TABLE 1 continued

Characteristic	Variable	Low BMI ($n = 35$)	Normal BMI ($n = 177$)	High BMI ($n = 31$)	*P* value	
					L vs. N	N vs. H
pN	0	13 (37.1)	95 (53.7)	13 (41.9)	0.25	0.62
	1	13 (37.1)	40 (22.6)	8 (25.8)		
	2	7 (20.0)	32 (18.1)	7 (22.6)		
	3	2 (5.7)	10 (5.6)	3 (9.7)		
pStage	0	1 (2.9)	4 (2.3)	1 (3.2)	0.011	0.24
	IA	5 (14.9)	68 (38.4)	7 (22.6)		
	IB	0	8 (4.5)	1 (3.2)		
	IIA	8 (22.9)	15 (8.5)	4 (12.9)		
	IIB	4 (11.4)	29 (16.4)	6 (19.4)		
	IIIA	7 (20.0)	25 (14.1)	3 (9.7)		
	IIIB	3 (8.6)	15 (8.5)	4 (12.9)		
	IIIC	7 (20.0)	13 (7.3)	4 (12.9)		
	IV	0	0	1 (3.2)		
Histologic grade	G1	10 (28.6)	40 (22.6)	8 (25.8)	0.18	0.75
	G2	15 (42.9)	61 (34.5)	12 (38.7)		
	G3	2 (5.7)	38 (21.5)	4 (12.9)		
	Unknown	8 (22.9)	38 (21.5)	7 (22.6)		
Residual disease	R0	27 (77.1)	165 (93.2)	28 (90.3)	0.012	0.26
	R1	2 (5.7)	3 (1.7)	2 (6.5)		
	R2	6 (17.1)	9 (5.1)	1 (3.2)		
Postoperative CT	Present	7 (20.0)	39 (22.0)	11 (35.5)	0.79	0.11

BMI body mass index, *CT* chemotherapy, *CRT* chemoradiotherapy, *dCRT* definitive chemoradiotherapy, *CVD* cerebrovascular diseases, *TT* transthoracic esophagectomy, *ILE* Ivor-Lewis esophagectomy, *TH* transhiatal esophagectomy, *L* low BMI, *N* normal BMI, *H* high BMI

cited as possible mechanisms through which diabetes may stimulate tumor growth.[18]

Obesity also has the potential to contribute to tumor progression through the upregulation of insulin signaling and chronic inflammation with altered regulation of cytokines and adipokines, including tumor necrosis factor alpha, interleukin-6, fatty acid synthase, resistin, leptin, and adiponectin.[19,20] Insulin-like growth factor (IGF) signaling is known to be associated with progression of esophageal cancer cells and mediates 5-fluorouracil chemoresistance.[21–23] Leptin is overexpressed in obese subjects and has been identified as a growth factor for cancers arising from the gastrointestinal epithelium.[20] To understand how high BMI negatively affects prognosis, it is necessary to investigate the effect of obesity on such signals or molecules. We are planning in vitro assay to evaluate the effect of different glucose concentration on the proliferation and the insulin-signaling in esophageal cancer cells. In addition, effect of antidiabetics or IGF targeting agents should be assessed in ESCC cell lines.[24]

Recently, FDG PET has become a useful tool to estimate the extent of certain tumors.[25] The rationale for the use of FDG-PET is based on the enhanced glucose metabolism of malignant tumor cells. Increased FDG uptake has been documented also in ESCC, suggesting the increased glucose metabolism in this tumor.[26] We have previously reported that glucose transporter type 1 (Glut 1) was overexpressed in esophageal cancer compared to the normal esophageal epithelium and the expression correlated with the FDG accumulation.[27] These findings suggest that enhanced glucose metabolism may have an important role in the progression of ESCC. Although we compared maximum standardized uptake values (SUV) among the groups stratified by BMI, no difference was found among the groups (data not shown). Simple comparison of SUV may not make sense to evaluate the glucose metabolism because blood sugar levels affect the SUV.

Definitions of obesity vary among countries. Japan Society for the study of obesity defines a BMI $\geq$25 kg/m^2 as obesity, while a BMI of $\geq$28 mg/m^2 is defined as obesity in China and a BMI between 25 and 30 mg/m^2 is diagnosed as overweight in Western countries. Therefore, results of this study cannot be generalized to different populations and should be validated according to the different definitions.

This study has several limitations. First, this study was a retrospective study conducted at a single institute, and the case number was limited. Obese patients with higher number of underlying comorbidities may have excluded from surgery and recommended definitive chemoradiotherapy without surgery. In order to exclude the possibility, we investigated BMIs of patients listed in our

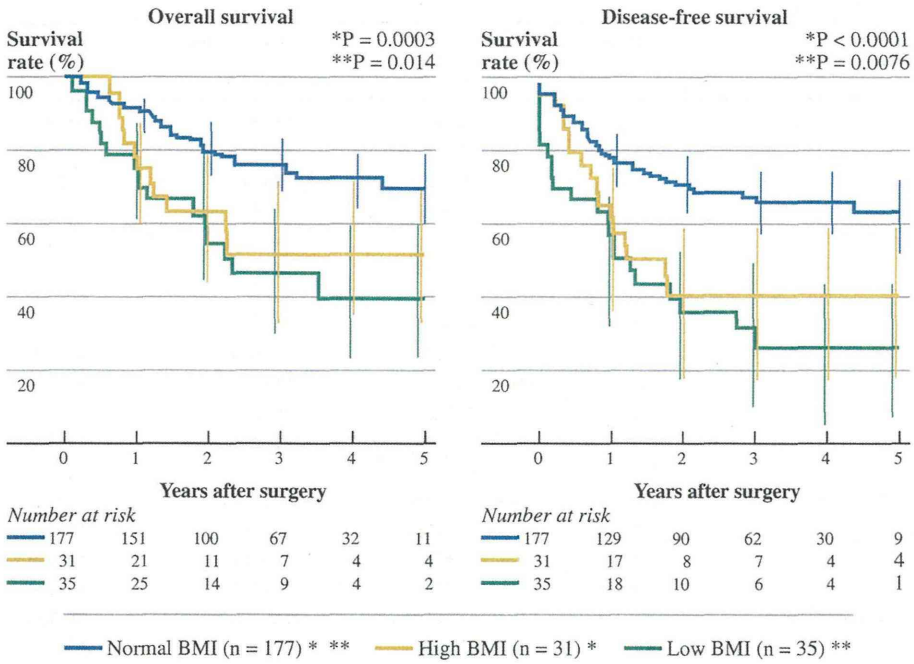

FIG. 1 Overall and disease-free survival, stratified by BMI. The median follow-up period was 25.7 months. Significantly worse overall and disease-free survival rates were observed in both the low and high BMI groups, compared to the normal BMI group

TABLE 2 Characteristics of patients who underwent esophagectomy in the propensity score-matching cohort

Characteristic	Variable	Normal BMI ($n = 27$)	High BMI ($n = 27$)	P value
Propensity score		0.86 ± 0.06	0.86 ± 0.06	0.33
Age (years)	Mean ± SD	69.1 ± 7.8	67.1 ± 7.2	0.24
Sex	Male	24 (88.9)	24 (88.9)	0.68
	Female	3 (11.1)	3 (11.1)	
BMI	Mean ± SD	22.0 ± 2.0	26.6 ± 1.1	<0.0001
Location	Upper/middle	18 (66.7)	18 (66.7)	0.75
	Middle	9 (33.3)	9 (33.3)	
cT	1/2	15 (55.6)	18 (66.7)	0.58
	3/4	12 (44.4)	9 (33.3)	
cN	0	13 (48.1)	12 (44.4)	1.00
	1–3	14 (51.9)	15 (55.6)	
cStage	I/II	16 (59.3)	17 (63.0)	1.00
	III	11 (40.7)	10 (37.0)	
Neoadjuvant treatment	Present	11 (40.7)	12 (44.4)	1.00
Comorbidity	Present	7 (25.9)	8 (29.6)	0.72
Postoperative complication	Present	18 (66.7)	18 (66.7)	0.75
No. of retrieved nodes		40.4 ± 18.8	41.6 ± 18.4	0.81
Residual tumor	R0	25 (92.6)	24 (88.8)	1.00
	R1/2	2 (7.4)	3 (11.2)	
Postoperative CT	Present	5 (18.5)	9 (33.3)	0.29

BMI body mass index, *CT* chemotherapy

chemoradiotherapy database, and found that percentage of high BMI patients who underwent definitive chemoradiotherapy was very similar to that of the high BMI group in this study. A large cohort is needed to confirm the result. Second, the follow-up period of 25.7 months may not be long enough. However, given that more than 86 % of

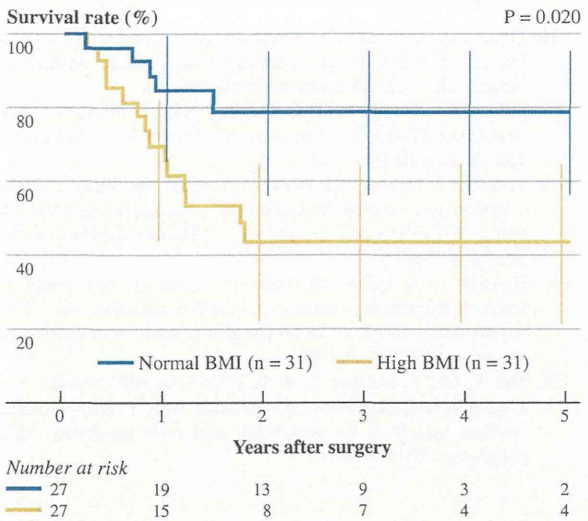

FIG. 2 Disease-free survival in the propensity score-matching cohort. Significantly worse survival rates were observed in high BMI group in comparison with the normal BMI group

TABLE 3 Multivariate analysis of disease-free survival in the propensity score-matching cohort

Characteristic	Variable	HR	95 % CI	*P* value
Age		1.006	0.933–1.084	0.88
Sex	Male	6.188	1.243–30.80	0.026
Location	Upper or middle	0.953	0.340–2.676	0.93
pStage	III, IV	11.110	3.690–33.45	<0.0001
Postoperative morbidity	Present	0.896	0.233–3.454	0.87
BMI group	H (vs. N)	2.949	1.132–7.683	0.027
Propensity score		0.001	2.09E-8–33.45	0.87

HR hazard ratio, *CI* confidence interval, *BMI* body mass index, *N* normal BMI, *H* high BMI

recurring tumors appear within 2 years after esophagectomy, the great difference in prognosis for normal and high BMI groups (Fig. 1) will not diminish over a longer follow-up period.[28] Third, we currently have no data supporting the hypothesis that tumors in overweight patients are more aggressive than those in patients with normal BMI. Further analysis on the expression levels of glucose metabolism-related molecules, such as IGF, leptin and Glut 1, is required to clarify the molecular basis of relationship between obesity and cancer.

In conclusion, both undernutrition and overweight were associated with poor prognosis of squamous cell carcinoma of the esophagus, due to different mechanisms. Advanced tumors are the reason for poor prognosis in patients with low BMI, while biological aggressiveness of tumors is possibly the reason for poor prognosis in patients with high BMI. Although further analysis is required to clarify the influence of overweight on the biological features of ESCC, glucose metabolism may be a therapeutic target for ESCC.

CONFLICT OF INTEREST The authors declare no conflict of interest.

REFERENCES

1. Calle EE, Rodriguez C, Walker-Thurmond K, et al. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. *N Engl J Med.* 2003;348:1625–38.
2. Protani M, Coory M, Martin JH. Effects of obesity on survival of women with breast cancer: systematic review and meta-analysis. *Breast Cancer Res Treat.* 2010;123:627–35.
3. Sinicrope FA, Foster NR, Sargent DJ, et al. Obesity is an independent prognostic variable in colon cancer survivors. *Clin Cancer Res.* 2010;16:1884–93.
4. Tokunaga M, Hiki N, Fukunaga T, et al. Better 5-year survival rate following curative gastrectomy in overweight patients. *Ann Surg Oncol.* 2009;16:3245–51.
5. Kamat AM, Shock RP, Rosser CJ, et al. Prognostic value of body mass index in patients undergoing nephrectomy for localized renal tumors. *Urology.* 2004;63:46–50.
6. Healy LA, Ryan AM, Gopinath B, et al. Impact of obesity on outcomes in the management of localized adenocarcinoma of the esophagus and esophagogastric junction. *J Thorac Cardiovasc Surg.* 2007;134:1284–91.
7. Morgan MA, Lewis WG, Hopper AN, et al. Prognostic significance of body mass indices for patients undergoing esophagectomy for cancer. *Dis Esophagus.* 2007;20:29–35.
8. Hayashi Y, Correa AM, Hofstetter WL, et al. The influence of high body mass index on the prognosis of patients with esophageal cancer after surgery as primary therapy. *Cancer.* 2010;116:5619–27.
9. Yoon HH, Lewis MA, Shi Q, et al. Prognostic impact of body mass index stratified by smoking status in patients with esophageal adenocarcinoma. *J Clin Oncol.* 2011;29:4561–7.
10. Sobin LH, GospodarowiczMK, Wittekind C. TNM classification of malignant tumors. 7th ed. Oxford: Wiley-Blackwell; 2010.
11. World Health Organization. WHO global database on body mass index. http://apps.who.int/bmi/index.jsp?introPage_intro_3.html. Accessed 21 Apr 2013.
12. Melis M, Weber JM, McLoughlin JM, et al. An elevated body mass index does not reduce survival after esophagectomy for cancer. *Ann Surg Oncol.* 2011;18:824–31.
13. Scarpa M, Cagol M, Bettini S, et al. Overweight patients operated on for cancer of the esophagus survive longer than normal-weight patients. *J Gastrointest Surg.* 2013;17:218–27.
14. Han-Geurts IJ, Hop WC, Tran TC, et al. Nutritional status as a risk factor in esophageal surgery. *Dig Surg.* 2006;23:159–63.
15. Swisher SG, Wynn P, Putnam JB, et al. Salvage esophagectomy for recurrent tumors after definitive chemoradiotherapy and radiotherapy. *J Thorac Cardiovasc Surg.* 2002;123:175–83.
16. Srokowski TP, Fang S, Hortobagyi GN, et al. Impact of diabetes mellitus on complications and outcomes of adjuvant chemotherapy in older patients with breast cancer. *J Clin Oncol.* 2009;27:2170–6.
17. Barone BB, Yeh HC, Snyder CF, et al. Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis. *JAMA.* 2008;300:2754–64.

18. Giovannucci E, Harlan DM, Archer MC, et al. Diabetes and cancer: a consensus report. *Diabetes Care.* 2010;60:207–21.
19. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive–leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 1996;334:292–5.
20. Ogunwobi O, Mutungi G, Beales IL. Leptin stimulates proliferation and inhibits apoptosis in Barrett's esophageal adenocarcinoma cells by cyclooxygenase-2-dependent, prostaglandin-E2-mediated transactivation of the epidermal growth factor receptor and c-Jun NH2-terminal kinase activation. *Endocrinology.* 2006;147:4505–16.
21. Chen SC, Chou CK, Wong FH, et al. Overexpression of epidermal growth factor and insulin-like growth factor receptors and autocrine stimulation in human esophageal carcinoma cells. *Cancer Res.* 1991;51:1898–903.
22. Sohda M, Kato H, Miyazaki T, et al. The role of insulin-like growth factor 1 and insulin-like growth factor binding protein 3 in human esophageal cancer. *Anticancer Res.* 2004;24:3029–34.
23. Juan HC, Tsai HT, Chang PH, et al. Insulin-like growth factor 1 mediates 5-fluorouracil chemoresistance in esophageal carcinoma cells through increasing survivin stability. *Apoptosis.* 2011;16:174–83.
24. Fidler MJ, Shersher DD, Borgia JA, et al. Targeting the insulin-like growth factor receptor pathway in lung cancer: problems and pitfalls. *Ther Adv Med Oncol.* 2012;4:51–60.
25. Brown RS, Goodman TM, Zasadny KR, et al. Expression of hexokinase II and Glut I in untreated human breast cancer. *Nucl Med Biol.* 2002;29:43–53.
26. Meyers BF, Downey RJ, Decker PA, et al. The utility of positron emission tomography in staging of potentially operable carcinoma of the thoracic esophagus. *J Thorac Cardiovasc Surg.* 2007;133:738–45.
27. Hiyoshi Y, Watanabe M, Imamura Y, et al. The relationship between the glucose transporter type 1 expression and ^{18}F-fluorodeoxyglucose uptake in esophageal squamous cell carcinoma. *Oncology.* 2008;76:286–92.
28. Toh Y, Oki E, Minami K, et al. Follow-up and recurrence after a curative esophagectomy for patients with esophageal cancer: the first indicators for recurrence and their prognostic values. *Esophagus.* 2010;7:37–43.

Preoperative Neutrophil-to-Lymphocyte Ratio Is a Predictor of Survival After Hepatectomy for Hepatocellular Carcinoma

A Retrospective Analysis

Yohei Mano, MD, Ken Shirabe, MD, PhD,* Yo-ichi Yamashita, MD, PhD,† Norifumi Harimoto, MD, PhD,‡ Eiji Tsujita, MD,† Kazuki Takeishi, MD,† Shinichi Aishima, MD, PhD,§ Toru Ikegami, MD, PhD,* Tomoharu Yoshizumi, MD, PhD, FACS,* Takeharu Yamanaka, PhD,¶ and Yoshihiko Maehara, MD, PhD, FACS**

Objective: To clarify the prognostic value of the preoperative blood neutrophil-to-lymphocyte ratio (NLR) in patients undergoing hepatectomy for hepatocellular carcinoma (HCC).

Background: Although a high NLR has been reported to be a predictor of poor survival in patients with various cancers, it has not been extensively examined in patients with HCC.

Methods: This retrospective study enrolled 958 patients who underwent hepatectomy without preoperative therapy for HCC from 1996 to 2009. Clinicopathological parameters, including NLR, were evaluated to identify predictors of overall and recurrence-free survival after hepatectomy. Univariate and multivariate analyses were performed, using the Cox proportional hazards model. The best cutoff was determined with time-dependent receiver operating characteristic curve. To determine the mechanism of NLR elevation, immunohistological examination using CD163 staining was performed in 150 patients.

Results: Univariate and multivariate analyses showed that NLR was an independent prognostic factor in overall and recurrence-free survival. The best cutoff of NLR was 2.81, and 238 of 958 patients (24.8%) had NLR of more than 2.81. The 5-year survival rate after hepatectomy was 72.9% in patients with NLR less than 2.81 and 51.5% in those with NLR 2.81 or more ($P < 0.0001$). CD163-positive cell counts were significantly higher in tumors in the group with NLR 2.81 or more than in the group with NLR less than 2.81 ($P = 0.0004$).

Conclusions: Our results show that NLR is an independent predictor of survival after hepatectomy in patients with HCC. Accumulation of tumor-associated macrophages in the tumor is associated with a high NLR.

Keywords: blood neutrophil-to-lymphocyte ratio, hepatocellular carcinoma, liver resection, prognosis, tumor-associated macrophage

(*Ann Surg* 2013;258: 301–305)

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide.[1] Hepatic resection is considered to be the treatment of choice for solitary lesions in patients with noncirrhotic livers or with Child-Pugh–grade cirrhosis, indicating well-preserved liver function.[2] However, the 5-year overall survival rate after hepatic resection is only 50% to 70%.[3–5]

The most significant factor affecting survival is the high postoperative recurrence rate. The reasons for this high recurrence rate remain unclear and seem to be complex and multifactorial.[6,7] One of the important causes of recurrence is metachronous carcinogenesis, caused by hepatic inflammation.[8] Another cause is the malignant potential of cancer cells. Pathological examination shows that microscopic portal vein invasion and intrahepatic metastasis are prognostic factors for survival.[9] Tumor-associated macrophages (TAMs) have been shown to have tumor-promoting effects, with a high density of TAMs in the tumor reported to be associated with a poor prognosis.[10,11] High serum *des-γ*-carboxy prothrombin level and expression of focal adhesion kinase have also been reported to reflect a high malignant potential in HCC.[12,13]

There is increasing evidence that increased systemic inflammation correlates with poorer cancer-specific survival in various cancers.[14–18] Recent studies have shown that the host's inflammatory response to cancer and/or the systemic effects exerted by the cancer cells lead to upregulation of the inflammatory process, predisposing the cancer to proliferation and metastasis through the inhibition of apoptosis, promotion of angiogenesis, and repair of DNA damage.[19,20] The presence of a systemic inflammatory response can be detected by the elevation of the C-reactive protein (CRP) level[21] and neutrophil-to-lymphocyte ratio (NLR)[22]. A high serum CRP level has been shown to be associated with portal vein invasion of cancer cells, and some reports have indicated that a high preoperative serum CRP level is associated with early recurrence of HCC and poorer survival after hepatic resection.[23] A high NLR has been reported to be a predictor of poor survival after hepatic resection, radio-frequency ablation, transarterial chemoembolization, and liver transplantation for HCC.[24–27] To our knowledge, only one relatively small study of fewer than 100 patients by Gomez et al[24] has reported that the preoperative NLR was a prognostic indicator of survival after hepatic resection for HCC.

This study aimed to evaluate the relationship between systemic inflammation and focal infiltration of inflammatory cells, represented by the preoperative NLR and TAMs, and outcome after hepatic resection in 958 patients in 3 high-volume centers in Japan.

METHODS

Patients

From January 1996 to December 2009, a total of 422 patients at the Second Department of Surgery, Kyushu University, 253 patients at the Department of Surgery, Hiroshima Red Cross Hospital, and 316 patients at the Department of Surgery, Iizuka Hospital, underwent hepatic resection for HCC. Thirty-three patients who underwent

From the *Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; †Department of Surgery, Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital, Hiroshima, Japan; ‡Department of Surgery, Iizuka Hospital, Fukuoka, Japan; §Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; and ¶Biostatistics Section, Research Center for Innovative Oncology, National Cancer Hospital East, Chiba, Japan.

Disclosure: Supported in part by a Grant-in Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 24390320). The authors declare no conflicts of interest.

Reprints: Ken Shirabe, MD, PhD, Department of Surgery and Sciences, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: kshirabe@surg2.med.kyushu-u.ac.jp.

ISSN: 0003-4932/13/25802-0301
DOI: 10.1097/SLA.0b013e318297ad6b

preoperative therapy, such as transarterial chemoembolization, radiofrequency ablation, or percutaneous ethanol injection, were excluded, and the remaining 958 patients (689 males, 269 females) were enrolled in this study. The mean age of patients was 67 years.

Curative resection was defined as complete macroscopic removal of the tumor and was performed in 874 patients (91.2%). Of these, 591 patients (61.7%) were seropositive for hepatitis C antibody (HCV-Ab), 161 (16.8%) were seropositive for hepatitis B surface antigen (HBs-Ag), 204 (21.3%) were seronegative for both HCV-Ab and HBs-Ag, and 5 (0.5%) were seropositive for both HBs-Ag and HCV-Ab. Of the 422 patients who underwent hepatic resection in Kyushu University, 150 consecutive patients who underwent resection from January 1997 to March 2005 were selected for immunohistological examination using CD163 staining.

Prognostic Factors in Overall and Recurrence-Free Survival After Hepatectomy

Neutrophil-to-lymphocyte ratios of all the patients in this study were calculated on the basis of preoperative blood value. Univariate analysis in overall survival and recurrence-free survival was performed, using the Cox proportional hazards model. The overall survival was evaluated in all the 958 patients, and the recurrence-free survival was evaluated only in 874 patients who underwent curative resection. The following variables were examined with respect to overall survival and recurrence-free survival rate: age, sex, serum albumin level, indocyanine green retention rate at 15 minutes (ICGR15), tumor size, serum α-fetoprotein (AFP) level, portal vein thrombus, number of tumors, TNM stage according to the Liver Cancer Study Group in Japan[28] (I or II vs III or IV), and curative resection (resection without remnant tumors). In the analysis of recurrence-free survival, variable: curative resection was excluded, because postoperative recurrence was defined only in the patients without remnant HCC who underwent curative resection. The contiguous variables were entered into the model.

The best cutoff of NLR was determined by receiver operating characteristic curve. The recurrence pattern of HCC was compared between patients with the best cutoff value of NLR. The recurrence pattern was defined as nodular ($\leq$3 nodules), multiple (>3 nodules), and extrahepatic metastasis (metastasis to organs other than the liver), as previously described.[29]

Follow-up Strategy and Recurrence Pattern

After discharge, all patients underwent monthly screening for recurrence, using ultrasonography and tumor markers such as AFP, and 6-monthly computerized tomography scanning. If recurrence was suspected, additional investigations such as hepatic angiography were performed.

Immunohistochemical Examination

Sections of resected specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained using the Envision+ system and DAB kit (DAKO, Grostrup, Denmark). Immunohistochemical staining was performed using CD163 antibodies (10D6, 1:200; Novocastra). Sections were pretreated before being incubated with primary antibodies in a microwave oven for 20 minutes. Serial sections were stained and examined by 2 pathologists (Y.M. and S.A.). The total number of cells with cytoplasmic or membrane staining in 3 high-power fields was counted.

Statistical Analysis

All data are expressed as the mean $\pm$ standard deviation. Independent χ^2 tests were used to compare categorical variables. Continuous variables were compared using unpaired t tests. Survival curves were analyzed using the Kaplan-Meier method and compared using the log-rank test. The Cox proportional hazards model was used for univariate and multivariate analyses. The best cutoff of NLR was determined by time-dependent receiver operating characteristic curve.[30] Adjustment for covariates and the Cox proportional hazards model was conducted using JMP software (SAS Institute, Cary, NC) on a Windows computer. P values of less than 0.05 were considered statistically significant.

RESULTS

NLR as an Independent Prognostic Factor

The statistically significant prognostic factors identified by univariate analyses are shown in Table 1. Indicators of poor liver function, such as low serum albumin level and high ICGR15, were identified as significant predictors of poor prognosis. Among tumor-related factors, large tumor size, high AFP level, presence of portal vein thrombus, multiple tumors, advanced clinical stage, and noncurative resection were identified as predictors of poor prognosis. Furthermore, NLR was also identified as a predictor of prognosis. Multivariate analyses identified low serum albumin level, large tumor size, high NLR level, presence of portal vein thrombus, multiple tumors, and advanced clinical stage as independent predictors of poor prognosis (Table 2).

The statistically significant factors in recurrence-free survival identified by univariate analyses are shown in Table 3. Indicators of poor liver function, such as low serum albumin level and high ICGR15, were identified as significant predictors of poor prognosis. Among tumor-related factors, large tumor size, high AFP level,

TABLE 1. Univariate Analyses of Factors in Relation to Overall Survival, Using the Cox Proportional Hazards Model

Prognostic Variables	Hazard Ratio	P	95% CI
Age	1.226	0.4145	0.993–1.018
Sex	1.964	0.6105	0.821–1.400
Albumin	7.813	<0.0001	0.271–0.457
ICGR15, %	3.274	0.0011	1.007–1.030
Tumor size	8.527	<0.0001	1.117–1.193
AFP	5.608	<0.0001	1.000–1.000
Portal vein thrombus	7.666	<0.0001	0.194–0.378
Multiple	5.520	<0.0001	0.375–0.627
Stage (I + II)	8.150	<0.0001	0.292–0.471
NLR	3.716	0.0002	1.022–1.074
Curative resection	2.392	0.0168	0.445–0.923

TABLE 2. Multivariate Analyses of Factors in Relation to Overall Survival, Using the Cox Proportional Hazards Model

Prognostic Variables	Hazard Ratio	P	95% CI
Albumin	6.779	<0.0001	0.279–0.495
NLR	3.745	0.0002	1.027–1.088
Tumor size	3.736	0.0002	1.036–1.122
Portal vein thrombus	3.445	0.0006	0.315–0.728
Stage (I + II)	2.603	0.0092	0.467–0.898
Multiple	2.211	0.0270	0.512–0.960
ICGR15	1.532	0.1254	0.997–1.022
Curative resection	1.044	0.2967	0.534–1.211
AFP	1.000	0.5100	1.000–1.000
Age	1.003	0.6721	0.990–1.016
Sex	1.058	0.6947	0.797–1.405

presence of portal vein thrombus, multiple tumors, and advanced clinical stage were identified as predictors of poor prognosis for recurrence-free survivals. Furthermore, NLR was also identified as a predictor of tumor recurrence. Multivariate analyses identified high AFP levels, low serum albumin level, high IGGR15, high NLR level, and presence of portal vein thrombus as independent predictors of tumor recurrence (Table 4).

Selection of the Best Cutoff Point for NLR

The best cutoff of NLR was determined for postoperative prognosis, using time-dependent receiver operating characteristic curve. An NLR of 2.81 was the best cutoff point for operative prognosis. All the patients were divided into 2 groups: a low (<2.81) NLR group (n = 720) and a high (≥2.81) NLR group (n = 238).

Prognostic Comparisons of the Low and High NLR Groups

The overall survival rates of patients in the low and high NLR groups are shown in Figure 1. The overall 1-, 3-, and 5-year survival rates were 95.5%, 83.9%, and 72.9% in the low (<2.81) NLR group and 87.1%, 68.9%, and 51.5% in the high (≥2.81) NLR group, which was a significant difference ($P < 0.0001$). The mean survival time was 8.0 ± 0.23 years in the low NLR group and 6.1 ± 0.38 years in the high NLR group.

The recurrence-free survival rates of patients in the low and high NLR groups are shown in Figure 2. The recurrence-free survival rate was significantly higher in the low NLR group than in the high NLR group ($P = 0.0272$).

Comparison of tumor recurrence patterns between the groups is shown in Table 5. Considering those patients with recurrence, multiple tumors in the liver were significantly more frequent in the high NLR group than in the low NLR group ($P = 0.0002$).

TABLE 3. Univariate Analyses of Factors in Relation to Recurrence Free Survival, Using the Cox Proportional Hazards Model

Prognostic Variables	Hazard Ratio	P	95% CI
Age	1.002	0.6467	0.993–1.011
Sex	1.121	0.2622	0.919–1.366
Albumin	3.928	<0.0001	0.546–0.817
ICGR15, %	3.603	0.0003	1.007–1.024
Tumor size	1.452	0.1465	0.991–1.063
AFP	6.271	<0.0001	1.000–1.000
Portal vein thrombus	2.659	0.0078	0.452–0.887
Multiple	2.657	0.0079	0.580–0.921
Stage (I + II)	3.438	0.0006	0.561–0.854
NLR	2.359	0.0183	1.005–1.059

TABLE 4. Multivariate Analyses of Factors in Relation to Recurrence free Survival, Using the Cox Proportional Hazards Model

Prognostic Variables	Hazard Ratio	P	95% CI
AFP	5.376	<0.0001	1.000–1.000
Albumin	3.517	0.0004	0.551–0.844
ICGR15	2.509	0.0121	1.003–1.021
NLR	2.096	0.0361	1.002–1.060
Portal vein thrombus	2.337	0.0194	0.487–1.032
Multiple	2.211	0.0728	0.512–0.960
Stage (I + II)	2.603	0.2673	0.659–1.123
Sex	1.096	0.368	0.892–1.345
Tumor size	1.008	0.8641	0.965–1.044
Age	1.003	0.9082	0.991–1.010

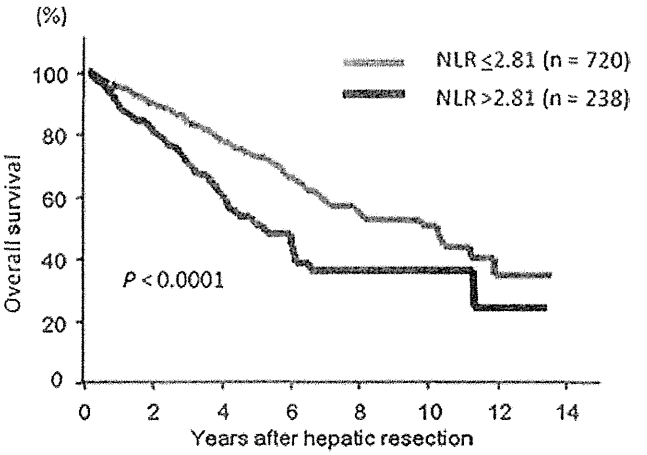

FIGURE 1. Comparison of overall survival rates in the low (<2.81) and high (≥2.81) blood NLR groups. The overall 1-, 3-, and 5-year survival rates were 95.5%, 83.9%, and 72.9% in the low (< 2.81) NLR group and 87.1%, 68.9%, and 51.5% in the high (≥2.81) NLR group, which was a significant difference ($P < 0.0001$).

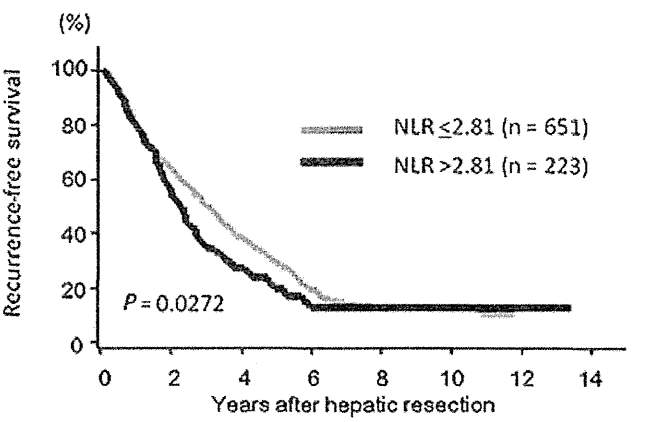

FIGURE 2. Comparison of recurrence-free survival rates in the low (<2.81) and high (≥2.81) NLR groups. The recurrence-free survival rate was significantly higher in the low NLR group than in the high NLR group ($P = 0.0272$).

TABLE 5. Comparison of Recurrence Patterns Between the Low and High NLR Groups

NLR	Nodular	Multiple	Extrahepatic	P
<2.81 (n = 351)	243 (69.2%)	86 (24.5%)	22 (6.3%)	0.0002
≥2.81 (n = 115)	55 (47.8%)	48 (41.7%)	12 (10.4%)	—

Nodular indicates fewer than 3 recurrent intrahepatic tumors; multiple, 3 or more recurrent intrahepatic tumors.

Immunohistochemical Examination

We performed immunohistochemical staining for CD163 in 150 consecutive cases at Kyushu University Hospital. Figure 3A

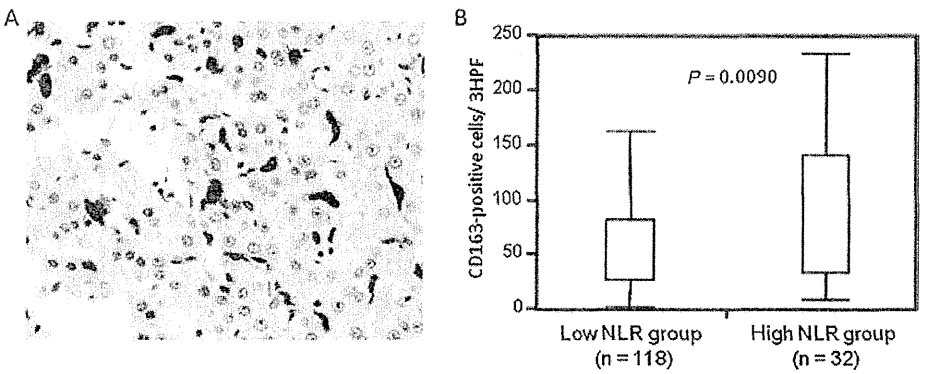

FIGURE 3. A, Immunohistochemical CD163 staining of a hepatocellular carcinoma specimen (×200). B, CD163-positive cell counts in the low and high NLR groups ($P = 0.0090$). HPF indicates high-power field.

shows CD163 staining of TAMs. We compared tumor infiltration by CD163-positive cells between the high and low NLR groups. CD163-positive cell counts were significantly higher in tumors in the high NLR group than in the low NLR group (91.0 ± 82.5 vs 61.2 ± 47.4, $P = 0.0090$; Fig. 3B).

DISCUSSION

Indicators of poor liver function, such as low serum albumin level and high ICGR15, and tumor invasion factors, such as large tumor size, presence of portal vein thrombosis, multiple HCC, and high serum AFP level, have previously been reported to be predictors of poor prognosis in patients with HCC.[31,32] The results of this study clearly show that the high preoperative NLR was an independent predictor of poor survival after hepatectomy in patients with HCC.

Although a high NLR is thought be associated with systemic inflammation, the cause of this inflammation remains unclear. Hashimoto et al[22] reported that a high CRP level was an independent prognostic factor in patients who underwent hepatectomy for HCC. Fever and high CRP level are suspected to be caused by humoral factors, especially inflammatory cytokines such as interleukin (IL)-6, IL-8, and tumor necrosis factor-α. However, fever is extremely rare in patients with HCC, and this mechanism cannot be applied to all patients with a high NLR.

Some reports have indicated that macrophage infiltration into HCC is related to the aggressiveness of the tumor.[10,11] Macrophages can assume a range of different phenotypes based on environmental stimuli. The extremes of this range in vitro are the M1 phenotype, associated with active microbial killing, and the M2 phenotype, associated with tissue remodeling and angiogenesis.[10,11,32] When monocytes in the tumor are exposed to tumor-derived anti-inflammatory molecules such as IL-4, IL-10, transforming growth factor-β1, and prostaglandin E2, they polarize into M2 macrophages.[11] The M2 phenotype macrophage seems to be the dominant type in tumors, with TAMs characterized by high expression of M2 macrophage antigens such as CD163 and high constitutive expression of IL-6 and IL-10.[33,34] Our immunohistochemical analysis showed that high infiltration of TAMs was associated with a high NLR. TAMs express some cytokines, such as IL-6 and IL-8, within the lesion, and these cytokines may promote systemic neutrophilia.[35–37] Ubukata et al[38] demonstrated that a high NLR is significantly correlated with high numbers of Th2 cells in patients with gastric cancer. Th2 cells express IL-4 and IL-10, which polarize macrophages to TAMs. A high NLR is associated with a high infiltration of TAMs and high inflammatory cytokine production in the tumor. On the contrary, our histological examination revealed that local accumulation of neutrophils into HCC might not play an important role in NLR elevation (date not shown). This phenomenon may be explained by complex expression of several cytokines. Kuang et al[39] demonstrated that intratumoral neutrophils did not have a critical role in tumor progression but peritumoral neutrophils did, and proinfilammatory IL-17 secreted by lymphocytes recruits neutrophils to peritumoral stroma. IL-17 is one of the proinflammatory cytokines. Peritumoral IL-17 may enhance systematic neutrophils in our study. Close relationship between TAMs and IL-17–producing cells was reported previously.[34,40] Thus, similar mechanism may be one of the cause of NLR elevation in HCC patients. From this point of view, a high infiltration of TAM is a first and important step of NLR elevation. Further examination is necessary to determine this clear mechanism.

There are a many reports regarding the promotion of distant metastasis of cancer cells by TAMs. Rolny et al[41] demonstrated that inhibition of TAM infiltration into tumors, by neutralizing antibodies to monocyte chemoattractants, reduces metastasis. Recent studies have provided evidence that TAMs and cytokines, such as IL-1, tumor necrosis factor, IL-6, and IL-8, increase metastasis. IL-6 levels are much higher in HCC patients than in healthy adults.[42] Harimoto et al[43] reported an HCC patient with a high IL-8 level, high CRP level, and pyrexia who had an extremely poor outcome after hepatectomy. Liu et al[44] demonstrated that IL-6 induced antiapoptotic activity via the STAT3 signaling pathway in human HCC cell lines. These phenomena may be related to TAMs, which can produce IL-6 and IL-8. Anti-inflammatory treatment may be beneficial in the treatment of HCC, and further study is necessary to investigate this.

CONCLUSIONS

Neutrophil-to-lymphocyte ratio is an easily measurable inflammatory biomarker. Our results show that NLR is an independent predictor of survival after hepatectomy in patients with HCC and that accumulation of TAMs in the tumor may be one of the causes of NLR elevation.

REFERENCES

1. Okuda K. Hepatocellular carcinoma: recent progress. *Hepatology*. 1992;15:948–963.
2. Kanematsu T, Matsumata T, Shirabe K, et al. A comparative study of hepatic resection and transcatheter arterial embolization for the treatment of primary hepatocellular carcinoma. *Cancer*. 1993;71:2181–2186.
3. Tsai JF, Jeng JE, Ho MS, et al. Effect of hepatitis C and B virus infection on risk of hepatocellular carcinoma: a prospective study. *Br J Cancer*. 1997;76:968–974.
4. Scherlock S. Viruses and hepatocellular carcinoma. *Gut*. 1994;35:828–832.
5. Taketomi A, Kitagawa D, Itoh S, et al. Trends in morbidity and mortality after hepatic resection for hepatocellular carcinoma: an institute's experience with 625 patients. *J Am Coll Surg*. 2007;204:580–587.
6. Adachi E, Maeda T, Matsumata T, et al. Risk factors for intrahepatic recurrence in human small hepatocellular carcinoma. *Gastroenterology*. 1995;108: 768–775.

7. Shirabe K, Takenaka K, Taketomi A, et al. Postoperative hepatitis status as a significant risk factor for recurrence in cirrhotic patients with small hepatocellular carcinoma. *Cancer*. 1996;77:1050–1055.
8. Shirabe K, Wakiyama S, Gion T, et al. Clinicopathological risk factors linked to recurrence pattern after curative hepatic resection for hepatocellular carcinoma—results of 152 resected cases. *Hepatogastroenterology*. 2007;54:2084–2087.
9. Shirabe K, Kajiyama K, Harimoto N, et al. Prognosis of hepatocellular carcinoma accompanied by microscopic portal vein invasion. *World J Gastroenterol*. 2009;15:2632–2637.
10. Li YW, Qiu SJ, Fan J, et al. Tumor-infiltrating macrophages can predict favorable prognosis in hepatocellular carcinoma after resection. *J Cancer Res Clin Oncol*. 2009;135:439–449.
11. Zhou J, Ding T, Pan W, et al. Increased intratumoral regulatory T cells are related to intratumoral macrophages and poor prognosis in hepatocellular carcinoma patients. *Int J Cancer*. 2009;125:1640–1648.
12. Shirabe K, Itoh S, Yoshizumi T, et al. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma—with special reference to the serum levels of des-gamma-carboxy prothrombin. *J Surg Oncol*. 2007;95:235–240.
13. Itoh S, Maeda T, Shimada M, et al. Role of expression of focal adhesion kinase in progression of hepatocellular carcinoma. *Clin Cancer Res*. 2004;10:2812–2817.
14. Proctor MJ, Talwar D, Balmar SM, et al. The relationship between the presence and site of cancer, an inflammation-based prognostic score and biochemical parameters. Initial results of the Glasgow Inflammation Outcome Study. *Br J Cancer*. 2010;103:870–876.
15. Proctor MJ, Morrison DS, Talwar D, et al. A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer*. 2011;47:2633–2641.
16. Gomez D, Morris-Stiff G, Toogood GJ, et al. Impact of systemic inflammation on outcome following resection for intrahepatic cholangiocarcinoma. *J Surg Oncol*. 2008;97:513–518.
17. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420:860–867.
18. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*. 2001;357:539–545.
19. Jaiswal M, LaRusso NF, Burgart LJ, et al. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res*. 2000;60:184–190.
20. McMillan DC, Canna K, McArdle CS. Systemic inflammatory response predicts survival following curative resection of colorectal cancer. *Br J Surg*. 2003;90:215–219.
21. Hashimoto K, Ikeda Y, Korenaga D, et al. The impact of preoperative serum C-reactive protein on the prognosis of patients with hepatocellular carcinoma. *Cancer*. 2001;103:1856–1864.
22. Zahorec R. Ratio of neutrophil to lymphocyte counts—rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lek Listy*. 2001;102:5–14.
23. Nagaoka S, Yoshida T, Akiyoshi J, et al. Serum C-reactive protein levels predict survival in hepatocellular carcinoma. *Liver Int*. 2007;27:1091–1097.
24. Gomez D, Farid S, Malik HZ, et al. Preoperative neutrophil-to-lymphocyte ratio as a prognostic predictor after curative resection for hepatocellular carcinoma. *World J Surg*. 2008;32:1757–1762.
25. Chen TM, Lin CC, Huang PT, et al. Neutrophil-to-lymphocyte ratio associated with mortality in early hepatocellular carcinoma patients after radiofrequency ablation. *J Gastroenterol Hepatol*. 2012;27:553–561.
26. Huang ZL, Luo J, Chen MS, et al. Blood neutrophil-to-lymphocyte ratio predicts survival in patients with unresectable hepatocellular carcinoma undergoing transarterial chemoembolization. *J Vasc Interv Radiol*. 2011;22:702–709.
27. Bertuzzo VR, Cescon M, Ravaioli M, et al. Analysis of factors affecting recurrence of hepatocellular carcinoma after liver transplantation with a special focus on inflammation markers. *Transplantation*. 2011;91:1279–1285.
28. Liver Cancer Study Group of Japan. *General Rules for the Clinical and Pathological Study of Primary Liver Cancer*. 4th Japanese ed. Tokyo, Japan: Kanehara; 2000.
29. Matono R, Yoshiya S, Motomura T, et al. Factors linked to long-term survival of patients with hepatocellular carcinoma accompanied by tumour thrombus in the major portal vein after surgical resection. *HPB (Oxford)*. 2012;14:247–253.
30. Heagerty PJ, Lumley T, Pepe MS. Time dependent ROC curves for censored survival data and a diagnostic marker. *Biometrics*. 2000;56:337–344.
31. Shirabe K, Takeishi K, Taketomi A, et al. Improvement of long-term outcomes in hepatitis C virus antibody-positive patients with hepatocellular carcinoma after hepatectomy in the modern era. *World J Surg*. 2011;35:1072–1084.
32. Biswas SK, Gangi L, Paul S, et al. A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). *Blood*. 2006;107:2112–2122.
33. Komohara Y, Hirahara J, Horikawa T, et al. AM-3K, an anti-macrophage antibody, recognizes CD163, a molecule associated with an anti-inflammatory macrophage phenotype. *J Histochem Cytochem*. 2006;54:763–771.
34. Kuang DM, Peng C, Zhao Q, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma promote expansion of memory T helper 17 cells. *Hepatology*. 2010;51:154–164.
35. Borish L, Rosenbaum R, Albury L, et al. Activation of neutrophils by recombinant interleukin 6. *Cell Immunol*. 1989;121:280–289.
36. Maniecki MB, Etzerodt A, Ulhøi BP, et al. Tumor-promoting macrophages induce the expression of the macrophage-specific receptor CD163 in malignant cells. *Int J Cancer*. 2012;131:2320–2331.
37. Varney ML, Olsen KJ, Mosley RL, et al. Monocyte/macrophage recruitment, activation and differentiation modulate interleukin-8 production: a paracrine role of tumor-associated macrophages in tumor angiogenesis. *In Vivo*. 2002;16:471–477.
38. Ubukata H, Motohashi G, Tabuchi T, et al. Evaluations of interferon-γ/interleukin-4 ratio and neutrophil/lymphocyte ratio as prognostic indicators in gastric cancer patients. *J Surg Oncol*. 2010;102:742–747.
39. Kuang DM, Zhao Q, Wu Y, et al. Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. *J Hepatol*. 2011;54:948–955.
40. Kuang DM, Peng C, Zhao Q, et al. Tumor-activated monocytes promote expansion of IL-17-producing $CD8^+$ T cells in hepatocellular carcinoma patients. *J Immunol*. 2010;185:1544–1549.
41. Rolny C, Capparuccia L, Casazza A, et al. The tumor suppressor semaphorin 3B triggers a prometastatic program mediated by interleukin 8 and the tumor microenvironment. *J Exp Med*. 2008;205:1155–1171.
42. Hsia CY, Huo TI, Chiang SY, et al. Evaluation of interleukin-6, interleukin-10 and human hepatocyte growth factor as tumor markers for hepatocellular carcinoma. *Eur J Surg Oncol*. 2007;33:208–212.
43. Harimoto N, Shirabe K, Abe T, et al. Interleukin-8 producing hepatocellular carcinoma with pyrexia. *HPB Surg*. 2009;2009:461492.
44. Liu Y, Fuchs J, Li C, et al. IL-6, a risk factor for hepatocellular carcinoma: FLLL32 inhibits IL-6-induced STAT3 phosphorylation in human hepatocellular cancer cells. *Cell Cycle*. 2010;9:3423–3427.

Solid tumors versus mixed tumors with a ground-glass opacity component in patients with clinical stage IA lung adenocarcinoma: Prognostic comparison using high-resolution computed tomography findings

Yasuhiro Tsutani, MD, PhD,[a] Yoshihiro Miyata, MD, PhD,[a] Takeharu Yamanaka, PhD,[b] Haruhiko Nakayama, MD, PhD,[c] Sakae Okumura, MD, PhD,[d] Shuji Adachi, MD, PhD,[e] Masahiro Yoshimura, MD, PhD,[f] and Morihito Okada, MD, PhD[a]

Objective: This study aimed to compare malignant behavior and prognosis between solid tumors and mixed tumors with a ground-glass opacity component on high-resolution computed tomography.

Methods: We examined 436 of 502 consecutive patients with clinical stage IA adenocarcinoma who had undergone preoperative high-resolution computed tomography and F-18-fluorodeoxyglucose positron emission tomography/computed tomography; 66 patients with tumors with pure ground-glass opacity components were excluded. Tumor type (solid, n = 137; mixed, n = 299) and surgical results were analyzed for all patients and their matched pairs.

Results: In all patients, solid tumors showed a significantly greater association ($P < .001$) with lymphatic, vascular, and pleural invasion and lymph node metastasis compared with mixed tumors. The disease-free survival was also worse in patients with solid tumors ($P = .0006$). Analysis of 97 pairs matched for solid component size confirmed that solid tumors were significantly associated with lymphatic, vascular, and pleural invasion ($P = .008$, $P = .029$, $P = .003$, respectively) and poor prognosis. When maximum standardized uptake value and solid component size were matched (n = 79), the differences in pathologic prognostic parameters and disease-free survivals between patients with solid and mixed tumors disappeared.

Conclusions: Solid tumors exhibit more malignant behavior and have a poorer prognosis compared with mixed tumors, even when the solid component size is the same in both tumor types. However, differences in malignant behavior can be identified using maximum standardized uptake values determined by F-18-fluorodeoxyglucose positron emission tomography/computed tomography. (J Thorac Cardiovasc Surg 2013;146:17-23)

The recent development of high-resolution computed tomography (HRCT) and low-dose computed tomography (CT) screening has improved the detection of small lung cancers, especially lung adenocarcinomas.[1-3] These often contain a nonsolid component that presents as a ground-glass opacity (GGO) on HRCT and is closely associated with bronchioloalveolar carcinoma.[4,5] We have previously reported the benefits of comparing solid component size (the maximum dimension of the solid component excluding GGO) on HRCT with whole tumor size for predicting the pathologic invasiveness of tumors or the prognosis of clinical stage IA lung adenocarcinomas.[6] It remains unclear whether GGO-containing tumors have the same malignant behavior and prognosis as pure solid tumors after matching for solid component size.

Whether or not differences exist in malignant behavior between pure solid tumors and mixed tumors with a GGO component on HRCT remains controversial. Therefore, we used HRCT to compare malignant behavior, including lymphatic, vascular, and pleural invasion, and prognosis between solid tumors and mixed tumors having a GGO component in patients with clinical stage IA lung adenocarcinoma.

PATIENTS AND METHODS

Between August 1, 2005, and December 31, 2009, we enrolled 502 patients with clinical T1N0M0 stage IA lung adenocarcinoma who were admitted to 1 of the following 4 institutions: Hiroshima University, Kanagawa Cancer Center, Cancer Institute Hospital, and Hyogo Cancer Center. HRCT and F-18-fluorodeoxyglucose positron emission tomography/CT

From the Department of Surgical Oncology,[a] Hiroshima University, Hiroshima, Japan; Research Center for Innovative Oncology,[b] National Cancer Center Hospital East, Kashiwa, Japan; Department of Thoracic Surgery,[c] Kanagawa Cancer Center, Yokohama, Japan; Department of Thoracic Surgery,[d] Cancer Institute Hospital, Tokyo, Japan; and Departments of Radiology[e] and Thoracic Surgery,[f] Hyogo Cancer Center, Akashi, Japan.

Disclosures: Authors have nothing to disclose with regard to commercial support.
Received for publication Jan 23, 2012; revisions received Sept 14, 2012; accepted for publication Nov 6, 2012; available ahead of print Dec 14, 2012.

Address for reprints: Morihito Okada, MD, PhD, Department of Surgical Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3-Kasumi, Minami-ku, Hiroshima City, Hiroshima 734-0037, Japan (E-mail: morihito@hiroshima-u.ac.jp).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2012.11.019