

Elevated neutrophil–lymphocyte ratio can be a biomarker for predicting the development of cervical intraepithelial neoplasia

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Abstract

Cervical intraepithelial neoplasia (CIN) is the abnormal growth of cells on the surface of the cervix that could potentially lead to cervical cancer. In the present study, we investigated whether measuring the neutrophil–lymphocyte ratio (NLR) can be useful for predicting the risks of developing cervical lesions.

This is a retrospective analysis of 212 women who were enrolled in this study. Among them, 106 patients with histologically confirmed CIN1–3 who were treated with loop electrosurgical excision procedure or cold knife cone in the Department of Gynecology, The Affiliated Hospital of Inner Mongolia Medical University between July 30th 2016 and January 30th 2019.

Among the 106 patients in the CIN group, cytology showed minor abnormality which included atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion in 42, high-grade squamous intraepithelial lesion in 62, and squamous cell carcinoma in 2 patients. We found that the NLR has no significant difference between the control group and the CIN1 group, while there were significant differences between CIN1 and CIN2, and CIN2 and CIN3 group. The median of the NLR was higher in the HPV16-persistent groups than in the HPV-negative group.

In conclusion, a high NLR value independently predicts CIN and the stage of CIN. The NLR may help doctors evaluate outcomes of patients received conization and choose alternative therapies for patients with high NLR value.

Abbreviations: ASCCP = American Society for Colposcopy and Cervical Pathology, ASCUS = atypical squamous cells of undetermined significance, CIN = Cervical intraepithelial neoplasia, LSIL = low-grade squamous intraepithelial lesion, ROC = receiver-operating characteristic.

Keywords: biomarker, CIN, NLR

1. Introduction

Although the mortality rates of cervical cancer have been drastically reduced since the introduction of the Pap smear test, it remains one of the leading causes of death in women worldwide. It is estimated that cervical cancer will occur 1 in 100 women in

developed countries and approximately 1 in 50 women during their lifetime in developing countries.^[1,2] Cervical lesions are classified into cervical intraepithelial neoplasia (CIN) 1, 2, and 3 based on its relationship with the prognosis. CIN 1 is mild dysplasia, which is mostly observed because it disappears as part of its natural course. CIN 3 includes severe dysplasia and carcinoma in situ, and management involves treatment because it is highly likely to develop into invasive cancer.^[3–5] CIN 2 is moderate dysplasia, and the choice of whether to observe or treat the patient depends on the patient's condition and wishes. The American Society for Colposcopy and Cervical Pathology recommends that women with a histological diagnosis of CIN 2–3 receive ablative or excisional treatment to eliminate CIN and associated HPV infection. Thus, the treatment decision should be carefully made.^[6–8] On this topic, many studies have been performed to appropriately evaluate the risk of developing cervical lesions. Although detection of high-risk HPV has a high negative predictive value, its positive predictive value should be discussed because of the aforementioned transient infection.

The neutrophil–lymphocyte ratio (NLR), which has been considered as a member of the marker of the systemic inflammation response, is valuable for predicting the prognosis of various cancers.^[9–11] Clearly, challenges remain in order to identify reliable, cost-effective biomarkers to identify which patients are most likely to develop to cervical cancer and need to receive pre-treatment. Inflammatory response plays a vital role in tumor progression including initiation, promotion, malignant conversion, invasion, and metastasis. Based on these factors, several inflammation and immune-based prognostic scores such

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as lymphocyte count, platelet-lymphocyte ratio (PLR), and NLR have been developed to predict the inflammatory response being associated with poor survival and recurrence in different types of cancer, including PDAC. An increasing body of evidence shows that systemic inflammation activation exerted by cancer cells anticipates tumor progression *via* inducing cancer proliferation and metastasis or promoting angiogenesis.^[12,13]

Taken together, we aimed to investigate whether NLR was useful for both distinguishing cervical cancer cells from normal cells and predicting the risk of developing cervical precancerous lesions.^[14] In other words, we hypothesized that NLR detected in patients with CIN would increase with the disease progression. In addition, we also hypothesized that NLR should help discriminate the pathologically normal cells if their prognoses differ.

In the present study, we investigated whether measuring the NLR can be useful for predicting the risks of developing cervical lesions. Further investigation is needed to determine how NLR changes in each patient; however, NLR was suggested to be a useful biomarker for predicting the development of cervical intraepithelial neoplasia.

2. Patients and methods

2.1. Study design and participants

This is a retrospective analysis of 212 women who were enrolled in this study. Among them, 106 patients with histologically confirmed CIN1-3 who were treated with cold knife cone or loop electrosurgical excision procedure in the Department of Gynecology, The Affiliated Hospital of Inner Mongolia Medical University between July 30th 2016 and January 30th 2019. The mean age of the patients at diagnosis was 44.5 years (range, 21–65 years). This study was approved by the institutional review board of the Inner Mongolia Medical University (IRB approval number: 201611945) and written informed consent was exempted owing the retrospective nature of the study. Selection of the conization method depends on the type of cervical transformation zone and the size of the lesion, with the aim of completely removing the lesion. All specimens were marked for orientation with a delayed absorbable suture at the 12-o'clock position for pathology examination. Cone specimens were formalin fixed, paraffin embedded, and processed sagittally in 2-mm thick sections. Slides with a thickness of 4 mm were cut at 250- μ m intervals and stained using hematoxylin and eosin. After examining all specimens, an experienced pathologist made a final histopathological diagnosis according to the 2012 Lower Anogenital Squamous Terminology. For uncertain cases, immunohistochemical staining was performed with labeling indices of Ki67 and p16 protein expression to evaluate the proliferative activity of the tumor and degree of CIN, respectively.

2.2. Pretreatment evaluation

Medical history and physical findings were documented in each patient. Each patient also had an ECG, computed tomography of the abdomen and pelvis (and thorax, if needed), blood routine test, serum chemistry, tumor biomarkers including CEA, CA-199, CA-125, and urine analysis.

2.3. Cervical cytology for all patients

For cervical cytology, cervical assessment was done with the BD SurePath Liquid-Based Pap Test (Becton, Dickinson and

Company, Franklin Lakes, NJ) and the specimens were evaluated following the 2001 Bethesda criteria. A positive result was defined as the presence of atypical squamous cells of uncertain significance, atypical squamous cells, which cannot rule out HSIL, low-grade squamous intraepithelial lesion, HSIL, atypical glandular cells, and invasive cervical cancer.

2.4. HPV DNA typing

HPV testing was performed using the clinically validated, real-time PCR-based, hpVIR test. This test detects and quantifies HPV16, 31, 35, 39, 51, 56, and 59 as individual genotypes, HPV18 and 45 in one group and HPV33, 52 and 58 as a second group. Negative HPV infection was defined as 3 consecutive negative HPV results. Only 1 positive HPV result (including $-/-/+$, $-/+/-$, and $+/-/-$) or 2 nonconsecutive positive HPV results (such as $+/-/+$) was defined as transient HPV infection. Two or more consecutive positive HPV results (including $+/+/+$, $-/+/+$, and $+/+/-$) were defined as persistent HPV infection. Two or more consecutive positive HPV results with the same HPV genotype(s) were defined as persistent HPV infection of the same HPV genotype. The test also detects and quantifies a human single copy nuclear gene (HMBS), which serves as a control for that the samples contain sufficient amounts of cellular material for the test to be informative, and a reference to which the HPV copy number can be related, that is, for normalization of the HPV copy number. The limit of detection for hpVIR was 10 copies per PCR for both the nuclear single copy gene HMBS and HPV.

2.5. Statistical methods

Continuous variables were expressed as mean \pm SD (standard deviation) and compared using a 2-tailed unpaired Student *t* test; categorical variables were compared using χ^2 or Fisher analysis. The predictive performance of NLR was measured using the area under ROC curve (AUC).^[15] All statistical evaluations were carried out using SPSS software (Statistical Package for the Social Science, version 15.0, SPSS Inc, Chicago, IL). A value of $P < .05$ was considered to be statistically significant in all the analyses.

3. Results

3.1. Patients' characteristics

Totally 212 patients were enrolled in this study. Among these patients, 106 patients with CINs were diagnosed by histology examination, which were defined as CIN group. The other 106 patients were defined as control group who performed routine physical examination and no diseases was observed. The mean age of the patients was 44.5 years (range, 21–65 years). Among the 106 patients in the CIN group, cytology showed minor abnormality which included atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion in 42, high-grade squamous intraepithelial lesion in 62, and squamous cell carcinoma in 2 patients, which were shown in Table 1. The histopathological finding was low-grade CIN in 39, high-grade CIN (CIN 2 and CIN 3) in 65 patients, and 2 patients had intraepithelial carcinoma *in situ*.

All patients in this study performed blood test and the NLR were calculated for each patient. The difference between the CIN group and control group were shown in Figure 1A. We then performed ROC curves analysis to stratify all patients into NLR

Table 1
Demographics and baseline characteristics of patients.

Variable	CIN group (N=106)	Control group (N=106)	P
Age	45.6±11.2	46.2±12.3	.742
Cytology abnormalities on follow-up			.001
Negative	0	106	
ASCUS or LSIL	42	0	
High-grade squamous intraepithelial lesion	62	0	
Intraepithelial carcinoma in situ	2	0	
Postconization HPV at any visit			.001
Positive	68	26	
negative	38	80	
Persistent HPV infection			.472
HPV 16+ genotype	35	11	
other HPV genotype	33	15	
Glandular involvement			.026
Positive	14	13	
Negative	4	16	
NLR levels			.021
NLR >2.3	78	40	
NLR ≤2.3	28	66	

ASCUS = atypical squamous cells of undetermined significance, CIN = Cervical intraepithelial neoplasia, NLR = neutrophil-lymphocyte ratio, LSIL = low-grade squamous intraepithelial lesion.

>2.3 (n=118) and NLR ≤2.3 (n=94) groups according to the diagnosis of CINs. The diagnostic value was demonstrated by the area under curves of ROC curve shown in Figure 1B.

3.2. NLR was a predictor for distinguish the levels of CINs

After further stratified the CINs into CIN1, CIN2 and CIN3, we found that the NLR has no significant difference between the control group and the CIN1 group (Fig. 2A), whereas there were significant differences between CIN1 and CIN2, and CIN2 and CIN3 group (Fig. 2A). These results demonstrated that NLR had closely relationship with the extent of CIN and had the potentials to be agg excellent predictor for CINs.

3.3. NLR is higher in the HPV-persistent groups than the HPV- negative group

The HPV status were detected and patients with HPV positive were then received HPV DNA detection by PCR. Enrolled women visited the hospital twice, 2 years apart (visits 1 and 2). Among women with normal cytology at visit 2, those who had HPV16 at both visits 1 and 2 were assigned to the HPV16-persistent groups, while those who did not have HPV at both visits 1 and 2 were assigned to the HPV-negative group. Finally, in the CINs group, there were 38 HPV-negative, 33 HPV16-persistent without progression, and 35 HPV16-persistent with progression. As shown in Figure 2B, the median of the NLR was higher in the HPV16-persistent groups than in the HPV-negative group. The group that was destined to progress to CIN3+, or the HPV16-persistent with progression group, especially tended to have a higher NLR than the HPV16-persistent without progression group. Thus, the NLR was shown to be able to be a useful biomarker for predicting the development of cervical intraepithelial neoplasia.

4. Discussion

Cervical cancer (CC) is one of the most common neoplasms among women worldwide, especially in developing countries. Although the vaccine protection is very high, it should be taken into account that the distribution of HPV genotypes is age-dependent. Serrano et al reported that the prevalence of HPV-16, -18, and -45 in CCs decreased with increasing age; otherwise, the incidence of HPV genotypes 35, 52, 53, 56, 59, and 73 showed a positive trend with increasing age in women affected by CCs.^[4,16,17] Finally, hr-HPV genotypes showed a negative trend with increasing age in women with cervical intraepithelial neoplasia (CIN) grade 3. Generally, conization, which is one of the most common treatments for cervical lesions, increases the risks of postoperative complications and pregnancy complications, such as abortion and preterm labor. Thus, the treatment decision should be carefully made. On this topic, many studies have been performed to appropriately evaluate the risk of

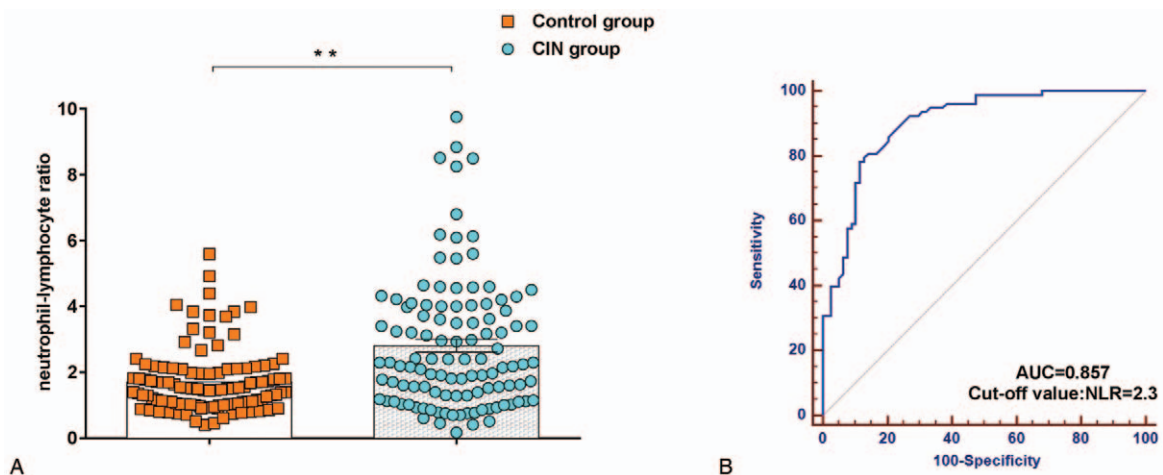


Figure 1. (A) The difference between the CIN group and control group were shown; (B) ROC curves analysis to stratify all patients into NLR >2.3 (n = 118) and NLR ≤2.3 (n=94) groups according to the diagnosis of CINs. CIN = cervical intraepithelial neoplasia, NLR = neutrophil-lymphocyte ratio.

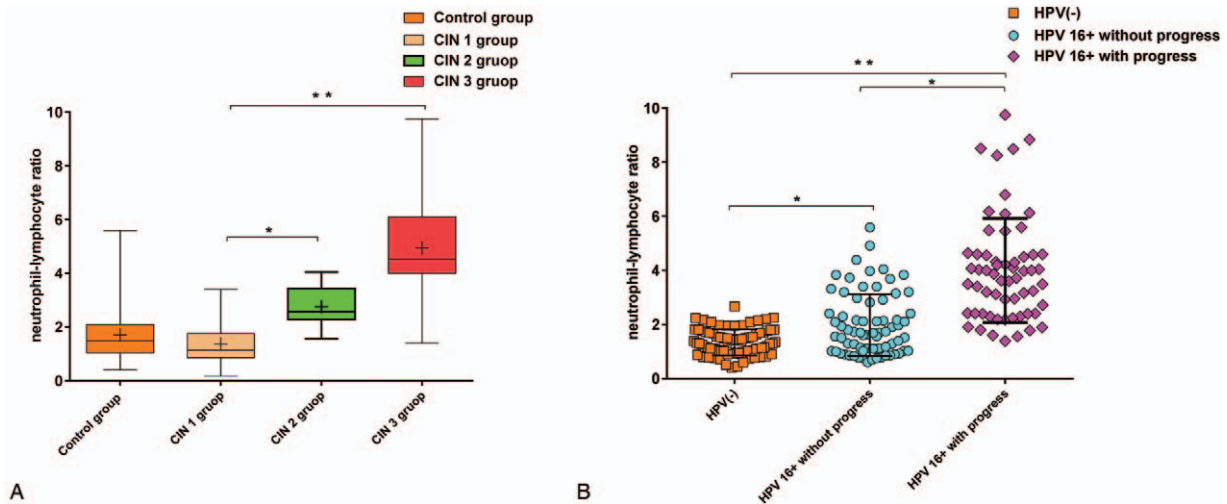


Figure 2. (A) the NLR has no significant difference between the control group and the CIN1 group (A), whereas there were significant differences between CIN1 and CIN2, and CIN2 and CIN3 group; (B) the median of the NLR was higher in the HPV16-persistent groups than in the HPV-negative group. CIN = cervical intraepithelial neoplasia, NLR = neutrophil-lymphocyte ratio.

developing cervical lesions. Although detection of high-risk HPV has a high negative predictive value, its positive predictive value should be discussed because of the aforementioned transient infection.^[18–21]

This study showed that assessment of the NLR calculated from complete blood counts before treatments in patients with CIN who would increase with the disease progression. The result is consistent with previously published articles displaying that high NLR with poor outcome in patients with cervical cancer.^[22,23] Yet, the cutoff value of the NLR is inconsistent in these above studies, which reduces its clinical applicability, we think the impact of the NLR has been explored as a continuous explanatory variable and it is affected by the patients baselines and therapeutic approaches. Consequently, we explored the pretreatment value of 2.3 as the most appropriate cutoff, not only the statistical sensitivity and specificity which were taken into account but also the clinical significance. Our results showed that there were significant differences between CIN1 and CIN2, and CIN2 and CIN3 group.

It is well known that lymphocyte depletion is likely reflection of an impaired T-lymphocyte-mediated antitumor response, which represents an adverse prognostic trait.^[24] In general, the relative ratio of elevated neutrophils and decreased lymphocytes could be a scientific marker for evaluating the systemic inflammatory response and outcome of individuals. And so, NLR is valuable as a potential indicator of prognosis to some degree. The potential mechanism underlying the significant value of NLR is mainly due to the significance of the infiltrated neutrophils and lymphocytes. The systemic inflammatory response from cancer cells promotes the infiltration of neutrophils, which benefits cancer progression via secreting interleukin-2 (IL-2), IL-6, IL-10, tumor necrosis factor α and vascular endothelia growth factor (VEGF).^[25,26] VEGF is a proangiogenic factor contributes to cancer development especially through angiogenesis. Moreover, increased tumor necrosis factor α and IL-10 issue in lymphocyte count decrease and lymphocyte dysfunction also.^[27]

By the way this study is in small size and retrospective design. On the other hand, the relationship between CIN and the

potential possibilities of CIN progressing to cervical cancer was not clearly explained by changing of NLR. Moreover, the change of NLR after treatment apart from pretreatment can be investigated in future studies.

In conclusion, a high NLR value independently predicts CIN and the stage of CIN. The NLR may help doctors evaluate outcomes of patients received conization and choose alternative therapies for patients with high NLR value.

Author contributions

Conceptualization: Lina Xu, Jiandong Song.
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Formal analysis: Lina Xu.
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