primary colorectal tumours using the pulse labelling technique, as reviewed elsewhere.[1] Dukes' stage and patient age and gender were found to be statistically significant predictors of patient outcome in a multi-variable analysis. The lack of association in this study between proliferation parameters including dynamic indices, and survival is consistent with the findings in a smaller cohort of breast cancers studied in a similar fashion with *in vivo* pulse labeling,[9] and is consistent with an earlier analysis from this cohort.[4]

In vivo dynamic proliferation indices are a research tool with no immediate clinical applications. Apoptosis, cell loss and exfoliation play a significant role in the volume growth of tumours *in vivo*. Proliferation data do not describe this important feature of tumour growth, nor of other components of the biological aggressiveness of colorectal carcinoma including metastatic potential and invasive characteristics. Nevertheless the BrdUrd/flow cytometry labelling technique has provided valuable and unique information about tumour behaviour from the quantitative study of time vectored cell proliferation parameters of clinical tumours *in vivo* in a safe and simple way.

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