Original Article

Evaluation of Pre-treatment Neutrophil to Lymphocyte Ratio (NLR) in Prediction of Disease Free Survival (DFS) in Patients with Operable Breast Cancers

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Abstract:

Introduction: *Breast cancer is the most common type of cancer in females. Despite the widespread adoption of adjuvant treatments, resulted in improved survival, nearly 20% of patients with early breast cancer still had recurrence of disease. As of the heterogeneity of disease, validated clinical, pathological and molecular prognostic factors currently used in decision-making to provide an optimal adjuvant treatment for these patients are somewhat inadequate and are needed to be further exploration. Increasing evidence suggests that cancer initiation, progression and dissemination are affected by host's immune response in both systemic and the local tumor milieu. The production and release of neutrophils are increased in the bone marrow in response to tumor-related/induced inflammation. This inflammation shows an inverse effect on peripheral blood lymphocyte count due to cytokines. The neutrophil count increases whilst lymphocyte count decreases in peripheral blood.* **Objective:** *To evaluate the pre-treatment neutrophil to lymphocyte ratio (NLR) in prediction of disease free survival (DFS) in patients with operable breast cancers.* **Materials and Methods:** *Retrospective analysis in 240 patients with early breast cancer who operated during January 2012 to December 2013. The study was ethical approved by Institutional Review Board, Royal Thai Army Medical Department. In this study, we evaluated the association of pre-treatment NLR and recurrence of disease in patients with operable breast cancers undergoing curative surgery and adjuvant therapy.* **Result:** *High level of pre-treatment NLR (≥ 4) was found to be an independent predictor for early recurrence in short term follow up (39 months).* **Conclusion:** *Our study has confirmed previous published findings and documented novel findings regarding the prognostic significant of pre-treatment NLR in patients with early (operable) breast cancers. We believe that the clinical application of pre-treatment NLR as one of major prognostic biomarker in treatment of operable breast cancer will be achieved in a very near future.*

Keywords: ● *Breast cancer* ● *Pre-treatment neutrophil to lymphocyte ratio* ● *Prognostic biomarker*

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นิพนธ์ต้นฉบับ

การประเมินค่าอัตราส่วนของเม็ดเลือดขาวนิวโทรฟิลต่อเม็ดเลือดขาวลิมโฟไซต์ในกระแสเลือดก่อนผ่าตัดในการทำนายระยะปลอดโรคของผู้ป่วยมะเร็งเต้านมภายหลังการรักษา

กาญจนา อารีรัตนเวช วิริยะ แก้วกังสดาล วิจิตรา อาสาฬห์ประกิต สุขไชย สาทถาพร และ วิชัย วาสนสิริ
กองศัลยกรรม โรงพยาบาลพระมงกุฎเกล้า

บทคัดย่อ

ความเป็นมา มะเร็งเต้านมเป็นมะเร็งที่พบมากที่สุดในผู้หญิง ปัจจุบันถึงแม้จะมีการพัฒนาของการรักษาเสริมหลังผ่าตัดทั้งยาเคมีบำบัด ยาต้านฮอร์โมน รวมทั้งการฉายรังสี หลังจากได้รับการรักษามะเร็งเต้านม ซึ่งเพิ่มอัตรารอดชีวิตของผู้ป่วยให้ยาวมากขึ้น แต่ก็โอกาสพบมะเร็งกลับมาใหม่ หรือตรวจพบมะเร็งแพร่กระจายได้ถึงร้อยละ 20 มีหลายปัจจัยพยากรณ์โรคที่จะนำมาช่วยในการตัดสินใจเลือกการรักษาเสริมที่เหมาะสมในผู้ป่วยแต่ละคน ***วัตถุประสงค์*** เพื่อประเมินความสามารถของค่าอัตราส่วนของเม็ดเลือดขาวนิวโทรฟิลต่อเม็ดเลือดขาวลิมโฟไซต์ในกระแสเลือดก่อนการรักษา ในการทำนายระยะปลอดโรคของผู้ป่วยมะเร็งเต้านมหลังได้รับการรักษาแล้ว ***วิธีการ*** รวบรวมข้อมูลผู้ป่วยมะเร็งเต้านมระยะแรกที่ได้รับการรักษาด้วยการผ่าตัด จำนวน 240 คน หาค่าอัตราส่วนของเม็ดเลือดขาวนิวโทรฟิลต่อเม็ดเลือดขาวลิมโฟไซต์ในกระแสเลือดก่อนการรักษาและการกลับเป็นซ้ำของมะเร็งเต้านมภายหลังการรักษาด้วยการผ่าตัดและการรักษาเสริม วิเคราะห์หาค่าความแตกต่างและความสัมพันธ์ของปัจจัยต่างๆ ระหว่างทั้งสองกลุ่ม ***ผลการศึกษา*** พบว่าค่าอัตราส่วนของเม็ดเลือดขาวนิวโทรฟิลต่อเม็ดเลือดขาวลิมโฟไซต์ในกระแสเลือดก่อนการรักษาในระดับที่สูง ≥ 4 เป็นปัจจัยพยากรณ์การกลับเป็นซ้ำของโรคในการติดตามช่วงระยะสั้น (39 เดือน)

คำสำคัญ: ● มะเร็งเต้านม ● ค่าอัตราส่วนของเม็ดเลือดขาวนิวโทรฟิลต่อเม็ดเลือดขาวลิมโฟไซต์
● การทำนายระยะปลอดโรคโดยดัชนีวัดทางชีวภาพ

เวชสารแพทย์ทหารบก 2560;70:119-26.

Introduction

Breast cancer is the most common type of cancer in female. In U.S., data from Seer Cancer Statistic Review, the number of new cases of female breast cancer was 125.0 per 100,000 women per year. The number of deaths was 21.5 per 100,000 women per year. These rates are age-adjusted and based on 2009-2013 cases and deaths. Lifetime risk of developing cancer approximately 12.4% of women in U.S.[1]. In Thailand incidence was 29.5 per 100,000 women per year and mortality rate was 11 per 100,000 women per year[2].

Despite the widespread adoption of adjuvant treatments, resulted in improved survival, nearly 20% of patients with breast cancer still had recurrence of disease, mostly seen in first 5 years[3]. The validated clinical and molecular prognostic factors used in treatment decision-making for breast cancer include tumor stage, number of involved regional lymph nodes, histology grade, presence of vascular invasion, age, endocrine receptor status, Her-2 status, Ki67 value. Additional biomarkers namely, tumor-infiltrating lymphocytes, urokinase plasminogen activator inhibitors and multi-gene signatures have recently been investigated. However, the ideal markers to apply in routine clinical use were still unmet[4].

Increasing evidence suggests that cancer progression and prognosis are affected by host's inflammatory response in the tumor microenvironment[5]. The production and release of neutrophils increase in the bone marrow in response to inflammation. The neutrophils are released from the bone marrow and accumulation of neutrophils in peripheral tissue. Inflammation shows an inverse effect on lymphocyte count due to cytokines, particularly TNF-alpha and IL-1beta. Due to all these effects of inflammation, the neutrophil count increases while lymphocyte count decreases in peripheral blood. Consequently, these changes increase the neutrophil to lymphocyte ratio (NLR). Since increased NLR may be a marker of cancer-related inflammation, it may also be associated with prognosis[6]. Several studies have shown that high NLR may be associated with poor prognosis in various types of cancer[7]. However, there are limited studies in the literature demonstrating the prognostic significance of NLR in breast cancer[8-12]. In the breast cancer, the results of recently published article evaluating the relationship between NLR and outcome of treatments are controversy[13].

The purpose of our study is to evaluate the association of pre-treatment NLR and disease free survival (DFS) in patients with operable breast cancers underwent curative surgery and adjuvant therapy.

Patients and Methods

Data from patients with operable breast cancers managed at Phramongkutklao Hospital between January 2012 and December 2013 were reviewed and collected from medical records. A total of 240 patients were included. Pre-operative complete blood counts (CBCs) were recorded. Patients with receiving breast cancer surgery from other hospitals, present with hematologic disorders/autoimmune diseases, using steroid therapy and history of other malignancies/previously diagnosed with breast cancer were excluded.

The medical records for each patient were reviewed for baseline characteristics including age, tumor size, lymph node status, type of surgery (breast conserving surgery/mastectomy), estrogen receptor (ER)/progesterone receptor (PR) status, HER 2 status. Laboratory blood values particularly NLR were obtained pre- and post-treatment. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count.

The primary endpoints which are all event outcomes (locoregional recurrence, distant recurrence and death) documenting during follow up period after completion of treatments, were identified from medical record.

In addition, the alteration of NLR between pre- and post-treatment (1 month post-operative NLR value) was observed. Furthermore, the differences of NLR comparing between patients with breast cancer (N = 240) and patients with benign breast lesions (N = 34) were also evaluated.

Statistical analysis

Statistical analyses were performed with the IBM SPSS statistics software, version 21 (SPSS Inc., Chicago, IL, USA). Where the data followed a normal distribution, parametric tests (Independent t-test [between two variables/groups]) were used to compare the groups based on disease recurrences. Univariate and multivariate logistic regression analyses were carried out to determine whether a factor/variable was associated with and significantly predicted a recurrence. To evaluate and compare the related-sample data between pre- and post-treatment NLR, paired t-tests were performed. Receiver operating characteristic (ROC) curve analysis was used to investigate whether NLR could distinguish between disease recurrence and non-recurrence at 3-year follow up [area under the ROC curve (AUC)]. NLR value with the best accuracy (the highest sensitivity and specificity) was selected as the NLR cut-off value. Survival curve was estimated between high and low NLR cut off values using log-rank test. Survival curves were plotted using the Kaplan-Meier method. A probability value (p value) of equal to or less than 0.05 (2-tailed) was considered statistically significant.

Results

Clinico-pathological characteristics of studied patients (n = 240)

A total of 240 patients with histological proven early breast cancer (T1-2, N0-1, non-metastasis) were included. The median age at diagnosis was 55 years. Eighty point four percent of patients presented with tumor size ≤ 2 cm, 7.9% with tumor size 2-5 cm, and 11.7% with DCIS. Fourty point four percent of patients had axillary lymph node involvement. NLR ranged from 0.57 to 14.35 (mean 2.33 ± 1.42, median 2.03). For treatments, 167 (72%) received adjuvant hormonal treatment along with histology confirming hormonal receptor positive, 125 (54.1%) received adjuvant chemotherapy followed with a standard guideline recommendation of treatment and 127 (54.7%) received adjuvant radiotherapy.

The majority of patients (220 patients) had NLR ≥ 4 were grouped as high NLR. NLR was low (< 4) in 20 patients. At the end of study, 8 (3.5%) patients had locoregional recurrences, 24 (10.6%) patients had distance recurrence and 14 (6.2%) patients died during the follow up period. Median time to follow up was 39 months (range 2-55 months). (Table 1)

No significant difference of NLR between patients with benign and malignant breast lesions

NLR of patients with benign breast lesions (2.18 ± 1.09) was lower than NLR of patients with breast cancers (2.33 ± 1.42) but not reach statistically different, p = 0.560.

NLR showed did not affect recurrences

In breast cancer patients studied, there was no significant difference of NLR (3.00 versus 2.24) between patients with and without event of recurrences during follow up period, p = 0.154. However, NLR was lower in event-free group.

Treatment associated with significant reduction of NLR (n = 119)

Post-surgery NLR demonstrated a significant alteration of NLR compared with pre-treatment NLR. By undergoing tumor removal, NLR was reduced from 2.39 ± 1.33 to 2.13 ± 0.95 (p < 0.05).

ROC curve was unable to show the cut-off value of NLR in distinguish of the occurrence of events

This model showed AUC = 0.548 (95%CI: 0.424-0.672), p = 0.404. Thus, NLR of 2.33 (mean) had not ability to

Table 1 Clinico-pathological characteristics of 240 breast cancer patients

Variables	N (%)
Sex	
Female	272 (99.3%)
Male	2 (0.7%)
Age	
< 60	180 (65.7%)
≥ 60	94 (34.3%)
Tumor size	
Tis	28 (11.7%)
T1 (≤ 2 CM)	193 (80.4%)
T2 (2-5 CM)	19 (7.9%)
Nodal metastases	
Positive	97 (40.4%)
Negative	143 (59.6%)
Type of surgery	
Breast conserving surgery	71 (29.6%)
Mastectomy	169 (70.4%)
ER status	
Positive	167 (72%)
Negative	73 (28%)
PR status	
Positive	115 (49.8%)
Negative	125 (50.2%)
HER2 status	
Positive	54 (24%)
Negative	186 (76%)
Ki67	
< 20%	117 (54.4%)
≥ 20%	98 (45.6%)
All events	29 (12.8%)
Local recurrence	8 (3.5%)
Distance recurrence	24 (10.6%)
Death	14 (6.2%)

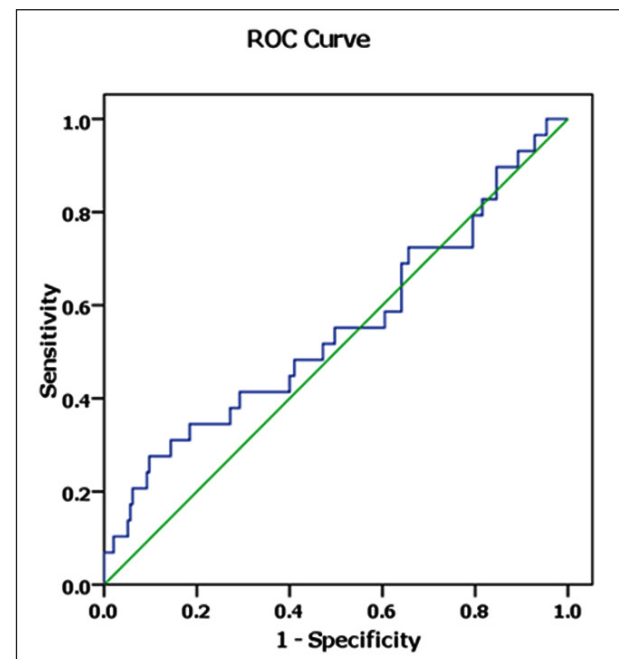Tis: carcinoma in situ, Events: at median follow up of 39 months (minimum 2 months, maximum 55 months)

distinguish the cut-off value between event and event-free patients as established by ROC curve analysis. As a consequence, we had to select the cut-off value of NLR of 4 to distinguish low and high NLR in further analyses. This cut-off point has been proposed by previously published study of Templeton et al, 2014[7] (Figure 1).

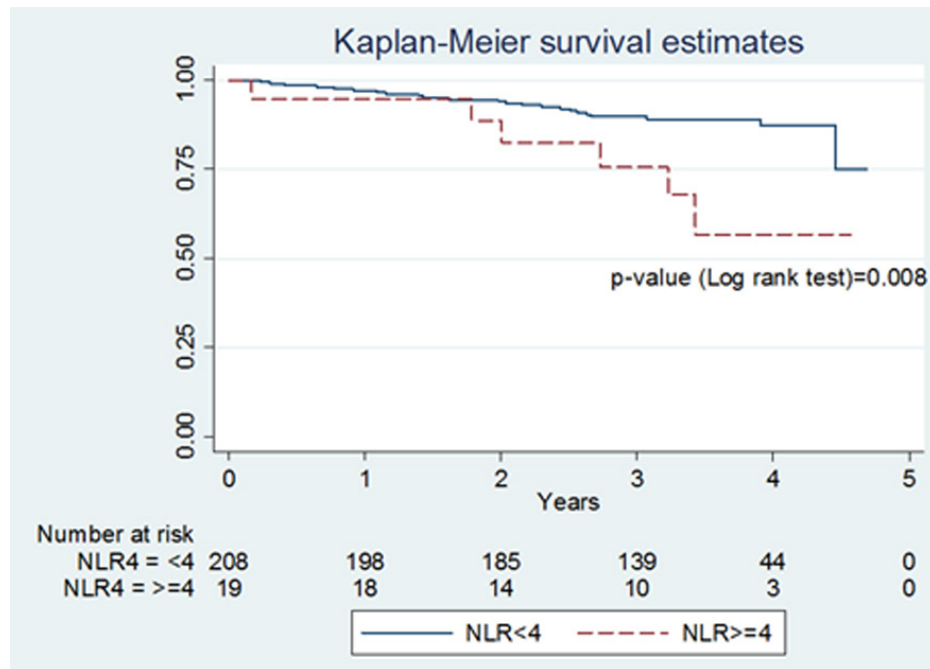**Patients with high NLR (≥ 4) had a significant increased recurrence.**

Kaplan - Meier curves showed significantly higher (log rank chi-square test, p < 0.008) 3-year all event rates in patients with high NLR compared with patients with low NLR (Figure 2).

High NLR was an independent predictive factor for recurrence

In univariate analysis, pre-treatment NLR ≥ 4 (high), Age ≥ 60, High Ki67 (≥ 20), Her-2 positive, triple nega-tive breast cancer were shown to be poor prognostic

Figure 1 ROC analysis based on NLR for DFS

ROC : receiver operating characteristics, AUC : area under curve, DFS : disease free survival

Figure 2 Kaplan - Meier survival curves for events outcome bases on NLR value

Table2 Univariate and multivariate analyses for recurrence of disease in patients with breast cancers (n=240)

	Univariate analysis		Multivariate analysis	
	HR (95%CI)	p-value	Adj HR (95%CI)	p-value
Pre NLR ≥ 4	3.18 (1.29, 7.81)	0.012*	3 (1.08, 8.35)	0.035*
Age				
< 60	Reference	1	Reference	1
≥ 60	2.13 (1.02, 4.43)	0.043*	2.91 (1.29, 6.54)	0.01*
Er+	0.31 (0.14, 0.65)	0.002*	0.85 (0.01, 71.88)	0.941
Pr+	0.31 (0.14, 0.71)	0.006*	0.45 (0.12, 1.7)	0.240
HER2+	1.79 (0.8, 3.98)	0.156		
Ki67 ≥ 20	3.51 (1.48, 8.36)	0.005*	3.68 (1.18, 11.48)	0.025*
Molecular subtype				
Luminal A	Reference	1	Reference	1
Luminal B/HER-	1.55 (0.44, 5.49)	0.499	0.72 (0.13, 4.01)	0.708
Luminal B/HER+	2.89 (0.71, 11.74)	0.138	2.29 (0.43, 12.39)	0.334
HER+	4.81 (1.27, 18.23)	0.021*	1.31 (0.01, 147.92)	0.911
Triple negative	5.47 (1.65, 18.11)	0.005*	2.71 (0.02, 306.95)	0.68

Values presented as Hazards ratio (95% Confident interval), p-value corresponds to Logistic regression analysis

NLR = neutrophil to lymphocyte ratio, ER = estrogen receptor, PR = progesterone receptor, HER2 = human epidermal growth

factor receptor2

factors related with significantly increased recurrent rate. Pre-treatment NLR ≥ 4 (high), age ≥ 60 and high Ki67 (≥ 20) were documented to be independent prognostic factors of poor outcomes in multivariate analysis. There was 3-time higher rate of recurrence in patients with high NLR than patients with low NLR (Table 2).

Discussion

In our study, high levels of pre-treatment NLR (≥ 4) were significantly associated with recurrence of disease and independently predicted a shorten DFS in patients with breast cancers.

Previous studies which evaluated the prognostic roles of the NLR, showed a different cut-off point with different methods of studies[8]. First, we evaluated receiver operating characteristic (ROC) curve analysis to decide whether NLR cut-off values of 2.33 (mean value of NLR from all studied patients) could distinguish between poor outcomes (recurrence/death) and event-free. Unfortunately, the ROC showed an inability to distinguish. Data from recent meta-analysis of 40,559 patients with solid tumors showed that NLR cut-off value at 4.00 was significantly associated with all causes of mortality (HR: 1.81, 95%CI: 1.67-1.97)[7,6]. Using this cut-off point, we demonstrated a significant association of shorten DFS and high NLR.

Accumulating evidence suggests that the systemic inflammatory response played an important role in cancer progression (enhancing angiogenesis, tumor growth and development of metastasis) and NLR represented cancer-related inflammation. However, our findings have failed to demonstrate the significant differences of NLR in patients with benign tumors and malignant tumors. Patients with malignant breast diseases still have a similar level of NLR to patients without malignant diseases. This finding emphasized that NLR was unable to identify patients with malignant diseases and

should not be used as screening tool for breast cancer. Comparing NLR between pre-treatment and post-surgery showed a significant reduction. This reduction may be the results of reversal of cancer-induced inflammation after curative removal of tumors. Nevertheless, there are a number of factors affecting an absolute number of neutrophil and lymphocyte in blood circulation which confound measurement of an accurate NLR. The prediction role of NLR is needed to be carefully interpretation and further exploration. In addition, our study relied on retrospective reviews, short-term follow up and relatively small number of events (recurrence) in studied patients. Further prospective cohorts with a larger sample size are necessary to confirm our findings and provide stronger evidence, such as a prediction of overall survival (OS), to support this biomarker in clinical usage for breast cancer.

Conclusion

Our study has confirmed previous published findings and documented novel findings regarding the prognostic significant of pre-treatment NLR in patients with early (operable) breast cancers. We believe that the clinical application of pre-treatment NLR as one of major prognostic biomarker in treatment of operable breast cancer will be achieved in a very near future.

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