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RESEARCH ARTICLE

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Combination of preoperative NLR, PLR and CEA could increase the diagnostic efficacy for I-III stage CRC

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Background: Inflammation plays an important role in the development and progression of CRC. The members of inflammatory biomarkers, preoperative NLR and PLR, have been proved by numerous studies to be promising prognostic biomarkers for CRC. However, the diagnostic value of the two biomarkers in CRC remains unknown, and no study reported the combined diagnostic efficacy of NLR, PLR and CEA.

Methods: Five hundred and fifty-nine patients with I-III stage CRC undergoing surgical resection and 559 gender- and age-matched healthy controls were enrolled in this retrospective study. NLR and PLR were calculated from preoperative peripheral blood cell count detected using white blood cell five classification by Sysmex XT-1800i Automated Hematology System and serum CEA were measured by electrochemiluminescence by ELECSYS 2010. The diagnostic performance of NLR, PLR and CEA for CRC was evaluated by ROC curve.

Results: Levels of NLR and PLR in the cases were significantly higher than them in the healthy controls. ROC curves comparison analyses showed that the diagnostic efficacy of NLR (AUC=.755, 95%CI=.728-.780) alone for CRC was significantly higher than PLR (AUC=.723, 95%CI=.696-.749, *P*=.037) and CEA (AUC=.690, 95%CI=.662-.717, *P*=.002) alone. In addition, the diagnostic efficacy of the combination of NLR, PLR and CEA(AUC=.831, 95%CI=.807-.852)for CRC was not only significantly higher than NLR alone but also higher than any combinations of the two of these three biomarkers (*P*<.05). Moreover, the NLR and PLR in the patients with TNM stage I/II was higher than that in the healthy controls, and patients with stage III had a higher NLR and PLR than those with stage I/II, but no significant difference was observed.

Conclusion: Our study indicated that preoperative NLR could be a CRC diagnostic biomarker, even for early stage CRC, and the combination of NLR, PLR and CEA could significantly improve the diagnostic efficacy.

KEYWORDS
colorectal cancer, diagnosis, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

1 | INTRODUCTION

Colorectal cancer (CRC) remains to be one of the most common cancers and leading causes of cancer related death worldwide.[1] In 2015, approximately 376.3 thousands newly diagnosed cases and 191.0 thousands CRC-related deaths had been predicted to occurrence in China.[2] Obvious improvements had been developed and applied

Abbreviations: 95%CI, 95% confidential interval; AUC, area under curve; CEA, carcinoembryonic antigen; CRC, colorectal cancer; CRP, C-reactive protein; NF-kB, nuclear factor-k-gene binding; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; ROC, receiver operating characteristic; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor.

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in diagnosis and treatment of CRC recently; however, most of the patients were still diagnosed in advanced stage leading to unsatisfactory prognosis for them. Thus, it is urgent for us to identify effective early diagnostic, treatment predicting, and prognostic biomarkers for survival improvement of CRC patients.

Till now colonoscopy is considered as the gold standard for CRC diagnosis.[3] However, it is invasive, painful, and expensive for patients. What is more, it is not safe because of complications such as bleeding, perforation, and infection. Alternatively, occult blood test (OB test) is a widely used screening biomarker for CRC. However, OB test with low sensitivity was usually detected for screening CRC patients who had alimentary tract hemorrhage, leading to unavoidable missed diagnosis.[4] Moreover, other biomarkers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA199), etc., are also used in clinical detection of CRC. Generally, they were routinely used for detecting CRC recurrence due to insufficient sensitivity and organ specificity.[5] Thus, a simple, non-invasive and high-diagnostic efficacy biomarker to detect CRC is urgently to be explored.

As we knew, cancers were correlated with systemic inflammatory response.[6] Meanwhile, accumulating evidences indicate that inflammation plays a fundamental role in the development and progression of various cancers, including CRC.[7,8] The inflammation could cause proliferation of CRC cells and promote angiogenesis of CRC.[9] Systemic inflammatory state could be measured by many biomarkers, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), C-reactive protein (CRP), CRP-to-albumin ratio, cytokines, and leukocyte and its subsets.[10-13] However, CRP and cytokines were not measured routinely in clinical treatment of CRC so that CRP, CRP-to-albumin ratio and cytokines could not be widely adopted. Inversely, NLR, PLR, leukocyte, and its subsets were ubiquitously available because they were the parameters of the simple and inexpensive full blood count which was routinely measured in outpatients and inpatients. Theoretically, NLR (neutrophil count divided by lymphocyte count) and PLR (platelet count divided by lymphocyte count), might be more reliable than neutrophil count, lymphocyte count or platelet count alone, because it was easy for individual count to be influenced by many factors. At the same time, NLR and PLR have been reported by numerous studies to be promising prognostic biomarkers in various cancers, including CRC.[14-18] For the diagnostic value of NLR and PLR for CRC, until now, there are only four small sized case–control studies published.[19-22] However, the patients sample sizes of these studies were all less than 200, and the cohort of their studies were from different population (three of them were from Turkey[19,21,22] and another one was from southern China[20]), which would lead to the instability of the results. Moreover, Emir[19] and Karaman's[22] studies only focused on one biomarker (NLR). In addition to the conclusion drawn from Emir's study,[19] Jia[20], and Kilincalp[21] put a little bit forward, analyzing the data from healthy control and CRC patient cohort focused on NLR and PLR and evaluated these two markers according to the tumor stage. However, the result was still at loggerheads. Kilincalp[21] thought NLR and PLR were not associated with TNM stage, yet Jia[20] took the opposite point of the view, even though they all indicated that pretreatment levels of NLR and PLR might be well in the early diagnosis. What is worse, there was no paper to clarify the diagnostic role of NLR and PLR in conjunction with other markers in patients with CRC, although it was widely recognized that biomarker combinations might have better diagnostic value than individual markers.[23,24] This study with large samples size was conducted in eastern Chinese population to comprehensively analyze the diagnostic value of NLR and PLR in CRC and the first attempt to explore whether this new index, combination of NLR, PLR, and CEA, could improve the diagnostic validity.

2 | MATERIALS AND METHODS

2.1 | Study population

A retrospective analysis was conducted in patients with newly diagnosed CRC who underwent surgical resection in Nanjing First hospital between 2005 and 2012. Patients with follow criteria were excluded: (1) preoperative anti-tumor therapy, such as chemotherapy or radiotherapy; (2) with infections, diseases of blood system or other intestinal diseases; (3) history of cancer in other organ; (4) mingled with other cancer; (5) with preoperative clinical parameter and laboratory results loss. At last, 559 patients and 559 healthy controls matched with gender and age were enrolled in this study and informed consents were obtained from all eligible patients. This study was approved by ethics committee of Southeast University.

2.2 | Clinical parameter and laboratory results

Eligible patients' clinical parameter including age, sex, tumor location, TNM classification, tumor grade, and treatment type were retrieved from medical records. At same time, laboratory results including hematology report (total neutrophil count, total lymphocyte count, and platelet count) detected using white blood cell five classification by Sysmex XT-1800i Automated Hematology System (Shanghai, China) and tumor biomarkers (carcinoembryonic antigen) measured using electrochemiluminescence by ELECSYS 2010 (Roche, Basel, Switzerland) were also collected from medical records. All enrolled patients' peripheral blood sample was collected in tubes at 6-8 clock in the morning before surgical operation.

2.3 | Statistical analysis

IBM SPSS Statistical 20.0 (SPSS Inc. Chicago, IL, USA), GraphPad Prism statistical program version 5 (GraphPad Software, San Diego, CA, USA) and MedCalc statistical software version 15.10 (MedCalc Software, Mariakerke, Belgium) were used for statistical analysis. Kolmogorov–Smirnow test was selected to assess the normality of calculated parameters. Student's *t*-test was used for normal distributed parameter, otherwise Mann–Whitney *U*-test was performed. Chi-square test was used to compare categorical variables. Receiver operator characteristic curve (ROC) analysis which was conducted by MedCalc statistical software version 15.10 was to calculate the

TABLE 1 The baseline characteristics of CRC and healthy controls

Characteristics	Cases (n=559)	Controls (n=559)	*P*-value
Age (y, M with R)	63 (27-97)	63 (27-96)	.994[a]
Sex (male/female)	356/203	356/203	1.00[b]
Location			
Colon	282 (50.4%)		
Rectal	277 (49.6%)		
TNM stage			
I	92 (16.5%)		
II	275 (49.2%)		
III	192 (34.3%)		
Differentiation level			
G1	49 (8.8%)		
G2	410 (73.3%)		
G3	100 (17.9%)		
Invasion depth			
T1	19 (3.4%)		
T2	87 (15.6%)		
T3	388 (69.4%)		
T4	65 (11.6%)		
Lymph node metastasis			
N0	367 (65.7%)		
N1	129 (23.1%)		
N2	63 (11.3%)		
NLR (ratio, M with R)	2.47 (0.57-38.72)	1.64 (0.57-38.72)	<.01[a]
PLR (ratio, M with R)	135.00 (22.90-1140.74)	96.00 (4.47-200.87)	<.01[a]
CEA (ng/mL, M with R)	4.78 (0.01-1500)	2.62 (0.21-9.14)	<.01[a]

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CEA, carcinoembryonic antigen; M, median; R, range.
[a]Difference between groups was tested by Mann–Whitney *U*-test.
[b]Difference between groups was tested by Chi-square test.

optimal cut-off values of NLR and PLR and their corresponding sensitivity, specificity and AUC.[25] The combined diagnostic value of NLR, PLR and CEA was carried out by binary logistic analysis. The comparisons of the AUC under different dependent ROC curves were performed by nonparametric method which was based on Mann–Whitney *U*-statistics.[26] *P*-value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics of enrolled participants

Baseline characteristics of the cases and the healthy controls were described in Table 1. As for CRC patients, a total of 282 (50.4%) were colon cases and 277 (49.6%) were rectal cancer patients, the numbers of patient in stage I, II, and III were 92 (16.5%), 275 (49.2%) and 192 (34.3%), respectively. The patients with good, median, and poor cell differentiation were 49 (8.8%), 410 (73.3%), and 100 (17.9%), respectively. The number of patients with T1, T2, T3, and T4 were 19 (3.4%), 87 (15.6%), 388 (69.4%), and 65 (11.6%), respectively. A total of 367 (65.7%) were identified without lymph node metastasis (N=0). There is no statistical significance between CRC and healthy controls in age and sex. However, NLR, PLR, and CEA in CRC patients were significantly higher than that in healthy controls (Table 1 and Figure 1).

3.2 | Diagnostic value of NLR, PLR, and CEA for CRC

Results of ROC curve analysis showed that the optimal cut-off values of NLR (AUC=.755, 95%CI=.728-.780, sensitivity, Se=51.88%, specificity, Sp=88.55%), PLR (AUC=.723, 95%CI=.696-.749, Se=59.93%, Sp=78.18%), and CEA (AUC=.723, 95%CI=.696-.749, Se=45.62%, Sp=93.56%) were 2.42, 120, and 5.81, respectively, which were summarized in the Table 2. The diagnostic value of NLR was better than PLR (*P*=.037) and CEA (*P*=.002) and there was no statistical significance between PLR and CEA (*P*=.1372; Table 2 and Figure 2), indicating NLR was the best one among these three markers, therefore, NLR in conjunction with other markers were further analyzed for combined detection.

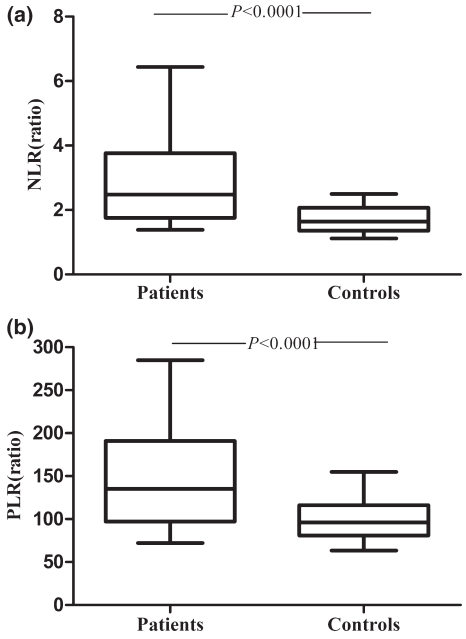

FIGURE 1 NLR and PLR in CRC patients in comparison with healthy controls. (A) NLR; (B) PLR

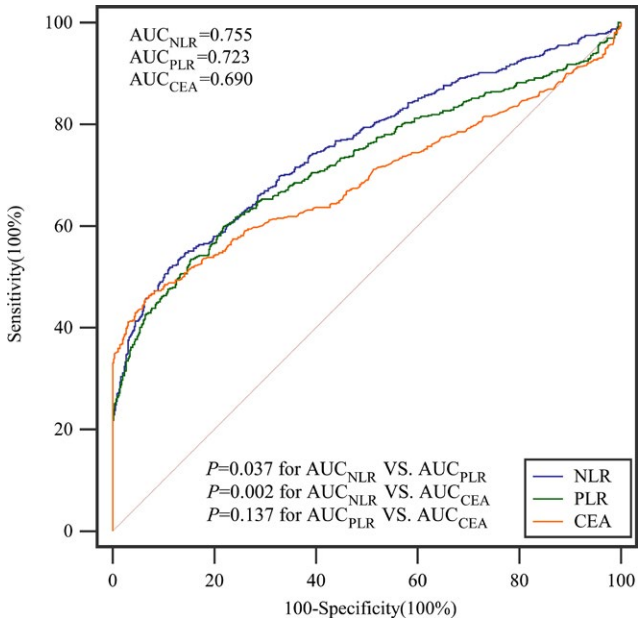

FIGURE 2 Diagnostic value of NLR, PLR, and CEA alone for CRC

3.3 | Combined diagnostic value of NLR, PLR, and CEA for CRC

As Table 2 and Figure 3 showed, the AUC for the combined detection of NLR and CEA was significantly higher than NLR (P<.001) and the combined detection of NLR and PLR (P<.001), but was significantly lower than the combined detection of NLR, PLR and CEA (P=.002). Therefore, the AUC for combined detection of NLR, PLR, and CEA (AUC=.831, 95%CI=.807-.852; Se= 62.97%, Sp=92.84%) was an optimal marker for CRC diagnosis.

3.4 | NLR and PLR for early CRC diagnosis

The stratifying analysis based on TNM stage (I-III) was performed to assess the NLR and PLR for the early CRC diagnosis (Figure 4). The results revealed that the level of NLR and PLR in patients with early tumor stage (stage I/II) and stage III were all significantly higher than that in the healthy controls (P<.0001), and patients with stage III had a higher NLR and PLR than those with stage I/II, but no significant difference was observed. These results indicated that NLR and PLR could act as early diagnostic markers for CRC.

4 | DISCUSSION

Persistent infections and inflammatory responses contributed up to 15% of all deaths from cancer worldwide.[27] Inflammation played a critical role in tumorigenesis. Waldner reported that inflammatory bowel disease could act as the trigger of choric inflammation and therefore increased the risk of CRC.[28] Interestingly, non-steroidal anti-inflammatory drug use could reduce the risk of CRC.[29] Thus, NLR and PLR, the members of systematic inflammatory response seem to be potential diagnostic factors for CRC.

TABLE 2 The results of preoperative NLR, PLR and CEA in diagnosis of I-III stage CRC

Markers	TP	FP	FN	TN	AUC (95%CI)	Cut-off value	Se (%)	Sp (%)	PPV (%)	NPV (%)	Accuracy (%)	YI	LR(+)	LR(−)	Kappa
NLR	290	64	269	495	.755 (.728-.780)	2.42	51.88	88.55	81.92	64.79	70.21	.406	4.53	.54	.404
PLR	335	122	224	437	.723 (.696-.749)	120	59.93	78.18	73.30	66.11	69.05	.381	2.75	.51	.381
CEA	255	36	304	523	.690 (.662-.717)	5.81	45.62	93.56	87.63	63.24	69.59	.392	7.08	.58	.392
NLR+PLR	310	64	249	495	.766 (.740-.790)	—	55.64	88.55	82.89	66.53	72.00	.442	4.84	.50	.440
NLR+CEA	368	75	191	484	.817 (.793-.839)	—	65.83	86.58	83.07	71.70	76.21	.526	4.97	.39	.524
NLR+PLR+CEA	352	40	207	519	.831 (.807-.852)	—	62.97	92.84	89.80	71.49	77.91	.564	8.80	.40	.558

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to- lymphocyte ratio; CEA, carcinoembryonic antigen; TP, true positive; FP, false positive; FN, false negative; TN, true negative; AUC, receiver operator characteristic; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; YI, youden index; LR, likelihood ratio.

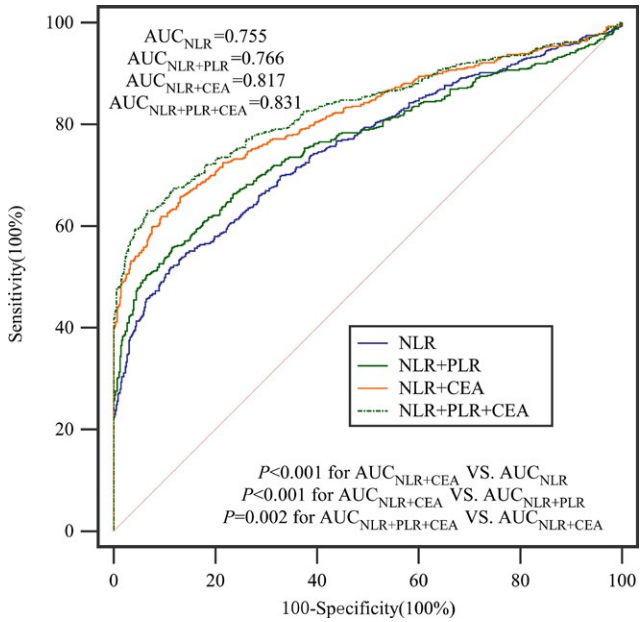

FIGURE 3 Combined diagnostic value of NLR, PLR, and CEA for CRC

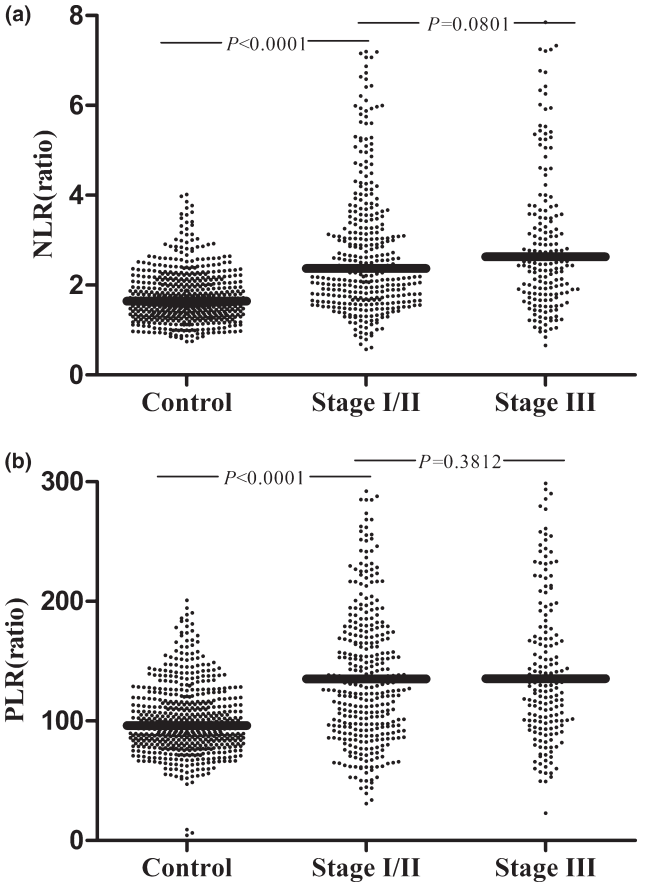

FIGURE 4 The association of NLR and PLR with TNM stage in patients with CRC. (A) NLR; (B) PLR

In our study, we showed that the elevated preoperative NLR and PLR were observed in the cases which were consistent with the recent publication by Kilincalp and Jia.[20,21] We also found both NLR and PLR were elevated in the early tumor stage compared with healthy controls, indicating that NLR and PLR could act as early diagnostic markers for CRC which was in line with Jia's study[20] and were also proved to be associated with the progression of CRC, which still need to be confirmed in further studies, though accumulating studies reported that NLR and PLR could considered as prognostic biomarkers for CRC. In addition, our results revealed that the CRC diagnostic value of NLR was superior to PLR and CEA and combination of them could increase the diagnostic efficacy for stage I-III CRC, suggesting the combination of NLR, PLR, and CEA was an optimal marker for CRC diagnosis. As far as we know, it was the first study to clarify the diagnostic role of NLR in conjunction with other markers in patients with CRC.

The following reasons maybe could explain our findings. First, neutrophils, main component of leukocyte, on the one hand, could be recruited from the peripheral circulation system to tumor tissues after chronic inflammation.[30] Hence, transcription factors of inflammatory cell and tumor cell such as nuclear factor-k-gene binding (NF-kB) and signal transducer and activator of transcription 3 (STAT3) were activated, thus promoting the production of inflammatory mediators including chemokine and cytokines. All of these inflammatory mediators would play a great role in tumor development, for example, IL-6, which was reported to be a crucial promoter of intestinal carcinogenesis and was involved in immune regulation, hematopoiesis, and carcinogenesis.[6] On the other hand, a good deal of reactive oxygen species (ROS) released by neutrophils induced cell DNA damage and genetic instability, leading to carcinogenesis.[31] Moreover, it was reported that less tissue influx of neutrophils followed $CD8^+$ T-cell depletion in infectious diseases.[32,33] Second, the infiltration of lymphocytes in tumor tissues was first observed by Rudolf Virchow and Lymphocyte, major immune cell triggered by cancer cells, playing great roles in cellular immunity. $CD4^+$ T cell was decreasing and $CD8^+$ T cell was increasing after inflammation, resulting in the immune escape of tumor, thus tumor developed. Third, platelet, also a major component of peripheral blood, could secret inflammatory mediators and growth factors, like vascular endothelial growth factor (VEGF), which could stimulate tumor angiogenesis, growth and metastasis.[34] As a result, cancer related inflammation made great contributions to the up-regulation of NLR and PLR.

Some advantages and limitations should be listed as follows: this study with a large population size conducted a strictly exclusive criterion so that our outcomes seemed to be more reliable. What is more, it is the first study, to our knowledge, that explores the diagnostic role of NLR and PLR combined with CEA in patients with CRC. However, we just compared the CRC patients to healthy controls, but whether the level of NLR and PLR were also significantly upregulated in colorectal adenoma is unknown, which need be proved in further study.

In summary, preoperative NLR, an easy and high efficient laboratory biomarker, could be a CRC diagnostic biomarker, even for early stage CRC, and the combination of NLR, PLR, and CEA could significantly improve the diagnostic efficacy.

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