ORIGINAL RESEARCH

The Impact of Hematologic Parameters on Histopathologic Features of Colorectal Cancer

Özcan Dere [ID][1], Yelda Dere [ID][2]

[1]Department of Surgery, Muğla Sıtkı Koçman University Faculty of Medicine, Muğla, Turkey; [2]Department of Pathology, Muğla Sıtkı Koçman University Faculty of Medicine, Muğla, Turkey

Correspondence: Özcan Dere, Muğla Sıtkı Koçman University Faculty of Medicine, Department of Surgery, Gülağzı mah Haliller Sokak No: 34/5 Daire E2, Menteşe, Muğla, 48050, Türkiye, Tel +90 (505) 4653197, Email ozcandere@gmail.com

Background: Colorectal cancers (CRC) are one of the most common tumors that are being researched for new biomarkers worldwide. In this context, studies are being carried out to estimate whether various hematological parameters can be used for predicting prognosis. In this study, our aim was to evaluate the relation between platelet (PLT) levels as well as neutrophil-to-lymphocyte ratio (NLR), platelet (PLT)-to-lymphocyte ratio (PLR), and Lymphocyte-to-CRP ratio (LCR) which are easily accessible inflammatory response indicators.

Methods: In this retrospective cross-sectional study, 111 patients diagnosed as colorectal adenocarcinoma were included. Patients with clinical evidence of an infection, recurrent colorectal cancer, previous history of a hematological disease, and a neoadjuvant chemo/radiotherapy were excluded. Demographic features such as age, gender, and histopathologic parameters such as tumor size, surgical margin status (proximal, distal, and radial), the presence of serosal inflammation, lymphovascular invasion (LVI), perineural invasion (PNI), lymph node metastasis (LNM) and distant metastasis, preoperative blood sample analysis, and CRP levels were noted. Statistical analysis for the association between hematologic parameters platelet (PLT) levels as well as neutrophil-to-lymphocyte ratio (NLR), platelet (PLT)-to-lymphocyte ratio (PLR), and Lymphocyte-to-CRP ratio (LCR) and histopathological features were done.

Results: Among 111 patients, the mean age was 65.37, and the mean tumor size was 5.41 cm. Lymphovascular invasion, perineural invasion, radial surgical margin positivity, lymph node metastasis, localization, and stage were statistically significantly related to the number of platelets. For NLR, PNI (p=0.001), LNM (p=0.048), and stage (early/advanced) (p=0.045) were significantly related. None of the parameters were related to PLR and LCR.

Conclusion: Perineural invasion, lymph node metastasis, and the stage of the tumor could be the major histopathological features that could be related to hematologic parameters; however, this should be researched by larger studies as if they can be used as prognostic markers.

Keywords: hematologic parameters, Lymphocyte-C-reactive protein ratio, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, colorectal cancer

Introduction

Colorectal cancer (CRC) ranks as the third most common malignancy worldwide, with an incidence of 12% in men and 13.1% in Europe.[1,2] While the incidence and mortality rates have decreased in the USA in recent years, developing countries are experiencing an upward trend in CRC incidence.[2,3] Consequently, there is a growing need for preoperative markers to predict prognosis and categorize patients for further therapies. The association between inflammation and cancer has been a subject of study for many years, dating back to Virchow's initial description in 1863.[4] Inflammation and the local immune response are strongly linked to the development and progression of various malignant tumors.[5]

Markers such as the neutrophil-to-lymphocyte ratio (NLR) and platelet (PLT)-to-lymphocyte ratio (PLR) have emerged as nonspecific indicators of inflammation. Lymphocyte-to-CRP ratio (LCR), a novel marker defined by Okugawa et al in a recent study,[6] has gained attention. Given their accessibility, cost-effectiveness, and simplicity, these biomarkers have been investigated worldwide for predicting tumoral behavior.[7] NLR and PLR have been identified

as prognostic factors associated with poor overall survival (OS) and disease-specific survival in various solid malignancies, including gastric cancer, lung cancer, renal cancer, and gynecological cancers.[8–11]

This study aims to assess platelet (PLT) levels, along with NLR, PLR, and LCR, as inflammatory response indicators in colorectal cancer. The goal is to explore their potential utility in classifying patients for different treatment modalities and predicting prognosis.

Materials and Methods

This retrospective cross-sectional study included 111 patients diagnosed with colorectal adenocarcinoma who had undergone radical surgery at our hospital between January 1, 2013, and December 31, 2019. Criteria for inclusion were as follows: 1) a confirmed diagnosis of colorectal adenocarcinoma at any age; 2) no prior administration of neoadjuvant chemotherapy; 3) initiation of treatment with surgery. Exclusion criteria encompassed 1) the presence of clinical evidence indicating infection; 2) recurrent colorectal cancer; 3) previous exposure to neoadjuvant chemotherapy or radiochemotherapy; 4) the existence of hematological disease; 5) loss to follow-up or missing blood count data.

Demographic features, histopathologic parameters, preoperative blood sample analyses (conducted just before the day of the operation), and C-reactive protein (CRP) levels were documented among the 111 patients. Tumors located in the cecum, ascending colon, and right-half transverse colon were categorized as right-sided CRCs, while those in the left-half transverse colon, descending colon, sigmoid colon, and rectum were classified as left-sided CRCs.

Histopathological processing of the surgical specimens was done by the same pathologist. The specimen was fixed by 10% formalin solution for 24 hours. After the fixation period, histopathologic samples for margins, tumor, depth of invasion, and dissection of lymph nodes were processed by a routine tissue processor in our pathology laboratory (Leica ASP300®). After tissue processing, tissue samples were embedded into the paraffin and 4–5-μm-thick sections were prepared from the paraffin embedded tissues, and the standard hematoxylin–eosin staining procedure was applied. After, hematoxylin-eosin stained samples were analysed by Nikon Eclipse Ni-U®. Microscopic evaluation was done by the same pathologist. Tumor grading, based on differentiation, included well differentiated, moderately differentiated, and poorly differentiated categories. Pathological staging adhered to the TNM classification for CRC, as defined by the World Health Organization (WHO) in 2010. Additionally, various parameters such as tumor size, surgical margin status (proximal, distal, and radial), the presence of serosal inflammation, lymphovascular invasion (LVI), perineural invasion (PNI), lymph node metastasis (LNM), and distant metastasis were meticulously documented.

Venous blood samples (10 cc) from each patient were analyzed using an automatic hemogram analysis device (Symex, XM-1000, SA01, Germany). Counts of lymphocytes, neutrophils, and platelets were recorded. All preoperative blood sampling and CRP level assessments were conducted within 1–5 days before surgery. Preoperative blood samples, comprising neutrophil (N), lymphocyte (L), and PLT levels, along with CRP levels, were subjected to analysis for calculating NLR, PLR, and LCR. The NLR value was determined by dividing the absolute count of neutrophils by the absolute lymphocyte count, while the PLR value was derived from the ratio of platelets to lymphocytes.

Patients underwent regular follow-ups every 3 months and 6 months during the first and second years post-operation, respectively. Subsequently, after two years post-operation, annual follow-ups were conducted. The follow-up data was expressed in months.

Clinical features of both groups were compared using SPSS v22.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was employed to assess the normality of data distribution. For normally distributed variables, differences between means were assessed using the two-sample independent t-test. Categorical variables were evaluated using the chi-square test. Receiver operating characteristic (ROC) curve analysis was employed to determine the cutoff values for NLR, PLR, and platelet levels. To identify the appropriate cutoff point for each variable, the Youden index (sensitivity + specificity − 1) was calculated, and the corresponding cutoff value with the highest Youden index was considered the optimal cutoff value. Due to retrospective design of the study, all the patients suitable were included in the study and this eliminated our chance of power analysis. The statistical analysis was carried out by an expert statistician at the expense of the authors. The level of significance was set at $p < 0.05$.

This study adhered to the principles outlined in the Declaration of Helsinki and received approval from the research ethics committee of Muğla Sıtkı Koçman University (Approval number: 120/20). No specific guideline was obtained due

to retrospective design of the study. An informed written consent was obtained from all patients, or their parent/legal guardian/next of kin, prior to their enrollment in this study.

Results

The mean age of the participants was 65.37 (±11.16) years, and the mean tumor size was 5.41 cm (±2.54). Table 1 provides the mean values of hematological parameters and other numeric data. Among the patients, 35 (31.5%) were ≤60 years old, and 39 (35.1%) were female. The majority of the tumors were located in the left colon (n=78), moderately differentiated (n=77), and had a T3 stage (n=51). Additional patient features are summarized in Table 2.

Table 1 Means and Standard Deviation of Numeric Data

	CRP	PLT (x10³/µL)	N (x10³/µL)	L (x10³/µL)	PLR	NLR	LCR	OS (mo)
Mean	28.29	306.09	6.79	1.59	243.25	6.46	479.57	24.68
Median	6.01	281.00	6.12	1.49	209.30	3.89	210.06	20.00
Std deviation	49.79	121.17	3.59	1.11	144.38	6.81	853.69	18.99
Minimum	0.36	137.00	1.76	0.27	40.93	0.72	1.72	1.00
Maximum	261.58	836.00	19.85	10.80	918.52	36.28	6666.67	78.00

Table 2 Demographic and Histopathologic Features of the Tumors

		Frequency (n)	Percentage (%)
Age	≤60	35	31.5
	>60	76	68.5
Localization			
	Right colon	33	29.7
	Left colon	78	70.3
Pathological stage (pT)			
	T1	3	2.7
	T2	16	14.4
	T3	51	45.9
	T4	41	36.9
Differentiation			
	Well-differentiated	24	21.6
	Moderately-differentiated	77	69.4
	Poorly-differentiated	10	9.0
Radial surgical margin			
	Negative	84	75.7
	Positive	27	24.3

(*Continued*)

Table 2 (Continued).

		Frequency (n)	Percentage (%)
Proximal surgical margin			
	Negative	110	99.1
	Positive	1	0.9
Distal surgical margin			
	Negative	111	100
	Positive	0	0
Serosal inflammation			
	Negative	67	60.4
	Positive	44	39.6
Lymph node metastasis (LNM)			
	Negative	54	48.6
	Positive	57	51.4
Lymphovascular invasion (LVI)			
	Negative	54	48.6
	Positive	57	51.4
Perineural invasion (PNI)			
	Negative	48	43.2
	Positive	63	56.8
Distant metastasis			
	Negative	85	76.6
	Positive	26	23.4
Survival			
	Alive	82	73.9
	Exitus	29	26.1

Serosal inflammation was observed in 44 patients (39.6%) and demonstrated a significant association with CRP levels (p=0.000). Moreover, CRP levels were significantly correlated with pathological stage and distant metastasis (p=0.001 and p=0.039, respectively).

Regarding PLT levels, significant associations were found with LVI (p=0.005), PNI (p=0.002), radial surgical margin (p=0.000), serosal inflammation (p=0.000), LNM (p=0.016), localization (p=0.000), and stage (p=0.000). However, distant metastasis, differentiation, and survival showed no significant association with PLT. ROC analysis revealed that PLT levels were discriminative for LVI and LNM. The cutoff values for PLT were evaluated as 280.5 (sensitivity 63.2%, specificity 63%) for LVI and 278 (sensitivity 64.9%, specificity 63%) for LNM (Figure 1A and B).

Concerning NLR, PNI (p=0.001), LNM (p=0.048), and stage (early/advanced) (p=0.045) demonstrated significant associations. LVI, radial surgical margin status, serosal inflammation, distant metastasis, survival, stage, differentiation, and localization showed no correlation. As previously defined, two distinct cutoff values were employed for NLR. Using
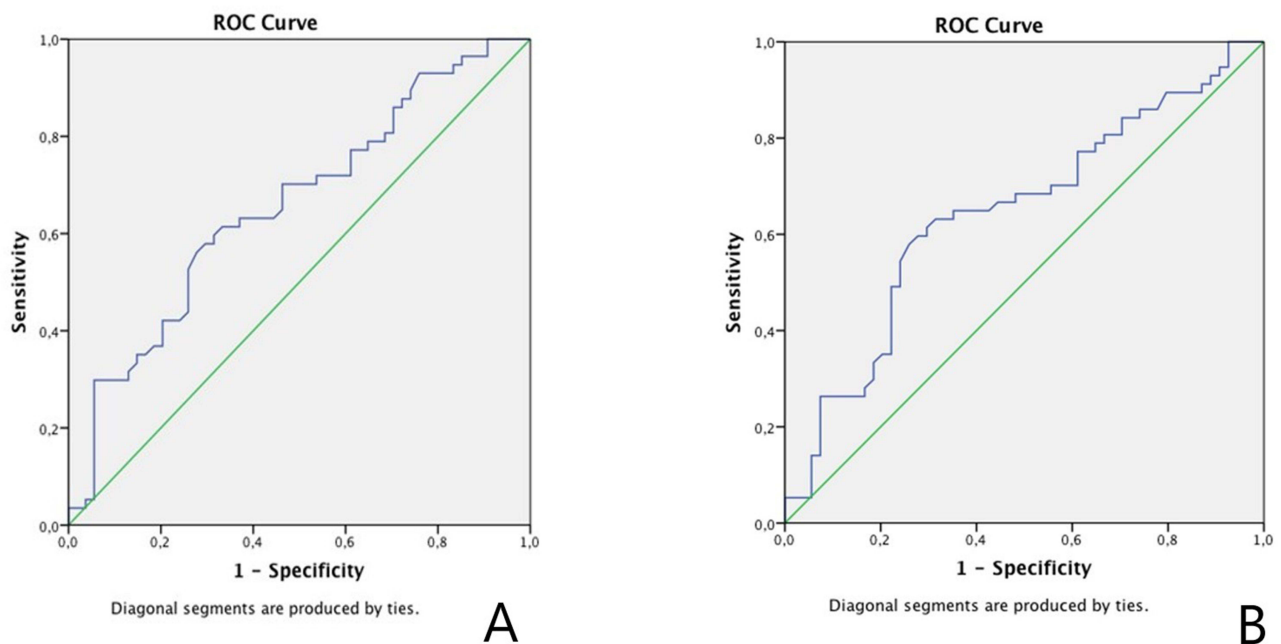
Figure 1 (**A**) ROC curve analysis of the relation between Platelets and lymphovascular invasion, (**B**) ROC curve analysis of the relation between Platelets and lymph node metastasis.

a cutoff value of 3, none of these parameters demonstrated an association with NLR. However, when the cutoff value of 5 was utilized, only PNI exhibited a significant correlation with NLR (p=0.001).

For PLR, none of the parameters exhibited a statistically significant correlation. Additionally, only distant metastasis (p=0.028) showed a significant association with LCR. Other parameters were not correlated (Table 3).

Among all cases, 29 individuals (26.1%) were deceased. The mean overall survival (OS) was 24.68 months (±18.99), ranging from a minimum of 1 month to a maximum of 78 months.

Discussion

Colonic adenocarcinomas rank among the most common tumors globally.[2] Given the variation in the clinical behavior of the tumor across patients, numerous researchers have explored potential prognostic parameters. Hematological parameters, offering easy accessibility and cost-effectiveness, have been scrutinized for their utility as prognostic indicators in various cancers.

Among these hematological parameters, NLR has been extensively investigated. Pine et al proposed that a lower NLR is associated with a more favorable prognosis.[12] Similarly, studies by Jankova et al and Shin et al suggested that higher NLR levels correlate with poorer OS and disease-free survival (DFS).[13,14] Our study aligns with this trend, revealing a significant association between NLR and adverse prognostic parameters such as PNI, LNM, and advanced stage.

Table 3 p values of Various Parameters and Their Relation to PLT, PLR, NLR, LCR

Parameter	Localization	Ex/ Alive	Distant Metastasis	LNM	Serosal Inflammation	Radial Surgical Margin	PNI	LVI	Tumor Size
PLT	0.000	0.363	0.515	0.016	0.000	0.000	0.002	0.005	0.000
PLR	0.825	0.900	0.485	0.311	0.375	0.942	0.097	0.485	0.989
NLR	0.328	0.689	0.285	0.048	0.960	0.253	0.001	0.269	0.318
LCR	0.208	0.424	0.028	0.289	0.192	0.715	0.701	0.689	0.123

In a study by Nagasaki et al, NLR was found to be associated with recurrence and shorter survival after recurrence in patients who underwent neoadjuvant chemotherapy.[15] However, to standardize patient groups, we excluded individuals who had undergone neoadjuvant chemotherapy. Furthermore, numerous studies establishing a correlation between NLR and other prognostic markers have deliberated on the optimal cutoff value for NLR. Some studies set the cutoff value at 5, while others evaluated it as 3.[14–16] Interestingly, the relationship between NLR and other prognostic parameters did not reach significance when grouped using the two cutoff values of NLR ≤3 and NLR >3.

PLR is commonly studied in comparison to NLR. In a study by Emir et al, which compared healthy individuals, those with polyps, and CRC, PLR was found to be statistically higher in CRCs.[17] Similarly, Jia et al established a correlation between PLR and tumor stage.[18] Studies by Kwon et al and Ozawa et al demonstrated that PLR is independently associated with OS and DFS.[19,20] Contrary to these results, our study revealed no significant associations between PLR and prognostic parameters or survival, aligning with the meta-analysis by Zhang et al.[21] The limited follow-up duration in our cases may contribute to the lack of such a correlation.

LCR was initially defined as a prognostic biomarker in the study by Okugawa et al.[6] In their study, a lower LCR level was significantly associated with poor DFS and OS, along with associations with advanced stage, LVI, LNM, and distant metastasis. In our study, however, none of the prognostic parameters correlated with LCR, suggesting that the prognostic effect of LCR may require validation through larger studies, given its recent introduction as a biomarker.

PLT has been investigated due to its role in angiogenesis and the protection of tumor cells from cytolysis.[22] In a study by Sasaki et al, thrombocytosis was identified as an independent indicator of cancer-specific survival.[23] Wan et al reported a significant association between increased platelets and poor survival and distant metastasis.[24] Similarly, in our group, PLT was significantly associated with LVI (p=0.005), PNI (p=0.002), LNM (p=0.016), and stage (p=0.000). However, no significant association was found between PLT and survival, possibly influenced by the variation in tumor stages and the relatively short median follow-up duration.

Our study results, particularly concerning the count of platelets, align with existing literature.

Nevertheless, it is essential to acknowledge the limitations of our study: no power analysis could be performed due to retrospective design of the study, including a low number of T1 cases and a relatively short follow-up period for a cancer characterized by a relatively long expected life span. However, the strength of the study is the same homogenous grouping for the type of treatment (neoadjuvant chemotherapy is excluded), the operation was done by the same surgeon, and the specimen was processed by the same pathologist.

Conclusion

Given their easy accessibility and cost-effectiveness, hematologic parameters merit attention in larger studies to more comprehensively elucidate the genuine impact of inflammatory response in carcinogenesis. A day in the future after understanding hematologic parameters and their role in the tumor microenvironment along with circulating tumor cells would improve their use in predicting prognosis due to the increase in the tendency to choose noninvasive prognostic tests for cancer.

Abbreviations

PLT, platelet; NLR, neutrophil-to-lymphocyte ratio (NLR); platelet (PLT)-to-lymphocyte ratio (PLR); Lymphocyte-to-CRP ratio (LCR); LVI, lymphovascular invasion; PNI, perineural invasion; LNM, lymph node metastasis; CRP, C-reactive protein; CRC, colorectal cancer; OS, overall survival; WHO, World Health Organization.

Statement of Ethics

The study was conducted in accordance with the Declaration of Helsinki and was approved by the research ethics committee of Muğla Sıtkı Koçman University with a number of 120/20. Informed written consent was obtained from all patients/their parent/legal guardian/next of kin prior to their enrollment in this study.

Acknowledgments

Funding

There is no funding to report.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87–108. doi:10.3322/caac.21262
2. Kanavos P, Schurer W. The dynamics of colorectal cancer management in 17 countries. *Eur J Health Econ*. 2010;10(S1):115–129. doi:10.1007/s10198-009-0201-2
3. Canadian Cancer Society's Advisory Committee on Cancer Statistics. Canadian Cancer Statistics 2011. 2011. Available from: https://publications.gc.ca/collections/collection_2011/statcan/CS2-37-2011-eng.pdf. Accessed May, 2011.
4. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*. 2001;357(9255):539–545. doi:10.1016/S0140-6736(00)04046-0
5. Crusz SM, Balkwill FR. Inflammation and cancer: advances and new agents. *Nat Rev Clin Oncol*. 2015;12(10):584–596. doi:10.1038/nrclinonc.2015.105
6. Okugawa Y, Toiyama Y, Yamamoto A, et al. Lymphocyte-C-reactive protein ratio as promising new marker for predicting surgical and oncological outcomes in colorectal cancer. *Ann Surg*. 2019;270(6):e104–e105. doi:10.1097/SLA.0000000000003239
7. Bozkurt Köseoğlu S, Dere Y. New predictive markers for benign endometrial lesions: platelet to lymphocyte and neutrophil to lymphocyte ratio. *Ege Klin Derg*. 2019;57(2):143–147.
8. Xin-Ji Z, Yong-Gang L, Xiao-Jun S, Xiao-Wu C, Dong Z, Da-jian Z. The prognostic role of neutrophils to lymphocytes ratio and platelet count in gastric cancer: a meta-analysis. *Int j Surg*. 2015;21:84–91. doi:10.1016/j.ijsu.2015.07.681
9. Zhang X, Ran Y. Prognostic role of elevated platelet count in patients with lung cancer: a systematic review and meta-analysis. *Int J Clin Exp Med*. 2015;8(4):5379–5387.
10. Men H, Liang C, Yu M. Thrombocytosis as a prognostic factor in patients with renal cell carcinoma: a meta-analysis of literature. *J Cancer Res Ther*. 2015;11(1):67–72. doi:10.4103/0973-1482.150345
11. Yu M, Liu L, Zhang BL, et al. Pretreatment thrombocytosis as a prognostic factor in women with gynecologic malignancies: a meta-analysis. *Asian Pac J Cancer Prev*. 2012;13(12):6077–6081. doi:10.7314/APJCP.2012.13.12.6077
12. Pine JK, Morris E, Hutchins GG, et al. Systemic neutrophil-to-lymphocyte ratio in colorectal cancer: the relationship to patient survival, tumour biology and local lymphocytic response to tumour. *Br J Cancer*. 2015;113(2):204–211. doi:10.1038/bjc.2015.87
13. Jankova L, Dent OF, Chan C, Chapuis P, Clarke SJ. Preoperative neutrophil/ lymphocyte ratio predicts overall survival but does not predict recurrence or cancer-specific survival after curative resection of node-positive colorectal cancer. *BMC Cancer*. 2013;13(1):442. doi:10.1186/1471-2407-13-442
14. Shin JS, Suh KW, Oh SY. Preoperative neutrophil to lymphocyte ratio predicts survival in patients with T1-2N0 colorectal cancer. *J Surg Oncol*. 2015;112(6):654–657. doi:10.1002/jso.24061
15. Nagasaki T, Akiyoshi T, Fujimoto Y, et al. Prognostic impact of neutrophil-to-lymphocyte ratio in patients with advanced low rectal cancer treated with preoperative chemoradiotherapy. *Dig Surg*. 2015;32(6):496–503. doi:10.1159/000441396
16. Palin RP, Devine AT, Hicks G, Burke D. Association of pretreatment neutrophil–lymphocyte ratio and outcome in emergency colorectal cancer care. *Ann R Coll Surg Engl*. 2018;100(4):308–315. doi:10.1308/rcsann.2017.0232
17. Emir S, Aydin M, Can G, et al. Comparison of colorectal neoplastic polyps and adenocarcinoma with regard to NLR and PLR. *Eur Rev Med Pharmacol Sci*. 2015;19(19):3613–3618.
18. Jia J, Zheng X, Chen Y, et al. Stage-dependent changes of preoperative neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in colorectal cancer. *Tumour Biol*. 2015;36(12):9319–9325. doi:10.1007/s13277-015-3667-9
19. Kwon HC, Kim SH, Oh SY, et al. Clinical significance of preoperative neutrophil-lymphocyte versus platelet-lymphocyte ratio in patients with operable colorectal cancer. *Biomarkers*. 2012;17(3):216–222. doi:10.3109/1354750X.2012.656705
20. Ozawa T, Ishihara S, Nishikawa T, et al. The preoperative platelet to lymphocyte ratio is a prognostic marker in patients with stage II colorectal cancer. *Int J Colorectal Dis*. 2015;30(9):1165–1171. doi:10.1007/s00384-015-2276-9
21. Zhang J, Zhang HY, Li J, Shao XY, Zhang CX. The elevated NLR, PLR and PLT may predict the prognosis of patients with colorectal cancer: a systematic review and meta-analysis. *Oncotarget*. 2017;8(40):68837–68846. doi:10.18632/oncotarget.18575
22. Nieswandt B, Hafner M, Echtenacher B, Mannel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Res*. 1999;59(6):1295–1300.
23. Sasaki K, Kawai K, Tsuno NH, Sunami E, Kitayama J. Impact of preoperative thrombocytosis on the survival of patients with primary colorectal cancer. *World J Surg*. 2012;36(1):192–200. doi:10.1007/s00268-011-1329-7
24. Wan S, Lai Y, Myers RE, et al. Preoperative platelet count associates with survival and distant metastasis in surgically resected colorectal cancer patients. *J Gastrointest Cancer*. 2013;44(3):293–304. doi:10.1007/s12029-013-9491-9