Proliferation in African Breast Cancer: Biology and Prognostication in Nigerian Breast Cancer Material

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Three hundred cases of invasive breast carcinoma from the University of Calabar Teaching Hospital, Nigeria were subjected to evaluation of proliferative activity by mitotic counts. The prognostic significance and association with other prognostic factors were evaluated. The mitotic activity was expressed as mitotic activity index (MAI), and standardized mitotic index (SMI). Pearson's correlation and univariate and multivariate Cox's regression were used. The mean follow-up time was 25.9 months. The mean values of SMI and MAI were 42.6 mitotic figures per square millimeter and 30.5 mitotic figures per 10 high-power fields, respectively, and these were much higher than values reported for Europe or other Western countries. The SMI had a positive correlation with tumor size ($r = 0.31$, $P < .0001$), histologic grade ($r = 0.68$, $P < .0001$), nuclear area ($r = 0.45$, $P < .0001$), and negative correlation with fraction of fields with tubular differentiation (FTD; $r = -0.56$, $P = <0.0001$). There was no statistically significant difference in the mitotic activity between the postmenopausal and the premenopausal patients. Also, lymph node–positive patients had higher counts than did lymph node–negative patients. Earlier determined grading associated decision thresholds divided the patients into groups of favorable and unfavorable prognosis. However, the statistically optimal thresholds for Nigerian material were different (32 and 92 mitotic figures per square millimeter for SMI). Tumor size of 5 cm, SMI, and MAI were independent prognostic factors. Nigerian breast cancers are high-grade, high-stage, and high-proliferating cancers occurring in a younger population than those of the Western countries. Proliferation is also more active. Evaluation of SMI or MAI can improve the distinction between aggressive and less aggressive variants of breast cancer.

KEY WORDS: Africa, Breast cancer, Mitotic activity, Mitotic index, Nigeria, Prognostication, Proliferation, Survival.

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Earlier studies suggest epidemiological differences between breast cancers in the Caucasian (European) and African populations (1–3). In a recent clinicopathological comparison of breast cancer in Nigeria and Finland, high histological grades, especially caused by high mitotic rates, characterized a significant proportion of the Nigerian breast cancers, suggesting a more aggressive nature (4). The proliferation markers are strong and reproducible prognosticators in invasive breast cancer (5–7). To study the situation further, this study assesses the proliferative activity of breast cancer in Nigerian breast cancers using the mitotic activity index (MAI; 8) and volume fraction–corrected standardized mitotic index (SMI; 9). Attempts are made at evaluating the relevant thresholds for a grading system that could be applied in Nigeria. The correlation of mitotic indices with other known prognostic factors and the importance of mitotic counts as prognosticators in different patient fractions are estimated.

MATERIALS AND METHODS

Nigeria

A previously described group (4) of 300 patients with histologically confirmed invasive breast cancers diagnosed at the University of Calabar Teaching Hospital, Nigeria, between 1983 and 1999 was examined. Patients with intraductal carcinoma were excluded. Those with either a recurrent or bilateral tumor were counted as one. Metastases were detected clinically with the assistance of ra-

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diological, laboratory, and histological examinations as appropriate. None of the patients had preoperative radiotherapy or any other form of preoperative adjuvant treatment.

Follow-up information was obtained from the hospital medical records. Follow-up history was available for 129 patients. A surgical team with a fairly uniform treatment protocol managed these patients. The follow-up period ranged from 2.0 to 60.0 months (mean, 25.9; median, 24.0 mo). These 129 patients were, therefore, available for survival studies.

All patients were treated with either simple or radical mastectomy with axillary evacuation. Chemotherapy was given after this to 85 (65.9%); 20 (15.5%) had endocrine therapy; and 19 (6.3%) had both chemotherapy and endocrine therapy. Twenty-four (18.6%) had radiotherapy in addition to the surgery.

The end points of the follow-up were the presence or absence of recurrence, metastasis recorded, and survival status. This study concentrates on survival analysis, which keenly followed the pattern of disease recurrence. Deaths from other causes than cancer were recorded when information was available, and only cancer-associated survival was evaluated as an end point in the survival analysis.

The characteristics of the patients are shown in Table 1.

Histological Methods

Perioperative biopsy specimens were fixed in buffered formalin (pH 7.0), embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin. The histologic typing (10) and grading (11) were done by one of the authors (OFRI), and unclear cases were reviewed after discussion and microscopy with another (YC). The criteria used in identifying mitotic figures were those described by Baak and Oort (12). Mitotic figures were characterized by an absent nuclear membrane with clear, hairy extensions of nuclear material (condensed chromosomes) that were clumped (beginning metaphase), in a plane (metaphase/anaphase), or in separate chromosomal aggregates (anaphase/telophase). The basic idea was that at least one chromosomal end was seen in a mitosis. Two parallel, clearly separate chromosome clumps were counted as one mitotic figure. The cytoplasm of the mitotic cells was often larger during mitosis than in the resting cells.

Counting was carried out in the most cellular region at tumor periphery, avoiding areas of necrosis, inflammation, calcification, and *in situ* carcinoma. If several areas met these criteria, the area with the highest number of mitotic figures, assessed subjectively, was chosen.

The first author carried mitotic count after a training period (13), using a standard laboratory microscope (objective, 40×; numerical aperture, 0.75; field diameter, 420 μm).

The number of mitotic figures in 10 consecutive fields from the most cellular area of the sample was the MAI.

The volume fraction–corrected mitotic index or SMI gives the mitotic count as the number of mitotic figures by the area of the neoplastic tissue in the microscopic fields. This is the number of mitoses in 10 consecutive fields corrected for the volume fraction and field size. In this method, the area fraction (as estimate of volume fraction) of neoplastic tissue in the microscopic field is evaluated simultaneously with the mitotic count (9):

$$\mathrm{SMI} = k(\Sigma \mathrm{MI})/(\Sigma \mathrm{Vv}),$$

where $k = 100/r^2$, r is the radius of the field, and MI = number of mitotic figures in the studied field. Vv is the volume fraction (estimated by the area fraction, as a percentage) of malignant epithelium in the studied field.

The Finnish material was studied earlier (7, 14), with the same methodology, and the results of these studies will be compared with findings from the Nigerian material in our discussion.

TABLE 1. Characteristics of the Studied Patients and Their Breast Cancer (n = 300)

Characteristic	Data
Age at diagnosis (y)	
Mean (SD)	42.7 (12.1)
Median	41
Range	18–85
Menopausal status, *n* (%)	
No. of premenopausal patients	223 (74.3)
Postmenopausal patients	77 (25.7)
Axillary lymph node status, *n* (%)	
No. of positive patients	236 (78.7)
No. of negative patients	64 (21.3)
Tumor size (cm)	
Mean (SD)	4.8 (2.4)
Median	5.3
Range	1.0–11.0
Follow-up time (mo)	
Mean (SD)	25.9
Range	2.0–60
Alive after follow-up	92 (71.3)
Causes of death during follow-up (*n*)	
Breast cancer	28
Other	9

Statistical Analysis

The SAS statistical package (SAS System for Windows, Release 6.12; SAS Institute Inc., Cary, NC) was used for analysis of both whole materials and the subgroups according to variables like age, axillary lymph node status, and tumor size at the time of diagnosis.

Previously determined (14) SMI threshold of 17 mitoses per square millimeter and MAI threshold of 10 mitotic figures per 10 high-power fields were used to compare the outcomes between the group of patients showing mitotic counts above and below the cut points using Kaplan-Meier curves and the log-rank test.

To obtain decision thresholds that could be expected to be more representative for the Nigerian material, diagrams of the χ^2 of log-rank tests were used to show variation of statistical significance associated with each tested cut point. Cut points yielding the most obvious rise in statistical significance were the best at separating good and poor prognostic groups and could therefore be used as thresholds for classification of patients based on mitotic activity.

To evaluate the prognostic significance of the mitotic counts, univariate and multivariate analyses based on Cox's regression were applied (15). The ratios indicating relative risk (RR) and their 95% confidence intervals (95% CI) showed associations between different prognostic factors and breast cancer survival.

RESULTS

The characteristics of the Nigerian breast cancer material are shown in Table 1. Descriptive statistics were performed on all 300 patients, whereas survival analysis was restricted to the 129 with a follow-up history.

The mean age at diagnosis of breast cancers among Nigerian patients was 42.7 (12.1) years. A large fraction of the patients were premenopausal (74.3%). A large tumor size with a mean (SD) of 4.8 (2.4) cm and of high frequency of lymph node involvement (78.7%) characterized the material. The average follow-up time was 25.9 months, and a survival rate of 71.3% was observed.

The mean values of SMI and MAI were 42.6 mitotic figures per square millimeter and 30.5 mitotic figures per 10 high-power fields, respectively, for the whole material of 300 patients. These mean values were not statistically different from those obtained for the 129 patients that were subsequently used for survival analysis (SMI, 45.5 ± 32.5, $P = .3437$; MAI, 33.2 ± 28.7, $P = .3288$).

The MAI and SMI values were higher in the postmenopausal patients than in the premenopausal group, but the difference was not statistically significant (MAI, $P = .1849$; SMI, $P = .4098$). On the other hand, the difference in mitotic counts between the lymph node–positive and -negative tumors was significant (MAI, $P = .0038$; SMI, $P = .0008$). Lymph node–positive tumors had higher values.

The proliferative indices in the whole material, and in different subgroups defined by the menopausal status, lymph node status, tumor size, clinical stage, histologic grade, and type are shown in

TABLE 2. Average Mitotic Activity as Expressed by MAI and SMI in Nigerian Breast Cancer Cases

Group	Number of Patients	MAI (SD)[a]	*P*	SMI (SD)[b]	*P*
Whole material	300	30.5 (25.1)		42.6 (27.5)	
Menopausal status					
Premenopausal	223	29.4 (22.1)	0.1849	41.9 (27.8)	0.4098
Postmenopausal	77	33.8 (24.9)		44.9 (26.6)	
Lymph node (LN) status					
LN−	65	22.6 (21.2)		32.6 (25.1)	0.0008
LN+	235	32.7 (25.6)	0.0038	45.4 (27.6)	
Tumor size (cm)					
<2	66	20.7 (20.4)	<0.0001	30.3 (24.6)	<0.0001
2–5	84	28.9 (23.4)		42.2 (27.2)	
>5	150	35.8 (26.5)		48.3 (27.2)	
Histologic grade			<0.0001		<0.0001
1	44	4.2 (3.8)		11.8 (11.4)	
2	119	16.8 (10.1)		31.7 (16.5)	
3	137	50.9 (21.9)		61.9 (24.5)	
Histologic type			<0.0001		<0.0001
Invasive ductal	242	34.3 (24.3)		47.7 (26.4)	
Invasive lobular	10	6.1 (7.0)		13.7 (14.1)	
Others	48	16.6 (23.6)		23.4 (22.8)	
Clinical stage			0.0201		0.0065
1	65	22.6 (21.2)		32.6 (25.1)	
2	75	28.3 (24.4)		41.9 (28.3)	
3	98	36.7 (26.8)		48.9 (28.8)	
4	72	31.9 (24.7)		44.3 (24.4)	

MAI, mitotic activity index; SMI, standardized mitotic index.
[a] Mitotic figures per 10 high-power fields.
[b] Mitotic figures per square millimeter of neoplastic epithelium.

Table 2. Higher values are seen in large tumors and those of higher histologic grade. The difference in the proliferative indices between invasive ductal carcinoma and lobular carcinoma was statistically significant ($P < .0001$ for both MAI and SMI).

A positive correlation between the SMI and MAI was observed (Pearson's $r = 0.80$, $P < .001$). Other statistically significant correlation coefficients between SMI and other variables were as follows: apoptotic index, $r = 0.28$; tumor size, $r = 0.31$; clinical stage, $r = 0.17$; histologic grade, $r = 0.68$; fraction of tubular differentiation, $r = -0.56$; nuclear area, $r = 0.41$; nuclear perimeter, $r = 0.42$; nuclear diameter, $r = 0.45$; and standard deviation of nuclear area, $r = 0.41$.

Table 3 shows the results of the univariate survival analysis performed in the whole material and subgroups of patients. Tumor size and nodal status clearly predicted breast cancer death in the whole material.

SMI and MAI were significant predictors of survival in the overall material. These proliferative indices did not come out as significant prognosticators in tumors of <2 cm in diameter. The reason for this was purely technical, as the algorithm did not calculate the prognostic influence if there were no deaths in the studied group.

Lymph node status as a predictive variable was significant in the whole data set ($P = .031$) and in premenopausal patients ($P = .052$).

Determination of decision cut points in the Nigerian material resulted in an obvious cut point at 92. This figure turned out to be the one with the greatest relevance for both SMI and MAI. The analysis detected only one cut point surrounded by less significant cut points. For morphometric grading, we propose SMI = 32 as the lower cut point for morphometric grading in the Nigerian material. Also at this threshold, the difference on both sides of the cut point is statistically significant.

Multivariate analyses were performed using tumor size (5-cm cut point), MAI, and/or SMI as prognosticators (Table 4). Because of limited number of deaths among lymph node– or <2-cm groups, the multivariate significance of lymph node status and the <2-cm cut point could not be analyzed. In multivariate analysis with the grading-associated cut points (17 mitotic figures per square millimeter for SMI, 10 mitotic figures per 10 high-power fields for MAI; 14) of SMI and MAI, tumor size at the 5-cm cut point was the most significant independent prognosticator (more significant than the mitotic counts). However, when the cut point of 92 was used, SMI (RR 6.9) and MAI (RR 7.5) had about the same significant risk ratio as the tumor size at 5-cm cut point (RR 5.5). Among the premenopausal patients, the importance of mitotic indices increased (SMI, RR 14.9). Mitotic indices were also significant prognosticators when treated as continuous variables among all patients and in the premenopausal patients.

Figure 1 demonstrates the SMI-associated survival curves as defined by the cut points at 32 mitotic figures per square millimeter of neoplastic

TABLE 3. Univariate Analysis on the Significance of the Most Important Prognosticators in the Nigerian Material

Patient Group	Prognostic Feature	*P*	Risk Ratio	95% Confidence Limit
All ($n = 129$)	SMI_{17}	0.0149	4.4	1.3–14.3
	SMI_{92}	<0.0001	48.4	14.8–158.5
	MAI_{10}	0.0110	3.4	1.3–8.9
	MAI_{92}	<0.0001	32.4	7.8–135.7
	Nodal status	0.031	8.9	1.2–64.9
	Tumor size of <2 cm	0.022	10.2	1.4–74.3
	Tumor size >5 cm	<0.0001	8.0	3.1–20.6
Premenopausal ($n = 97$)	SMI_{17}	0.0465	4.4	1.0–18.5
	SMI_{92}	<0.0001	123.1	13.9–1090
	MAI_{10}	0.0412	3.1	1.1–9.0
	MAI_{92}	<0.0001	39.4	8.5–182.7
	Nodal status	0.052	7.3	1.0–53.7
	Tumor size of <2 cm	0.037	8.4	1.2–62.1
	Tumor size >5 cm	<0.0001	9.5	3.3–27.7
Postmenopausal ($n = 32$)	SMI_{17}	0.1940	3.9	0.5–31.1
	SMI_{92}	0.0130	12.1	1.7–86.3
	MAI_{10}	0.2086	3.8	0.5–30.0
	MAI_{92}	0.9968	0.0	0.0
	Tumor size of >5 cm	0.335	2.7	0.4–21.3
Node positive ($n = 76$)	SMI_{92}	<0.0001	35.6	10.8–117.1
	MAI_{92}	<0.0001	23.4	5.6–97.9
	Tumor size of >5 cm	0.436	2.4	0.3–21.4
Tumor size of 5 cm ($n = 66$)	SMI_{17}	0.0117	3.2	0.7–13.7
	SMI_{92}	<0.0001	5.5	4.5–53.6
	MAI_{17}	0.0412	3.1	1.1–9.2
	MAI_{92}	0.0152	8.7	1.5–50.5
	Nodal status	0.436	2.4	0.3–21.4

Thresholds are 17 and 92 mitotic figures per square millimeter and 10 and 92 mitotic figures per 10 high-power fields for SMI and MAI, respectively.

TABLE 4. Multivariate (Cox's Regression) Analyses with Different Prognosticators and Cut Points

Variable	*P*	Risk Ratio	95% CI	Data Group
Tumor size, 5 cm	0.0009	5.8	2.1–16.5	All data
MAI (cut point of 10)	0.0335	2.9	1.1–7.6	
Tumor size, 5 cm	0.0023	5.1	2.1–16.5	All data
SMI (cut point of 17)	0.0864	2.9	0.9–9.6	
Tumor size, 5 cm	0.0015	5.5	1.9–15.5	All data
MAI (cut point of 92)	0.0023	7.5	2.1–27.5	
SMI (cut point of 92)	0.0005	6.9	2.3–19.4	
Tumor size, 5 cm	0.0025	6.6	1.9–22.3	Premenopausal patients
MAI (cut point of 92)	0.1532	3.6	0.6–20.9	
SMI (cut point of 92)	0.0047	14.9	2.3–97.1	

MAI expressed as mitotic figures per 10 high-power fields. SMI expressed as mitotic figures per square millimeter of neoplastic epithelium. CI, confidence interval; MAI, mitotic activity index; SMI, standardized mitotic index.

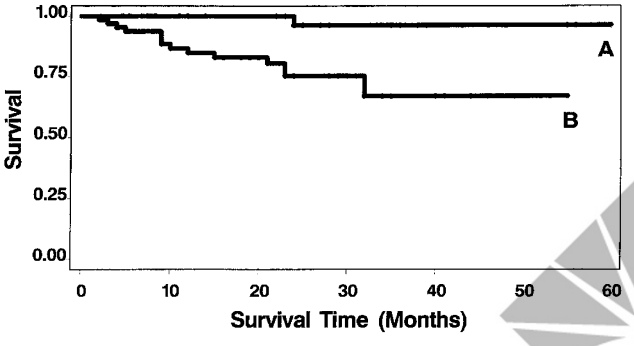

FIGURE 1. Survival of patients divided according to SMI values among 102 patients with Stage 1–3 tumors. The used cut point is 32, proposed as a relevant cut point for grading Nigerian breast cancer at the present time. The ***upper curve*** represents survival of patients with SMI of <32 (n = 37; 1 dead) whereas the ***lowest curve*** incorporates patients with SMI of ≥32 (n = 65, 15 dead). There is a clear and significant survival difference (log-rank P = .006).

tissue in the materials from the 129 patients available for survival studies.

Figure 2 shows survival curves at the cut point of 92 mitotic figures per square millimeter in the whole data set (129 patients). There is a dramatic survival difference at this cut point.

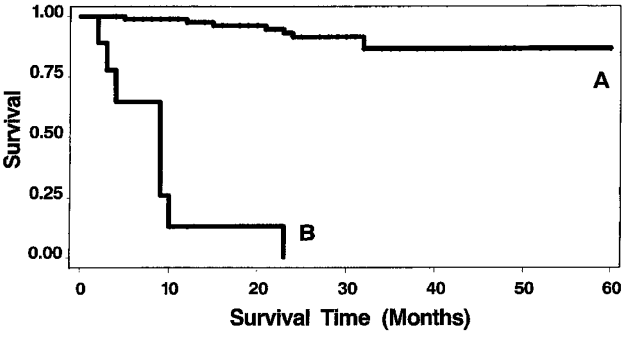

FIGURE 2. Survival of 102 patients with infiltrating breast carcinoma, with Stage 1–3 tumors (Stage 4 excluded from the follow-up material of 129 patients), divided by standardized mitotic index (SMI, also called volume fraction–corrected mitotic index, M/Vv-index). Patients with mitotic counts of >92 (n = 9, 8 dead) have a dramatically worse survival than patients with SMI of < 92 (n = 93, 8 dead). The survival difference is highly significant (log-rank P < .0001).

DISCUSSION

Differences in the age at presentation and histological dissimilarities between breast cancer in Nigeria and Finland had been previously described (4, 27).

Although demographic differences between the Nigerian and Finnish population may contribute to the lower age at presentation, dietary, genetic, and environmental factors may independently or simultaneously influence the biological and clinical patterns observed (16–19). The reproductive factors seem to influence the occurrence of breast cancer in a similar fashion in the two countries.

The proliferative activity is an established prognostic feature in breast carcinomas (5–9). Studies have suggested that prognostic variables may have different prognostic association at different time periods during the follow-up (10). The significant factors observed in the Nigerian material will, therefore, reflect the prognostic variables in the early stages of follow-up in Nigerian breast carcinomas. The limited follow-up time is a potential weakness in this study, and future studies with longer follow-up times will be beneficial.

The mean values of SMI and MAI were 42.64 mitotic figures per square millimeter and 30.53 mitotic figures per 10 high-power fields, respectively in the Nigerian material. The corresponding values in the Finnish material were 13.8 mitotic figures per square millimeter and 10.7 mitotic figures per 10 high-power fields (14). Proliferative differences between the Nigerian and Finnish tumors were significant in the overall material and in the different subgroups studied ($P < .001$). Because there were no Stage 4 cases in the Finnish material, a comparison was made using the Nigerian material of Stages 1 to 3. Although the proliferative indices of the Finnish material were within ranges observed in other studies (5–8), the values in the Nigerian material are much higher.

It is important to note that material used in the other studies was from Western countries. Our study is in concordance with observations in the United States, where after adjusting for age, stage,

socioeconomic status, reproductive experience, and health care access, African-American patients had significantly higher mitotic activity and grade nuclear atypia than did their Caucasian counterparts (28–30).

The Nigerian material was processed after variable fixation time. Despite this (20, 21), the higher proliferative activity could still be determined without practical problems. Considering the little information available on the proliferative activities of epithelial tissues in the normal breast and benign mammary conditions in the Nigerian female, the interpretation of these findings is problematic. However, several explanations can be presented.

The prevalence of obesity in the Nigerian female (22, 23) is 22.3% (versus 18% among Finnish women; 24). Obesity is usually associated with higher plasma estrogen levels (25). This, together with other exogenous sources of dietary estrogens, may contribute to the observed increase in proliferative rates. Estrogen as a carcinogen may have permissive, promotional, and/or tumor growth–inducing influences in the multistage development process of breast carcinoma (25). However, differences in the prevalence of obesity alone are not sufficient to account for the significant differences in proliferative activity of the tumors in the two studied populations. Certain mutations in the growth-regulating genes may contribute to the high mitotic activity seen (26). However, there is no evidence of corresponding influences in the Nigerian material.

Finnish premenopausal patients (14) had higher values of the proliferative indices than postmenopausal patients. In the Nigerian material, there was no statistically significant difference between premenopausal and postmenopausal patients. This finding may reflect the more advanced nature of the disease in the postmenopausal patients.

Aaltomaa *et al.* (7) suggested that all breast tumors could be graded using the same principles when the mitotic indices are determined. This was based on their observing insignificant proliferative activity differences between ductal carcinomas and the special forms of breast cancers (10).

The finding of a significant correlation observed between the SMI and other prognostic factors, including nuclear measurements, in the Nigerian material also exists in the Finnish (7, 9, 14) and other studies (5, 6, 8). This is understandable because the nuclear size (8) is also dependent on the activity of the cell cycle.

Our results show that the earlier proposed cut points (14) for MAI and SMI are also applicable in the Nigerian material in separating patients with favorable and unfavorable outcome of the disease when used independently or in combination with other prognosticators as observed in earlier Finnish studies (7, 9, 14).

However, more significant cut points (SMI as the example) are 32 and 92 mitotic figures per square millimeter. At 92 mitotic figures per square millimeter, there is a true cut point. At 32 mitotic figures per square millimeter, the significance of the cut point is still increasing towards 92 mitotic figures per square millimeter, which is the only true ditch in a curve of *P* values over the range of Nigerian SMI values. The cut points could be used for morphometric grading in Nigeria, though the system outlined by Kronqvist *et al.* (14) is still applicable in the Nigerian material. It is therefore necessary to validate the determined cut points in separate Nigerian materials after an adequate follow-up period, as the conventional Western grading parameters may be suboptimal in African tumors.

Counting of mitotic figures can be performed with good reproducibility, and it is a relatively cheap process (8, 11). The use of this technique in the prognostic evaluation of African breast cancers, especially in lymph node–positive premenopausal patients with large tumors, should be encouraged. This is because the availability of other methodologies, like flow cytometry or immunohistochemistry, is still limited in Africa.

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