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Original Research Article

Tissue polypeptide specific antigen and cancer antigen-15.3 as predictive biomarkers for metastasis in breast cancer

Omobolanle T. Akinbami[1]*, Anthonia C. Sowunmi[1], Abiodun O. Popoola[1], Temitope A. Olatunji[1], Omolara A. Fatiregun[1], Adewumi Alabi[1], Ademola O. Oyekan[1], Oladunni R. Yususf-Oduyoye[1], Oluwatosin T. Ogunsanwo[1], Tolulope O. Idowu[1], Bolaji M. Okedairo[1], Serifat Asunmo-Salaudeen[1], Mariam A. Bashir[2], Akinbami A. Akinsegun[3]

[1]Department of Oncology, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria
[2]Department of Oncology, Lagos State University Teaching Hospital, Idiaraba, Lagos, Nigeria
[3]Department of Haematology and Blood Transfusion, Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

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***Correspondence:**
Dr. Omobolanle T. Akinbami,
E-mail: omobolanleakinbami@gmail.com

ABSTRACT

Background: The management of metastatic breast cancer patients reflects the heterogeneous nature of the disease. Distant metastasis of breast cancer is the main reason for the increasing mortality of breast cancer patients due to failure of breast cancer treatment. This study aimed at determining the association between levels of tissue polypeptide-specific antigen (TPS) compared with cancer antigen-15.3 (CA-15.3) as biomarkers for metastasis in breast cancers.
Methods: This is a cross-sectional study involving one-hundred and fifty (150) female participants who presented with histologically confirmed new cases of breast cancer at the Oncology Clinic of Lagos State University Teaching Hospital Ikeja. Blood samples were collected and run using enzyme-linked immunosorbent assay (ELISA), for TPS and CA-15.3. Data were analyzed using statistical package for the social sciences (SPSS) version 26.0 (Inc., Chicago, Ill). The comparison of median TPS and CA-15.3 between two groups was evaluated using the Mann-Whitney U test. Statistical significance was considered when the p value was ≤0.05.
Results: The most represented age group was 50 to 59 years (36.6%) with overall mean age, age and at diagnosis of 49.21±10.1 years, and 48.76±10.1 years respectively. There was statistically significant increase in CA-15.3 with increasing clinical stage of the participants ($p<0.001$). However, no significant relationship was noted between median TPS and all breast cancer related characteristics assessed ($p>0.05$). The AUC for CA-15.3 and TPS were 0.679 (95% CI=0.593-0.766) and 0.524 (95% CI=0.430-0.618) respectively.
Conclusions: CA-15.3 has a better diagnostic ability for predicting distant metastasis in breast cancer.

Keywords: Breast cancer, Metastasis, Tissue polypeptide specific antigen, Cancer antigen-15.3

INTRODUCTION

Globally, breast cancer is the most commonly diagnosed cancer, surpassing lung and prostate cancers.[1] In 2020, breast cancer (BC) accounted for about 12% of all new cancer cases diagnosed in women worldwide.[1] Breast cancer is more common in industrialized countries, with an incidence rate of 54.5 per 100,000 female population compared to 31.3 in poor countries. It has the highest mortality rate among females and is the leading cause of female cancer mortality.[2] In contrast, in whites, where BC is more frequently diagnosed in women over 50 years, African incidence rates are increasing in younger premenopausal women ages 30–49.[3] This can be attributed to various factors, including the relatively shorter life

expectancy in women, which is 66 years, compared to the global female life expectancy of 71 years.[3] Consequently, the incidence rates of breast cancer appear to be higher among younger African women due to this disparity in overall life expectancy.[3] The low median age of the African female population results in a higher prevalence of BC diagnoses among younger women.[3]

Although various criteria and classifications are possible, the most representative types of breast cancer can be divided into four types based on molecular subtypes: luminal A, luminal B, human epidermal growth factor receptor 2 (Her2) positive, and triple-negative breast cancer (TNBC).[4] These subtypes have very different metastases, prognoses, and treatment methods.[4] The major cause of death in patients with breast cancer is metastasis. Also, patients with different molecular types tend to have preferential sites of distant metastases.[5] Those with hormone receptor-positive (HR+)/HER2− subtype have a higher probability of bone metastases. HER2-riched cancers have a propensity to give rise to liver and triple-negative subtypes in the lung and brain. In general, the commonest site of metastases with all subtypes is the bone, followed by the lung, liver, and brain.[5] Breast cancer metastasis accounts for the majority of deaths and about 10%–15% of breast cancer patients eventually develop distant metastases within three years after the initial detection of the primary tumour.[6] Given the deadly consequence of metastatic breast cancer, it is imperative to detect metastasis at its earliest stage.[6]

Several different tumour-specific antigens are usually generated by tumor cells or by host cells in response to tumourigenesis. These unique antigens are termed tumour markers and can be used in cancer screening and monitoring. Due to its benefits of easy clinical availability, dynamic monitoring, little invasiveness, and low cost of testing, serum tumor marker (STM) has become a strong tool for patient follow-up in many tumour species. As a result, STM is employed in periodic monitoring of patients during follow-up because STM elevation can occur 4-6 months before imaging diagnosis of tumour metastasis, allowing for early detection of metastasis without clinical symptoms.[7]

More studies are needed especially in the area of tumour metastasis, tumour biology, the pathway of spread, and, angiogenesis. Tumour-specific characteristics, biomarkers, and other factors in the tumour microenvironment that influences tumour cell metastasis need to be researched extensively for effective management of this condition. The study of specific biochemical markers toward breast cancer and metastatic disease in the blood is necessary to enable early diagnosis and treatment. Tumour biomarkers for screening metastatic disease must be both highly sensitive and specific. They need also to be safe, cheap, and widely accepted. The sensitivity and specificity of an individual tumour marker may be low, but the combination of multiple tumour markers can be very helpful as a clinical tool in oncology. CA15-3 and CEA are frequently used in the clinical treatment of breast cancer.[8]

Aside from all the known biomarkers for breast cancer could tissue polypeptide specific antigen (TPS) be a reliable biomarker for metastatic breast cancers? This study aimed at determining the association between levels of tissue polypeptide-specific antigen compared with cancer antigen-15.3 as biomarkers for metastasis in breast cancers.

METHODS

Study location

The oncology clinic of Lagos State University Teaching Hospital (LASUTH) was used.

Study population

The participants of the study were recruited from the oncology out-patient clinic, LASUTH. All consenting, histologically confirmed treatment-naive breast cancer cases were recruited and their socio-demographic, and clinical data obtained.

Study design

This study is a descriptive, cross-sectional study of breast cancer cases.

Study duration

This study was carried out for three months between December 2023 and March 2024.

Sampling technique

Non-probability consecutive recruitment sampling technique was used.

Inclusion criteria

Patients with histological diagnosis of breast cancer, adults 18 years and above, consenting metastatic and non-metastatic breast cancer patients, and chemotherapy-naive breast cancer patients were included.

Exclusion criteria

Critically ill breast cancer patients, newly referred who have undergone radiotherapy, and post-mastectomy breast cancer patients were excluded.

Sample size calculation

The minimum sample size was calculated using Cochran's formula.[9]

$$n_o = \frac{Z^2 Pq}{d^2}$$

Here n_o=minimum sample size required, Z=standard normal deviation set at 1.96 which corresponds to the 95% confidence level, and P=expected prevalence rate (%)=15.3%, and d=degree of accuracy desired set at 0.05.[10]

$$q = 1 - p(1 - 0.153 = 0.847)$$

$$n_o = \frac{1.96^2 \times 0.153 \times 0.847}{0.05^2} = \frac{3.8416 \times 0.129591}{0.0025} = 199.13 = approximately\ 199$$

The total number of adult new patients diagnosed with breast cancer in the oncology clinic of LASUTH every month is about sixty (60). Hence, the expected number of new breast cancer patients for the study duration (three months) was 180.

However, since this sample size exceeded 5% of the population (199×0.05=9.95), Cochran's correction formula was used to calculate the final sample size.[9] These calculations are as follows, where: n=minimum sample size when it exceeds 5% of the population, N=population size, n_o=199, and N=180.

$$n = n_o/(1 + n_o/N) = 199/(1 + 199/180) = 94.51$$

$$10\%\ attrition = \frac{10 \times 94.51}{100} = 9.451$$

$$Total\ sample\ size = 94.51 + 9.451 = 103.961 = approximately\ 104$$

However, to make result more generalizable a total sample size of 150 was used.

Data collection

With the use of an interviewer-administered questionnaire, each participant was interviewed by a well-trained research assistant to obtain relevant socio-demographic, and anthropometric data of participants including weight, height, were documented and clinical data like age at diagnosis, histological type, radiotherapy, and drug history were obtained. All imaging reports and clinical information were reviewed to determine their clinical stage, identify clients with metastatic and non-metastatic breast cancer as well as sites of metastases at diagnosis.

Sample collection

All newly enrolled consenting breast cancer patients were bled before the commencement of treatment by a phlebotomist engaged. These samples were used for TPS and CA-15.3 estimation by the enzyme-linked immunosorbent assay method (ELISA). All samples were collected before the commencement of treatment.

Laboratory used

The laboratory used for the ELISA was LASUTH's haematology laboratory.

Confidentiality

Hospital registration numbers rather than the name were used for data collection. Written consents were obtained from all participants. Electronic data were pass-worded and confidentiality of the hard copy data was maintained by keeping it in a secured place.

Statistical analysis

Data were analyzed using statistical package for social sciences (SPSS) version 26.0 Inc., Chicago, Ill. The continuous variables with normal distribution e.g. age were presented using mean and standard deviation while skewed data including CA-153 and TPS were presented using median and interquartile range. The data were tested for normality by performing skewness and Kolmogorov-Smirnov tests.

Median comparisons of TPS and CA-15.3 between two groups were assessed using Mann Whitney U, while Kruskal Wallis was used to compare more than two medians. Receiver operating characteristics (ROC) curve was used to determine cut off values of TPS and CA-15.3 in predicting metastases among participants with breast cancer. Youden index was used to determine optimal cut-off of TPS and CA-15.3. The p value was considered to be statistically significant when at $p \leq 0.05$ at 95% confidence interval. Graphical representation including bar, pie and box plot was used for data presentation where appropriate.

RESULTS

Socio-demographics characteristics of study participant

A total of one-hundred and fifty (150) female participants were recruited into the study. The most represented age group was 50 to 59 years (36.6%) with overall mean age, age at diagnosis and BMI of 49.21±10.1 years, 48.76±10.1 years and 27.20±5.1 kg/m^2 respectively.

Also, 38% of these participants were either underweight or with normal weight while 28.7% were overweight and 33.3% classified as obese.

Majority of the participants were married (82.7%) and most were from Yoruba ethnic group (70%). About 50 (33.3%) of the participants were obese and 59 (39.4%) had secondary level of education. Over 90% of the participants denied positive family history of cancer or history of alcohol consumption.

Cancer-related characteristics in study participants

About 17% (26) of the participants were diagnosed with early stage breast cancer while 35.3% (53) and 47.3% (71) were diagnosed with locally advanced and distant metastases respectively. Also, almost 50% (74) of the participants exhibited high grade disease with invasive ductal carcinoma (87.3%) being the most predominant histological type. This study, revealed that majority of participants had breast cancer arising from the left (58.7%), while 38.0 % of the participants had theirs on the right and only a few bilateral breast cancers (3.3%). Triple negative breast cancer accounted for slightly over 50% (79) of the participants while hormone receptor positive, HER-2 positive and triple positive accounted for 27.3% (41), 15.7% (25) and 3.3% (5) respectively.

Proportion of metastatic and non-metastatic study participants

The proportion of metastatic and non-metastatic study participants is presented in Figure 1.

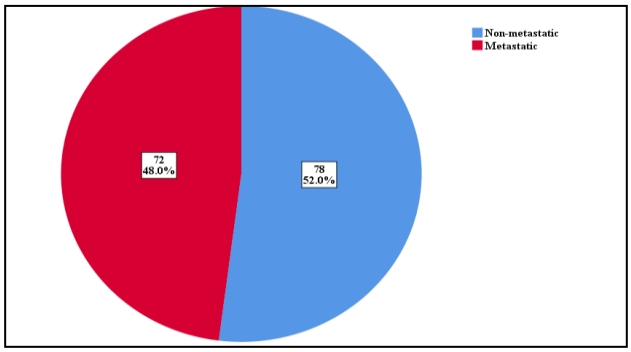

Figure 1: Proportion of metastatic and non-metastatic breast cancer among participants.

Lymph node status among study participants

The lymph node status of study participants is presented in Figure 2.

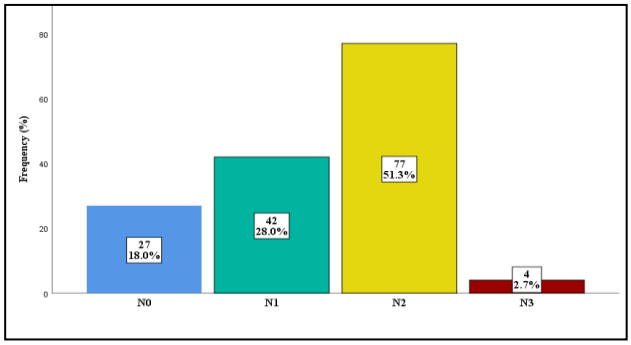

Figure 2: Bar chart showing clinical lymph nodes status among participants.
N0=no palpable axillary lymph node, N1=mobile ipsilateral axillary lymph node, N2=fixed and matted ipsilateral axillary lymph node, and N3=metastases in ipsilateral infra clavicular or supraclavicular lymph nodes.

Median comparison of CA-15.3 values in metastatic and non-metastatic study participants

Median CA-15.3 value was 87.80 U/ml with first and third quartile of 27.0 and 216.9 respectively in metastatic participants while median CA-15.3 was 29.30 with first and third quartile of 15.2 U/ml to 76.9 in non-metastatic participants. The CA-15.3 ranged from 1.6 U/ml to 1580 U/ml in metastatic participants while lowest and highest ranges were 0.0 U/ml and 462.8 U/ml respectively in non-metastatic participants. The CA-15.3 was statistically different between the two groups (p<0.001) as shown in the box and whisker plot below.

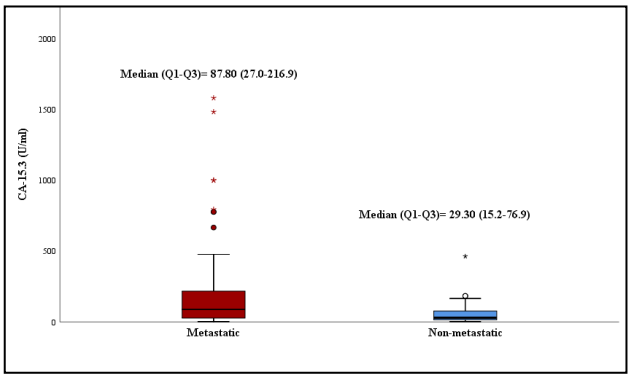

Figure 3: Median comparison of CA-15.3 values between metastatic and non-metastatic participants.
Mann-Whitney U=3.826, p<0.001*

Median comparison of TPS values in metastatic and non-metastatic study participants

With regards to the values of TPS in the metastatic group, 50% of the participants had their values between 46.3 U/l and 80.0 U/l with a median value of 68.75 U/l. On the contrary, 50% of participants in the non-metastatic group had their values between 49.0 U/l and 77.5 U/l with a median value of 62.50 U/l. This comparison showed that there was no statistical significance in the values of TPS in the metastatic setting compared to the non-metastatic arm; with a p value of 0.619.

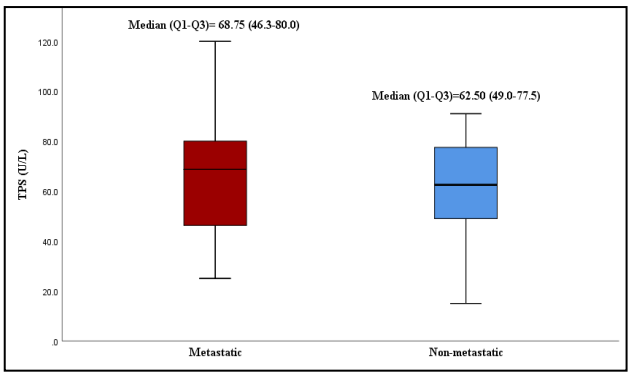

Figure 4: Median comparison of TPS values between metastatic and non-metastatic participants.
Mann-whitney U=0.498, p=0.619*

Median comparison of Ca-15.3 and TPS according to cancer-related characteristics of study participants

There was statistically significant increase in CA-15.3 with increasing clinical stage of the participants (p<0.001). Participants with bilateral tumor location had significantly higher median CA-15.3 compared to participants with unilateral location (p=0.047). There was significant difference in median CA-15.3 according to histology type (p=0.037). In addition, median CA-15.3 was significantly related to the immunohistochemistry subtype and lymph node status among participants (p<0.05). However, no significant relationship was noted between median TPS and all breast cancer related characteristics assessed (p>0.05)

Receiver operating characteristic curve for comparing the diagnostic ability between CA-15.3 and TPS

The graph below shows the ROC curve for CA-15.3 with a larger AUC compared to that of TPS depicting a better accuracy.

These comparisons are as stated in the box and whisker plot below.

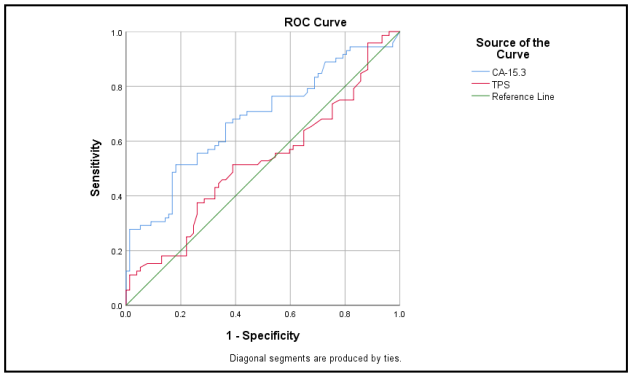

Figure 5: Receiver operating characteristics curve for determining the predictive ability of CA-15.3 and TPS.

Diagnostic accuracy of CA-15.3 and TPS in predicting metastases among study participants

The AUC for CA-15.3 and TPS were 0.679 (95% CI=0.593-0.766) and 0.524(95% CI=0.430-0.618) respectively. The optimal cut-off levels for distant metastasis determined for each biomarker were 86.55 U/ml for CA-15.3 and 68.25 U/l for TPS.

DISCUSSION

This study initially recruited 170 female breast cancer patients, but 20 were excluded due to missing immunohistochemistry results. Final analysis was conducted on 150 participants with confirmed histology and IHC subtypes. Staging was based on clinical data, imaging, and American Joint Committee on Cancer (AJCC) TNM criteria, with a slight bias toward non-metastatic cases.

The most represented age group was 50–59 years (36.6%), with a mean age of 49.21±10.1 years, indicating that breast cancer commonly affects middle-aged women in Africa as reported by Abdul-Salam et al.[11] Compared to Western data, diagnosis occurs 10–15 years earlier in African populations.[12,13] Additionally, 62% of participants were overweight or obese, Ogundiran et al reported a possible inverse association between BMI and breast cancer.[14]

Invasive ductal carcinoma was the predominant subtype (87.3%), in this study, followed by lobular (7.3%), mucinous (2.7%), cribiform (1.3%), and medullary (0.7%) types, aligning with global trends, though Ntekim et al reported medullary as the most common rare type.[15-17] Most cases were high grade (grade 3: 49.3%) and presented at late stages III and IV (82.6%) similar to another study, which is likely due to delayed presentation and sociocultural factors.[18-20] Unilateral breast cancer (96.7%) and left-sided involvement (58.7%) were more common, consistent with findings from Ibadan and Oguntola et al.[20,21]

Metastasis was observed in 48% of patients, with the lungs (55.6%) being the most common site, followed by bones (18.1%), similar to other Nigerian studies, although some reported bone metastasis as more frequent.[20,22,23] Palpable axillary lymph nodes were found in 82.9%, mostly fixed (51.3%), which is in keeping with Ali-Gombe et al's findings, but lower than Elumelu et al's (17.2%).[24,25] CA15-3 levels were significantly higher in metastatic cases (p<0.001) and correlated with clinical stage, lymph node status, histology, and IHC, while TPS levels showed no significant difference (p=0.619).[26]

The highest median CA-15.3 levels were seen in Luminal B (380.0 U/ml), followed by HER-2 positive (107.7 U/ml), TNBC (36.6 U/ml), and Luminal A (26.1 U/ml), with HR+ tumors having significantly higher levels (73.3%) compared to HER2+ (41.7%) and TNBC (42.3%).[26,27] TPS showed no significant correlation with clinical stage, lymph node status, grade, or IHC.[28,29] CA-15.3 demonstrated better diagnostic ability for predicting distant metastasis (AUC 0.68) compared to TPS (AUC 0.52), with optimal cutoff values of 86.55 U/ml for CA-15.3 and 68.25 U/l for TPS, though Sliwowska et al found TPS to be a better predictor.[30,31]

Metastatic breast cancer poses a significant challenge in Nigeria, with poor prognosis and limited access to affordable treatment options. This study found CA-15.3 to be a more reliable biomarker for predicting distant metastasis, with positive correlations to clinical stage, IHC, and lymph node status. In contrast, TPS showed negative predictive ability and lacked associations with tumor grade, IHC, or lymph node status. This research, the first to explore TPS in Nigerian breast cancer patients to the best of our knowledge, did not support previous

findings linking TPS to distant metastasis. Despite CA-15.3's value in staging, tumor markers like CA-15.3 can also be elevated in benign conditions, posing a potential confounding factor.

Limitations

Tumour markers cannot be considered completely reliable since they can also exhibit elevated levels in certain benign conditions. For instance, benign breast or ovarian diseases and endometriosis could potentially contribute to an increase in CA-15.3, which might act as a confounding factor in this research. The utilization of convenience sampling in the data collection process may present with a limitation that may potentially impact on the quality of data.

CONCLUSION

CA-15.3 has a better diagnostic ability for predicting distant metastasis in breast cancer.

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