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ORIGINAL ARTICLE

A prognostic index for colorectal cancer based on preoperative absolute lymphocyte, monocyte, and neutrophil counts

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Abstract

Purpose Associations between the preoperative absolute neutrophil count (NC), lymphocyte count (LC), and monocyte count (MC) in the peripheral blood and the prognosis of colorectal cancer (CRC) patients have not been widely studied.

Methods We enrolled 361 patients who underwent surgery for CRC between January 2007 and December 2013 to analyze correlations among the LC, MC, and NC and prognosis.

Results Based on cut-off values determined by a receiver operating characteristic analysis, patients were subgrouped as $Lymph^{High}$ or $Lymph^{Low}$ (cut-off: LC = 1460 cells/μL); as $Mono^{High}$ or $Mono^{Low}$ (cut-off: MC = 421 cells/μL); and as $Neut^{High}$ or $Neut^{Low}$ (cut-off: NC = 3247 cells/μL). Patients were then given lymphocyte–monocyte–neutrophil (LMN) scores by adding the points of their different subgroups (1 point each for $Lymph^{Low}$, $Mono^{High}$ and $Neut^{High}$; 0 points for $Lymph^{High}$, $Mono^{Low}$ and $Neut^{Low}$). The 5-year overall survival rates significantly differed by the LMN score (0: 89.7%, 1: 80.6%, 2: 68.8%, and 3: 57.4%; $P < 0.0001$). In the multivariate analysis, the LMN score was found to be an independent prognostic indicator.

Conclusions The combination of the preoperative absolute number of lymphocytes, monocytes, and neutrophils is a useful prognostic indicator in CRC patients.

Keywords Colorectal cancer · Lymphocyte · Monocyte · Neutrophil · Prognosis

Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide. Although the prognosis of CRC has improved because of the greater availability of diagnostic techniques and advances in intraoperative and postoperative care, it still ranks fourth among all cancer-related deaths worldwide [1]. Therefore, identifying postoperative factors that help predict the prognosis in CRC patients is important—ideally, these would be factors that can be measured quickly, easily, and non-invasively in an ordinary clinical setting. Serum tumor markers (TMs) meet these requirements. Carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 are the most commonly used TMs in diagnosing, monitoring, and predicting the prognosis in CRC [2, 3].

TMs in CRC patients mainly originate from the cancer itself. However, recent studies have shown that prognoses in various cancer types are also affected by patient-related factors, including inflammation, immunocompetence, and nutrition. The peripheral-blood immune cells (neutrophils, monocytes, and lymphocytes) are considered in the prognostic nutritional index (PNI), neutrophil-to-lymphocyte ratio (NLR), and monocyte-to-lymphocyte ratio (MLR), which are used to evaluate patients' inflammation, immunocompetence, and nutritional status. These indices are reportedly correlated with the prognosis in various cancer types [4–6], which indicates that the concentrations of these cells are closely related to the prognosis of cancer patients. Therefore, we speculated that evaluating these three cell types would facilitate a more precise prediction of the CRC prognosis.

This study investigated the prognostic significance of the preoperative absolute number of neutrophils, lymphocytes, and monocytes in CRC patients.

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Materials and methods

Patients

We retrospectively analyzed the records of 361 patients with colorectal adenocarcinoma who underwent proctocolectomies at our institution between January 2007 and December 2013. The clinicopathologic findings were determined by the Japanese Classification of Colorectal Carcinoma [7]. No patient received preoperative chemotherapy or radiation therapy. No patients had a history of inflammatory bowel disease in this study. Eight patients underwent emergency operation for an ileus caused by cancer. Of the 361 patients included in the current study, 136 underwent adjuvant chemotherapy with 5-fluorouracil (5-FU)-based regimens, such as S-1, tegafur/uracil, 5-FU plus leucovorin with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI), and tegafur/uracil plus leucovorin.

Patients were periodically checked for early recurrence by diagnostic imaging (chest X-ray, colonoscopy, ultrasonography, computed tomography, and magnetic resonance imaging). Causes of death and patterns of recurrence were determined by reviewing medical records, including laboratory data, ultrasonography, computed tomography, scintigrams, peritoneal punctures, and laparotomies, or by direct inquiry with family members. At the time of the analysis, the median follow-up of the 248 surviving patients was 64.5 months. Of the 113 deaths, 50 were related to recurrence of colorectal cancer and 63 to an unrelated malignancy and an unrelated disease or accident.

Clinicopathologic data, such as the age, sex, tumor localization, tumor size, depth of invasion, lymph node metastasis, distant metastasis, lymphatic invasion, and vascular invasion, were obtained from the database. We also collected data on the absolute numbers of lymphocytes (lymphocyte count; LC), monocytes (monocyte count; MC), neutrophils (neutrophil count; NC), serum albumin level, serum CEA level, and serum C-reactive protein (CRP) level from patients' preoperative (within 1 month before surgery) blood test results. The body mass index (BMI) was calculated as the weight (kg)/height^2 (m^2). Our study was approved by the institutional review board and the informed consent requirement was waived for this retrospective study.

Statistical analyses

Differences between two groups were evaluated using the Mann–Whitney *U* test. Correlations among the LC, MC, NC, and CEA were analyzed using Spearman's rank correlation coefficient. A receiver operating characteristic (ROC) analysis for the status of the 5-year overall survival (OS) was performed, and the Youden index was calculated to determine the optimal cut-off values for the LC, MC, and NC for use in the survival analysis. The follow-up periods of the 73 surviving patients were less than 5 years at the time of the analysis. Those patients were considered to be alive in the ROC analysis in this study. The survival curves were calculated according to the Kaplan–Meier method. For the disease-specific survival (DSS), patients who died from causes other than CRC were considered lost to follow-up at the time of death. Differences between the survival curves were examined with the log-rank test. We used a multivariate analysis of factors considered prognostic of the OS, with Cox's proportional hazards model and a stepwise procedure. $P < 0.05$ was considered significant. The GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS statistics version 24.0 (SPSS Inc, Chicago, IL, USA) software programs were used for the statistical analyses.

Results

The median concentrations (cells/μL) of cell types of interest in peripheral blood were as follows: LC, 1535.4 ± 550.2; MC, 487.6 ± 185.9; and NC, 3992.7 ± 1710. We found significant correlations between the LC and MC ($r = 0.31$, $P < 0.001$, Fig. 1a) and between the MC and NC ($r = 0.43$, $P < 0.001$, Fig. 1b). However, there was no significant correlation between the NC and LC ($r = 0.02$, $P = 0.76$, Fig. 1c).

CEA is the most frequently used TM in CRC patients and is associated with their prognosis. In fact, the 5-year OS rates differed significantly between the CEA^{High} group (≥ 5.0 ng/ml, 53.2%) and the CEA^{Low} group (< 5.0 ng/ml, 81.2%, $P < 0.001$; Fig. 2) in this study. We found no significant correlation between CEA and the LC (Fig. 3a) or between CEA and the MC (Fig. 3b), but we did note a significant but weak correlation between CEA and the NC ($r = 0.13$, $P = 0.014$; Fig. 3c).

Table 1 shows relationships among the clinicopathologic characteristics and the LC, MC, and NC. Patients with larger tumors (≥ 4.0 cm), a low BMI (< 22), a low serum albumin level (< 4.1 g/dL), and distant metastasis had significantly lower LCs than did patients with smaller tumors (< 4.0 cm; $P = 0.032$), those with a high BMI (≥ 22; $P < 0.001$), those with high serum albumin level (≥ 4.1 g/dL; $P = 0.017$), and those without distant metastasis ($P = 0.029$), respectively. Male patients, those with a low serum albumin level (< 4.1 g/dL), and those with high serum CRP level (≥ 0.15 mg/dL) had significantly higher MCs than did female patients ($P < 0.001$), those with a high serum albumin level (≥ 4.1 g/dL; $P = 0.012$), and those with a low serum CRP

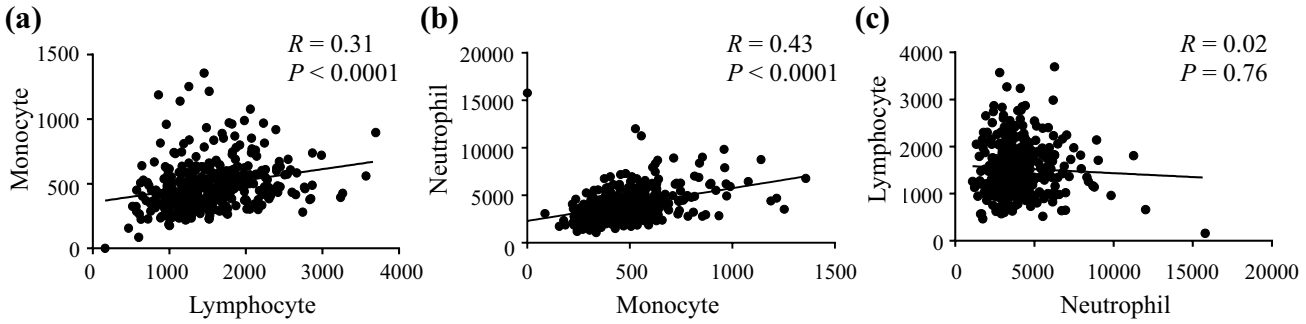

Fig. 1 Correlations between the lymphocyte count (LC) and monocyte count (MC) (**a**), the MC and neutrophil count (NC) (**b**), and the NC and LC (**c**)

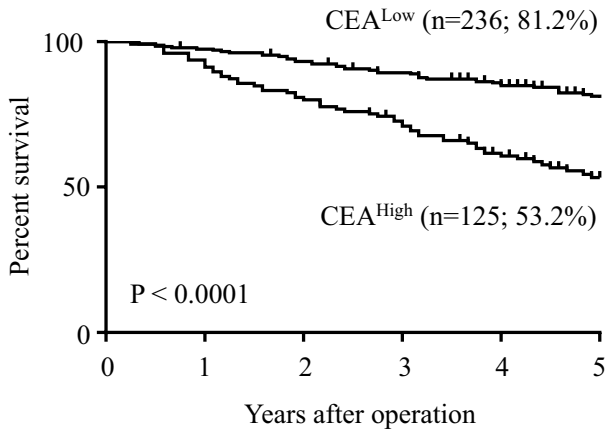

Fig. 2 Overall survival curves based on the serum concentrations of CEA

level (< 0.15 mg/dL; $P < 0.001$), respectively. Patients with larger tumors, with a low serum albumin level, with a high serum CRP level, with T3/T4 disease, with a high serum CEA level, and who underwent emergency operation had a significantly higher NC than did those with smaller tumors ($P < 0.001$), with a high serum albumin level ($P = 0.019$), with a low serum CRP level ($P < 0.001$), with T1/T2 tumors ($P < 0.001$), with a low serum CEA level ($P = 0.012$), and who underwent elective operation ($P = 0.0037$), respectively.

An ROC analysis with respect to the OS indicated that the highest Youden indices (sensitivity + specificity − 1) showed the optimal cut-off values to be as follows: LC, 1460/μl [$P = 0.14$, area under the curve (AUC): 0.549, 95% confidence interval (CI): 0.484–0.613]; MC, 421.5/μl ($P = 0.005$, AUC: 0.592, 95% CI: 0.529–0.654); and NC, 3247/μl ($P = 0.006$, AUC: 0.589, 95% CI: 0.527–0.652). Based on these results, patients were subgrouped by cell counts above or below the respective cut-off values as follows: $Lymph^{High}$ ($n = 183$) or $Lymph^{Low}$ ($n = 178$); $Mono^{High}$ ($n = 220$) or $Mono^{Low}$ ($n = 141$); and $Neut^{High}$ ($n = 226$) or $Neut^{Low}$ ($n = 135$). The 5-year OS rates differed significantly between the $Lymph^{High}$ group (76.5%) and $Lymph^{Low}$ group (66.8%, $P = 0.032$; Fig. 4a); between the $Mono^{High}$ group (66.7%) and $Mono^{Low}$ group (79.6%, $P = 0.0053$; Fig. 4b); and between the $Neut^{High}$ group (67.9%) and $Neut^{Low}$ group (78.2%, $P = 0.006$; Fig. 4c).

Patients were given lymphocyte–monocyte–neutrophil (LMN) scores by adding the points of their different subgroups: 1 point each for $Lymph^{Low}$, $Mono^{High}$ and $Neut^{High}$; and 0 points each for $Lymph^{High}$, $Mono^{Low}$ and $Neut^{Low}$. We

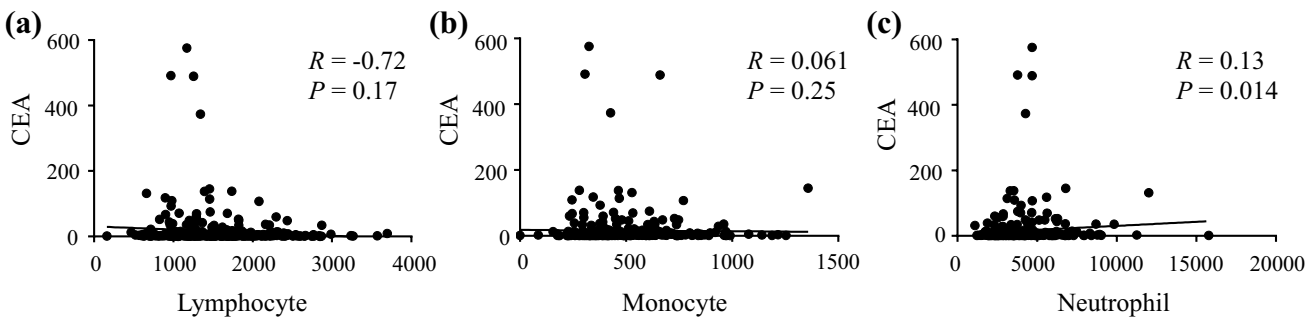

Fig. 3 Correlations between the serum concentrations of CEA and the LC (**a**), MC (**b**), and NC (**c**)

Table 1 Lymphocyte, monocyte and neutrophil counts by patient characteristics

Variables	Lymphocyte	*P* value	Monocyte	*P* value	Neutrophil	*P* value
Age (years)		0.247		0.2		0.494
< 70 (*n* = 181)	1508.0 (1168.0–1898.0)		450.0 (351.0–574.0)		3774.0 (2890.5–4790.0)	
≥ 70 (*n* = 180)	1440.0 (1097.3–1828.5)		468.0(381.0–580.0)		3582.5 (2891.0–5931.5)	
Gender		0.432		< 0.001		0.833
Male (*n* = 199)	1479.0 (1166.0–1863.0)		504.0 (420.0–603.0)		3720.0 (2914.0–4680.0)	
Female (*n* = 162)	1425.0 (1087.8–1859.0)		404.0 (324.8–507.5)		3593.5 (2856.0–4859.3)	
Body mass index		< 0.001		0.342		0.245
< 22 (*n* = 177)	1476.0 (1179.0–1860.0)		470.0 (378.0–593.0)		3577.0 (2868.5–4623.0)	
≥ 22 (*n* = 184)	1425.0 (1127.3–1861.3)		450.0 (361.0–553.0)		3718.5 (2916.5–4752.0)	
Serum albumin level (g/dL)		0.017		0.012		0.019
< 4.1 (*n* = 190)	1404.0 (1055.3–1821.0)		470.0 (383.3–610.5)		3822.0 (2955.5–4975.5)	
≥ 4.1 (*n* = 171)	1512.0 (1240.0–1978.0)		448.0 (357.0–534.0)		3519.0 (2856.0–4380.0)	
Serum CRP level (mg/dL)		0.508		< 0.001		< 0.001
< 0.15 (*n* = 174)	1482.0 (1175.3–1851.5)		429.0 (330.0–518.5)		3240.0 (2574.0–4030.0)	
≥ 0.15 (*n* = 187)	1400.0 (1100.0–1872.0)		483.0 (405.0–630.0)		4266.0 (3264.0–5472.0)	
Serum CEA level (ng/mL)		0.525		0.435		0.012
< 5.0 (*n* = 236)	1475.0 (1173.8–1902.0)		450.5 (366.0–560.0)		3531.0 (2851.5–4533.5)	
≥ 5.0 (*n* = 125)	1455.0 (1135.5–1807.5)		469.0 (370.0–596.0)		3922.0 (3079.5–4986.0)	
Location		0.983		0.144		0.511
Colon (*n* = 238)	1469.0 (1143.0–1898.0)		470.0 (368.3–585.0)		3663.0 (2906.0–4692.5)	
Rectum (*n* = 123)	1470.0 (1188.0–1800.0)		450.0 (364.0–536.0)		3600.0 (2867.0–4662.0)	
Tumor size (cm)		0.032		0.323		< 0.001
< 4.0 (*n* = 182)	1508.0 (1223.8–1935.3)		460.5 (343.8–562.5)		3412.0 (2753.5–4264.5)	
≥ 4.0 (*n* = 179)	1422.0 (1035.0–1800.0)		459.0 (390.0–583.0)		3960.0 (3100.0–5002.0)	
Histology[a]		0.555		0.154		0.094
Differentiated (*n* = 321)	1474.0 (1159.5–1859.5)		456.0 (362.0–565.0)		3599.0 (2880.0–4611.5)	
Undifferentiated (*n* = 40)	1330.0 (1033.8–1938.0)		491.0 (378.5–632.3)		4211.0 (2952.5–5267.8)	
Depth of invasion[b]		0.381		0.125		< 0.001
T1/T2 (*n* = 118)	1510.0 (1194.0–1873.0)		450.0 (343.8–541.5)		3204.0 (2536.3–4082.0)	
T3/T4 (*n* = 243)	1440.0 (1113.0–1848.0)		468.0 (378.0–592.0)		3950.0 (3096.0–4896.0)	
Lymph node metastasis		0.455		0.39		0.76
Absent (*n* = 236)	1504.5 (1163.0–1911.5)		469.0 (364.0–576.0)		3654.0 (2919.0–4705.5)	
Present (*n* = 125)	1440.0 (1130.0–1791.0)		448.0 (371.0–574.0)		3600.0 (2861.0–4680.0)	
Lymphatic invasion[c]		0.579		0.214		0.842
Ly 0/1 (*n* = 190)	1474.0 (1170.0–1878.5)		480.0 (367.8–583.3)		3608.5(2885.8–4692.5)	
Ly 2/3 (*n* = 171)	1455.0(1100.0–1862.0)		448.0(366.0–560.0)		3654.0(2891.0–4680.0)	
Vascular invasion[d]		0.345		0.826		0.289
V 0/1 (*n* = 235)	1512.0 (1159.0–1896.0)		468.0 (364.0–574.0)		3577.0 (2860.0–4623.0)	
V 2/3 (*n* = 126)	1366.5 (1163.5–1810.3)		452.5 (376.5–571.5)		3730.0 (2958.0–4722.0)	
Distant metastasis		0.029		0.548		0.205
Absent (*n* = 327)	1508.0 (1223.0–1935.0)		460.0 (343.0–562.0)		3412.0 (2753.0–4264.0)	
Present (*n* = 34)	1422.0 (1035.0–1800.0)		459.0 (390.0–583.0)		3960.0 (3100.0–5002.0)	
Stage of disease		0.284		0.540		0.622
Stage I/II (*n* = 226)	1508.0 (1170.0–1894.5)		468.0 (364.0–574.5)		3654.0 (2955.5–4712.0)	
Stage III/IV (*n* = 135)	1440.0 (1100.0–1800.0)		448.0 (366.0–574.0)		3600.0 (2856.0–4680.0)	
Operation		0.138		0.515		0.0037
Emergency (*n* = 8)	1463.0 (1145.5–1853.0)		459.0 (366.0–574.0)		3654.0 (2901.0–4683.0)	
Elective (*n* = 353)	1622.0 (1343.5–1944.0)		561.0 (363.0–611.5)		3090.0 (2776.5–4748.3)	

Table 1 (continued)

Data are presented as the median (25th–75th percentile)

[a]Histology: differentiated, well- or moderately differentiated adenocarcinoma; undifferentiated, poorly differentiated adenocarcinoma, signet ring cell carcinoma or mucinous carcinoma

[b]Depth of invasion: T1, tumor invasion of the lamina propria or submucosa; T2, tumor invasion of the muscularis propria; T3, tumor invasion of the sub serosa or within adventitia; T4, tumor penetration of the serosa or tumor invasion of adjacent organs

[c]Lymphatic invasion: ly0–ly3, grade of lymphatic invasion

[d]Vascular invasion: v0–v3, grade of vascular invasion

then performed an ROC analysis of the LMN score with respect to the OS and compared the AUC values of the LMN score to those of the LC, MC, and NC. The AUC for the LMN scores was 0.642 ($P < 0.001$, 95% CI 0.581–0.702), which was higher than those of the LC, MC, or NC and indicated that the LMN score was more accurate than the other indicators used separately in predicting the prognosis of CRC patients.

We next investigated the prognostic significance of the LMN score in patients with CRC. The 5-year OS rates by LMN score differed significantly (0: 89.7%, 1: 80.6%, 2: 68.8%, and 3: 57.4%; $P < 0.0001$, Fig. 5a). Furthermore, the 5-year DSS rates also differed significantly by LMN score (0: 96.6%, 1: 92.2%, 2: 82.3%, and 3: 75.4%; $P = 0.0013$, Fig. 5b). Figure 6 shows the OS curves of stage I/II and stage III/IV according to the LMN score. The 5-year OS rates by LMN score differed significantly in stage I/II CRC patients (0: 100%, 1: 84.5%, 2: 80.6%, and 3: 65.0%; $P = 0.0019$, Fig. 6a). Furthermore, the 5-year OS rates also differed significantly by LMN score in stage III/IV CRC patients (0: 80.0%, 1: 73.0%, 2: 47.5%, and 3: 46.2%; $P = 0.013$, Fig. 6b).

Finally, we performed univariate and multivariate analyses of clinicopathological factors considered prognostic of the OS. The univariate analysis indicated that the age, tumor size, depth of invasion, lymph node metastasis, lymphatic invasion, vascular invasion, distant metastasis, serum CEA

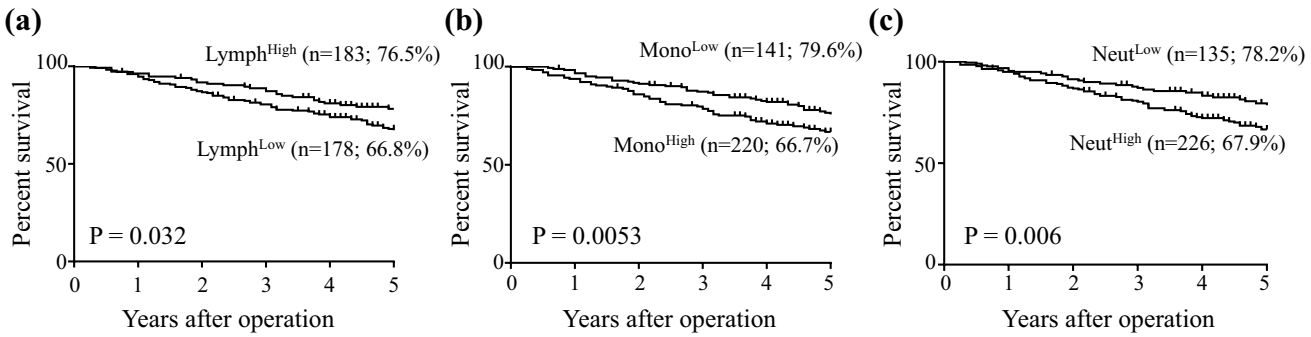

Fig. 4 Overall survival curves based on the LC (**a**), MC (**b**), and NC (**c**)

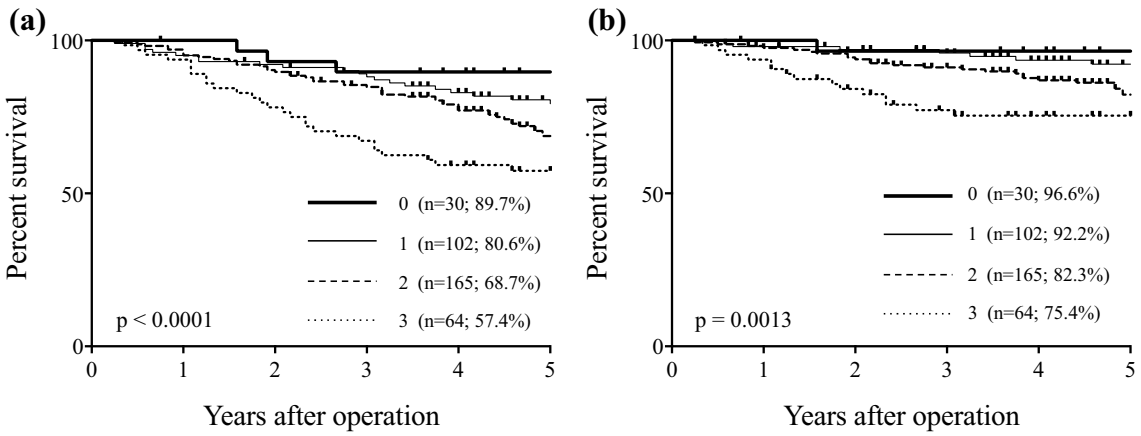

Fig. 5 Survival curves based on the lymphocyte–monocyte–neutrophil score (LMN score). **a** The OS, **b** the DSS

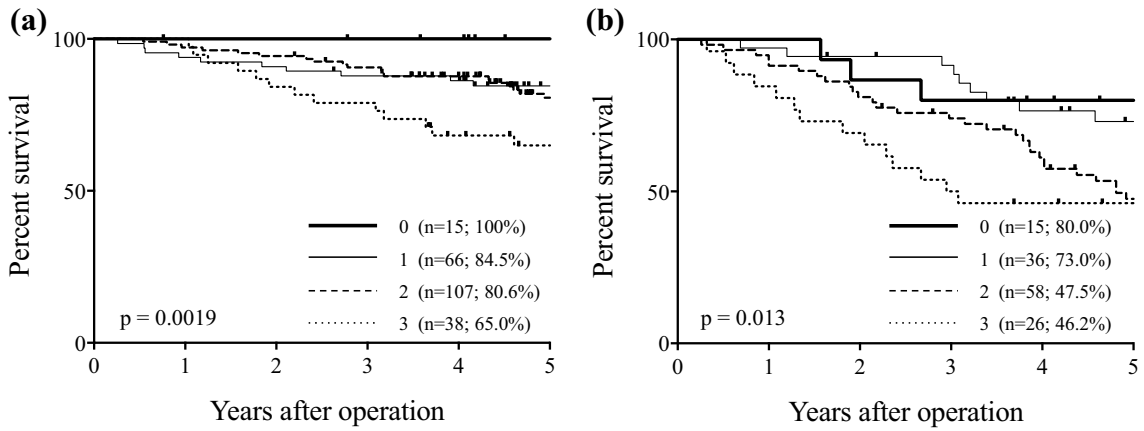

Fig. 6 Overall survival curves based on the lymphocyte–monocyte–neutrophil score (LMN score) according to the stage of disease. **a** Stage I/II, **b** stage III/IV

Table 2 Results of univariate and multivariate analyses of clinicopathological factors in patients with colorectal cancer

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age (≥ 70 years vs. <70 years)	1.515	1.049–2.195	0.0275	1.727	1.171–2.547	0.0058
Gender (male vs. female)	1.163	0.803–1.684	0.4267			
Location (colon vs. rectum)	1.230	0.828–1.806	0.3145			
Tumor size (≥ 4.0 cm vs. <4.0 cm)	1.736	1.198–2.507	0.0037			
Histology (undifferentiated vs. differentiated)	1.400	0.808–2.684	0.2086			
Depth of invasion (T3/T4 vs. T1/ T2)	2.843	1.583–3.430	< 0.0001	3.057	1.062–3.057	0.0289
Lymph node metastasis (present vs. absent)	1.900	1.367–3.035	0.0005			
Lymphatic invasion (ly 2/3 vs. ly 0/1)	1.588	1.101–2.306	0.0139			
Vascular invasion (v 2/3 vs. v 0/1)	1.667	1.173–2.570	0.0060			
Distant metastasis (present vs. absent)	4.063	6.269–28.85	< 0.0001	2.909	1.767–4.788	< 0.0001
CEA (≥ 5.0 ng/mL vs. <5.0 ng/mL)	2.667	2.063–4.619	< 0.0001	1.885	1.260–2.819	0.0020
LMN score (0–3)	1.728	1.360–2.197	< 0.0001	1.509	1.185–1.920	0.0008

See Table 1 for the details of histology, depth of invasion, lymphatic invasion, and vascular invasion

CI confidence interval

level, and LMN score were prognostic indicators. Furthermore, in the multivariate analysis of LMN scores, along with the age, gender, tumor location, tumor size, histology, depth of invasion, lymph node metastasis, lymphatic invasion, vascular invasion, distant metastasis, and serum CEA level, we found that the LMN score was an independent prognostic indicator, as was the age, depth of invasion, distant metastasis, and serum CEA level (Table 2).

Discussion

In the current study, we demonstrated that the LC, MC, and NC were significantly associated with prognosis in CRC patients. Several other studies have also found a low preoperative LC to be associated with a poor prognosis in various types of cancer, including pancreatic cancer [8], esophageal cancer [9], renal cancer [10], and sarcoma and lymphoma [11]. Lymphocytes include $CD4^+$ and $CD8^+$ T-cells, NK cells, NKT cells, gamma-delta T-cells, and B-cells, which are closely associated with tumor immunity. Therefore, an association between fewer lymphocytes and impaired tumor immunity, leading to tumor progression seems logical. In fact, several studies have found that the levels of tumor-infiltrating lymphocytes (such as $CD4^+$ and $CD8^+$ T-cells) are associated with the prognosis in some cancers [12–14], while low levels of immune cells (such as NK cells, B-cells, and gamma-delta T-cells) in peripheral blood and cancer tissue are associated with a poor prognosis in other cancer types [15–17]. Therefore,

the peripheral LC might be a good indicator of the cell-mediated immune status, including both acquired and adaptive immunity, and the humoral immune status against CRC. The LC is also included in the PNI and is considered to reflect patients' nutritional status. In this regard, a low LC was significantly associated with a low BMI and low serum albumin level in this study.

Although preoperative lymphopenia is frequently observed in advanced cancers, details of its mechanism remain unclear. However, we previously reported that up-regulated Fas expression in $CD8^+$ T-cells is largely involved in increased apoptosis of circulating $CD8^+$ T-cells in patients with gastric cancer [18]. Lymphopenia might be induced by a similar mechanism in CRC.

High NC and MC values were also significantly related to a poor prognosis of CRC patients in this study. Neutrophils and monocytes are important components of the inflammatory response. In fact, both high MC and NC were shown to be significantly related to a high serum CRP level in this study. Furthermore, the MC and NC were significantly higher in patients with large tumors (≥ 4 cm) than in those with small tumors (< 4 cm) in this study. Since the tumor size is likely associated with the extent of cancer-related inflammation, the close correlations among the MC, NC, and tumor size indicate that neutrophils and monocytes are important components of the cancer-induced inflammatory response. In this regard, the LC was significantly lower in patients with large tumors (≥ 4 cm) than in those with small tumors (< 4 cm) in the present study, which emphasizes the close relationship between cancer-induced inflammation and peripheral blood cells, including neutrophils, monocytes, and lymphocytes.

Neutrophils have dual roles in tumor development and metastasis. In response to stimulation of various cytokines, neutrophils can become polarized towards antitumor (N1) or protumor (N2) phenotypes [19]. Acute inflammation activates neutrophils to exert antitumor effects, whereas chronic inflammation activates neutrophils to promote tumor growth and metastasis. Inflammatory cytokines, such as G-CSF, IL-6, and TGF-β1, can induce the N2 neutrophil phenotype in bone marrow and tumor microenvironments [20]. Conversely, priming with IFN-γ and TNF-α can convert the neutrophil phenotype from N2 to N1 [21]. As serum concentrations of IL-6 and TGF-β1 are reportedly increased in CRC patients [22, 23], the majority of neutrophils in CRC patients are likely to be of the N2 phenotype, which promotes tumor growth and metastasis and leads to a poor prognosis.

Accumulating evidence suggests that an increased MC may be an indicator of various inflammatory diseases. In addition, monocytes suppress the host immune response to cancer. In this regard, we previously demonstrated that peripheral monocytes up-regulated the PD-L1 expression, which is a ligand for PD-1, in gastric cancer patients [24]. Mounting evidence suggests that the PD-L1 expression by solid tumors dampens the antitumor T-cell responses [25–30] because PD-L1 delivers a co-inhibitory signal to T-cell upon binding to PD-1, which results in an impaired function of T-cells. It has been demonstrated that cytotoxic tumor-infiltrating T-lymphocytes and tumor-infiltrating CD45RO + memory T-cells are closely associated with the prognosis in cancer patients [31, 32]. PD-L1-expressing monocytes likely dampen their function, which results in a poor prognosis. Furthermore, a recent study showed that tumor-associated macrophages (TAMs) expressed PD-1 as well as PD-L1 and TAM PD-1 expression correlates negatively with the phagocytic potency against tumor cells, which has been reported to be closely associated with an impaired tumor immunity [33]. These findings indicate that monocytes are closely associated with both the inflammation status and immunosuppression in cancer patients and support the association between high monocyte levels and a poor prognosis observed in this study.

The prediction of the postoperative prognosis in CRC patients is important for determining the treatment strategy. The tumor size, depth of invasion, presence or absence of lymph node metastasis, and differentiation have been shown to be significant prognostic indicators in CRC patients [34]. Our results indicate that the LC, MC, and NC are also useful predictors of the CRC prognosis. Interestingly, however, their correlation with the serum CEA concentration—the most frequently used TM in CRC patients—was extremely weak. Therefore, these markers can be used as prognostic indicators independently of CEA.

However, a significant positive correlation was noted between the MC and NC. This seems to be reasonable, as both monocytes and neutrophils are components of the inflammatory response. We also observed a significant positive correlation between the MC and LC. It has been demonstrated that monocytes can differentiate into dendritic cells, which are antigen-presenting cells, and can stimulate T-cells [35–37]. This might explain why there was a significant positive correlation between the MC and LC in this study. Although there were some significant positive correlations among the LC, MC, and NC in this study, these correlations were also weak. We therefore hypothesized that their combination would be a more useful prognostic predictor than the any of them alone. To determine their combined prognostic significance, we developed the LMN score in the current study and showed that the AUC of the LMN score was much higher than those of the component blood counts, which indicates its greater utility in predicting the prognosis of CRC patients. Furthermore, our results clearly showed the LMN score, but not the serum CEA level, to be an independent prognostic indicator. Its use facilitates the assessment of

a patient's inflammatory and immune statuses, which are considered to be patient-related prognostic factors.

Several limitations associated with the present study warrant mention. First, this was a retrospective analysis, which should generate some bias. Second, lymphocytes include some regulatory cells (e.g. regulatory T-cells), which reportedly have negative effects on the cancer prognosis [38]; however, this study did not address those effects. Third, our study cohort was relatively small; a large-scale, prospective study is therefore needed to verify our results.

In conclusion, the LMN score—a combination of LC, MC, and NC—appears to be a useful indicator of the CRC prognosis. Because the peripheral blood cell count is a quick, easy, and non-invasive assay, the LMN score can be used to predict the prognosis of CRC patients in routine clinical settings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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