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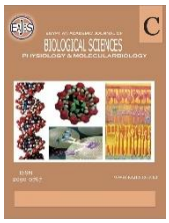

Neutrophil Lymphocyte Ratio In Breast Cancer: Prognostic Value

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ABSTRACT

Introduction: Breast cancer (BC) is a common female cancer worldwide including in Sudan. Despite the improvement in diagnosis, still more effort is needed for prognostic biomarkers. **Objectives**: To assess the predictive value of neutrophils-lymphocytes ratio (NLR) in BC disease-free survival (DFS) and overall survival (OS). **Method:** This was a retrospective hospital-based study including 65 stage-III BC female patients treated at the National Cancer Institute -University of Gezira (NCI-UG), Sudan between 1st January 2013 and 31st December 2014 and followed up to 2019. Data were retrieved from medical records and SPSS software was used for statistical analysis. X^2 test was used to demonstrate the association of NLR with demographic, clinicopathological and therapeutic variables. Cox regression models were used to determine the hazard ratios and 95% confidence intervals (CIs) of DFS and OS in relation to pre-treatment NLR. **Results:** Analysis showed that patients with high NLR had a high rate of postoperative recurrence and death. From the 65 investigated BC patients, 20 (31%) have abnormally higher than normal NLR (> 3%). Of those 31% BC patients, 70% were in the age group > 40 years old, 75% were histologically grade III, 95% were invasive ductal carcinoma (IDC) histological type, 55% were postmenopausal, 89.5% were lymph node-positive, and 70% not underwent radiotherapy. NLR was identified as an independent prognostic factor for DFS and OS in stage III BC ($P < 0.05$). **Conclusion**: It had been concluded that high NLR was associated with decreased DFS and OS and a higher rate of postoperative recurrence and death in BC.

INTRODUCTION

BC is the most common cancer in women. Despite much progress in treatment, it is still the second most common cause of death in women in the United States (Jemal *et al.*, 2006). Incidence is higher in the developed countries than in the developing, and in urban areas versus rural (Parkin *et al.*, 2005; Igene, 2008). BC prognosis depends on the stage of the tumor and molecular subtypes. Additional predictive and prognostic biomarkers are needed to better adapt the treatment to each individual patient (Cardoso *et al.*, 2018; Cardoso *et al.*, 2019). Nowadays, there is evidence that the systemic immune inflammation index and neutrophil-lymphocyte ratio are useful in predicting the prognosis of many cancers (Fu *et al.*, 2018; Gkika *et al.*, 2018). Activated inflammatory cells are sources of reactive oxygen species and reactive nitrogen intermediates that can induce DNA damage and genome instability, thus promoting cancer initiation (Grivennikov *et al.*, 2010; Greten and Grivennikov, 2019), or interfere with the DNA repair systems (Colotta *et al.*, 2009).

Neutrophils promote the growth of the tumor by secreting factors that facilitate tumor proliferation, invasion, and distant metastasis. In contrast, lymphocytes, especially cytotoxic T cells have an important role in the anti-tumor response by promoting apoptosis and suppressing tumor growth (McCourt *et al.,* 2001; Mahmoud *et al.,* 2011). NLR, which has a comprehensive evaluation of the balance between systemic inflammation and immunity, plays a necessary role in the prognostic prediction of various malignancies (Gregory and McGarry Houghton, 2011; Guthrie *et al.,* 2013). In Sudan, BC is the most common cancer, yet there is no previous study on the prognostic value of NLR on the disease.

MATERIALS AND METHODS

Study Design and Settings:

This was a retrospective hospital-based study to evaluate the prognostic value of NLR in patients mostly diagnosed with stage III BC treated at NCI-UG between January 2013 and December 2014. The study was conducted in the NCI-UG, Wad Medani city, Sudan. In Sudan, there are only three specialized oncology centers and NCI-UG is one of them. It consists of the oncology department, nuclear medicine, molecular biology, laboratories, a blood bank, and an outpatient clinic. The institute is attended by patients from various areas in Sudan which makes it a suitable place to conduct the study.

Study Population:

They were BC patients who attended NCI-UG from January 2013 to December 2014, then followed up to 2019. The inclusion criteria were patients with BC of the age of 18 – 85 years old, while the exclusion criteria were patients with prior anti-cancer treatment, patients with inflammatory, immune, coronary artery, and hematological diseases, early and metastatic BC, patients with inflammatory BC, and patients using anti-inflammatory or immunosuppressive drugs.

Data Collection Tool:

The data was collected in a pre-designed form (questionnaire) from patients' medical records. The data included personal data (age and gender), past medical and drug history, current acute and chronic disease other than BC, histopathology and grade of tumor, and setting of treatment.

Data Management and Statistical Analysis:

The collected data was analyzed using the Statistical Package for Social Science (SPSS) version (25) computer program, ANOVA style. The association of demographic, clinical pathological, and therapeutic characteristics between testing cohorts and validation cohorts was analyzed using the X^2 test. The Cox proportional hazards regression model was used. ($P \leq 0.5$) was considered statistically significant.

RESULTS

From Table 1, of the 65-BC women patients, the majority (89.2%) were married and 73.8% were at the age of 40 years old and older. 95.4% of the patients had invasive ductal carcinoma (IDC) histological type of BC, while 55.4% of patients were grade III BC. The major type of surgery (73.9%) was breast cancer sectioning (BCS), while modified radical mastectomy (MRM) was only 24.6%. In our study, only 37 (65.9%) of women had available hormonal receptor (HR) status, in those whose HR status was available, about 21 (32.3%) had negative HR (both estrogen receptor (ER) and progesterone receptor (PR)).

From the 65 investigated BC patients, 20 (31%) had abnormally higher than normal NLR ($\geq$ 3%). Of those 31% BC patients whose NLR was higher than normal, 70% were in the age group > 40 years old, 75% were histologically grade III BC, 95% were IDC histological type, 75% were BCS surgery, 60% were HR negative, 55% were menopausal, 89.5% were lymph node-positive, and 70% not underwent radiotherapy (Table 2).

Table 1: Tumor and treatment characteristics in women with breast cancer (BC).

Characteristic		Frequency	Percent
Age at diagnosis (year)	<40 Years	17	26.2
	≥ 40 Years	48	73.8
	Total	65	100
Marital status	Married	58	89.2
	Un-Married	7	10.8
	Total	65	100
Histological grade	G I & II	29	44.6
	G III	36	55.4
	Total	65	100
Histological type	IDC	62	95.4
	Other	3	4.6
	Total	65	100
Type of surgery	BCS	48	73.9
	MRM	16	24.6
	Not Done	1	1.5
	Total	65	100.0
Hormone Status	HR Negative	21	32.3
	HR Positive	16	24.6
	Not Done	28	43.1
	Total	65	100

Table 2: NLR among Stage III BC patients in association with demographic, clinical-pathological, and treatment variables.

Characteristic		NLR				P-value
		<3 NLR		≥3 NLR		
		Patients	%	Patients	%	
Age Group	<40 Years	11	24.4	6	30	0.472
	> 40 Years	34	75.6	14	70	
	Total	45	100.0	20	100	
Marital status	Married	42	93.3	16	80	0.123
	Un-Married	3	6.7	4	20	
	Total	45	100.0	20	100	
Histological grade	GI&II	24	53.	5	25	0.098
	G III	21	46.7	15	75	
	Total	45	100.0	20	100	
Histological type	IDC	43	95.6	19	95	0.208
	Others	2	4.4	1	5	
	Total	45	100.0	20	100	
Type of surgery	BCS	33	73.3	15	75	0.798
	MRM	11	24.4	5	25	
	Total	45	100.0	20	100	
Hormone Status	HR Negative	15	55.6	6	60	0.555
	HR Positive	12	44.4	4	40	
	Total	27	100.0	10	100	
Menopausal status	Premenopausal	11	25.0	9	45	0.096
	Postmenopausal	33	75.0	11	55	
	Total	44	100.0	20	100	
Lymph node	Positive	39	88.6	17	89.5	0.647
	Negative	5	11.4	2	10.5	
	Total	44	100.0	19	100	
Radiotherapy	Yes	26	57.8	6	30	0.035
	No	19	42.2	14	70	
	Total	45	100.0	20	100	

Table 3: Relation between NLR and disease-free survival (DFS) in stage III BC.

NLR	Mean			
	Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
<3 NLR	44.439	4.373	35.869	53.010
≥3 NLR	11.625	2.569	6.591	16.659
Overall	37.375	4.331	28.887	45.863

Patients with lower (<3) NLR showed significantly longer DFS (44.5 months) than those with higher (≥3) NLR (11.6 months) (P = 0.001).

Table 4: Relation between NLR and OS in stage III BC.

NLR	Mean			
	Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
<3 NLR	54.781	5.057	44.870	64.692
≥3NLR	26.455	6.717	13.290	39.619
Overall	47.535	4.546	38.625	56.445

The results also showed that even in patients with grade III BC, the overall survival (OS) was significantly higher with low (<3) NLR (54.8 months) compared to patients with high (≥3) NLR (26.5 months) (P = 0.002) (Table4). In our study, it was found that NLR was a significant predictor for OS (P = 0.033).

DISCUSSION

The immune system has a significant impact on the tumour occurrence and progression. Our study showed an increase in NLR in 31% of our BC patients' population. The higher NLR was associated with decreased DFS and OS. These obtained results were supported by several researchers who indicated that NLR is a useful biomarker for predicting the survival outcome of breast cancer (Guthrie *et al.,* 2013; Koh *et al.,* 2015; Ethier *et al.,* 2017). A meta-analysis of 39 trials including 17079 breast cancer patients showed that the increase of NLR before treatment was correlated with poorer overall survival (OS) (Swierczak *et al.,* 2015; Guo *et al.,* 2019). It has been concluded that NLR is an independent prognostic factor for overall survival in oligometastatic BC and a prognostic model, including the NLR, will help identify patients with a favorable prognosis (Yuka *et al.,* 2022). Preoperative NLR can be a useful prognostic marker, especially in postmenopausal breast cancer patients (Hideki *et al.,* 2024). In another meta-analysis study, although NLR hold clinical promise in their association with poor cancer prognosis, further research is required to provide clear-cut evidence, assess causality and test clinical utility (Meghan *et al.,* 2020). A growing body of researchers has shown that inflammation predisposes the development of cancer and promotes all stages of tumorigenesis (Greten and Grivennikov, 2019; Maiorino and Egeblad, 2019; McAndrew *et al.,* 2021). In recent years, the elevated peripheral blood neutrophil-to-lymphocyte ratio (NLR) has been proven to be an effective indicator for predicting the poorer prognosis of various cancers (Guthrie *et al.,* 2013).

Conclusion:

From the results, it had been concluded that high NLR was associated with decreased DFS and OS and a higher rate of postoperative recurrence and death in BC.

Declarations:

Ethical Consideration: Ethical approval was obtained from the research ethical committee (REC) of the NCI-UG. A code had been used to ensure patients' confidentiality. Written consent was obtained from the dean of NCI-UG.

Conflict of interests: The authors declare no conflicts of interest.

Authors Contributions: All authors contributed equally, and have read and

agreed to the published version of the manuscript.

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Availability of Data and Materials: The data presented in this study are available on request from the corresponding author.

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ARABIC SUMMARY

نسبة العدلات الى الخلايا اللمفاوية في سرطان الثدي: دراسة تنبؤية

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مقدمة: يعد سرطان الثدي الأكثر أنتشارا ضمن السرطانات الأنثوية على مستوى العالم و السودان. رغم التحسن الملحوظ في التشخيص يجب بذل المزيد من الجهد لدراسة المؤشرات الحيوية المتعلقة بالتنبؤ بحالة المرض.

الأهداف: دراسة القيمة التنبؤية لنسبة العدلات الي الخلايا اللمفاوية في في فترة العيش الخالية من الأمراض و فترة العيش الكلية في سرطان الثدي. **الطريقة:** هذه دراسة ذات أثر رجعي مقرها المستشفي أجريت على 65 أمرأة مريضة بسرطان الثدي تخضع للعلاج في المعهد القومي للأورام – جامعة الجزيرة – السودان في الفترة ما بين الأول من يناير 2013م و حتي الحادي و الثلاثين من ديسمبر 2014م و من ثم المتابعة حتي 2019م. تم أستخلاص البيانات المطلوبة من التقارير الطبية المحفوظة و من ثم تم أستخدام برنامج الحزم الأحصائية للعلوم الأجتماعية للتحليل الأحصائي. تم أستخدام أختبار مربع كاي لتوضيح علاقة نسبة العدلات الى الخلايا اللمفاوية مع متغيرات ديموغرافية، سريرية أمراضية و علاجية. تم أستخدام نماذج أنحدار كوكس لتحديد نسب الخطر كما تم أستخدام 95% نطاق ثقة لفترة البقاء على قيد الحياة بدون مرض آخر و معدل البقاء على قيد الحياة بصورة عامة و علاقة ذلك بنسبة العدلات الى الخلايا اللمفاوية. **النتائج:** أوضح التحليل أن المرضى ذوي نسبة العدلات الي الخلايا اللمفاوية العالية لهن نسبة تكرار حدوث للمرض بعد التدخل الجراحي و موت أعلى. 20 (31%) من جملة ال 65 مريضة بسرطان الثدي و اللائي تمت دراستهن لهن نسبة عدلات الي خلايا لمفاوية أعلى من الطبيعي (3% <). من أولئك ال 31% مريضة يسرطان الثدي، 70% كن في الفئة العمرية أكثر من 40 عام، 75% حسب التصنيف النسيجي الأمراضي من الدرجة III ، 95% كن نسيجيا حالات سرطان فصيصي غزوي، 55% كن في حالات أنقطاع طمث، 89,5 % كن أيجابيات العقد اللمفاوية للمرض، و 70% لم يخضعن لعلاج أشعاعي. نسبة العدلات الي الخلايا اللمفاوية عرفت كعامل تنبؤي مستقل لفترة البقاء على قيد الحياة بدون مرض آخر و معدل البقاء على قيد الحياة بصورة عامة في الدرجة III في سرطان الثدي ($P< 0.05$). **الأستنتاج:** لقد تم أستنتاج أن نسبة العدلات الى الخلايا اللمفاوية العالية متعلقة بقصر فترة البقاء على قيد الحياة بدون مرض آخر و معدل البقاء على قيد الحياة بصورة عامة و أرتفاع نسبة تكرار حدوث للمرض بعد التدخل الجراحي و موت أعلى.