Post-operative Lymphocyte Count May Predict the Outcome of Radical Resection for Gallbladder Carcinoma

RYOTA IWASE[1,2], HIROAKI SHIBA[1], KOICHIRO HARUKI[1], YUKI FUJIWARA[1], KENEI FURUKAWA[1], YASURO FUTAGAWA[1], SHIGEKI WAKIYAMA[1], TAKEYUKI MISAWA[1] and KATSUHIKO YANAGA[1]

[1]Department of Surgery, and [2]Department of Gene Therapy, Institute of DNA Medicine, The Jikei University School of Medicine, Tokyo, Japan

Abstract. *Background: Gallbladder carcinoma (GBC) is a cancer of the digestive tract with poor prognosis, for which surgical resection is the only potentially curative therapy. The prognostic value of postoperative peripheral blood leukocyte subset count in patients with cancer has not been fully investigated. Therefore, we retrospectively investigated the relation-ship between postoperative peripheral blood lymphocyte count and disease-free as well as overall survival after radical resection of GBC. Patients and Methods: The study subjects were 34 patients who underwent radical resection for GBC between January 2005 and April 2010. We retrospectively investigated the relation-ship between clinicopathological variables, including postoperative peripheral blood lymphocyte count, and disease-free as well as overall survival. Results: In univariate analysis, disease-free survival was worse in patients with intraoperative blood transfusion (p=0.0285), tumor node metastasis (TNM) stage ≥II (p<0.0001), and lymphocyte count of less than 1,000/μl (p=0.0002). Overall survival was worse in patients with TNM stage ≥II (p=0.0002) and lymphocyte count of less than 1,000/μl (p=0.0151). In multivariate analysis, TNM stage ≥II (p<0.0089) and peripheral blood lymphocyte count of less than 1,000/μl (p=0.0365) were independent predictors of poor disease-free survival. For overall survival, TNM stage ≥II (p=0.0230) was the only independent predictor. Moreover, lymphocyte counts of less than 1,000/μl correlated significantly with TNM stage ≥II, duration of operation, greater blood loss, and presence of intraoperative blood transfusion. Conclusion: Postoperative peripheral blood lymphocyte count correlates with outcome of patients with GBC treated by radical resection.*

Correspondence to: Ryota Iwase, MD, Department of Surgery, The Jikei University School of Medicine, 3-25-8, Nishi-Shimbashi, Minato-ku, Tokyo 105-8461, Japan. Tel: +81 334331111 ext. 3401, Fax: +81 354724140, e-mail: ryotaiwa@jikei.ac.jp

Key Words: Gallbladder carcinoma, lymphocyte count, prognosis, operation.

Gallbladder carcinoma (GBC) is a cancer of the digestive tract with poor prognosis and is the most common cancer of the biliary tract. The prognosis of GBC remains dismal even with recent advances in diagnostic modalities and surgical techniques (1-3). It is well-known that surgical resection is the only treatment which can achieve long-term survival in patients with GBC. The prognosis for patients with early GBC is associated with a 5-year survival rate ranging from 90% to 100% (4-6). On the other hand, advanced GBC is characterized by a very poor prognosis, with the 5-year survival rate below 30%. Therefore, assessment of prognostic predictors is important for the management of patients with GBC.

Recent studies reported that perioperative immunological and inflammatory response findings correlate with tumor recurrence and prognosis in various types of malignant tumors, including GBC. Decreased preoperative lymphocyte count, increased preoperative monocyte count, and elevated preoperative neutrophil-to-lymphocyte ratio (NLR) are related to poor prognosis in patients with cancer (9-12). Several investigators reported that perioperative changes in immune response are one of the predictors of therapeutic outcome after curative resection for digestive-tract cancer (13, 14). Lymphocytes play an important role in antitumor immune responses, through tumor recognition and immunological elimination of local and metastatic tumor cells (15-17). Therefore, we hypothesized that the postoperative peripheral blood lymphocyte count might be related to prognosis after radical resection for GBC. In this study, we retrospectively investigated the relation between postoperative peripheral blood lymphocyte count and disease-free, as well as overall, survival in patients with GBC after radical resection.

Patients and Methods

Between January 2004 and December 2010, 39 patients underwent radical resection for GBC at the Department of Surgery, Jikei University Hospital, Tokyo, Japan. Out of these, five patients were excluded: one patient due to insufficient data, and four patients who were lost to follow-up, leaving the remaining 34 (15 male and 19 female; mean age=67.0 years, range=38-88 years) patients for this

study. All patients underwent macroscopically curative resection for GBC. Hemogram and chemistry profile were routinely measured for each patient preoperatively and on postoperative day (POD) 1. Absolute white blood cell (WBC) count, lymphocyte, monocyte, and each subset were routinely determined in peripheral venous samples.

First, we investigated the relation between clinicopathological variables and disease-free and overall survival after radical resection for GBC by univariate and multivariate analyses. These consisted of the following 10 factors: age, gender, duration of operation, intraoperative blood loss, presence or absence of intraoperative blood transfusion, presence or absence of gallstones, tumor stage based on tumor pathology, and postoperative neutrophil, lymphocyte, and monocyte counts on POD 1. Clinicopathological continuous variables were classified into two groups for the log-rank test and the Cox proportional hazard regression model as follows: age <60 or ≥60 years, duration of operation <300 or ≥300 min, and blood loss <1,000 or ≥1,000 g. Tumor staging was performed according to the pathological tumor node metastasis (pTNM) classification based on the guidelines of the TNM Classification of Malignant Tumors 6th edition in 2002 (18) and TNM stage was classified into two groups: stage ≤I or ≥II. The postoperative WBC subsets counts were classified as follows: neutrophil count <10,000 or ≥10,000/μl, lymphocyte count <1,000 or ≥1,000/μl, and monocyte count <300 or ≥300/μl, according to previous reports (19, 20).

Next, to assess the risk factor for decrement of postoperative peripheral blood lymphocyte count on POD 1, we analyzed the relation between patient characteristics and postoperative peripheral blood lymphocyte count, using the following nine factors: age, gender, duration of operation, intraoperative blood loss, intraoperative blood transfusion, presence of gallstones, TNM stage, and postoperative neutrophil and monocyte counts on POD 1.

Recurrence of GBC was defined as newly-detected abdominal or extra-abdominal tumors by computed tomography, magnetic resonance image, with or without an increase in serum carcinoembryonic antigen (CEA), or carbohydrate antigen 19-9 (CA19-9). For recurrence of GBC, chemotherapies or conversion to other chemotherapy were given based on performance status. For patients with poor performance status or refusal, best supportive care was given.

This retrospective study was approved by the Ethics Committee of the Jikei University School of Medicine (#21-121).

Statistical analysis. Data are expressed as the mean±standard deviation (SD). Univariate analysis was performed using non-paired Student's t-test and Chi-square test. Analysis of disease-free and overall survival was performed using the log-rank test. Factors that significantly influenced disease-free or overall survival were then used in the Cox proportional regression model for multivariate analysis. All *p*-values were considered statistically significant when the associated probability was less than 0.05.

Results

Patients' characteristics and clinicopathological variables. Patients' characteristics and clinicopathological variables are outlined in Table I. Among the study population, the mean age was 67.0, with a range from 38 to 88 years, and fifteen patients were male. Twenty-two out of 34 patients had TMN stage ≤I disease on tumor pathology. In this study, the five-year disease-free and overall survival rates after elective resection for gallbladder carcinoma were 45.4% (Figure 1A) and 74.3% (Figure 1B), respectively.

Table I. *Patients' characteristics*.

Factor	Mean±SD or Rate	Range
Age (years)	67.0±11.0	38-88
Gender (M:F)	15:19	
Procedure (Lap-C:OC:EC:CSH)	(12:8:12:2)	
Operation time (min)	187.1±168.8	45-660
Blood loss (g)	522.1±706.5	0-3,270
Blood transfusion (yes:no)	10:24	
Gallstones (Present:absent)	14:20	
TNM stage (≤I:≥II)	22:12	
Curability (R0:R1)	29:5	

Lap-C: Laparoscopic cholecystectomy, OC: open cholecystectomy, EC: extended cholecystectomy, CSH: cholecystectomy with subsegmental hepatectomy, M: male, F: female.

Comparison of clinical variables in relation to disease-free survival after radical resection for gallbladder carcinoma. Table II lists the relationship between the clinicopathological variables and disease-free survival after resection. In univariate analysis, disease-free survival was worse in patients with TNM stage ≥II ($p<0.0001$), intraoperative blood transfusion ($p=0.0285$), and postoperative lymphocyte count of less than 1,000/μl ($p=0.0002$). In multivariate analysis, TNM stage ≥II ($p<0.0089$) and postoperative lymphocyte count of less than 1,000/μl ($p=0.0365$) were independent and significant predictors of poorer disease-free survival.

Comparison of clinical variables in relation to overall survival after radical resection for gallbladder carcinoma. Table III lists the relationship between the clinicopathological variables and overall survival after resection. In univariate analysis, overall survival was worse in patients with TNM stage ≥II ($p=0.0002$) and lymphocyte count of less than 1,000/μl ($p=0.0151$). In multivariate analysis, TNM stage ≥II ($p=0.0230$) was the only independent and significant predictor of poor survival. Patients with a postoperative lymphocyte count of less than 1,000/μl tended to have worse overall survival than those with more than 1,000/μl postoperative lymphocyte count, which however did not achieve statistical significance ($p=0.2995$).

Univariate analysis of clinicopathological variables in relation to postoperative lymphocyte count after radical resection for gallbladder carcinoma. Table IV lists the relationship between clinicopathological variables and postoperative lymphocyte count. In univariate analysis,

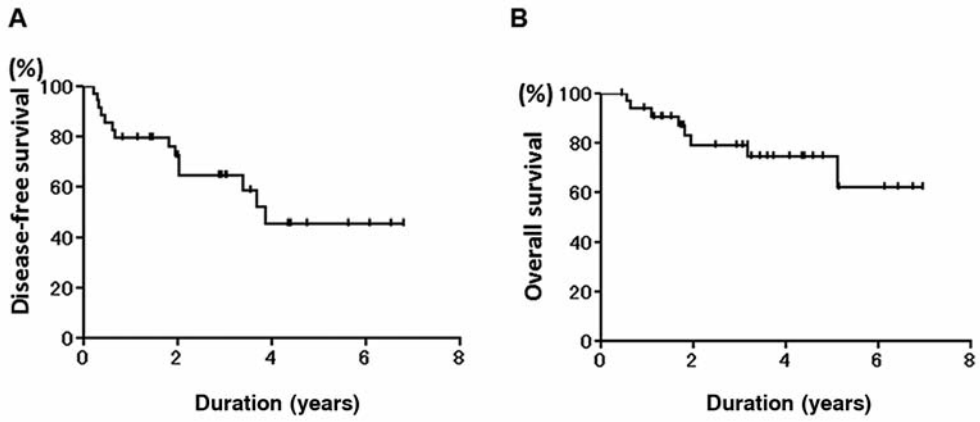

Figure 1. *The five-year disease-free and overall survival rates for the patient group overall were 45.4% (A) and 74.3% (B), respectively.*

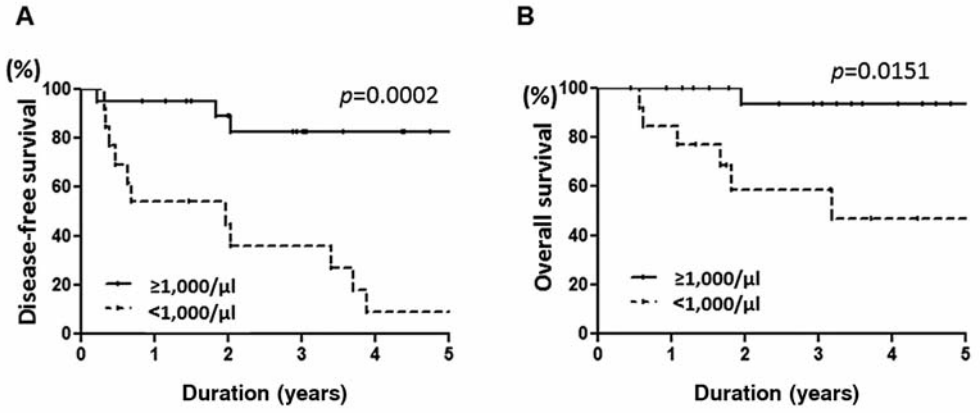

Figure 2. *Patients with a postoperative lymphocyte count of less than 1,000/μl had significantly worse disease-free (p=0.0002) (A) and overall (p=0.0151) (B) survival.*

duration of operation (p=0.0025), intraoperative blood loss (p=0.0120), intraoperative blood transfusion (p=0.0139), and TNM stage ≥II (p=0.0011) negatively correlated with postoperative peripheral blood lymphocyte count.

Discussion

Recently, reported prognostic factors for patients with GBC included early TNM stage, extent of surgical resection, microscopic curative resection, negative perineural invasion, well-differentiated tumor, adenocarcinoma, younger age, and female gender (21-24). Preoperative immunological factors have also been reported as predictors of prognosis in patients with GBC after cholecystectomy (12, 21). Ong *et al*. indicated that elevated preoperative NLR is a predictor of survival for curative resection in GBC (12).

Lymphocytes play an important role in antitumor immune responses and a persistent immunosuppressive state is associated with a high probability of recurrence and poor prognosis (15-17, 25, 26). A single malignant cell may have multiple tumor-specific antigens (27, 28). Cluster of differentiation-4 $(CD4)^+$ and $CD8^+$ T-cells have antitumor effect *via* recognizing these tumor-specific antigens. Activation of both types of T-cells requires the presentation of antigenic peptides on professional antigen-presenting cells (APCs). Dendritic cells (DCs) are the most potent APCs and play a central role in antitumor immunity by engulfing tumor antigens to facilitate the stimulation of antigen-specific T-cells (29-32). Actually, Nakakubo *et al*. indicated that a low presence $CD4^+$ and $CD8^+$ T-cells and DC infiltration in the tumor correlate with poor prognosis after surgery for GBC (33). Such infiltration and immune response could be considered to reflect a preoperative immunological and inflammatory status in patients with GBC.

In the present study, the postoperative peripheral lymphocyte count was shown to be a significant and an

Table II. *Comparison of clinical variables in relation to disease-free survival after radical resection for gallbladder carcinoma.*

Factor	N	Univariate analysys		Multivariate analysis	
		Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value
Age (years)					
≥60	10	0.7139	0.6112		
<60	24	(0.1947 to 2.618)			
Gender					
Female	19	1.237	0.6964		
Male	15	(0.4247 to 3.604)			
Operation time (min)					
≥300	13	1.552	0.4222		
<300	21	(0.5308 to 4.536)			
Blood loss (g)					
≥1,000	7	1.259	0.7165		
<1,000	17	(0.3638 to 4.354)			
Blood transfusion					
Yes	10	3.902	0.0285	2.378	0.2459
No	24	(1.154 to 13.20)		(0.550 to 10.273)	
Gallstones					
Absent	20	2.776	0.0537		
Present	14	(0.9689 to 7.953)			
TNM stage					
≥II	12	16.85	<0.0001	11.011	0.0061
≤I	22	(4.760 to 59.62)		(1.985 to 61.090)	
Neutrophils					
≥10,000	10	1.984	0.2476		
<10,000	24	(0.6211 to 6.335)			
Lymphocytes					
<1,000	13	8.506	0.0002	4.400	0.0323
≥1,000	21	(2.759 to 26.22)		(1.133 to 17.090)	
Monocytes					
<300	7	2.084	0.2943		
≥300	27	(0.5284 to 8.216)			

CI: Confidence interval.

independent factor of disease-free survival and a significant prognostic factor of overall survival. Moreover, the postoperative lymphocyte count had a negative association with intraoperative blood loss and blood transfusion. Sugita *et al*. reported that the absolute count of the peripheral blood lymphocyte in the early postoperative period was significantly decreased in patients who underwent intraoperative blood transfusion as compared to that in those who did not (34). Dietz *et al*. have shown that depression of the lymphocyte count after surgery correlates with the duration of operation and the volume of intraoperative blood loss (35). Postoperative immunosuppression can be exacerbated by blood transfusion, which can induce a shift toward a Th2 phenotype associated with a fall in lymphocyte count and down-regulation of APCs (36). Reduction of intraoperative blood transfusion may maintain postoperative immune response and improve therapeutic outcome after radical resection for GBC.

Conclusion

The postoperative peripheral blood lymphocyte count positively correlates with tumor recurrence and survival in patients with GBC after resection. Measurement of the postoperative peripheral blood lymphocyte count may help risk stratification and decision making in the postoperative management of patients with GBC after resection.

Acknowledgements

None declared.

References

1 Zhu AX, Hong TS, Hezel AF and Kooby DA: Current management of gallbladder carcinoma. Oncologist *15*: 168-181, 2010.

2 Chijiiwa K, Kai M, Nagano M, Hiyoshi M, Ohuchida J and Kondo K: Outcome of radical surgery for stage IV gallbladder carcinoma. J Hepatobiliary Pancreat Surg *14*: 345-350, 2007.

Table III. *Comparison of clinical variables in relation to overall survival after radical resection for gallbladder carcinoma.*

Factor	N	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p*-Value	Hazard ratio (95% CI)	*p*-Value
Age (years)					
≥60	24	0.5511	0.4625		
<60	10	(0.1125 to 2.701)			
Gender					
Female	19	1.939	0.3597		
Male	15	(0.4701 to 7.995)			
Operation time (min)					
≥300	13	0.7533	0.6913		
<300	21	(0.1861 to 3.050)			
Blood loss (g)					
≥1,000	7	1.138	0.8785		
<1,000	17	(0.2159 to 6.004)			
Blood transfusion					
Yes	10	5.107	0.0570		
No	24	(0.9529 to 27.37)			
Gallstones					
Absent	20	2.547	0.1915		
Present	14	(0.6263 to 10.36)			
TNM stage					
≥II	12	26.73	0.0002	13.706	0.0230
≤I	22	(4.752 to 150.3)		(1.435 to 130.914)	
Neutrophils					
≥10,000	10	4.008	0.0667		
<10,000	24	(0.9087 to 17.68)			
Lymphocytes					
<1,000	13	6.137	0.0151	2.493	0.2995
≥1,000	21	(1.420 to 26.52)		(0.444 to 14.009)	
Monocytes					
<300	7	0.8005	0.8231		
≥300	27	(0.1138 to 5.633)			

CI: Confidence interval.

Table IV. *Univariate analysis of clinicopathological variables in relation to postoperative lymphocyte count after radical resection for gallbladder carcinoma.*

Factor	Postoperative lymphocytes		*p*-Value
	≥1,000 (n=21)	<1,000 (n=13)	
Age (years)	65.6±11.6	69.2±10.0	0.3607
Gender (male:female)	10:11	5:8	0.6012
TNM stage (≤I:≥II)	18:3	4:9	0.0011
Operation time (min)	187.1±122.6	358.8±183.2	0.0025
Bood loss (g)	289.8±430.0	897.3±904.1	0.012
Blood transfusion (yes:no)	3:18	7:6	0.0139
Gallstones (present:absent)	11:9	3:11	0.0698
Neutrophils (≥10,000:<10,000)	6:15	4:9	0.8915
Monocytes (<300:≥300)	4:17	3:10	0.7777

Data are the mean±SD, or absolute frequency.

3 Nagakawa T, Kayahara M, Ikeda S, Futakawa S, Kakita A, Kawarada H, Matsuno M, Takada T, Takasaki K, Tanimura H, Tashiro S and Yamaoka Y: Biliary tract cancer treatment: Results from the Biliary Tract Cancer Statistics Registry in Japan. J Hepatobiliary Pancreat Surg *9*: 569-575, 2002.

4 Toyonaga T, Chijiiwa K, Nakano K, Noshiro H, Yamaguchi K, Sada M, Terasaka R, Konomi K, Nishikata F and Tanaka M: Completion radical surgery after cholecystectomy for accidentally undiagnosed gallbladder carcinoma. World J Surg *27*: 266-271, 2003.

5 Kokudo N, Makuuchi M, Natori T, Sakamoto Y, Yamamoto J, Seki M, Noie T, Sugawara Y, Imamura H, Asahara S and Ikari T: Strategies for surgical treatment of gallbladder carcinoma based on information available before resection. Arch Surg *138*: 741-750, 2003.

6 Frena A, Guardia G and Martin F: Outcome of radical surgery for carcinoma of the gallbladder according to the tumor node matastasis and Japanese Society of Biliary Surgery stages. J Gastrointest Surg *8*: 580-590, 2004.

7 Kayahara M and Nagakawa T: Recent trends of gallbladder cancer in Japan: An analysis of 4,770 patients. Cancer *110*: 572-580, 2007.

8 Puhalla H, Wild T, Bareck E, Pokorny H, Ploner M, Soliman T, Stremitzer S, Depisch D, Laengle F and Gruenberger T: Long-term follow-up of surgically treated gallbladder cancer patients. Eur J Surg Oncol *28*: 857-863, 2002.

9 Bishara S, Griffin M, Cargill A, Bali A, Gore ME, Kaye SB, Shepherd JH and Van Trappen PO: Pre-treatment white blood cell subtypes as prognostic indicators in ovarian cancer. Eur J Obstet Gynecol Reprod Biol *138*: 71-75, 2008.

10 Chen MH, Chang PM, Chen PM, Tzeng CH, Chu PY, Chang SY and Yang MH: Prognostic significance of a pretreatment hematologic profile in patients with head and neck cancer. J Cancer Res Clin Oncol *135*: 1783-1790, 2009.

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

12 Ong SL, Garcea G, Thomasset SC, Neal CP, Lloyd DM, Berry DP and Dennison AR: Ten-year experience in the management of gallbladder cancer from a single hepatobiliary and pancreatic centre with review of the literature. HPB *10*: 446-458, 2008.

13 Fujiwara Y, Shiba H, Furukawa K, Iida T, Sakamoto T, Gocho T, Wakiyama S, Hirohara S, Ishida Y, Misawa T, Ohashi T and Yanaga K: Perioperative change in white blood cell count predicts outcome of hepatic resection for hepatocellular carcinoma. J Hepatobiliary Pancreat Sci *17*: 892-897, 2010.

14 Haruki K, Shiba H, Fujiwara Y, Furukawa K, Wakiyama S, Ogawa M, Ishida Y, Misawa T and Yanaga K: Perioperative change in peripheral blood monocyte count may predict prognosis in patients with colorectal liver metastasis after hepatic resection. J Surg Oncol *106*: 31-35, 2012.

15 Boon T, Cerottini JC, Van den Eynde B, van der Bruggen P and Van Pel A: Tumor antigens recognized by T-lymphocytes. Annu Rev Immunol *12*: 337-365, 1994.

16 Brändle D, Brasseur F, Weynants P, Boon T and Van den Eynde B: A mutated HLA-A2 molecule recognized by autologous cytotoxic T-lymphocytes on a human renal cell carcinoma. J Exp Med *183*: 2501-2508, 1996.

17 Tanaka F, Abe M, Akiyoshi T, Nomura T, Sugimachi K, Kishimoto T, Suzuki T and Okada M: The anti-human tumor effect and generation of human cytotoxic T-cells in SCID mice given human peripheral blood lymphocytes by the *in vivo* transfer of the interleukin-6 gene using adenovirus vector. Cancer Res *57*: 1335-1343, 1997.

18 Sobin LH, Wittekind C: TNM classification of Malignant Tumors (6th edition). International Union Against Cancer, 2002.

19 De Giorgi U, Mego M, Scarpi E, Giuliano M, Giordano A, Reuben JM, Valero V, Ueno NT, Hortobagyi GN and Cristofanilli M: Relationship between lymphocytopenia and circulating tumor cells as prognostic factors for overall survival in metastatic breast cancer. Clin Breast Cancer *12*: 264-269, 2012.

20 Sasaki A, Kai S, Endo Y, Iwaki K, Uchida H, Tominaga M, Okunaga R, Shibata K, Ohta M and Kitano S: Prognostic value of preoperative peripheral blood monocyte count in patients with colorectal liver metastasis after liver resection. J Gastrointest Surg *11*: 596-602, 2007.

21 Han HS, Cho JY, Yoon YS, Ahn KS and Kim H: Preoperative inflammation is a prognostic factor for gallbladder carcinoma. Br J Surg *98*: 111-116, 2011.

22 Sakata J, Shirai Y, Wakai T, Ajioka Y and Hatakeyama K: Number of positive lymph nodes independently determines the prognosis after resection in patients with gallbladder carcinoma. Ann Surg Oncol *17*: 1831-1840, 2010.

23 Yamaguchi R, Nagino M, Oda K, Kamiya J, Uesaka K and Nimura Y: Perineural invasion has a negative impact on survival of patients with gallbladder carcinoma. Br J Surg *89*: 1130-1136, 2002.

24 Kayahara M, Nagakawa T, Nakagawara H, Kitagawa H and Ohta T: Prognostic factors for gallbladder cancer in Japan. Ann Surg *248*: 807-814, 2008.

25 Shi JS, Zhou LS, Han Y, Zhu AJ, Sun XJ and Yang YJ: Expression of tumor necrosis factor and its receptor in gallstone and gallbladder carcinoma tissue. Hepatobiliary Pancreat Dis Int *3*: 448-452, 2004.

26 Sun XJ, Shi JS, Han Y, Wang JS and Ren H: Diagnosis and treatment of polypoid lesions of the gallbladder: Report of 194 cases. Hepatobiliary Pancreat Dis Int *3*: 591-594, 2004.

27 Wortzel RD, Philipps C and Schreiber H: Multiple tumour-specific antigens expressed on a single tumour cell. Nature *304*: 165-167, 1983.

28 Van den Eynde B, Hainaut P, Hérin M, Knuth A, Lemoine C, Weynants P, van der Bruggen P, Fauchet R and Boon T: Presence on a human melanoma of multiple antigens recognized by autologous CTL. Int J Cancer *44*: 634-640, 1989.

29 Larsson M, Fonteneau JF and Bhardwaj N: Dendritic cells resurrect antigens from dead cells. Trends Immunol *22*: 141-148, 2001.

30 Wang RF: The role of MHC class II-restricted tumor antigens and CD4+ T-cells in antitumor immunity. Trends Immunol *22*: 269-276, 2001.

31 Marzo AL, Kinnear BF, Lake RA, Frelinger JJ, Collins EJ, Robinson BW and Scott B: Tumor-specific CD4+ T-cells have a major "post-licensing" role in CTL mediated antitumor immunity. J Immunol *165*: 6047-6055, 2000.

32 Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, Kwon ED, Carbone DP and Gabrilovich DI: Clinical significance of defective dendritic cell differentiation in cancer. Clin Cancer Res *6*: 1755-1766, 2000.

33 Nakakubo Y, Miyamoto M, Cho Y, Hida Y, Oshikiri T, Suzuoki M, Hiraoka K, Itoh T, Kondo S and Katoh H: Clinical significance of immune cell infiltration within gallbladder cancer. Br J Cancer *89*: 1736-1742, 2003.

34 Sugita S, Sasaki A, Iwaki K, Uchida H, Kai S, Shibata K, Ohta M and Kitano S: Prognosis and postoperative lymphocyte count in patients with hepatocellular carcinoma who received intraoperative allogenic blood transfusion: A retrospective study. Eur J Surg Oncol *34*: 339-345, 2008.

35 Dietz A, Heimlich F, Daniel V, Polarz H, Weidauer H and Maier H: Immunomodulating effects of surgical intervention in tumors of the head and neck. Otolaryngol Head Neck Surg *123*: 132-139, 2000.

36 Klein HG: Immunomodulatory aspects of transfusion: A once and future risk? Anesthesiology *91*: 861-865, 1999.

Received May 17, 2013
Revised June 26, 2013
Accepted June 28, 2013