Role of Neutrophil to Lymphocyte Ratio as a Prognostic Indicator for Epithelial Ovarian Cancer

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Objective: *To determine whether level of preoperative peripheral blood neutrophil to lymphocyte ratio (NLR) was a prognostic indicator for epithelial ovarian cancer (EOC) patient. The present study further aimed to evaluate the impact of the change of preoperative/ postoperative NLR on the survival.*

Material and Method: *Medical records of the patients undergoing an operation for EOC in Vajira Hospital between January 1, 2004 and December 31, 2009 were reviewed. Demographic, clinicopathological, and laboratory data were assessed. Preoperative NLR level was divided into high (more than 2.6) and low (2.6 or less) groups and their association with survival were determined. The survival of the subjects according to their preoperative NLR compared to postoperative level was also evaluated.*

Results: *Mean age of 129 subjects was 49.6 $\pm$ 12.5 years. Median pre- and post-operative NLR were 2.8 (range 0.86 to 30.0) and 2.7 (range 0.16 to 31.3), respectively. Seventy-one patients (55.0%) had high preoperative NLR and 64 (53.8%) had high postoperative value. High preoperative and pre- and post-operative NLR were significantly associated with advanced stage and suboptimal surgery. While high post-operative NLR was significantly associated with advanced stage but not suboptimal surgery. In univariable analysis, preoperative/postoperative high/high NLR tended to associate with poor progression free survival (PFS) but not overall survival (OS), with p = 0.019 and p = 0.052 respectively. By multivariable analysis, the advanced stage and suboptimal surgery, but not preoperative and postoperative NLR, were independent poor prognostic factors for PFS and OS.*

Conclusion: *The level of preoperative NLR and the change of preoperative/postoperative NLR level tended to associate with PFS more than OS of EOC patient.*

Keywords: *Epithelial ovarian cancer, Neutrophil to lymphocyte ratio, Prognostic indicator, Survival*

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Epithelial ovarian cancer (EOC) is the third leading cause of death among women with gynecological cancers worldwide[1]. One important reason for a high mortality rate is that the majority of patients frequently have advanced stage of diseases at presentation. This is due to a lack of symptoms at early stage as well as an absence of effective screening for ovarian cancer. Primary surgery is the mainstay of treatment while adjuvant chemotherapy is indicated for advanced stage diseases or some early stages with risk of recurrence[2]. Despite standard therapy, outcomes of the patient are still unsatisfactory with a 5-year survival rate of only 20 to 40%[3,4].

To improve the prognosis of ovarian cancer patients, many prognostic factors are searched in an attempt to improve treatment outcomes, such as age, size of residual tumor, and level of tumor marker, etc[1,2]. Recently, a few inflammatory markers were studied to evaluate their possible prognostic role. A number of studies demonstrated that the host's immune response to cancer is expressed through the process of inflammation[5-7]. This inflammatory response involves in the process of tumor progression by releasing leukocytic and other phagocytic mediators or inflammatory cytokines that would induce damage to cellular DNA, inhibit apoptosis and promote angiogenesis around the cancer area. These would ultimately result in tumor growth and progression.

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On the contrary, whenever the inflammatory process is inhibited or suppressed, tumor growth will be impeded[5-10].

C-reactive protein (CRP) is a biomarker generally used to assess an event of chronic inflammation. Although it was also demonstrated to be a prognostic factor for the survival in colorectal cancer[10,11], CRP is rarely used in a routine clinical practice for preoperative evaluation or for post treatment surveillance in cancer patients due to its extra cost and low sensitivity. Apart from CRP, abnormal hematologic profile particularly leukocyte and its differential counts are also well recognized in association with the inflammatory process. Some studies also showed a relationship of these abnormal hematologic value and survival of cancer patients, for example, increased neutrophil count, decreased lymphocyte count, and elevated neutrophil to lymphocyte ratio (NLR) were found to be associated with poor prognosis of patients with colorectal, hepatic, gastric, and uterine cancer[12-17]. To date, there has been only one study that reported NLR as a prognostic factor for EOC, and pointed out the relationship between high level of preoperative NLR (exceeded 2.6) and poor survival of EOC[18].

The present study aimed to determine whether the level of preoperative NLR is related to the prognosis of EOC patients. The second objective was to evaluate the impact of the change of preoperative/postoperative NLR on the survival.

Material and Method

Permission to conduct the present study was granted by the Ethic Committee for Researches Involving Human Subjects, Bangkok Metropolitan Administration. The medical records of the women who underwent gynecologic surgery in Vajira Hospital due to ovarian cancer between January 1, 2004 and December 31, 2009 were collected. Inclusion criteria were those who had pathological diagnosis of EOC and had available pre- and post-operative complete blood count (CBC). The test had to be done within a month before and after the surgical intervention. In case where several pre- and/or post- operative CBC testing were available, the one that was investigated on the nearest date before surgery and the one prior to adjuvant chemotherapy were taken. Exclusion criteria were those who had any condition that may affect the number of leukocyte or proportion of differential count such as immediate past, current history and sign or symptoms of infection, bone marrow, hematologic or autoimmune disease, recent (14 days or less) steroid or nonsteroidal anti-inflammatory drug intake, smoking, receiving blood transfusion, and those with incomplete clinical data.

A complete blood profile, including total and differential leukocyte count, was measured using an automatic counter model LH 750 (Beckman Coulter, Inc., Brea, CA, USA). The laboratory personnel regularly calibrated the machine once daily, in the morning as quality control. The intra-assay coefficient of variation for WBC was 2.5%, and the standard error of differential count was less than 3%, which fall within an acceptable range.

Data collection included age, FIGO stage, histological type, debulking surgery (optimum or sub-optimum), NLR and treatment response (progression free survival-PFS and overall survival-OS).

The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count; the proportion of more than 2.6 and 2.6 or less was classified as high and low NLR respectively[18]. The change of NLR as preoperative/postoperative level was categorized into four subgroups: high/high, high/low, low/high and low/low.

Optimal debulking surgery was defined when the residual of cancer size was 2.0 centimeters or less. The clinical response was determined according to the Gynecologic Oncology Group response criteria based on findings from the physical examinations, radiologic imaging, or cancer antigen (CA 125)[19].

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) software package version 11.5 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean with standard deviation (SD) or median with range and categorical variables as number with percentage. The χ^2 test was used for categorical variables. The PFS and OS were analyzed with the Kaplan-Meier method and were compared by log-rank test in univariable analysis and by Cox-regression model in multivariable analysis. The p-value of ≤ 0.05 was considered statistically significant.

Results

One hundred thirty four women with EOC underwent gynecologic surgery. Five clients were excluded because of unavailable preoperative CBC, thus 129 clients remained.

For the stage and optimal surgery, one subject had no surgical information, so 128 subjects were analyzed. The mean age of subjects was 49.6 ± 12.5 years.

As clinicopathological characteristics, more than half of the patients (52.4%) had early stage diseases (stage I, II). The most common histopathology was serous carcinoma, comprising approximately one third (34.1%), while mucinous, clear cell, endometrioid carcinoma, mixed epithelial, and adenocarcinoma not otherwise specified were less common with 19.4%, 16.3%, 15.5%, 4.6%, and 10.1% respectively. The majority of the surgical intervention was optimal (80.5%). The subjects in early stage with high-grade tumor or tumor spillage, and those in advanced stages had adjuvant chemotherapy (83.7%). The numbers of the chemotherapy treatment ranged from 1 to 6 cycles (median 6 cycles).

Out of 129 patients, 119 had postoperative CBC determination with available NLR value. Median pre- and post-operative NLR were 2.8 (range 0.86 to 30.0) and 2.7 (range 0.16 to 31.3), respectively. Defining the NLR more than 2.6 as high value, 71 patients (55.0%) had high preoperative NLR and 64 (53.8%) had high postoperative values.

The association of NLR and the clinico-pathological factors of age, stage, surgery and histology were evaluated. The number and percentage of subjects with preoperative high, postoperative high, and high/high NLR level were evaluated toward the clinicopathological features such as age, stage, surgery, and histology, as demonstrated in Table 1. The high preoperative, and pre- and post-operative NLR were significantly associated with advanced stage and suboptimal surgery. While high postoperative NLR was significantly associated with advanced stage but not suboptimal surgery. There was no relation between NLR and histology.

The 119 patients who had complete data of preoperative/postoperative NLR level were divided into four groups: group 1 high/high (n = 46, 38.7%); group 2 high/low (n = 23, 19.3%); group 3 low/high (n = 18, 15.1%), and group 4 low/low (n = 32, 26.9%), as shown in Table 2. In univariable analysis, high postoperative, high NLR, and preoperative/postoperative high/high NLR tended to associate with poor PFS but not OS, with p = 0.019 and 0.052 respectively. While high preoperative NLR was not related with both poor PFS and OS.

The survival rate associated to characteristic features of: age, stage, results of primary surgery, and histology, as shown in Table 3. The PFS and OS were highly significant associated with advanced stage and suboptimal surgery. By multivariable analysis, the advanced stage and suboptimal surgery, but not preoperative and postoperative NLR, were

Table 1. The clinicopathological characteristics associated with high NLR

Characteristics	High level of NLR					
	Preoperative (n = 129)		Postoperative (n = 119)**		Pre- and post-operative NLR (n = 119)**	
	No. (%)	p-value	No. (%)	p-value	No. (%)	p-value
Age (years)		0.740		1.000		0.629
≤ 40	15/25 (60.0)		12/22 (54.5)		10/22 (45.5)	
> 40	56/104 (53.8)		52/97 (53.6)		36/97 (37.1)	
Stage*		0.029		0.010		0.008
Early	30/67 (44.8)		24/59 (40.7)		15/59 (25.4)	
Advanced	40/61 (65.6)		39/59 (61.1)		30/59 (50.8)	
Surgery*		0.003		0.155		0.008
Optimum	50/103 (48.5)		46/93 (49.5)		30/93 (32.3)	
Suboptimum	21/25 (84.0)		17/25 (68.0)		16/25 (64.0)	
Histology		0.652		0.387		0.760
Non-clear cell	58/108 (53.7)		55/98 (56.1)		39/98 (39.8)	
Clear cell	13/21 (61.9)		9/21 (42.9)		7/21 (33.3)	
Total	71/129 (55.0)		64/119 (53.8)		46/119 (38.7)	

* One patient had no data of stage and optimum of surgery
** Ten patients had no postoperative CBC or NLR data

Table 2. Distribution of high and low NLR related to different year of progression free survival and overall survival

Year	Progression free survival at year (%)					Overall survival at year (%)				
	1	2	3	5	p-value	1	2	3	5	p-value
All patients (n = 129)	66.2	57.4	52.2	49.0		83.2	71.1	65.6	63.3	
Preoperative NLR (n = 129)					0.140					0.282
High (n = 71)	61.9	52.1	44.4	44.4		77.6	67.6	64.3	60.8	
Low (n = 58)	71.5	64.1	64.1	54.9		90.4	75.6	66.8	66.8	
Postoperative NLR (n = 119**)					0.039					0.063
High (n = 64)	59.4	47.2	40.5	40.5		77.0	64.6	57.6	53.2	
Low (n = 55)	72.3	69.7	65.4	58.8		88.2	78.6	74.0	74.0	
Pre- and post-operative NLR (n = 119**)					0.019*					0.052*
High/high (n = 46)	55.7	44.6	37.1	37.1		72.8	61.4	56.7	51.5	
High/low (n = 23)	75.9	69.0	61.3	61.3		85.9	79.7	79.7	79.7	
Low/high (n = 18)	69.0	53.6	53.6	53.6		87.7	72.4	62.0	62.0	
Low/low (n = 32)	69.3	69.3	69.3	57.7		89.8	77.1	69.1	69.1	

* p-value of high preoperative and postoperative NLR compared to other groups
** Ten patients had no postoperative CBC or NLR data

Table 3. Progression free survival and overall survival associated with the clinicopathogical characteristics

Year	Progression free survival (%)					Overall survival (%)				
	1	2	3	5	p-value	1	2	3	5	p-value
Age (years)					0.807					0.385
≤ 40 (n = 25)	79.0	79.0	69.1	69.1		87.7	75.9	75.9	75.9	
> 40 (n = 104)	63.0	52.2	48.0	43.6		82.1	69.8	62.8	59.9	
Stage*					<0.001					<0.001
Early (n = 67)	95.3	90.6	90.6	90.6		98.4	96.1	96.1	91.7	
Advanced (n = 61)	36.5	23.7	14.8	7.4		68.0	45.8	34.8	34.8	
Surgery*					<0.001					<0.001
Optimum (n = 103)	76.6	68.6	64.4	60.4		92.6	79.7	75.4	72.6	
Suboptimum (n = 25)	23.6	11.8	5.9	5.9		47.7	38.1	25.4	25.4	
Histology					0.900					0.605
Non-clear cell (n = 108)	65.9	55.6	51.6	51.6		85.2	72.1	65.6	63.0	
Clear cell (n = 21)	68.1	68.1	54.5	27.2		72.1	65.5	65.5	65.5	

* One patient had no data of stage and result of surgery

independent poor prognostic factors for PFS and OS, as demonstrated in Table 4.

Discussion

Recently, Cho et al reported that preoperative NLR, in combination with CA 125, may represent a simple and cost effective method of identifying ovarian cancer, and an elevated NLR may predict an adverse outcome in ovarian cancer[18]. In the present study, the high preoperative, and pre- and post-operative NLR were significantly associated with advance stage and suboptimal surgery, while high postoperative NLR was significantly related to advanced stage but not suboptimal surgery.

In postoperative, high NLR may be a poor prognostic indicator for suboptimal surgery. Nevertheless, the ovarian cancer is often fatal simply because it is usually not detected until it is in the advanced stage. Therefore, the benefit for early detection is clinically helpful. These two recent studies raised the prospect that the preoperative and/or postoperative NLR measurement in EOC patient

Table 4. Multivariable analysis of stage, optimum of surgery, pre- and post-operative NLR as prognostic factors for progression free survival (PFS) and overall survival (OS)

Prognostic factors	Adjusted hazard ratio (HR) of PFS* (95% CI)	p-value	Adjusted HR of OS* (95% CI)	p-value
Stage (early**/advanced)	16.3 (5.6-47.5)	<0.001	10.1 (2.9-35.1)	<0.001
Surgery (optimum**/suboptimum)	2.1 (1.1-4.2)	0.027	2.2 (1.0-4.9)	0.045
Preoperative NLR (low**/high)	0.7 (0.3-1.4)	0.344	0.7 (0.3-1.6)	0.399
Postoperative NLR (low**/high)	1.1 (0.6-2.1)	0.699	1.4 (0.7-3.3)	0.356

* Adjusted for other variables in the Table
** Reference group

provide a simple method of identifying patients with a poorer prognosis and aid in guiding treatment effectively.

The finding of preoperative/postoperative high/high NLR tended to associate with poor PFS with $p = 0.019$, but not OS with $p = 0.052$. Furthermore, the advanced stage and suboptimal surgery, but not preoperative and postoperative NLR, were independent poor prognostic factors for PFS and OS. The clinical implication may use the elevated NLR, indicating that NLR measurement could become the part of routine cancer follow-up and routine diagnosis of early stage of ovarian cancer with other prognostic indicators.

Several possible reasons have been proposed to explain a relationship between high blood NLR and the poor prognosis of cancer patients. Basically, the immune response of host to cancer is lymphocyte dependent. In addition, circulating neutrophils contain and secrete the vast majority of circulating vascular endothelial growth factor or a pro-angiogenic factor that is thought to play an integral role in tumor development and progression[20,21].

In addition, the change of high preoperative NLR level declined to low postoperative NLR after surgery had the highest 5-year PFS and OS may be a prognostic indicator against the response to cancer therapy. The application of NLR for pre- and post- surgical intervention and/or pre- and post-chemotherapy may be a challenging issue for further study with a large number of subjects.

In conclusion, NLR could be a promising parameter for prognostic indicator of effective ovarian cancer management, with and without other tumor parameters. The level of preoperative NLR and the change of preoperative/postoperative NLR level tend to be associated with PFS more than OS of EOC patients.

Potential conflicts of interest

None.

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บทบาทของอัตราส่วนนิวโทรฟิลต่อลิมโฟไซต์เป็นตัววัดพยากรณ์มะเร็งรังไข่ชนิดเยื่อบุผิว

เถาวลัย ถาวรามร, ชาดากานต์ ผโลประการ, ศิริวรรณ ตั้งจิตกมล, สุมนมาลย์ มนัสศิริวิทยา

วัตถุประสงค์: *เพื่อศึกษาอัตราส่วนของเม็ดเลือดขาวชนิดนิวโทรฟิลต่อลิมโฟไซต์ (neutrophil to lymphocyte ratio: NLR) ในเลือดก่อนผ่าตัด เป็นปัจจัยพยากรณ์ในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิวหรือไม่การศึกษาเพิ่มเติมมุ่งประเมินการเปลี่ยนแปลงของ NLR ก่อนและหลังผ่าตัดต่ออัตราการอยู่รอด*

วัสดุและวิธีการ: *ทบทวนเวชระเบียนผู้ป่วยที่ได้รับการผ่าตัดมะเร็งรังไข่ชนิดเยื่อบุผิวที่ ระหว่างวันที่ 1 มกราคม พ.ศ. 2547 ถึง วันที่ 31 ธันวาคม พ.ศ. 2552 ณ โรงพยาบาลวชิรพยาบาล ประกอบด้วยข้อมูลทั่วไป ข้อมูลพยาธิคลินิก และการตรวจทางห้องปฏิบัติการ ค่า NLR ก่อนผ่าตัดแบ่งเป็น กลุ่มสูง (อัตราส่วนมากกว่า 2.6) และกลุ่มต่ำ (อัตราส่วนเท่ากับหรือน้อยกว่า 2.6) เพื่อศึกษาความเกี่ยวข้องกับการอยู่รอด และประเมินการอยู่รอดต่อค่า NLR ก่อนผ่าตัด เปรียบเทียบกับค่าหลังผ่าตัด*

ผลการศึกษา: *อายุเฉลี่ยของผู้ป่วย 129 ราย เท่ากับ 49.6 $\pm$ 12.5 ปี ค่ามัธยฐาน NLR ก่อนและหลังผ่าตัดเท่ากับ 2.8 (พิสัย 0.86 ถึง 30.0) และ 2.7 (พิสัย 0.16 ถึง 31.3) ตามลำดับ ผู้ป่วย 71 ราย (ร้อยละ 55.0) มี NLR ก่อนผ่าตัดสูง และ 64 ราย (ร้อยละ 53.8) มี NLR หลังผ่าตัดสูง พบว่า NLR สูงก่อนผ่าตัดมีความเกี่ยวข้องอย่างมีนัยสำคัญกับโรคระยะลุกลามและการผ่าตัดต่ำกว่าระดับพอเหมาะ (suboptimal) การวิเคราะห์แบบ univariable ค่า NLR สูงก่อนกับก่อนและหลังผ่าตัดเกี่ยวข้องอย่างมีนัยสำคัญกับโรคระยะลุกลาม และการผ่าตัดต่ำกว่าระดับพอเหมาะ ขณะที่ค่า NLR สูงหลังผ่าตัดเกี่ยวข้องอย่างมีนัยสำคัญกับโรคระยะลุกลามแต่ไม่เกี่ยวกับการผ่าตัดต่ำกว่าระดับพอเหมาะ การวิเคราะห์แบบ univariable ค่า NLR ทั้งก่อน/หลังผ่าตัดสูง/สูง มีแนวโน้มเกี่ยวข้องกับการอยู่รอดปราศจากโครุดหน้า (p = 0.019) แต่ไม่เกี่ยวข้องกับการอยู่รอดโดยรวม (p = 0.052) ส่วนการวิเคราะห์แบบ multivariable โรคระยะลุกลาม และการผ่าตัดระดับพอเหมาะเป็นปัจจัยพยากรณ์ที่เป็นอิสระด้านลบ สำหรับการอยู่รอดปราศจากโครุดหน้าและการอยู่รอดโดยรวม*

สรุป: *ระดับของ NLR ก่อนผ่าตัดและการเปลี่ยนแปลงของ NLR ก่อนและหลังผ่าตัดมีแนวโน้มที่จะเกี่ยวข้องกับการอยู่รอดปราศจากโครุดหน้ามากกว่าการอยู่รอดโดยรวมของผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว*