FULL PAPER

BJC

British Journal of Cancer (2013) 109, 401–407 | doi: 10.1038/bjc.2013.350

Keywords: combination of platelet count and neutrophil to lymphocyte ratio; inflammation-based prognostic system; neutrophil to lymphocyte ratio; survival after surgery; systemic inflammatory response; thrombocytosis

Combination of platelet count and neutrophil to lymphocyte ratio is a useful predictor of postoperative survival in patients with colorectal cancer

M Ishizuka*[,1], H Nagata[1], K Takagi[1], Y Iwasaki[1] and K Kubota[1]

[1]*Department of Gastroenterological Surgery, Dokkyo Medical University, Mibu, Tochigi 321-0293, Japan*

Background: This study investigated the usefulness of a novel inflammation-based prognostic system, named the COP-NLR (COmbination of Platelet count and Neutrophil to Lymphocyte Ratio), for predicting the postoperative survival of patients with colorectal cancer (CRC).

Methods: The COP-NLR was calculated on the basis of data obtained on the day of admission: patients with both an elevated platelet count ($>30\times10^4\,mm^{-3}$) and an elevated NLR (>3) were allocated a score of 2, and patients showing one or neither were allocated a score of 1 or 0, respectively.

Results: Four-hundred and eighty patients were enrolled. Multivariate analysis of clinical characteristics selected by univariate analysis showed that the COP-NLR (1, 2/0) (odds ratio, 0.464; 95% confidence interval, 0.267–0.807; $P=0.007$) had an association with cancer-specific survival, along with pathology, lymph node metastasis, the serum levels of carcinoembryonic antigen, C-reactive protein and albumin, and the Glasgow Prognostic Score. Kaplan–Meier analysis and log-rank test revealed that the COP-NLR was able to divide such patients into three independent groups ($P<0.001$).

Conclusion: The COP-NLR is considered to be a useful predictor of postoperative survival in patients with CRC.

Recent studies have demonstrated that the systemic inflammatory response (SIR) (McMillan *et al*, 2003) is associated with postoperative survival in patients with several types of cancer (Forrest *et al*, 2004; Ishizuka *et al*, 2007; Ramsey *et al*, 2007). Although there are a number of inflammation-based prognostic systems and clinical measures based on SIR, such as the Glasgow Prognostic Score (GPS) (Forrest *et al*, 2004; Ramsey *et al*, 2007; Ishizuka *et al*, 2012), neutrophil to lymphocyte ratio (NLR) (Bhatti *et al*, 2010; Kim *et al*, 2010; Shimada *et al*, 2010; Chua *et al*, 2011), and reactive thrombocytosis (Silvis *et al*, 1970; Monreal *et al*, 1998; Shimada *et al*, 2004), their systems of evaluation differ. Although the GPS is based on estimation of two types of protein – the serum levels of C-reactive protein (CRP) and albumin, which are regulated by inflammatory cytokines – the NLR and thrombocytosis are based on cellular components that are also regulated by such cytokines, especially interleukin-6 (IL-6) (Ohsugi, 2007).

Among the inflammatory cytokines, IL-6 is known to be a multifunctional cytokine that acts on a variety of cells, including immune-competent cells and hematopoietic cells, to trigger proliferation and differentiation. From a clinical viewpoint, it is also important to consider that IL-6 stimulates hepatocytes to induce acute-phase proteins, including CRP, and decrease the serum albumin level (Ramadori *et al*, 1998; Ohsugi, 2007). This phenomenon is merely part of the mechanism reflected in the GPS. Similarly, IL-6 elicits not only neutrophil proliferation but also differentiation of megakaryocytes to platelets (Imai *et al*, 1991; Ruscetti, 1994), and these phenomena are also related to the mechanisms underlying SIR.

*Correspondence: Dr M Ishizuka; E-mail: mm-ishizuka@umin.ac.jp

Received 16 November 2012; revised 13 February 2013; accepted 14 June 2013; published online 2 July 2013

There is an increasing evidence that the GPS, reactive thrombocytosis, and the NLR can be used for prognostication in patients with several types of cancer (Shimada *et al*, 2004, 2010). Because various combinations of acute-phase proteins can be employed for this purpose, it would be expected that estimation of reactive thrombocytosis (Shimada *et al*, 2004) and NLR (Shimada *et al*, 2010) would also be potentially applicable for prognostication.

In the present study, therefore, we evaluated the prognostic utility of a novel inflammation-based prognostic system, named the COP-NLR (COmbination of Platelet count and Neutrophil to Lymphocyte Ratio), in patients undergoing surgery for colorectal cancer (CRC).

MATERIALS AND METHODS

We conducted a retrospective review of a database comprising 490 patients who had undergone elective surgery for CRC. All procedures had been performed by the same surgical team at the Department of Gastroenterological Surgery, Dokkyo Medical University Hospital, between January 2000 and August 2009. Among these patients, 480 were enrolled in this study on the basis of specific criteria. Patients for whom insufficient laboratory data were available in the record, or those who had idiopathic thrombocytopenic purpura ($n=2$), were excluded.

Routine laboratory measurements including the serum levels of CRP, albumin, and tumour markers such as carcinoembryonic antigen (CEA; upper physiological value $5\,ng\,ml^{-1}$) and carbohydrate antigen 19-9 (upper physiological value $37\,U\,ml^{-1}$) were carried out on the day of admission in order to exclude any effects attributable to inflammation associated with sequential preoperative examinations. None of the patients had clinical evidence of infection or other inflammatory conditions, and none had received preoperative chemotherapy or irradiation.

The recommended cut-off values of the preoperative NLR and platelet count were decided using receiver operating characteristic (ROC) curve analyses. The recommended cut-off value of the NLR was based on the most prominent point on the ROC curve for sensitivity (0.492) and specificity (0.742), respectively. Because these two parameters indicated a cut-off value of 2.902, the recommended NLR cut-off value was defined as 3.0. The area under the ROC curve was 0.619. Similarly, the recommended cut-off value of the preoperative platelet count was defined as $30\times10^4\,mm^{-3}$, because the most prominent point on the ROC curve indicated a cut-off value of $28.85\times10^4\,mm^{-3}$ for sensitivity (0.413) and specificity (0.742). The area under the ROC curve was 0.589.

The COP-NLR was calculated on the basis of data obtained on the day of admission as follows: patients with both an elevated platelet count ($>30\times10^4\,mm^{-3}$) and an elevated NLR (>3) were allocated a score of 2, and patients showing one or neither were allocated a score of 1 or 0, respectively.

Univariate analysis was performed to evaluate clinical characteristics including age (year), sex (male/female), number of tumours ($\geqslant2/1$), maximum tumour diameter ($>40/\leqslant40$ mm), tumour location (rectum/colon), tumour type (3, 4, and 5/0, 1, and 2), pathology (others/tub1, 2), lymphatic invasion (presence/absence), venous invasion (presence/absence), lymph node metastasis (presence/absence), white blood cell (WBC) count ($\times10^3\,mm^{-3}$), platelet count ($\times10^4\,mm^{-3}$), neutrophil ratio (%), lymphocyte ratio (%), NLR, the serum levels of CRP ($mg\,dl^{-1}$), albumin ($g\,dl^{-1}$), CEA ($ng\,ml^{-1}$), and CA19-9 ($U\,ml^{-1}$), body mass index (BMI) ($kg\,m^{-2}$), the GPS (2/0, 1), and the COP-NLR (1, 2/0) relative to cancer-specific survival.

Multivariate analysis was then performed using clinical characteristics with a P-value <0.05 selected in the univariate analysis to assess those that were predictive of cancer-specific survival.

Kaplan–Meier analysis and log-rank test were used to compare the survival curves of the three groups divided according to the COP-NLR scores of 0, 1, and 2.

Estimation of the GPS. The GPS was estimated as described previously. Briefly, patients with both an elevated CRP level ($>1.0\,mg\,dl^{-1}$) and hypoalbuminemia ($<3.5\,g\,dl^{-1}$) were allocated a score of 2. Patients in whom only one of these biochemical abnormalities was present were allocated a score of 1, and those in whom neither of these abnormalities was present were allocated a score of 0 (Forrest *et al*, 2004).

Definition of operative curability. Based on the Japanese Classification of Colorectal Carcinoma (Japanese Society for Cancer of the Colon and Rectum, Second English Edition), residual tumour is diagnosed as follows: R0, no residual tumour; R1, no residual tumour, but tumour suspected at the resection margin; and R2, macroscopically evident residual tumour. Based on this definition, operative curability is defined as follows: curability A, R0 in TNM stage I, II, or III; curability B, R0 in TNM stage IV or R1 in any TNM stage; curability C, R2 in any TNM stage.

Definition of macroscopic tumour types and histological findings. Similarly, macroscopic tumour types are classified as follows: type 0, superficial; type 1, polypoid; type 2, ulcerated with clear margin; type 3, ulcerated with infiltration; type 4, diffusely infiltrating; and type 5, unclassified. On the basis of these definitions, we classified the patients into two groups: those with noninvasive tumours (types 0, 1, and 2) and those with invasive tumours (types 3, 4, and 5).

The pathological types of tumours are defined as follows: tub1, well-differentiated adenocarcinoma; tub2, moderately differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma; and sig, signet ring cell carcinoma. On the basis of these definitions, the patients were divided into two groups: those with differentiated adenocarcinoma (tub1 and tub2) and those with other types of carcinoma (por, muc, and sig).

Invasion of vessels – that is, lymphatic invasion (ly) and venous invasion (v) – was diagnosed as follows: ly0 (v0), no invasion; ly1 (v1), minimal invasion; ly2 (v2), moderate invasion; and ly3 (v3), severe invasion. On the basis of these definitions, the patients were divided into two groups: those without (ly0, v0) and those with (ly1–3, v1–3) vessel invasion.

Administration of chemotherapy. Most of the stage IV patients undergoing surgery were considered for postoperative chemotherapy. Because, in our department, recently developed chemotherapy regimens such as FOLFIRI (FOLinic acid (leucovorin)/Fluorouracil (5-FU)/IRInotecan (Camptosar)) (Tournigand *et al*, 2004) and FOLFOX (FOLinic acid (leucovorin)/Fluorouracil (5-FU)/OXaliplatin (Eloxatin)) (de Gramont *et al*, 2000) were introduced in January 2005, patients who had undergone surgery before January 2005 had been administered oral anticancer drugs based on 5-FU as postoperative chemotherapy (Hernandez *et al*, 1992).

Similarly, most of the stage III patients undergoing surgery were considered for postoperative chemotherapy. Because, in our department, recently developed chemotherapy regimens such as capecitabine (Twelves *et al*, 2005) were introduced in October 2008, patients who had undergone surgery before October 2008 were administered the same drugs as those for stage IV patients (Kaser *et al*, 2001). In addition, most of the stage II patients undergoing surgery were not considered for postoperative chemotherapy.

Statistical analysis. Data are presented as mean ± s.d. Differences among the three groups were analysed using the χ^2- test and the Kruskal–Wallis test. Odds ratios (OR) with 95% confidence interval (CI) were calculated by uni- or multivariate Cox proportional hazards model analyses.

Kaplan–Meier analysis and log-rank test were used to compare survival curves. Deaths before 31 March 2009 were included in this analysis.

Statistical analyses were performed using the SPSS statistical software package, version 16.0 (SPSS Inc., Chicago, IL, USA) at a significance level of $P<0.05$.

RESULTS

A total of 480 patients were enrolled (male : female = 309 (64.4%) : 171 (35.6%)). There were 356 (74.2%) patients with a platelet count of $\leqslant 30\times10^4\,mm^{-3}$ and 124 (25.8%) patients with a platelet count of $>30\times10^4\,mm^{-3}$. Among these patients, 338 (70.4%) patients had a NLR of $\leqslant 3$ and 142 (29.6%) patients had a NLR of >3.

Table 1 shows the distribution of the clinical background characteristics of the studied patients in the three groups divided according to the COP-NLR. There were no significant differences among the groups, except for maximum tumour diameter ($\leqslant 40$/ >40, mm) ($P<0.001$), tumour type (0, 1, 2/3, 4, and 5) ($P=0.016$), lymphatic invasion (absence/presence) ($P<0.001$), venous invasion (absence/presence) ($P=0.004$), operative curability (A/B/C) ($P<0.001$), GPS (0/1/2) ($P<0.001$), and TNM stage (0/I/II/III/IV) ($P<0.001$) (χ^2-test).

Table 2 shows the relationships between clinicolaboratory characteristics and the three groups of patients. There were no significant inter-group differences, except for maximum tumour diameter (mm; $P<0.001$), WBC count ($P<0.001$), platelet count ($P<0.001$), neutrophil ratio ($P<0.001$), lymphocyte ratio ($P<0.001$), NLR ($P<0.001$), the serum levels of CRP ($mg\,dl^{-1}$; $P<0.001$), albumin ($g\,dl^{-1}$; $P<0.001$) and CEA ($ng\,ml^{-1}$; $P<0.001$), BMI ($kg\,m^{-2}$; $P=0.002$), and survival period ($P=0.001$) (Kruskal–Wallis test).

During the observation period, 150 (31.3%) patients died, among whom 30 (20.0%) died of intercurrent disease. Univariate and multivariate analyses were performed to evaluate the relationship between clinical characteristics and cancer-specific survival.

The results of univariate analyses demonstrated that maximum tumour diameter ($\leqslant 40$/ >40 mm) (OR, 0.517; 95% CI, 0.349–0.767; $P=0.001$), pathology (others/tub1, 2) (OR, 0.338; 95% CI, 0.205–0.558; $P<0.001$), lymphatic invasion (presence/absence) (OR, 0.167; 95% CI, 0.068–0.409; $P<0.001$), venous invasion (presence/absence) (OR, 0.376; 95% CI, 0.207–0.683; $P=0.001$), lymph node metastasis (presence/absence) (OR, 0.299; 95% CI, 0.201–0.444; $P<0.001$), WBC count ($\times10^3\,mm^{-3}$) (OR, 1.108; 95% CI, 1.060–1.159; $P<0.001$), platelet count ($\times10^4\,mm^{-3}$) (OR, 1.027; 95% CI, 1.011–1.043; $P=0.001$), neutrophil ratio (%) (OR, 1.041; 95% CI, 1.024–1.059; $P<0.001$), lymphocyte ratio (%) (OR, 0.951; 95% CI, 0.933–0.970; $P<0.001$), NLR (OR, 1.126; 95% CI, 1.074–1.179; $P<0.001$), the serum levels of CRP ($mg\,dl^{-1}$) (OR, 1.224; 95% CI, 1.159–1.292; $P<0.001$), albumin ($g\,dl^{-1}$) (OR, 0.530; 95% CI, 0.394–0.714; $P<0.001$), CEA ($ng\,ml^{-1}$) (OR, 1.001; 95% CI, 1.001–1.002; $P<0.001$), and CA19-9 ($U\,ml^{-1}$) (OR, 1.000; 95% CI, 1.000–1.000; $P<0.001$), the GPS (2/0, 1) (OR, 0.321; 95% CI, 0.208–0.497; $P<0.001$), and the COP-NLR (1, 2/0) (OR, 0.349; 95% CI, 0.240–0.507; $P<0.001$) were associated with cancer-specific survival (Table 3).

Multivariate analysis was performed using the characteristics shown to have statistical significance ($P<0.05$) by univariate analysis. This indicated that the COP-NLR (1, 2/0) was associated

Table 1. Relationships between clinical characteristics and the COP-NLR in patients with CRC

Variable	COP-NLR 0 ($n=271$)	COP-NLR 1 ($n=152$)	COP-NLR 2 ($n=57$)	*P*-value
Age (year)				
⩽70	162	81	33	
>70	109	71	24	0.431
Sex				
Male	170	102	37	
Female	101	50	20	0.663
Number of tumours				
1	248	136	52	
⩾2	23	16	5	0.785
Maximum tumour diameter (mm)				
⩽40	145	42	10	
>40	126	110	47	**<0.001**
Tumour location				
Colon	166	99	42	
Rectum	105	53	15	0.193
Tumour type				
0, 1, 2	240	136	43	
3, 4, 5	31	16	14	**0.016**
Pathology				
tub 1, 2	255	138	49	
Others	16	14	8	0.092
Lymphatic invasion				
Absence	66	17	4	
Presence	205	135	53	**<0.001**
Venous invasion				
Absence	66	28	3	
Presence	205	124	54	**0.004**
Lymph node metastasis				
Absence	156	81	26	
Presence	111	70	28	
Not available	4	1	3	0.100
Operative curability				
A	226	103	30	
B	25	18	9	
C	20	31	18	**<0.001**
GPS				
0	182	74	14	
1	82	46	22	
2	7	32	21	**<0.001**
TNM stage				
0	15	3	0	
I	60	15	3	
II	71	52	17	
III	86	39	11	
IV	39	43	26	**<0.001**

Abbreviations: COP-NLR = COmbination of Platelet count and Neutrophil to Lymphocyte Ratio; GPS = Glasgow Prognostic Score; TNM = tumour nodes metastasis. χ^2-test tub1: well-differentiated adenocarcinoma, tub2: moderately differentiated adenocarcinoma. Bold entries indicate statistical significance $P<0.05$.

Table 2. Relationships between clinicolaboratory characteristics and the COP-NLR in patients with CRC

Variable	COP-NLR 0 (*n* = 271)	COP-NLR 1 (*n* = 152)	COP-NLR 2 (*n* = 57)	*P*-value
Age (year)	67 ± 10	68 ± 12	67 ± 12	0.587
Number of tumours	1.1 ± 0.5	1.1 ± 0.5	1.1 ± 0.4	0.946
Maximum tumour diameter (mm)	43 ± 21	56 ± 20	68 ± 26	**<0.001**
WBC count ($\times 10^3\,mm^{-3}$)	5.8 ± 1.6	7.1 ± 2.4	10.0 ± 4.2	**<0.001**
Platelet count ($\times 10^4\,mm^{-3}$)	22 ± 5	29 ± 10	40 ± 9	**<0.001**
Neutrophil ratio (%)	57 ± 7	67 ± 11	75 ± 7	**<0.001**
Lymphocyte ratio (%)	33 ± 6	23 ± 9	16 ± 5	**<0.001**
NLR	1.8 ± 0.6	3.7 ± 2.6	6.2 ± 4.8	**<0.001**
CRP ($mg\,dl^{-1}$)	0.5 ± 1.2	1.2 ± 1.9	3.1 ± 4.1	**<0.001**
Albumin ($g\,dl^{-1}$)	3.7 ± 0.5	3.5 ± 0.6	3.2 ± 0.7	**<0.001**
CEA ($ng\,ml^{-1}$)	25 ± 148	116 ± 441	68 ± 193	**<0.001**
CA19-9 ($U\,ml^{-1}$)	104 ± 595	268 ± 1202	302 ± 820	0.094
Body mass index ($kg\,m^{-2}$)	23 ± 3.2	22 ± 3.3	22 ± 3.8	**0.002**
Survival period (days)	1414 ± 841	1253 ± 1058	1104 ± 1031	**0.001**

Abbreviations: CA19-9 = carbohydrate antigen 19-9; CEA = carcinoembryonic antigen; COP-NLR = COmbination of Platelet count and Neutrophil to Lymphocyte Ratio; CRP = C-reactive protein; NLR = neutrophil to lymphocyte ratio; WBC = white blood cell. Mean ± s.d., Kruskal–Wallis test. Bold entries indicate statistical significance $P<0.05$.

Table 3. Univariate analyses in relation to cancer-specific survival

Variable	*P*-value	Odds ratio	95% CI
Age (year)	0.784	0.998	0.981–1.014
Sex (male/female)	0.530	0.885	0.605–1.295
Number of tumours (⩾2/1)	0.205	0.697	0.399–1.218
Maximum tumour diameter (>40/⩽40, mm)	**0.001**	0.517	0.349–0.767
Tumour location (rectum/colon)	0.944	0.987	0.680–1.432
Tumour type (3, 4, 5/0, 1, 2)	0.195	0.723	0.442–1.180
Pathology (others/tub1, 2)	**<0.001**	0.338	0.205–0.558
Lymphatic invasion (presence/absence)	**<0.001**	0.167	0.068–0.409
Venous invasion (presence/absence)	**0.001**	0.376	0.207–0.683
Lymph node metastasis (presence/absence)	**<0.001**	0.299	0.201–0.444
WBC count ($\times 10^3\,mm^{-3}$)	**<0.001**	1.108	1.060–1.159
Platelet count ($\times 10^4\,mm^{-3}$)	**0.001**	1.027	1.011–1.043
Neutrophil ratio (%)	**<0.001**	1.041	1.024–1.059
Lymphocyte ratio (%)	**<0.001**	0.951	0.933–0.970
NLR	**<0.001**	1.126	1.074–1.179
CRP ($mg\,dl^{-1}$)	**<0.001**	1.224	1.159–1.292
Albumin ($g\,dl^{-1}$)	**<0.001**	0.530	0.394–0.714
CEA ($ng\,ml^{-1}$)	**<0.001**	1.001	1.001–1.002
CA19-9 ($U\,ml^{-1}$)	**<0.001**	1.000	1.000–1.000
BMI ($kg\,m^{-2}$)	0.350	0.975	0.923–1.029
GPS (2/0, 1)	**<0.001**	0.321	0.208–0.497
COP-NLR (1, 2/0)	**<0.001**	0.349	0.240–0.507

Abbreviations: BMI = body mass index; CA19-9 = carbohydrate antigen 19-9; CEA = carcinoembryonic antigen; CI = confidence interval; COP-NLR = COmbination of Platelet count and Neutrophil to Lymphocyte Ratio; CRP = C-reactive protein; GPS = Glasgow Prognostic Score; NLR = neutrophil to lymphocyte ratio; WBC = white blood cell. Bold entries indicate statistical significance $P<0.05$.

with cancer-specific survival (OR, 0.464; 95% CI, 0.267–0.807; $P = 0.007$), along with pathology (others/tub1, 2) (OR, 0.377; 95% CI, 0.217–0.655; $P<0.001$), lymph node metastasis (presence/absence) (OR, 0.377; 95% CI, 0.241–0.591; $P<0.001$), the serum levels of CRP ($mg\,dl^{-1}$) (OR, 1.189; 95% CI, 1.081–1.308; $P<0.001$), albumin ($g\,dl^{-1}$) (OR, 0.547; 95% CI, 0.376–0.794; $P = 0.002$), and CEA (OR, 1.001; 95% CI, 1.001–1.001; $P<0.001$), and the GPS (2/0, 1) (OR, 2.604; 95% CI, 1.242–5.456; $P = 0.011$) (Table 4).

The median and maximum follow-up periods for survivors were 1169 and 3936 days, respectively, and the mean follow-up period was 1326 ± 943 days (mean ± s.d.). There were significant differences in the periods after surgery among the COP-NLR 0 (1414 ± 841 days), 1 (1253 ± 1058 days) and 2 (1104 ± 1031 days) groups (mean ± s.d., $P = 0.001$, Kruskal–Wallis test).

Kaplan–Meier analysis and log-rank test demonstrated that there were significant differences in cancer-specific survival among the three groups ($P<0.001$) (Figure 1). Thus, the COP-NLR was able to clearly classify such patients into three independent groups.

DISCUSSION

Because the COP-NLR consists of two SIR-related characteristics – NLR and platelet count – it is reasonable that a higher proportion of patients with COP-NLR 2 or 1 would have large tumours, invasive-type tumours, presence of lymphatic invasion, presence of venous invasion, operative curability C, GPS 2, and TNM stage IV than patients with COP-NLR 0. Similarly, the COP-NLR showed close relationships with not only tumour-related characteristics, such as maximum tumour diameter and the serum level of CEA, but also SIR-related characteristics such as WBC count, platelet count, neutrophil ratio, lymphocyte ratio, NLR, the serum levels of CRP and albumin, and BMI. The latter characteristics are related to nutritional status, because an elevated CRP level, hypoalbuminemia, and low BMI reflect cachexia due to hypercytokinemia resulting from tumour progression. These characteristics were retained after uni- and multivariate analyses. Univariate analysis also selected tumour-related characteristics including maximum tumour diameter, pathology, lymphatic invasion, venous invasion, lymph node metastasis, CEA, and CA19-9, and SIR-related characteristics including WBC count, platelet count, neutrophil ratio, lymphocyte ratio, NLR, the serum levels of CRP and albumin, the GPS, and the COP-NLR. Among these clinical characteristics, multivariate analysis disclosed that the COP-NLR was associated with cancer-specific survival, along with not only tumour-related characteristics, such as pathology, lymph node metastasis, and CEA, but also SIR-related characteristics such as

Table 4. Multivariate analyses in relation to cancer-specific survival

Variable	*P*-value	Odds ratio	95% CI
Maximum tumour diameter (>40/⩽40, mm)	0.595	0.889	0.575–1.373
Pathology (others/tub1, 2)	<**0.001**	0.377	0.217–0.655
Lymphatic invasion (presence/absence)	0.260	0.564	0.208–1.530
Venous invasion (presence/absence)	0.305	0.706	0.363–1.374
Lymph node metastasis (presence/absence)	<**0.001**	0.377	0.241–0.591
WBC count ($\times 10^3\,mm^{-3}$)	0.301	1.050	0.957–1.152
Platelet count ($\times 10^4\,mm^{-3}$)	0.585	0.994	0.973–1.016
Neutrophil ratio (%)	0.361	0.973	0.919–1.031
Lymphocyte ratio (%)	0.295	0.965	0.903–1.032
NLR	0.554	0.961	0.843–1.096
CRP ($mg\,dl^{-1}$)	<**0.001**	1.189	1.081–1.308
Albumin ($g\,dl^{-1}$)	**0.002**	0.547	0.376–0.794
CEA ($ng\,ml^{-1}$)	<**0.001**	1.001	1.001–1.001
CA19-9 ($U\,ml^{-1}$)	0.084	1.000	1.000–1.000
GPS (2/0, 1)	**0.011**	2.604	1.242–5.456
COP-NLR (1, 2/0)	**0.007**	0.464	0.267–0.807

Abbreviations: CA19-9 = carbohydrate antigen 19-9; CEA = carcinoembryonic antigen; CI = confidence interval; COP-NLR = COmbination of Platelet count and Neutrophil to Lymphocyte Ratio; CRP = C-reactive protein; GPS = Glasgow Prognostic Score; NLR = neutrophil to lymphocyte ratio; WBC = white blood cell. Bold entries indicate statistical significance $P<0.05$.

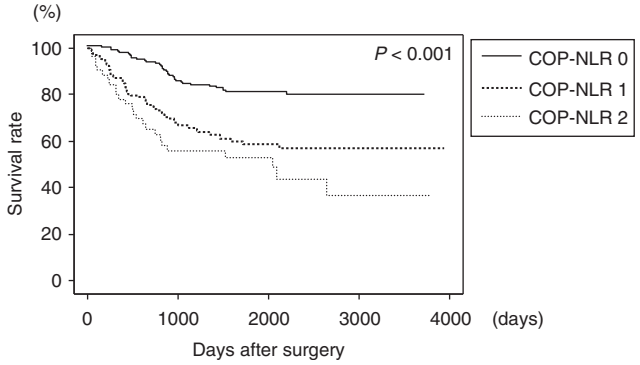

Figure 1. Relationship between the COP-NLR (COP-NLR 0, 1, and 2 from top to bottom) and cancer-specific survival in patients with CRC undergoing surgery.

CRP, albumin, and the GPS. Therefore, the COP-NLR appears to have potential utility for predicting the postoperative survival of patients with CRC, as well as the GPS.

Recent studies have demonstrated that reactive thrombocytosis is associated with survival after surgery for several types of cancer (Silvis *et al*, 1970; Monreal *et al*, 1998; Takahashi *et al*, 1998; Browder *et al*, 2000; Shimada *et al*, 2010). There are two explanations for this phenomenon.

First, reactive thrombocytosis is induced in a background of hypercytokinemia through tumour *vs* host interaction. Among several inflammatory cytokines, IL-6 has an important role in reactive thrombocytosis (Sierko and Wojtukiewicz, 2004), as it is a multifunctional cytokine with a number of physiological actions, stimulating not only CRP upregulation but also albumin down-regulation in the liver, as well as protein synthesis (Ramadori *et al*, 1998). Similarly, IL-6 has a cell-proliferative effect, triggering the differentiation of megakaryocytes to platelets in the bone marrow (Imai *et al*, 1991; Ruscetti, 1994). Therefore, it is reasonable that reactive thrombocytosis would be associated with survival after surgery, because this phenomenon is based on the same mechanism as that reflected in the GPS, which is determined from the serum levels of CRP and albumin.

Second, thrombocytosis is also induced from the tumour itself (Mohle *et al*, 1997; Kohrana and Fine, 2004). Generally, thrombocytosis is a feature in 10–57% of patients with malignancy, as a variety of neoplastic cells can stimulate platelet activation (George *et al*, 2000; Gunsilius *et al*, 2000). Several studies have revealed that cancer cells secrete vascular endothelial growth factor (VEGF), which also stimulates megakaryocyte differentiation (Troxler *et al*, 2007). Because VEGF induction promotes tumour growth (Mohle *et al*, 1997; Khorana and Fine, 2004; Gorelick *et al*, 2009), thrombocytosis indirectly reflects tumour progression. In fact, a high level of VEGF is found in serum, platelets, and leukocytes of patients with malignant disease (Lavie *et al*, 1999), and platelet interactions with malignant cells promote metastasis (Gislason and Nou, 1985).

With regard to the platelet count, most previous studies have used a cut-off value of $30–40 \times 10^4\,mm^{-3}$ (Symbas *et al*, 2000; Ishizuka *et al*, 2012). Although the normal platelet count is $15–30 \times 10^4\,mm^{-3}$, the cut-off value for reactive thrombocytosis is not clearly defined. Therefore, the cut-off value of the preoperative platelet count used in this study, $30 \times 10^4\,mm^{-3}$, based on ROC curve analysis, was acceptable.

Previous studies have also demonstrated a relationship between the NLR and postoperative survival in patients with several types of cancer (Shimada *et al*, 2003; Walsh *et al*, 2005; Malik *et al*, 2007; Halazun *et al*, 2008, 2009; Shimada *et al*, 2010; Chiang *et al*, 2012). Because the NLR is based on neutrophils and lymphocytes, it would also be affected by SIR. Although most studies have recommended a NLR cut-off value of 5 (Shimada *et al*, 2003; Szczepanik *et al*, 2011), a recent report has recommended a value of 3 (Chiang *et al*, 2012). Therefore, the NLR cut-off value of 3.0 that we adopted in the present study was acceptable and in line with a previous study that derived a similar value based on ROC curve analysis (Cho *et al*, 2008).

Shimada *et al* (2010) have reported that a high NLR was associated with a high platelet count, and selected it as an independent indicator of reduced postoperative survival in patients with gastric cancer. They also reported that not only an elevated CRP level (Shimada *et al*, 2003) but also reactive thrombocytosis (Shimada *et al*, 2004) was associated with postoperative survival in patients with oesophageal cancer. These results lend strong support to the use of a combination of reactive thrombocytosis and the NLR for prediction of postoperative survival, along with the GPS.

There are now a number of well-established systemic inflammation-based prognostic scores for patients with CRC. In particular, the GPS and the NLR have been well validated (McMillan, 2013). However, the possibility that additional measures of the SIR might add further significance to these scores has been suggested, as the parameters comprising these scores, that is, CRP, albumin, neutrophils, and lymphocytes, are all correlated with each other (Table 2). To investigate this issue, we examined the ability of neutrophil, lymphocyte, and platelet counts to further discriminate patients in individual groups divided according to whether they had a GPS of 0 or 1, or a GPS of 2. The results obtained from Cox proportional hazards model analyses using the recommended cut-off values from ROC curve analyses revealed that only the platelet count was able to divide both patients with a GPS of 0 or 1, and those with a GPS of 2, into two independent groups (data not shown). These results demonstrated that the

platelet count was able to add additional discriminatory ability to not only the GPS but also the NLR; combination of the NLR and the platelet count created a novel inflammation-based prognostic score, the COP-NLR. This is considered to be a meaningful advance in the development of an optimal, routinely available, systemic inflammation-based prognostic score that is able to complement tumour staging.

Theoretically, direct measurement of the serum IL-6 level is the best way to estimate SIR based on tumour *vs* host interaction. However, there are many unsolved problems associated with the routine measurement of IL-6 in cancer patients. Although a recent study has revealed that the serum level of IL-6 is associated with the postoperative survival of patients with gastric cancer (Szczepanik *et al*, 2011), routine measurement of IL-6 is difficult in a clinical setting because of its high cost and inconvenience.

On the other hand, the COP-NLR is easy to measure routinely because of its low cost and convenience. In addition, in comparison with the GPS, the COP-NLR may have higher applicability for estimation of SIR, because proliferation and differentiation of cellular components occurs much faster than protein synthesis when inflammatory cytokines are released. Moreover, because repeat measurements of the COP-NLR can be performed more easily, not only before but also after surgery, than those of the GPS and tumour markers, it would deliver solid data based on well-known tumour markers.

Thus, the COP-NLR not only appears capable of classifying patients with CRC into three independent groups before surgery but also has potential as a novel predictor of postoperative survival in such patients.

ACKNOWLEDGEMENTS

We received no funding/grant support for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Bhatti I, Peacock O, Lloyd G, Larvin M, Hall RI (2010) Preoperative hematologic markers as independent predictors of prognosis in resected pancreatic ductal adenocarcinoma: neutrophil-lymphocyte versus platelet-lymphocyte ratio. *Am J Surg* **200**: 197–203.

Browder T, Folkman J, Pirie-Shepherd S (2000) The hemostatic system as a regulator of angiogenesis. *J Biol Chem* **275**: 1521–1524.

Chiang SF, Hung HY, Tang R, Changchien CR, Chen JS, You YT, Chiang JM, Lin JR (2012) Can neutrophil-to-lymphocyte ratio predict the survival of colorectal cancer patients who have received curative surgery electively? *Int J Colorectal Dis* **27**: 1347–1357.

Cho S, Cho H, Nam A, Kim HY, Choi YS, Park KH, Cho DJ, Lee BS (2008) Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. *Fertil Steril* **90**: 2073–2079.

Chua W, Charles KA, Baracos VE, Clarke SJ (2011) Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer. *Br J Cancer* **104**: 1288–1295.

de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A (2000) Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* **18**: 2938–2947.

Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dunlop DJ (2004) Comparison of an inflammation-based prognostic score (GPS) with performance status (ECOG) in patients receiving platinum-based chemotherapy for inoperable non-small-cell lung cancer. *Br J Cancer* **90**: 1704–1706.

George ML, Eccles SA, Tutton MG, Abulafi AM, Swift RI (2000) Correlation of plasma and serum vascular endothelial growth factor levels with platelet count in colorectal cancer: clinical evidence of platelet scavenging? *Clin Cancer Res* **6**: 3147–3152.

Gislason T, Nou E (1985) Sedimentation rate, leucocytes, platelet count and haemoglobin in bronchial carcinoma: an epidemiological study. *Eur J Respir Dis* **66**: 141–146.

Gorelick C, Andikyan V, Mack M, Lee YC, Abulafia O (2009) Prognostic significance of preoperative thrombocytosis in patients with endometrial carcinoma in an inner-city population. *Int J Gynecol Cancer* **19**: 1384–1389.

Gunsilius E, Petzer A, Stockhammer G, Nussbaumer W, Schumacher P, Clausen J, Gastl G (2000) Thrombocytes are the major source for soluble vascular endothelial growth factor in peripheral blood. *Oncology* **58**: 169–174.

Halazun KJ, Aldoori A, Malik HZ, Al-Mukhtar A, Prasad KR, Toogood GJ, Lodge JP (2008) Elevated preoperative neutrophil to lymphocyte ratio predicts survival following hepatic resection for colorectal liver metastases. *Eur J Surg Oncol* **34**: 55–60.

Halazun KJ, Hardy MA, Rana AA, Woodland 4th DC, Luyten EJ, Mahadev S, Witkowski P, Siegel AB, Brown Jr RS, Emond JC (2009) Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. *Ann Surg* **250**: 141–151.

Hernandez E, Lavine M, Dunton CJ, Gracely E, Parker J (1992) Poor prognosis associated with thrombocytosis in patients with cervical cancer. *Cancer* **69**: 2975–2977.

Imai T, Koike K, Kubo T, Kikuchi T, Amano Y, Takagi M, Okumura N, Nakahata T (1991) Interleukin-6 supports human megakaryocytic proliferation and differentiation in vitro. *Blood* **78**: 1969–1974.

Ishizuka M, Nagata H, Takagi K, Horie T, Kubota K (2007) Inflammation-based prognostic score is a novel predictor of postoperative outcome in patients with colorectal cancer. *Ann Surg* **246**: 1047–1051.

Ishizuka M, Nagata H, Takagi K, Iwasaki Y, Kubota K (2012) Preoperative thrombocytosis is associated with survival after surgery for colorectal cancer. *J Surg Oncol* **106**: 887–891.

Kaser A, Brandacher G, Steurer W, Kaser S, Offner FA, Zoller H, Theurl I, Widder W, Molnar C, Ludwiczek O, Atkins MB, Mier JW, Tilg H (2001) Interleukin-6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis. *Blood* **98**: 2720–2725.

Khorana AA, Fine RL (2004) Pancreatic cancer and thromboembolic disease. *Lancet Oncol* **5**: 655–663.

Kim HS, Han KH, Chung HH, Kim JW, Park NH, Song YS, Kang SB (2010) Neutrophil to lymphocyte ratio for preoperative diagnosis of uterine sarcomas: a case-matched comparison. *Eur J Surg Oncol* **36**: 691–698.

Lavie O, Comerci G, Daras V, Bolger BS, Lopes A, Monaghan JM (1999) Thrombocytosis in women with vulvar carcinoma. *Gynecol Oncol* **72**: 82–86.

Malik HZ, Prasad KR, Halazun KJ, Aldoori A, Al-Mukhtar A, Gomez D, Lodge JP, Toogood GJ (2007) Preoperative prognostic score for predicting survival after hepatic resection for colorectal liver metastases. *Ann Surg* **246**: 806–814.

McMillan DC (2013) The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer. *Cancer Treat Rev* **39**(5): 534–540.

McMillan DC, Canna K, McArdle CS (2003) Systemic inflammatory response predicts survival following curative resection of colorectal cancer. *Br J Surg* **90**: 215–219.

Mohle R, Green D, Moore MA, Nachman RL, Rafii S (1997) Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc Natl Acad Sci USA* **94**: 663–668.

Monreal M, Fernandez-Llamazares J, Pinol M, Julian JF, Broggi M, Escola D, Abad A (1998) Platelet count and survival in patients with colorectal cancer: a preliminary study. *Thromb Haemost* **79**: 916–918.

Ohsugi Y (2007) Recent advances in immunopathophysiology of interleukin-6: an innovative therapeutic drug, tocilizumab (recombinant humanized anti-human interleukin-6 receptor antibody), unveils the mysterious etiology of immune-mediated inflammatory diseases. *Biol Pharm Bull* **30**: 2001–2006.

Ramadori G, Van Damme J, Rieder H, Meyer zum Buschenfelde KH (1998) Interleukin 6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1 beta and tumor necrosis factor-alpha. *Eur J Immunol* **18**: 1259–1264.

Ramsey S, Lamb GW, Aitchison M, Graham J, McMillan DC (2007) Evaluation of an inflammation-based prognostic score in patients with metastatic renal cancer. *Cancer* **109**: 205–212.

Ruscetti FW (1994) Hematologic effects of interleukin-1 and interleukin-6. *Curr Opin Hematol* **1**: 210–215.

Shimada H, Nabeya Y, Okazumi S, Matsubara H, Shiratori T, Aoki T, Sugaya M, Miyazawa Y, Hayashi H, Miyazaki S, Ochiai T (2003) Elevation of preoperative serum C-reactive protein level is related to poor prognosis in oesophageal squamous cell carcinoma. *J Surg Oncol* **83**: 248–252.

Shimada H, Oohira G, Okazumi S, Matsubara H, Nabeya Y, Hayashi H, Takeda A, Gunji Y, Ochiai T (2004) Thrombocytosis associated with poor prognosis in patients with oesophageal carcinoma. *J Am Coll Surg* **198**: 737–741.

Shimada H, Takiguchi N, Kainuma O, Soda H, Ikeda A, Cho A, Miyazaki A, Gunji H, Yamamoto H, Nagata M (2010) High preoperative neutrophil-lymphocyte ratio predicts poor survival in patients with gastric cancer. *Gastric Cancer* **13**: 170–176.

Sierko E, Wojtukiewicz MZ (2004) Platelets and angiogenesis in malignancy. *Semin Thromb Hemost* **30**: 95–108.

Silvis SE, Turkbas N, Doscherholmen A (1970) Thrombocytosis in patients with lung cancer. *JAMA* **211**: 1852–1853.

Symbas NP, Townsend MF, El-Galley R, Keane TE, Graham SD, Petros JA (2000) Poor prognosis associated with thrombocytosis in patients with renal cell carcinoma. *BJU Int* **86**: 203–207.

Szczepanik AM, Scislo L, Scully T, Walewska E, Siedlar M, Kolodziejczyk P, Lenart M, Rutkowska M, Galas A, Czupryna A, Kulig J (2011) IL-6 serum levels predict postoperative morbidity in gastric cancer patients. *Gastric Cancer* **14**: 266–273.

Takahashi Y, Bucana CD, Akagi Y, Liu W, Cleary KR, Mai M, Ellis LM (1998) Significance of platelet-derived endothelial cell growth factor in the angiogenesis of human gastric cancer. *Clin Cancer Res* **4**: 429–434.

Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A (2004) FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* **22**: 229–237.

Troxler M, Dickinson K, Homer-Vanniasinkam S (2007) Platelet function and antiplatelet therapy. *Br J Surg* **94**: 674–682.

Twelves C, Wong A, Nowacki MP, Abt M, Burris 3rd H, Carrato A, Cassidy J, Cervantes A, Fagerberg J, Georgoulias V, Husseini F, Jodrell D, Koralewski P, Kroning H, Maroun J, Marschner N, Mckendrick J, Pawlicki M, Rosso R, Schuller J, Seitz JF, Stabuc B, Tujakowski J, Van Hazel G, Zaulski J, Scheithauer W (2005) Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med* **352**: 2696–2704.

Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ (2005) Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. *J Surg Oncol* **91**: 181–184.