Pretreatment Proliferation Parameters Do Not Add Predictive Power to Clinical Factors in Cervical Cancer Treated with Definitive Radiation Therapy

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ABSTRACT

Purpose: **To examine the prognostic value of tumor proliferation measurements in women with carcinoma of the uterine cervix. We report an update of a prospective study focusing on whether pretreatment proliferation parameters are associated with clinical outcome, relative to other established clinical factors.**

Materials and Method: **One hundred and one patients were recruited into the study from years 1991 to 1999. The LI for *in vivo* bromodeoxyuridine incorporation by the tumor and the potential doubling time (T_{pot}) were determined by flow cytometry (fc). LI and its staining pattern were also assessed by immunohistochemistry (ih) using tissue sections. Apoptosis was assessed histologically using morphological criteria. Patients were treated with definitive radiation therapy.**

Results: **A successful fc measurement for LI-fc and T_{pot} was possible in 95 patients (94%). The median/mean LI-fc was 6.6/7.6% (range 1.4–36.1%), and for LI-ih, 10.8/11.5%. To date, 43 patients have died of disease, and the median follow-up for alive patients is 6.2 years (range 1.3–9.3 years). Among 88 patients who completely responded to treatment, 40 patients have relapsed (14 pelvic, 23 distant, and 3 pelvic and distant). In univariate analysis, the significant factors for adverse disease-free survival were large tumor size ($P = 0.0001$), low hemoglobin ($P = 0.001$), pelvic lymph node status ($P = 0.004$), stage ($P = 0.013$), and overall treatment time ($P = 0.0008$). In multivariate analysis, only tumor size, pelvic lymph node status, and overall treatment time remained significant for disease-free survival. LI-fc, LI-ih, T_{pot}, ploidy, pattern of bromodeoxyuridine staining, and apoptosis were not significantly associated with clinical outcome in univariate or multivariate analyses.**

Conclusions: **These mature data indicate that none of the pretreatment proliferation parameters have prognostic significance in the radical radiotherapy of carcinoma of the uterine cervix, despite the significance of overall treatment time for treatment outcome.**

INTRODUCTION

The traditional clinical features used to assess prognosis in cervix cancer have been based on the extent of disease (tumor size, pelvic lymph node status, and stage) and histological type (1). In the late 1980s to early 1990s, published data supported the hypothesis that rapid tumor cell proliferation may be a significant cause of failure in the RT[2] of several types of cancers (2–4). Tumor proliferation during RT has been inferred as the mechanism for the observation that prolongation of OTT was associated with poor tumor control in carcinomas of the head and neck (2, 5) and uterine cervix (6–9). Pretreatment measurement of tumor proliferation rate, as a surrogate parameter reflecting the extent of tumor cell repopulation during RT (3, 10, 11), was proposed as an attractive strategy to identify poor prognosis patients who might then be treated with alternate regimens, such as accelerated radiotherapy (5, 12–15). Preliminary work on the BrdUrd labeling method to measure proliferation from our group (10, 16, 17) and those of others (18) showed that LI and/or the T_{pot} were potentially useful as predictive assays for patients with invasive carcinoma of the cervix. We now report an updated analysis on this prospective study, with inclusion of a larger number of patients with mature follow-up. Results of an analysis of BrdUrd staining pattern, using the method described by Bennett *et al.* (19), and of morphological tumor cell apoptosis are also included in this report.

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[2] The abbreviations used are: RT, radiation therapy; BrdUrd, bromodeoxyuridine; LI, labeling index; IFP, interstitial fluid pressure; EUA, examination under anesthesia; MI, mitotic index; LI-fc, labeling index by flow cytometry; OTT, overall treatment time; AI, apoptotic index; PMH, Princess Margaret Hospital; T_{pot}, potential doubling time; DFS, disease-free survival; LI-ih, labeling index by immunohistologic assessment.

Table 1 Patient characteristics (n = 101)

Characteristic		Number (%)
Histology:	squamous	78 (77.2%)
	adeno	17 (16.8%)
	adenosquamous	6 (5.9%)
Differentiation:	well	7 (6.9%)
	moderately-well	41 (40.6%)
	poor	33 (32.7%)
	not recorded	20 (19.8%)
FIGO stage:	IB, IIA	26 (25.7%)
	IIB	41 (40.6%)
	IIIB	32 (31.7%)
	IIIA, IVA	1 each (2.0%)
Pelvic lymph node:	Positive	33 (32.7%)
	Negative	56 (55.5%)
	Equivocal	12 (11.9%)
Maximum tumor Diameter:	≤4 cm	28 (27.7%)
	4.1–6.0 cm	35 (34.7%)
	6.1–8.0 cm	29 (28.7%)
	8.1–10.0 cm	5 (5.0%)
	>10.0 cm	1 (1.0%)
	not recorded	3 (3.0%)

Table 2 Parameters of tumor proliferation and apoptosis

Parameter	n	Mean	CV	Median	Range
Flow cytometry parameters					
LI-fc	95	7.6%	68%	6.6%	1.4–36.1%
T_s	95	11.1 h	37%	10.0 h	5.6–29.2 h
T_{pot}	95	7.2 d	86%	5.6 d	1.3–42.1 d
$1/T_{pot}$	95	0.21	62%	0.18	0.02–0.75
SPF	99	16.9%	64%	15.1%	0.6–64.7%
Histology parameters					
LI-ih	88	11.5%	46%	10.8%	1.9–26.6%
MI	62	0.7%	58%	0.7%	0–1.8%
AI	62	1.5%	97%	0.9%	0–6.8%

SPF = S-phase fraction, CV = coefficient of variation.

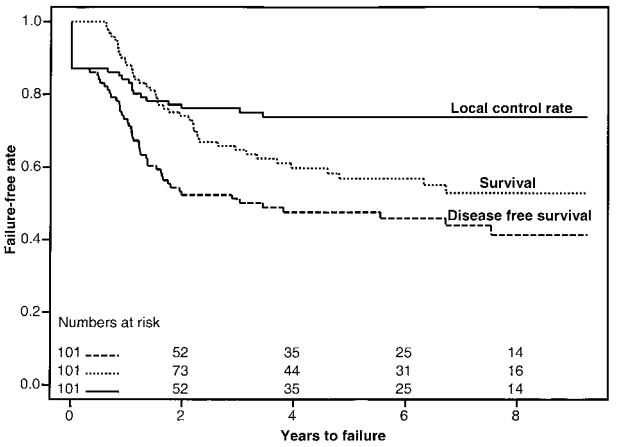

Fig. 1 Plots of overall survival (OS), DFS, and local disease-free rates for the entire 101 patients in the study.

MATERIALS AND METHODS

Patients. One hundred and fourteen patients untreated previously referred to the PMH with a diagnosis of carcinoma of the uterine cervix were studied prospectively from March 1991 to June 1999. The protocol was approved by the Institutional Review Boards of the PMH and the University of Toronto. Informed consent was obtained from all patients. Patients were given a 200 mg i.v. infusion of BrdUrd (Investigational Drug Branch, National Cancer Institute, Bethesda, MD) over 10 min, and a biopsy of the tumor was obtained ~4–10 h thereafter during an EUA. Tumor specimens were processed for flow cytometric, histological, and immunohistochemical analysis. From March 1994 onwards, these same patients were also recruited into studies of tumor hypoxia and IFP, with the results reported separately (20–23).

The tumor size and stage of the patients were assessed prospectively. Tumor staging was done using the Fédération Internationale de Gynécologie et d'Obstétrique system by the attending radiation oncologist using information from the EUA. Lymph nodes were assessed with one or more of the following: lymphangiography, computed tomography scan, and magnetic resonance imaging, with more frequent use of magnetic resonance imaging in the later part of the study. Pelvic lymph nodes were classified as positive, negative, or equivocal for metastatic disease with positive nodes being those >1 cm in transverse dimension, as reported previously (20, 24). Thirteen patients were excluded from analysis: 2 had surgery; 5 had metastatic disease at presentation; and 6 received chemotherapy. The remaining 101 patients, all of whom received definitive RT with curative intent, form the basis of this report.

Tumor Proliferation and Apoptosis Assays. The technical procedures for flow cytometry, histopathology, and immunohistochemistry were as described previously (10, 17). Parameters obtained were LI-fc, S phase duration (T_s), T_{pot}, S phase fraction, and LI-ih. On the basis of the same slide assessed for LI-ih, the distribution of BrdUrd-stained cells was also classified into marginal, intermediate, mixed, and random patterns, according to the methods described by Bennett *et al.* (19). AI and MI were obtained by manual counting based on morphological criteria as detailed previously (17). There was technical failure in the flow cytometry analysis in two patients, and an additional four patients did not receive BrdUrd as scheduled, resulting in a total of 95 patients with a successful determination of the LI-fc, T_s, and T_{pot}. There were a smaller number of patients completing histological assessments (LI-ih, 88 patients; AI and MI, 62 patients) because they were performed in the later period of the study, with some specimen sizes being inadequate after the previous analysis by flow cytometry. All individual laboratory assessments were performed in isolation based on a unique identification number for the specimen with no knowledge of the value of other laboratory or clinical parameters.

RT. All patients received external beam RT followed by brachytherapy. The standard fractionation schedule for external beam RT to the pelvis was a total dose of 45–50 Gy in 25–28 fractions, 5 days a week over 5–5.5 weeks. Five patients received a total external beam dose > 50 Gy (maximum 52.8 Gy, with a partially hyperfractionated course as part of a clinical trial), whereas 4 patients received total dose < 45 Gy. Treatments were delivered with a four-field box technique using

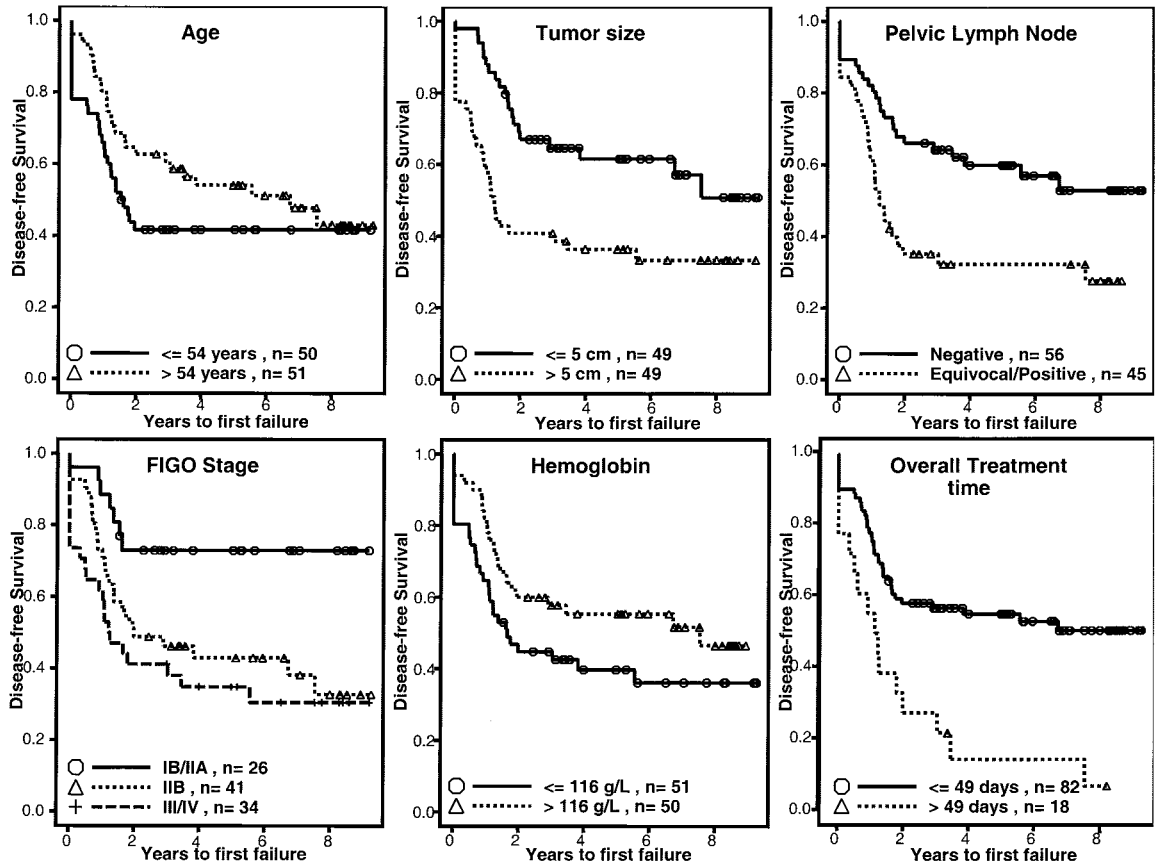

Fig. 2 The effects of age, tumor size, pelvic lymph node status, Fédération Internationale de Gynécologie et d'Obstétrique stage, hemoglobin level, and OTT on DFS. The statistical significance of these factors in univariate and multivariate analyses is shown in Tables 3 and 4, respectively.

linear accelerators with photon energies of 18–25 MV. External beam RT was followed by an intracavitary insertion using a remote afterloading stem (low-dose rate with ^{137}Cesium or pulse dose rate with ^{192}Iridium) as a single line source delivering 36–40 Gy to a point 2 cm lateral to the active length of the stem (90 patients) at an average dose rate of 0.69 Gy/h. Seven patients received brachytherapy doses under 36 Gy (25.5–35 Gy) at the discretion of the treating physician. No vaginal ovoids were used. Four patients did not receive intracavitary treatment because of previous hysterectomy (cervical stump carcinoma) or inability to insert a stem, and they were given an external beam RT boost of an additional 5–20 Gy. The overall total dose ("mixed" external + brachytherapy) was ≥85 Gy in 90 patients (89%). The OTT was the number of elapsed days from the first external radiation treatment to the completion of brachytherapy. The median OTT was 42.5 days (range 34–73 days).

Seventeen patients received concurrent para-aortic nodal RT (median dose 45 Gy, range 40–50 Gy) because of imaging evidence of nodal disease in the pelvis ($n = 10$) and/or paraaortic areas ($n = 5$). Twenty-four patients (24%) received packed red cell transfusion for anemia during the course of RT.

Statistical Analysis. There were three types of outcome considered in this study: survival, for which only death was considered as failure, DFS, for which any relapse or death was a failure, and local failure, for which only local relapse (within the pelvic irradiated volume) was considered as failure. The patients with residual or progressive disease at 3 months after completion of RT were considered as treatment failures at time zero for DFS and local failure. The probabilities for event-free survival and DFS were calculated using Kaplan-Meier method (25). The local relapse rate was calculated using cumulative incidence (26). The statistical analysis was done treating potential prognostic factors as continuous variables where appropriate. However, to illustrate the effect of individual prognostic factors graphically, the curves for the continuous variables (age, tumor size, hemoglobin, and the laboratory parameters) were drawn for the two groups formed by dichotomizing at the median (Figs. 2–5). For the OTT, a total duration of ≥7 weeks was regarded as prolonged (Fig. 2). This was chosen because it was common practice to deliver the external beam treatment over 5–5.5 weeks, with a ≤1-week gap between its completion and the intracavitary insertion, and then 2–4-day duration for the brachytherapy. The statistical significance of each of the laboratory parameters (as continuous variable, where appropriate) was tested using Wald test (univariate analysis) within the Cox proportional hazard model (27). The laboratory parameters were also tested in the presence of clinical factors: (*a*) the clinical factors were considered in the model, and using step-

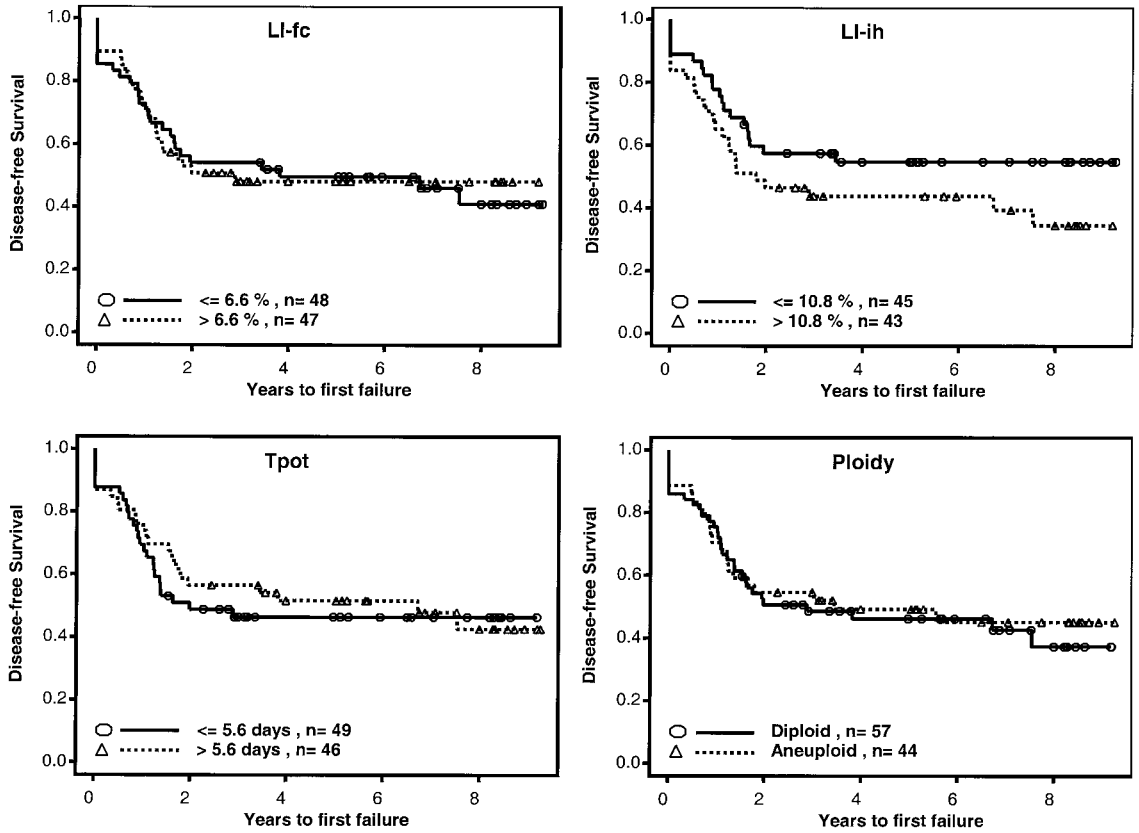

Fig. 3 The effects of BrdUrd LI-fc, immunohistochemistry (LI-ih), T_{pot}, and ploidy on DFS. The statistical significance of these factors in univariate and multivariate analyses is shown in Table 5.

wise selection technique, the significant ones ($P < 0.05$) were chosen in the model (Table 4); and (*b*) the laboratory parameters were added one at a time to the clinical factor(s) already chosen (Table 5). The inverse of T_{pot} ($1/T_{pot}$) was also analyzed because of its direct relationship with the growth constant (λ) and because this analysis may minimize the effects of outliers in the dataset.

RESULTS

Patient Characteristics. The clinical and pathological characteristics of the patients are detailed in Table 1. The median age at diagnosis was 54 years (range 23–83 years). The maximum tumor diameter assessed clinically at EUA ranged from 2 to 12 cm, with median value of 5.3 cm. The median hemoglobin concentration was 116 grams/liter (range 70–146 grams/liter). The median follow-up for alive patients was 6.2 years (range 1.3–9.3 years).

Tumor Proliferation Parameters and AI. The median BrdUrd LI-fc was 6.6% (range 1.4–36.1%). The median AI was 0.94% (range 0–6.8%). A summary of the results of the laboratory parameters is given in Table 2. The BrdUrd staining pattern was assessed in 88 biopsies: 22 had a marginal pattern (25%), 20 were intermediate (22.7%), 35 were mixed (39.8%), and 11 exhibited a random pattern (12.5%). There were 57 tumors with diploid DNA content, and 44 were aneuploid.

Treatment Outcome. After treatment, 88 patients (87%) had a complete response, whereas 13 patients had resistant/progressive pelvic disease. Patients with complete response had a median T_{pot} of 5.6 days, not significantly different from the 5.3 days for nonresponders (Wilcoxon test, $P = 0.82$). Among the 88 complete responders, 40 patients have relapsed at the following sites: 14 pelvic, 23 distant sites, and 3 pelvic and distant. To date, 43 patients died of disease, 12 patients who had residual or relapsed disease remain alive, and 46 patients are alive with no disease. The 5- and 8-year clinical outcomes were: overall survival of 57 and 53%, DFS of 48 and 41%, and local relapse of 26 and 26%, respectively (Fig. 1). There was no significant correlation of LI-fc, T_{pot}, or $1/T_{pot}$ with relapse of disease (data not shown), and because there were only two deaths unrelated to disease, further detailed analysis focused on DFS as the main end point.

Analysis of Prognostic Factors. Prognostic factors for survival, DFS, and local control were assessed in a univariate analysis, and the DFS results are presented in Fig. 2 (clinical factors) and Figs. 3 and 4 (laboratory factors). In the univariate analysis, the significant clinical factors for a lower DFS were large tumor size, pelvic lymph node status, higher stage, low hemoglobin level, and long OTT. Age, histological type, and grade were not significant for DFS. The results for overall survival, DFS, and local control are summarized in Table 3.

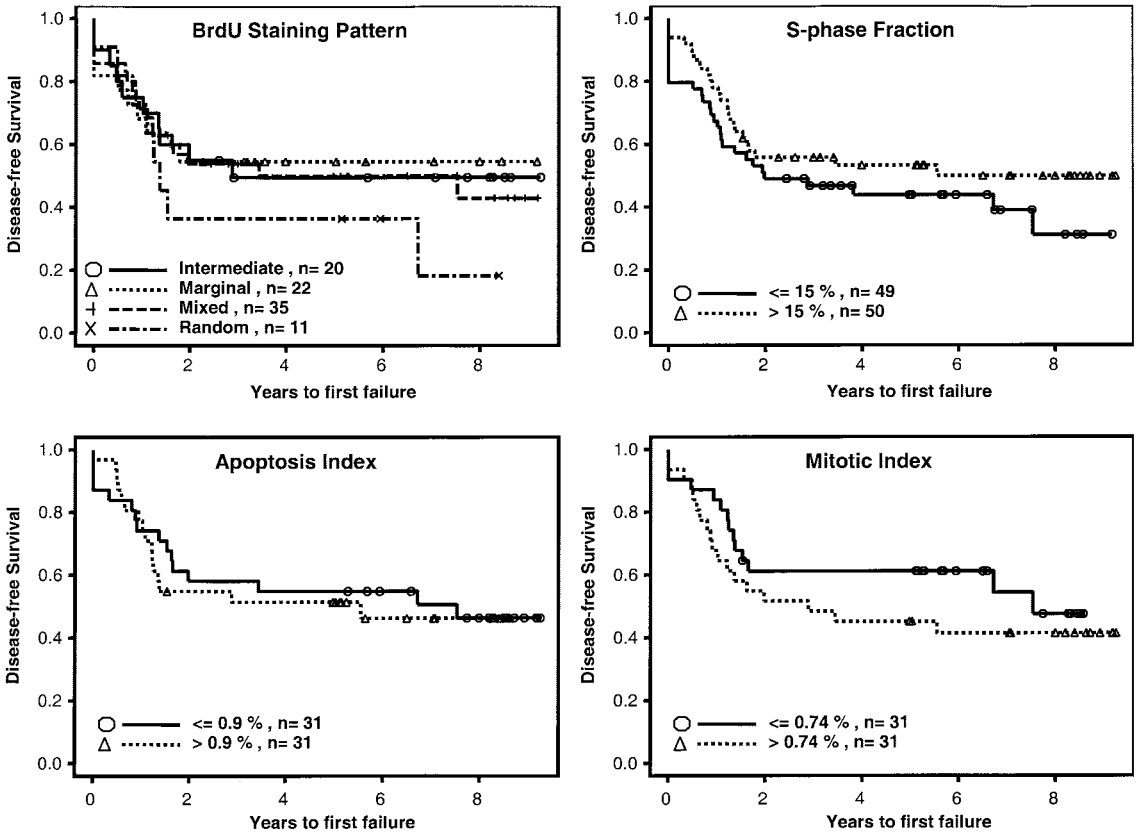

Fig. 4 The effects of BrdUrd staining pattern, S phase fraction by flow cytometry, AI, and MI on DFS. The statistical significance of these factors in univariate and multivariate analyses is shown in Table 5.

Table 3 Effect of clinical factors on Overall Survival, Disease-free Survival, and Local Control Rate: Univariate analyses

	Survival		Disease-free survival		Local control	
Univariate analysis	Hazard ratio (95% CL)	p-value	Hazard ratio (95% CL)	p-value	Hazard ratio (95% CL)	p-value
Age	0.99 (0.97–1.01)	0.23	0.99 (0.97–1.00)	0.12	0.98 (0.95–1.01)	0.12
Tumor size	1.30 (1.13–1.49)	0.0003	1.28 (1.13–1.45)	0.0001	1.41 (1.18–1.69)	0.0002
Pelvic LN (positive vs. negative)	1.78 (0.98–3.25)	0.06	2.19 (1.28–3.74)	0.0044	1.84 (0.85–4.01)	0.12
Stage						
IB/IIA vs. III/IV	0.30 (0.12–0.75)	0.01	0.28 (0.12–0.65)	0.003	0.13 (0.03–0.572)	0.0069
IIB vs. III/IV	0.68 (0.36–1.29)	0.24	0.75 (0.43–1.32)	0.32	0.43 (0.19–0.98)	0.044
Histologic grade (well and moderately well)	0.73 (0.37–1.45)	0.37	0.77 (0.42–1.40)	0.39	1.34 (0.50–3.58)	0.56
Histologic type (SCC vs. others)	1.37 (0.64–2.96)	0.42	0.88 (0.47–1.64)	0.69	0.76 (0.32–1.81)	0.54
Hb level	0.98 (0.96–1.00)	0.044	0.97 (0.96–0.99)	0.0012	0.97 (0.94–0.99)	0.0045
OTT	2.96 (1.53–5.72)	0.0013	2.73 (1.52–4.91)	0.0008	3.90 (1.74–8.72)	0.0009

CL: confidence limits.

Hemoglobin level was correlated with tumor size (Pearson $r = -0.58$), and a more advanced stage was positively correlated with tumor size (Kruskal-Wallis, $P < 0.0001$). In the final clinical model (multivariate analysis), tumor size and OTT were the only significant factors for overall survival and local control, whereas tumor size, pelvic lymph node status, and OTT were significant for DFS (Table 4).

The laboratory parameters were not significant in univariate analysis (Figs. 3 and 4), nor when adjusted for the significant clinical factors (Table 5). The hazard ratios and *P*s for all of the laboratory factors are given for DFS in Table 5. The results for overall survival and local control are similar to that of DFS (Table 5), with no statistically significant effect for any of the laboratory parameters (data not shown). The interaction term of LI with tumor size was not significant for survival ($P = 0.75$), DFS ($P = 0.65$), and local control ($P = 0.5$), suggesting that the effect of LI was not different in small tumors as compared with larger tumors. This is illustrated in Fig. 5, where small tumors

Table 4 Multivariate analyses showing the effect of significant clinical factors on overall survival, disease-free survival, and local control rate

Final clinical model	Survival	DFS	Local control
Tumor Size:			
Hazard Ratio	1.2	1.2	1.3
95% CL	1.1–1.4	1.1–1.4	1.1–1.6
p-value	0.0022	0.0011	0.0014
Pelvic LN Positive:			
Hazard Ratio	—	1.8	—
95% CL	—	1.1–3.2	—
p-value	—	0.0329	—
Overall Treatment Time:			
Hazard Ratio	2.9	2.2	3.1
95% CL	1.6–5.5	1.2–4.0	1.3–7.3
p-value	0.0007	0.0143	0.0091

Note: Pelvic lymph node status was tested but not selected in the multivariate model for survival and local control.

fared better than larger tumors, and LI-fc did not have additional prognostic value in either subgroup. Neither LI-fc nor LI-ih were significantly correlated with tumor size (Pearson $r = 0.33$ for LI-fc, and $r = 0.01$ for LI-ih). Neither LI nor T_{pot} were significantly correlated with pelvic lymph node status (χ^2 $P > 0.9$). There were no statistically significant correlations between LI and hypoxia measurements (22) or LI and IFP of the primary tumor (LI-fc and IFP, $r = 0.05$; LI-ih and IFP, $r = 0.23$, data not shown).

DISCUSSION

When this study started in 1991, there was evidence that prolongation of OTT in radical radiotherapy was associated with a higher risk of treatment failure in some human tumors. This effect has been best described in squamous cell carcinomas of the head and neck (2, 3) and uterine cervix (6–9). The proposed underlying hypothesis was that a rapidly proliferating tumor accelerates further in growth rate after 3–4 weeks of conventional fractionated RT contributing to a treatment failure (2).

In theory, tumor proliferation is balanced by cell loss, and the T_{pot} measures the growth potential of the tumor assuming no cell loss (28). The *in vivo* labeling of the tumor by BrdUrd is a dynamic method of assessing proliferation rate and results in estimates of both LI-fc and T_{pot} (17, 29–31). However, at the time this study was initiated, it was largely unknown whether it is a better predictor of clinical outcome compared with the LI or detection of other proliferation antigens, such as Ki-67 (32), MIB-1 (33), and proliferating cell nuclear antigen (34). Flow cytometric analysis is subject to contamination by nontumor cells, particularly for diploid tumors, and it also requires correction for cell division for cells that had passed through mitoses (17, 29, 35). It also does not allow for a morphological structural assessment of the tumor. Because of this, it has been suggested that LI-ih may be a more accurate reflection of tumor cell proliferation (36).

Our previous analysis showed that BrdUrd LI-fc predicted DFS in a univariate analysis but not independent of the clinical factors consisting of tumor size and OTT (16). However, LI-fc seemed to have a significant effect on the 30 small tumors (3-year DFS 81% for LI $< 7\%$ *versus* 30% for LI $\geq 7\%$; Ref. 16). In the present study, tumor size remained a strong and highly significant factor in predicting survival, DFS, and local failure. Although the hemoglobin level was significant, it was highly correlated with tumor size and suggested the possibility that it was a surrogate of local tumor extent. The only significant factors for DFS in the multivariate analysis were tumor size, pelvic lymph node status, and OTT. LI-fc and T_{pot} were not significant in univariate analysis, nor were the other laboratory parameters, including LI-ih and BrdUrd staining pattern, with nearly overlapping curves for DFS (Figs. 3 and 4). This represented a significant difference from our previous report, chiefly because of a larger number of patients in the present analyses (101 patients *versus* the previous 77) and a longer duration of follow-up, presently, a median of 6.2 *versus* 3.2 years from our last report (16). In a study of a very similar patient population as in the present study, Bolger *et al.* found tumor size to be the strongest prognostic factor, but in their multivariate analysis, LI remained statistically significant with a hazard ratio of 1.1 (confidence interval = 1.01–1.2, $P = 0.034$; Ref. 18). However, the median follow-up was 34 months (18), very similar to our initial report. It would be of interest to determine whether their data are different with a longer follow-up duration.

The staining patterns of BrdUrd in tissue sections did not correlate with clinical outcome (Fig. 4). In the original description of staining pattern of BrdUrd by Wilson *et al.* (5), mixed and random patterns were associated with poorer outcome in head and neck cancer compared with marginal and intermediate patterns. In the present study, the random pattern did have a lower DFS, but this was not significant.

In cervix cancer, there have been a number of correlative studies in the literature focusing on proliferation rate, as measured by static methods of proliferation antigens, such as Ki-67 (32, 37–42) and MIB-1 (33, 43, 44), with clinical outcome. The majority of these reported a lack of association (32, 33, 37, 38, 43, 45). Two studies reporting a positive correlation of high tumor proliferation rate associating with a worse clinical outcome only included a small number of patients (<40 patients each; Refs. 41 and 44). An additional study reported a better response to radiotherapy for those patients with a high proliferative index as measured by Ki67 and argyrophilic nucleolar organizer region counts (42). Our data confirm that pretreatment LI, either by flow cytometry or immunohistochemistry, has no advantage over other methods in trying to identify patients with a worse prognosis when treated with radical radiotherapy. It is likely that the LI and T_{pot} measurements do not reflect the true "stimulated" proliferative capacity of the tumor clonogenic cells during fractionated conventional radiotherapy. In addition, the extent to which clonogenic tumor cells respond to cellular depletion because of cytotoxic therapy by accelerating their proliferation rate is largely unknown. One study showed that the proliferation rate with the MIB-1 index can increase after 1–2 weeks of fractionated RT in cervical carcinoma (46), and in a more recent report, this was shown to be paradoxically associated with a good prognosis (47). An accurate measure of the rate of proliferation of clonogenic tumor cells during RT (repopulation) is still not available (36). A study addressing whether tumor proliferation measurements predicted clinical outcome in the setting of radical radiotherapy for head and neck cancer had

Table 5 Multivariate analysis of the effect of tumor kinetics parameters on disease-free Survival (DFS). The laboratory parameters were tested one at a time, with and without adjustments for clinically important prognostic factors

	Unadjusted		Adjusted*		Adjusted**	
Disease-free survival	Hazard ratio (95% CL)	p-value	Hazard ratio (95% CL)	p-value	Hazard ratio (95% CL)	p-value
LI-fc	1.04 (0.98–1.1)	0.22	1.01 (0.95–1.08)	0.70	1.03 (0.96–1.09)	0.43
T_{pot}	1.01 (0.97–1.06)	0.61	1.02 (0.98–1.07)	0.37	1.02 (0.97–1.07)	0.45
$1/T_{pot}$	1.69 (0.22–13.25)	0.62	0.99 (0.12–7.86)	0.99	1.38 (0.16–11.9)	0.77
T_s	1.02 (0.95–1.08)	0.67	1.00 (0.93–1.07)	0.94	1.00 (0.93–1.08)	0.95
Ploidy	0.78 (0.37–1.65)	0.51	0.63 (0.31–1.32)	0.22	0.54 (0.25–1.17)	0.12
SPF	1.00 (0.97–1.03)	0.91	0.99 (0.95–1.02)	0.39	0.99 (0.95–1.02)	0.39
LI-ih	1.04 (0.98–1.09)	0.21	1.03 (0.97–1.09)	0.35	1.04 (0.98–1.10)	0.2
BrdU staining pattern (mixed/random)	1.21 (0.67–2.16)	0.53	1.39 (0.75–2.60)	0.30	1.61 (0.85–3.05)	0.14
AI	1.13 (0.89–1.42)	0.32	1.18 (0.92–1.52)	0.20	1.24 (0.98–1.57)	0.077
MI	1.51 (0.70–3.24)	0.29	1.31 (0.59–2.92)	0.51	1.11 (0.48–2.58)	0.81

* Adjusted for tumor size and pelvic lymph node status.
** Adjusted for tumor size, pelvic lymph node status and overall treatment time.
CL: confidence limits.

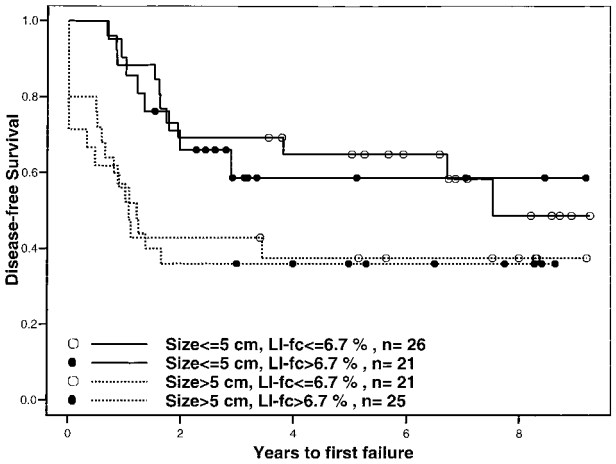

Fig. 5 The effects of BrdUrd LI-fc on DFS, for patients with small and large tumors dichotomized at the median maximum tumor diameter of 5 cm.

also yielded negative results (48). The nonsignificant findings for T_{pot}, $1/T_{pot}$, and LI, despite an observed treatment time effect in this study, would suggest that other factors in the cervix cancer situation may override the negative impact of repopulation. One might expect a rapidly proliferating tumor to also respond rapidly to the external beam treatment, hence putting the residual disease in a more favorable position to receive full dose brachytherapy, thereby counteracting the repopulation effect. However, the fact that the complete responders in our series did not have significantly different T_{pot} or LI values seem to argue against this explanation. Of course, other biological reasons for a prolonged OTT, such as inability to tolerate continuous treatment because of comorbid medical conditions, treatment toxicity, or other unknown reasons, may also be important. These require further investigation.

Even if tumor repopulation during RT is a significant problem, it is only one of many factors that contribute to treatment failure. The observed strong effect of tumor size on prognosis in this study suggests that clonogenic cell number or tumor microenvironmental features, such as hypoxia and elevated IFP, may be dominant factors for predicting outcome in cervix cancer. In analyses of a similar group of patients treated at the PMH, tumor hypoxia (20, 49) and high IFP (21) were significant adverse factors for poor DFS in multivariate analyses. There was no significant association between LI and tumor hypoxia (22) or between LI and IFP (data not shown). A high level of tumor vascularity reflecting increased angiogenesis has been reported to associate with a poor prognosis (50). Another important radiobiological predictive parameter is intrinsic radiosensitivity with *in vitro* assessment of survival fraction at 2 Gy (51). The morphological assessment of pretreatment apoptosis in this study showed no correlation with clinical outcome, similar to a recent study of 146 patients (52). Previously, a high AI was either reported to be associated with a good prognosis (53, 54) or a poor prognosis (45) in smaller studies. However, a limitation of the present study is the sample size. Although larger than in some published series, it could still result in a small effect being missed, particularly for AI and MI, where a significant proportion of biopsy specimens was not available for analysis.

In conclusion, tumor size, pelvic lymph node status, and OTT are the dominant clinical prognostic factors in cervix carcinoma. The pretreatment proliferation parameters studied were not associated with survival, DFS, or local disease control. We are continuing to study other measurements of tumor microenvironment, such as hypoxia and IFP, to devise individualized treatment strategies in cervical carcinoma to improve clinical outcome.

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Correction

In the article by R. W. Tsang *et al.*, which appeared in the October 1, 2003 issue of *Clinical Cancer Research* (pp. 4387–4395), two author names were inadvertently omitted: Wilfred Levin and Lee A. Manchul. The correct author list is: Richard W. Tsang, Stephen Juvet, Melania Pintilie, Richard P. Hill, C. Shun Wong, Michael Milosevic, William Chapman, Wilfred Levin, Lee A. Manchul, and Anthony W. Fyles.

Clinical Cancer Research

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