Importance of Nuclear Morphology in Breast Cancer Prognosis

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ABSTRACT

The purpose of this study is to define prognostic relationships between computer-derived nuclear morphological features, lymph node status, and tumor size in breast cancer. Computer-derived nuclear size, shape, and texture features were determined in fine-needle aspirates obtained at the time of diagnosis from 253 consecutive patients with invasive breast cancer. Tumor size and lymph node status were determined at the time of surgery. Median follow-up time was 61.5 months for patients without distant recurrence. In univariate analysis, tumor size, nuclear features, and the number of metastatic nodes were of decreasing significance for distant disease-free survival. Nuclear features, tumor size, and the number of metastatic nodes were of decreasing significance for overall survival. In multivariate analysis, the morphological size feature, largest perimeter, was more predictive of disease-free and overall survival than were either tumor size or the number of axillary lymph node metastases. This morphological feature, when combined with tumor size, identified more patients at both the good and poor ends of the prognostic spectrum than did the combination of tumor size and axillary lymph node status. Our data indicate that computer analysis of nuclear features has the potential to replace axillary lymph node status for staging of breast cancer. If confirmed by others, axillary dissection for breast cancer staging, estimating prognosis, and selecting patients for adjunctive therapy could be eliminated.

INTRODUCTION

Our original goal was to develop a method to assist diagnosis of breast fine-needle aspirates (1, 2) based on computer-generated morphological features. Subsequently, we found that these features were prognostically stronger than lymph node status for disease-free survival (3). The previous end point of disease-free survival may have underestimated the significance of lymph node involvement because only patients with positive axillary lymph nodes were given adjunctive chemotherapy. Therefore, disease-free survival in node-positive patients could approach that of node-negative patients. However, overall survival, which is used as an end point in this study, was not affected by the adjunctive therapies given to our patients (4–6).

PATIENTS AND METHODS

Patients

In 1984, we started patient accrual and FNA[2] sample acquisition. In 1992, we developed our computer-based nuclear analytic system and analyzed the 184 previously acquired FNAs. After 1992, we analyzed samples at the time of collection. The samples were obtained from 253 consecutive patients who had invasive breast cancer with no evidence of distant metastases at the time of diagnosis, and for whom follow-up data are available. A total of 240 patients had preoperative FNAs of palpable masses for diagnosis, and 13 had FNAs on the surgically excised specimens. All histological tumor types were represented because the study included all patients with palpable masses. Pathologists determined tumor size on surgical specimens. A total of 238 patients had lymph node dissection and pathological staging. The median number of nodes examined was 15 (range, 1–38 nodes). The mean number ($\pm$ SD) of axillary nodes obtained was 8.23 ± 4.23 in the 13 patients who had axillary sampling and 16.92 ± 6.16 in the 225 patients who had level I and II axillary dissection. Fifteen patients did not have axillary surgery and were clinically staged as node negative. Of these, five patients did not have axillary surgery because of tubular carcinoma, one patient did not have axillary surgery because of an 8-mm low-grade infiltrating ductal carcinoma, and nine patients did not have axillary surgery because of complicating medical conditions. None of the clinically staged patients received adjunctive drug therapy, none developed axillary recurrences, and one developed distant recurrence after 53 months. Positive nodes were obtained in two of the six patients in whom axillary dissection or sampling yielded fewer than five nodes, and no axillary recurrences developed in these patients or in the other four patients.

All five patients who were in the good prognostic group and who recurred had a full axillary dissection with 10–18 lymph nodes examined; three patients were node negative, one patient had 1 positive lymph node, and the other patient had 4 positive nodes. All had infiltrating ductal carcinomas, four had breast conservation (tumor excision with histologically negative margins and breast irradiation), and one opted for mastectomy. The two patients who had positive nodes received adjunctive chemotherapy.

We compared our patients' outcomes with those of 24,000 similar patients from the SEER program of the National Cancer

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[2] The abbreviations used are: FNA, fine-needle aspiration; SEER, Surveillance, Epidemiology, and End Results.

Institute (3) to determine whether our patients represented the population of the United States. Both groups had invasive cancer and did not have distant metastases at the time of diagnosis. For comparison, we stratified axillary lymph node involvement at 0, 1–3, and ≥4 positive nodes (7, 8).

FNA Preparation

A physician aspirated palpable breast masses with a 23-gauge needle by multiple passes while maintaining suction and longitudinally rotating a 30-ml syringe. The aspirated material was expressed onto two glass slides. The slides were coapted face-to-face, and the aspirate was spread by separating the slides with a horizontal motion. Preparations were immediately fixed in 95% ethanol and stained with H&E.

Computer Analysis

Selection of Nuclei for Analysis. An operator used a microscope with a ×2.5 ocular and a ×63 objective to visually select a field for analysis that was deemed to be most atypical. He avoided areas where the preparation distorted nuclei or where nuclei overlapped considerably. A 640 × 400 pixel digital image of this field was produced by a video camera on the microscope and a framegrabber card in a PC. Data storage accommodated only a single image, so analysis was performed on 10–20 nuclei/patient.

Digital Assessment Process. The operator used a mouse button to outline each cell nucleus on the computer monitor. Beginning with this user-defined approximate border, a deformable spline technique (9, 10) precisely located the actual nuclear border.

Nuclear Features. The computer calculated the following 10 nuclear features for each nucleus (11) using the values contained within the border defined by the deformable spline technique.

(*a*) Radius was computed by averaging the length of radial line segments from the center of the nuclear mass to each of the points of the nuclear border.

(*b*) Perimeter was measured as the distance around the nuclear border.

(*c*) Area was measured by counting the number of pixels in the interior of the nuclear border and adding one-half of the pixels on the perimeter.

(*d*) Perimeter and area were combined to give a measure of the compactness of the cell nuclei using the following formula: $\text{perimeter}^2/\text{area}$.

(*e*) Smoothness was quantified by measuring the difference between the length of each radius and the mean length of adjacent radii.

(*f*) Concavity was determined by measuring the size of any indentations in the nuclear border.

(*g*) Concave points counted the number of points on the nuclear border that lie on an indentation.

(*h*) Symmetry was measured by finding the relative difference in length between line segments perpendicular to and on either side of the major axis.

(*i*) Fractal dimension was approximated using the "coastline approximation" described by Mandelbrot (12) that measured nuclear border irregularity.

Table 1 Comparison of breast cancer-specific survival (%) in the SEER database and our series stratified by the extent of lymph node metastases[a]

	5-year survival		10-year survival	
Lymph node metastasis	SEER	Our study	SEER	Our study
Node negative	90.6	89.4	82.2	82.1
1–3 positive nodes	80.9	82.0	67.5	75.7
4 or more positive nodes	59.8	63.0	42.6	49.0

[a] For the same lymph node grouping and at the same time, none of the differences are statistically significant.

(*j*) Texture was measured by finding the variance of the gray scale intensities in the component pixels.

The computer calculated the mean value, the "largest" value, and the SE for each nuclear feature, resulting in a total of 30 features. The largest value for each feature was the mean of the three largest values for all nuclei in the analyzed image. Three was chosen as the smallest number that would guard against numerical instability in the shape features.

We assessed the reproducibility of nuclear border determination by independent analysis of five images by one of the authors (W. H. W.) and an accomplished cytopathologist and by analysis of 39 images by W. H. W. and a cytopathology fellow.

Statistical Analysis

We assessed two end points, time to distant recurrence and overall breast cancer-specific survival, using SPSS software (13, 14).

RESULTS

Nuclear feature assay results were consistent between observers. The interobserver Pearson correlation coefficients with three observers were between 0.90 and 0.99 for the nuclear size features (radius, perimeter, area, and compactness) and were about 0.6 for the shape features (smoothness, concavity, concave points, symmetry and fractal dimension). Specifically for largest perimeter, the Pearson correlation coefficient was 0.959 between W. H. W. and the accomplished cytopathologist and 0.946 between W. H. W. and the cytopathology fellow.

The median follow-up was 61.5 months for the 184 cases without recurrence, and the median recurrence time was 19 months for the 69 cases with distant recurrence. Breast cancer-specific survival, stratified by the extent of lymph node metastasis, was similar for our series and that of the SEER database (Table 1).

The computer-derived nuclear feature largest perimeter was the strongest prognostic indicator for breast cancer-specific survival and was second to tumor size for distant disease-free survival by Cox univariate analysis (Table 2). Therefore, largest perimeter and tumor size, together with the number of metastatic lymph nodes, were selected for a three-factor Cox multivariate analysis. In this model, largest perimeter was the strongest prognostic factor for both distant disease-free survival and breast cancer-specific survival (Table 3).

Life table analysis was done for each pair of the three prognostic features: (*a*) tumor size; (*b*) largest perimeter; and (*c*)

Table 2 Cox univariate analysis of prognostically significant factors

Factor	Distant disease-free survival		Breast cancer-specific survival	
	Wald statistic	*P*	Wald statistic	*P*
Tumor size	18.24	<0.0001	16.82	<0.0001
Largest perimeter	13.68	0.0002	24.96	<0.0001
No. of metastatic nodes	11.91	0.0006	14.77	<0.0001
Largest radius	12.53	0.0004	22.42	<0.0001
Mean area	12.22	0.0005	22.00	<0.0001
Mean perimeter	12.16	0.0005	21.73	<0.0001
Mean radius	11.98	0.0005	20.61	<0.0001
Largest area	11.50	0.0007	21.90	<0.0001
SE of area	3.85	0.0497	10.19	0.0014
SE of perimeter	3.81	0.0508	10.91	0.0010
SE of radius	3.24	0.0720	9.35	0.0022

Table 3 *P*s obtained from Cox multivariate analysis of the three-factor model using the number of metastatic nodes, tumor size, and largest perimeter

Factor	Distant disease-free survival		Breast cancer-specific survival	
	Wald statistic	*P*	Wald statistic	*P*
No. of metastatic nodes	3.7829	0.0518	6.5123	0.0107
Tumor size	6.5108	0.0107	4.4071	0.358
Largest perimeter	11.8163	0.0006	24.9303	<0.0001

lymph node positivity. Patients were assigned to groups based on the median split for tumor size (2.4 cm), largest perimeter (38.6 μm), and lymph node positivity. This created four groups for tumor size and largest perimeter: (*a*) small size, small largest perimeter (SS/SP); (*b*) small size, large largest perimeter (SS/LP); (*c*) large size, small largest perimeter (LS/SP); and (*d*) large size, large largest perimeter (LS/LP). This is illustrated in Fig. 1, where individual values for patients recurring or nonrecurring relative to the median value cut points for tumor size and largest perimeter are shown. Similarly, patients above and below the median split values for tumor size and largest perimeter were paired according to node-positive (Node +) or node-negative (Node −) status to give four groups each. Life table analyses of disease-free survival and breast cancer-specific overall survival showed no differences at either 5 or 10 years between SS/LP and LS/SP, between Node +/SS and Node −/LS, or between Node +/SP and Node −/LP. Therefore, these were consolidated into "intermediate" groups. This left a "good" group consisting of SS/SP, Node −/SP, or Node −/SS, and a "poor" group consisting of LS/LP, Node +/LP, or Node +/LS.

Life table analyses showed similar disease-free survival and breast cancer-specific overall survival at both 5 and 10 years when patients were stratified according to all criteria pairs, tumor size/node status, node status/largest perimeter, and tumor size/largest perimeter (Tables 4 and 5). The Wilcoxon (Gehan) test statistics for the difference in separation between the good, intermediate, and poor groups shows that the best results were obtained from the tumor size/largest perimeter combination (Table 6). Kaplan-Meier plots are shown in Figs. 2 and 3.

The independence of the prognostic factors was demonstrated by the overlap of patients in the various groups when stratified by the three criteria. Fifty patients were classified as good, and 50 patients were classified as poor by all three stratification criteria. However, 75 patients were classified as good by one stratification criterion and classified as intermediate by two stratification criteria. An additional 78 patients were classified as poor by one stratification criterion and classified as intermediate by two stratification criteria. Further demonstration of the prognostic independence between largest perimeter and nodal status was shown by the Wilcoxon test, in which no significant difference was demonstrated between the largest perimeter values for node-negative and node-positive patients. The mean largest perimeter was 39.1 ± 8.6 for node-negative patients and 41.3 ± 9.4 for node-positive patients. Additionally, there was a poor correlation between tumor size and largest perimeter (Pearson correlation coefficient = 0.1426; $P = 0.023$). However, tumor size and the number of lymph nodes containing metastatic tumor were correlated (Pearson correlation coefficient = 0.4537; $P < 0.001$).

DISCUSSION

The major objectives in staging breast cancer are to estimate prognosis and determine the need for adjunctive therapy. The size of the primary tumor, metastases to the axillary lymph nodes, and the presence or absence of known distant metastases are the basis for the classical tumor-node-metastasis (TNM) system for breast cancer staging. Axillary lymph nodes are removed for staging because of their prognostic importance (15, 16). Sampling procedures (17–20) that require less than a complete axillary dissection are of current interest. Although this is a single-institution study, we believe our findings are generally applicable because our patients' breast cancer-specific survival was comparable to that of the large, multi-institutional SEER study. This indicates that our patient population behaved in a manner similar to the global breast cancer population and is probably a representative subset.

Our data include invasive cancers of various histological types because we were interested in determining how cytological features predicted breast cancer outcome rather than exploring differences between various histological types. Moreover, histological grade and type are somewhat intermingled (21), and different typing criteria can create significant differences between histological groupings (22).

Histological grading of tumor differentiation is prognostically important. The grading system of Bloom and Richardson (23) combines cytological and histological criteria. This system has been modified by Elston and Ellis and incorporated, together with tumor size and axillary lymph node status, in the Nottingham index (24). Prospectively, the Nottingham index has been shown to reflect prognosis (25–27). The Nottingham histological tumor grading method evaluates nuclear size/pleomorphism, tubule formation, and mitotic count. On multivariate analysis, tumor grade emerged as the most powerful and the only significant prognostic factor (28). Tumor grading systems depend on subjective assessment of various features, with nuclear pleomorphism being the least reproducible feature (29). Computer technology objectively assesses these nuclear size/pleomorphism

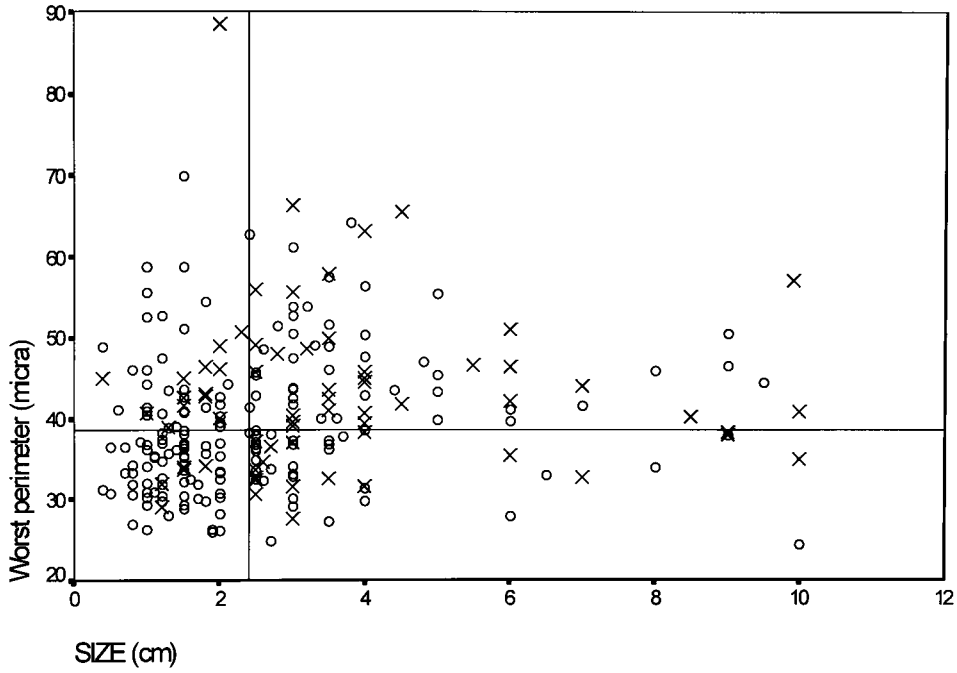

Fig. 1 Individual values for patients recurring (×) or not recurring (○) relative to the median value cut points for tumor size and largest perimeter.

Table 4 Distant disease-free survival ± SE (%)

	Groups					
	5-year survival			10-year survival		
Stratification[a]	Good	Interm[b]	Poor	Good	Interm	Poor
Node/size	85.1 ± 4.6	77.3 ± 4.8	55.1 ± 5.8	77.4 ± 6.7	71.5 ± 6.0	42.9 ± 6.6
Node/LP	87.4 ± 4.5	74.2 ± 4.6	55.0 ± 6.2	79.8 ± 6.6	64.7 ± 6.0	45.0 ± 7.3
Size/LP	94.8 ± 2.9	68.2 ± 5.0	55.9 ± 6.2	87.6 ± 5.6	58.1 ± 6.3	46.3 ± 7.2

[a] Node/size, axillary lymph node positivity and tumor size; Node/LP, axillary lymph node positivity and the nuclear feature largest perimeter; Size/LP, tumor size and the nuclear feature largest perimeter.
[b] Interm, intermediate.

Table 5 Breast cancer-specific survival ± SE (%)

	Groups					
	5-year survival			10-year survival		
Stratification[a]	Good	Interm[b]	Poor	Good	Interm	Poor
Node/size	89.9 ± 3.9	90.3 ± 3.5	62.5 ± 5.7	85.8 ± 5.5	78.3 ± 6.4	54.7 ± 6.5
Node/LP	98.2 ± 1.8	81.6 ± 4.1	63.5 ± 6.1	90.1 ± 5.7	76.6 ± 5.2	50.1 ± 7.6
Size/LP	96.5 ± 2.4	88.4 ± 4.0	60.6 ± 6.1	92.8 ± 4.3	73.4 ± 6.2	51.3 ± 7.2

[a] Node/size, axillary lymph node positivity and tumor size; Node/LP, axillary lymph node positivity and the nuclear feature largest perimeter; Size/LP, tumor size and the nuclear feature largest perimeter.
[b] Interm, intermediate.

features and should be more reproducible than visual grading. Although we analyzed the nuclear morphology of cytological preparations, others have shown that both visual and image-analyzed cytological characteristics are related to histological grade (30–32). Therefore, we believe that similar results will be obtained when our methods are applied to histological analyses.

Five-year survival and the percentage of patients in each group (in parentheses) reported for the Nottingham good, intermediate, and poor groups are 88% (27.1%), 69% (53.9%), and 22% (19.0%), respectively (25). For comparison, our results when patients were stratified by largest perimeter and tumor size were 96.5% (29.6%), 84.3% (41.5%), and 60.6% (28.9%).

Table 6 Wilcoxon (Gehan) *P*s for significance between groups

	Groups					
	Distant disease-free survival			Breast cancer-specific survival		
Stratification[a]	Good *vs* poor	Good *vs* interm[b]	Interm *vs* poor	Good *vs* poor	Good *vs* interm	Interm *vs* poor
Node/size	<0.0001	0.1877	0.0002	0.0002	0.9124	0.0001
Node/LP	<0.0001	0.0393	0.0021	<0.0001	0.0093	0.0006
Size/LP	<0.0001	0.0001	0.0114	<0.0001	0.0151	<0.0001

[a] Node/size, axillary lymph node positivity and tumor size; Node/LP, axillary lymph node positivity and the nuclear feature largest perimeter; Size/LP, tumor size and the nuclear feature largest perimeter.
[b] Interm, intermediate.

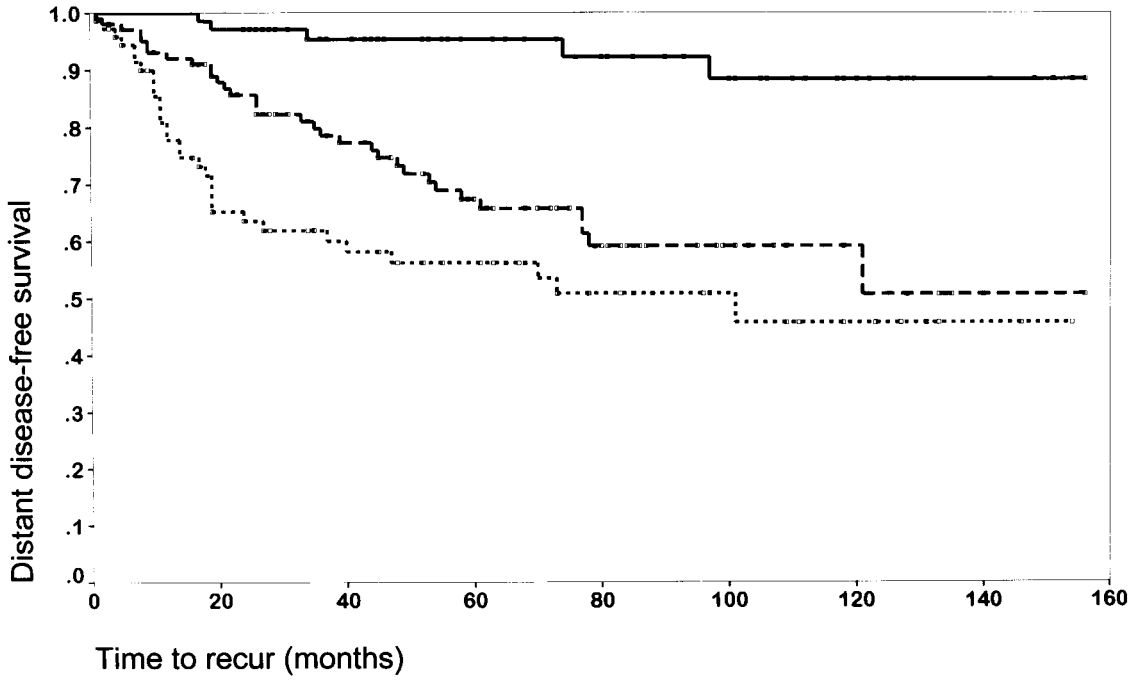

Fig. 2 Kaplan-Meier curves for distant recurrence for the three groups determined by the median cut points for tumor size and largest perimeter. Good (size ≤ 2.4 cm/largest perimeter ≤ 38.6 μm), ———; intermediate (size ≤ 2.4 cm/largest perimeter > 38.6 μm and size > 2.4 cm/largest perimeter ≤ 38.6 μm), – – – – – –; and poor (size > 2.4 cm/largest perimeter > 38.6 μm), ·····.

Therefore, our system classified more patients with a better prognosis than did the Nottingham system and did so without including axillary lymph node information.

Our results are provocative because they indicate that the nuclear feature largest perimeter, when combined with tumor size, was a better predictor of distant disease-free survival and breast cancer-specific overall survival than the classical combination of tumor size and lymph node status. In support of our data, it should be noted that of the three features used in the Nottingham index (tumor size, axillary lymph node stage, and tumor grade), tumor grade emerged as the most indicative and the only significant prognostic factor (28). Other computer-based studies demonstrated that larger nuclear size portends a poor prognosis (33–37). As in our study, other investigators (33, 34) have found that variation in nuclear size is prognostically unfavorable. Currently, a large trial is comparing nuclear morphometry (nuclear area and axes ratio) to other prognostic factors (38).

In our analyses, an operator selected nuclei from an area deemed to be the most atypical. Such selection may be subject to operator bias when compared with random selection. However, a study by Baak *et al.* (39) supports our approach. In their series of breast cancers, an operator made nuclear size measurements from areas selected as maximally atypical and found that these measurements correlated closely with systematic random measurements over the entire slide. Our interoperator reproducibility was very good for nuclear size features and specifically for largest perimeter. We attribute the differences in nuclear shape feature measurements to the way that different operators traced irregular nuclear borders during initialization. Differences between operators in initialization can produce substantially different results. We developed automated segmenta-

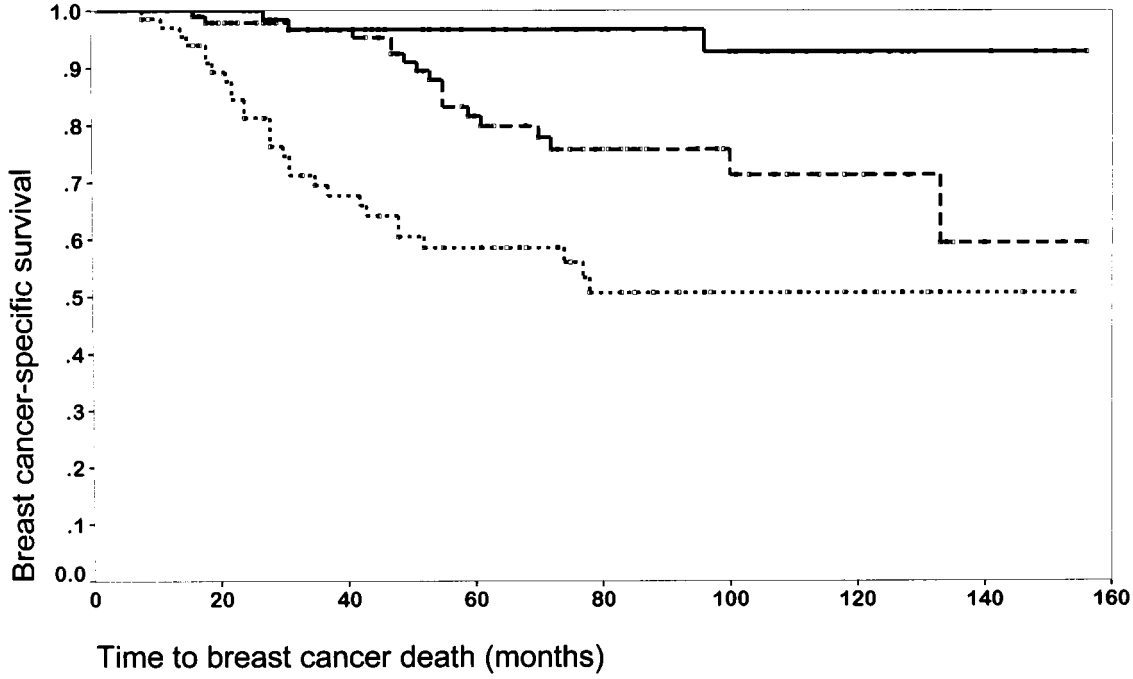

Fig. 3 Kaplan-Meier curves for breast cancer-specific survival for the three groups determined by the median cut points for tumor size and largest perimeter. Good (size $\leq$ 2.4 cm/largest perimeter $\leq$ 38.6 μm), ————; intermediate (size $\leq$ 2.4 cm/largest perimeter $>$ 38.6 μm and size $>$ 2.4 cm/largest perimeter $\leq$ 38.6 μm), – – – – –; and poor (size $>$ 2.4 cm/largest perimeter $>$ 38.6 μm), ·····.

tion to overcome this problem (40–42). After automated segmentation is incorporated into our program, a group of blinded FNAs will be sent to collaborators to assess the influence of field selection.

Our study was not controlled for the use of adjuvant chemotherapy. Axillary lymph node-positive patients were generally given adjunctive chemotherapy, whereas node-negative patients were not. However, it is unlikely that our conclusions are affected because lymph node positivity and largest perimeter were demonstrated to be independent. Moreover, the protocol adjunctive chemotherapy that was given to our node-positive patients was shown to prolong distant disease-free survival but did not increase overall survival (4–6).

The medical community is justifiably wary of new prognostic indicators because most fail to retain significance in subsequent trials. A frequent cause for failure is selecting cut points that present data in the most favorable light (43). We guarded against this by using median values as cut points. Additionally, the results were consistent with previous results obtained by cross-validated machine learning techniques (3). Nevertheless, our data require confirmation by others. We are addressing that issue. Although special equipment was not required, testing at other sites was limited because our program operated under Unix. Now it has been recoded in Java and operates on the Web. The program will be available to collaborators, pending the resolution of security issues.

Computer-derived nuclear features may be more prognostically powerful than demonstrated in this study. We do not have sufficient patients to attempt to optimize cut points or to use combinations of nuclear features to find the best way to categorize patients. Our goal was accomplished by demonstrating that a single computer-derived feature, largest perimeter, when combined with tumor size, has the potential for replacing axillary lymph node status for prognostic staging of breast cancer. Because survival is unaltered by removing the affected lymph nodes only if they become clinically apparent (44, 45), many women could avoid lymph node dissection and its attendant morbidities, expense, and recovery time. This may avoid axillary surgery and help identify patients, even preoperatively, who do not need adjunctive chemotherapy.

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