



Predictive and prognostic relevance of immunohistochemical testing of estrogen and progesterone receptors in breast cancer in South East Nigeria: A review of 417 cases

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Abstract

ER/PgR testing are now routinely performed in breast cancer evaluation in Southeastern Nigeria. ER is predictive to show beneficiaries of hormonal therapy and a prognostic marker to establish tumors that will resist paclitaxel induced apoptosis so a cost effective combination of anthracyclines can be used as treatment in our low resource setting thus improving survival, reducing recurrence, and cost. Four hundred seventeen cases of breast cancer seen over a period of 3 years were routinely tested for ER/PgR. ER positivity was defined as nuclear positivity of 1% in the presence of internal and external controls. Four hundred seventeen patients with Ductal Carcinoma participated. Majority were females 98.3%. Majority 60.2% were between 31 and 50 years old. Mean age was 33.5 ± 6.4 years. Two hundred fifty-seven (61.6%) were positive both for ER/PgR. 70.3% of age group 41–50 years had positive ER, age groups 20–30, and >70 years had positive ER also. ER positive cancer was 60.2%. Fifty-seven were 1%–9% positive. Most positive estrogen receptors were seen between 41 and 50 years at 70.3%. Least was seen at 31–40 years at 51.4%. Study provides an objective basis for using hormonal manipulation and makes cost affordable with appropriate chemotherapeutic agents in our low resource setting. Presentations were typically late. Seventy-six percent of stage 2 disease survived after 6 years compared with only 56% of stage 2 disease prior to immunotyping and radiotherapy in 2007. Both stage 3 and 4 had remarkable survival too at 55% and 33% respectively when compared with 2007 figures at 33% for stage 3 and 9.2% at stage 4.

Keywords

Predictive, prognostic, markers, breast cancer

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Introduction

Breast cancer is a heterogeneous complex of diseases, with a spectrum of many subtypes with distinct biological features that lead to differences in response patterns to various treatment modalities and clinical outcomes.^{1,2}

Estrogen receptor assessment is key in the management of breast cancer.³ Its relevance as a predictive and prognostic factor is well documented.⁴ The relevance of progesterone receptor however is uncertain, though it is usually recommended. It is pertinent to note that the basis of estrogen receptor positivity in these cases was an algorithm of at least 1% nuclear positivity as recommended by the American Society of Clinical Oncologists/College of American Pathologist guidelines recommendation for immunohistochemically testing of estrogen and progesterone receptor of 2010.⁵ Interestingly also estrogen receptor alpha positivity signaling, regulates the expression of taxane biomarker PRP4K thus reducing the sensitivity of these cells to taxanes if estrogen antagonists Tamoxifen is incorporated in the treatment, as well as providing an important regulator of cancer sensitivity to paclitaxel in neoadjuvant chemotherapy for breast cancer mediating resistance.⁶⁻⁸ In view of the above paclitaxel was not recommended for treatment in addition to its cost. We rather opted for the more readily available and cheaper anthracyclines as a first drug of choice if tamoxifen is also used.

On this basis we advised nonuse of paclitaxel because we operate in a low resource economy opting rather for the use of anthracycline based combination with longer lasting effects hopefully as estrogen activates ERa to suppress PC3 cell proliferation and mediates resistance to paclitaxel.⁹

The group of 1%–9% ER positive cancers were treated with Adriamycin but were not subjected to hormonal manipulations with tamoxifen because they are proven in previous studies to behave much like estrogen receptor negative tumors.¹⁷

Materials and methods

Four hundred seventeen cases of breast cancer seen over a period of 3 years from January 2014 to December 2016 were routinely tested for estrogen and progesterone positivity. Estrogen positivity was defined as nuclear positivity of at least 1% in the presence of internal (normal epithelial elements and external controls).

Recruited Patients gave their consent to use their tissue blocks for this study. An approval was first secured from the relevant agency at The University of Nigeria and Enugu State Teaching Hospital to meet the ethical guidelines to enable the study to be carried out. Results of these samples were and slides were collated and subsequently analyzed.

The sample size population was 500 patients was obtained but due to improper age documentations, 83 were dropped. Inclusive criteria are (1) Established breast cancer diagnosis whose diagnosis has been verified by at least two

pathologist. (2) Consent from patients to use their tissue blocks for the study as well as proper documentation of patient's details including age. (3) Those who were typed has their tissue blocks obtained using rabbit monoclonal antibodies from Bio SB Inc.

All monoclonal Antibodies used were procured from Bio-Sb Inc. USA as predilute ready to use and stored at a cold chain of 2–8°C until they were used for typing.

Estrogen receptor description

Estrogen receptor (ER) is a nuclear receptor for estrogens such as estradiol (the main endogenous human estrogen). Two different estrogen receptor proteins are produced from the ESR1 and ESR2 genes are usually called alpha and beta receptors. This ER antibody recognizes a protein of 67kDa, which is identified as estrogen receptor alpha. It is expressed in normal breast tissue and some breast cancers.

Estrogen receptor clone RB11 is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized, and diluted in buffer pH 7.5, containing BSA and sodium azide as preservative. Storage is at 2–8°C.¹⁰

Quality controlled procedural steps involved in this typing includes.

1. Microtomy to produce 2–4 µm thick sections.
2. Heating at 64° for 2 h.
3. Heat epitope retrieval using citrate in a Bios SB pressure cooker at 100°C for 25 min.
4. Peroxidase blocker application for 5 min.
5. Application of primary Antibody 30 min.
6. Secondary Biotylated link 10 min.
7. Application of HRP label 10 min.
8. Application of DAB substrate chromogen 5 min.
9. Application of Hematoxylin counter staining 1 min.¹¹⁻¹⁵

Results

A total of 417 patients with Invasive Ductal Carcinoma of the breast participated, because we have largely unscreened populations with poor mammography screening penetration, most cases came in as invasive ductal carcinoma. Majority were females 98.3%. More than half of respondents 60.2% were between 31 and 50 years old (Table 1). The mean age was 44.6 ± 6.4 years. Two hundred and fifty seven 61.6% are positive both for estrogen and progesterone receptor (Table 2). 70.3% of those in the age group 41–50 years had positive estrogen receptor, those in the age groups 20–30 and >70 years had positive estrogen receptor also (Table 3).

The Estrogen receptor assessment using American Society of Oncology guidelines by Hammond of at least 1% nuclear positive algorithm only not only determined basis for exclusion of paclitaxel as a chemotherapeutic agent.⁴

Table 1. Socio-demographic characteristics of the patients.

| Variables | Categories | Frequency | % |
|-------------|------------|-----------|------|
| Sex | Male | 7 | 1.7 |
| | Female | 410 | 98.3 |
| Age (years) | 20–30 | 30 | 7.2 |
| | 31–40 | 133 | 31.9 |
| | 41–50 | 118 | 28.3 |
| | 51–60 | 87 | 20.9 |
| | 61–70 | 39 | 9.4 |
| | >70 | 10 | 2.3 |

Table 2. Status of estrogen receptor, progesterone receptor.

| Variables | Positive | Negative |
|-----------------------|------------|------------|
| | N (%) | N (%) |
| Estrogen receptor | 257 (61.6) | 160 (38.4) |
| Progesterone receptor | 257 (61.6) | 160 (38.4) |

Table 3. Status of estrogen receptor and age relationship.

| Age of the patients (years) | Estrogen receptor | | X^2 | <i>p</i> |
|-----------------------------|-------------------|-----------|---------|----------|
| | Positive | Negative | | |
| | N (%) | N (%) | | |
| 20–30 | 18 (60.0) | 12 (40.0) | 228.131 | 0.886 |
| 31–40 | 72 (54.1) | 61 (45.9) | | |
| 41–50 | 83 (70.3) | 35 (29.7) | | |
| 51–60 | 51 (58.6) | 36 (41.4) | | |
| 61–70 | 27 (69.2) | 12 (30.8) | | |
| >70 | 6 (60.0) | 4 (40.0) | | |

Table 4. Staging of breast cancer seen using Manchester staging.

| Stage | Frequency | % |
|---------|-----------|------|
| Stage 1 | 18 | 4.3 |
| Stage 2 | 95 | 22.8 |
| Stage 3 | 162 | 38.8 |
| Stage 4 | 142 | 34.1 |
| Total | 417 | 100 |

The first time this recommendation is being applied in sub-Saharan Africa to type breast cancer.¹⁷ At this time all malignant breast lesions were treated with chemotherapy without immunohistochemistry neither were they subtyped.

Discussion

Nzegwu et al.¹⁶ had reported malignant breast lesions in Eastern Nigeria. It is the most common malignant lesion

affecting females in Nigeria.^{17,18} At that time all breast cancer lesions were treated the same with the same chemotherapeutic agent followed by tamoxifen administration because immunohistochemical typing was not available in our center. Most breast cancers were reported to be premenopausal with majority having a reported mean age of 41.8 years SD 11.4.¹⁶ This scenario was also seen in this data with majority of the cancers seen from patients aged 31 to 50 years (60.2%). An overwhelming majority 98.3% were females. 1.7% was found in males. Breast cancer was also reported to be the most common malignant lesion in female by Nzegwu et al.¹⁷ with the prevalence rising ever since. Currently all breast cancers are typed using monoclonal antibodies ER, PgR, and Her-2 which is used to sub-classify them as.

Either Lumina B her-2 negative, Lumina B her-2 positive, Triple negative basal like, Her-2 type.^{19,20} Only the Her-2 type had amplification of the Her-2 gene. Her-2 type was treated by incorporating trastuzumab in the neoadjuvant chemotherapy where the patients could afford it, because of obvious amplification. Another report will evaluate the efficacy of trastuzumab therapy where they could be afforded. This report shows that ER and PgR positive breast cancers were 257 cases or 61.2%. Comparatively at Ibadan they found a prevalence rate of 52.1% of 192 cases as ER positive.²¹ We believe that our more sensitive system of admitting 1%–9% of cases may have contributed to this regional variation. We however found this system of approximation extremely useful in our low resource setting enabling us to use hormonal manipulation with tamoxifen as well as the choice of chemotherapeutic agent as well as the basis of avoidance of paclitaxel as we had previously hinted. Besides it obviating the need for genetic typing which is unavailable in Nigeria as at today.

Using this method some breast cancers that would have been categorized as ER- in the past using the current threshold for ER positivity (1%) lower than that used by many labs in the past arose in our circumstance naturally. A scenario that was painted in the document published by the United States and Canadian Academy of Pathology.¹⁹ Although these breast cancer with ER positivity from 1% to 9% were categorized to behave differently from other ER positive tumors with regards to hormonal manipulation outcome, we had left them here as positive because of the lack of facilities for genetic typing and limited radiotherapy in our low resource setting. They were therefore treated with Adriamycin but were exempted from hormonal manipulation because retrospective studies has shown outcomes similar to ER negative tumors with no benefit. They were postulated also to be a heterogeneous group.¹⁷

Using this newer criteria we have institutionalized data is now being collected over 5 years to see how these group will compare with those in the past where every patient had the same chemotherapy and hormonal manipulation without recourse to immunophenotyping although preliminary data points to a markedly improved outcome. Because we

Table 5. Mortality after 6 years.

| Stage | Frequency | Mortality | % Of mortality after 6 years | Survival (%) |
|---------|-----------|-----------|------------------------------|--------------|
| Stage 1 | 18 | 0 | 0 | 100 |
| Stage 2 | 95 | 23 | 24 | 76 |
| Stage 3 | 162 | 73 | 45 | 55 |
| Stage 4 | 142 | 100 | 87 | 13 |
| Total | 417 | 196 | 47 | 53 |

Table 6. Previously in 2007 when there was no ER, PgR, and Her-2 neu antibody typing.

| Stage | Frequency | Mortality | % Mortality after 6 years | Survival (%) |
|---------|-----------|-----------|---------------------------|--------------|
| Stage 1 | 6 | 2 | 33.3 | 66.7 |
| Stage 2 | 25 | 11 | 44 | 56 |
| Stage 3 | 45 | 30 | 67 | 33 |
| Stage 4 | 35 | 31 | 88.8 | 9.2 |
| Total | 111 | 74 | 66.7 | 33.3 |

lack good health insurance schemes most breast cancers comes at a late stage ranging from stage 2 to 3 to even stage 4 with fungating lesions frequently seen.

Neoadjuvant chemotherapy (NAC) has become a standard treatment for advanced breast cancer.⁸ Several prognostic factors including estrogen receptor alpha are used to predict the response to NAC. Paclitaxel is a key drug in NAC is a microtubule stabilizing agent. Tokuda et al. found that ER alpha regulates the sensitivity to paclitaxel in NAC for breast cancer via its effect in microtubule stability. We therefore advised against the use of paclitaxel for the treatment of all ER positive tumors and instead used anthracyclines especially Adriamycin in its treatment.^{22,23} Cisplatin based combination chemotherapy was used for triple negative while Trastuzumab was added to the Her-2 type where it was affordable in addition to mastectomy in late diseases as well as pathologically complete resections in limited diseases with breast conservation and radiotherapy. Follow up is being done to ascertain if the findings are statistically significant when compared with the older method of using one chemotherapeutic agent and applying tamoxifen without immunophenotyping and radiotherapy was used. But in summary most cases recorded shorter recovery time and less morbidity with shorter periods of stay in the hospital.

Table 5 shows the persistent problem of late and very late presentation of breast cancer patients in our environment, with 162 cases (38.8%) showing up with stage 3 with multiple glandular involvement and glands over 5 cm in diameter disease (using the Manchester classification), while 142 cases or 34.1% showed up with skin involvement and distant metastases at stage 4 disease.

No lump less breast cancer was seen buttressing the near absence of mammographic screening. The reason for this late presentation has to do with poor mammographic screening,

a lack of breast cancer education, and absence of a comprehensive national health insurance scheme. Only a paltry 18 cases which represents the very elite showed up as a stage 1 disease.

Prior to the era of breast cancer antibody typing in 2007 breast cancer mortality was abysmal with only 33% alive after 6 years. Only 33% of those with stage 3 disease and 9.2% of those with stage 4 disease were alive after 6 years.

With a combination of neoadjuvant chemotherapy, radiotherapy and hormone replacement where available survival was markedly increased. Stage I were all alive after 6 years, compare with 66% survival in 2007. Seventy-six percent of stage 2 disease survived after 6 years compared with only 56% of stage 2 disease prior to immunotyping and radiotherapy in 2007. Both stage 3 and 4 had remarkable survival too at 55% and 33% respectively when compared with 2007 figures at 33% for stage 3 and 9.2% at stage 4.

Another review is underway to compare the outcome and follow up of those patients' treated with Adriamycin for Lumina b and those who had paclitaxel to establish if there is any advantage of using adrimycin those subgroups.

Author contributions

Martin Nzegwu: writer/concept developer/pathologist. Joseph Uzoigwe: writer/pathologist. Babatunde Omotowo: statistician. Anthony Ugochukwu, Emmanuel Sule, and Emmanuel Ezeome: surgeon. Okechukwu Okafor, Daniel Olusina, Chidi Eluke, and Francis I Ukekwe: pathologist. Christie Nzegwu and Victor Nzegwu: lab work.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

University of Nigeria Teaching Hospital Ethical Committee. NHREC/05/01/2008B. FWA00002458-IRB00002323.

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Informed consent

Was sought and given.

Trial registration

Not applicable.

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