JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Population Differences in Breast Cancer: Survey in Indigenous African Women Reveals Over-Representation of Triple-Negative Breast Cancer

Dezheng Huo, Francis Ikpatt, Andrey Khramtsov, Jean-Marie Dangou, Rita Nanda, James Dignam, Bifeng Zhang, Tatyana Grushko, Chunling Zhang, Olayiwola Oluwasola, David Malaka, Sani Malami, Abayomi Odetunde, Adewumi O. Adeoye, Festus Iyare, Adeyinka Falusi, Charles M. Perou, and Olufunmilayo I. Olopade

ABSTRACT

Purpose
Compared with white women, black women experience a disproportionate burden of aggressive breast cancer for reasons that remain unknown and understudied. In the first study of its kind, we determined the distribution of molecular subtypes of invasive breast tumors in indigenous black women in West Africa.

Patients and Methods
The study comprised 507 patients diagnosed with breast cancer between 1996 and 2007 at six geographic regions in Nigeria and Senegal. Formalin-fixed and paraffin-embedded sections were constructed into tissue microarrays and immunostained with 15 antibodies. Five molecular subtypes were determined, and hierarchical cluster analysis was conducted to explore subgroups for unclassified cases.

Results
The mean ($\pm$ standard deviation) age of 378 patients in the first cohort was 44.8 ± 11.8 years, with the majority of women presenting with large (4.4 ± 2.0 cm) high-grade tumors (83%) in advanced stages (72% node positive). The proportions of estrogen receptor (ER) –positive, progesterone receptor–positive, and human epidermal growth factor receptor 2 (HER2) –positive tumors were 24%, 20%, and 17%, respectively. Triple negativity for these markers was predominant, including basal-like (27%) and unclassified subtype (28%). Other subtypes were luminal A (27%), luminal B (2%), and HER2 positive/ER negative (15%). The findings were replicated in the second cohort of 129 patients. The unclassified cases could be grouped into a bad prognosis branch, with expression of vascular endothelial growth factor, B-cell lymphoma extra-large protein, and Cyclin E, and a good prognosis branch, with expression of B-cell lymphoma protein 2 and Cyclin D1.

Conclusion
These findings underscore the urgent need for research into the etiology and treatment of the aggressive molecular subtypes that disproportionately affect young women in the African diaspora.

J Clin Oncol 27:4515-4521. © 2009 by American Society of Clinical Oncology

INTRODUCTION

Among women born and raised in the United States, black women have a lower incidence rate of breast cancer but poorer survival than white women.[1] Socioeconomic factors that lead to later stage at diagnosis and limited access to quality health care contribute substantially to this disparity.[2,3] However, differences in outcomes were still observed between black and white patients after accounting for stage, socioeconomic status, and age.[4,5] Breast cancer in African Americans is more likely to be early-onset, higher grade, and estrogen receptor (ER) negative compared with breast cancer in white Americans.[2,3,6] A British study also found that black patients presented at a younger age with a higher frequency of grade 3, ER-negative tumors and had poorer outcomes than white patients with breast cancer.[7] Additional tumor features that differ between black and white patients may explain these differences in outcomes, but there is paucity of data. Women of African ancestry remain understudied, despite the significant scientific advances of the past decade.

Gene expression profiling studies have identified at least four breast cancer subtypes and

demonstrated the ability to predict clinical outcomes independent of other prognostic factors.[8,9] Luminal A and B subtypes are hormone receptor positive and have favorable clinical outcomes. Human epidermal growth factor receptor 2 (HER2) –positive/ER-negative subtype is characterized by overexpression of HER2, and basal-like subtype is negative for ER, progesterone receptor (PR), and HER2; both subtypes had poor outcomes before the advent of trastuzumab as molecularly targeted therapy for HER2-positive breast cancer. Immunohistochemical (IHC) markers have been used to define these subtypes with similar prognostic value,[10,11] which allows for breast cancer subtype assignment in large-scale epidemiologic studies and clinical practice. Basal-like, or more generally triple-negative (ER negative/PR negative/HER2 negative) breast cancer, is reportedly more prevalent in African Americans than in their white counterparts.[11-14]

Although the age-standardized incidence rate of breast cancer in Africa is only a quarter of the rate in North America, the mortality rate in Africa is close to that in North America.[15] In West Africa, the founder population of most African Americans, breast cancer is a virulent disease of young women.[16,17] Unfortunately, there has been minimal research output to guide cancer control policies in impoverished African countries. To our knowledge, this is the first international study to examine the proportion of breast cancer molecular subtypes in a large survey of indigenous West African women from six geographic regions.

PATIENTS AND METHODS

Sample Collection

Archival materials from patients with breast cancer were initially obtained from four institutions in West Africa between 1996 and 2004: University of Calabar Teaching Hospital, Calabar, Nigeria; Usman Danfodio University Teaching Hospital, Sokoto, Nigeria; Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria; and Institut Pasteur, Dakar, Sénégal (from three local pathology laboratories in Dakar). We identified all patients with histologically confirmed breast cancer who were consecutively treated in these institutions or whose samples were received in these pathology laboratories. All samples were formalin-fixed and paraffin-embedded (FFPE) according to routine surgical pathology practice. In total, 378 eligible cases were included in the final analysis.

In 2005, we established a breast cancer laboratory within the Institute for Medical Research and Training at the University of Ibadan, Nigeria, to provide core research support and clinical services to all Nigerian medical institutions. Through this laboratory, FFPE tissues of patients with breast cancer were collected using standard tissue handling guidelines to maintain integrity of the tissue blocks. This report includes a replicate sample of 129 patients with histologically confirmed breast cancer who were received in the laboratory by the end of 2007 and were treated mainly at the University College Hospital at Ibadan (47%) and Ebonyi State University Teaching Hospital at Abakaliki (42%). The study was approved by institutional review boards of the coordinating institutions in Ibadan and Calabar and the University of Chicago.

Pathologic Assessment and Construction of Tissue Microarray

Pathologic features, including histologic diagnosis, grade, tumor size, and axillary lymph node metastasis, were abstracted from pathologic reports and evaluated separately by the two study pathologists (F. Ikpatt, A.K.). Whole sections of archival slides stained with hematoxylin and eosin were evaluated for volume-corrected mitotic index expressed as mitoses per square millimeter, mean nuclear area, and fraction of fields with tubular differentiation. The histology grading of invasive breast cancer was performed using the modified Scarff-Bloom-Richardson system.[18]

Tissue microarrays were constructed from FFPE tumor samples and adjacent histologic normal epithelium, which serve as an internal positive control. Cores were precisely arrayed into a new recipient paraffin block using the automated tissue microarrayer ATA-27 (Beecher Instruments, Silver Spring, MD) with the method described by Kononen et al.[19]

Immunohistochemistry

Paraffin specimens were cut into 4-μm sections and mounted on positively charged slides. The slides were deparaffinized and rehydrated in xylene followed by graded alcohols, then washed in Tris-buffered saline.

Antibody	Clone	Dilution	Source	Pretreatment
ER	SP1	1:50	NeoMarkers, Fremont, CA	Microwave 30 minutes, citrate buffer (pH 6.0)
PR	SP2	1:50	NeoMarkers	Microwave 30 minutes, citrate buffer (pH 6.0)
HER2/neu	HercepTest	Ready to use	DAKO, Carpinteria, CA	Microwave 15 minutes, Epitope retrieval solution (HercepTest cat K5207)
EGFR	2-18C9	Ready to use	DAKO	Proteinase K (DAKO, PharmDX, Code K1494)
Cytokeratin 5/6	D5/16 B4	1:100	DAKO	Microwave 30 minutes, citrate buffer (pH 6.0)
VEGF-A	A-20, sc-152	1:500	Santa Cruz Biotechnology, Santa Cruz, CA	Microwave 30 minutes, citrate buffer (pH 6.0)
VEGF-C	Z-CVC7, 18-2255	1:25	Zymed Laboratories, South San Francisco, CA	Microwave 30 minutes, citrate buffer (pH 6.0)
Ki-67	Ki-S5	1:50	DAKO	Microwave 15 minutes, Target Retrieval Solution (DAKO, cat S3308)
P53	DO1	1:100	Oncogene, Cambridge, MA	Microwave 30 minutes, citrate buffer (pH 6.0)
P63	4A4 + Y4A3	1:100	NeoMarkers	Microwave 15 minutes, Target Retrieval Solution (DAKO, cat S3308)
BCL2	124	1:100	DAKO	Microwave 30 minutes, citrate buffer (pH 6.0)
BCLX$_L$	Polyclonal rabbit	1:500	BD Pharmingen, San Diego, CA	Microwave 30 minutes, citrate buffer (pH 6.0)
Cyclin D1	DSC-6	1:300	DAKO	Microwave 30 minutes, citrate buffer (pH 6.0)
Cyclin E	13A3	1:100	Novocastra, Newcastle upon Tyne, United Kingdom	Microwave 30 minutes, citrate buffer (pH 6.0)
IGF-1R	24-31	1:50	NeoMarkers	Microwave 30 minutes, citrate buffer (pH 6.0)
Vimentin	V9	1:50	DAKO	None

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; cat, catalog number; BCL2, B-cell lymphoma protein 2; BCLX$_L$, B-cell lymphoma extra large protein; IGF-1R, insulin-like growth factor 1 receptor.

Immunohistochemical assays were performed using a DAKO immunostainer (DAKO, Carpinteria, CA) with antibodies and antigen unmasking as detailed in Table 1. Slides were incubated in 0.03% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity, followed by incubation for 20 minutes in a protein-blocking solution (Protein Block Serum-free solution, DAKO) to reduce nonspecific background. Envision+ reagents (DAKO) were used as a detection system. Slides were then treated for 5 minutes with 3-3'-diaminobenzidine chromogen, counterstained with hematoxylin, and coverslipped. Appropriate negative controls for the immunostaining were prepared by omitting the primary antibody step. The results of immunostainings were scored semiquantitatively by two pathologists using Reiner's four-point scale based on intensity and percentage of IHC reaction.[20] Epidermal growth factor receptor (EGFR) and HER2 stainings were evaluated according to manufacturer's instructions (DAKO).

Consistent with previous publication,[11] breast cancer subtypes were defined as luminal A (ER positive and/or PR positive, HER2 negative), luminal B (ER positive and/or PR positive, HER2 positive), basal-like (ER negative, PR negative, HER2 negative, CK5/6 positive, and/or EGFR positive), HER2 positive/ER negative (HER2 positive, ER negative, PR negative), and unclassified (negative for all five markers).

Statistical Analysis

One-way analysis of variance was conducted to compare the difference in age at diagnosis between breast cancer subtypes. Kruskal-Wallis tests were used to compare tumor size, histologic grade, and morphometric measures between subtypes. Fisher's exact tests were used to examine the relationship between categorical pathologic characteristics and subtypes. Hierarchical cluster analysis with average linkage algorithms[21] was performed to explore tumor subtypes based on immunohistochemical data. Clustering analysis was done with Cluster.[21] Other statistical analyses were done with STATA 9.2 (STATA, College Station, TX).

	All Cases (N = 378)		Luminal A (n = 102)		Luminal B (n = 9)		Basal-Like (n = 103)		HER2 Positive/ER Negative (n = 57)		Unclassified (n = 107)		
Characteristic	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	P
Age, years													
< 50	251	66	70	69	6	67	69	67	35	61	71	66	.92
≥ 50	127	34	32	31	3	33	34	33	22	39	36	34	
Mean	44.8		44.2		44.8		44.4		45.9		45.1		.91
SD	11.8		12.2		10.5		11.0		11.7		12.4		
Site													.14
Calabar, Nigeria	148	39	42	41	3	33	34	33	26	46	43	40	
Ile-Ife, Nigeria	54	14	13	13	0		23	22	9	16	9	8	
Sokoto, Nigeria	30	8	9	9	1	11	11	11	4	7	5	5	
Senegal	146	39	38	37	5	56	35	34	18	32	50	47	
Positive lymph nodes	271	72	64	63	4	44	82	80	41	72	80	75	.03
Tumor size, cm													
≤ 2.0	21	6	5	5	0		6	6	4	7	6	6	.96
2.1-4.0	209	55	57	56	4	44	55	53	27	47	66	62	
> 4.0	148	39	40	39	5	56	42	41	26	46	35	33	
Histologic grade													< .0001
Well differentiated	65	17	44	43	1	11	2	2	2	4	16	15	
Moderately differentiated	145	38	48	47	4	44	32	31	7	12	54	51	
Poorly differentiated	168	44	10	10	4	44	69	67	48	84	37	35	
Histologic type													.0003
Ductal	328	87	85	83	8	89	92	89	54	95	89	83	
Medullary	13	3	0		0		6	6	1	2	6	6	
Metaplastic	14	4	0		0		3	3	2	4	9	8	
Lobular	14	4	13	13	0		0		0		1	1	
Tubular	5	1	4	4	1	11	0		0		0		
Colloid	4	1	0		0		2	2	0		2	2	
Mitotic index													< .0001
Median	27		18		30		31		44		25		
IQR	19-34		10-22		26-44		27-37		39-46		18-30		
MNA													< .0001
Median	54		34		57		60		73		49		
IQR	40-63		25-45		49-75		55-66		63-83		39-57		
FTD													< .0001
Median	10		25		10		7		5		10		
IQR	5-20		15-50		0-15		5-10		0-5		7-20		
MVD													< .0001
Median	6.1		5.2		8.8		6.5		8.7		5.5		
IQR	5.1-7.4		3.8-6.7		5.3-10.4		5.9-7.4		7.6-10.5		4.8-6.2		

Table 2. Patient Demographic and Clinical Characteristics by Established Subtypes

Abbreviations: HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; SD, standard deviation; IQR, interquartile range; MNA, mean nuclear area; FTD, fraction of fields with tubular differentiation; MVD, microvessel density.

RESULTS

A total of 507 patients with invasive breast cancer from Nigeria and Senegal were included in the study. In the first cohort of 378 patients, the mean age was 44.8 years, and only 12% were older than 60 years. Almost all tumors were larger than 2.0 cm, and more than two thirds of the cases were lymph node positive. More than 80% of the cases were of intermediate and high grade, with the majority being of ductal histology. IHC analysis revealed that 24%, 20%, and 17% of tumors were positive for ER, PR, and HER2, respectively. The majority of tumors were basal-like or unclassified subtypes (27% and 28%, respectively). Only 29% were luminal A or B, and 15% were HER2-positive/ER-negative subtype.

Table 2 shows the distribution of clinicopathologic characteristics by the five molecular subtypes. Prevalence of these molecular subtypes was independent of study site, age, and tumor size. Lack of association with age may due to narrow range of age distribution in this relatively young cohort. Subtype was highly significantly associated with histologic grade and the four tumor morphometric parameters (all $P < .0001$). HER2-positive/ER-negative subtype had the highest grade, followed by basal-like. Luminal tumors had the lowest grade, with unclassified tumors in between. The molecular subtypes also differed by histologic type: metaplastic and medullary tumors were in the basal-like, HER2-positive/ER-negative, or unclassified categories, whereas almost all lobular tumors were classified into luminal A and B subtypes. The association between subtype and lymph node metastasis was marginally significant in the crude analysis but not significant after adjusting for histologic grade.

Because of concern for antigen degradation of archived specimens, 10 additional IHC markers were examined. We found at least one of the 10 markers was positive for all hormone receptor–negative tumors, and at least five markers were positive for 83% of the hormone receptor–negative tumors. Table 3 shows the distribution of all these

markers was significantly different between molecular subtypes. Specifically, luminal A subtype tumors were less likely to express proliferation markers, such as Ki67, than other subtypes. P53 mutation was more likely to be present among basal-like, HER2-positive/ER-negative, and luminal B subtypes. BCL2 overexpression was observed among luminal A tumors, whereas $BCLX_L$ overexpression was observed among basal-like and HER2-positive/ER-negative tumors.

Hierarchical clustering analysis of all 15 IHC markers demonstrated that the established IHC-based subtype classification could be validated among tumors from West Africa, but nearly one third of the tumors were unclassified (Fig 1). The two large branches probably represent tumors with good and bad prognosis. The dendrogram also suggests that the unclassified tumors can be further divided into two distinct clusters. Interestingly, one cluster was under the "good" prognosis branch, characterized as BCL2 positive and Cyclin D1 positive, and the other cluster was under the "bad" prognosis branch, with a molecular portrait of vascular endothelial growth factor (VEGF) –A positive, VEGF-C positive, $BCLX_L$ positive, and Cyclin E positive.

On the basis of the cluster analysis, we categorized the unclassified cases into two subgroups: 86 patients in the VEGF-positive subgroup (VEGF-A positive or VEGF-C positive) and 21 patients in the VEGF-negative subgroup (negative for both VEGF-A and VEGF-C). The VEGF-positive subgroup had significantly worse histologic grade (42% grade 3 and 56% grade 2) than the VEGF-negative subgroup (5% grade 3 and 29% grade 2; $P < .0001$). The VEGF-positive subgroup also had significantly higher mitotic index, mean nuclear area, and microvessel density and lower fraction of fields with tubular differentiation than the VEGF-negative subgroup (all $P < .0001$). The two subgroups were similar in tumor size, lymph node status, and histologic type. Interestingly, the VEGF-negative subgroup had better grade, mitotic index, mean nuclear area, and microvessel density than

Positive Biomarkers	All Cases (N = 378)		Luminal A (n = 102)		Luminal B (n = 9)		Basal-Like (n = 103)		HER2 Positive/ER Negative (n = 57)		Unclassified (n = 107)		P
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
ER	92	24	85	83	7	78	0		0		0		
PR	76	20	72	71	4	44	0		0		0		
HER2	66	17	0		9	100	0		57	100	0		
Cytokeratin 5/6	123	33	5	5	3	33	82	80	33	58	0		
EGFR	136	36	4	4	2	22	96	93	34	60	0		
VEGF-A	281	74	58	57	9	100	86	84	50	88	78	73	< .0001
VEGF-C	255	67	46	45	6	67	76	74	48	84	79	74	< .0001
Ki67	328	87	66	65	9	100	102	99	55	96	96	90	< .0001
P53	133	35	15	15	7	78	53	51	38	67	20	19	< .0001
P63	43	11	0		2	22	9	9	22	39	10	9	< .0001
BCL2	130	34	83	81	2	22	8	8	1	2	36	34	< .0001
$BCLX_L$	243	64	22	22	3	33	98	95	54	95	66	62	< .0001
Cyclin D1	171	45	87	85	1	11	19	19	7	12	57	53	< .0001
Cyclin E	226	60	22	22	5	56	91	88	52	91	56	52	< .0001
IGF-1R	257	68	73	72	8	89	73	71	45	79	58	54	.005

Table 3. Immunohistologic Chemistry Markers by Established Subtypes

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; BCL2, B-cell lymphoma protein 2; $BCLX_L$, B-cell lymphoma extra-large protein; IGF-1R, insulin-like growth factor 1 receptor.
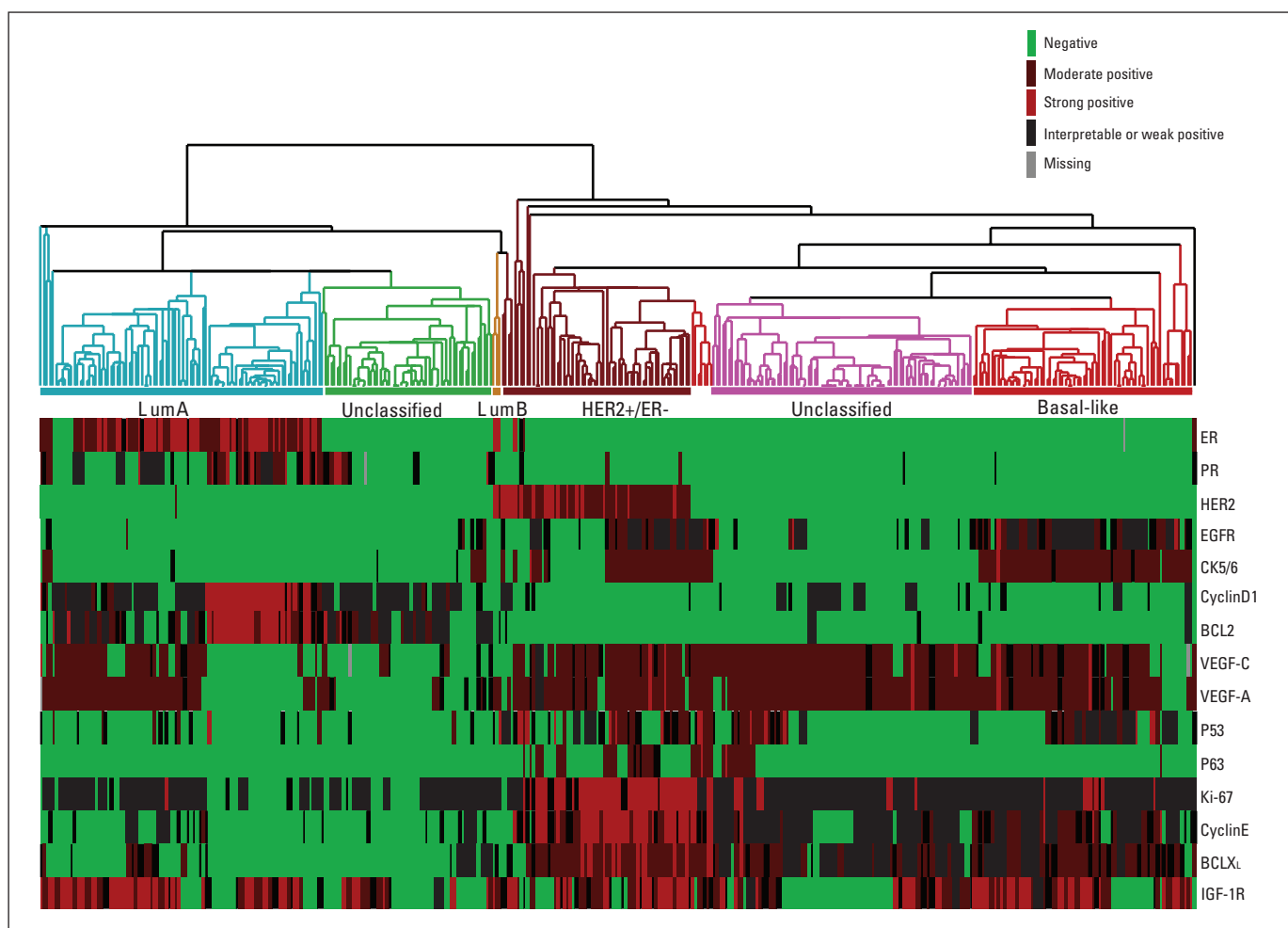
Fig 1. Hierarchical clustering of 378 invasive breast tumors from West Africa using 15 immunohistochemical markers. LumA, luminal A; LumB, luminal B; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; EGFR, epidermal growth factor receptor; BCL2, B-cell lymphoma protein 2; VEGF, vascular endothelial growth factor; BCLX$_L$, B-cell lymphoma extra-large protein; IGF-1R, insulin-like growth factor 1 receptor.

the luminal A subtype (all $P \leq .05$), suggesting that these VEGF-negative tumors are less likely to represent ER-positive cases that were false negatives.

To replicate our initial findings, an additional 129 African tumors were studied. The proportion of ER-positive, PR-positive, and HER2-positive tumors was 27%, 17%, and 16%, respectively. In this second cohort, the proportion of the five molecular subtypes was 33% luminal A, 3% luminal B, 23% basal-like, 14% HER2 positive/ER negative, and 27% unclassified, which were quite similar to those in the first cohort.

DISCUSSION

In this study of more than 500 patients with breast cancer from different geographic regions in West Africa, we found that hormone receptor–negative breast cancer is predominant, and only 25% were ER positive. This finding is consistent with two studies conducted in Nigeria, which reported 25% and 24% ER positive in 178 and 124 cases, respectively,[22,23] but different from another Nigerian study, which reported 71% ER positive in 177 cases.[24] The proportion of

ER-positive breast cancer in blacks living in the United States and United Kingdom has been consistently reported between 61% and 66% in most studies and the Surveillance, Epidemiology, and End Results (SEER) database.[6,7] To have a fair comparison, we calculated the age-standardized proportion of ER-positive tumors for African Americans by applying the age-specific proportions of ER-positive tumors from the SEER program[25] to age distribution of the study cohort in West Africans. We found the expected proportion of ER-positive tumors for African Americans would be 55% if they had the same age distribution as the African cohort. Thus although the African cohort is young as a result of the relatively short life expectancy of the population (49.1 years in Nigeria and 60.7 years in Senegal), the observed 25% is much lower than expected based on SEER data.

We used hierarchical clustering analysis of IHC markers to demonstrate that the predefined subtype classification is concordant with the self-organized clusters. The distribution of breast cancer subtypes seems to vary across populations based on our results and published literature for unknown reasons.[14,26,27] Luminal A breast cancers were predominant in Asian, white, and postmenopausal African American populations (all > 50%),

approximately 40% in premenopausal African Americans, and only 27% in indigenous Africans. In contrast, the proportion of basal-like subtype was 27% in indigenous Africans and premenopausal African Americans, approximately 15% in postmenopausal African Americans and premenopausal European Americans, and only approximately 10% in other populations. There is also a clear gradient in the proportion of unclassified breast tumors across populations, with Africans having the highest proportion. Interestingly, the proportion of HER2-positive tumors (luminal B and HER2-positive/ER-negative subtypes combined) seems similar in all populations.

Our findings have implications for breast cancer prevention and treatment. First, the striking difference in subtype distribution across populations suggests heterogeneity in etiology. Tumor subtype is strongly associated with grade, but not or only weakly associated with lymph node metastasis and tumor size, suggesting that subtype is "intrinsic" and predetermined. The comparison of subtype spectrum between whites, African Americans, and indigenous Africans suggests that both environmental exposures and genetic background determine breast cancer subtypes. Indeed, recent studies have found that breast cancer risk factors vary by tumor subtypes.[14,27] Second, our study findings suggest that triple-negative and basal-like breast cancer are not synonymous. Basal markers, such as basal cytokeratins and EGFR, are useful to identify true basal-like breast cancer.[10,11,28] In fact, unclassified tumors (nonbasal, triple-negative tumors) are histologically less aggressive than basal-like tumors but more aggressive than luminal A tumors in our study and those of others.[11,14] Using hierarchical clustering analysis, we also showed that unclassified tumors are not homogenous, and two subgroups (VEGF-positive and VEGF-negative subgroup) emerged with distinct patterns of tumor aggressiveness.

Caution should be exercised when interpreting our finding of high frequency of hormone receptor negativity. Antigen degradation of archival materials may cause false-negative results,[24] but we took several steps to address this issue. First, IHC analysis of the second cohort confirmed findings from the initial cohort. Second, vimentin served as an internal control to monitor quality of tissue fixation in archival tumors. Specimens with negative vimentin stromal staining (approximately 1%) were excluded in the final analysis. Consistent with previous study,[29] vimentin expression in tumors was associated with the basal-like phenotype (data not shown). Third, tissue microarrays were constructed from FFPE tumor samples and adjacent histologic normal epithelium (where available), which serve as an internal positive control. Fourth, tumor blocks with inadequate or questionable material were excluded. As an indirect validation of our assessment of ER status, we found ER-negative breast cancer subtypes were associated with higher grade, unfavorable histologic phenotype, and higher proliferative capacity, which is consistent with previous studies.[11,14,26] Taken together, it is unlikely that the low ER-positive proportion we observed is purely due to poor antigen retrieval but rather reflects the biology of these tumors.

Although we acknowledge that the prevalence of breast cancer subtypes observed in this study may not reflect the diversity in the entire African breast cancer patient population, unselected consecutive samples from several institutions were assayed. This study was conducted in two impoverished African countries where access to health care is limited, so underdiagnosis and delayed diagnosis are possible. On one hand, patients with rapidly progressive breast cancer are more likely to die before going to the hospital, generating bias toward a higher proportion of ER-positive tumors. However, patients with aggressive disease may be more likely to be referred to tertiary hospitals for cancer diagnosis and treatment, which generates bias toward a lower proportion of ER-positive tumors. Given the relatively poor health infrastructure in both countries, it is unlikely that there is a referral bias, as most community providers lack the infrastructure to treat patients with cancer. It is also unlikely that delayed diagnosis is an important factor because breast cancer subtype was only weakly associated with tumor stage. Because hospital-based cases referred to pathology departments were the only source of reliable data for studying cancer molecular subtype in these countries, it is reassuring that the age distribution of our study cohort was similar to that in the Ibadan Cancer Registry in Nigeria (mean age ± standard deviation, 46.5 ± 12.1 years), which suggests selection or referral bias is minimal.

In summary, our study suggests that hormone receptor–negative breast cancer is predominant, and triple-negative tumors, including basal-like and unclassified subtypes, represent the majority of tumors in West African patients with breast cancer. These findings partly explain the poor prognosis of breast cancer in African women and have important clinical and policy implications for breast cancer control in Africa. Mammographic screening may not work and low resource treatments such as oophorectomy or tamoxifen may be ineffective without knowledge of the patient's hormone receptor status. It underscores the urgent need for research into the etiology and pathogenesis of the aggressive molecular subtypes that disproportionately affect young women of African ancestry. Only then can we begin to close the gaps in the global disparities in breast cancer outcomes across populations.

AUTHOR CONTRIBUTIONS

Conception and design: Francis Ikpatt, Olufunmilayo I. Olopade
Financial support: Olufunmilayo I. Olopade
Administrative support: Olufunmilayo I. Olopade
Provision of study materials or patients: Francis Ikpatt, Jean-Marie Dangou, Olayiwola Oluwasola, Sani Malami, Abayomi Odetunde, Adewumi O. Adeoye, Festus Iyare, Adeyinka Falusi
Collection and assembly of data: Francis Ikpatt, Andrey Khramtsov, Jean-Marie Dangou, Bifeng Zhang, Tatyana Grushko, Olayiwola Oluwasola, David Malaka, Sani Malami, Abayomi Odetunde, Adewumi O. Adeoye, Festus Iyare, Adeyinka Falusi
Data analysis and interpretation: Dezheng Huo, Rita Nanda, James Dignam, Chunling Zhang, Charles M. Perou, Olufunmilayo I. Olopade
Manuscript writing: Dezheng Huo, Andrey Khramtsov, James Dignam, Olufunmilayo I. Olopade
Final approval of manuscript: Dezheng Huo, Francis Ikpatt, Andrey Khramtsov, Jean-Marie Dangou, Rita Nanda, James Dignam, Bifeng Zhang, Tatyana Grushko, Chunling Zhang, Olayiwola Oluwasola, David Malaka, Sani Malami, Abayomi Odetunde, Adewumi O. Adeoye, Festus Iyare, Adeyinka Falusi, Charles M. Perou, Olufunmilayo I. Olopade

REFERENCES

1. Smigal C, Jemal A, Ward E, et al: Trends in breast cancer by race and ethnicity: Update 2006. CA Cancer J Clin 56:168-183, 2006

2. Eley JW, Hill HA, Chen VW, et al: Racial differences in survival from breast cancer: Results of the National Cancer Institute Black/White Cancer Survival Study. JAMA 272:947-954, 1994

3. Smith-Bindman R, Miglioretti DL, Lurie N, et al: Does utilization of screening mammography explain racial and ethnic differences in breast cancer? Ann Intern Med 144:541-553, 2006

4. Newman LA, Griffith KA, Jatoi I, et al: Meta-analysis of survival in African American and white American patients with breast cancer: Ethnicity compared with socioeconomic status. J Clin Oncol 24:1342-1349, 2006

5. Chlebowski RT, Chen Z, Anderson GL, et al: Ethnicity and breast cancer: Factors influencing differences in incidence and outcome. J Natl Cancer Inst 97:439-448, 2005

6. Chu KC, Anderson WF: Rates for breast cancer characteristics by estrogen and progesterone receptor status in the major racial/ethnic groups. Breast Cancer Res Treat 74:199-211, 2002

7. Bowen RL, Duffy SW, Ryan DA, et al: Early onset of breast cancer in a group of British black women. Br J Cancer 98:277-281, 2008

8. Perou CM, Sorlie T, Eisen MB, et al: Molecular portraits of human breast tumours. Nature 406:747-752, 2000

9. Fan C, Oh DS, Wessels L, et al: Concordance among gene-expression-based predictors for breast cancer. N Engl J Med 355:560-569, 2006

10. Nielsen TO, Hsu FD, Jensen K, et al: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 10:5367-5374, 2004

11. Carey LA, Perou CM, Livasy CA, et al: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 295:2492-2502, 2006

12. Bauer KR, Brown M, Cress RD, et al: Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: A population-based study from the California cancer Registry. Cancer 109:1721-1728, 2007

13. Ihemelandu CU, Leffall LD Jr, Dewitty RL, et al: Molecular breast cancer subtypes in premeno-pausal and postmenopausal African-American women: Age-specific prevalence and survival. J Surg Res 143:109-118, 2007

14. Yang XR, Sherman ME, Rimm DL, et al: Differences in risk factors for breast cancer molecular subtypes in a population-based study. Cancer Epidemiol Biomarkers Prev 16:439-443, 2007

15. Kamangar F, Dores GM, Anderson WF: Patterns of cancer incidence, mortality, and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 24:2137-2150, 2006

16. Adebamowo CA, Adekunle OO: Case-controlled study of the epidemiological risk factors for breast cancer in Nigeria. Br J Surg 86:665-668, 1999

17. Ihekwaba FN: Breast cancer in Nigerian women. Br J Surg 79:771-775, 1992

18. Elston CW, Ellis IO: Pathological prognostic factors in breast cancer: I. The value of histological grade in breast cancer—Experience from a large study with long-term follow-up. Histopathology 19:403-410, 1991

19. Kononen J, Bubendorf L, Kallioniemi A, et al: Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4:844-847, 1998

20. Reiner A, Neumeister B, Spona J, et al: Immunocytochemical localization of estrogen and progesterone receptor and prognosis in human primary breast cancer. Cancer Res 50:7057-7061, 1990

21. Eisen MB, Spellman PT, Brown PO, et al: Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 95:14863-14868, 1998

22. Gukas ID, Jennings BA, Mandong BM, et al: Clinicopathological features and molecular markers of breast cancer in Jos, Nigeria. West Afr J Med 24:209-213, 2005

23. Ikpatt OF, Ndoma-Egba R: Oestrogen and progesterone receptors in Nigerian breast cancer: Relationship to tumour histopathology and survival of patients. Cent Afr J Med 49:122-126, 2003

24. Adebamowo CA, Famooto A, Ogundiran TO, et al: Immunohistochemical and molecular subtypes of breast cancer in Nigeria. Breast Cancer Res Treat 110:183-188, 2008

25. Ries L, Melbert D, Krapcho M, et al: SEER Cancer Statistics Review, 1975-2005. Bethesda, MD, National Cancer Institute, 2008

26. Kurebayashi J, Moriya T, Ishida T, et al: The prevalence of intrinsic subtypes and prognosis in breast cancer patients of different races. Breast 16:S72-S77, 2007 (suppl 2)

27. Millikan RC, Newman B, Tse CK, et al: Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 109:123-139, 2008

28. Rakha EA, El-Sayed ME, Green AR, et al: Prognostic markers in triple-negative breast cancer. Cancer 109:25-32, 2007

29. Livasy CA, Karaca G, Nanda R, et al: Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol 19:264-271, 2006