Breast Cancer Research and Treatment 12: 191–204 (1988)

Report

Proliferative index of breast carcinoma by thymidine labeling: prognostic power independent of stage, estrogen and progesterone receptors

John Strauch Meyer[1] and Michael Province[2]
[1] *Department of Pathology, Washington University School of Medicine, St. Louis, Mo., St. Luke's Hospital, Chesterfield, Mo., and The Jewish Hospital of St. Louis, USA;* [2] *Division of Biostatistics, Washington University School of Medicine, St. Louis, Mo., USA*

Key words: breast cancer, cell kinetics, estrogen receptor, progesterone receptor, prognosis, proliferative index, stage, thymidine labeling index

Summary

We studied cellular proliferation by measuring the tritiated thymidine labeling index (TLI) in slices of primary invasive breast carcinomas. Estrogen receptor (ER) and progesterone receptor (PgR) were measured by ligand-binding assay.

The TLI was a strong independent predictor of survival and relapse-free survival in women with or without axillary lymph nodal metastases and in American Joint Committee stage I. In operable node-negative women treated surgically, predicted survival at 5 years was 89 ± 4% (probability±standard error) for 81 patients with low TLI (≤3%), 64 ± 7% for 101 with mid TLI (3.1 – 8%), and 66 ± 6% for 86 with high TLI (>8%) (P = 0.001). Probabilities of survival for patients with positive axillary nodes were 79 ± 6% for 86 with low, 71 ± 7% for 71 with mid, and 52 ± 6% for 89 with high TLI (P = 0.0002). In stage I patients (tumor diameter not exceeding 2 cm), 5-year survival probabilities were 93 ± 4% in 70 with low, 72 ± 8% in 43 with mid, and 58 ± 10% in 35 with high TLI, (P = 0.0005). The TLI was predictive for survival and relapse-free survival within subgroups positive and negative for ER and positive for PgR (P<0.05) in stage I patients, and a predictive trend was observed in the PgR-negative subgroup (P = 0.16). TLI also predicted within different categories of vascular invasion and nuclear grade.

A stepwise Cox proportional hazards model selected TLI, number of positive axillary lymph nodes, and maximum diameter of the breast carcinoma as independent variables predictive of relapse, and added ER as a fourth variable for prediction of survival.

Introduction

The pathologic stage of breast carcinoma as determined by the degree of spread beyond the primary site to the axillary lymph nodes and other local or distant sites has remained the most important prognostic indicator. In the last ten years, measurements of estrogen and progesterone receptors (ER and PgR, respectively) in the neoplastic tissue [1, 2] and the thymidine labeling index (TLI) [3–6], have been recognized as having prognostic significance independent of the clinical or pathological stage of the tumor. These measurements have the advantage of yielding quantifiable results. The TLI is a measure of the rate of cellular proliferation, and relates to the growth rate of the tumor, although it

Address for offprints: J.S. Meyer, St. Luke's Hospital, 232 S. Woods Mill Road, Chesterfield, MO 63017, USA

is not the only determinant of growth. ER and PgR are measurements of hormonal responsiveness but not necessarily of growth. In this presentation we provide evidence that the TLI is a stage-independent predictor of relapse-free survival and overall survival of breast carcinoma patients, that it takes precedence over the receptor measurements in predicting the outcome over a period of five years, and that it is particularly predictive in stage I patients.

Methods

Patients studied, evaluation and staging

Primary, invasive breast carcinomas were accessioned into the study from July, 1975, through December, 1984. During this time approximately 90% of the primary breast carcinoma patients at Jewish Hospital were studied. In addition, for a year beginning in July, 1975, tissues were received from Barnes Hospital, St. Louis, Mo. During accessions at both hospitals, we attempted to study all available patients. Two percent of the patients came from other hospitals in the St. Louis area.

The number of patients in the study-group was 718, of whom 514 were initially operable, received potentially adequate local surgical therapy of the breast carcinoma together with axillary lymph nodal dissection yielding at least 5 lymph nodes for evaluation, and had no confounding second neoplasm during or prior to the period of observation. One hundred seventy-one in the latter group were stage I, and 148 of them received no adjuvant therapy (local surgical ablation only). Two hundred ninety-four were stage II, of whom 153 received no adjuvant therapy. TLI and histopathologic data were available for all patients, ER for 471, and PgR for 416. In 414 patients, both ER and PgR were available. The mean age of the 718 patients was 60 ± 0.55, median 60 yr, the mean maximum diameter of tumor 2.5 ± 0.078, median 2.6 cm, the mean number of axillary lymph nodes examined histologically 15.6 ± 0.34, median 16, the mean number of positive (metastatic) nodes 2.9 ± 0.23, median 0, and the mean TLI $7.3 \pm 0.24\%$, median 5.2%.

Patients entered the study at the time of initial definitive surgical treatment or, if inoperable, at the time of pathological diagnosis. Jewish Hospital patients were followed by a Tumor Registry. Data pertaining to other patients was available from physician's office records and hospital charts which were examined during 1986. When information was otherwise unavailable, attempts were made to contact patients by mail or by telephone. Tumor registry information pertinent to relapse or death was verified and extended by review of hospital and doctor's office records whenever such records were available. The review was conducted by one of the authors (JSM) without reference to TLI data. More than 50 physicians participated in the care of the patients. Their diagnoses of relapse of tumor or death as a result of tumor were accepted, but over 95% of relapses were clearly documented by physical examination, radiography, or tissue examination. In evaluation of survival, death from any cause counted the same as death from cancer. In evaluation of relapse-free survival, patients who died of causes other than breast cancer were censored at time of death. The mean and median durations of observation from entry to death for all the patients studied were 2.8 and 2.4 yr respectively, and for entry to last observation of survivors 4.4 and 3.6 yr. Corresponding figures for operable, evaluable patients were 3.6 and 3.1. yr for those who died and 4.7 and 4.1 yr for survivors.

Patients were staged on entry according to the American Joint Committee for Cancer Staging and End-Results Reporting, 1978 [7]. When excisional surgery was performed, measurements of tumor-size and axillary lymph nodal status were based on pathologic study. This system specifies that stage I includes patients with primary tumors no greater than 2.0 cm in diameter and either histologically tumor-free axillary lymph nodes or micrometastases. We used a diameter of 2 mm as the upper limit of micrometastasis. Seventeen percent of the stage I patients had axillary micrometastases. Patients staged as I, II, or III were considered operable and eligible for evaluation of course after potentially curative local therapy. An additional four patients, stage IV because of local extension of the primary tumor that was considered surgically excisable, were included in the operable group.

For evaluation of survival and relapse patterns, we defined patients as operable and qualified for evaluation if they were judged to be potentially curable by surgical treatment of the breast and axillary lymph nodes, and if total mastectomy and axillary dissection were performed, at least five axillary lymph nodes were examined histologically, and no confounding neoplasm was diagnosed at any time.

Adjuvant therapy

Of the patients who were treated with potentially curable surgical procedures and were evaluable, 194 received adjuvant therapy and 320 did not. The type of adjuvant therapy was cytotoxic in 60.6%, irradiation in 10.1%, hormonal in 5.6%, cytotoxic + irradiation in 18.2%, irradiation + hormonal in 3.0%, cytotoxic + irradiation + hormonal in 2.0%, and immunotherapy in 0.5%. Altogether 80.8% received cytotoxic therapy. Twenty-three Stage I patients received some form of adjuvant therapy, chemotherapy in 13 patients, irradiation in 8, hormonal therapy in 1, and immunotherapy in 1. One hundred forty-eight Stage I patients received no adjuvant therapy. One hundred forty-one Stage II patients received some form of adjuvant therapy which was cytotoxic in 62.2%, irradiation in 7.0%, hormonal in 5.6%, cytotoxic + irradiation in 19.6%, irradiation + hormonal in 2.8%, and cytotoxic + irradiation + hormonal in 2.8%. Altogether 84.6% received cytotoxic therapy.

Details of adjuvant therapy

Cytotoxic therapy was administered by many physicians and without central control. Eighty percent of patients treated received a combination of cyclophosphamide, methotrexate, and 5-fluorouracil [8], 8% received L-phenylalanine mustard [9], 6% received the latter together with 5-fluorouracil [9], 5% received doxorubicin with or without other agents, and 1% received some other regimen. We were not able to determine accurately the doses received. With very few exceptions, hormonal therapy was in the form of tamoxifen.

Thymidine labeling and steroidal receptor assays

Fresh tissue received directly from the operating rooms was sliced at less than 1 mm thickness for incubation with tritiated thymidine by a procedure previously reported [10]. To enhance labeling of cells in the phase of nuclear DNA synthesis, the incubation medium contained 1 μmole/liter 5-fluoro-2′-deoxyuridine to inhibit synthesis of thymidylic acid, and incubations were for 2 hours at 37°C under 3 to 4 atmospheres oxygen tension. The TLI was determined by a count of 2000 carcinomatous cells selected in groups of 400 from five areas of the autoradiographed microsections stained with hematoxylin and eosin, and was expressed as per cent labeled cells [11].

Estrogen and progesterone receptor assays were performed on tumor cytosols obtained by centrifugation at 100,000 × g for 1 hour by tritiated ligand binding with correction for nonspecific binding by excess nonlabeled ligand. Dextran-coated charcoal was used to separate bound from unbound ligand. The level of receptor present was taken as the specific binding resulting from a nearly saturating concentration of tritiated ligand. Tritiated estradiol-17β was the ligand for the estrogen receptor (ER) assay [12], and the progesterone receptor (PgR) assay was run with both tritiated progesterone and R-5020 with use of hydrocortisone to block binding by cortisol-binding globulin [13]. The level of progesterone receptor was taken as the mean of the results with both ligands. All points in each assay were run in duplicate as previously described [12]. Positive (proliferative-phase endometrium) and negative (renal cortex) cytosols were prepared in bulk as standards and were run with each assay. Coefficients of variation for the positive cytosol ER controls averaged approximately 12%, whereas for the positive PgR controls they were approximately 18%. The receptor laboratory consistently met the quality assurance standards required for participation in the Southeastern Cancer Study Group and the National Surgical Adjuvant Breast and Bowel Project.

Selection of cutoffs for grouping by TLI

We used data from 757 primary, infiltrating breast carcinomas, including the patients in the current study, to define the median (5.2%), low (0–3.0%), mid (3.1–8.0%), and high (8.1–36%) third TLI ranges [14]. These cutoffs were selected prior to beginning the analysis of survival rates.

Histologic evaluation

Histologic grade 1 indicated well-developed tubules or glands, grade 3 indicated little or no formation of lumens, and grade 2 was intermediate. Nuclear grade 1 indicated small nuclei, grade 3 indicated large nuclei, and grade 2 was intermediate. Criteria for histologic classification are defined in detail in a previous publication [14].

Analysis of data

Survival and recurrence time distribution estimates were made using the Kaplan-Meier product limit method [15]. Differences between the survival (and/or tumor recurrence times) in patient groups were assessed using primarily the logrank test, but confirmed with the Wilcoxon test [16]. We decided in advance to divide patients by lower, middle, and upper third TLI values when groups tested were large, and to use the median TLI for smaller groups. Comparisons between the lower third and median cutoffs showed little difference in survival analyses. Initial attempts to identify the effects of multiple factors on survival involved subdividing the patients into subgroups by one factor and testing for the significance of strata by another factor. Based on the univariate analyses, a subset of prognostic variables was selected for inclusion into a stepwise Cox proportional hazards model [17] to attempt to find the most parsimonious model for survival.

Results

Traditional prognostic variables behaved as expected in a population of women with breast carcinoma. The stage of the disease was strongly prognostic for absolute survival ($P<0.0001$) and relapse-free survival ($P<0.0001$), both for all patients considered together and for operable patients. In the operable, evaluable group, age was not prognostic for either survival ($P = 0.23$) or relapse-free survival ($P = 0.65$). The size of the primary tumor was strongly prognostic for survival ($P<0.0001$) and relapse-free survival ($P<0.0001$), as was the number of positive axillary lymph nodes ($P<0.0001$). Results for some other variables of particular interest to this study are given in Table 1.

The TLI was strongly prognostic for patients of all stages taken together for both survival and relapse-free survival ($P<0.0001$). The probabilities of survival at 5 years ± standard error of estimate were 79 ± 4% for 245 low, 61 ± 4% for 238 mid, and 51 ± 4% for 235 high TLI patients, and the corresponding relapse-free survival probabilities were 69 ± 4%, 49 ± 4%, and 41 ± 4%. The TLI predicted more strongly for patients with negative axillary lymph nodes and small tumors (diameter less than 2.6 cm) than for those with larger tumors, but in the node-positive group it predicted more strongly in patients with large tumors. Further results are presented in Table 2 and the figures.

The TLI predicted strongly for operable patients of combined stages whether or not they received adjuvant therapy (Fig. 1), for patients either with or without axillary lymph nodal metastases (Fig. 2), for American Joint Committee stage I patients who did not receive adjuvant chemotherapy (Fig. 3), and for stage II patients who did receive adjuvant therapy, but not for stage II patients who received no adjuvant therapy (Fig. 3). Too few stage I patients received adjuvant therapy for evaluation of the predictive power of the TLI in that group.

A distinctive group, the medullary carcinomas, reduced the predictivity of the TLI in stage II patients. Atypical medullary carcinoma, like medullary carcinoma, is well circumscribed, undifferentiated, and has high grade nuclei, but it differs in

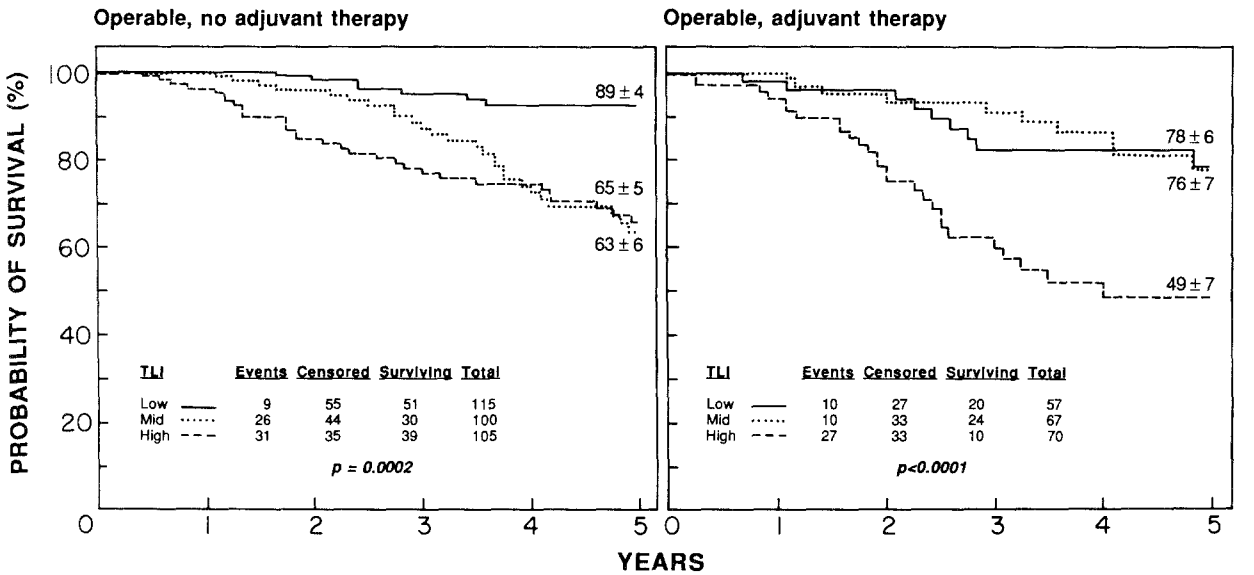

Fig. 1. Survival of operable, qualified patients with breast carcinoma of any stage who did or did not receive adjuvant therapy. Patients are stratified by TLI ranges that divided the 757 primary invasive breast carcinomas studied in this laboratory into three equal groups. Low TLI = 0 to 3%, intermediate (mid) TLI = 3.1 to 8%, high TLI = >8%. The probability of survival ± standard error at 5 years is posted at the end of the plot. Left panel: Survival of 320 patients who received no adjuvant therapy. Right panel: Survival of 194 patients who received adjuvant therapy.

Table 1. Comparison of prognostic variables other than TLI at five years.

Class of patients and variable	No. patients	Absolute survival % ± SE*	P	Relapse-free survival % ± SE*	P
Histologic grade					
1	27	86 ± 8	0.06	72 ± 11	0.06
2	170	76 ± 4		66 ± 4	
3	510	67 ± 3		56 ± 3	
Nuclear grade					
1	133	86 ± 4	0.0002	80 ± 4	0.0004
2	215	72 ± 4		55 ± 4	
3	162	60 ± 5		54 ± 4	
Venous or lymphatic invasion at tumor-margin					
Present	139	58 ± 5	< 0.0001	45 ± 5	< 0.0001
Absent	379	77 ± 3		66 ± 3	
Estrogen receptor**					
0– 9 fm/mg	177	57 ± 5	0.001	54 ± 4	0.006
10–49 fm/mg	124	85 ± 4		67 ± 5	
50+ fm/mg	170	75 ± 4		62 ± 5	
Progesterone receptor**					
0– 9 fm/mg	140	68 ± 5	0.06 (NS)	62 ± 6	0.78 (NS)
10–49 fm/mg	66	78 ± 7		63 ± 8	
50+ fm/mg	210	78 ± 7		61 ± 8	

* Percent probability of survival ± standard error.

** In fmoles/mg cytosol protein.

Table 2. Predictive value of TLI at five years.

Class of patients and variable	No. patients	Absolute survival % ± SE*	P	Relapse-free survival % ± SE*	P
Operable, treated for cure					
All stages, adjuvant treatment or not					
TLI low	172	85 ± 3	<0.0001	74 ± 4	<0.0001
TLI mid	167	69 ± 4		56 ± 5	
TLI high	175	59 ± 4		50 ± 4	
All stages, no adjuvant treatment					
TLI low	115	89 ± 4	0.0002	78 ± 5	0.001
TLI mid	100	63 ± 6		55 ± 5	
TLI high	105	65 ± 5		55 ± 5	
All stages, adjuvant treatment					
TLI low	57	76 ± 7	<0.0001	63 ± 8	0.004
TLI mid	67	78 ± 6		58 ± 7	
TLI high	70	49 ± 7		43 ± 7	
Vascular invasion at tumor-margin absent, all stages					
TLI low	138	85 ± 4	0.006	75 ± 5	0.01
TLI mid	113	73 ± 6		62 ± 6	
TLI high	125	69 ± 5		59 ± 5	
Vascular invasion at tumor-margin present, all stages					
TLI low	34	81 ± 7	<0.0001	68 ± 9	0.002
TLI mid	54	62 ± 7		44 ± 7	
TLI high	50	37 ± 7		30 ± 7	
Stage 1, no adjuvant therapy					
TLI low	70	93 ± 4	0.0005	86 ± 5	0.02
TLI mid	43	72 ± 8		61 ± 9	
TLI high	35	58 ± 10		58 ± 9	
Stage 2, no adjuvant therapy					
TLI low	40	86 ± 6	0.22 (NS)	69 ± 8	0.43 (NS)
TLI mid	51	60 ± 9		53 ± 8	
TLI high	62	74 ± 6		62 ± 7	
Stage 2, adjuvant therapy					
TLI low	43	78 ± 8	0.0025	64 ± 9	0.02
TLI mid	52	79 ± 7		62 ± 8	
TLI high	46	51 ± 9		44 ± 9	
Stage 1, ER-positive, adjuvant therapy or not					
TLI below median	77	91 ± 4	0.002	86 ± 5	0.005
TLI above median	23	66 ± 12		48 ± 13	
Stage 1, ER-negative, adjuvant therapy or not					
TLI below median	12	100	0.03	100	0.03
TLI above median	39	61 ± 9		64 ± 9	
Stage 1, PgR-positive, adjuvant therapy or not					
TLI below median	45	100	0.003	91 ± 7	0.01
TLI above median	11	47 ± 23		60 ± 16	
Stage 1, PgR-negative, adjuvant therapy or not					
TLI below median	10	100	0.16 (NS)	100	0.08 (NS)
TLI above median	26	75 ± 11		53 ± 18	

* Percent probability of survival ± standard error.

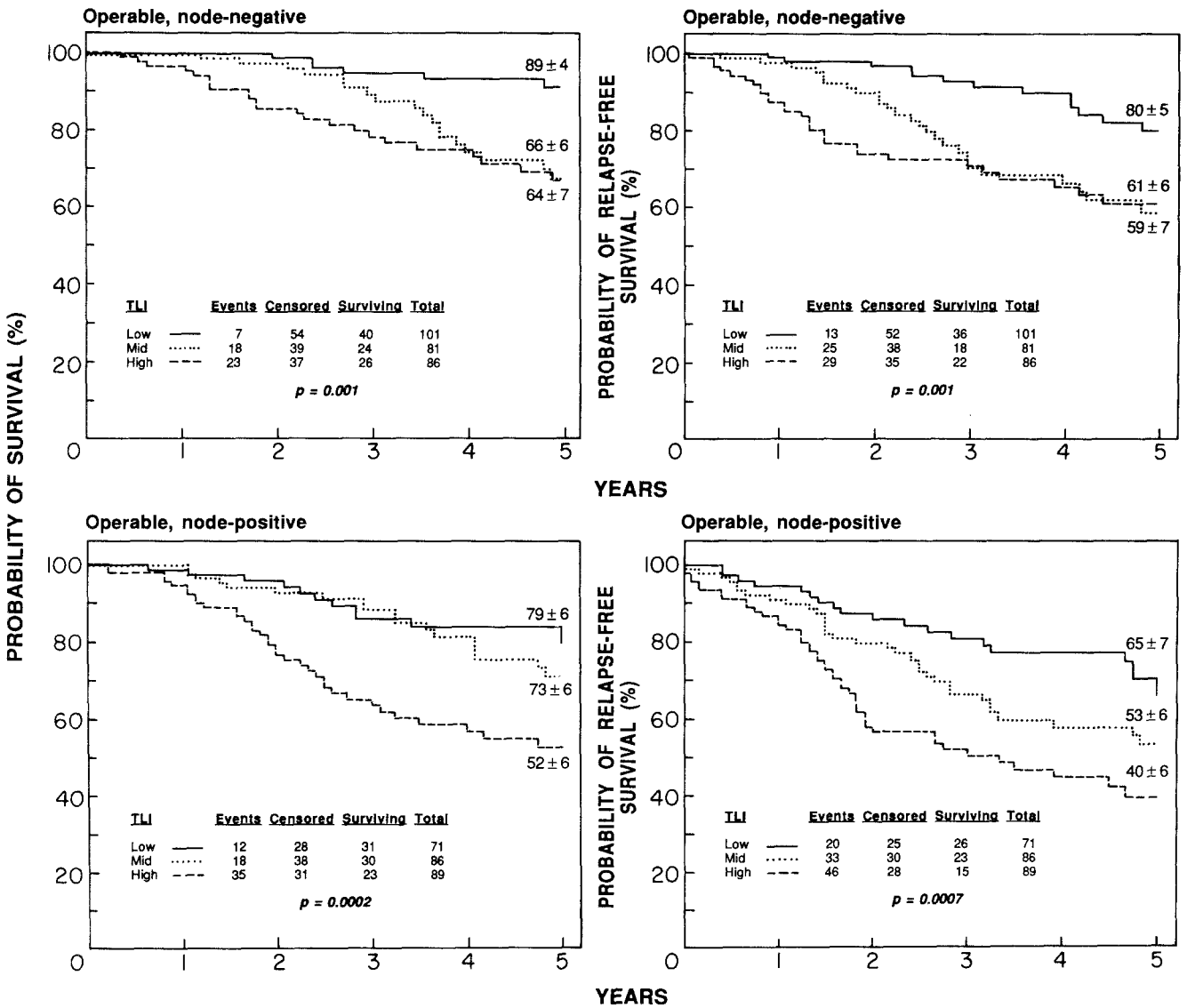

Fig. 2. Survival and relapse-free survival of operable, qualified patients stratified by TLI as in Fig. 1 according to axillary lymph nodal status. Stratification by TLI is as in Fig. 1. The probability of survival or relapse-free survival ± standard error at 5 years is posted at the end of the plot. Upper panel, left: Survival of 268 operable patients without axillary metastases. Upper right: Relapse-free survival of 268 operable patients without axillary metastases. Lower left: Survival of 246 operable patients with axillary metastases. Lower right: Relapse-free survival of 246 operable patients with axillary metastases.

lacking intense inflammatory cellular infiltrate and it may have foci of fibrosis. The stage II, no adjuvant cytotoxic group contained 18 of the 24 stage II medullary and atypical medullary carcinomas. Each of these 24 tumors had TLI above the median for all breast carcinomas. Only 5 relapses and 2 deaths occurred among the 24 operable stage II medullary and atypical medullary carcinoma patients within 5 years of treatment, despite their high TLIs. The medullary carcinomas of all stages had a probability of survival of 82 ± 18% and the atypical medullary carcinomas of 55 ± 14% at 5 years. The TLI did not predict the probability of survival or relapse-free survival in the combined group of medullary and atypical medullary carcinomas.

In survival and relapse-free survival plots of pa-

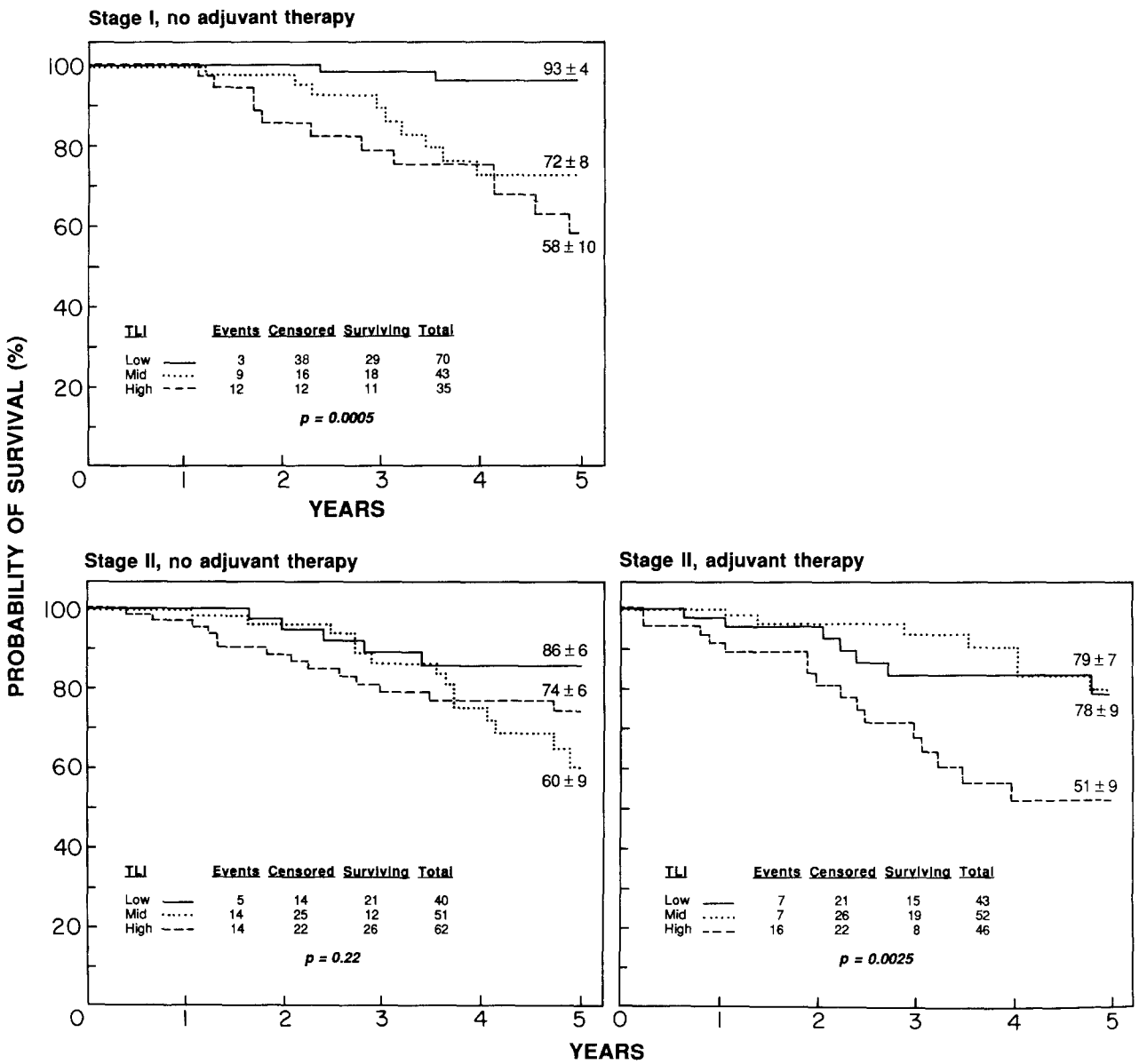

Fig. 3. Survival of Joint Committee stage I and II operable and qualified patients stratified by TLI and classified according to whether they received adjuvant therapy. Stratification by TLI is as in Fig. 1. The probability of survival ± standard error at 5 years is posted at the end of the plot. Too few stage I patients received adjuvant therapy for evaluation. Upper panel: Survival of 148 stage I patients who received no adjuvant therapy. Lower left: Survival of 153 stage II patients who received no adjuvant therapy. Lower right: Survival of 141 stage II patients who received adjuvant therapy.

tients who received adjuvant therapy, the intermediate TLI group behaved like the low TLI group, whereas in the stage I and II patients who received no adjuvant therapy the intermediate TLI stratum showed better survival and relapse-free survival for the first 3 years, but by 5 years resembled the high TLI stratum (Fig. 1). Adjuvant therapy does not seem to have affected the high TLI stratum (Figs 1, 3). But selection of patients to receive adjuvant therapy was not random. In addition to presence of most medullary carcinomas in the high TLI group, an age bias existed. Thirty-seven percent of the patients who received adjuvant therapy were more than 60 year old in comparison to 51% of those who did not receive it.

The TLI was inversely related to both ER ($r = -0.20$, $P<0.0001$ by Spearman rank correlation) and PgR ($r \pm -0.19$, $P<0.0001$) contents of the breast carcinomas, indicating that the TLI tended to be low in receptor-positive patients and high in negative patients. Nonetheless, the TLI predicted the survival and relapse-free survival in three of the stage I subgroups divided according to ER or PgR: ER-positive, PgR-positive, and ER-negative. The plots for the PgR-negative patients showed the same strong trends toward increased survival and relapse-free survival with low TLI, but the number of patients was small and the P values exceeded 0.05 (Table 2, Fig. 4). Only 1.4% of the stage 1 patients received adjuvant hormonal therapy and 8.8% received cytotoxic therapy, so adjuvant therapy did not likely affect the interaction between TLI, receptor status, and end results.

The ER itself predicted only among stage II patients. PgR did not predict significantly in either stage (Table 1). Because most reported studies used R5020 as the ligand for PgR measurement, we reanalyzed our results for that ligand alone and found no significant predictive power of PgR measured that way. In 205 operable patients in whom PgR measurements were recorded for both ligands, the Pearson correlation coefficient was 0.99, the median for progesterone was 12 fm/mg cytosol protein, and the median for R5020 was 14 fmol/mg cytosol protein.

In stage 2 patients, of whom 41% received adjuvant cytotoxic therapy and 16% received adjuvant hormonal therapy, the TLI was nonpredictive within subgroups divided according to ER or PgR results. The only difference that approached significance was in the ER-positive group in which 95 patients with below median TLIs had a probability of survival of $85 \pm 4\%$ at 5 years in comparison to $74 \pm 6\%$ for 73 patients with TLI above median ($P = 0.09$). Interactions between the receptor status, adjuvant therapy, and TLI could account for the failure of the latter to predict. We chose not to proceed with analysis of further subgroups because assignment of adjuvant therapy was nonrandom and the therapy was not standardized.

The presence or absence of blood vascular invasion or lymphatic invasion at the periphery of the primary tumor in operable patients did not relate significantly to the TLI. Since the presence of either type of vascular invasion was associated with decreased probability of survival or relapse-free survival, we examined the patients with vascular invasion to ascertain the effect of TLI in this group. The TLI was strongly predictive, so that the low TLI subgroup had a high probability of survival or relapse-free survival at 5 years despite the vascular invasion, whereas the probability of survival or relapse-free survival if the TLI was high was very poor (Table 2).

We fit a multivariate Cox proportional hazards model to assess which objectively quantifiable variables contributed independently to the prognosis. Histologic observations were not included because they are subject to observer bias with poor reproducibility from one observer to another [18], and are in some instances, particularly tumor-type and histologic and nuclear grades, highly correlated with the TLI [14]. The variables selected were age of patient, size of the primary tumor, number of axillary lymph nodal metastases, log ER, log PgR and log TLI. These variables had all been measured in 414 operable, evaluable patients. The univariate predictive power of each variable and the results of the Cox model analysis are listed in Tables 3 and 4. The number of positive axillary lymph nodes, log TLI, and tumor-size proved to be strong independent predictors both for absolute survival and for relapse-free survival. Of three quantifiable non stage related variables, log ER, log PgR, and log TLI, only log TLI showed independent prognostic significance for relapse-free survival. Log ER contributed to the prognosis of absolute survival, perhaps because of prolongation of lives of patients with ER-positive tumors by hormonal therapy after relapse. When the nuclear grade was added as a seventh variable and the Cox model analysis was repeated, log TLI remained a significant independent predictor of both survival ($P = 0.0032$) and relapse-free survival ($P = 0.014$). The model did not attribute independent prognostic signficance to the nuclear grade.

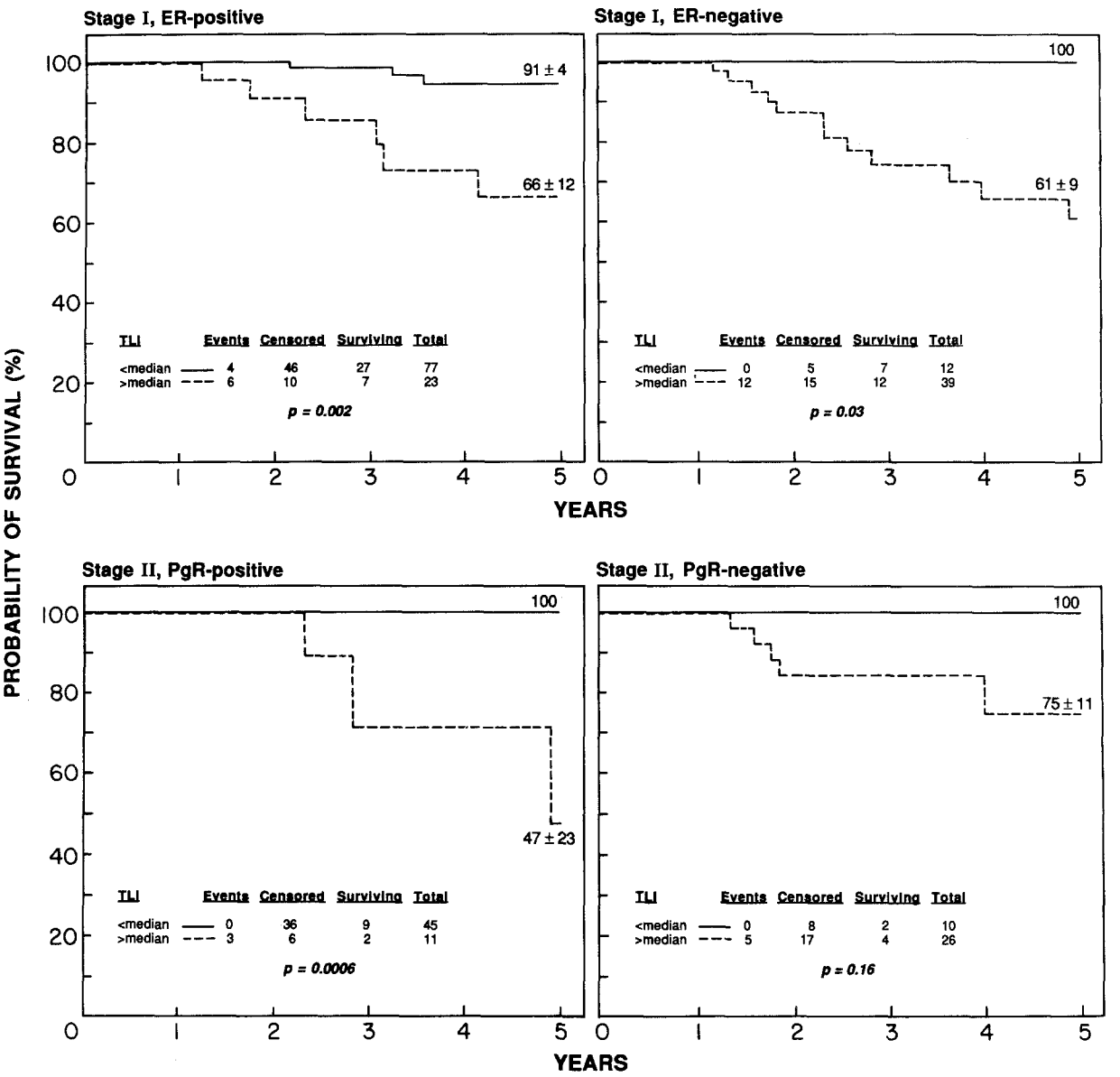

Fig. 4. Survival of Joint Committee stage I patients stratified according to median TLI (5.2%) and status for estrogen receptor (ER) or progesterone receptor (PgR). The probability of survival ± standard error is posted at the end of the plot. Upper panel, left: Survival of 100 patients with ER-positive tumors stratified according to TLI. Upper right: Survival of 51 patients with ER-negative tumors stratified according to TLI. Lower panel, left: Survival of 56 patients with PgR-positive tumors stratified according to TLI. Lower right: Survival of 36 stage I patients with PgR-negative tumors stratified according to TLI.

Discussion

The current study confirms prior reports that the TLI is a stage-independent predictor of survival and relapse-free survival in breast carcinoma patients [3–5, 7, 19]. With larger numbers of patients and longer observation it also confirms our prior report [4] and that of Silvestrini and associates [6] that the TLI predicts independently of ER status. In addition, we find that the TLI is predictive with-

in the separate set of PgR-positive tumors, probably also for PgR-negative tumors, and in patients with or without vascular invasion by the primary tumor.

The TLI is a measure of DNA synthesis within the carcinoma, and therefore measures the potential growth rate of the tumor. The actual growth rate is not measured by the TLI because growth rate depends also on the rate of cell-loss, which can exceed 90% [20, 21, 22]. The TLI is not significantly correlated with the lymph nodal status. Means, medians, and ranges of TLI in patients with or without axillary lymph nodal metastases are similar, and it is only weakly correlated with the size of the carcinoma [14]. Therefore, TLI is a stage-independent prognostic variable, and it is useful for identifying groups of patients without lymph nodal metastases who have high or low probabilities of relapse after primary treatment.

Patients with negative lymph nodes have had relapse rates of approximately 18% at 5 years and 25% at 10 years [23, 24]. The rate we observed was 33% (95% confidence limits 26% to 38%). We have no explanation for this higher than expected result. The size distribution of the primary tumors was not unusual; nearly one half were 2 cm in diameter or less. The relatively high overall relapse rate in the node-negative group could have exaggerated the relapse rates for the mid and high TLI groups, but could not explain the distinct difference between them and the low TLI group. The survival rates at 5 years differed but little for groups of patients with zero, 1–3, or 4–9 positive lymph nodes, although the relapse-free survival rates showed the expected relationships (data not shown). The surprisingly high probability of survival at 5 years in the group with four to nine positive nodes (75 ± 7%) could have resulted from relatively intensive adjuvant and post-relapse therapy, factors that we could not evaluate. Similarity between patients with one to three *vs* no positive nodes could relate to efficacy of adjuvant therapy used in more than half of the node-positive patients and in only a few of the node-negative patients.

The toxicity to efficacy ratio of current adjuvant cytotoxic regimens does not justify treating breast

Table 3. Cox proportional hazards model of absolute survival (N = 414).

Risk factor	Univariate P	Multivariate P	(beta ± SE)
Number of positive nodes	0.0001	0.024	(0.06 ± 0.02)
Tumor size	0.0001	0.0008	(0.35 ± 0.10)
Log TLI	0.0001	0.0047	(1.37 ± 0.48)
Log ER	0.0005	0.044	(− 0.44 ± 0.22
Log PgR	0.32	N.S.	
Age	0.16	N.S.	

Table 4. Cox proportional hazards model of relapse-free survival (N = 414).

Risk factor	Univariate P	Multivariate P	(Beta ± SE)
Number of positive nodes	0.0001	0.0024	(0.08 ± 0.02)
Tumor size	0.0001	0.0004	(0.29 ± 0.08)
Log TLI	0.0001	0.0052	(0.95 ± 0.34)
Log ER	0.002	N.S.	
Log PgR	0.92	N.S.	
Age	0.93	N.S.	

carcinoma patients indiscriminately. The TLI allows selection of a subset of node-negative or stage I patients with a probability of relapse of approximately 40% at 5 years, according to our findings and those of Silvestrini and associates [5]. Silvestrini's group used the median TLI as a cutoff to define groups with good and bad prognosis. We found that when three divisions were defined by the TLI the mid group resembled the low group for a short time, but by five years it was like the high group. Within this time-span, a TLI in excess of 3% implied a high risk of relapse, leaving one-third of the patients in a low risk group. These low risk patients could be excluded from randomized studies of adjuvant cytotoxic therapy, thereby sparing them risks and side effects when little benefit could be expected. The residual group, representing those patients with high probability of relapse within 5 years, would provide enhanced sensitivity for the evaluation of therapeutic effects in the short term.

Our data indicate that the TLI is a better means of identifying risk in low stage patients than the results of ER or PgR assays. The data further suggest that receptor assays are prognostic in patients not treated with hormonal agents largely or entirely because of the inverse correlation between specific ligand-binding and proliferative rate. The ER results predicted survival (but not relapse-free survival) independent of the TLI, a result that could be explained by prolongation of life after relapse by hormonal therapy when the tumor was ER-positive. Many of our patients received some form of hormonal therapy after relapse, but we do not have data necessary to analyze the efficacy of the therapy.

Most published reports indicate that ER and PgR predict the course of breast carcinoma, but some studies have failed to confirm predictivity [25], particularly in node-negative patients [26–28], and early differences may disappear prior to five years of observation [29, 30]. Our results differ from those of Clark, McGuire and associates, who found that ER predicted well for stage I breast carcinoma, whereas PgR predicted better for stage II breast carcinoma [31], but agree more closely with the results of Vollenweider-Zerargui *et al* who found that ER was a better predictor than PgR and that neither receptor predicted for patients with fewer than 4 axillary lymph nodal metastases [28]. In the patients currently studied, both ER and PgR levels correlated inversely with the TLI; in fact the relationship between PgR and TLI was more consistent than between ER and TLI [13].

The nuclear grade was a significant univariate predictor for both survival and relapse-free survival. It is closely related to the TLI, which increases with increasing nuclear size [11]. Since the nuclear grade did not contribute as an independent prognostic variable in Cox model analysis, its predictive power can be attributed to the correlation of nuclear grade with TLI.

The TLI of different tumors of various organs parallels the aggressiveness of those tumors. It is very low in carcinoid tumors, low in papillary and follicular carcinomas of the thyroid gland and well differentiated and nodular malignant lymphomas, high in most carcinomas of the lung and gastrointestinal tract and diffuse large-cell lymphomas, and very high in germ cell tumors of the testis [32]. Breast carcinoma is a heterogeneous entity in which the TLI defines prognostic groups.

Since the TLI is a measure of the cellular proliferative rate, it presumably reflects the activity of growth factors, some of which are oncogene products [33, 34]. Slamon and associates have recently related relapse and survival of breast carcinoma patients to amplification of the HER-2/*neu* oncogene in DNA extracted from primary breast carcinomas [35]. They showed that HER-2/*neu* amplification was predictive for both survival and relapse-free survival in both univariate and multivariate analyses. If the TLI measures the effects of growth-regulating products of several different oncogenes, it may be a good screening test for the presence of these stimulators. The TLI currently can be measured with less expense than the cost of screening for amplification of a series of oncogenes or for specific products of oncogenes. The TLI has shown little tendency to change in breast carcinomas over the passage of time, and consistent results were obtained when multiple samples of a single tumor were taken [36]. It is a marker for aggressiveness in breast carcinoma with sound biological rationale that can be used to identify high-risk and low-risk patients for adjuvant therapy protocols.

We were disappointed to observe no evident effect of adjuvant therapy, which usually included cytotoxic drugs, on the high TLI tumors. The data did suggest an effect on the tumors with intermediate TLI. Because adjuvant therapy was neither standardized nor randomly assigned, these results are not definitive. Comparison of response rates within kinetic classes to standardized cytotoxic regimens in randomized studies could demonstrate whether the replicative rate is a determinant of response, and whether knowledge of the cell kinetics of breast carcinoma can be used effectively in design of protocols for adjuvant therapy.

Acknowledgements

Walter C. Bauer, M.D., Division of Surgical Pathology, made tissue available from Barnes Hospital for kinetic and receptor assays and histologic study. He and Marianne U. Prey, M.D., assisted in histologic classification of tumors. Robert W. McDivitt, M.D., Department of Pathology, Jewish Hospital of St. Louis, provided consultation. Excellent technical assistance was furnished by Rhoda Facher, Laura Googin, Jean Dale, Nancy McPherson, Jeanne Ruperto and Harold Briggs. Receptor assays were performed in the laboratory of Sue C. Stevens, Ph.D., and Roland Valdes, Ph.D., Division of Clinical Chemistry, Jewish Hospital of St. Louis. Phyllis Todd, R.N., director of the Tumor Registry of Jewish Hospital, and Judy Herron, Medical Records Services, Barnes Hospital, provided information on treatment and course of patients. Information was also provided by Leo A. Sachar, M.D., Richard G. Sisson, M.D., Alan P. Lyss, M.D., Gary A. Ratkin, M.D., Albert L. VanAmburg, M.D., Joel L. Zeffren, M.D., George L. Tucker, M.D., Kenneth J. Bennett, M.D., Harvey R. Butcher, M.D., Richard V. Bradley, M.D., Richard H. Fallon, M.D., Alvin Goldfarb, M.D., Ralph J. Graff, M.D., Heinz Haffner, M.D., Fleming B. Harper, M.D., Harvey Liebhaber, M.D., Marvin E. Levin, M.D., Jerome F. Levy, M.D., Morton A. Levy, M.D., Virgil Loeb, Jr., M.D., Stanley L. London, M.D., Herbert Lubowitz, M.D., Robert H. Lund, M.D., Sherwin Malt, M.D., Julian C. Mosley, M.D., Lawrence W. O'Neal, M.D., Michael Orgel, M.D., M. Norman Orgel, M.D., Gordon W. Philpott, M.D., Jacob G. Probestein, M.D., Frank O. Richards, M.D., Shale M. Rifkin, M.D., Donald C. Sauer, M.D., William Shieber, M.D., Willard B. Walker, M.D., and others.

Supported by research funds of The Jewish Hospital of St. Louis, American Cancer Society grant 42681M, and Public Health Service grant RO1 CA42154

References

1. Knight WA, Livingston RB, Gregory EJ, McGuire WL: Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. Cancer Res 37: 4669–4671, 1977
2. Clark GM, McGuire WL, Hubay CA, Pearson OH, Marshall JS: Progesterone receptors as a prognostic factor in Stage II breast cancer. N Engl J Med 309: 1343–1347, 1983
3. Tubiana M, Pejovic MH, Chavaudra N, Contesso G, Malaise EP: The long-term prognostic significance of the thymidine labelling index in breast cancer. Int J Cancer 33: 441–445, 1984
4. Meyer JS, Friedman E, McCrate MM, Bauer WC: Prediction of early course of breast carcinoma by thymidine labeling. Cancer 51: 1879–1886, 1983
5. Silvestrini R, Daidone MG, Gasparini G: Cell kinetics as a prognostic marker in node-negative breast cancer. Cancer 56: 1982–1987, 1985
6. Silvestrini R, Daidone MG, Di Fronzo G, Morabito A, Valagussa P, Bonadonna G: Prognostic implication of labeling index versus estrogen receptors and tumor size in node-negative breast cancer. Breast Cancer Res Treat 7: 161–169, 1986
7. American Joint Committee for Cancer Staging and End-Results Reporting. Manual for Staging of Cancer 1978. Whiting Press, Chicago, 1978, pp 101–107
8. Bonadonna G, Brusamolino E, Valagussa P, Rossi A, Brugnatelli L, Brambilla C, De Lena M, Tancini G, Bajetta E, Musumeci R, Veronesi U: Combination chemotherapy as an adjuvant treatment in operable breast cancer. N Engl J Med 294: 405–410, 1976
9. Fisher B, Redmond C, Wolmark N, Wieand HS: Disease-free survival at intervals during and following completion of adjuvant chemotherapy: the NSABP experience. Cancer 48: 1273–1280, 1981
10. Meyer JS, Connor RE: *In vitro* labeling of solid tissues with tritiated thymidine for autoradiographic detection of S-phase nuclei. Stain Tech 52: 185–195, 1977
11. Meyer JS, Bauer WC, Rao BR: Subpopulations of breast

carcinoma defined by S-phase fraction, morphology, and estrogen receptor content. Lab Invest 39: 225–235, 1978
12. Meyer JS, Stevens SC, White WL, Hixon B: Estrogen receptor assay of carcinomas of the breast by a simplified dextran-charcoal method. Am J Clin Pathol 70: 655–664, 1978
13. Pichon MF, Milgrom E: Characterization and assay of progesterone receptor in human mammary carcinoma. Cancer Res 37: 464–471, 1977
14. Meyer JS, Prey MU, Babcock DS, McDivitt RW: Breast carcinoma cell kinetics, morphology, stage, and host characteristics. Lab Invest 54: 41–51, 1986
15. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53: 457–481, 1958
16. Kalbfleish JD, Prentice RL: The Statistical Analysis of Failure Time Data. John Wiley & Sons, New York, 1980
17. Cox DR, Oates D: Analysis of Survival Data. Chapman and Hall, London, 1984
18. Gilchrist KW, Kalish L, Gould VE, Hirschl S, Imbriglia JE, Levy WM, Patchefsky AS, Penner DW, Pickren J, Roth JA, Schinella RA, Schwartz IS, Wheeler JE: Interobserver reproducibility of histopathological features in stage II breast cancer. An ECOG Study. Breast Cancer Res Treat 5: 3–10, 1985
19. Meyer JS, Lee JY: Relationships of S-phase fraction of breast carcinoma in relapse to duration of remission, estrogen receptor content, therapeutic responsiveness, and duration of survival. Cancer Res 40: 1890–1896, 1980
20. Steel GG: Cell loss as a factor in the growth of human tumours. Eur J Cancer 3: 381–387, 1967
21. Steel GG: Cytokinetics of Neoplasia. In: Holland JF, Frei E III, (eds) Cancer Medicine. Lea & Febiger, Philadelphia, 1982, pp 177–189
22. Meyer JS: Growth and cell kinetic measurements in human tumors. Path Annu 16(2): 53–81, 1981
23. Fisher B, Slack N, Katrych D, Wolmark N: Ten year follow-up results of patients with carcinoma of the breast in a cooperative clinical trial evaluating surgical adjuvant chemotherapy. Surg Gynec Obstet 140: 528–534, 1975
24. Nemoto T, Vana J, Bedwani RN, Baker HW, McGregor FH, Murphy GP: Management and survival of female breast cancer: results of a national survey by the American College of Surgeons. Cancer 45: 2917–2924, 1980
25. McGuire WL, Clark GM, Dressler LG, Owens MA: Role of steroid hormone receptors as prognostic factors in primary breast cancer. NCI Monogr 1: 19–23, 1986
26. Sears HF, Janus C, Levy W, Hopson R, Creech R, Grotzinger P: Breast cancer without axillary metastases. Are there high-risk biologic subpopulations? Cancer 50: 1820–1827, 1982
27. Stewart JF, Rubens RD, Millis RR, King RJB, Hayward JL: Steroid receptors and prognosis in operable (stage I and II) breast cancer. Eur J Cancer Clin Oncol 19: 1381–1387, 1983
28. Vollenweider-Zerargui L, Barrelet L, Wong Y, Lemarchand-Beraud T, Gomez F: The predictive value of estrogen and progesterone receptors' concentrations on the clinical behavior of breast cancer in women. Clinical correlation on 547 patients. Cancer 57: 1171–1180, 1986
29. Aamdal S, Bormer O, Jorgensen O, Host H, Eliassen G, Kaalhus O, Ing DR, Pihl A: Estrogen receptors and long-term prognosis in breast cancer. Cancer 53: 2525–2529, 1984
30. Howat JMT, Barnes DM, Harris M, Swindell R: The association of cytosol oestrogen and progesterone receptors with histological features of breast cancer and early recurrence of disease. Brit J Cancer 47: 629–640, 1983
31. Clark GM, Osborne CK, McGuire WL: Estrogen receptor status and tumor size identify a subgroup from 1461 node-negative breast cancer patients at high risk for recurrence and death. J Clin Oncol, 1987, in press
32. Meyer JS: Potential value of cell kinetics in management of cancers of unknown origin. In: Fer MF, Greco FA, Oldham RK, (eds) Poorly Differentiated Neoplasms and Tumors of Unknown Origin. Grune and Stratton, Orlando, 1986, pp 519–539
33. Land H, Parada LF, Weinberg RA: Cellular oncogenes and multistep carcinogenesis. Science 222: 771–778, 1983
34. Slamon DJ, diKernion JB, Verma IM, Cline MJ: Expression of cellular oncogenes in human malignancies. Science 224: 256–262, 1984
35. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. Science 235: 177–182, 1987
36. Meyer JS, McDivitt RW: Reliability and stability of the thymidine labeling index of breast carcinoma. Lab Invest 54: 160–164, 1986