ELEVATED PRE-TREATMENT SYSTEMIC IMMUNO-INFLAMMATORY INDICES, TRIPLE-NEGATIVE BREAST CANCER, AND P53 MUTATION ARE ASSOCIATED WITH EARLY-ONSET BREAST CANCER IN SOUTH-EASTERN NIGERIA

Okoye, J.O.*[1] Samuel, D.O.[1] Ezidiegwu, K.S.[1] Chiemeka, M.E.[2] Okoye, O.A.[3] Eleje G.U.[4]

[1]Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Nigeria.

[2]Department of Morbid Anatomy and Forensic Medicine, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria.

[3]Department of Surgery, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria.

[4]Department of Obstetrics & Gynecology, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria.

***Correspondence Author:** E-mail: jog.okoye@unizik.edu.ng; +2347031119994

Received: 9th November, 2023 **Accepted**: 18th November, 2023 **Published:** 1st December 2023

ABSTRACT

Background: In West Africa, breast cancer (BC) patients have a mortality rate that is three times higher than those in North America and Northwestern Europe. **Aim:** This study aimed to identify high-risk patients by evaluating the pre-treatment systemic inflammatory indices, p53, and BRCA2 expressions in molecular sub-types of BC in South-Eastern Nigeria.

Methods: This retrospective cohort study included 152 BC tissues, diagnosed between January 2017 and December 2022. The tissue sections were immunohistochemically stained for p53, BRCA2, hormone receptors, and human epidermal growth factor receptor 2 (HER2), scored, and analyzed accordingly. Statistical significance was set at $p \leq 0.05$.

Results: The frequency of early-onset BC (≤ 49 years) was 58.6% while the frequency of early-onset BC among patients with a family history of cancer was 76.5%. The frequency of late-stage BC was 84.9%. The frequency of luminal A and triple-negative BC (TNBC) was 1.7 times higher in early-onset BC. In comparison, the frequency of Luminal B/B-like and HER2-enriched BC was 1.9 times higher in late-onset BC ($p= 0.022$). The frequency of p53 and BRCA2 mutation was 1.6 times and 1.2 times higher in early-onset BC than in late-onset BC ($p= 0.003$ and $p= 0.843$, respectively). Significant differences in pre-treatment systemic inflammatory index were observed between patients with early-onset and late-onset BC, and ≤ 6 months survival and > 12 months survival ($p< 0.05$).

Conclusion: This study found a high incidence of early-onset BC, p53 mutation, and TNBC. Additionally, it suggests that pre-treatment systemic inflammatory indices can identify high-mortality-risk patients and early-onset BC.

Keywords: Female breast cancer, BRCA2, Luminal A, Body mass index, Neoadjuvant chemotherapy**,** White cell count

INTRODUCTION

Female breast cancer (BC) is the most common cancer in the world with over 2 million new cases and over 600,000 deaths annually (Sung *et al*., 2021). The age-standardized incident-to-mortality ratio of BC is lower in West Africa (1.47) compared to North America and Northwestern Europe (5.12 and 4.13, respectively) (Sung *et al*., 2021). This suggests that the annual fatality rate is higher in West Africa than in North America and Northwestern Europe

Breast cancer is a multifaceted disease that can be divided into different classifications. One of these classifications is referred to as triple-negative breast cancer (TNBC). TNBC is identified by the lack of estrogen receptor (ER) and progesterone receptor (PR) expression, and the overexpression of human epidermal growth factor receptor 2 (HER2) (Ensenyat-Mendez *et al*., 2021). Research has found that TNBC and Luminal A are more prevalent in premenopausal African-American women (39% and 36%) compared to postmenopausal African-American women (14% and 59%) and non-African-American women (16% and 54%, respectively) of all ages (Carey *et al.*, 2006; Bauer *et al*., 2007). Factors attributed to the high mortality rate include poor health infrastructure, low uptake of screening, late detection, and late-stage presentation and the high prevalence of triple-negative breast cancer and DNA mismatch repair mutation, especially p53 mutation (Scully, 2001; Nwagu *et al*., 2021). Interestingly, TNBC has been linked to a greater incidence of TP53 mutations and shorter survival rates (Carey *et al.*, 2006) while BRCA1/2 mutation is associated with tumour aggressiveness and 31-50% of hereditary breast cancer (Sardanelli *et al*., 2010). In mice, the inactivation of both BRCA2 and p53 resulted in mammary tumour formation and detected using an immunohistochemical technique with an accuracy of approximately 80-90% (Scully, 2001; Garg *et al*., 2013). Loss of p53 has been linked to WNT-dependent systemic inflammation (Wellenstein *et al*., 2019). Inflammatory cells, when chronically activated, can contribute to DNA damage and inflammation-driven cancer. The interplay between these factors is particularly relevant in the context of early-onset cancer, where genetic predispositions may converge with DNA repair processes and inflammatory responses to promote tumorigenesis at a younger age which in the long run determines patients' survival rates (Jiang *et al*., 2020). A correlation has been observed between elevated counts of neutrophils and monocytes and reduced counts of lymphocytes with the onset and advancement of tumours (Zhu *et al*., 2022). Quantifying the inflammatory response can be used as an affordable prognostic tool to assess disease outcomes. This study is the first to compare the systemic inflammatory indices, p53, and BRCA2 expressions, hormone receptors and HER2 in early-onset and late-onset breast cancer in Southern Nigeria.

MATERIALS AND METHODS

Study Population

This retrospective study included 152 cases of BC diagnosed from January 2017 to December 2022 at the Department of Gynaecology of a tertiary institution and private clinics in Nnewi and Onitsha, Nigeria. Patients with secondary breast cancer were excluded from the study. Some patients received Cyclophosphamide, Doxorubicin/Adriamycin, and 5-fluorouracil as neoadjuvant chemotherapy. Some patients received second-line chemotherapy (Paclitaxel/Docetaxel and carboplatin/capecitabine). The patient's medical records were accessed for socio-clinical demographics such as age, gender, comorbidities, and time of presentation. All analyses were performed by the ethical standards laid down in the Declaration of Helsinki. Patients were grouped into early-onset BC and late-onset BC.

Ethics Statement

The protocol of this retrospective study was approved by the Institutional Review Board (NAUTH/CS/66/VOL.14/VER.3/159/2021/ 124 and NAUTH/CS/66/VOL.14/VER.3/106/ 2021/109). The study was conducted according to the Declaration of Helsinki by the World Medical Association (WMA) General Assembly.

Sample collection and handling

Each patient provided two samples of 5 ml of venous whole blood, which were collected and discharged into EDTA containers - one a week before the first chemotherapy and the other a week before discharge. The whole blood samples were analyzed using a Hematology analyzer to obtain full blood counts.

After ultrasound investigations, biopsy, and surgery, the resected tissues were sent to the Department of Morbid Anatomy and Forensic Medicine for histological investigation. At least two pathologists evaluated the tissues for evidence of malignancy and the disease was staged accordingly (Cserni *et al*., 2018). The following parameters were calculated for the subgroups: total white cell count (TWBC 10^9/L), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), platelets-neutrophils to lymphocytes ratio (PNLR; [Platelet count x Neutrophil count]/Lymphocyte count), and neutrophils-to-lymphocytes-platelets ratio (NLPR; [Neutrophil count x 100]/Lymphocyte count x platelet count).

Immunohistochemical technique

Cases of BC were subclassified into 3 groups based on keratinization, invasiveness, and extent of differentiation. The presence of ER, PR, HER2, BRCA2, and p53 proteins in the tissue sections was determined by immunohistochemical technique as described by Buchwalow and Bocker (Buchwalow and Bocker, 2010). Sections from confirmed positive and negative cases were used as controls. Sections of 3 microns from formalin-fixed paraffin-embedded tissue samples were mounted on charged slides and air-dried for 2 hours at 60^{o}C. Sections were deparaffinized in three changes of xylene and hydrated through grades of alcohol. Thereafter, tissue sections were subjected to heat epitope retrieval using citrate buffer at pH 6.0 at 95^{o}C. The retrieval solution was heated in a water bath to 65^{o}C before the slides were introduced and heated to 95^{o}C. The sections and buffer were further heated at 95^{o}C for 20 mins. Sections were then allowed to cool at room temperature for 20 mins and adequately washed using phosphate-buffered saline (PBS) at pH 7.4. This was followed by placing the tissue sections in a peroxidase blocker, allowing them to stand for 5 minutes, and subsequently washing them using PBS. The circumferences of tissue sections on slides were marked round with a grease pencil and subsequently covered with primary antibodies (diluted with immunodetection protein blocker/antibody diluent), allowed to stand for 60 mins, and adequately washed using PBS. Tissue sections further were covered with a biotin link, allowed to stand for 10 minutes, and washed using PBS. Subsequently, tissue sections were covered with a horseradish peroxidase label, incubated for 10 mins, and washed with deionized water. Tissue sections were covered with DAB substrate-chromogen solution (one drop of DAB chromogen in one ml of immunodetection DAB buffer), allowed to stand for 5 mins, and rinsed with deionized water. The sections were counterstained with haematoxylin for 2 mins, rinsed in PBS and water, dehydrated, dealcoholized in xylene, and permanently mounted using coverslips. Photomicrographs were taken for documentation. Immunoglobin G1-based primary antibodies included ER, PR, HER2, BRCA2, and p53. Positive staining (brown) in the tissues was scored using a scale of 0, +1, +2, and +3, based on intensity. Scores 0 and +1 were considered negative while scores +2 and +3 were considered positive (Okoye *et al*., 2015). Immunohistochemical classification of the disease include Luminal A (ER+, PR+ and HER2-), Luminal B (ER+, PR-HER-), Luminal B-like (ER+, PR+/-, and HER2+), Her2 enriched (ER-, PR- and HER2+) and Triple-negative (ER-, PR-, and HER2+) BCs.

Statistical Analysis

Chi-square/Fisher was used to determine the association between the socio-clinical demographics of patients who were ≤ 49 years old and those > 49 years of age. Pearson's correlation was used to determine the relationship between the variables (NLR, PLR, PNLR, NLPR, and LMR) before treatment. A T-test was used to compare data of patients aged ≤ 49 years old (early onset) and those > 49 years (late-onset). ANOVA was used to compare patients' data based on duration in care (DIC); ≤ 6 months, 7 -12 months, and > 12 months.

RESULTS

The mean and median ages of the patients were 48.93 ± 13.89 years and 45.10 years, respectively. According to the results, the highest number of BC cases were diagnosed in 2019, specifically within the age group of 40 to 49 years (as illustrated in Figure 1). The survival rates of patients before the COVID-19 pandemic declaration (2017-2019) and after (2020-2021) were 299.0 ± 31.8 days and 205.5 ± 23.8 days, respectively (p= 0.022). The frequency of early-onset cases was lower in the former (56.2%) than in the latter (63.9%), but this difference was not statistically significant (p= 0.408). Figure 1A shows that breast lump and axilla lymphadenopathy were the most frequent signs present by the patients (80.9% and 33.6%, respectively). Figure 1B indicates that the year 2019 had the highest frequency of BC diagnoses, followed by 2020. Figure 1C revealed that the occurrence of BC was most prevalent in the age group of 40 to 49 years. Additionally, early-onset BC (20 to 49 years) was found to be more frequent than late-onset BC (50 to 99 years).

Socio-demographic features

The age range was 23 to 96 years. Results in Table 1 show that the unemployment rate was 2.0 times lower among patients aged 49 years old and below (Group A) compared with patients who were over 49 years old (Group B) at p> 0.05. The frequency of secondary and tertiary education was 1.7 times higher in Group A compared with Group B (p< 0.05). The uptake of a complete course of first-line and second-line chemotherapy was lower among unemployed patients (13.6% and 13.6%) compared with their employed counterparts (42.4% and 25.6%) at p= 0.016 and p= 0.4100, respectively. The alcohol consumption rate was slightly higher in Group A compared to Group B (p>0.05), while tobacco use was 10.1 times more prevalent in Group B than in Group A (p<0.05). The frequency of hypertension and diabetes was higher in Group B than in Group A (1.1 and 3.7 times, respectively; p>0.05 and <0.05). In contrast, a history of pre-hypertension was 1.4 times higher in Group A compared with Group B (p> 0.05). The consumption of herbal therapy was slightly higher in Group B than in Group A (p>0.05). The history of miscarriage or fetal death was 1.7 times higher in Group A compared to Group B (p> 0.05). The history of oral conceptive uptake was 1.8 times higher in Group A compared to Group B (p> 0.05). The frequency of obesity and early menarche (between ages 12 and 13) were 1.2 and 1.6 times higher, respectively, in Group B than in Group A (p < 0.05). Additionally, the frequency of early-age pregnancy (between ages 10 and 19) was 2.0 times higher in Group B compared to Group A (p < 0.05). The occurrence of multiple births was 1.3 times greater in Group B compared to Group A (p< 0.05). Moreover, the frequency of breastfeeding for more than 6 months was 1.2 times higher in Group A than in Group B (p< 0.05).

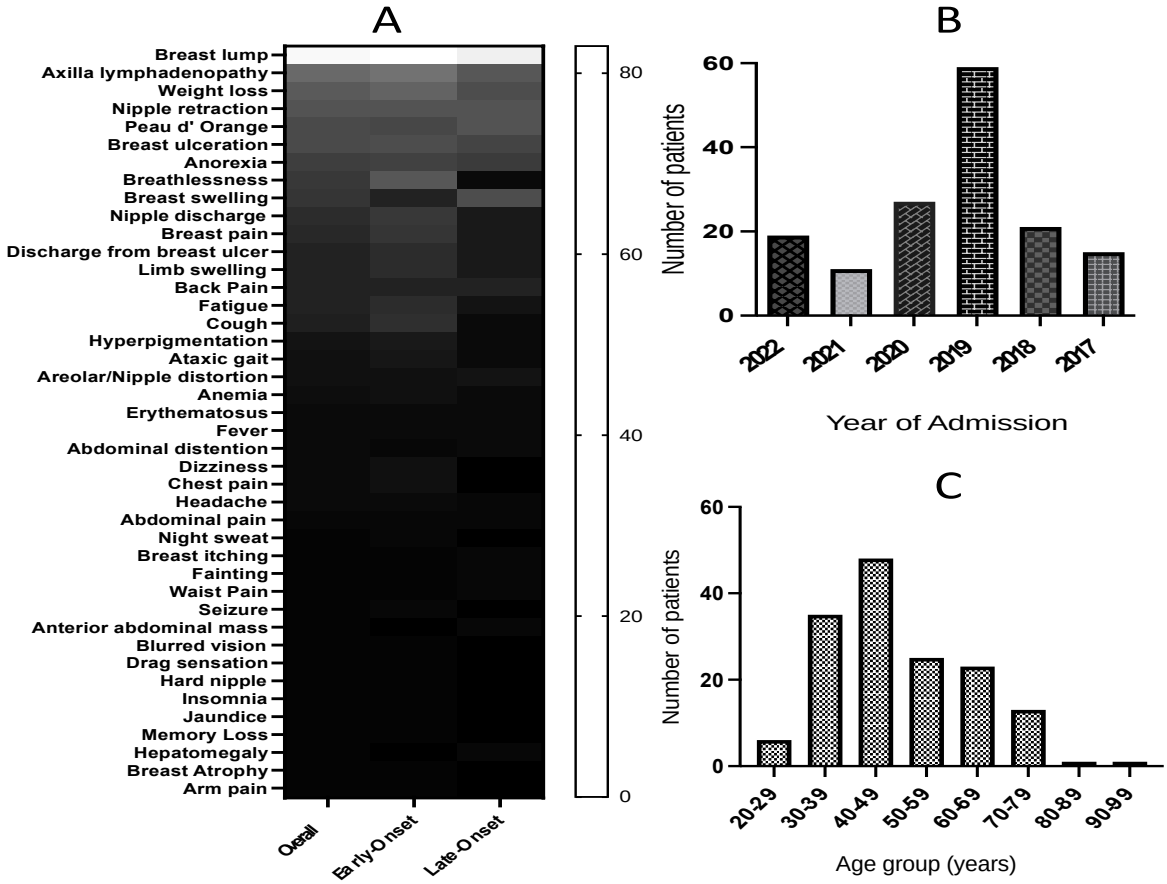

Figure 1: Features, year of diagnosis and age distribution of patients diagnosed with BC

Figure 1A shows frequency of signs and symptoms between early and late-onset BC. Figure 1B shows the patient distribution from 2017 through 2022. Figure IC shows the patient distribution across different age groups.

Table 1: Socio-demographic characteristics of patients diagnosed with breast cancer

Variables	No. (%)	≤ 49 years	> 49 years	X^2	p- value
	N= 152	n= 89 (%)	n= 63 (%)		
Employment status:				5.232	0.073
Civil servant	33 (21.7)	18 (20.2)	15 (23.8)		
Self employed	96 (63.2)	62 (69.7)	34 (54.0)		
Unemployed	23 (15.1)	9 (10.1)	14 (22.2)		
Level of Education:				28.21	<0.001*
No formal education	4 (2.6)	0 (0.0)	4 (6.3)		
Primary education	35 (23.0)	9 (10.1)	26 (41.3)		
Secondary education	63 (41.4)	45 (50.6)	18 (28.6)		
Tertiary education	50 (32.9)	35 (39.3)	15 (23.8)		
Alcohol consumption:				0.186	0.666
No	118 (77.6)	68 (76.4)	50 (79.4)		
Yes	34 (22.4)	21 (23.6)	13 (20.6)		
Tobacco Use:				7.380	0.007*
No	144 (94.7)	88 (98.9)	56 (88.9)		
Yes	8 (5.3)	1 (1.1)	7 (11.1)		
History of Hypertension:				0.885	0.643
None	65 (42.8)	38 (42.7)	27 (42.9)		
Pre-HTN	24 (15.8)	16 (18.0)	8 (12.7)		
HTN	63 (41.4)	35 (39.3)	28 (44.4)		

Table 1: Cont

Diabetes Mellitus				4.781	0.029*
No	141(92.8)	86 (96.6)	55 (87.3)		
Yes	11 (7.2)	3 (3.4)	8 (12.7)		
History of Herbal therapy:				0.0281	0.867
No	124 (81.6)	73 (82.0)	51 (81.0)		
Yes	28 (18.4)	16 (18.0)	12 (19.0)		
History of Miscarriage/fetal death				0.982	0.322
No	141 (92.8)	81 (91.0)	60 (95.2)		
Yes	11 (7.2)	8 (8.0)	3 (4.8)		
History of Oral contraceptives				0.501	0.479
No	145 (95.4)	84 (94.4)	61 (96.8)		
Yes	7 (4.6)	5 (5.6)	2 (3.2)		
Body Mass Index (BMI)				8.101	0.004*
≤ 24.9	53 (34.9)	35 (39.3)	18 (28.6)		
>24.9	99 (65.1)	54 (60.7)	45 (71.4)		
Menarche:				8.068	0.018*
12-13 years	60 (39.5)	28 (31.5)	32 (50.8)		
14-15 years	58 (38.2)	35 (39.3)	23 (36.5)		
≥16 years	34 (22.4)	26 (29.2)	8 (12.7)		
Age at first confinement				9.316	0.010*
10 – 19	18 (11.8)	8 (8.0)	10 (15.9)		
20 – 29	90 (59.2)	47 (52.8)	43 (68.2)		
≥ 30	42 (27.6)	34 (38.2)	10 (15.9)		
Parity:				22.25	<0.001
0	23 (15.1)	17 (19.1)	6 (9.5)		
≤ 2	24 (15.8)	18 (20.2)	6 (9.5)		
3 – 4	49 (32.2)	35 (39.3)	14 (22.2)		
> 4	56 (36.8)	19 (21.3)	37 (58.7)		
Average breast feeding					
≤ 6 months	49 (32.2)	25 (28.1)	24 (38.1)	6.449	0.040*
7 – 12 months	55 (36.2)	39 (43.8)	16 (25.4)		
> 12 months	48 (31.6)	25 (28.1)	23 (36.5)		

TSMP: Time of symptom manifestation to presentation. Descriptive analysis and Chi-square/Fisher's exact test. *Significance was set at $p < 0.05$.

Clinical Characteristics

Table 2 shows that early-onset breast cancer had a 1.4 times higher incidence rate than late-onset BC. The table also demonstrates that late-stage BC was 5.6 times more common than early-stage BC. The frequency of family history of (breast and prostate) cancer was 1.7 times higher in Group A (patients 49 years or under) compared with Group B (patients older than 49 years) at p> 0.05. In other words, 76.5% of patients with a family history of any cancer were of early-onset type. Among the cohort, the frequency of late presentation (symptom-wise) was high although Group B exhibited a higher frequency of early presentation at the clinic when compared to Group A (p> 0.05). Additionally, it was found that the frequency of right breast cancer was higher than that of left breast cancer. Interestingly, the frequency of bilateral breast cancer was 5.6 times higher in Group A than in Group B (p= 0.080). The frequency of poorly differentiated and high-score BC (6 to 9) of Group A were 1.2 and 1.1 times higher than in Group B, respectively (p> 0.05). The frequency of invasive BC was 1.1 times higher in Group B than in Group A (p> 0.05). The study also found that the frequency of Luminal A and triple-negative breast cancer was 1.7 times higher in Group A compared to Group B.

On the other hand, the frequency of Luminal B/B-like and HER2-enriched cancer was 1.9 times higher in Group B than in Group A ($p< 0.05$). The frequency of p53 and BRCA2 mutations in Group A was 1.6 times and 1.2 times higher than in Group B, respectively ($p<0.05$ and $p>0.05$). The average age of patients diagnosed with TNBC was found to be 48.92 ± 13.61 years, which is lower than that of patients diagnosed with Luminal B-like cancer (53.0 ± 11.01 years). The prevalence of p53/BRCA2 loss in TNBCs and Luminal B-like cancer was 68.7%/46.9% and 81.0%/33.3%, respectively indicating that p53 loss precedes BRCA2 loss. The mutation of both p53 and BRCA2 were higher in TNBCs (15.6%) than in Luminal B-like cancer (11.9%). The frequency of stage 4 BC was 1.2 times higher in Group A compared to Group B ($p= 0.497$). Only 47% of the 117 patients who received chemotherapy completed six courses. Group A had a 1.2 times higher number of chemotherapy-naïve patients than Group B ($p> 0.05$). Group A and Group B had similar in-hospital death rates of 19.1% and 18.0%, respectively. Approximately 49% and 62% of patients in Groups A and B, respectively, received care for over 6 months. Elevated platelet-related systemic immune-inflammatory indices were observed among patients with early-onset BC compared to patients with late-onset BC (Figure 2).

Table 2: Clinical characteristics of patients diagnosed with breast cancer

Variables	No. (%)	≤ 49 years	> 49 years	X^2	p- value
	N= 152	n= 89 (%)	n= 63 (%)		
Family History of Cancer				1.142	0.285
No	135 (88.8)	77 (86.5)	58 (92.1)		
Yes	17 (11.2)	12 (13.5)	5 (7.9)		
TSMP:				2.875	0.238
≤ 6 months	54 (35.5)	27 (30.3)	27 (42.9)		
7 – 12 months	42 (27.6)	28 (31.5)	14 (22.2)		
> 12 months	56 (36.8)	34 (38.2)	22 (34.9)		
Site of tumour				3.655	0.161
Left breast	68 (44.7)	39 (43.8)	29 (46.0)		
Right breast	75 (49.3)	42 (47.2)	33 (52.4)		
Left and right breasts	9 (5.9)	8 (9.0)	1 (1.6)		
Tumour Score				2.782	0.095
2 – 5	29 (19.1)	13 (14.6)	16 (25.4)		
6 – 9	123 (80.9)	76 (85.4)	47 (74.6)		
Tumour grade				5.726	0.057
Well-differentiated (Grade I)	47 (30.9)	32 (36.0)	15 (23.8)		
Moderately differentiated (Grade II)	84 (55.3)	42 (47.2)	42 (66.7)		
Poorly differentiated (Grade III)	21 (13.8)	15 (16.9)	6 (9.5)		
Histologic type				3.791	0.285
Invasive Ductal carcinoma	128 (84.2)	71 (79.8)	57 (90.5)		
Invasive Lobular Carcinoma	2 (1.3)	2 (2.2)	0 (0.0)		
Mixed type	3 (2.0)	2 (2.2)	1 (1.6)		
Unspecified	19 (12.5)	14 (15.7)	5 (7.9)		
Sub-molecular				11.43	0.022*
Luminal A	35 (23.0)	24 (27.0)	11 (17.5)		
Luminal B	17 (11.2)	7 (7.9)	10 (15.9)		
Luminal B-like	26 (17.1)	12 (13.5)	14 (22.2)		
HER2 Enriched	22 (14.5)	9 (10.1)	13 (20.6)		
TNBC	52 (34.2)	37 (41.6)	15 (23.8)		
BRCA2 mutation				0.710	0.843
Negative	88 (57.9)	49 (55.1)	39 (61.9)		
Positive	64 (42.1)	40 (44.9)	24 (38.1)		

Table 2: Cont

P53 mutation				2.969	0.003*
No	70 (46.1)	32 (36.0)	38 (60.3)		
Yes	82 (53.9)	57 (64.0)	25 (39.7)		
Disease Stage				0.060	0.807
Stages 1 and 2 (Early stage)	23 (15.1)	14 (15.7)	9 (14.3)		
1A	3 (2.0)	2 (2.2)	1 (1.6)		
1B	7 (4.6)	4 (4.5)	3 (4.8)		
2A	7 (4.6)	6 (6.7)	1 (1.6)		
2B	6 (3.9)	2 (2.2)	4 (6.3)		
Stages 3 and 4 (Late stage)	129 (84.9)	75 (84.3)	54 (85.7)		
3A	17 (11.2)	13 (14.6)	4 (6.3)		
3B	19 (12.5)	6 (6.7)	13 (20.6)		
3C	37 (24.3)	21 (23.6)	16 (25.4)		
4	56 (36.8	35 (39.3)	21 (33.3)		
Chemotherapy				0.882	0.643
naïve	35 (23.0)	22 (24.0)	13 (20.6)		
Experience: 1 – 3 courses First Line	37 (24.3)	23 (25.8)	14 (22.2)		
Experience: 4 – 6 courses First line	80 (52.6)	44 (49.4)	36 (57.1)		
Experience: Second Line				0.037	0.847
No	117 (77.0)	69 (77.5)	48 (76.2)		
Yes	35 (23.0)	20 (22.5)	15 (23.8)		

Key: TNBC= Triple-negative breast cancer, Chi-square/Fisher's exact test. *Significance was set at p < 0.05.

Correlation between Socio-clinical characteristics

The study found that there were direct relationships between parity and late-onset BC (r= 0.415, p= 0.000), late-stage BC and age of first pregnancy (r= 0.251, p= 0.048), and a higher level of education and employment rate (r= 0.241, p= 0.015). On the other hand, there were inverse relationships between parity and high tumour score (r= -0.319, p= 0.023), increasing level of education and parity (r= -0.420, p= 0.000), increasing level of education and late-onset BC (r= -0.368, p= 0.000), age at first pregnancy and late-onset BC (r= -0.264, p= 0.029), and employment rate and late-onset BC (r= -0.169, p= 0.042). The findings of this study revealed direct relationships between late-stage BC and high-grade tumours (r= 0.271, p= 0.028), as well as late-stage BC and in-hospital death (r= 0.396, p = 0.002). Additionally, there was a correlation between taking oral contraceptives and developing high-grade tumours (r= 0.313, p= 0.008), as well as between having an elevated TWBC before treatment and having a high body mass index (r = 0.286, p = 0.008). Furthermore, the study revealed that having an elevated neutrophil-to-lymphocyte ratio before treatment was associated with having an elevated platelet-to-lymphocyte ratio and recurrent BC (r= 0.476, p= 0.000), while there was an inverse relationship between age and the frequency of Luminal A and TNBC (r= -0.367, p= 0.030). The study shows that in-hospital death was inversely related to the number of chemotherapy courses (r= -0.310, p= 0.016), and having an elevated lymphocyte-to-monocyte ratio before treatment was inversely associated with the frequency of Luminal A and TNBC (r= -0.425, p= 0.048). Figure 2 shows significantly lower pre-treatment (BT) PNLR and PLR, and insignificant lower pre-treatment NLPR and TWBC among patients with late-onset (L-onset) BC compared to their counterparts with early-onset (E-onset) BC at p< 0.05, < 0.05, > 0.05 and > 0.05, respectively. Contrastingly, elevated pre-treatment NLR and LMR were also observed among patients with late-onset BC compared to patients with early-onset BC (p< 0.05 and p> 0.05, respectively. The pre-treatment PNLR, NLR, and TWBC were significantly higher among patients who received care for 6 months or less compared to patients who received care for more than 12 months (p< 0.05). Contrastingly, the pre-treatment LMR was insignificantly lower among patients who received care for 6 months or less compared to patients who received care for more than 12 months (p> 0.05).

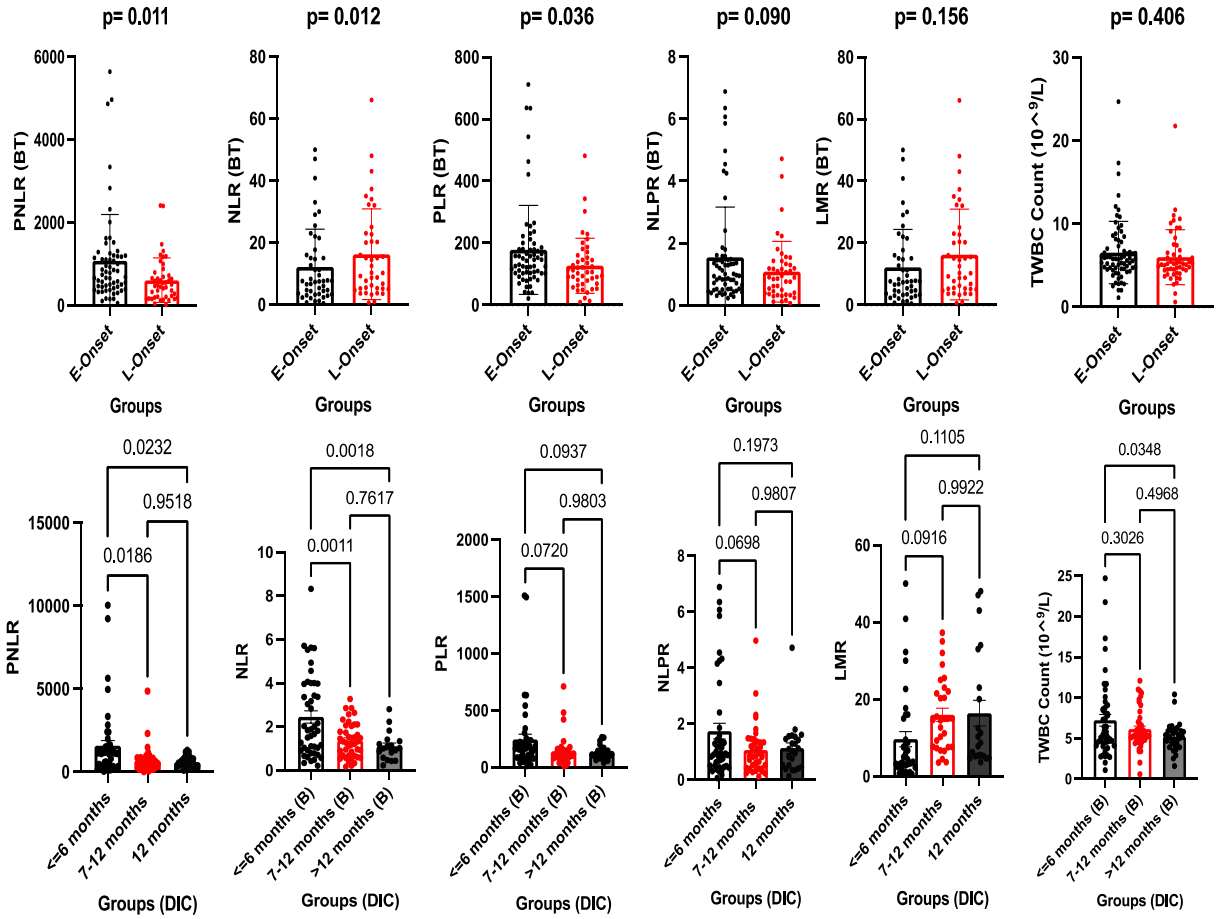

Figure 2: Comparison of pre-treatment white cell count in early-onset and late-onset BC
Figure 2 compared the total counts and ratio of white blood cells among patients with early- and late-onset BC. It also compared the total counts and ratio of white blood cells among patients based on duration of survival.

DISCUSSION

To identify factors associated with the high mortality rate in West Africa and identify prognostic tools, this study compared the sociodemographic and clinicopathologic features, p53 and BRCA2 expressions, hormone receptors and HER2, and systemic inflammatory indices in early-onset and late-onset BC in West Africa. This study found that the number of BC diagnosed in 2019 was twice or more than the number diagnosed in other years. The reason for the increased diagnosis is unknown. A systematic review showed that there was a decrease in cancer diagnoses during the COVID-19 pandemic (Angelini *et al.*, 2023). Another study reported an increased mortality rate of breast cancer patients from 2019 to 2021 due to reduced screening during the COVID-19 pandemic (Concepcion *et al.*, 2023). This might be the explanation for the lower survival duration among the patients from 2020 to 2022 compared to 2017 to 2019. Additionally, the reduction in screening during the pandemic may be the reason for the higher number of early-onset BC observed from 2020 to 2022.

This study also found that patients who developed breast cancer at an earlier age had a higher level of education as compared to those who developed it later in life. This might explain the lower incidence of advanced stage III/IV breast cancer (1.4%) in the former group than the latter. The findings emphasize the impact of education on breast cancer screening uptake (Damiani *et al.*, 2015).

The noteworthy observation here is the elevated rate of unemployment among the patient population, particularly within the subset of patients with late-onset breast cancer. Furthermore, there is a notable disparity in the utilization of treatment options, specifically the completion of full courses of chemotherapy and the uptake of second-line chemotherapy, between unemployed patients and their employed counterparts. Collectively, it can be posited that the heightened unemployment rate among patients with late-onset BC may contribute to the reduced adherence to both initial and subsequent chemotherapy treatments, ultimately diminishing overall patient survival rates. While there was no discernible distinction in alcohol consumption between patients with early-onset and late-onset breast cancer, a noteworthy contrast emerged concerning tobacco usage. Notably, the patients had ceased smoking before their cancer diagnosis, suggesting that early-life exposure to tobacco may heighten the risk of breast cancer development in later years. The finding is consistent with prior studies that associated a personal history of smoking with the prediction of breast cancer incidence and mortality (Croghan *et al*., 2009; Sollie and Bille, 2017). It is essential to highlight that a significant 80% of the patients were overweight (≥24.9 kg/m^2), A high percentage of the patients in this study were obese (≥30 kg/m^2; 35.6%), especially the patients with late-onset BC. Although the prevalence of diabetes mellitus was low, it was relatively high among patients with late-onset breast cancer. It is worth noting that obesity and diabetes are known to be strongly associated with an increased risk of developing breast cancer, as highlighted by Kang et al. (2018). This study also revealed that the risk of early-onset BC was higher in women who experience late menarche, delay their first pregnancy, and have low parity, compared to patients with late-onset. These risk factors are associated with prolonged exposure to estrogen, a known contributor to breast cancer development (Clemons and Goss, 2001; Russo and Russo, 2006).

In this study, the prevalence of family history of cancer exceeds the rates reported for African-American women diagnosed with breast cancer (10.3%) in the study conducted by Bethea et al. (2016). Similarly, the observed prevalence is higher than the 6.6% reported in Uganda and Cameroon, as noted by Adedokun et al. (2020). Among the subset of patients with a family history of cancer, a substantial 76.5% had first-degree relatives affected, while 23.5% had second-degree relatives with a history of cancer. Furthermore, within this group, the prevalence of early-onset breast cancer stood at 70.6%, indicating that inherited gene mutations likely contribute to an elevated risk of early-onset breast cancer. Additionally, it is noteworthy that the frequency of BRCA2 loss in this study surpasses the 5.6% prevalence recorded among patients in Uganda and Cameroon according to the findings of Adedokun et al. (2020). These results underscore the significance of genetic factors in breast cancer susceptibility, particularly in the context of familial cancer histories. Although the prevalence of oral contraceptive use in this study is low, literature has shown that it increases the risk of developing breast cancer (Kumle *et al*., 2002).

In this study, a high frequency of TNBC was observed compared with other molecular subtypes of BC, especially among patients with early-onset BC. The frequency of TNBC recorded in this study (34.2%) is higher than the frequency of 24.7% and 27% reported by Akakpo et al. (2023) and Huo et al. (2009) in Ghana and African women, respectively but lower than the frequency (82%) reported by Stark et al. (2010) among Ghanaians. The reason for the difference is unknown but could be associated with the mutation of DNA repair genes such as BRCA2 and p53 or social characteristics. Additionally, the frequency of TNBC and Luminal A were higher among patients with early-onset BC compared with late-onset BC. This agrees with the findings of Akakpo et al. (2023) in Ghana. In this study, the highest frequency of obesity was found among patients with Luminal A breast cancer (85.7%).

In contrast, Vona-Davis et al. (2008) observed higher cases of obesity among patients with TNBC (50%) than non-TNBC cases (16%). The frequency of p53 expression in TNBC cases is like the prevalence rate reported in Ghana (31.0%) by Ameh-Mensah et al. (2021) but lower than the frequency rate reported in Morocco (41.0%) by Jouli et al. (2020). Here, the frequency of BRCA2 loss in TNBC is higher than the prevalence rate reported by Jiagge et al. (2015) and Xie et al. (2017) in Ghana (40%) and China (20%), respectively. The difference in BRCA2 expression in TNBC could be race and lifestyle-related. Studies have shown that a high expression of the p53 gene is associated with high-grade large-size and highly proliferative tumours with lymph node involvement, and risk of recurrence (Pan *et al*., 2017; Jouli *et al*., 2020; Ameh-Mensah *et al*., 2021). Thus, the concomitant high expression of p53 and moderate to low expression of BRCA2 in this study could be an early immune response to aggressive tumours. According to Lee et al. (2012), individuals with loss of both BRCA2 and p53 function are at risk of early-onset BC. The latter might be an explanation for the lower mean age of TNBCs compared with Luminal B-like cancer. The absence or low expression of p53 and BRCA2 in a malignant breast tumour suggests poor prognosis.

This study revealed elevated pre-treatment PLR among patients with early-onset BC compared with late-onset BC. This study also found that elevated pre-treatment NLR, PNLR and TWBC were associated with shorter survival rates. Cho et al. (2018) revealed that the PLR, an indicator of systemic inflammation as a part of the host immune response, was an independent marker for poor disease-free survival in patients with lymph node metastasis and luminal subtype. Finally, our study is limited by a small sample size and single-center origin. Future studies should collect data from multiple tertiary healthcare facilities and a larger patient sample.

CONCLUSION

This study found a high incidence of early-onset BC, p53 mutation, and TNBC. Additionally, it suggests that pre-treatment systemic inflammatory indices can identify high-mortality-risk patients and early-onset BC. These findings can inform strategies for early detection, treatment, and support for individuals facing BC in the region.

Recommendation

Total white blood cell count, PNLR, and NLR should be used to monitor patients to improve treatment outcomes.

Acknowledgements

Special thanks are to the Histopathology Unit, Medical Laboratory Services, University of Benin University Teaching Hospital and Nnamdi Azikiwe University Teaching Hospital staff for their technical assistance.

REFERENCES

Adedokun, B., Zheng, Y., Ndom, P., Gakwaya, A., Makumbi, T., Zhou, A.Y., Yoshimatsu, T.F., Rodriguez, A., Madduri, R.K., Foster, I.T. and Sallam, A. (2020). Prevalence of inherited mutations in breast cancer predisposition genes among women in Uganda and Cameroon. *Cancer Epidemiology, Biomarkers & Prevention*, ***29***(2), 359-367.

Akakpo, P.K., Imbeah, E.G., Edusei, L., Naporo, S., Ulzen-Appiah, K., Clegg-Lamptey, J.N., Dedey, F., Nsaful, J., Affram, N., Wiafe, B. and Mensah, S. (2023). Clinicopathologic characteristics of early-onset breast cancer: a comparative analysis of cases from across Ghana. *BMC Women's Health*, ***23***(1), 5.

Ameh-Mensah, C., Duduyemi, B.M., Bedu-Addo, K., Atta Manu, E., Opoku, F. and Titiloye, N. (2021). The Analysis of bcl-2 in Association with p53 and Ki-67 in Triple Negative Breast Cancer and Other Molecular Subtypes in Ghana. *Journal of Oncology*, ***2021***, *2021*.

Angelini, M., Teglia, F., Astolfi, L., Casolari, G. and Boffetta, P. (2023). Decrease of cancer diagnosis during COVID-19 pandemic: a systematic review and meta-analysis. *European Journal of Epidemiology*, ***38***(1), pp.31-38.

Bauer, K.R., Brown, M., Cress, R.D., Parise, C.A. and Caggiano, V. (2007). 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*****(**9), pp.1721-1728.

Bethea, T.N., Rosenberg, L., Castro-Webb, N., Lunetta, K.L., Sucheston-Campbell, L.E., Ruiz-Narváez, E.A., Charlot, M., Park, S.Y., Bandera, E.V., Troester, M.A. and Ambrosone, C.B. (2016). Family history of cancer in relation to breast cancer subtypes in African American women. *Cancer epidemiology, biomarkers & prevention*, ***25*****(**2), pp.366-373.

Buchwalow, I.B., Böcker, W. (2010). Immunohistochemistry: basics and methods. Springer Science & Business Media. P. 19-77.

Carey, L.A., Perou, C.M., Livasy, C.A., Dressler, L.G., Cowan, D., Conway, K., Karaca, G., Troester, M.A., Tse, C.K., Edmiston, S. and Deming, S.L. (2006). Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *Jama*, ***295***(21), 2492-2502.

Cho, U., Park, H.S., Im, S.Y., Yoo, C.Y., Jung, J.H., Suh, Y.J. and Choi, H.J. (2018). Prognostic value of systemic inflammatory markers and development of a nomogram in breast cancer. *PloS one*, ***13***(7), p.e0200936.

Clemons, M. and Goss, P. (2001). Estrogen and the risk of breast cancer. *New England Journal of Medicine*, ***344***(4), 276-285.

Concepcion, J., Yeager, M., Alfaro, S., Newsome, K., Ibrahim, J., Bilski, T. and Elkbuli, A. (2023). Trends of cancer screenings, diagnoses, and mortalities during the COVID-19 pandemic: implications and future recommendations. *The American Surgeon™*, ***89***(6), pp.2276-2283.

Croghan, I.T., Pruthi, S., Hays, J.T., Cha, S., Johnson, R.E., Kosel, M., Morris, R. and Hurt, R.D., (2009). The role of smoking in breast cancer development: an analysis of a Mayo Clinic cohort. *The Breast Journal*, ***15*****(**5), 489-495.

Cserni, G., Chmielik, E., Cserni, B., & Tot, T. (2018). The new TNM-based staging of breast cancer. *Virchows Archive*, **472**, 697-703.

Damiani, G., Basso, D., Acampora, A., Bianchi, C.B., Silvestrini, G., Frisicale, E.M., Sassi, F. and Ricciardi, W. (2015). The impact of level of education on adherence to breast and cervical cancer screening: evidence from a systematic review and meta-analysis. *Preventive medicine*, ***81***, 281-289.

Ensenyat-Mendez, M., Llinàs-Arias, P., Orozco, J.I., Íñiguez-Muñoz, S., Salomon, M.P., Sesé, B., DiNome, M.L. and Marzese, D.M. (2021). Current triple-negative breast cancer subtypes: dissecting the most aggressive form of breast cancer. *Frontiers in oncology*, ***11***, 681476.

Garg, K., Levine, D.A., Olvera, N., Dao, F., Bisogna, M., Secord, A.A., Berchuck, A., Cerami, E., Schultz, N. and Soslow, R.A. (2013). BRCA1 immunohistochemistry in a molecularly characterized cohort of ovarian carcinomas. *The American journal of surgical pathology*, ***37*****(**1), p.138.

Huo, D., Ikpatt, F., Khramtsov, A., Dangou, J.M., Nanda, R., Dignam, J., Zhang, B., Grushko, T., Zhang, C., Oluwasola, O. and Malaka, D. (2009). Population differences in breast cancer: survey in indigenous African women reveals over-representation of triple-negative breast cancer. *Journal of Clinical Oncology*, ***27***(27), 4515.

Jiagge, E.M., Wong, S., Lupu, G., Qiao, M., Dziubinski, M., Newman, L.A., Carpten, J., Wicha, M. and Merajver, S.D. (2015). Prevalent loss of BRCA1 and BRCA2 expression in African TNBC suggests their prominent role in sporadic carcinogenesis. *Cancer Research*, ***75*****(**15_Suppl), 3852-3852.

Jiang, C., Lu, Y., Zhang, S. and Huang, Y. (2020). Systemic immune-inflammation index is superior to neutrophil to lymphocyte ratio in prognostic assessment of breast cancer patients undergoing neoadjuvant chemotherapy. *BioMed Research International*, ***2020,*** 1-10.

Jouali, F., El Ansari, F.Z., Marchoudi, N., Barakat, A., Zmaimita, H., Samlali, H. and Fekkak, J. (2020). EGFR, BRCA1, BRCA2 and TP53 genetic profile in Moroccan triple negative breast cancer cases. International journal of molecular epidemiology and genetics, **11**(1), 16.

Kang, C., LeRoith, D. and Gallagher, E.J. (2018). Diabetes, obesity, and breast cancer. *Endocrinology*, ***159***(11), 3801-3812.

Kumle, M., Weiderpass, E., Braaten, T., Persson, I., Adami, H.O. and Lund, E., (2002). Use of oral contraceptives and breast cancer risk: The Norwegian-Swedish Women's Lifestyle and Health Cohort Study. *Cancer Epidemiology Biomarkers & Prevention*, ***11***(11), pp.1375-1381.

Lee, D.S., Yoon, S.Y., Looi, L.M., Kang, P., Kang, I.N., Sivanandan, K., Ariffin, H., Thong, M.K., Chin, K.F., Mohd Taib, N.A. and Yip, C.H., (2012). Comparable frequency of BRCA1, BRCA2 and TP53 germline mutations in a multi-ethnic Asian cohort suggests TP53 screening should be offered together with BRCA1/2 screening to early-onset breast cancer patients. *Breast Cancer Research*, ***14***(2), 1-8.

Nwagu, G. C., Bhattarai, S., Swahn, M., Ahmed, S., and Aneja, R. (2021). Prevalence and mortality of triple-negative breast cancer in West Africa: biologic and sociocultural factors. *JCO Global Oncology*, 7, 1129-1140.

Okoye, J.O., Nnatuanya, I. N., and Okoye, J. O. (2015). Immunohistochemistry: a revolutionary technique in laboratory medicine. Clinical Medicine and Diagnosis, **5**: 60-9.

Pan, Y., Yuan, Y., Liu, G. and Wei, Y. (2017). P53 and Ki-67 as prognostic markers in triple-negative breast cancer patients. *PloS one*, ***12***(2), e0172324.

Russo, J.I.H.R. and Russo, I.H. (2006). The role of estrogen in the initiation of breast cancer. *The Journal of steroid biochemistry and molecular biology*, ***102***(1-5), 89-96.

Sardanelli, F., Carbonaro, L.A., Santoro, F. and Podo, F. (2010). Sorveglianza RM nelle donne ad alto rischio di carcinoma mammario. In *Imaging RM nella donna* (pp. 47-72). Idelson-Gnocchi.

Scully, R. (2001). Interactions between BRCA proteins and DNA structure. *Experimental cell research*, ***264***(1), 67-73.

Sollie, M. and Bille, C. (2017). Smoking and mortality in women diagnosed with breast cancer—a systematic review with meta-analysis based on 400,944 breast cancer cases. *Gland surgery*, ***6***(4), 385.

Stark, A., Kleer, C.G., Martin, I., Awuah, B., Nsiah-Asare, A., Takyi, V., Braman, M., E. Quayson, S., Zarbo, R., Wicha, M. and Newman, L. (2010). African ancestry and higher prevalence of triple-negative breast cancer: findings from an international study. *Cancer*, ***116***(21), 4926-4932.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, **71**(3): 209-249.

Vona-Davis, L., Rose, D.P., Hazard, H., Howard-McNatt, M., Adkins, F., Partin, J. and Hobbs, G. (2008). Triple-negative breast cancer and obesity in a rural Appalachian population. *Cancer Epidemiology Biomarkers & Prevention*, ***17***(12), 3319-3324.

Wellenstein, M.D., Coffelt, S.B., Duits, D.E., van Miltenburg, M.H., Slagter, M., de Rink, I., Henneman, L., Kas, S.M., Prekovic, S., Hau, C.S. and Vrijland, K., (2019). Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature*, **572**(7770), 538-542.

Xie, Y., Gou, Q., Wang, Q., Zhong, X. and Zheng, H., 2017. The role of BRCA status on prognosis in patients with triple-negative breast cancer. *Oncotarget*, ***8***(50), 87151.

Zhu, M., Chen, L., Kong, X., Wang, X., Li, X., Fang, Y., and Wang, J. (2022). The systemic immune-inflammation index is an independent predictor of survival in breast cancer patients. Cancer Management and Research. **14**, 775-820.