Values of applying white blood cell counts in the prognostic evaluation of resectable colorectal cancer

JING WU[1*], XIN-XIN GE[1*], WENYU ZHU[2*], QIAOMING ZHI[3*], MENG-DAN XU[1], WEIMING DUAN[1], KAI CHEN[1], FEI-RAN GONG[4], MIN TAO[1,5], LIU-MEI SHOU[6], MENG-YAO WU[1] and WEN-JIE WANG[1,7]

[1]Department of Oncology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006; [2]Department of Oncology, The Affiliated Changzhou No. 2 People's Hospital, Nanjing Medical University, Changzhou, Jiangsu 213000; Departments of [3]General Surgery and [4]Hematology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006; [5]PREMED Key Laboratory for Precision Medicine, Soochow University, Suzhou, Jiangsu 215021; [6]Department of Oncology, The First Affiliated Hospital of Zhejiang Chinese Medicine University, Hangzhou, Zhejiang 310006; [7]Department of Radio-Oncology, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, Jiangsu 215001, P.R. China

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Abstract. The count and classification of white blood cells (WBCs) may be used as prognostic markers in certain types of cancer. The present study investigated the prognostic potential of the counts of WBCs, including lymphocytes (LYs), monocytes (MOs), neutrophils (NEs), eosinophils (EOs) and basophils (BAs), in the prognosis of resectable colorectal cancer. The present study recruited 153 resectable colorectal cancer cases retrospectively, which were pathologically confirmed. All patients were divided into two groups, according to the median value of LY (low LY, ≤1.632x10^9/l or high LY, >1.632x10^9/l), MO (low MO, ≤0.330x10^9/l or high MO, >0.330x10^9/l), NE (low NE, ≤3.600x10^9/l or high NE, >3.600x10^9/l), EO (low EO, ≤0.085x10^9/l or high EO, >0.085x10^9/l), BA (low BA, ≤0.010x10^9/l or high BA, >0.010x10^9/l), or WBC (low WBC, ≤5.780x10^9/l or high WBC, >5.780x10^9/l). To evaluate the alterations in WBC counts following surgery and adjuvant chemotherapy; all samples received oxiplatin and capecitabine (XELOX) for 6-8 cycles or 5-fluorouracil, leucovorin and oxaliplatin (mFOLFOX6) for 10-12 cycles. XELOX included oxaliplatin administered intravenously at a dose of 130 mg/m^2 on day 1 and 850-1,250 mg/m^2 capecitabine twice daily for days 1-14, repeated every 3 weeks. mFOLFOX6 included oxaliplatin administered intravenously at a dose of 85 mg/m^2, 400 mg/m^2 leucovorin and 400 mg/m^2 5-FU on day 1 followed by 1,200 mg/m^2/days continuous infusion for 2 days (in total, 2,400 mg/m^2 over 46-48 h), repeated every 2 weeks. The present study investigated the post/pre-treatment of LY, MO, NE, EO, BA and WBC ratios (≤1 indicated that LY, MO, NE, EO, BA and WBC counts were not increased following therapy; whereas, >1 suggested increased counts). Kaplan-Meier curves were constructed to demonstrate overall survival (OS). A multivariate and univariate logistic regression analyses model was employed to identify the independent risk factors. Low pre-treatment BA counts were associated with larger tumor size (>5 cm); pre-treatment BA levels were positively associated with OS. Surgery significantly decreased the count of BAs and increased the count of EOs; whereas, no effect was observed on LYs, MOs, NEs or WBCs. Adjuvant chemotherapy markedly decreased the counts of LY, NE and WBC; whereas, no notable effects on MOs, EOs or BAs were observed. Whole course treatment (surgery combined with adjuvant chemotherapy) significantly decreased the values of LY, NE and WBC; however, increased the value of EO; no effects on the MO or BA counts were observed. An increased post-/pre-treatment NE ratio suggested poorer prognosis. Multivariate Cox regression analysis revealed that sex, tumor size, pre-treatment BA count and the post-/pre-treatment NE ratio were independent prognostic factors affecting OS. The results of the present study suggested that the pre-treatment BA count and post-/pre-treatment NE ratio may be potential prognostic factors for resectable colorectal cancer.

Correspondence to: Dr Wen-Jie Wang, Department of Radio-Oncology, The Affiliated Suzhou Hospital of Nanjing Medical University, 26 Daoqian Road, Suzhou, Jiangsu 215001, P.R. China
E-mail: doctor.wjwang@gmail.com

Dr Meng-Yao Wu, Department of Oncology, The First Affiliated Hospital of Soochow University, 899 Pinghai Road, Suzhou, Jiangsu 215006, P.R. China
E-mail: mywu@suda.edu.cn

*Contributed equally

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Introduction

Colorectal cancer (CRC), which is the third most commonly diagnosed cancer, is the second and third leading cause of

cancer-associated mortality in men and women, respectively (1). The prognosis of CRC is associated with the time of diagnosis, the depth of tumor invasion, metastasis to regional lymph nodes and distant metastasis (2). It is widely reported that surgical resection and post-operative adjuvant chemotherapy are the principal therapies used for patients with resectable CRC (3).

Inflammation is one of the most common pathological reactions in the body and is closely associated with tumorigenesis (4). The classification and the count of white blood cells (WBCs) may alter when inflammation occurs and may be associated with particular types of cancer, including bladder cancer (5), breast cancer (6) and prostate cancer (7). WBCs may be categorized into eosinophils (EOs), basophils (BAs), neutrophils (NEs), lymphocytes (LYs) and monocytes (MOs), based on the morphology of cells and nuclei (8). Among these, EOs serve important roles in allergic diseases and helminth infections (9). In addition, as multifunctional leukocytes, EOs have additionally been demonstrated to be involved in anticancer immunity (10). BAs, as the least abundant type of granulocytes, account for <1% of circulating leukocytes and were first described as a type of immune cell responsible for immediate hypersensitivity (11).

Numerous previous studies have demonstrated the prognostic value of the NE-LY ratio (NLR) in patients with CRC (12,13). The NLR reflects the activity of the innate (NEs) and adaptive (LYs) aspects of the immune system (14); however, it is unclear whether components of the NLR contribute equally to the prognostic value (14). Peripheral blood LYs have a vital role in the tumor immune response, via the recognition and elimination of tumor cells (15). NEs are crucial for tumor progression by releasing various pro-angiogenic factors, including vascular endothelial growth factor, interleukin (IL)-8 and matrix metalloproteinase 9 (14).

In addition, MOs are markers of inflammation and associated with tumor prognosis (16). The aim of the present study was to investigate whether the count and classifications of WBCs serve as markers with high sensitivity and as prognostic factors in patients with resectable CRC.

Patients and methods

Subjects and inclusion criteria. The present study was conducted as a retrospective investigation of patients with CRC that had been referred to The First Affiliated Hospital of Soochow University (Suzhou, China) between June 2006 and July 2017. Approval for the study was granted by the Medical Ethics Committees of The First Affiliated Hospital of Soochow University. Written informed consent was obtained from all patients. The inclusion criteria were as follows: i) Those with histologically or cytologically confirmed resectable colorectal cancer; ii) age 18-70 years; iii) Karnofsky performance status score of ≥70 (17); iv) those with a predicted survival of ≥6 months; v) either naive to anti-tumor treatment or the postoperative adjuvant chemotherapy was performed ≥6 months after the last dose of chemotherapy; and vi) those who met the following laboratory criteria: WBCs $\geq 4.0 \times 10^9$/l; absolute NE count $\geq 2.0 \times 10^9$/l; and PLT $\geq 100 \times 10^9$/l. The exclusion criterion was that the patient failed to complete adjuvant chemotherapy following surgery. Clinical and pathological records of all the patients participating in the study were reviewed periodically, the first follow-up was 3 months following adjuvant chemotherapy and the last follow-up was conducted in July 2017.

In total, 153 patients with CRC were included in the present study. All cases were confirmed by surgery and pathological analysis. In the present study, all patients underwent surgery and adjuvant chemotherapy. Patient characteristics are presented in Table I. The median age of the 153 patients was 56-years old (range of 27-85 years); 71 patients were male and 82 were female. Pathological staging of the patients was conducted according to the tumor-node-metastases classification system and classified via the American Joint Committee on Cancer (AJCC) recommendations (18); prognostic analyses were performed regarding overall survival (OS).

Blood samples. Peripheral venous blood (5-7 ml) was collected into a sterile EDTA tube; patients fasted for 8 h and samples were obtained from the elbow veins between 6:30 and 7:30 a.m. in order to standardize the known impact of circulating hormones (circadian rhythm) on the number and subtype distribution of the various WBC indices. LY, MO, NE, EO, BA and WBC were analyzed within 30 min following collection using a hematology analyzer (Sysmex XE-2100; Sysmex Corporation, Kobe, Japan). LY, MO, NE, EO, BA and WBC levels were recorded. The patients were divided into two groups according to the median values of LY, MO, NE, EO, BA or WBC. The post-/pre-treatment ratios were defined as the rate of pre-treatment blood parameters values and the corresponding values obtained following therapy. Blood samples from all patients were obtained 1 month following surgery and 3 months following adjuvant chemotherapy. In the present study, all patients underwent surgery and adjuvant chemotherapy. Surgery was performed for conventional mesocolic excision or complete mesocolic excision in patients with colon cancer, conventional mesocolic excision or total mesocolic excision in patients with colon cancer. Adjuvant chemotherapy included oxiplatin and capecitabine (XELOX) for 6-8 cycles or 5-fluorouracil, leucovorin and oxaliplatin (mFOLFOX6) for 10-12 cycles. XELOX included oxaliplatin administered intravenously at a dose of 130 mg/m^2 on day 1 and 850-1,250 mg/m^2 capecitabine twice daily for days 1-14, repeated every 3 weeks. mFOLFOX6 included oxaliplatin administered intravenously at a dose of 85 mg/m^2, 400 mg/m^2 leucovorin and 400 mg/m^2 5-FU on day 1 followed by 1,200 mg/m^2/days continuous infusion for 2 days (in total, 2,400 mg/m^2 over 46-48 h), repeated every 2 weeks.

Evaluation. Computed tomography scans were performed for the assessment of response to treatment every 2 months with all treatments lasting for 6 months, and evaluated according to the criteria of Response Evaluation Criteria in Solid Tumors 1.1 (19).

Follow-up. Survival time was measured from the date of chemotherapy administration until mortality or the last clinical evaluation. The prognostic analyses were performed regarding OS, which was defined as the duration between the date of diagnosis and mortality due to any cause.

Statistical analysis. All statistical analyses were performed using SPSS 19.0 software (IBM Corp., Armonk, NY, USA). For the analysis of survival data, Kaplan-Meier curves were

Table I. Clinicopathological features.

Clinicopathological features	n	LY				MO				NE				BA				WBC				EO			
		L (n)	H (n)	χ^2	P-value	L (n)	H (n)	χ^2	P-value	L (n)	H (n)	χ^2	P-value	L (n)	H (n)	χ^2	P-value	L (n)	H (n)	χ^2	P-value	L (n)	H (n)	χ^2	P-value
Sex	153			0.330	0.565			1.498	0.221			0.330	0.565			0.132	0.717			1.098	0.295			0.159	0.690
Men	70	33	37			39	31			37	33			40	30			32	38			34	36		
Women	83	43	40			38	45			40	43			45	38			45	38			43	40		
Age (years)	153			0.327	0.568			1.487	0.223			1.487	0.222			2.656	0.103			0.802	0.370			0.801	0.370
≤56	81	42	39			37	44			37	44			50	31			38	43			38	43		
>56	72	34	38			40	32			40	32			35	37			39	33			39	33		
Tumor size (cm)	153			3.474	0.062			3.474	0.062			0.314	0.575			4.94	0.026[a]			0.055	0.815			0.174	0.676
≤5	86	37	49			49	37			45	41			41	45			44	42			42	44		
>5	67	39	28			28	39			32	35			44	23			33	34			35	32		
Depth of invasion	153			0.052	0.819			0.552	0.457			1.729	0.189			0.053	0.818			1.729	0.189			1.729	0.189
T1, T2	17	8	9			10	7			6	11			9	8			6	11			6	11		
T3, T4	136	68	68			67	69			71	65			76	60			71	65			71	65		
Lymphonodus metastasis	153			2.247	0.134			1.959	0.162			0.456	0.500			1.477	0.224			3.104	0.078			0.600	0.439
N0, N1	111	51	60			52	59			54	57			65	46			51	60			58	53		
N2	42	25	17			25	17			23	19			20	22			26	16			19	23		
AJCC stage (14)	153			1.642	0.200			0.158	0.691			0.537	0.464			1.104	0.293			1.142	0.285			0.537	0.464
I, II	56	24	32			27	29			26	30			28	28			25	31			26	30		
III	97	52	45			50	47			51	46			57	40			52	45			51	46		

[a]P<0.05; AJCC, American Joint Committee on Cancer; LY, lymphocyte; BA, basophil; EO, eosinophil; H, high; L, low; MO, monocyte; NE, neutrophil; WBC, white blood cell.

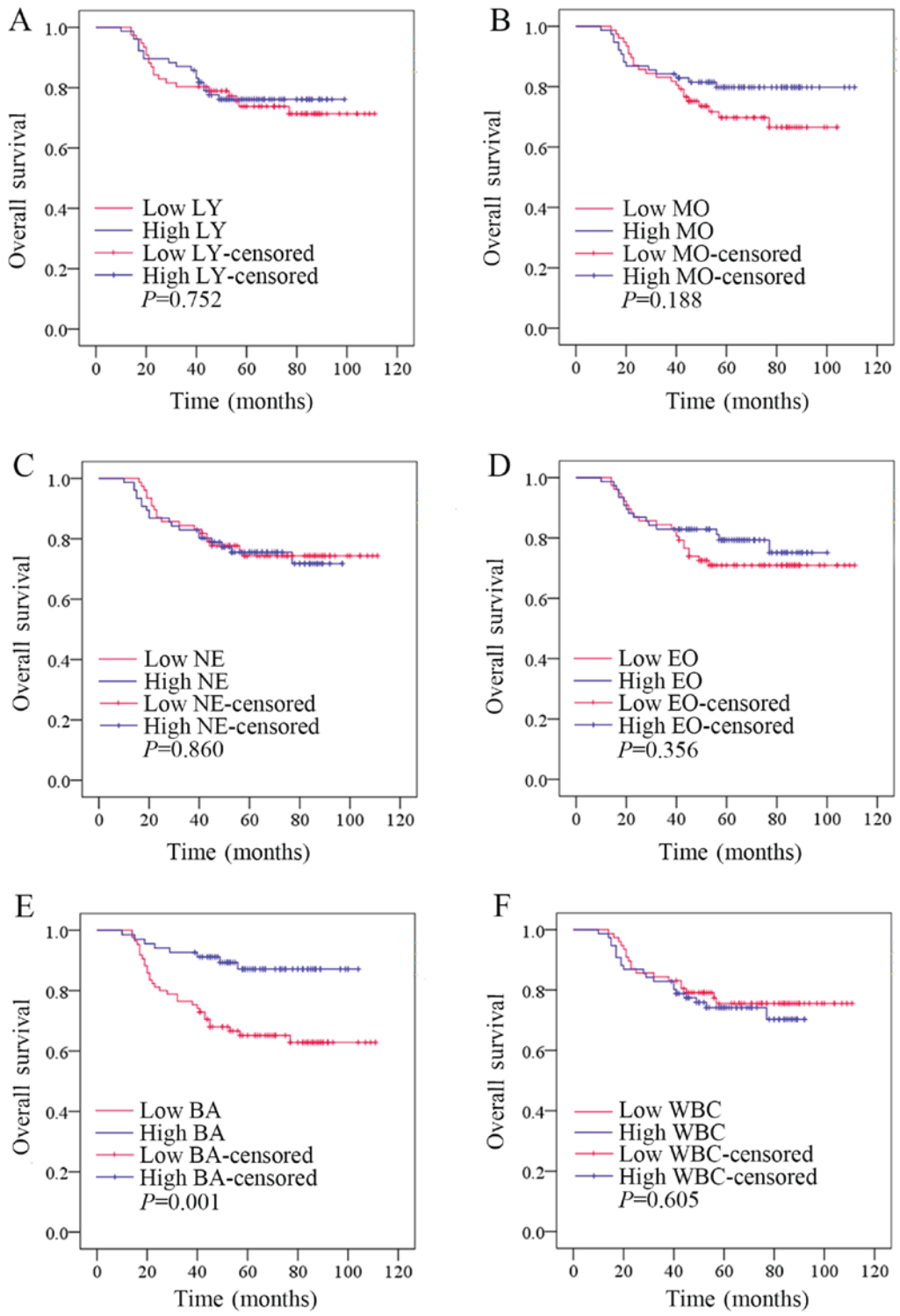

Figure 1. Association between the status of pre-treatment WBC counts and patient outcomes. (A) OS according to LY. (B) OS according to MO. (C) OS according to NE. (D) OS according to EO. (E) OS according to BA. (F) OS according to WBC. n=153. BA, basophil; EO, eosinophil; LY, lymphocyte; MO, monocyte; NE, neutrophil; OS, overall survival; WBC, white blood cell.

constructed and statistical analysis was conducted with a log-rank test. The associations between the status of blood parameters and clinicopathological features were analyzed using χ^2 test. The associations between alterations in the status of the blood parameters and surgery or chemotherapy were assessed by Paired Samples t-test. As the BA values were relatively small compared with other indicators, the P-value demonstrated statistical significance; however, it was difficult to illustrate these differences on graphs. Multivariate and univariate logistic regression analyses were conducted to identify the independent risk factors associated with CRC using a backwards elimination technique, which involves including all the independent variables into the equation and gradually eliminating the non-statistical independent variables, to derive a potentially suitable set of predictors (20). OS was defined as the time from the initiation of surgery to the patient succumbing to any cause. Numerical data

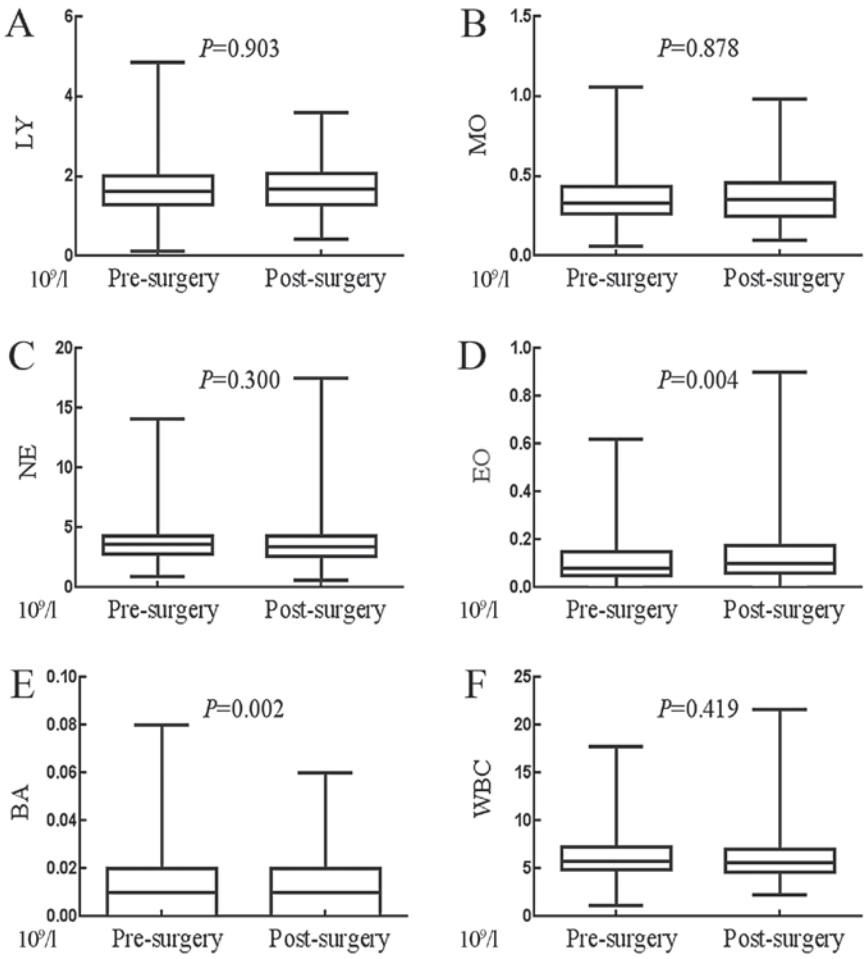

Figure 2. Association between alterations in the status of WBC counts and surgery. (A) Surgery had no effect on LY counts. (B) Surgery had no effect on MO counts. (C) Surgery decreased NE counts. (D) Surgery increased EO counts. (E) Surgery decreased BA counts. (F) Surgery had no effect on WBC counts. n=153. BA, basophil; EO, eosinophil; LY, lymphocyte; MO, monocyte; NE, neutrophil; WBC, white blood cell.

are presented as the mean ± standard error. P<0.05 was considered to indicate a statistically significant difference.

Results

Pre-treatment BA levels are associated with the outcomes of patients with CRC. The patients were divided into two groups according to the median values of LY (low LY, ≤1.632x10^9/l; or high LY, >1.632x10^9/l), MO (low MO, ≤0.330x10^9/l; or high MO, >0.330x10^9/l), NE (low NE, ≤3.600x10^9/l; or high NE, >3.600x10^9/l), EO (low EO, ≤0.085x10^9/l; or high EO, >0.085x10^9/l), BA (low BA, ≤0.010x10^9/l; or high BA, >0.010x10^9/l), or WBC (low WBC, ≤5.780x10^9/l; or high WBC, >5.780x10^9/l). Kaplan-Meier plots were produced to determine the association between LY, MO, NE, EO, BA and WBC status and OS (Fig. 1). The mean OS of the high LY group was 82.438 months [95% confidence interval (CI), 75.635-89.241], while that of the low LY group was 88.670 months (95% CI, 80.209-97.130; P=0.752). The mean OS was 93.644 months (95% CI, 85.712-101.576) in the high MO group and 81.539 months (95% CI, 73.795-89.282) in the low MO group (P=0.188). The mean OS of the high NE group was 79.186 months (95% CI, 72.153-86.220), while that of the low NE group was 90.586 months (95% CI, 82.554-98.618; P=0.860). The mean OS was 83.867 months (95% CI, 76.806-90.928) in the high EO group and 87.778 months (95% CI, 79.469-96.088) in the low EO group (P=0.356). The mean OS of the high BA group was 94.753 months (95% CI, 88.688-100.817), while that of the low BA group was 81.506 months (95% CI, 72.919-90.092; P=0.001). The mean OS was 74.923 months (95% CI, 68.366-81.479) in the high WBC group and 91.433 months (95% CI, 83.461-81.479) in the low WBC group (P=0.605). Therefore, higher pre-treatment BA levels were associated with improved prognosis; however, the pre-treatment levels of LY, MO, NE, EO or WBC were not significantly associated with OS.

Effects of surgery on the counts of WBCs. The effects of surgery on the counts of the various types of WBCs are presented in Fig. 2. The median value of LY was 1.630x10^9/l (95% CI, 1.530-1.730x10^9/l) pre-surgery and 1.670x10^9/l (95% CI, 1.540-1.780x10^9/l) post-surgery (P=0.903). The median value of MO was 0.330x10^9/l (95% CI, 0.3100.360x10^9/l) pre-surgery and 0.350x10^9/l (95% CI, 0.320-0.370x10^9/l) post-surgery (P=0.878). The median value of NE was 3.600x10^9/l (95% CI, 3.410-3.790x10^9/l) pre-surgery and 3.400x10^9/l (95% CI, 3.170-3.800x10^9/l) post-surgery (P=0.300). The median value of EO was 0.080x10^9/l (95% CI, 0.080-0.100x10^9/l) pre-surgery and 0.100x10^9/l (95% CI, 0.090-0.110x10^9/l) post-surgery (P=0.004). The median value of BA was 0.010x10^9/l

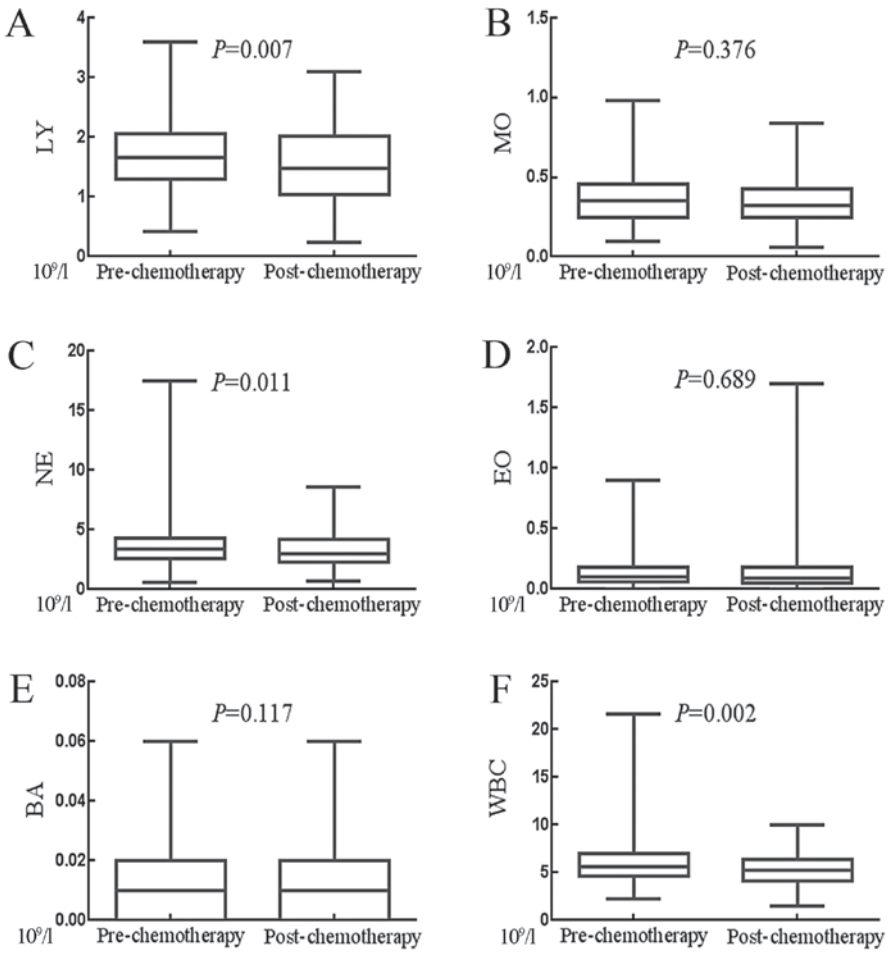

Figure 3. Association between alterations in the number of WBCs and adjuvant chemotherapy. (A) Chemotherapy decreased the levels of LY. (B) Chemotherapy had no effect on the levels of MO. (C) Chemotherapy decreased the levels of NE. (D) Chemotherapy had no effect on the levels of EO. (E) Chemotherapy had no effect on the value of BA. (F) Chemotherapy decreased the value of WBC. n=153. BA, basophil; EO, eosinophil; LY, lymphocyte; MO, monocyte; NE, neutrophil; WBC, white blood cell.

(95% CI, 0.010-0.010x10^9/l) pre-surgery and 0.010x10^9/l (95% CI, 0.000-0.010x10^9/l) post-surgery (P=0.002). The median value of WBC was 5.780x10^9/l (95% CI, 5.480-5.940x10^9/l) pre-surgery and 5.680x10^9/l (95% CI, 5.490-5.900x10^9/l) post-surgery (P=0.419). The results suggested that surgery increased the levels of EO and BA; however, exhibited no effect on the values of LY, MO, NE or WBC.

Effects of adjuvant chemotherapy on the counts of WBCs. The effects of adjuvant chemotherapy on the counts of WBCs are presented in Fig. 3. The median value of LYs was 1.670x10^9/l (95% CI, 1.540-1.780x10^9/l) pre-adjuvant chemotherapy and 1.470x10^9/l (95% CI, 1.370-1.610x10^9/l) post-adjuvant chemotherapy (P=0.007). The median value of MO was 0.350x10^9/l (95% CI, 0.320-0.370x10^9/l) pre-adjuvant chemotherapy and 0.320x10^9/l (95% CI, 0.290-0.340x10^9/l) post-adjuvant chemotherapy (P=0.376). The median value of NE was 3.400x10^9/l (95% CI, 3.170-3.800x10^9/l) pre-adjuvant chemotherapy and 3.010x10^9/l (95% CI, 2.880-3.340x10^9/l) post-adjuvant chemotherapy (P=0.011). The median value of EO was 0.100x10^9/l (95% CI, 0.090-0.110x10^9/l) pre-adjuvant chemotherapy and 0.090x10^9/l (95% CI, 0.070-0.120x10^9/l) post-adjuvant chemotherapy (P=0.689). The median value of BA was 0.010x10^9/l (95% CI, 0.000-0.010x10^9/l) pre-adjuvant chemotherapy and 0.016x10^9/l (95% CI, 0.011-0.023x10^9/l) post-adjuvant chemotherapy (P=0.117). The median value of WBC was 5.680x10^9/l (95% CI, 5.490-5.900x10^9/l) pre-adjuvant chemotherapy and 5.280x10^9/l (95% CI, 4.790-5.700x10^9/l) post-adjuvant chemotherapy (P=0.002). Therefore, adjuvant chemotherapy may have decreased the levels of LY, NE and WBC; however, exhibited no significant impact on the values of MO, EO or BA.

Effects of whole course treatment on the counts of WBCs. The impacts of whole course treatment (surgery and adjuvant chemotherapy) on the counts of WBCs are presented in Fig. 4. The median value of LY was 1.630x10^9/l (95% CI, 1.530-1.730x10^9/l) pre-treatment and 1.470x10^9/l (95% CI, 1.370-1.610x10^9/l) post-treatment (P=0.017). The median value of MO was 0.330x10^9/l (95% CI, 0.310-0.360x10^9/l) pre-treatment and 0.320x10^9/l (95% CI, 0.290-0.340x10^9/l) post-treatment (P=0.474). The median value of NE was 3.600x10^9/l (95% CI, 3.410-3.790x10^9/l) pre-treatment and 3.010x10^9/l (95% CI, 2.880-3.340x10^9/l) post-treatment (P<0.001). The median value of EO was 0.085x10^9/l (95% CI, 0.080-0.100x10^9/l) pre-treatment and 0.090x10^9/l (95% CI, 0.070-0.120x10^9/l) post-treatment (P=0.045). The median value of BA was 0.010x10^9/l (95% CI, 0.010-0.010x10^9/l) pre-treatment and 0.016x10^9/l (95% CI, 0.011-0.023x10^9/l) post-treatment

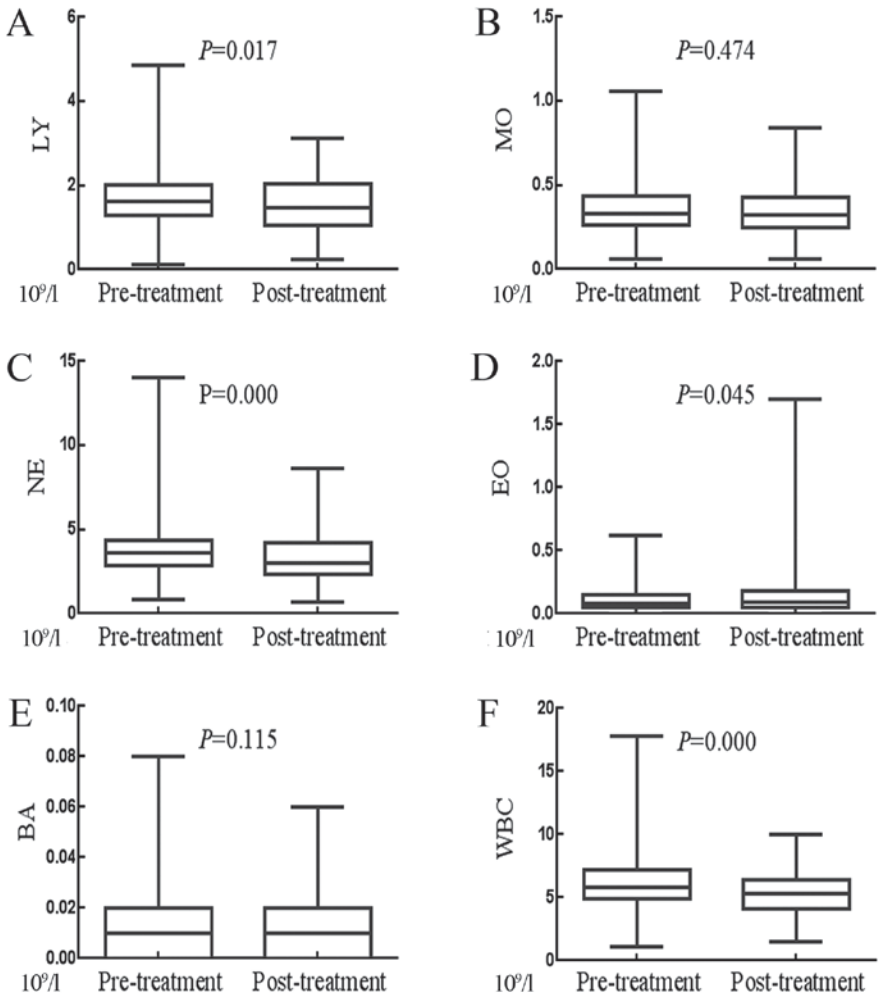

Figure 4. Association between alterations in the status WBC counts and whole course treatment. (A) Whole course treatment decreased the levels of LY. (B) Whole course treatment had no effect on the levels of MO. (C) Whole course treatment decreased the NE counts. (D) Whole course treatment increased the EO count. (E) Whole course of treatment had no influence on the BA counts. (F) Whole course of treatment decreased the value of WBC. n=153. BA, basophil; EO, eosinophil; LY, lymphocyte; MO, monocyte; NE, neutrophil; WBC, white blood cell.

(P=0.115). The median value of WBC was 5.780x10^9/l (95% CI, 5.480-5.94x10^9/l) pre-treatment and 5.280x10^9/l (95% CI, 4.790-5.700x10^9/l) post-treatment (P<0.001). Therefore, whole course treatment significantly decreased the values of LY, NE and WBC; however increased the value of EO, whereas no significant effects on the levels of MO or BA were observed.

Alterations in NE levels following whole course treatment are associated with the outcomes of patients with resectable CRC. Kaplan-Meier plots were used to determine the effects of alterations of LY, MO, NE, EO, BA and WBC status on OS (Fig. 5). The mean OS of patients with increased LY levels following whole course treatment was 87.782 months (95% CI, 79.055-96.508); whereas, that of the unaltered LY group was 86.107 months (95% CI, 78.763-93.450; P=0.516). The mean OS of patients with increased MO levels following whole course treatment was 84.173 months (95% CI, 77.050-91.297); whereas, that of the unaltered MO group was 88.142 months (95% CI, 80.062-96.222; P=0.528). The mean OS of patients with increased NE levels following whole course of treatment was 70.872 months (95% CI, 60.825-80.920), while that of the unaltered NE group was 95.664 months (95% CI, 89.379-101.948; P=0.005). The mean OS of patients with increased EO levels following whole course treatment was 84.245 months (95% CI, 75.092-93.398), while that of the unaltered EO group was 89.443 months (95% CI, 82.799-96.087; P=0.066). The mean OS of patients with increased BA levels following whole course of treatment was 79.730 months (95% CI, 69.770-89.691), while that of the unaltered BA group was 92.373 months (95% CI, 85.453-99.293; P=0.205). The mean OS of patients with increased WBC levels following whole course of treatment was 76.339 months (95% CI, 66.959-85.720), while that of the unaltered WBC group was 93.180 months (95% CI, 86.355-100.005; P=0.137). Therefore, the patients with increased NE levels post-therapy had decreased survival; however, alterations in LY, MO, EO, BA and WBC levels exhibited no marked effects on OS.

Prognostic factors for resectable CRC. Univariate analyses demonstrated that sex [women; hazard ratio (HR) 2.911; 95% CI, 1.413-5.998; P=0.004], tumor size (>5 cm; HR, 2.613; 95% CI, 1.351-5.054; P=0.004), lymphonodus metastasis (N2; HR, 2.197; 95% CI, 1.153-4.187; P=0.017), AJCC stage (III; HR, 2.408; 95% CI, 1.103-5.254; P=0.027), low pre-treatment BA levels (HR, 3.333; 95% CI, 1.524-7.246; P=0.003) and increased post-/pre-treatment NE ratio (HR, 2.410; 95% CI, 1.275-4.554; P=0.007) were significant risk factors for a poor

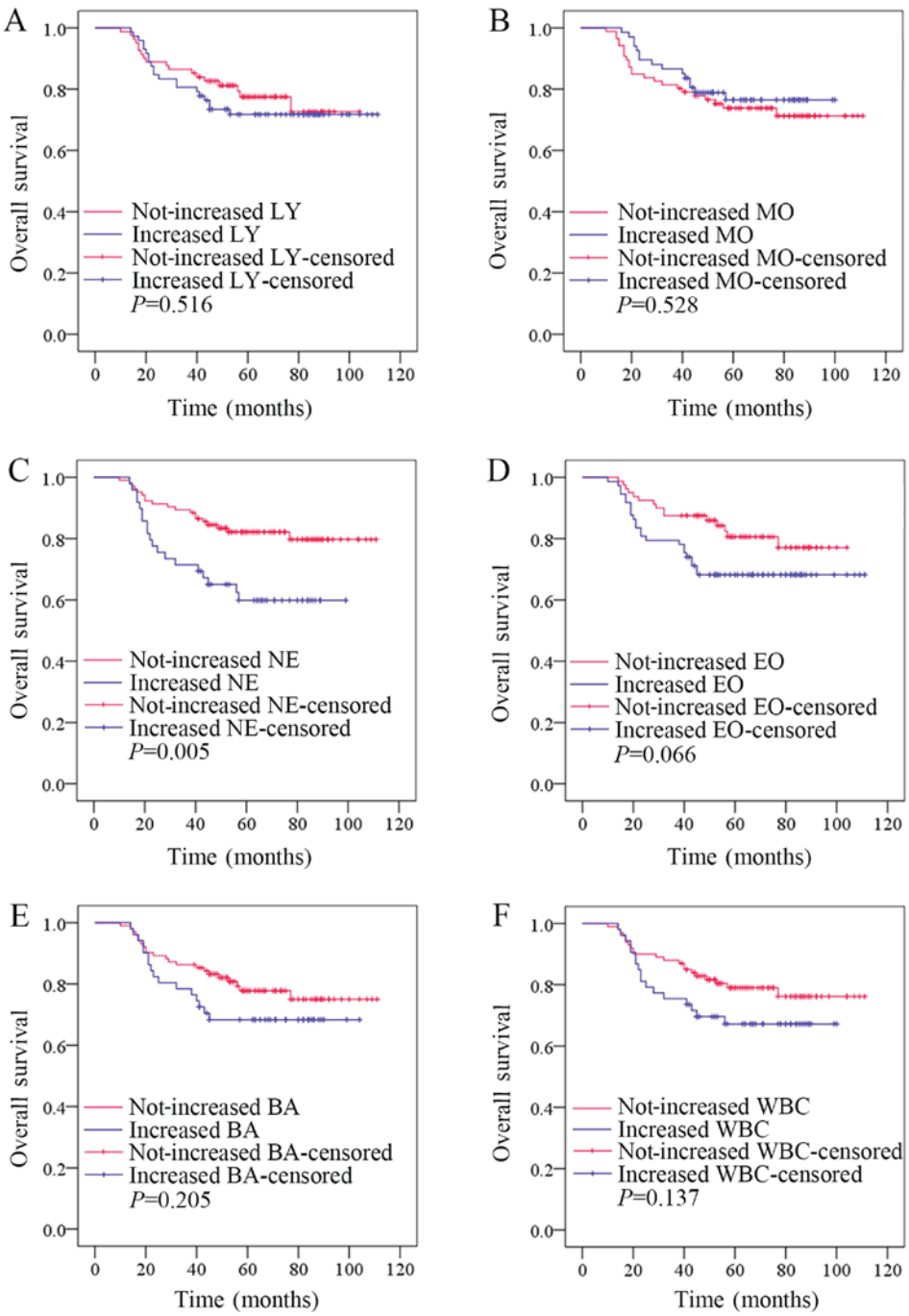

Figure 5. Association between alterations in the status of post-/pre-treatment counts of WBCs and the outcomes. (A) OS according to alterations in LY. (B) OS according to alterations in MO. (C) OS according to alterations in NE. (D) OS according to alterations in EO. (E) OS according to alterations in BA. (F) OS according to alterations in WBC. n=153. BA, basophil; EO, eosinophil; LY, lymphocyte; MO, monocyte; NE, neutrophil; OS, overall survival; WBC, white blood cell.

prognosis (Table II). In multivariate analysis, sex (women; HR, 3.503; 95% CI, 1.620-7.576; P=0.001), tumor size (>5 cm; HR, 2.329; 95% CI, 1.101-4.930; P=0.027), low pre-treatment BA (HR, 3.984; 95% CI, 1.751-9.009; P=0.001) and increased post-/pre-treatment NE ratio (HR, 2.444; 95% CI, 1.240-4.814; P=0.010) were observed to be independently associated with poor survival.

Discussion

WBCs, produced in the bone marrow, are indicators of inflammation, trauma, allergies, leukemia or infections; WBC infiltration is frequently observed in numerous types of cancer (21). WBCs may be classified into five forms, LY, MO, NE, EO and BA, each with various different roles.

In the present study, the LY count was not associated with prognosis; however, previous studies have demonstrated that the LY level is a prognostic factor for non-small-cell lung cancer, gallbladder carcinoma, cervical cancer and hepatocellular carcinoma (15,22-25). This may be due to tumor-infiltrating LYs, which activate $CD8^+$ T LYs by inducing the apoptosis of tumor cells (26). In addition, the primary influencing factors of the anti-tumor immune response are T LYs (27). Tumor-infiltrating LYs are core components of the

Table II. Univariate and multivariate logistic regression analysis of risk factors.

	Overall survival			
	Univariate analysis		Multivariate analysis	
Risk factors	OR (95% CI)	P-value	OR (95% CI)	P-value
Sex (women or men)	2.911 (1.413-5.998)	0.004[a]	3.503 (1.620-7.576)	0.001[a]
Age (>56 years or ≤56 years)	1.003 (0.974-1.034)	0.825	-	-
Tumor size (>5 or ≤5 cm)	2.613 (1.351-5.054)	0.004[a]	2.329 (1.101-4.930)	0.027[b]
Depth of invasion (T3-4 or T1-2)	2.591 (0.624-10.77)	0.190	-	-
Lymphonodus metastasis (N2 or N0-1)	2.197 (1.153-4.187)	0.017[b]	2.100 (0.955-4.619)	0.065
American Joint Committee on Cancer stage (III or I-II)	2.408 (1.103-5.254)	0.027[b]	1.328 (0.524-3.366)	0.550
Pre-treatment LY level (>1.632x10^9/l or ≤1.632x10^9/l)	0.903 (0.477-1.708)	0.753	-	-
Pre-treatment MO level (>0.330x10^9/l or ≤0.330x10^9/l)	0.649 (0.338-1.244)	0.193	-	-
Pre-treatment NE level (>3.600x10^9/l or ≤3.600x10^9/l)	1.059 (0.560-2.000)	0.860	-	-
Pre-treatment EO level (>0.085x10^9/l or ≤0.085x10^9/l)	0.740 (0.388-1.409)	0.359	-	-
Pre-treatment BA level (≤0.010x10^9/l or >0.010x10^9/l)	3.333 (1.524-7.246)	0.003[a]	3.984 (1.751-9.009)	0.001[a]
Pre-treatment WBC level (>5.780x10^9/l or ≤5.780x10^9/l)	1.182 (0.625-2.235)	0.606	-	-
Post-/pre-treatment LY ratio (>1 or ≤1)	1.234 (0.652-2.336)	0.518	-	-
Post-/pre-treatment MO ratio (>1 or ≤1)	0.812 (0.423-1.557)	0.530	-	-
Post-/pre-treatment NE ratio (>1 or ≤1)	2.410 (1.275-4.554)	0.007[a]	2.444 (1.240-4.814)	0.010[b]
Post-/pre-treatment EO ratio (>1 or ≤1)	1.820 (0.949-3.489)	0.071	-	-
Post-/pre-treatment BA ratio (>1 or ≤1)	1.511 (0.793-2.880)	0.209	-	-
Post-/pre-treatment WBC ratio (>1 or ≤1)	1.615 (0.852-3.063)	0.142	-	-

[a]P<0.01; [b]P<0.05; BA, basophil; CI, confidence interval; LY, lymphocyte; EO, eosinophil; MO, monocyte; NE, neutrophil; OR, odds ratio; WBC, white blood cell.

immune response to tumors, with cytotoxic T LYs serving a key role in the eradication of tumors; whereas, regulating T cells may inhibit the immune response (28,29).

Numerous mechanisms mediate the association between elevated NE counts and poor prognosis. Cancer cells are involved in the production of NEs via the release of myeloid growth factors (30). In feedback mechanisms, elevated NE levels promote angiogenesis by secreting factors, including vascular endothelial growth factor, interleukin (IL)-8 and matrix metallopeptidase, which contribute to the progression of cancer (31). Additionally, numerous previous studies have revealed an important association between the NLR and survival in a variety of malignancies, including CRC (32-34). In the present study, it was demonstrated that the NE count may be reduced in response to chemotherapy. As surgery exhibited no effect on NE status, the contribution of reduced NE levels may be primarily associated with chemotherapy. In addition, based on alterations in individual NE levels, the increased post-/pre-treatment NE ratio was associated with reduced OS, and demonstrated to be an independent prognostic marker of poor survival via univariate and multivariate analyses. This suggested that NEs may be a poor marker of prognosis for patients with resectable CRC. As the LY count was not determined to be associated with prognosis, the present study proposed that the association between NLR and survival of patients with resectable CRC maybe primarily due to the association between NE and prognosis.

MOs have been proposed as a prognostic factor in numerous types of cancer, including cervical cancer, melanoma and colorectal cancer (35); cancer-associated inflammation contributes to tumor proliferation, angiogenesis, metastasis and drug resistance (36). MOs, as a part of systemic inflammation responses, serve a critical role in tumorigenesis (37). Tumor-associated macrophages (TAMs) originate from MOs and have an important role in the tumor microenvironment (37). TAMs exhibit pro-tumoral activity by accelerating tumor proliferation and metastasis, immunosuppression and angiogenesis (38-40). Chan *et al* (41) reported that myeloid-derived suppressor cells, which possess immunosuppressive activity, are a subset of circulating leucocytes. High MO counts have been associated with the poor prognosis of cancer via elevated monocytic myeloid-derived suppressor cell counts (41); however, in the present study, surgery and/or chemotherapy did not affect the MO count. In addition, MOs were not observed to be associated with prognosis.

The prognostic role of EO in patients with cancer remains controversial (10). Previous studies have demonstrated that EO was associated with a better prognosis in a number of types of cancer, including laryngeal and colon cancer (42,43). Two mechanisms may be involved in the anti-tumor effects of EOs. EOs are recruited into tumor tissues, and establish contact with tumor cells, inducing a tumoricidal effect (10). EOs may additionally secrete granule proteins and cytokines, including EO cationic protein, EO-derived neurotoxin, tumor necrosis factor-α, 2B4 and cross-linked 2B4, which are involved in tumor cell death (42,44). Conversely, previous studies have demonstrated that EOs had no association or were negatively associated with the prognosis of patients with stage III and IV laryngeal squamous cell carcinoma or nodular sclerosis Hodgkin's disease, respectively (45,46). The present study revealed that EOs were not associated with age, sex, tumor size, depth of invasion, lymphonodus metastasis or AJCC stage; however, surgery increased the levels of

EO. The EO count was not observed to be associated with the prognosis of patients with resectable CRC in the present study.

BA is the least abundant type of WBC in the circulation and has not been studied extensively (47). A number of previous studies have investigated the prognostic potential of BA in cancer progression (47-49). As a type of immune cell, BA has been demonstrated to be negatively associated with tumor prognosis in pancreatic cancer (47). De Monte *et al (*47) revealed that the infiltration of BAs in tumor-draining lymph nodes was associated with the Th2/Th1 cell ratio in tumors and was responsible for the presence of IL-4 in pancreatic cancer. In addition, the counts of circulating BAs in patients with non-small cell lung cancer were higher compared with in healthy individuals (50). Furthermore, in a mouse model of metastatic breast cancer generated by implanting 4T1 cells into the mammary fat pads, Wang *et al (*21) reported that circulating BAs were negatively associated with the number of lung metastases. In the present study, the BA count was negatively associated with tumor size and was proposed to be an independent prognostic indicator for CRC; no effects on age, sex, depth of invasion, lymphonodus metastasis or AJCC stage were observed. Additionally, the present study reported that the pre-treatment BA count was positively associated with OS, suggesting BA may be a prognostic indicator of resectable CRC. Additionally, surgery may increase the BA count; however, differences in the BA levels between the pre- and post-surgery groups were difficult to determine as the BA count was low and was not distinguishable.

The limitations of the present study include its retrospective design, data obtained from a single center and an insufficient number of cases for study. Future studies may aim to investigate larger sample sizes and use multiple approaches.

In conclusion, the results of the present study revealed that a low pre-treatment BA count and an increased post-/pre-treatment NE ratio were positively associated with poorer outcomes in patients with resectable CRC. These noninvasive, simple and low-cost biomarkers may serve as useful prognostic indicators for patients with resectable CRC.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WJW and MYW made substantial contributions to the conception and design of the study; JW, QZ, WZ and XXG revised the manuscript critically for important intellectual content and acquired the data; MDX, WD, KC, FRG, MT and LMS analyzed and interpreted the data for the present study. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committees of The First Affiliated Hospital of Soochow University (Suzhou, China). Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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