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Haemogram Pattern and Khorana Score of Breast Cancer Patients in A Tertiary Centre in Nigeria

Ogochukwu O. Izuegbuna[1]*, Hannah O. Olawumi[2], Samuel A. Olatoke[3], Olayide S. Agodirin[3]

[1]Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria
[2]Department of Haematology and Blood Transfusion, University of Ilorin Teaching Hospital, Ilorin, Nigeria
[3]Department of Surgery, University of Ilorin Teaching Hospital, Ilorin, Nigeria

***Corresponding author:**
Ogochukwu O. Izuegbuna
University of Ilorin Teaching Hospital
Ilorin, Nigeria
Email: ogoizu@gmail.com

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Abstract

Background

Malignancies are characterized by changes in the complete blood count. This study assessed the various derangements of complete blood count of female breast cancer patients when compared to controls, and their prognostic significance especially in relation to thrombosis using the Khorana Score.

Methods

This was a cross-sectional study. About 4.5mls of blood each was collected in an EDTA bottle from 45 breast cancer patients and 50 apparently normal controls in this cross-sectional study. Analysis was done on each sample on same day of collection on the Sysmex KN- 21N haemato- analyzer.

Results

In this study, 73.9% of the breast cancer patients had anaemia (Hb concentration < 12.5g/dl). The mean values of the Hb, PCV, RBC (11.26 ± 1.94 g/dl, 33.56 ±4.85%,4.14 ± 0.60) were significantly lower than the controls (12.67 ± 1.11 g/dl, 37.42 ± 3.08%,4.58 ± 0.42; p< 0.001, 0.001 and 0.002) respectively. Lymphocyte percentage and ALC were also significantly lower in the breast cancer patients. The mean values of the platelets, neutrophil percentage, NLR and PLR were significantly higher in the breast cancer patients. Only platelets and PLR were significantly positively correlated with tumour stage and size (Platelets. r= 0.448, p<0.002; r= 0.480 p<0.001. PLR r= 0.445, p<0.002; r=0.331, p<0.027). WBC and PLR were significant as a measure of disease progression across the stages of disease (p< 0.048 and 0.038 respectively), while platelets, WBC, and ANC were significant as a measure of tumour size (p < 0.005, 0.021 and 0.038 respectively). The Khorana Score showed that 2 (4.4%) of the patients were at a high risk of having a thrombosis.

Conclusion

Breast cancer patients have deranged CBC pattern when compared to normal controls, and with clinicopathologic significance including increased risk of thrombosis.

Keywords: *complete blood count, chemotherapy, cancer, haematological ratios, Khorana score.*

Introduction

Breast cancer is a major cause of morbidity and mortality among Nigerian women. It is estimated that worldwide about 1.7 million women were diagnosed with Breast cancer, and more than 521,000 women died of the disease in 2012(1). It is also known to be associated with social, psychological, economic and public health consequences (2). The mortality rate of breast cancer is known to be higher in developing countries than in developed countries as a result of late presentation and treatment (3). Many factors are known to influence morbidity and mortality in breast cancer patients, including stage and type of treatment. Haematological parameters have been shown to be associated with prognosis in different malignancies, and are a prerequisite before the commencement of any treatment. Blood cell count and ratios such as absolute lymphocyte counts (ALC), and neutrophil-to-lymphocyte ratio (NLR), are also known to be prognostic and predictive markers in several cancers (4,5).

Anaemia is reported to be prevalent in breast cancer patients (6), and haemoglobin levels is linked with treatment outcomes in breast cancer patients (7,8). Studies also show that patients with absolute granulocyte count of 6000/mm3 or more have a shorter survival than patients with less than 6000/mm3. Neutrophils on the other hand have been found to play a role in both cancer progression and cancer inhibition (9). Neutrophils ratios like NLR have been reported severally to be a prognostic indicator (10). Tumour associated neutrophils have been linked to poor outcomes and progression in solid cancers (9,11). Higher lymphocyte count has been linked to lower mortality in early stage breast cancer (12). Peripheral lymphocyte count has been shown to have both a predictive and a prognostic value in breast cancer patients treated with chemotherapy (13). The prognostic importance of lymphocytes in cancers generally have led to the development of the Galon immunoscore as a biomarker to aid in the classification of cancers and prediction of response to therapy (14).

A raised platelet count has been shown to be an adverse factor in some cancers. A relationship between platelets and breast cancer have been noted since 1968(15). Platelets are known to play a role in breast cancer progression including increased survival of disseminated cancer cells within the circulation, tumor cell adhesion to the endothelium, extravasation into the parenchyma of distant tissues, and ultimately the growth of tumor cells

at metastatic sites (16). Platelet ratios like the platelet lymphocyte ratio is also known to be a prognostic marker in both pre and post-chemotherapy breast cancer patients (17).

An increased risk of thrombosis is also associated with platelets levels. This along with leukocytosis and anaemia has been linked to the increased risk of thrombosis in cancer patients, and validated in an algorithm known as the Khorana Score. The Khorana Score details a risk score protocol that helps predict a cancer patient's risk of venous thromboembolism. It predicts thrombosis risk based on a collection of simple variables — type of cancer, body mass index (BMI) and complete blood count (platelet, leukocyte, haemoglobin). It was developed for a cancer outpatient setting, and equally easy to use. Each variable in the score is assigned a value, and from the sum of the values they are categorized as either low risk, intermediate risk or high risk.

Routine peripheral blood counts may thus be a useful prognostic tool in treatment in patients with cancers. The objective of the study was to determine and highlight the degree of derangements of haematological parameters in both pre and post-chemotherapy breast cancer patients when compared with apparently normal controls and evaluate their risk of thromboembolism using the Khorana Score.

Methods

This was a cross-sectional study carried out between January 2018 and June 2018 among the breast cancer patients attending Oncology clinic of University of Ilorin Teaching Hospital, Ilorin and health workers of the institution as controls. There were two groups of patients. The first group consists of histologically confirmed treatment naïve breast cancer patients, and the second group were made up of patients treated with chemotherapy only (<4 cycles), and were about to commence another cycle. A third group consist of apparently healthy, age matched adults as controls. Sample size was adequately powered with the power of the test at 80%, and a moderate effect size taken at 60% (0.6). The patients were staged clinically according to the tumor, node, and metastasis (TNM) classification. Information regarding age, education, marital status, chemotherapy, dietary habits, occupation and other relevant clinical information were also gathered through a semi structured questionnaire. Patients with Eastern Cooperative Oncology Group (ECOG) status higher than 3, patients who were pregnant, on anticoagulant or antiplatelet therapy, on growth factors, patients diagnosed of any other cancer, with any overt infection, and those hospitalized or bedridden were

excluded from this study. Ethical approval was obtained from the Institution's Ethics and Research Committee. A written informed consent was obtained from all participants.

Study procedure

Blood sample analysis

Venous blood of about 4.5 ml was collected from each participant into an EDTA bottle for a complete blood count (CBC) analysis. The CBC was determined using a Sysmex KN-21N (manufactured by Sysmex corporation Kobe, Japan) a three- part auto analyzer able to run 19 parameters per sample. Fifteen CBC parameters were considered for this study and they included: 'haemoglobin' (Hb in g/dl), 'white blood cells' (WBC in 10^9/l), 'platelet-count' (PLT in 10^9/l), 'haematocrit' (HCT in%),'red-blood cells' (RBC in 10^{12}/l), 'mean cell volume' (MCV in fl)'mean corpuscular haemoglobin' (MCH in g/dl), 'mean corpuscular haemoglobin concentration' (MCHC in g/dl), 'lymphocytes' (LYM in%), 'neutrophils' (NEUT in%), 'mean platelet volume' (MPV in fl), absolute neutrophil count (ANC in 10^9/l), absolute lymphocyte count (ALC in10^9/l). Neutrophil- lymphocyte ratio (NLR) and platelet lymphocyte ratio(PLR) were derived. NLR was defined as follows: the absolute neutrophil count divided by the absolute lymphocyte count. PLR was defined as follows: the absolute platelet count divided by the absolute lymphocyte count. On the basis of previous studies, an NLR value of 3.5(18) was used as the cutoff value to discriminate between high-NLR (>3.5) and low-NLR (<3.5), while a PLR value of 150(17) was used to equally discriminate between high PLR (>150) and low PLR (<150). Blood samples collected from the chemotherapy group were collected 48h before their next cycle of chemotherapy. The Khorana score was determined based on a collection of simple variables i.e. site of cancer, platelet count of 350 x 10^9/L or more, hemoglobin less than 10 g/dL and/or use of erythropoiesis stimulating agents, WBC count more than 11 x 10^9/L, and body mass index of 35 kg/m2. Each variable in the score is assigned a value, and from the sum of the values they are categorized as either low risk, intermediate risk or high risk (Figure 2).

Chemotherapy drugs

The following chemotherapeutic drugs were used for the treatment of cancer patients in this study: Doxorubicin, cyclophosphamide, epirubicin, 5-fluorouracil, capacetabine, paclitaxel, and docetaxel.

Data analysis

The results of 45 histologically diagnosed, consenting, breast cancer patients and 50 age-matched control were analyzed. Statistical analysis was performed using the SPSS version 23.0 statistical software package. The descriptive data were given as mean ±SD. The Pearson chi-square test and analysis of variance were used for the analytic assessment and the differences were considered to be statistically significant when the p-value obtained was <0.05. A 'Bivariate correlation' analysis conducted to see the correlation between all CBC parameters with an 'independent variable'. The r-values through 'Pearson coefficients' were obtained to observe the strength and direction of the correlation. Box-whisker plots were generated to compare mean values of all CBC parameters between treatment naive and chemotherapy breast cancer patients.

Results

In this study, 15 breast cancer patients were treatment naïve, 30 were on some form of chemotherapy, and 50 were age-matched controls. Other characteristics of the study group and control is shown in table 1. About two-third (66%) of the study group were below the age of 50 years, while 72% of the controls were in that age bracket too. Some of the study group had at least a secondary education (24.4%); comparatively 50% of the controls had a tertiary education. Of the 45 breast cancer patients, 2 (4.4%), 10 (22.2%), 22 (49%), and 11 (24.4%) were diagnosed with stages I, II, III and IV respectively.

The data in Table 2 display haematological parameters of healthy controls and patients with cancer. The mean platelet count was higher in breast cancer patients than in healthy controls. By contrast, patients with breast cancer had significantly lower mean haemoglobin (Hb) level, PCV, RBC and ALC than the controls. Compared with controls, mean NLR, and PLR were also significantly elevated in patients with breast cancer. However, when grouped as treatment naïve against those on chemotherapy, Platelet count, mean platelet volume (MPV), neutrophil %, NLR and PLR were higher in those on chemotherapy. When the control group was matched against each of treatment naïve and chemotherapy groups separately, there was a significant difference ($p < 0.05$) between the mean values of Hb, PCV, RBC, platelets, the neutrophil and lymphocyte %, ALC, NLR and PLR as displayed in Table 2.

A Bivariate correlation analysis was performed for the CBC parameters using the Spearman correlation test to know the correlations between the haematological parameters and some clinicopathological features (Table 3). Our results showed that none of the CBC parameters had any significant association with age, BMI and lymph node status. However, platelet count, WBC, ANC, NLR and PLR had significant positive correlation with ECOG status. Only platelet count and PLR were significantly correlated with tumour stage. RBC was significantly negatively correlated with both the ECOG status and tumour stage. The tumour size had significant positive correlation with platelet count and PLR only.

Some CBC parameters for different stages of breast cancer are given in table 4. The overall mean Hb, RBC, platelets, ANC, ALC and NLR of cases of breast cancer were 11.41g/dl, 4.24/l, 288/l, 3.2/µL, 1.86/µL, 1.01 respectively with no significant correlation with staging (p value 0.548, p value 0.25, p value 0.85, p value 0.915, 0.307, 0.615 respectively). While the mean WBC count and PLR were 5.55, and 177.36. Significant positive correlation was observed in both parameters (p value 0.048, and 0.038).

The Khorana score of each breast cancer patient was calculated (Figure 2) and represented on a histogram according to the risk level. More than half of the patients were low risk (53.3%), 42.2% were intermediate risk, and only 4.4% were high risk patients.

Graphical comparisons were made by box-whisker plots. Larger box lengths ranges and greater spread of data are observed in the following CBC parameters (HB, PLT, ANC, NLR, PLR) in patients on chemotherapy as compared to treatment naïve breast cancer patients. Large box length ranges and greater spread of data were observed in the RBC and WBC of treatment naïve breast cancer patients compared to patients on chemotherapy. The ALC showed almost equal box length range and spread of data in both the treatment naïve and the chemotherapy group. The medians, minimum and maximum values and upper & lower quartiles of the parameters in both groups can be noted from Figs 1: a–h. The box plots' centrality, symmetry and tail length are also observables from Figures 1: a–h.

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Table 1: Socio-demographic Characteristics of the Study Population

Variables	Catchments		χ^2	ρ
	Study (%)	Control (%)		
Age groups (Years)			**4.775**	**0.189**
< 35	4 (8.9)	13 (26.0)		
35 – 50	26 (57.8)	23 (46.0)		
51 – 65	8 (17.8)	8 (16.0)		
≥ 66	7 (15.6)	6 (12.0)		
Level of education			**5.148**	**0.161**
None	10 (22.2)	4 (8.0)		
Primary	6 (13.3)	4 (8.0)		
Secondary	11 (24.4)	17 (34.0)		
Tertiary	18 (40.0)	25 (50.0)		
Occupation			**2.014**	**0.116**
White collar	16 (35.6)	25 (50.0)		
Others	29 (64.4)	25 (50.0)		
BMI			**8.845**	**0.031**
Underweight	1 (2.2)	0 (0.0)		
Normal	17 (37.8)	31 (62.0)		
Overweight	15 (33.3)	15 (30.0)		
Obese	12 (26.7)	4 (8.0)		
Stage (n,%)				
1	02 (4.4)			
2	10 (22.2)			
3	22 (48.9)			
4	11 (24.4)			
Histologic type (n,%)				
Invasive ductal	43 (95.6)			
Lobular	2 (4.4)			
Tumour size (cm)				
< 10	25 (55.6)			
> 10	20 (44.4)			
Menopausal				
Yes	18 (40%)	16(32%)		
No	27 (60%)	34(68%)		

Table 2: Mean differences in Hematological findings of breast cancer patients and controls

Variable	Study, n=45	Chemotherapy, n=30	Treatment naive, n=15	Control, n= 50
	Mean ± (SD)	Mean ± (SD)	Mean ± (SD)	Mean ± (SD)
Hb (g/dl)	11.26 ± (1.94)‡**	10.80 ± (2.02)/*	12.18 ± (1.47)	12.67 ± (1.11)||**
PCV (%)	33.56 ± (4.85)‡**	32.54 ± (5.14) /*	35.61 ± (3.53)	37.42 ± (3.08)||**
RBC (x10^6/µL)	4.14 ± (0.60)‡**	4.02 ± (0.65) /*	4.39 ± (0.42)	4.58 ± (0.42)||**
MCV (fL)	81.17 ± (6.07)	81.19 ± (6.46)	81.12 ± (5.40)	81.62 ± (4.64)
MCH (pg)	27.25 ± (3.08)	26.90 ± (3.29) /*	27.94 ± (1.53)	27.66 ± (1.45)
MCHC (g/dl)	33.52 ± (2.01)	33.06 ± (2.08)	34.43 ± (1.54)	33.91 ± (1.02)
WBC (x10^3/µL)	5.92 ± (2.64)	5.72 ± (2.99)	6.31 ± (1.79)	6.06 ± (1.25)
PLT (x10^3/µL)	315.87 ± (132.22)‡**	315.87 ± (132.22)	286.33 ± (74.23)+*	239.84 ± (55.05)||**
MPV (fL)	10.13 ± (1.08)	10.20 ± (1.15)	9.98 ± (0.94)	10.51 ± (1.14)
NEUTR (%)	54.00 ± (14.13)‡**	54.02 ± (16.52)	53.60 ± (7.87)+**	44.05 ± (1.14)||**
LYMPH (%)	36.45 ± (13.22)‡**	36.15 ± (15.12)	37.06 ± (8.70)+**	45.32 ± (6.75)||**
ANC (x10^3/µL)	3.42 ± (2.14)	3.39 ± (2.47)	3.48 ± (1.35)+*	2.64 ± (0.66)
ALC(x10^3/µL)	1.96 ± (0.73)‡**	1.81 ± (0.76)	2.24 ± (0.58)+*	2.75 ± (0.68)||**
NLR	1.91 ± (1.34)‡**	2.04 ± (1.51)	1.64 ± (0.93)+**	1.00 ± (0.28)||**
PLR	190.83 ± (128.65)‡**	219.41 ± (143.36) /*	133.67 ± (64.75)+*	93.97 ± (44.11)||**

** p< 0.05 ** p< 0.01 ‡ Study vs Control + Treatment naïve vs Control \|\| Chemotherapy vs Control / Chemotherapy vs Treatment naïve*

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Table 3: Correlation between hematological indices of study and clinicopathologic features

Factors	Hb	PCV	RBC	WBC	PLATELET	ANC	ALC	NLR	PLR
	r_s	r_s	r_s	r_s	r_s	r_s	r_s	r_s	r_s
Age	-0.075	-0.125	-0.228	-0.123	0.062	-0.062	-0.071	-0.004	0.119
ECOG	-0.173	-0.249	-0.322*	0.427**	0.647**	0.454**	0.067	0.411**	0.357*
Stage	-0.158	-0.262	-0.325*	0.020	0.448**	0.008	-0.169	0.092	0.445**
Lymph node	0.039	0.080	0.097	0.150	0.198	0.142	-0.058	0.206	0.226
Tumour size	-0.030	-0.105	-0.207	0.283	0.480**	0.266	0.035	0.213	0.331*

** p< 0.05 ** p< 0.01 r_s Spearman rho*

Table 4: Haematological indices of study by Stage, and tumour size

Variable	Stage 1	Stage 2	Stage 3	Stage 4	Tumour size	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**< 10cm (n=25)**	**≥ 10cm (n=20)**
Hb	11.55 ± 1.48	12.03 ± 1.25	10.96 ± 1.34	11.11 ± 1.66	**Mean ± SD**	**Mean ± SD**
RBC	4.44 ± 0.47	4.45 ± 0.43	4.05 ± 0.74	4.00 ± 0.35	11.37 ± 1.79	11.12 ± 2.16
WBC*	4.25 ± 0.49	6.03 ± 1.72	6.01 ± 3.08	5.93 ± 2.75	4.21 ± 0.61*	4.06 ± 0.59
PLT	187.00 ± 41.01	257.30 ± 55.79	315.50 ± 137.21	393.27 ± 145.17	5.34 ± 1.74*	7.11 ± 3.02
ANC	2.35 ± 0.35	3.47 ± 1.49	3.44 ± 2.51	3.55 ± 2.19	268.00 ± 100.06*	375.70 ± 145.11
ALC	1.60 ± 0.14	2.12 ± 0.83	2.07 ± 0.74	1.64 ± 0.62	2.84 ± 1.50	4.16 ± 2.61
NLR	1.46 ± 0.09	1.98 ± 1.46	1.69 ± 1.17	2.33 ± 1.67	1.95 ± 0.78	1.96 ± 0.67
PLR*	118.45 ± 36.13	131.64 ± 57.35	180.11 ± 122.02	279.24 ± 158.30	1.65 ± 1.16	2.22 ± 1.52

** p< 0.05 ** p< 0.01*

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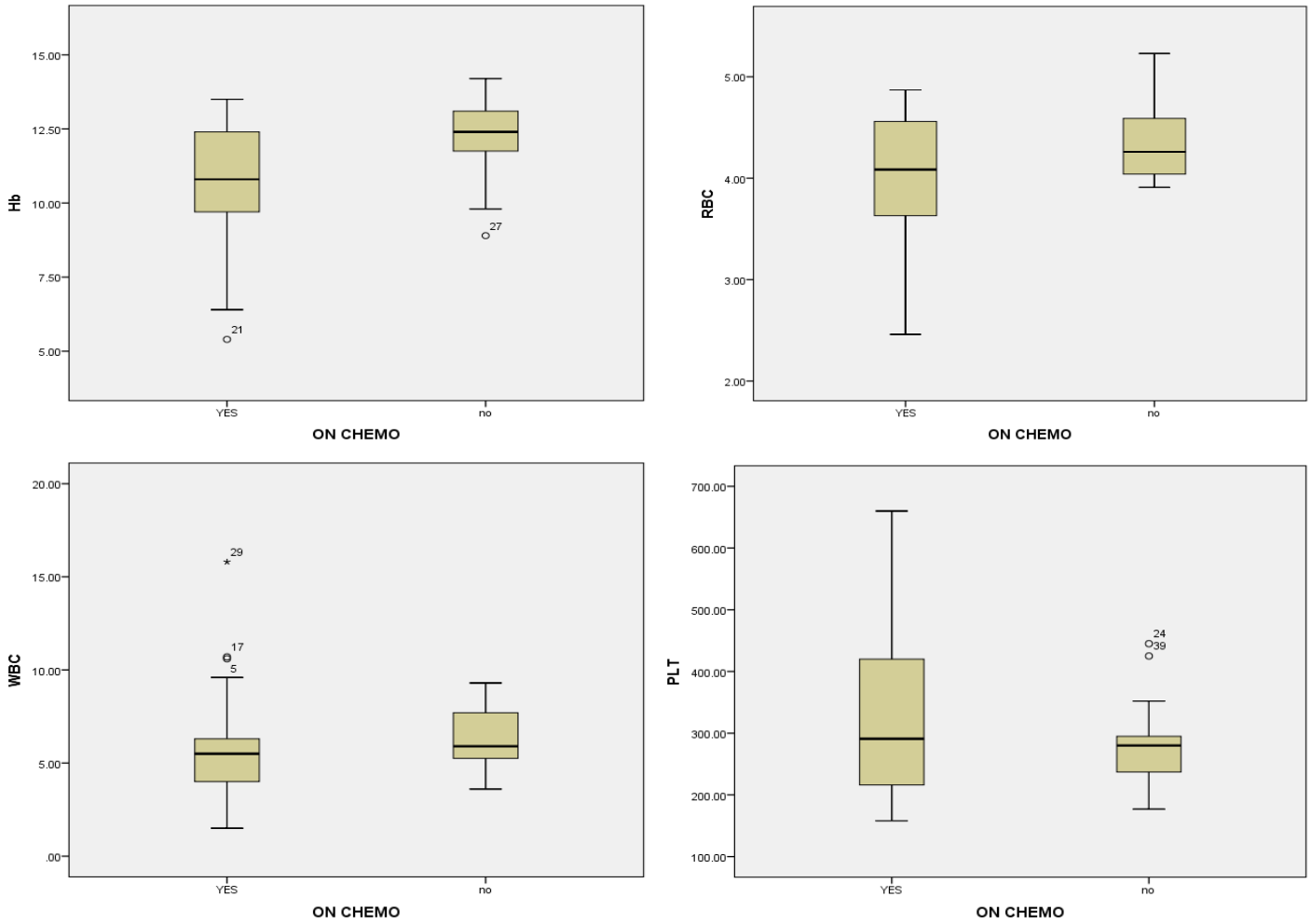

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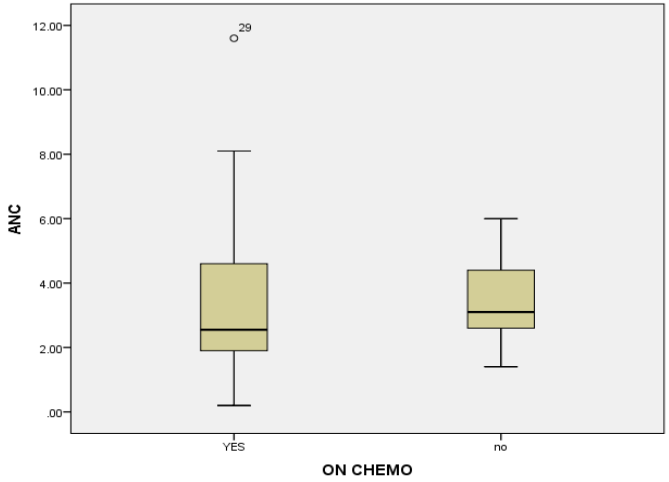

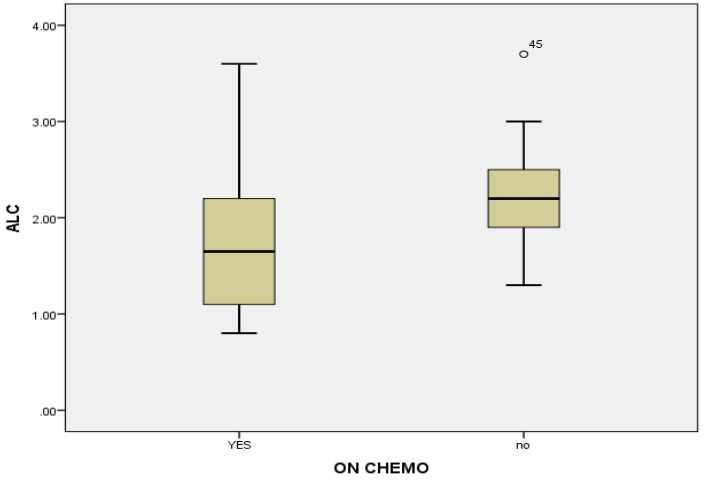

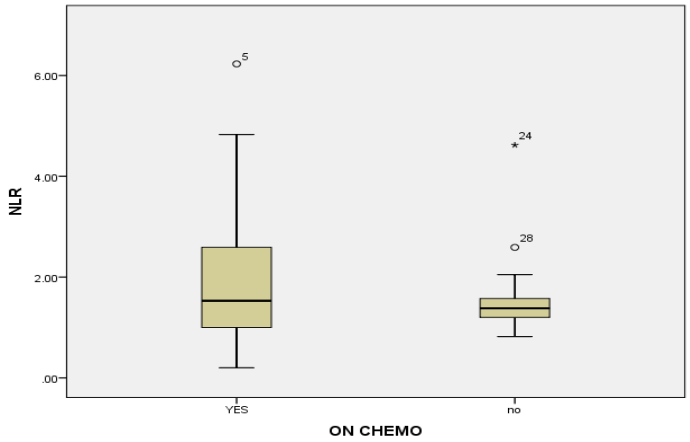

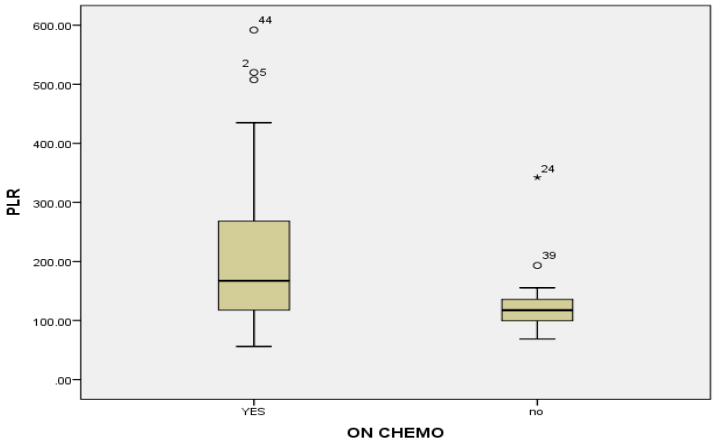

Figure 1. (a) Graphical comparison of mean Hb levels between patients on chemotherapy and treatment naïve. b) Graphical comparison of mean RBC levels between patients on chemotherapy and treatment naïve c) Graphical comparison of mean WBC levels between patients on chemotherapy and treatment naïve d) Graphical comparison of mean Plt levels between patients on chemotherapy and treatment naïve e) Graphical comparison of mean ANC levels between patients on chemotherapy and treatment naïve f) Graphical comparison of mean ALC levels between patients on chemotherapy and treatment naïve. g) Graphical comparison of mean NLR levels between patients on chemotherapy and treatment naïve h) Graphical comparison of mean PLR levels between patients on chemotherapy and treatment naïve.

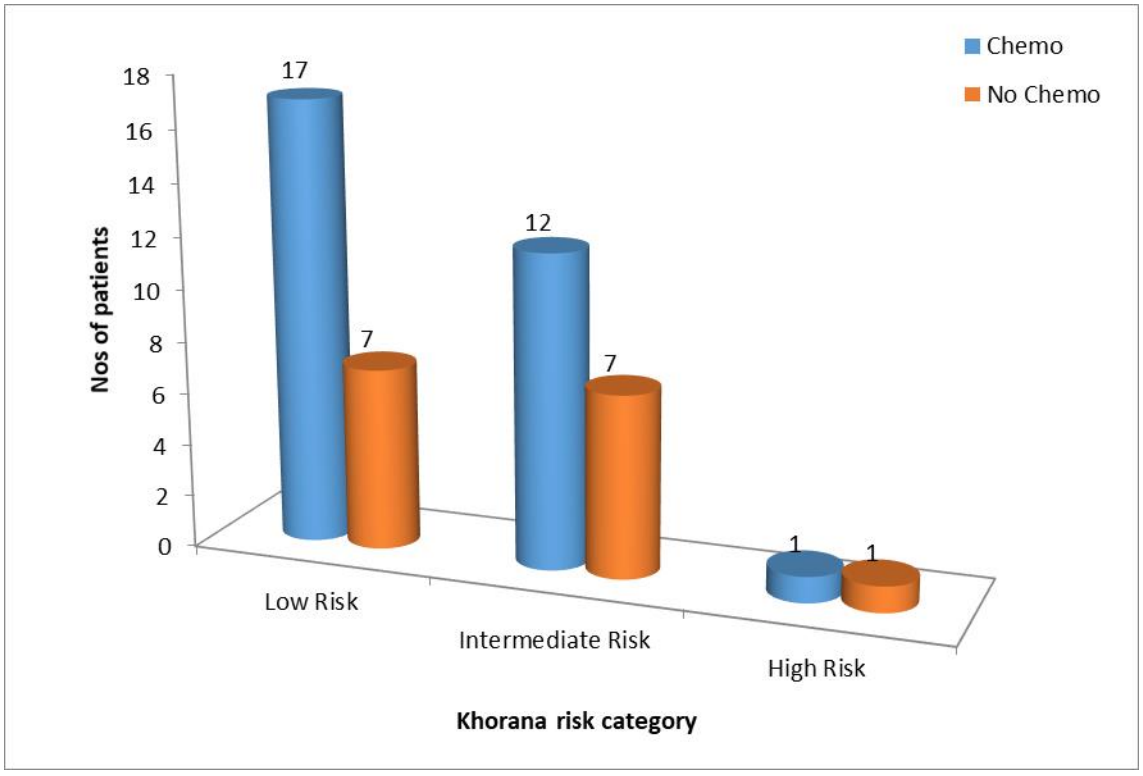

Figure 2: The risk score category of the study group using the Khorana score.

(site of cancer (breast = 0), platelet count of 350 x 10^9/L or more (1), hemoglobin less than 10 g/dL and/or use of erythropoiesis stimulating agents (1), WBC count more than 11 x 10^9/L (1), and body mass index of 35 kg/m2 (1). Each of the parameters is scored 0 or 1 if </ > than the specified variable. High risk >=3 points; Intermediate risk, 1 to 2 points; Low risk, 0 points).

Discussion

In this study, we determined the haematological indices of breast cancer patients and their thrombosis risk using the Khorana score. in this study, 73.4% of patients presented in the advanced stages III and IV. This result is consistent with what was reported by Akinbami et al who reported 71%, and Ihekwaba who reported 73.8% presented in stage III (19). Lately Ayoade et al reported 82.9% presented in stage III (20). This report like other studies show that most Nigerian breast cancer patient present late. The mean age of presentation was 49.27 ± 14.85 years. 57.8% of the patients were between the ages of 35 and 50 years - an age range where women are involved in raising children and providing for their family. Ayoade et al reported a mean age of 48 years.

In this study, the majority of the mean of the haematological indices of the breast cancer patients were lower than the controls. The mean value of Hb was 11.26 ± 1.94g/dl, which reflected an anaemic state; 73.9% of the breast cancer patients had Hb <12.5g.dl which is consistent with reported incidence rates. This was comparable to what was reported by other studies in Nigeria. The mean PCV value which is also a reflection of the Hb was equally low, and was comparable to what was reported by Akanni et al (21). Anaemia is known to be a constant feature of many types of cancers. Although there may be different causes of anaemia in cancers, it usually arises secondary to the disease ascribed as 'anaemia of chronic disease' or from the myelosuppressive effects of chemotherapy used in the management (22). Low Hb levels is known to have a prognostic impact on breast cancer, and it equally affects the quality of life, and overall survival (23). In a study done by Caro et al (24), it was reported that the overall mortality risk in cancer patients who were anaemic was 65%.

White blood cells are a diverse population of cells that play a role in immune response against tumours. They are made up of primarily granulocytes and lymphocytes. In this study the total WBC count was slightly lower than the control. This was lower than what was reported in some other studies, but while the neutrophil % and ANC were higher in breast cancer patients, the lymphocyte % and ALC were significantly lower compared to controls. These results may have clinical significance because of the role neutrophils and lymphocytes play in cancer.

The ability of cancer cells to progress and metastasize is not entirely dependent on some intrinsic mechanism within them, but also on their interactions with immune cells such as neutrophils and lymphocytes. A negative correlation has been observed between a high

ANC and breast cancer (25). Neutrophils have been reported to suppress the cytolytic activity of lymphocytes, natural killer cells, and activated T-cells when it is co-cultured with lymphocytes from normal healthy donor, and the myeloperoxidase from activated neutrophils also impairs lymphocyte function. Intratumoural neutrophils- tumour associated neutrophils (TANs) have been associated with unfavourable survival (26). Thus high neutrophils count in cancer have been implicated in cancer proliferation and metastasis (9). Though neutrophils equally play a role in cancer cell death. On the other hand, ALC have been reported to be a predictive factor in survival of breast cancer patients (27). Tumour-infiltrating lymphocytes (TIL) are known to be associated with improved survival in breast cancer patients especially in human epidermal growth factor receptor 2 (HER2) positive and triple negative breast cancers (TNBC), and high intratumoral CD8+ TIL expression was an independent prognostic factor for improved overall survival (28). Increasing evidence have shown that the immune architecture plays a role in tumour development and progression. It is known that though the AJCC/UICC-TNM classification which relies on tumour characteristics is comprehensive enough, it fails to provide a more detailed prognostic information. Clinical outcome has been known to vary significantly among patients within the same histologic tumour stage. Thus, an immune status based on a newly formed immunoscore classification have been suggested (14,29). Gabrielson et al. are reported to have applied the Galon Immunoscore to hepatocellular carcinoma and confirmed its prognostic value (30). While no research on the prognostic value of the immunoscore in cancer patients have been done here in Nigeria, it is believed that findings from this study can pioneer it.

Lately, hematological markers of inflammation, such as NLR, and PLR have been shown to have prognostic values in some cancers. A recent study shows that NLR and PLR have a predictive role in the response of breast cancer patients to neoadjuvant chemotherapy (10). In this present study, we found a significant difference in the NLR and PLR values of breast cancer patients compared to controls. This result is consistent with previous studies that concluded that NLR and PLR were higher in breast cancer patients than in controls (17). However, between treatment naïve and patients on chemotherapy in this study, the mean NLR was higher in the chemotherapy group, but was not significant (p= 0.357). PLR was higher in the chemotherapy group, and showed significance (p= 0.033). There was equally significant correlation between PLR and stage, ECOG, but there was no significant correlation between NLR and stage; only with ECOG. The mean values of NLR and PLR at various disease stage in this study, showed that the stage 4 disease has higher values than

other stages, and again PLR was the only marker that was significant. From this study it can be inferred that PLR is a more sensitive prognostic marker in breast cancer patients than NLR.

The relationship between platelets and cancer have been well documented, and also demonstrated, in an animal model; there was a 50% reduction in tumour metastasis after experimental thrombocytopenia induced with neuraminidase and antiplatelet serum (15). This anti-metastatic effect was effectively reversed with infusion of platelet-rich plasma (blood plasma enriched with platelets obtained by centrifugation and removal of red blood cells). Along with its role as a mediator of haemostasis, platelets are known to play a role in immune and inflammatory responses. Thrombocytosis is a known feature of malignancies, and it has been shown to be a prognostic factor in breast cancer. In this study, platelet count was significantly higher in breast cancer patients than in controls, and except for stage 1 disease, it was also higher in stage II-IV. In this report, thrombocytosis was defined as platelets more than 400×10^9/L and was present in 22.2% of all breast cancer patients. The mean platelet count was highest in patients with stage IV disease (Table 4; 393.27 ± 145.17). This was also reported by Elyasinia et al (10) where the highest stage in their study, stage 3C also had the highest platelet count. This shows a close association between platelets and cancer progression. Platelets are known to shield tumour cells from shear forces and assault of NK cells, recruit myeloid cells by secretion of chemokines, and mediate an arrest of the tumour cell platelet embolus at the vascular wall (31). The ability of cancer cells to metastasize is dependent on their ability to activate platelets. Platelet activation is achieved by triggering an aggregation of platelets called tumour cell induced platelet aggregation (TCIPA). TCIPA can occur via direct contact with tumour cells or by various mediators like ADP, thromboxane A2, or serine proteinases, including thrombi. ADP released from MCF-7 breast cancer cells has been found to induce platelet aggregates via activation of the platelet P2Y12 receptor (21). The use of anti-platelet drug has been muted as a way to attenuate metastatic spread. The Nurses' Health Study, which reported an association between aspirin use and a decrease in distant recurrence and improved survival in women with breast cancer who had survived a minimum of one year following a cancer diagnosis have often been cited as a potential study towards the use of anti-platelets in breast cancer management (32).

Cancer is known to be an acquired thrombophilic state. About 15 to 20% of all cancer patients develop thrombosis during the course of their disease, which can be as a result of

the cancer itself, or the drugs used in the management. Women with breast cancer are reported to have a three- to fourfold increased risk of venous thromboembolism (VTE) compared with women of an equivalent age without cancer (33). On the other hand, breast cancer patients with metastatic disease or those receiving chemotherapy have a 10-fold increase in VTE risk when compared with the breast cancer population as a whole. The identified risk factors include metastatic disease, chemotherapy and tamoxifen treatment (34). As a result of the increased risk of developing VTE, a risk model was derived and validated in an independent cohort of 1365 patients. This risk model was developed based on a number of predictive variables which included site of cancer, platelet count of 350 x 10^9/L or more, hemoglobin less than 10 g/dL and/or use of erythropoiesis stimulating agents, WBC count more than 11 x 10^9/L, and body mass index of 35 kg/m2 and became known as the Khorana score (35). It became the basis for selecting cancer outpatients at risk of VTE for thromboprophylaxis. The high risk category are the beneficiary of thromboprophylaxis.

Although breast cancer patients are regarded as low risk patients to develop VTE, but due to the large number of breast cancer patients compared to other cancers, breast cancer–associated VTE accounts for approximately 17% of cancer-related VTEs (36). In this present study, using the Khorana score only 4.4% of all the study were in the high-risk category (Figure 2). This means most of the breast cancer patient in this study are not at a high risk of venous thromboembolism. The rate of VTE from the validated study were 0.8% and 0.3% in low-risk (score 0), 1.8% and 2% in intermediate-risk (score1-2), and 7.1% and 6.7% in high-risk (score> 3) category over a median of 2.5 months. A search of the literature reveal that this study is first to report the risk of VTE in breast cancer patients using the validated Khorana score among Nigerian cancer patients. It is expected that this will spur more clinical works in searching out cancer patients that will benefit from thromboprophylaxis in Nigeria, and perhaps identify other risk factors.

Conclusion

In summary, in this study the CBC of breast cancer patients were compared with normal controls. It was observed that anaemia, elevated platelet count, higher neutrophil count, low lymphocyte count were common features seen in breast cancer patients. Haematologic markers of inflammation like NLR and PLR were also elevated in breast cancer patients. PLR, which is easily calculated, readily accessible and inexpensive, could be a predictor of severity of breast cancer and might be used to manage the disease. The Khorana score shows that not many breast cancer patients are at a high risk of developing VTE.

Limitation

The study has some limitations. First, the limited number of breast cancer patients and the data from only one centre may affect the conclusion in our study. Due to cost and limited expertise, most patients did not carry out immunohistochemistry tests such as estrogen, progesterone receptors, HER-2, and Ki- 67, so they could not be reported.

Ethical statement

This study received all the necessary ethical clearance from the hospital ethical review committee; informed consent of subjects was obtained.

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

OI was involved in the design of the study, data acquisition, analysis and writing of the first draft manuscript. HO, SO were involved in the design of the study, data analysis, coordination, and corrections of the draft manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, [OI], upon reasonable request.

Abbreviations

Hb	Haemoglobin
CBC	Complete Blood Count
Plt	Platelets

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NLR	Neutrophil Lymphocyte Ratio
PLR	Platelet Lymphocyte Ratio
ALC	Absolute Lymphocyte Count
ANC	Absolute Neutrophil Count
LYMPH	Lymphocyte
NEUTR	Neutrophil
MPV	Mean Platelet Volume
PCV	Packed Cell Volume
RBC	Red Blood Cell Count
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
BMI	Body Mass Index
ECOG	Eastern Cooperative Oncology Group

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