The postoperative peak number of leukocytes after hepatectomy is a significant prognostic factor for cholangiocarcinoma

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Abstract. Cholangiocarcinoma (CCA) is a lethal disease. A new predictive factor to identify patients suitable for adjuvant chemotherapy is needed. The relationship between the long-term prognosis and the perioperative immune responses in patients with CCA remains unclear. We therefore investigated the clinical impact of perioperative immune responses on the long-term prognosis in patients receiving hepatectomy for CCA. We investigated 81 patients who underwent hepatectomy between February 2000 and October 2012: 57 intra-hepatic CCA (iCCA) patients and 24 extra-hepatic CCA (eCCA) patients. We checked the postoperative level of C-reactive protein and the numbers of leukocytes. A multivariate analysis of the clinicopathological factors identified 2 significant risk factors for the overall survival: The postoperative maximum number of leukocytes (PNL) among patient factors (P=0.0406) and the TNM-stage among tumor factors (P=0.0059). On evaluating the distribution of each kind of leukocyte with a multivariate analysis, both the postoperative maximum number of neutrophils (PNN) and the postoperative maximum number of eosinophils (PNE) were detected as significant factors among leukocytes (PNN/PNE, P=0.0367/0.0083). In conclusion, the PNL after hepatectomy was significantly associated with the long-term prognosis in patients with CCA. Changes in the numbers of leukocytes after hepatectomy may be a marker on treatment for CCA.

Introduction

Cholangiocarcinoma (CCA) is a fatal neoplasm with a poor prognosis. Despite developments in its detection and treatment, only approximately 5-15% of CCA patients survive for 5 years after the diagnosis (1-4). Surgery is the only curative treatment for CCA; however, the overall survival (OS) is not satisfactory, with a 5-year OS of 40% in patients treated with surgery (5-7). To improve the OS, several adjuvant chemotherapies after surgery for CCA have been performed. However, despite a number of studies demonstrating the potential efficacy of adjuvant chemotherapies (8-10), there is no secure evidence on which to highly recommend adjuvant chemotherapy be administered for CCA. One reason for this is that patients are unlikely to tolerate chemotherapy after surgery, especially after hepatectomy. We therefore need to identify those patients who will receive a substantial clinical benefit from adjuvant chemotherapy.

Among preoperative systemic immunological and inflammatory clinical variables, the neutrophil-to-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and prognostic nutritional index (PNI) have been reported as predictors of the therapeutic outcome in cancer, including CCA (11-13). The postoperative immune response was also reported as a risk factor of the prognosis among patients with other cancers (14-16). For hepatocellular carcinoma after hepatectomy, it was reported that the perioperative change in the leukocyte number and levels of postoperative C-reactive protein (CRP) were associated with the survival (17,18). However, the relationship between the long-term prognosis and the perioperative immune responses in patients with CCA has remained unclear.

In this study, we investigated the clinical impact of the perioperative immune response on the long-term prognosis in patients receiving hepatectomy for CCA.

Materials and methods

Rules for CCA used in the present study. In this study, we used the 6th edition of the general rules for clinical and pathological studies on cancer of the biliary tract for extra-hepatic CCA (eCCA) and the 6th edition of the general rules for the clinical and pathological study of primary liver cancer for intra-hepatic CCA (iCCA) (19,20). Because R0 resection in the

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6th edition of the general rules for clinical and pathological studies on cancer of the biliary tract are equivalent to Cur A and B in the 6th edition of the general rules for the clinical and pathological study of primary liver cancer, we defined Cur A and B as R0 in this article.

Patients. Between February 2000 and October 2012, 109 patients diagnosed with iCCA or eCCA underwent R0 resection in the Department of Gastroenterological Surgery at Osaka University Hospital. After a routine examination, including a blood test, computed tomography, endoscopic retrograde cholangiography and/or percutaneous transhepatic cholangiography, 81 patients (57 iCCA patients and 24 eCCA patients) underwent hepatectomy.

In this study, written informed consent to receive perioperative management was obtained from all of the patients.

The criteria used for surgery were based on three factors; the preoperative liver function, the cut margin and the size of the remnant liver. In brief, the cutting line of the biliary duct should be 5 mm away from the CCA, and the estimated remnant liver volume should be >30% of the total liver volume with a normal liver function. If needed, percutaneous transhepatic portal embolization was performed before surgery.

Adjuvant chemotherapy and follow-up. Several kinds of adjuvant chemotherapies following hepatectomy were performed for some patients in this cohort as clinical trials in 2011-2012 (3,9). Patients with major hepatectomy were assigned to KHBO 1003, 'Phase I study of adjuvant gemcitabine or S-1 in patients with biliary tract cancers undergoing major hepatectomy' (Clinical-Trials.gov ID NCT01291615; UMIN ID 000004682), and were treated with GEM or S-1 chemotherapy. Patients with small hepatectomy were assigned to KHBO 1004, 'A Phase I study of adjuvant chemotherapy with gemcitabine plus cisplatin in patients with biliary tract cancer undergoing curative resection without major hepatectomy' (Clinical-Trials.gov ID NCT01297998; UMIN ID 000004622), and were treated with GEM plus cisplatin therapy. The median follow-up period of all patients was 50.6±38.1 months. Patients received regular follow-up with abdominal computed tomography and measurement of the serum CEA and CA19-9 levels every three months for the first two years and every six months thereafter. The last follow-up date of this study was November 2017.

Treatment for recurrence. Almost all of patients with recurrence were first treated by gemcitabine (GEM). The chemotherapy comprised oral 5-fulorouracil (5FU) and/or tegafur or the combination of 5FU, adriamycin, and cisplatin until June 2006, when GEM for CCA was approved in Japan.

Evaluations of the clinicopathological features. We collected all information related to three types of factors: patient factors, tumor factors and treatment factors. Regarding patient factors, we evaluated each patient's condition from multiple perspectives. Because several reports have suggested that the nutritional state or inflammation condition affects the prognosis of CCA, we evaluated whether or not these factors were associated with the long-term prognosis. The nutritional state of the patients before surgery was evaluated with Onodera's PNI. The inflammation-associated status at the same time point was also evaluated with two scores: the NLR and the PLR. These statuses were evaluated based on blood tests within one week before surgery. We checked the postoperative first peak of the level of CRP and the numbers of each kind of leukocyte (neutrophils, lymphocytes, eosinophils, monocytes and basophils). Each kind of leukocytes reached the peak at the various date after surgery; the median date of each kind of leukocytes (leukocytes/neutrophils/lymphocytes/eosinophils/monocytes/basophils) was 2nd/1st/13th/14th/6th/11th date, respectively. Regarding to neutrophils, they usually reached the peak around the same time as leukocytes, but the peak of eosinophils was usually delayed several days from the peak of leukocytes.

Regarding tumor factors, we collected all tumor information relevant to the pathological diagnosis. The TNM and stage of tumors were classified by different rules between eCCA and iCCA. The 6th edition of the general rules for clinical and pathological studies on cancer of the biliary tract was used for eCCA, and the 6th edition of the general rules for the clinical and pathological study of primary liver cancer was used for iCCA. The levels of the tumor markers CEA and CA19-9 were evaluated the day before surgery. Treatment factors consisted of information concerning the surgery type, the complications following surgery and the presence of adjuvant therapy. All morbidities were judged as clinically relevant postoperative complications when a surgical or interventional radiology approach was required (Table I).

Statistical analyses. All data are expressed as the mean ± standard deviation. The chi-squared test and Fisher's exact test were used to compare categorical variables, when appropriate. The Kaplan-Meier analysis and the log-rank test were used to construct the survival curve and to evaluate differences for the univariate analysis. A multivariate analysis of the detected factors was performed with a Cox regression analysis. All analyses were conducted with the JMP 13 software program (SAS Institute, Cary, NC, USA). Statistical significance was defined as a P-value of 0.05.

Ethical guidelines followed in this study. This study was conducted in accordance with the Declaration of Helsinki. This study was performed at Osaka University Hospital, Japan, and approved by the local ethics committee (no. 18261).

Results

The peak number of leukocytes after hepatectomy was an individual significant risk factor for the OS in CCA patients. Eighty-one patients of CCA received liver resection; 57 of them had intrahepatic CCA, and 24 had perihilar CCA (Table I). The univariate analysis detected several risk factors for the OS in CCA patients receiving hepatectomy, including 2 among the patient factors (the PLR (P=0.0251) and the postoperative maximum number of leukocytes (PNL, P=0.0111)); 6 among the tumor features [TNM classification (T/N/M, P=0.0091/0.0003/0.0002), historical vascular invasion (P<0.0001), staging (P=0.0030) and level of CA19-9 (P=0.0383)]; and 4 among the treatment features (operation time (P=0.0113), blood loss (P=0.0372), morbidity (P=0.0040)

Table I. Clinicopathological features in 81 CCA patients by each factor type.

Variable	n=81
Patient factors	
Age (years)	63.7±10.9
Sex (male:female)	52:29
Jaundice (present:absent)	6:75
PNI	44.1±4.8
NLR	2.8±1.5
PLR	174.6±89.5
Postoperative maximum number of leukocytes (/μl)	12182±3748
Postoperative maximum number of neutrophils (/μl)	9986±3730
Postoperative maximum number of lymphocytes (/μl)	1542±529
Postoperative maximum number of eosinophils (/μl)	466±419
Postoperative maximum number of monocytes (/μl)	879±316
Postoperative maximum number of basophils (/μl)	106±364
Postoperative peak value of CRP (mg/dl)	10.2±4.2
Tumor factors	
Tumor type (intrahepatic CCA:perihilar CCA)	57:24
Differentiation (tub1:tub2:por:muc:small cell:unknown)	8:51:12:6:1:1:2
pT[a](1:2:3:4)	7:38:27:8
Historical vascular invasion (present absent)	35:44
Tumor size (cm)	4.1±2.7
pN[a](1:0)	24:56
pMN[a](1:0)	1:80
pStageN[a](I:II:III:IV)	7:33:19:21
CEA (>5:<5 ng/ml)	10:71
CA19-9 (>37:<37 U/ml)	36:45
Treatment factors	
Adjuvant therapy (yes:no)	29:52
Treatment after recurrence with gemcitabine (yes:no:unknown:no recurrence)	24:36:2:19
Operative method (HPD: trisectionectomy:hemihepatectomy: segmentectomy:subseqmentectomy:partial hepatectomy)	1:8:49:5:2:16
Operation time (min)	536.8±202.2
Blood loss (ml)	1451.1±1148.8
Morbidity (present:absent)	14:67
Resected liver weight (g)	420.4±316.1

CCA, cholangiocarcinoma; HPD, hepatopancreatoduodenectomy; CRP, C-reactive protein; PNI, prognostic nutritional index, NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; tub1, well differentiated type; tub2, moderately differentiated type; por, poorly differentiated type; muc, mucinous type. [a]Stage and TNM for iCCAs and pCCAs were classified by the 6th edition of the general rules for the clinical and pathological study of primary liver cancer and the 6th edition of general rules for clinical and pathological studies on cancer of the biliary tract, respectively.

and resected liver weight (P=0.0103)]. A multivariate analysis of those risk factors revealed 2 significant risk factors for the OS: The PNL among patient factors (P=0.0406) and the TNM-stage among tumor factors (P=0.0059). There were no significant risk factors for the OS among treatment factors (Table II).

To clarify which kinds of leukocytes were significantly associated with the OS, we evaluated the distribution of each kind among neutrophils, lymphocytes, eosinophils, monocytes and basophils. A multivariate analysis was performed, and both the postoperative maximum number of neutrophils (PNN) and postoperative maximum number of eosinophils (PNE) were detected as significant factors among leukocytes (PNN/PNE, P=0.0367/0.0083, Table III).

PNN. We divided patients into a high-neutrophil group (high-PNN group, n=40) and a Lowl-neutrophil group (low-PNN group, n=41) based on the median PNN (9807/μl). The high-PNN group showed a poorer prognosis than the low-PNN group with regard to the OS (P=0.0406). To assess the influence of the increase in neutrophils after hepatectomy, a sub-analysis for the OS was performed. The high-PNN group

Table II. Results of univariate and multivariate analyses for the OS.

Variable	MST (months)	Univariate analysis P-value	Multivariate analysis HR	(95% CI)	P-value
Patient factors					
Age (<65:≥65 years)	39.4:69.2	0.3968			
Sex (male:female)	69.9:43.1	0.2617			
Jaundice (present:absent)	56.5:51.8	0.9873			
PNI (≤45:>45)	52.2:51.8	0.8540			
NLR (≤2.5:>2.5)	69.2:39.4	0.1215			
PLR (≤150:>150)	74.8:43.1	0.0251	0.59	(0.32-1.08)	0.0906
Postoperative maximum number of leukocytes (≤11580:>11580/μl)	74.8:32.7	0.0111	0.54	(0.29-0.97)	0.0406
Postoperative peak value of CRP (≤9.7:>9.7 mg/dl)	59.2:43.1	0.5203			
Tumor factors					
Tumor type (intrahepatic CCA:perihilar CCA)	53.8:43.7	0.1210			
Differentiation (tub1, tub2: others)	47.7:-	0.1818			
pT[a](1,2:3,4)	70.5:30.3	0.0091			N.A.
Historical vascular invasion (present: absent)	29.9:-	<0.0001			N.A.
Tumor size (≤2:>2 cm)	69.9:51.5	0.5559			
pN[a](0:1)	69.9:18.8	0.0003			N.A.
pM[a](1:0)	6.4:51.8	0.0002			N.A.
pStage[a](I, II:III, IV)	78.3:29.9	0.0030	0.44	(0.24-0.79)	0.0059
CEA (≤5:>5 ng/ml)	52.2:27.2	0.0688			
CA19-9 (≤37:>37 U/ml)	69.2:30.8	0.0383	0.60	(0.33-1.08)	0.0888
Treatment factors					
Adjuvant therapy (yes:no)	39.4:59.2	0.9716			
Treatment after recurrence with gemcitabine (yes:no)	43.7:27.9	0.3503			
Operative method of hepatectomy (major:minor)	47.7:-	0.1265			
Operation time (≤545:>545 min)	74.8:27.4	0.0113	0.66	(0.32-1.29)	0.2238
Blood loss (<1,000:≥1,000 ml)	70.5:30.3	0.0372	0.92	(0.44-1.89)	0.8249
Morbidity (present:absent)	20.4:59.2	0.0040	1.86	(0.82-3.97)	0.1346
Resected liver weight (≤340:>340 g)	-: 39.4	0.0103	0.64	(0.30-1.34)	0.2365

OS, overall survival; MST, median survival time; N.A., not available; PNI, prognostic nutritional index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein; CCA, cholangiocarcinoma; tub1, well differentiated type; tub2, moderately differentiated type; major hepatectomy, >3 segments; minor hepatectomy, ≤3 segments. P-value <0.05 was considered statistically significant. [a]Stage and TNM for iCCAs and pCCAs were classified by the 6th edition of the general rules for the clinical and pathological study of primary liver cancer and the 6th edition of general rules for clinical and pathological studies on cancer of the biliary tract, respectively.

showed a poorer prognosis than the low-PNN group (P=0.0318) in the survival time after recurrence (SAR). However, no significant difference in the disease-free survival (DFS) was noted between the groups (P=0.8809) (Fig. 1A).

To investigate the factors associated with the PNN, we compared the clinicopathological factors between the two groups. The comparison revealed significant differences in three factors: the level of CA19-9, CCA tumor type and the PLR. The high-PNN group contained more patients showing a higher preoperative CA19-9 level (P=0.0259) and high PLR (P=0.0018). In addition, the number of neutrophils in the serum was higher in iCCA patients than in eCCA patients after hepatectomy (P=0.0035) (Table IV).

PNE. When patients were divided into a high-eosinophil group (high-PNE group, n=40) and a low-eosinophil group (low-PNE group, n=41) based on the median PNE (356/μl), the low-PNE group showed a poorer prognosis with regard to the OS (P=0.0111) and the SAR (P=0.0086) (Fig. 1B). However, there was no significant difference in the DFS between the groups (P=0.2334) (Fig. 1B).

We investigated the factors associated with the PNE and determined that only the preoperative serum level of CA19-9 was associated with the PNE (P=0.0445) (Table V).

The rule of peak number of leukocytes after hepatectomy for the SAR in CCA patients. Because both PNN and PNE

Table III. Results of a multivariate analysis for the OS in leukocytes.

	Multivariate analysis		
Variable	HR	(95% CI)	P-value
Postoperative maximum number of leukocytes (≤11580:>11580/μl)			N.A.
Postoperative maximum number of neutrophils (≤9807:>9807/μl)	0.53	(0.28-0.96)	0.0367
Postoperative maximum number of lymphocytes (≤1513:>1513/μl)	1.06	(0.56-2.01)	0.8549
Postoperative maximum number of eosinophils (≤356:>356/μl)	2.20	(1.22-4.04)	0.0083
Postoperative maximum number of monocytes (≤815:>815/μl)	0.95	(0.52-1.74)	0.8752
Postoperative maximum number of basophils (≤58:>58/μl)	0.94	(0.50-1.77)	0.8527

OS, overall survival; N.A., not available. P-value <0.05 was considered statistically significant.

Table IV. Results of a univariate analysis of patient characteristics and postoperative peak number of neutrophils.

Variable	n	High neutrophils (n=40)	Low neutrophils (n=41)	P-value
Age (<65:≥65 years)	37:44	19:21	18:23	0.8249
Sex (male:female)	52:29	24:16	28:13	0.4919
CEA (≤5:>5 ng/ml)	71:10	34:6	37:4	0.5187
CA19-9 (≤37:>37 U/ml)	45:36	17:23	28:13	0.0259
Jaundice (present:absent)	6:75	5:35	1:40	0.1088
Adjuvant therapy (yes:no)	29:52	14:26	15:26	0.1394
Tumor type (intrahepatic CCA:perihilar CCA)	57:24	22:18	35:6	0.0035
Differentiation (tub1, tub2:others)	59:20	27:11	32:9	0.6059
Operation time (≤545:>545 min)	41:39	16:23	25:16	0.1168
Blood loss (<1,000:≥1,000 ml)	36:45	18:22	18:23	1.0000
Morbidity (present:absent)	14:67	10:30	4:37	0.0843
Resected liver weight (≤340:>340 g)	37:39	15:23	22:16	0.1681
Operative method of hepatectomy (major:minor)	58:23	32:8	26:15	0.1394
pT[a](1, 2:3, 4)	45:35	22 :17	23:18	1.0000
Historical vascular invasion (present:absent)	35:44	21:17	32:9	0.6059
Tumor size (≤2:>2 cm)	18:62	11:28	7:34	0.2890
pN[a](0:1)	56:24	26:13	30:11	0.6276
pM[a](1:0)	1:80	1:39	0:41	0.4938
pStage[a](I, II:III, IV)	40:40	18:21	22:19	0.6549
PNI (≤45:>45)	49:32	27:13	22:19	0.2576
NLR (≤2.5:>2.5)	40:41	18:22	22:19	0.5077
PLR (≤150:>150)	39:42	12:28	27:14	0.0018
Postoperative maximum number of lymphocytes (≤1513:>1513/μl)	41 :40	18:22	23:18	0.3771
Postoperative maximum number of eosinophils (≤356:>356/μl)	41:40	18:22	23:18	0.3771
Postoperative maximum number of monocytes (≤815:>815/μl)	41:40	17:23	24:17	0.1848
Postoperative maximum number of basophils (≤58:>58/μl)	41:40	16:24	25:16	0.0766
Postoperative peak value of CRP (≤9.7:>9.7 mg/dl)	41:40	19:21	22:19	0.6590

OS, overall survival; CCA, cholangiocarcinoma; tub1, well differentiated type; tub2, moderately differentiated type; PNI, prognostic nutritional index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein; major hepatectomy, >3 segments; minor hepatectomy, ≤3 segments. P-value <0.05 was considered statistically significant. [a]Stage and TNM for iCCAs and pCCAs were classified by the 6th edition of the general rules for the clinical and pathological study of primary liver cancer and the 6th edition of general rules for clinical and pathological studies on cancer of the biliary tract, respectively.

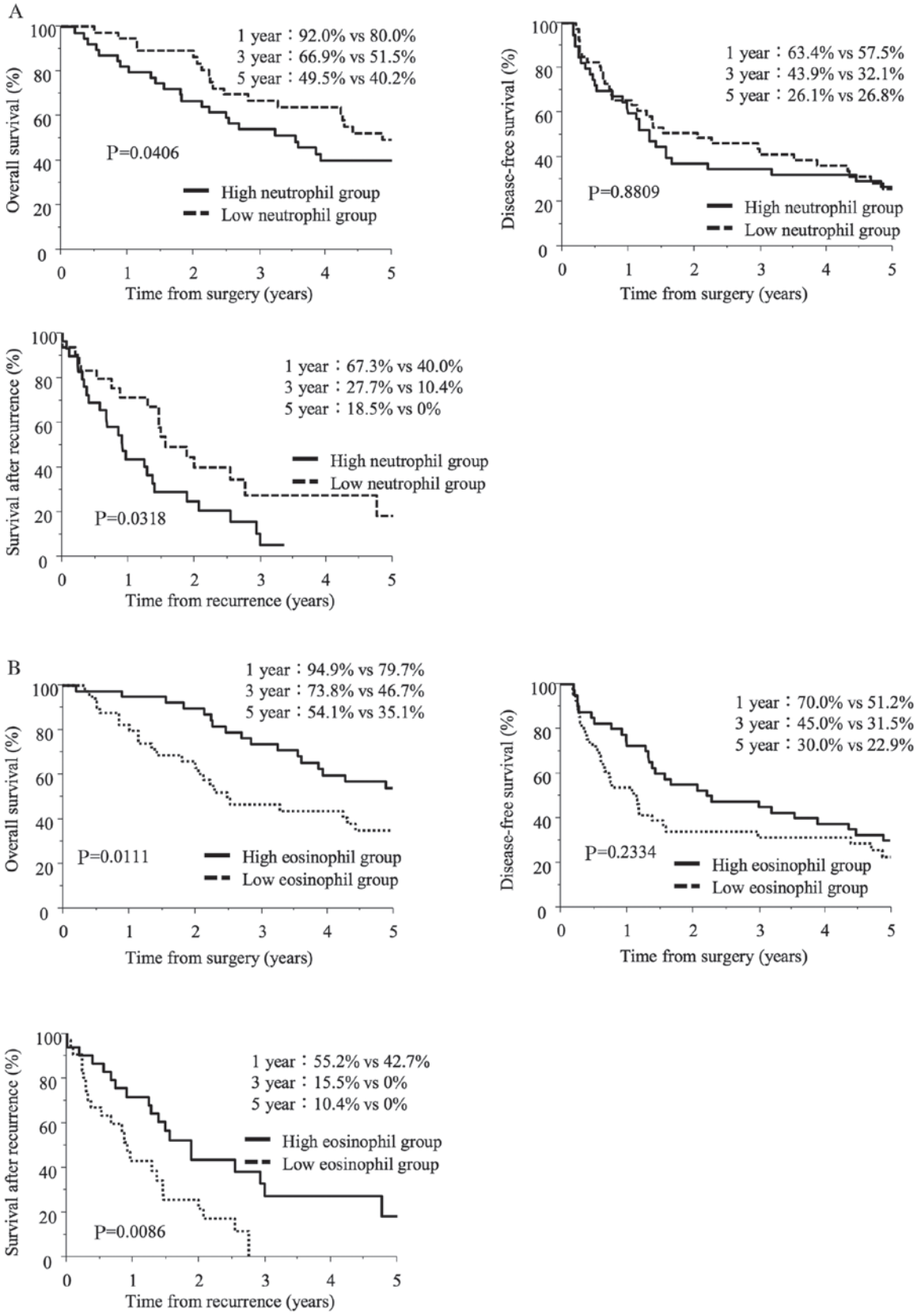

Figure 1. The long-term prognosis of patients after hepatectomy for cholangiocarcinoma (CCA). (A) The overall survival (OS, upper left), disease-free survival (DFS, upper right) and survival after recurrence (SAR, lower left) curves after surgery for 81 patients with CCA. Patients were divided into two groups according to the median postoperative peak number of neutrophils. The median OS in the high-neutrophil group (n=40) and low-neutrophil group (n=41) was 43.1 and 59.2 months, respectively; P=0.0406. The median DFS in the high-neutrophil group and low-neutrophil group was 16.1 and 25. months, respectively; P=0.8809. The median SAR in the high-neutrophil group and low-neutrophil group was 11.0 and 18.8 months, respectively; 0.0318. (B) The OS (upper left), DFS (upper right) and SAR (lower left) curves after surgery for 81 patients with CCA. Patients were divided into two groups according to the median postoperative peak number of eosinophils. The median OS in the high-eosinophil group (n=40) and low-eosinophil group (n=41) was 74.8 and 30.3 months, respectively; P=0.0111. The median DFS in the high-eosinophil group and low-eosinophil group was 27.3 and 13.8 months, respectively; P=0.2334. The median SAR in the high-eosinophil group and low-eosinophil group was 22.8 and 11.0 months, respectively; P=0.0086.

Table V. Results of a univariate analysis of patient characteristics and postoperative peak number of eosinophils.

Variable	n	High eosinophils (n=40)	Low eosinophils (n=41)	P-value
Age (<65:≥65 years)	37:44	17:23	20:21	0.6575
Sex (male:female)	52:29	27:13	25:16	0.6445
CEA (≤5:>5 ng/ml)	71:10	34:6	37:4	0.5187
CA19-9 (≤37:>37 U/ml)	45:36	27:13	18:23	0.0445
Jaundice (present:absent)	6:75	4:36	2:39	0.4321
Adjuvant therapy (yes:no)	29:52	16:24	13:28	0.4919
Tumor type (intrahepatic CCA:perihilar CCA)	57:24	28:12	29:12	1.0000
Differentiation (tub1, tub2:others)	59:20	32:8	27:12	0.3095
Operation time (≤545:>545 min)	41:39	23:17	18:22	0.3711
Blood loss (<1,000:≥1,000 ml)	36:45	20:20	16:25	0.3749
Morbidity (present:absent)	14:67	7:33	7:34	1.0000
Resected liver weight (≤340:>340 g)	37:39	22:17	15:22	0.1786
Operative method of hepatectomy (major:minor)	58:23	27:13	31:10	0.4670
pT[a](1, 2:3, 4)	45:35	26:14	19:21	0.1759
Historical vascular invasion (present:absent)	35:44	16:24	19:20	0.5005
Tumor size (≤2:>2 cm)	18:62	9:31	9:31	1.0000
pN[a](0:1)	56:24	32:8	24:16	0.0866
pM[a](1:0)	1:80	0:40	1:40	1.0000
pStage[a](I, II:III, IV)	40:40	24:16	16:24	0.1170
PNI (≤45:>45)	49:32	21:19	28:13	0.1763
NLR (≤2.5:>2.5)	40:41	22:18	18:23	0.3771
PLR (≤150:>150)	39:42	22:18	17:24	0.2692
Postoperative maximum number of neutrophils (≤9807:>9807/μl)	41:40	18:22	23:18	0.3771
Postoperative maximum number of lymphocytes (≤1513:>1513/μl)	41:40	19:21	22:19	0.6590
Postoperative maximum number of monocytes (≤815:>815/μl)	41:40	18:22	23:18	0.3771
Postoperative maximum number of basophils (≤58:>58/μl)	41:40	18:22	23:18	0.3771
Postoperative peak value of CRP (≤9.7:>9.7 mg/dl)	41:40	19:21	22:19	0.6590

OS, overall survival; CCA, cholangiocarcinoma; tub1, well differentiated type; tub2, moderately differentiated type; PNI, prognostic nutritional index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein; major hepatectomy: >3 segments; minor hepatectomy: ≤3 segments. P-value <0.05 was considered statistically significant. [a]Stage and TNM for iCCAs and pCCAs were classified by the 6th edition of the general rules for the clinical and pathological study of primary liver cancer and the 6th edition of general rules for clinical and pathological studies on cancer of the biliary tract, respectively.

were associated with the SAR, we investigated the univariate analysis for the SAR including other factors (Table SI). The univariate analysis detected several risk factors for the SAR in CCA patients receiving hepatectomy, including the postoperative maximum number of leukocytes (PNL, P=0.0076). Among leukocytes, PNN and PNE were risk factors for the SAR (PNN/PNE, P=0.0039/0.0008, Table SII).

Regarding to the treatment after recurrence, there were 16 patients without treatments after recurrence and 44 patients treated by mainly chemotherapy. Evaluating with PNN and PNE, there was no significant difference among high/low-PNN group in the presence of treatment after recurrence (respectively, 64.3%/81.3%, P=0.1569), and among high/low-PNE group (respectively, 83.9%/62.1%, P=0.0807). Whereas, we investigated clinical impact of the site of recurrence on SAR. There were 62 recurrence cases; 29 cases in the liver, 8 cases at the peritoneal dissemination, 7 cases in the lymph node, and 7 cases in other sites. There was no significant association with the site of recurrence among between high and low-PNN/PNE groups (respectively, P=0.7418/P=0.7311).

Discussion

We revealed that the postoperative peak number of leukocytes for CCA patients after hepatectomy was associated with the long-term prognosis, especially with regard to the OS and SAR, and the PNN and PNE were dominant components in the increase in leukocyte numbers. We also investigated the prognosis for patients of eCCA who underwent surgery without hepatectomy. There was no significant difference in OS, DFS and SAR with PNN (OS/DFS/SAR, P=0.6514/0.6630/0.6817) and PNE (OS/DFS/SAR, P=0.5980/05254/0.6372) (Fig. S1).

Because the clinical impact of postoperative leukocytes was observed in only CCA patients with hepatectomy, we considered that the immune response might be different between patients with hepatectomy and patients without hepatectomy. Thus, we focused on the immune response for CCA patients after hepatectomy in this study.

The immune response following liver resection has been shown to influence various immune functions represented by several cytokines and growth factors (21). As we previously reported, these reactions and changes in levels of cytokines/growth factors facilitate CCA progression (22-26). Thus, we assumed that the postoperative peak number of leukocytes/CRP would be good surrogate markers and useful prediction tools for the OS.

The postoperative level of CRP, which has been described as a postoperative risk marker in other tumors (18,27) was not associated with the prognosis in CCA. In contrast, an increased peak number of leukocytes was associated with a poorer OS, showing some degree of contradiction between these two findings. We therefore considered that the liver may not fully react and produce CRP sufficiently after hepatectomy in some cases, as CRP is produced in mainly by the liver.

The PNN was a significant risk factor of the OS. Historically, neutrophils were considered to have no effect on chronic or progressive diseases, such as cancer, due to the relatively short survival of such patients. Recently, however, several studies have revealed that the presence of neutrophils in tumors is associated with a poor prognosis in some tumors, such as bronchoalveolar carcinoma, melanoma, renal carcinoma and head and neck squamous cell carcinoma (28-32). Regarding a tumor-promoting role, neutrophils in inflammatory response product cytokines, proteases, and reactive oxygen species (ROS) (33). Interleukin-6 (IL-6) in particular is a marker of inflammation and the neuroendocrine stress response after surgery for primary biliary cancer and is a dominant inflammatory cytokine in the postoperative period (34,35). We previously revealed that IL-6 was associated with malignant features in CCA (23,25). The IL-6 expression was found to induce chemoresistance in CCA cells through epithelial-mesenchymal transition (EMT) (23). Thus, neutrophils might be induced in the acute inflammation period after hepatectomy by cytokines, including IL-6, and CCA exacerbated by IL-6 exposure carries a poor prognosis for patients.

In contrast to findings concerning neutrophils, eosinophils have rarely shown any particular association with cancer. Albeit in only a few reports, eosinophils have been found to be associated with both favorable and unfavorable prognoses. Most reports on eosinophils in cancer have demonstrated the presence of eosinophil infiltration in tissues surrounding tumors, such as nasopharyngeal carcinoma, colorectal tumor, oral squamous cell carcinoma, pulmonary adenocarcinoma and laryngeal carcinoma (36-38). Increased eosinophil counts in patients with prostate cancer and metastatic colon cancers have been shown to be associated with a prolonged survival (39,40). Recently, there have been reports that the short-term administration of IL-33 facilitates the development of a murine genetic model of CCA (22). However, the clinical role of IL-33 in CCA has not been investigated. IL-33, a member of the IL-1 family, has been shown to be a crucial costimulator of the adaptive immune response, exerting effects on antiviral $CD8^+$ cytotoxic T lymphocytes, $CD4^+$ T helper 1 cell reactions and immune regulation by regulatory T cells. We therefore initially assumed that an increase in eosinophils might be a surrogate marker of an increase in the expression of IL-33 and thus a risk factor of the prognosis of CCA patients. However, we observed the opposite results. Although these findings seemed to conflict with our hypothesis, several reports have shown that IL-33 does not increase the number of eosinophils in all cases. Tjota and Stolarski suggested that IL-33 was involved in both increases and decreases in the number of eosinophils. In contrast, Dyer *et al* determined that IL-33 antagonized IL-5-dependent eosinophilopoiesis (41-43). Those previous results may partially explain the findings in the present study.

After getting the result, we reanalyzed the prognosis dividing CCA patients into iCCA patients and eCCA patients. Particularly, OS and SAR for the high-PNN group in iCCA patients seemed to be inferior comparing to the low-PNN group in iCCA patients, although there was no significant different, maybe because of the small number (OS/SAR, P=0.1124/0.1629) (Fig. S2). We need to accumulate more cases for further investigation.

In conclusion, the postoperative peak number of leukocytes after hepatectomy was significantly associated with the long-term prognosis in patients with CCA. Although studies in a larger group are still needed, changes in the numbers of leukocytes after hepatectomy may be a marker on treatment for CCA.

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Availability of data and materials

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

Authors' contributions

GS made substantial contributions to acquisition of data and drafting the manuscript. DY and HE made substantial contributions to conception, design, revising the manuscript. HE, YI, HA, TA, TN and KG made contribution to acquisition of data and revising manuscript. SK, YT and MT made substantial contributions to analysis and interpretation of data. YD and MM revising it critically for important intellectual content, given final approval of the version. And All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all participants. This retrospective study protocol was approved by the

institutional reviewer board of the Osaka University Graduate School of Medicine (Suita, Japan) (no. 18261).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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