RESEARCH ARTICLE

Editorial Process: Submission:10/24/2023 Acceptance:02/29/2024

Evaluation of the Cutoff Point and Diagnostic Value of the Neutrophil-to-Lymphocyte Ratio in Predicting Ovarian Cancer Compared to Pathological Findings

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Abstract

Purpose: This research aims to establish a neutrophil-to-lymphocyte ratio (NLR) threshold and evaluate its diagnostic accuracy compared to pathological criteria for diagnosing Epithelial Ovarian Cancer (EOC). **Methods:** We conducted a cross-sectional study at Imam Hossein Hospital involving 204 women aged 18 and older with confirmed ovarian mass based on pathology. We recorded clinical, pathological, and preoperative blood count data, including neutrophil-to-lymphocyte ratio (NLR). Patients were categorized into malignant and benign ovarian mass groups based on postoperative pathology. The power of NLR to diagnosis of EOC was evaluated using ROC curve. **Results:** At total, 204 patients (Benign 75.5% vs. Malignant 24.5%) were included in the analysis with mean age of 54.26 ±12.04 yrs in malignant and 46.31±13.21 in benign. In all cases, the proportion of patients with the following tumor markers HE4 (>140 Pm), CA 125 (> 35U/Ml) and CEA (>5 ng/Ml) were 52.45%, 41.67% and 3.43%, respectively, and proportion of abnormal tumor markers was statistically higher in malignant group compared to benign mass ($p < 0.05$). Odds of having higher NLR levels in the malignancy group was higher than benign group (e.g., OR of 4.45 for NLR in quartile 4 vs. quartile 1). According to model selection criteria, the full model with including NLR level and age, BMI and tumor markers has best performance for diagnosis of malignancy (AUC =0.87). **Conclusion:** High NLR in combination with tumor markers including CA125, HE4 and CEA were associated with malignancy in patients with ovarian mass. More attention and further examinations should be devoted for patients with ovarian mass having high NLR and abnormal tumor markers levels to detect the probable malignancy as soon as possible.

Keywords: Neutrophil-to-lymphocyte ratio- epithelial ovarian cancer- tumor marker

Asian Pac J Cancer Prev, **25 (3)**, 971-976

Introduction

Ovarian cancer (EOC) ranks as the seventh most prevalent cancer globally and is the deadliest among gynecological malignancies [1]. Most patients are diagnosed with advanced-stage disease, which is associated with significant morbidity and mortality [2]. EOC is a silent malignancy, and survival rates strongly depend on early detection. The discovery of reliable EOC biomarkers plays a crucial role in disease management and greatly impacts patient prognosis [3]. Population-based screening has been ineffective, but new approaches for early detection and prevention are currently being developed [4]. Following its discovery about 40 years ago, carbohydrate antigen 125 (CA125) has been widely used but remains the most concerning biomarker in EOC screening. However, there is still controversy regarding its role in clinical practice. CA125 is not sufficiently reliable for diagnosing early-stage EOC. On the other hand, CA125 has been a valuable marker for assessing treatment efficacy and prognosis. We still have limited knowledge about its biological role, and multiple studies have shown its involvement in EOC development and progression [5]. While CA125 is a classical EOC biomarker, current research aims to find alternative biomarkers using proteomic or metabolomic approaches, utilizing non-invasive or minimally invasive sources such as urine, serum, plasma, tissue, ascites, or

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exosomes. Metabolism and metabolites play key roles in cancer biology, and their significance in the discovery of biomarkers cannot be overlooked [3]. Inflammatory markers, such as the neutrophil-to-lymphocyte ratio (NLR), have been studied in various cancers prior to surgery [6]. NLR is a useful factor in evaluating immune status [7], and is linked to epithelial ovarian cancers (EOCs), making it a useful tool for predicting post-treatment follow-up. Elevated NLR levels are correlated with an unfavorable prognosis. The results of conducted studies have shown that NLR may be a good marker for use in EOC screening [6].

Hence, it is crucial to find a solution to avoid false-negative outcomes resulting from low CA125 levels, specifically in the detection of EOC during regular physical examinations. Building upon the information mentioned above, this research project seeks to establish a threshold value for the neutrophil-to-lymphocyte ratio, in comparison to pathological criteria, for diagnosing EOC in patients with suspected EOC. Subsequently, the study aims to assess the sensitivity, specificity, positive predictive value, and negative predictive value of the determined threshold in relation to the pathological criteria for diagnosing EOC.

Materials and Methods

This cross-sectional study was conducted in 2022 at Imam Hossein Hospital and included 204 women aged 18 and above who were diagnosed with ovarian mass confirmed by pathology. The inclusion criteria consisted of women 18 years and older with an ovarian mass who required surgery and had malignant or benign disease confirmed by pathology. The exclusion criteria were concurrent infectious diseases, autoimmune diseases, current or history of extra-ovarian malignancy, systemic hematological disorders and thrombosis as criteria for withdrawal from the study.

After obtaining the ethical code from Shahid Beheshti University of Medical Sciences, and receiving approval for the study protocol, informed consent was obtained from the patients. At the time of initial diagnosis, clinical and pathological information of the patients was recorded. After surgery, the pathology diagnosis determined the benign or malignant nature of their ovarian mass. Additionally, preoperative blood count values, including absolute neutrophil count, absolute lymphocyte count and NLR (neutrophil-to-lymphocyte ratio), were recorded and calculated for the purpose of this study. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The study variables were determined based on the patients' clinical records, which included age, gravidity, BMI, menstruation, involvement of the right or left ovary, family history of cancer, diabetes, hypertension, and hypothyroidism morbidity. In addition, laboratory tests were conducted to measure complete blood cell count (platelet, neutrophil, and lymphocyte counts), as well as Ca_125, CEA, and HE4 tumor markers. These variables were considered important for the analysis and evaluation of the study.

Based on the postoperative pathological results reviewed by a trained pathologist, enrolled patients were divided into two groups, including 50 malignant and 154 benign ovaraion cancer.

Continuous variable data were reported as means ± standard deviation, while categorical variable data were presented as frequencies and percentage. For comparisons between two groups of benign and malignant masses, Student's t-test was employed. The chi-square test was used to compare rates or frequencies. The association between NLR and postoperative pathological diagnosis were assessed using univariate and multivariable logistic regression model. The power of NLR to diagnosis of EOC was evaluated using ROC curve, sensitivity and specificity. Data were analysed using Stata-17 software. The significant level was considered less than 0.05.

Results

At total, 204 patients were included in the analysis with mean (SD) age of 48.25 (13.35) years. Malignant and benign tumors composed 50 (24.51%) and 154 (75.49%) of the patients, respectively. High-grade serous carcinoma (70%), mucinous ovarian cancer (14%) and endometrioid carcinomas (12%) were most common subtypes of EOC, while serous cystadenoma (35.1%), mucinous cystadenomas (20.8%) and mature cystic teratoma (18.2%) were most common bengin ovarian masses, respectively (Figure 1).

Patient characteristics according to the type of tumor as well as resulting odds ratio (95% confidence interval) are presented in Table 1. Compared to benign mass group, patients with ovaian malignancy were significantly older, with lower BMI, reduced Hb level, increased platelets level and higher proportion of both ovaries' involvement (p-value<0.05). The proportion of patients with HE4 (>140 Pm), CA 125 (> 35U/Ml) and CEA (>5 ng/Ml) were 52.45%, 41.67% and 3.43%, respectively and proportion of abnormal tumor markers was statistically higher in malignant group compared to benign tumor (p<0.05). Odds of having higher NLR levels in the malignancy group was higher than benign group (e.g., OR of 4.45 for NLR in quartile 4 vs. quartile 1).

The multivariable analysis for the association between NLR level and ovarian malignancy are shown in Table 2, in which we showed ORs (95% CI) for the quartile 2, 3 and 4 vs. quartile 1 after adjusting for age, BMI and tumor markers. The predictive performance of the developed models is shown in Figure 2. In all developed models higher NLR levels (quartile 4) was identified as significant predictor of malignancy, however, in the model 2, NLR exhibited no significant association with malignancy after adjusting CA125. Odds of malignancy in patients with NLR of 3.87 to 55.41 compred to those with NLR of 0.14 to 1.60 were 7.08, 4.14 and 4.32 after adjusting age and BMI, HE4 and CEA, respectively. The corsponding OR (95% CI) for NLR quartile 4 vs quartile 1 in the model 5 (full model) was 3.66 (95% CI: 1.00, 13.49). According to model selection criteria, the full model with including NLR level and age, BMI and cancer tumor markers has best performance for diagnosis of tumor malignancy (AIC=166.46, BIC=196.33, AUC =0.87) (Table 2 and

Table 1. Patient Characteristics According the Type of Tumor as Well as Resulting Odds Ratio (95% Confidence Interval)

Characteristics	Malignant (N=50)	Benign (N=154)	OR (95% CI)	p-value
Age (yrs)	54.26 (12.04)	46.31 (13.21)	1.05 (1.02, 1.07)	<0.001
Gravidity				
0	10 (20.00)	33 (21.43)	Reference	
1	3 (6.00)	14 (9.09)	0.71 (0.17, 2.96)	0.63
2	8 (16.00)	37 (24.03)	0.71 (0.25, 2.02)	0.52
3	13 (26.00)	29 (18.83)	1.47 (0.56, 3.87)	0.42
>3	16 (32.00)	41 (26.62)	1.29 (0.51, 3.21)	0.59
BMI (kg/m^2)	25.19 (3.70)	27.84 (4.28)	0.84 (0.76, 0.92)	<0.001
Menstruation				
Regular periods	8 (16.00)	50 (32.47)	Reference	
Irregular periods	11 (22.00)	45 (29.22)	1.50 (0.56, 3.97)	0.41
Hysterectomy	0	12 (7.79)	0.23 (0.01, 4.40)	0.33
Menopause	31 (62.00)	47 (30.52)	3.94 (1.67, 9.26)	0.002
Rh blood group				
Positive	43 (87.76)	139 (90.26)	Reference	
Negative	6 (12.24)	15 (9.74)	1.29 (0.47, 3.53)	0.62
Diabetes				
No	43 (86.00)	135 (87.66)	Reference	
Yes	7 (14.00)	19 (12.34)	1.15 (0.45, 2.93)	0.76
Hypertension				
No	35 (70.00)	119 (77.27)	Reference	
Yes	15 (30.00)	35 (22.73)	1.45 (0.71, 2.97)	0.3
Hypothyroidism				
No	46 (92.00)	137 (88.96)	Reference	
Yes	4 (8.00)	17 (11.04)	0.70 (0.22, 2.19)	0.54
Family history				
No	38 (76.00)	134 (87.01)	Reference	
Yes	12 (24.00)	20 (12.99)	2.11 (0.95, 4.71)	0.07
Tumor size	87.12 (6.55)	80.18 (4.07)	1.002 (0.99, 1.008)	0.39
Ovarian involvement,				
Left	12 (24.00)	83 (53.90)	Reference	
Right	21 (42.00)	58 (37.66)	2.50 (1.14, 5.48)	0.02
Both	17 (34.00)	13 (8.44)	9.04 (3.52, 23.20)	<0.001
HE4				
<140	16 (32.00)	81 (52.60)		
>140	34 (68.00)	73 (47.70)	2.36 (1.20, 4.62)	0.01
CA125				
<35	13 (26.00)	106 (68.83)		
>35	37 (74.00)	48 (31.17)	6.28 (3.06, 12.88)	<0.001
CEA				
<5	44 (88.00)	153 (99.35)		
>5	6 (12.00)	1 (0.65)	20.86 (2.44, 177.93)	<0.005
NLR				
Q1 (0.14-1.60)	6 (12.00)	45 (29.22)	Reference	
Q2 (1.61-2.10)	13 (26.00)	38 (24.68)	2.56 (0.89, 7.40)	0.08
Q3 (2.17-3.75)	12 (24.00)	39 (25.32)	2.30 (0.79, 6.72)	0.12
Q4 (3.87-55.41)	19 (38.00)	32 (20.78)	4.45 (1.59, 12.39)	0.004

Presented data in the cells are mean (standard deviation) for age and BMI, otherwise are number (%)

Table 2. The Effect of NLR as Categorical Variable on Ovarian Malignant Tumor in Combination with General Characteristics and Tumor Markers

NLR	Median NLR	Model 1	Model 2	Model 3	Model 4	Model 5
Q1 (0.14-1.60)	1.35	Reference	Reference	Reference	Reference	Reference
Q2 (1.61-2.10)	1.87	3.19 (0.99, 10.28)	2.32 (0.76, 7.12)	2.79 (0.95, 8.20)	2.35 (0.77, 7.11)	2.85 (0.75, 10.81)
Q3 (2.17-3.75)	2.73	2.49 (0.77, 8.02)	1.52 (0.49, 4.74)	2.35 (0.79, 6.93)	2.59 (0.86, 7.79)	1.75 (0.46, 6.58)
Q4 (3.87-55.41)	7.05	7.08 (2.22, 22.61)	2.62 (0.88, 7.82)	4.14 (1.47, 11.67)	4.32 (1.49, 12.52)	3.66 (1.00, 13.49)
AIC, BIC		191.72, 211.63	204.50, 221.10	222.26, 238.85	216.68, 233.27	166.46, 196.33

Model 1, age and BMI adjusted; Model 2, CA125 adjusted; Model 3, HE4 adjusted; Model 4, CEA adjusted; Model 5, Full model

Figure 2).

The effect of NLR as continuous variable on ovarian malignant tumor in combination with tumor markers is shown in the Table 3. In the naïve model, one-unit increase in NLR is associated with odds of ovarian malignant tumor by 4%, however, the diagnositic criteria is lower than acceptable cutoff of 0.70. The highest diagnostic performance is expected to be yielded from NLR with cutpoint 13.82 and CA125 with cutpoint 149.65 (AUC=0.82 and maximum youden index=0.53).

Discussion

This study provides evidence about the prognostic value of NLR alone and in combination with patient characteristics (e.g., age and BMI) and tumor markers (CA125, HE4 and CEA) for malignancy among patients with ovarian mass. We found that high level of NLR in combination with age, BMI and tumor markers can reach to high accuracy 87% to discriminate malignancy from bengin tumor among patient with ovarian mass.

The effect of NLR on prognosis of EOC has been well documented in the literature. Higher level of NLR might be a surrogate of poor lymphocyte-mediated immune response against tumors and also association between neutrophils and transforming growth factor β (TGF-β) and then it is expected that this phenomenon contribute with significant impact on tumor cell proliferation and poor prognosis [8]. One other study has showed that the role of NLR on the prognosis in EOC is function of T cells and B cells counts [9]. Low T and B cells and elevated NLR are related with lower response to treatment and poor prognosis in EOC [9].

The predictive value of systemic inflammatory markers such as NLR in prognosis of EOC [10] and combination tumor markers (e.g., CA125, CA199 and CEA) have been previously suggested [11], however, the independent effect of NLR alone and in combination with tumor markers still require further studies. It has been noted that tumor markers stimulate inflammatory markers immunosuppressive in EOC [12]. Also, it argued that tumor marker (e.g., CA125) and NLR is linked by thrombocytes and an immunosuppressive cytokine-profile and then constellation of these factors can infelence the survaival rate of EOC patients [13]. We did not found significant association between NLR and EOC and this

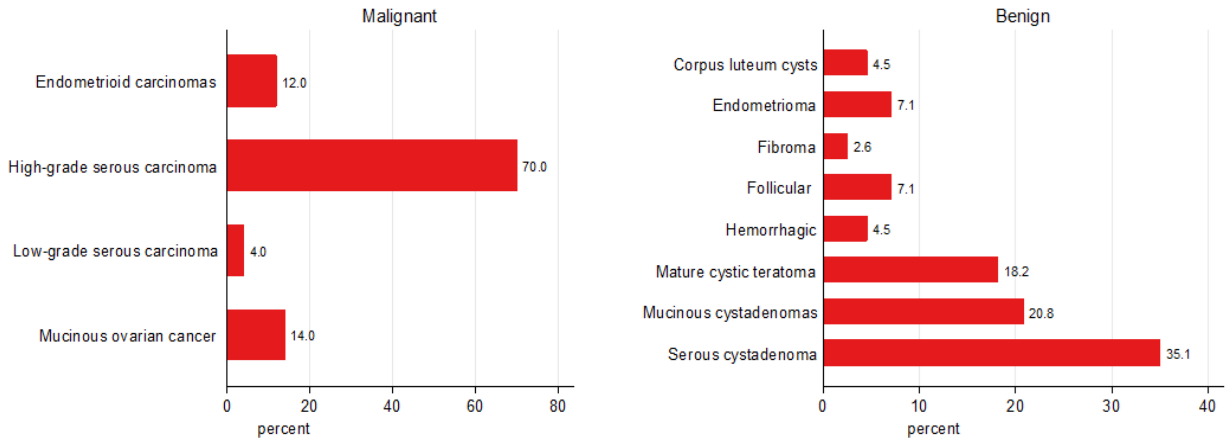

Figure 1. The Distribution of most Common Type of Ovarian Cancer

Tble 3. The Effect of NLR as Continuous Variable on Ovarian Malignant Tumor in Combination with Tumor Markers

		Optimal cut point					
Model	OR (95% CI)	NLR	Tumor marker	AUC	Maximum youden index	Sensitivity	Specificity
NLR	1.04 (1.00, 1.09)	2.61	-	0.62	0.23	0.6	0.63
NLR+CA125	1.03 (0.98, 1.08)	13.82	149.65	0.82	0.53	0.56	0.97
NLR+HE4	1.04 (1.00, 1.09)	4.92	146.85	0.71	0.33	0.46	0.87
NLR+CEA	1.04 (1.00, 1.09)	4.53	2.56	0.56	0.2	0.38	0.82

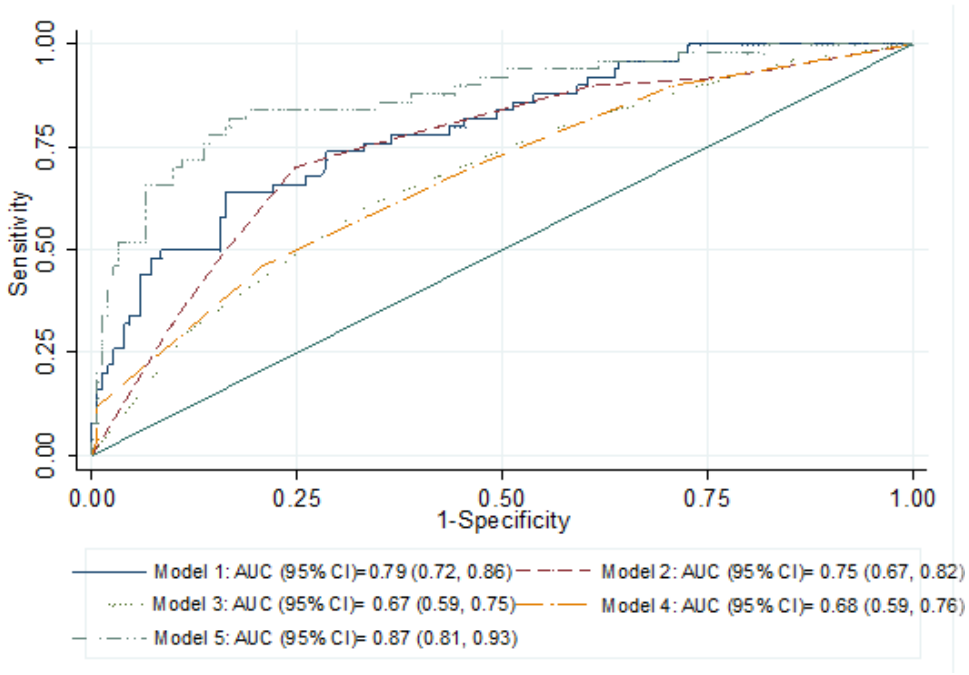

Figure 2. Area under the Curve (AUC) for Evaluating Power of NLR to Diagnosis Ovarian Cancer Maligna

results was inconsistent with study by Li et al. that showed patients with low CA125 level, elevated NLR is related with the risk of EOC [14].

Consistent with our findings, Cho et al. examined the diagnostic utility of NLR in cases of epithelial ovarian cancer and discovered that the preoperative NLR in EOC patients was markedly elevated when compared to that of patients with benign ovarian tumors [15]. Moreover, Yildirim et al. in their study found that NLR alone has not enough power for differentiation between benign and malignant ovarian masses in the preoperative period, which was in line with our findings [16]. Considering these findings, NLR appears to be a promising indicator for EOC. Moreover, since NLR results can be easily obtained, makes it a cost-effective choice that aligns well with the requirements of routine screening markers for regular physical examinations.

Several limitations should be considered when interpreting the results; some part of prognostic value of NLR may be due to inflammatory factors (e.g., Monocyte-to-Lymphocyte Ratio, lymphocyte-to-monocyte ratio, IL-6, IL-8, IL-10), however, data on these factors was not available to include in the analysis. The interaction between the NLR and tumor mrkers may varies depending on the type of treatment (e.g., chemotherapy) and this important issue should be considered in the mind. Limited sample size and lack of survival data precludes us to evaluate the associations with higher statistical power.

In conclusion, while NLR by itself may lack significant predictive power, the precence of high NLR in combination with tumor markers including CA125, HE4 and CEA was associated with malignancy in patients with ovarian mass. These findings suggest that NLR, along with these factors, could serve as a valuable tool in clinical practice for better risk assessment and decision-making in the management of EOC patients. More pay attention and further examinations should be performed for EOC patients with elevated NLR and abnormal tumor markers level for detecting malignancy in EOC patients as early as possible.

Author Contribution Statement

MSH and FA conceived and designed the study. MSH, TA, FF, MA, MT, RA and FA performed data collection and analysis. MR contributed to interpretation of the results. FA drafted the initial manuscript. All authors critically reviewed and approved the final version of the manuscript

Acknowledgements

Deputy of Research and Technology of Shahid Beheshti University of Medical Sciences approved our study and financially supported the study. We would like gratefully acknowledge the medical staff of the Imam Hussain Hospitol.

Funding statement

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Ethics

The ethics committee of Shahid Beheshti University of Medical Sciences approved the study (IR.SBMU.RETECH.REC.1402.134)

Availability of data

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors claimed no conflict of interest.

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