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Report

The prognostic value of proliferation indices: a study with *in vivo* bromodeoxyuridine and Ki-67

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Summary

Proliferation indices are intended to help patients and clinicians make treatment decisions. We have previously demonstrated that a proliferation index based on *in vivo* labeling of S-phase cells with bromodeoxyuridine (BrdUrd) correlates with Ki-67 labeling index (LI). We now compare the prognostic value of these indices.

With written consent, we gave 129 women with biopsy confirmed breast cancer 200 mg/M^2 BrdUrd during 30 min immediately preceding surgery. We used IU-4 anti BrdUrd antibody to count the immunohistochemical labeling index (LI) of DNA-incorporated BrdUrd in 2,000 cells and MIB-1 to count Ki-67 (118 cases). Patients received standard surgical and adjuvant treatment. No patients were lost to follow-up and patients were followed a minimum of 2 (median 5.1) years. We compared survival and recurrence in tumors with high vs low labeling indices. We found that women in the low BrdUrd LI group had better disease free survival (92% vs 67% 5-yr DFS $p = 0.001$) and overall survival (94% vs 70% 5-yr OS, $p = 0.0001$) than those with a high LI. In comparison, a low Ki-67 index predicted better OS (87% vs 80% 5-yr OS, $p = 0.020$) and a trend for better DFS (84% vs 72% DFS $p = 0.055$). The apparent superiority of BrdUrd LI over Ki-67 LI is likely due to chance ($p = 0.18$). In multivariate survival analyses we found that BrdUrd LI proliferative index significantly improves prediction of DFS or OS even when node status, age or tumor size is in the model. We conclude that markers of proliferation are useful adjuncts in predicting patient prognosis.

Introduction

Clinicians use prognostic information to estimate the absolute benefit of adjuvant therapy and patients use prognostic information to understand the threat cancer poses to their health as they make informed decisions about their own treatment. Pathologic staging is widely used to estimate prognosis, but pathologic staging '...although of considerable value does not take into full account the nature of the tumor itself' [1].

Innumerable factors have been proposed to supplement pathologic stage. Bloom and Richardson proposed a histologic grading system [1]. Others have proposed markers of hormone responsiveness, genetic instability, secreted proteases, vascularity, programmed cell death, growth fraction, etc.

Measures of cell proliferation have prognostic value for human breast cancer [2–11], but the importance of measures of cell proliferation relative to pathologic staging is still debated [12, 13]. Synthesis of the thymidine analog bromodeoxyuridine (BrdUrd) and isolation of antibodies specific for DNA-incorporated BrdUrd have allowed development of a proliferation index based on dynamic, *in vivo* labeling of proliferating cells. A brief *in vivo* infusion of BrdUrd just before tumor removal labels cells in S-phase in a manner analogous to *in vitro* 3H-thymidine exposure [14]. These cells can be identified with a monoclonal antibody specific for BrdUrd incorporated into DNA. We,

and others, have studied patients with breast [14–18] brain [19], colon [20], ovarian [21], head and neck [22], lung [23], and bladder [24] cancers using *in vivo* BrdUrd.

Growth fraction, another measure of cell proliferation, can be measured by repeated direct labeling [24], but it is more commonly estimated by the presence of the Ki-67 antigen. The function of Ki-67 is poorly understood. It was first isolated from a Hodgkin's disease cell line; and it is located in the nucleus, possibly associated with the nucleolus and/ or fibrillar components [25].

In continuously cycling cells, Ki-67 it is thought to be present in all cells in the growth fraction. However, its specific expression may vary in the cell cycle [26, 27]. Previous studies showed that Ki-67 LI has prognostic value in breast cancer, but less than nodes, tumor size, or estrogen receptor status [11, 28].

This paper presents the clinical outcome of patients we have previously reported [15]. We demonstrate the predictive value of a proliferation index based on *in vivo* infusion of bromodeoxyuridine. We compare this proliferation index to the predictive value of node status, growth fraction measured with the MIB-1 antibody to Ki-67, and other factors. We conclude that proliferation indices based both on BrdUrd labeling and on Ki-67, with or without nodal status information, are useful prognostic markers to help understand the 'nature of the tumor itself' for individual patients, and that such factors will have increased practical importance as surgical practice shifts toward removal of fewer axillary nodes for staging purposes.

Methods

Patients with breast cancer confirmed by fine needle aspiration, core biopsy, or incisional biopsy were asked to participate in this study. Accrual began in August 1986. Between January 1, 1988 and December 31, 1994, all patients with primary operable breast cancer in the surgical practice of one of us (William H. Goodson III) were asked to participate.

With written informed consent and approval from the Committee on Human Research at the University of California, San Francisco, subjects received 200 mg/ M^2 of body surface area of bromodeoxyuridine (BrdUrd, recently renamed broxuridine; National Cancer Institute, Bethesda, Maryland; now available only from NeoPharm Inc., Lakeforest, Illinois) in 250 ml of normal saline by intravenous infusion during the 30 min immediately before induction of general anesthesia. Anesthesia, surgery, and all pre- and post operative care were customary. Treatment recommendations were not based on BrdUrd labeling index.

Tissue was prepared, stained, and counted as described previously [14, 15, 29]. During routine cutting of fresh tissue, portions of the tumor were excised, fixed in 70% alcohol or 10% neutral buffered formalin and embedded in paraffin. Tissue sections were treated 1 h with 2N HCl to denature the DNA. Cross-linked proteins in the formalin fixed tissue sections were cleaved for 1 min with 0.1% Protease XXIV (Sigma Chemical Company, St. Louis, Mo.) before denaturing with HCl. The Elite ABC kit (Vector Laboratories, Burlingame, Ca.) was used for indirect immunoperoxidase staining using the lU4 antibody (45 min; 1:250 dilution with 5% horse serum; courtesy of Dr. Joe Gray, University of California, San Francisco, California) for DNA-incorporated Brd Urd. Light counterstaining with hematoxylin highlighted BrdUrd-negative cells.

To identify Ki-67 positive cells, 4 μm formalin fixed sections were cut, washed with xylene to remove paraffin, denatured with 0.25% trypsin, briefly exposed to microwave treatment in 10 mM citrate buffer (pH 6.0) to enhance antigen retrieval, and incubated overnight with MIB-1 antibody to Ki-67 (1:200; Immunotech, Westbrook, ME [29]. Immunoreactive cells were identified with standard techniques using biotinylated anti-mouse antibody (1:200; 30 min at room temperature; Vector Labs, Burlingame, CA) followed by Streptavidin-HRP (1:200; 30 min; Zymed Labs, South San Francisco, CA), DAB (Sigma, St. Louis, MO), and counterstained with hematoxylin.

BrdUrd labeling index and MIB-1 indices were determined as the percentage of positive cells among 1,000–2,000 tumor cells in multiple microscopic high-powered fields (×400). Early in our studies we found that 2000 cells was not statistically different from 1000 cells [14]. The number of positively stained nuclei was divided by the total number of tumor nuclei and expressed as a percent. Fields were selected from areas with the greatest proportion of cells staining positive which gives higher values than randomly selected fields [17, 30].

Pathology was reviewed independently on all patients. All ductal cancers were graded according to Scarff–Bloom–Richardson. Other breast cancer variants, including mucinous and lobular, were graded by assessing nuclear features and mitotic figures.

Patients were followed every 6 months. Assessment was on the basis of history, physical examination, and routine mammography. Patients unable to return for follow-up were contacted by telephone, a current history obtained, and records obtained from their local physicians when possible. Final clinical records or death certificates were reviewed for all patients who died.

Number of months to the identification of systemic disease and number of months to death were the dependent variables. Local recurrence in the breast after breast conserving surgery but without systemic disease was not counted as systemic disease. BrdUrd and Ki-67 labeling indices were considered both as continuous variables and as dichotomous variables with LI above or below the median; the number of positive nodes was considered as a continuous variable, as positive or negative, and as groups of 0, 1–3 and ≥ 4 positive nodes; hormone receptors were grouped positive or negative; and other variables were used as continuous values.

We compared the overall survival of our patients to cases matched from the surveillance epidemiology and end results (SEER) public use data base. For each case, all women in the SEER public use data base for northern California with similar age ($\pm$ 5 years), same tumor size category (T-1, T-2, T-3), node category (0, 1–3, $>$ 4), and year of diagnosis ($+$ 1 year) were selected. From each set, the three best match cases were selected to form a comparison population. The survival of our patients was compared with that of the matched SEER cases by Kaplan–Meier analysis and the log rank test.

In univariate analysis Kaplan–Meier survival curves were used to compare the different groups for each variable and the difference between curves compared with the log rank test. For multivariate analysis, a Cox proportional hazard model was fit to survival data. In fitting the Cox model to the data, we considered all combinations of pairs of factors that were univariately significant. We also explored prediction based on the Cox model with three factors, where a third factor was added to the 'best' two-factor model. The number of events in this dataset are small enough that it is unreasonable to expect more than two factors to be statistically significant [44]. We used a chi-square statistic equal to twice the difference in log likelihood ratios to judge statistical significance in making comparisons between nested models (i.e. models which contain factors added stepwise).

We also used a randomization test to determine whether the predictive value of BrdUrd and Ki-67 labeling indices were significantly different [31]. The null hypothesis for this test is that it makes no difference whether the BrdUrd LI or the Ki-67 LI is used to assign patients into the high or low labeling index groups and, therefore, that it makes no difference whether the BrdUrd LI or the Ki-67 LI is used to predict prognosis. We tested the null hypothesis by randomly reassigning the BrdUrd and Ki-67 LI's as the basis to determine if a patient was in the high or the low LI group. In the majority of cases BrdUrd and Ki-67 LI's were in agreement, that is, both have high LI or both have low LI and group assignment would not change if the other LI was used. Therefore, random reassignment did not change whether most cases were in the high or low group and the difference between high and low labeling index groups remained significant. For each case in the disagreement group, the identity of the LI index used in predicting outcome was randomly switched, or not switched, according to a random number generated by the computer. For example, if patient A's true BrdUrd LI was above the median and her true Ki-67 LI was below the median, a random number was used to decide whether to switch her LI results. Repeating this process for each patient in the LI disagreement group creates one randomization sample. The difference in p-values of the log rank test for this randomization sample of BrdUrd and Ki-67 LI's is then computed. By creating a set of 1000 randomization samples, we can determine the distribution of the difference in p-values when BrdUrd and Ki-67 LI are assigned randomly. We then compare the observed difference in p-value (based on the true LI assignments) to the distribution of differences in p-values generated by the randomization test to determine statistical significance under the null hypothesis of no difference between the two LIs.

Statistics were performed using a standard statistical package (StatView 5.0.1, SAS Institute, Inc.) which includes capabilities for plotting Kaplan–Meier survival curves, forming log rank test statistics and the ability to fit a Cox proportional hazard model to survival data.

Results

A total of 129 women with primary tumors consented. Four eligible patients declined participation. (Patients with previous breast cancer, extensive *in situ* cancer

but no more than micro invasion, preoperative chemo or hormone therapy, stage IV disease, one patient with a sarcoma, three men with invasive breast cancer, and three women with no identifiable residual cancer after an incisional biopsy, received BrdUrd but are not included in this paper.) There were no complications from bromodeoxyuridine infusion.

There were 129 women ranging from 25 to 87 years of age (mean 55 years median 51 years). Tumors were from 0.3 to 12 cm in diameter (mean 2.54 cm median 2.0 cm). There were 105 invasive ductal, 16 invasive lobular, two mixed invasive ductal and lobular; and four colloid/mucinous cancers.

There were two inflammatory cancers as defined by both dermal lymphatic invasion and clinical appearance of the breast. Sixty-five had lymph node metastases and 57 did not. Six patients did not have nodes removed, five because of advanced age, and one in accordance with the patient's choice. Initial node status could not be determined on one patient because neoadjuvant chemotherapy was given after biopsy and before node dissection.

Patients were treated in accord with standard clinical protocols at the University of California Medical Center. Patients with positive nodes received either chemotherapy or hormone therapy. Thirty-nine of 57 who did not have positive nodes received adjuvant therapy based on physician assessment of histology, vascular invasion, hormone receptors, and tumor size. Six patients had autologous bone marrow transplant. BrdUrd labeling index was not used for treatment decisions in this study.

Follow up was available on every patient. All patients had a minimum of 2 years of follow-up and the status of all patients was ascertained within the last year of this report. Mean follow-up was 5.3 years and the median follow-up was 5.1 years. Twenty-six patients have had systemic recurrence of disease. One patient had an in-breast local recurrence after a partial mastectomy and radiation therapy but a complete metastatic work up was negative, and she had a mastectomy with negative margins. She was not included as a systemic recurrence. Nineteen patients have died of breast cancer. One patient had exacerbation of scleroderma after adjuvant chemotherapy and died of pulmonary insufficiency 25 months after surgery. One patient died of myocardial infarction and one patient died of complications of a renal biopsy unrelated to her cancer. These three patients were censored as lost to follow up at the time of death.

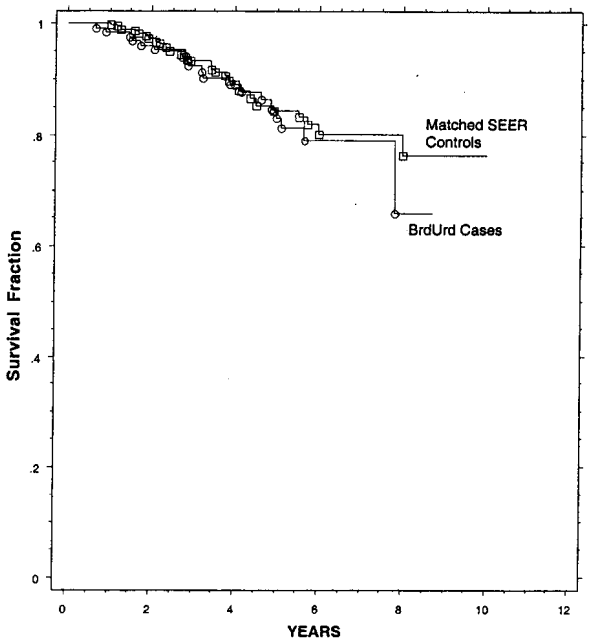

Figure 1. Comparison of overall survival of our patients to cases from the SEER data base matched for age, year of diagnosis, tumor size, and number of positive nodes.

Comparison with the SEER data base

For each of our patients, three San Francisco-Bay Area SEER cases were matched for age, tumor size, number of positive nodes, and year of diagnosis. There were no matches for four women who were treated before the SEER registry. There was no difference in survival between our patients and the matched cases from the SEER registry (Figure 1).

Proliferation indices

BrdUrd labeling was successful in all patients. The BrdUrd labeling index ranged from 0.6 to 34% (mean 9.6% median 8.1% standard deviation 7.2, Figure 2). Sufficient material was available to stain 118 cases with MIB-1 antibody. For these patients Ki-67 LI ranged from 1.4 to 77.1% (mean 24.9%, median 23.2%). The patients without Ki-67 data were significantly older, all in the lower BrdUrd LI group, and all positive for progesterone receptors. Comparison of BrdUrd and Ki-67 indices was in the subset for which both indices were available.

Univariate results

Univariate analyses are summarized in Table 1. Statistically significant factors for both disease free survival

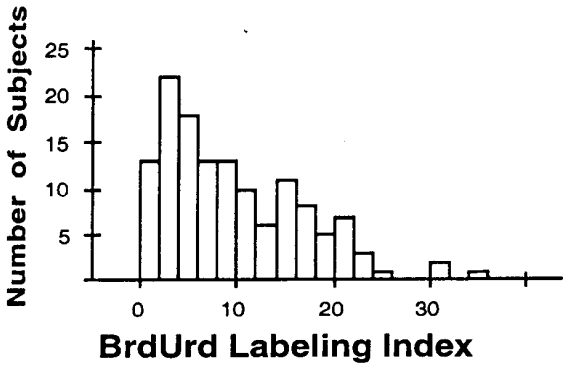

Figure 2. Frequency distribution of BrdUrd labeling index in 129 patients.

and overall survival were BrdUrd labeling index (Figures 3A and 4A), node status (both as positive vs negative nodes, Figures 3B and 4B, and grouped as 0, 1–3, and ≥ 4 nodes positive), presence of vascular invasion, age above or below 50 years, and tumor size. Ki-67 LI was predictive of overall survival ($p = 0.020$ Figure 3C) and marginally significant for disease free survival ($p = 0.055$ Figure 4C).

There was a strong correlation of BrdUrd LI and Ki-67 LI ($r = 0.76$, 95% CI 0.67 to 0.820 Figure 5). As we have observed previously [29], this correlation was driven by the agreement of the extremes of high and low values with overlap in the mid-range values. Seventeen percent of cases were shifted above or below median values according to which index was used. Note that unlike Figures 3 and 4, Figure 5 does not present time data.

This high correlation translates to roughly equal prognostic significance. In the 112 cases (with nodal status) for which Ki-67 could be determined, separation of patients into groups above and below the median achieved similar prognosis for disease free survival and overall survival ($p = 0.065$ and 0.012, respectively Figures 3C and 4C). In this same subset of patients, BrdUrd LI also predicted DFS and OS ($p = 0.006$ and $p = 0.0003$, respectively).

We then used a randomization test to compare survival prediction based on BrdUrd LI with that based on Ki-67 in the same 112 patients. In 179 of 1000 randomized trials (where BrdUrd LI or Ki-67 score was selected at random for each patient and used to predict survival) the observed difference in log rank statistics was larger than that observed (where true BrdUrd LI or Ki67 were used to classify all patients). Thus, the observed difference in log rank was found to occur by chance 179 times out of 1000.

Multivariate results

Results from fitting Cox proportional hazards models to pairs of factors univariately significant are summarized in Table 2. The table shows prognostic factors grouped by univariately significant factors. The first line in each grouping shows the chi-square statistic, equal to twice the log likelihood for the model with the factor. Subsequent lines show changes in chi-square from adding a second factor to each model. Thus, the prognostic significance of adding a second factor to the Cox model can be seen from the p-value associated with the change in chi-square value.

We explored alternative ways to analyze the data and discovered that the log-likelihood (a statistical measure of model appropriateness) was not significantly different when both number of positive nodes and BrdUrd LI were dichotomized. There was little difference over the cut-point range 6–9% for BrdUrd LI; thus the median seems to be a reasonable choice. Categorization of 0–3 vs ≥4 positive nodes provided better discrimination for both disease free and overall survival than did negative vs positive nodes. A cut-point between three and four positive nodes has been used by other authors [32]. However, we have focused on the distinction of positive versus negative nodes since that is more commonly used clinically.

For disease free survival, these data suggest that tumors with four or more positive nodes are 3.1 (95%, CI 1.4–6.7) times as likely to recur as those tumors with three or fewer positive nodes. If the BrdUrd labeling index is above 8% the chance of recurrence is 3.1 (95%, CI 1.2–7.8) times that for tumors with BrdU LI below the median. If we dichotomize the node status at positive vs negative nodes, the risk ratios decrease to 2.4 (95%, CI 1.0–5.7) for positive nodes and 2.7 (95%, CI 1.1–6.6) for higher BrdUrd LI. (Note that changing the cutpoint for positive nodes also affects the risk ratio for BrdUrd LI in a multivariate model.)

For overall survival, four or more positive nodes increases the risk by 4.3 (95%, CI 1.6–11.3) and BrdU LI above the median increases it by 7.9 (95%, CI 2.1–29.4). For a model with positive vs negative nodes, the risk ratio is 3.6 (95%, CI 1.0–12.8) for positive nodes and 6.0 (95%, CI 1.7–21.9) for a higher BrdUrd LI.

Discussion

We have demonstrated that an *in vivo* BrdUrd labeling index correlates with the clinical outcome of women

Table 1. Univariate survival analysis

Factor	Number	Disease free survival		Overall survival	
		5-yr surv (%)	*p*-value	5-yr surv (%)	*p*-value
Age					
Under 50	61	74		80	
Over 50	68	85	0.003	87	0.029
Tumor size					
T-1	55	87		89	
T-2	59	83		85	
T-3	15	47	0.002	60	0.002
Nodal status					
Negative	57	87		94	
Positive	65	73	0.012	76	0.001
Negative	57	90		94	
1–3 pos	29	86		86	
4 or more	36	62	0.004	74	0.0003
Grade					
Well	26	93		92	
Mod	62	83		84	
Poor	40	74	0.19	77	0.18
ER status					
Negative	40	80		80	
Positive	85	82	0.27	87	0.47
PR status					
Negative	40	80		80	
Positive	82	82	0.11	87	0.08
Vascular Inv					
Absent	84	87		89	
Present	38	58	0.002	70	0.0002
BrdUrd LI					
Below med	66	92		94	
Above med	63	67	0.001	70	0.0001
Ki-67					
Below med	60	84		87	
Above med	58	72	0.055	80	0.020

*p values based on logrank test.

with operable primary breast cancer. BrdUrd LI dichotomized women into groups with higher or lower risk of survival at a level of significance similar to the risk predicted both by node status and by age.

As expected, BrdUrd labeling index correlated with a Ki-67 LI based on MIB-1 antibody. Ki-67 LI was a significant predictor for overall survival and a marginally significant predictor for disease free survival.

Although our data suggest that BrdUrd LI is a more effective prognostic factor than Ki-67, these data are not sufficient to exclude statistical variation as the cause of the outcome prediction difference between BrdUrd LI and Ki-67 LI. Specifically, this difference was not significant in a randomization test.

Our conclusions are preliminary because this is a small series and because the majority of the patients received adjuvant treatment. We plan to study

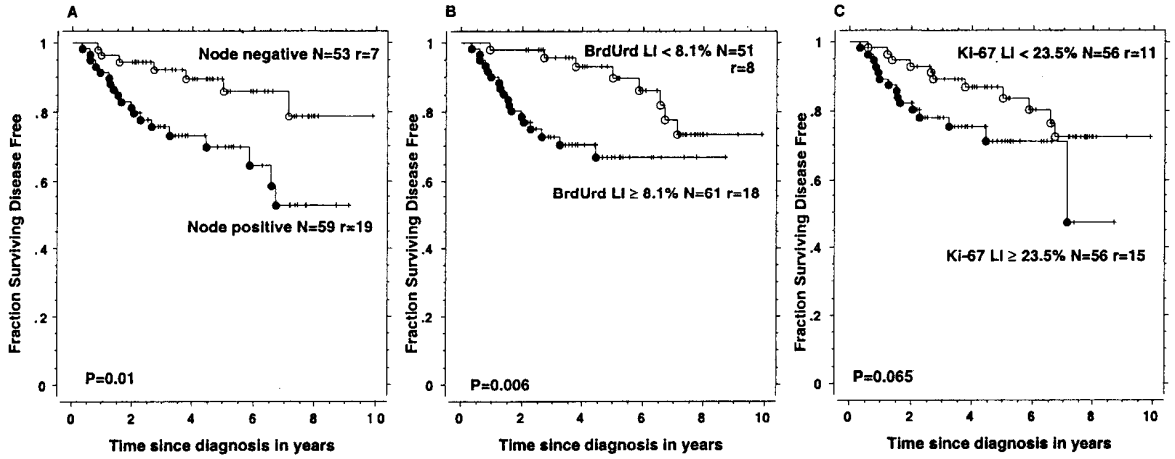

Figure 3. Disease free survival (A) according to node status, positive versus negative, (B) according to BrdUrd labeling index above and below the median, and (C) according to Ki-67 proliferation index above or below the median as measured with the MIB-1 antibody. Figure shows number of cases (N), number of recurrences of breast cancer (r) and log-rank p-value for cases with both BrdUrd and Ki-67 Lis and node status. Medians are for all cases so the BrdUrd LI split is uneven in the subset considered here. Circles indicate recurrences, tick marks indicate patients disease free at the end of followup.

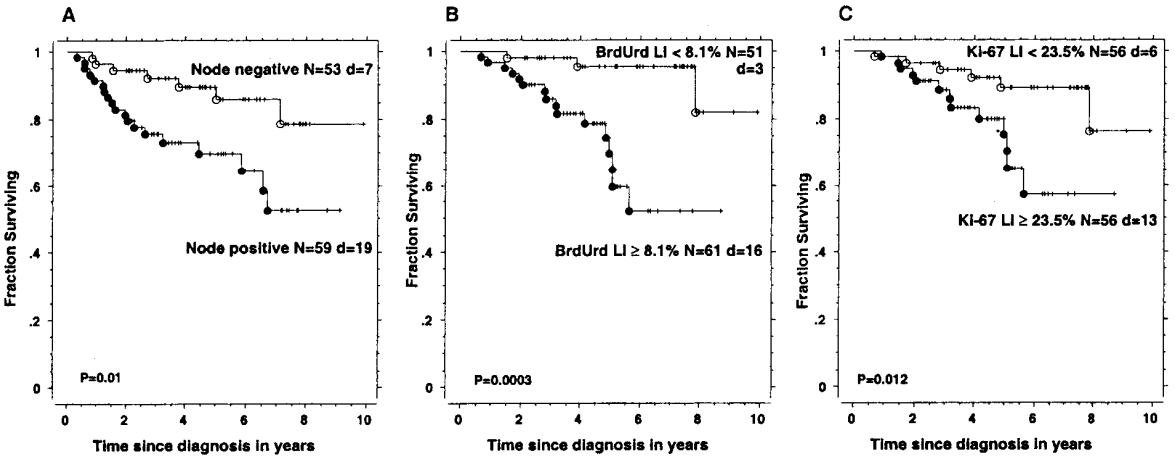

Figure 4. Overall survival (A) according to node status, positive versus negative, (B) according to BrdUrd labeling index above and below the median, and (C) according to Ki-67 proliferation index above or below the median as measured with the MIB-1 antibody. Figure shows number of cases (N), number of deaths due to breast cancer (d) and log-rank p-value for cases with both BrdUrd and Ki-67 Lis and node status. Medians are for all cases so the BrdUrd LI split is uneven in the subset considered here. Circles indicate deaths, tick marks indicate patients alive at the end of followup.

more patients but it is already impossible to develop a prospective series of patients with both positive and negative nodes who have not received adjuvant therapy unless one deviates from the standard of care. Adjuvant therapy is appropriate for all women with positive nodes, but it is not yet the standard of care for all women with negative nodes. This is probably a moot point, however, since adjuvant therapy is beneficial in virtually all settings, and women with high BrdUrd LI and/or positive nodes did less well than women with low BrdUrd LI and negative nodes despite adjuvant therapy.

Our study has several strengths – over half of our patients have been followed more than five years. No patients have been lost to follow-up. Ours is a prospective, consecutive series. The only eligible patients not included were the four who declined participation. A consecutive series is important to identify the interaction of node status with a prognostic factor. Retrospective selection influences results because the

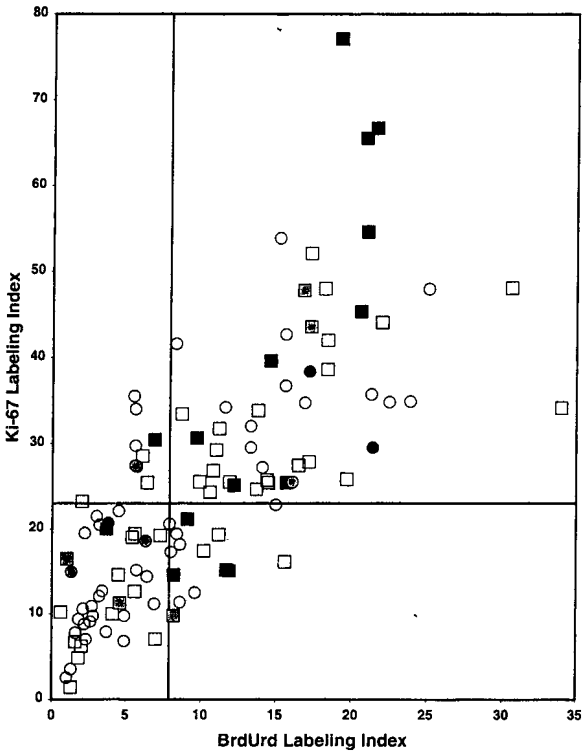

Figure 5. Scatter plot of BrdUrd labeling index versus Ki-67 proliferation index. Circles are patients with negative nodes. Squares are patients with positive nodes. Open symbols are patients who are disease free. Grey symbols are patients with metastatic disease. Black symbols are patients who have died. (Note: some datapoints are overlapped.)

number of cases in each specific category is determined by tissue availability.

It is important to consider several methodology issues. First, we establish that our subjects are not inherently unusual by comparing their survival, as a group, to age and stage matched SEER subjects. Because the outcome of our entire sample is typical for similar women, we are able to compare the ability of BrdUrd LI, Ki-67 LI, and node status to stratify risk.

Second, we have previously demonstrated in our laboratory that counts between individual observers and repeat counts by the same individual are reproducible for both BrdUrd and Ki-67 LI's ($r > 0.90$ for all pairwise comparisons) [29]. In this study, we confirm a strong correlation between BrdUrd LI and Ki-67 LI [29]. Figure 5 demonstrates both the correlation of these indices and that breast cancer metastasis and mortality are much more common in patients with higher labeling indices and positive nodes.

Third, we systematically count the areas of highest labeling for both BrdUrd and Ki-67 indices which gives higher counts than does random sampling. The merits of counting random areas vs areas of highest labeling have been debated [17, 30] but significant correlation with outcome using our selective counting of the areas of highest labeling proves that this method does work. Counting the areas with highest labeling is consistent with Bloom and Richardson who demonstrated that risk is predicted by the highest grade of a cancer, not the average grade [1].

Fourth, BrdUrd LI correlates with 3H-thymidine LI because, as analogues of naturally occurring thymidine, both are incorporated into S-phase cells which are synthesizing DNA [14]. The BrdUrd LI is a simplification of 3H-thymidine labeling index. *In vivo* incorporation of BrdUrd is a dynamic measure of DNA synthesis and agrees with previous work by Silvestrini et al. [2, 3], Meyer et al. [4, 33], and Tubiana et al. [5] who demonstrated with *in vitro* 3H-thymidine that dynamic measures of DNA synthesis are excellent indices of prognosis.

Our literature review found two direct comparisons between BrdUrd and Ki-67 indices. Both studies used *in vitro* incubation of tumor cells with BrdUrd.

Gagli et al. compared *in vitro* BrdUrd to Ki-67 LI in 385 patients [34]. Both indices were significant in univariate analysis but only Ki-67 LI and node status were significant in multivariate analysis.

Thor et al. compared *in vitro* BrdUrd LI, Ki-67 LI, and a visually counted mitotic index [35]. All were significant in univariate analysis. In multivariate analysis, proliferation indices added little except in node negative patients and Ki-67 had a smaller p-value (was more predictive) than *in vitro* BrdUrd LI.

In these studies using *in vitro* BrdUrd incubation, the mean and median labeling indices were lower than in our results. Also, the correlation of BrdUrd LI with the Ki-67 index, though significant, was lower ($r = 0.349$ and $r = 0.29$, respectively) than in our results ($r = 0.76$). We believe that there is a difference between *in vivo* and *in vitro* exposure of tumor cells to BrdUrd.

Fifth, we have focused our analysis on LI vs node status rather than LI as a complement to node status, because changing surgical practice is reducing the number of nodes available for study by histology.

This is in contrast to current ASCO guidelines which focus on the potential of proliferation indices from flow cytometry to refine information available from node dissection and suggest use only to identify low risk, node-negative patients [13] as has recently been reported [36]. Inherent in this opinion is the assumption that node status will be determined by node dissection in all patients.

Table 2. Multivariate survival analyses

Mode	Disease free survival [112/26][1]			Overall survival [112/19]		
	Chi-sq	ΔChi-sq[2]	*p*-value	Chi-sq	ΔChi-sq	*p*-value
Positive nodes	6.90			11.26		
+ size2[3]	11.30	4.41	0.036	14.77	3.51	0.061
+ size3	11.32	4.42	0.109	14.77	3.51	0.173
+ age<50	13.06	6.17	0.013	15.11	3.85	0.050
+ BrdU	11.77	4.88	0.027	20.08	8.82	0.003
+ Ki-67	8.76	1.86	0.173	14.66	3.40	0.065
Size2	7.55			7.94		
+ age<50	13.51	5.96	0.015	11.32	3.38	0.066
+ BrdU	12.20	4.65	0.031	18.10	10.16	0.001
+ Ki-67	11.01	3.46	0.063	13.32	5.39	0.020
Age< 50	7.01			5.13		
+ BrdU	12.01	5.00	0.025	16.62	11.48	0.001
+ Ki-67	8.97	1.97	0.161	9.82	4.69	0.030
BrdU	7.59			14.72		
+ nodes	11.77	4.18	0.041	20.08	5.36	0.021
Ki-67	3.34			6.23		
+ nodes	8.76	5.42	0.020	14.66	8.43	0.004
Vasc Inv*	6.63			11.48		
+ nodes	9.76	3.13	0.077	15.79	4.31	0.038
+ size2	11.36	4.73	0.030	15.24	3.77	0.052
+ age<50	14.05	7.42	0.006	16.90	5.42	0.020
+ BrdU	10.14	3.51	0.061	17.73	6.25	0.012
+ Ki-67	8.29	1.67	0.197	13.81	2.33	0.127

[1] First number is number of tumors, second is number of events (recurrences, deaths).
[2] ΔChisq is the change in chi-square due to adding a second factor to the prediction equation.
[3] Size2 contrasts large size (≥5 cm) vs small or med size (<5 cm); Size 3 allows 3 size categories (<2, 2–5, ≥ cm).
*For vascular invasion: number of tumors 106, number of recurrences 26, number of deaths 19. For 6 tumors presence of absence of vascular invasion could not be stated definitely.

Many groups have assumed that node status will continue to be determined surgically and have concentrated on proliferation indices in either node-positive or node-negative cases [2, 3, 7, 37, 38]. There are, however, studies which have shown in multivariate analysis that proliferation indices provide prognostic information independent of node status [4, 10, 39, 40].

We believe that increased use of 'sentinel' node biopsy will make the significance of proliferation indices in multivariate analysis (when compared to positive vs negative-node status) a more important characteristic. Giuliano et al. [41] and others [42, 43] have proposed that when the sentinel node is negative, no further nodes be removed. This reduces the morbidity of axillary dissection. However, although infrequent, a positive node can be missed [41–43].

Labeling indices can complement the information obtained by sentinel node biopsy. A high index of proliferation, such as that obtainable with either an *in vivo* BrdUrd LI or a Ki-67 index, would alert the clinician to an increased risk of recurrence even if there was a false negative sentinel lymph node.

We conclude that a high proliferation index based on *in vivo* labeling of breast cancer cells with bromodeoxyuridine predicts a higher risk of systemic recurrence and death with significance similar to, and independent of, that of positive-node status. Results with Ki-67 proliferation index are similar to results with BrdUrd LI, but we cannot establish the

superiority of either of these indices at this time. As sentinel node biopsy supplants axillary dissection in treatment of early breast cancer, proliferation indices will provide important confirmation of the prognostic information derived from node status.

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