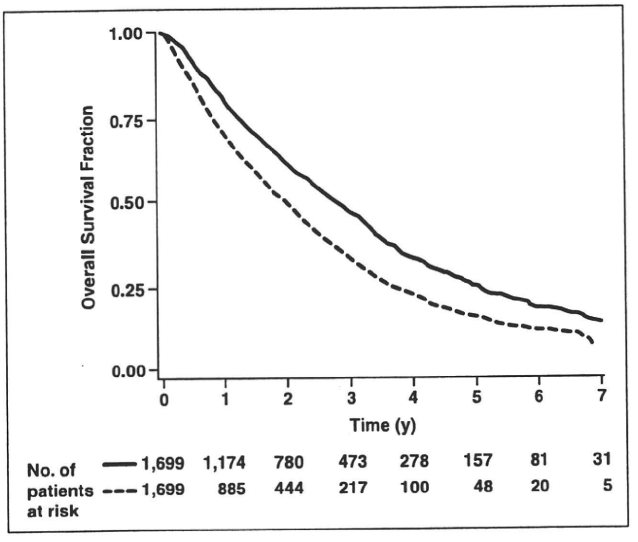

Fig. 1—Graph shows comparison of survival rates among patients with unresectable hepatocellular carcinoma treated with iodized oil transarterial chemoembolization (TACE) (n = 1,699 patients) (*solid line*) and those treated with iodized oil transarterial infusion therapy without embolization (n = 1,699) (*dotted line*) and matched by propensity score. TACE had significantly higher survival rate than therapy without embolization (hazard ratio, 0.70; 95% CI, 0.63–0.76; p = 0.0001).

TABLE 4: Results of Cox Proportional Hazards Model Multivariate Analysis of Factors Affecting Time to Death (n = 11,030)

Variable	Estimate	Standard Error	p	Hazard Ratio	
				Ratio	95% CI
Treatment (TACE vs no embolization)	−0.4556	0.0385	0.0001	0.63	0.59–0.68
Sex (male vs female)	0.0731	0.0383	0.056	1.08	0.99–1.16
Age (y) (≥ 60 vs < 60)	0.0551	0.0386	0.15	1.06	0.98–1.14
Liver damage					
Grade B vs A	0.3711	0.0358	0.0001	1.45	1.35–1.56
Grade C vs A	0.8566	0.0508	0.0001	2.36	2.13–2.60
Maximum tumor size (cm)					
2.1–3 vs ≤ 2	0.2076	0.0523	0.0001	1.23	1.11–1.36
3.1–5 vs ≤ 2	0.3802	0.0499	0.0001	1.46	1.33–1.61
≥ 5.1 vs ≤ 2	0.6689	0.0533	0.0001	1.95	1.76–2.17
No. of tumors					
2–3 vs 1	0.2593	0.0396	0.0001	1.30	1.20–1.40
≥ 4 vs 1	0.4990	0.0416	0.0001	1.65	1.52–1.79
Vascular invasion					
Vp1–≥ 3 vs Vp0	0.6137	0.0520	0.0001	1.85	1.67–2.05
≥ Vv1 vs Vv0	0.2649	0.0806	0.001	1.30	1.11–1.53
α-Fetoprotein (ng/mL)					
21–400 vs ≤ 20	0.2562	0.0412	0.0001	1.29	1.19–1.40
≥ 401 vs ≤ 20	0.7338	0.0454	0.0001	2.08	1.91–2.28

Note—TACE = transarterial iodized oil chemoembolization; no embolization = transarterial iodized oil infusion chemotherapy without embolization.

sponge particles was developed as a variation of TACE in the mid-1980s in Japan mainly to prevent posttherapeutic hepatic failure and to delay death among patients with poorer liver function and a more advanced stage of cancer than would be managed with TACE. Therapy without embolization continues to account for approximately one fourth of transarterial chemotherapeutic procedures [5].

The survival of patients who have undergone TACE and transarterial infusion therapy without embolization has stood in delicate balance between therapeutic effect against HCC and inadvertent injury to the noncancerous hepatic parenchyma. Pathologic study of resected specimens of HCC managed with TACE and with therapy without embolization revealed that TACE was associated with significantly more extensive tumor necrosis than was therapy without embolization [30, 31], whereas injury to noncancerous hepatic parenchyma has seldom been reported pathologically and clinically. An animal study [32] showed that intraarterial injection of iodized oil followed by gelatin sponge particles caused necrosis in the normal hepatic parenchyma that occurred in parallel with an increased dose of iodized oil, whereas injection of iodized oil alone did not induce necrosis. These findings are consistent with our impression of these therapies. TACE causes postembolization syndrome more frequently than does iodized oil infusion chemotherapy without embolization [19]. One serial clinical study of emulsion of iodized oil and zinostatin stimalamer, a lipophilic chemotherapeutic agent, with and without gelatin sponge particles showed that the former induced a higher response rate for HCC and more frequent impairment of hepatic function [33] than did the latter [34].

In our study of crude survival, TACE had a significantly higher overall survival rate than did therapy without embolization (HR, 0.60; 95% CI, 0.56–0.64; p = 0.0001). The median survival time and overall survival rates of therapy without embolization at 1-, 2-, 3-, and 5 years were 1.69 years and 66%, 45%, 31%, and 15%. The results in the literature are widely different from one series to another: a median survival time of 45 days [35], a 1-year survival rate of 25–82% [15, 19], a 2-year survival rate of 6–54% [15, 17], a 3-year survival rate of 24–40% [13, 19], and a 5-year survival rate of 18% [16]. The 1- to 5-year survival rates in our study were not inconsistent with those in other studies. In our study, patients who underwent TACE had better survival rates than patients in European [10, 11] and other Asian [7] series. The results may be due to the more preferable patient characteristics in our study for undergoing either transarterial therapy than was found in the other studies. More than 40% of patients in our study had a solitary HCC, and one fourth of them had HCCs smaller than 2 cm in diameter (Table 1).

Adjustment with multivariate analysis and the Cox proportional hazards model showed that TACE was associated with a better survival rate than was therapy without embolization (HR, 0.63; 95% CI, 0.59–0.68). We

TABLE 5: Baseline Characteristics of Patients in Two Groups Matched by Propensity Score (n = 3,398)

Background Factor	Transarterial Chemoembolization With Iodized Oil (n = 1,699)		Transarterial Iodized Oil Infusion Chemotherapy Without Embolization (n = 1,699)		p
	No. of Patients	%	No. of Patients	%	
Age (y)					0.75
< 60	422	25	414	24	
≥ 60	1,277	75	1,285	76	
Sex					0.52
Men	1,232	73	1,215	72	
Women	467	27	484	28	
Degree of liver damage					0.81
A	782	46	778	46	
B	696	41	694	41	
C	221	13	227	13	
Hepatitis B and C virus status					0.95
Hepatitis B surface antigen negative, hepatitis C virus antibody positive	1,282	75	1,269	75	
Hepatitis B surface antigen positive, hepatitis C virus antibody negative	165	10	172	10	
Both positive	36	2	39	2	
Both negative	216	13	219	13	
Maximum tumor size (cm)					0.59
< 2	475	28	463	27	
2.1–3	431	25	422	25	
3.1–5	394	23	413	24	
≥ 5.1	399	24	401	24	
No. of tumors					0.77
1	772	45	754	44	
2–3	472	28	494	29	
≥ 4	455	27	451	27	
Degree of portal vein invasion					0.03
Vp0	1,432	84	1,428	84	
Vp1	91	5	47	3	
Vp2	81	5	68	4	
≥ Vp3	95	6	156	9	
Degree of hepatic vein invasion					0.25
Vv0	1,630	96	1,616	95	
≥ Vv1	69	4	83	5	
α-Fetoprotein level (ng/mL)					0.19
< 20	560	33	533	31	
21–400	724	43	720	42	
> 401	415	24	446	26	
TNM stage					0.44
I (T1N0M0)	259	15	252	15	
II (T2N0M0)	636	37	628	37	
III (T3N0M0)	616	36	626	37	
IVA (T4N0M0)	188	11	193	11	

Note—Some percentages do not total 100 due to rounding.

compared the survival rates by performing patient-to-patient matching and computing the propensity score by logistic regression of the independent prognostic factors with all of the variables in Table 1 except TNM stage. As a result, the hazard ratio for death in the TACE compared with the therapy without embolization group was 0.70 (95% CI, 0.63–0.76; $p = 0.0001$), suggesting that TACE significantly reduced the overall risk of death 30%. This finding means embolization may be indispensable to better survival among patients with unresectable HCC. That is, the more intensive therapeutic effect of TACE may take precedence over the lower risk of inadvertent liver injury associated with therapy without embolization. Caturelli et al. [36] reported that the worsening of liver function expected in the long term with TACE did not occur. Results of phase 2 studies of transcatheter arterial therapy for HCC with drug-eluting beads with doxorubicin [37] and ^{90}Y-microspheres [38] and a cohort study of bland embolization with trisacryl gelatin microspheres without an anticancer agent and iodized oil [39] have been reported.

There were limitations to our study. The propensity score analysis might have matched the background of patients to have the same possibility of receiving one of the two therapies. This method, however, includes factors for insufficiency of treatment protocol among institutions and laboratory data that might affect survival. Another limitation was incomplete information about the doses of anticancer agents and iodized oil used, the total number of treatments, and Child-Pugh class because questions were overlooked on the questionnaire of the registration sheet.

Although a randomized controlled trial remains the reference standard, our analysis of an entire sample and of matched patients with a propensity score showed that in the care of patients with unresectable HCC, the survival rate associated with TACE was significantly higher than that associated with iodized oil infusion chemotherapy without embolization. These results may enhance or change decision-making about the strategy for transcatheter arterial therapy for HCC.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; 94:153–156
2. Kiyosawa K, Umemura T, Ichijo T, et al. Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; 127:S17–S26
3. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003; 139:817–823
4. Taylor-Robinson SD, Foster GR, Arora S, Hargreaves S, Thomas HC. Increase in primary liver cancer in the UK, 1979–94. *Lancet* 1997; 350:1142–1143
5. Ikai I, Arii S, Ichida T, et al. Report of the 16th follow-up survey of primary liver cancer. *Hepatol Res* 2005; 32:163–172
6. Llovet JM, Real MI, Montana X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; 359:1734–1739
7. Lo CM, Ngan H, Tso WK, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; 35:1164–1171
8. Camma C, Schepis F, Orlando A, et al. Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. *Radiology* 2002; 224:47–54
9. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003; 37:429–442
10. [No authors listed]. A comparison of lipiodol chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire. *N Engl J Med* 1995; 332:1256–1261
11. Pelletier G, Ducreux M, Gay F, et al. Treatment of unresectable hepatocellular carcinoma with lipiodol chemoembolization: a multicenter randomized trial. *J Hepatol* 1998; 29:129–134
12. Kanematsu T, Inokuchi K, Sugimachi K, et al. Selective effects of lipiodolized antitumor agents. *J Surg Oncol* 1984; 25:218–226
13. Ikeda K, Inoue H, Yano T, Kobayashi H, Nakajo M. Comparison of the anticancer effect of ADMOS alone and ADMOS with CDDP in the treatment of hepatocellular carcinoma by intra-arterial injection. *Cancer Chemother Pharmacol* 1992; 31[suppl]:S65–S68
14. Lu CD, Qi YG, Peng SY. Lipiodolization with or without gelatin sponge in hepatic arterial chemoembolization for hepatocellular carcinoma. *Chin Med J (Engl)* 1994; 107:209–215
15. Bhattacharya S, Novell JR, Dusheiko GM, Hilson AJ, Dick R, Hobbs KE. Epirubicin-lipiodol chemotherapy versus 131iodine-lipiodol radiotherapy in the treatment of unresectable hepatocellular carcinoma. *Cancer* 1995; 76:2202–2210
16. Hatanaka Y, Yamashita Y, Takahashi M, et al. Unresectable hepatocellular carcinoma: analysis of prognostic factors in transcatheter management. *Radiology* 1995; 195:747–752
17. Kawai S, Tani M, Okamura J, et al. Prospective and randomized trial of lipiodol-transcatheter arterial chemoembolization for treatment of hepatocellular carcinoma: a comparison of epirubicin and doxorubicin (second cooperative study). The Cooperative Study Group for Liver Cancer Treatment of Japan. *Semin Oncol* 1997;24[2 suppl 6]:S6-38–S6-45
18. Maeda S, Fujiyama S, Tanaka M, Ashihara H, Hirata R, Tomita K. Survival and local recurrence rates of hepatocellular carcinoma patients treated by transarterial chemolipiodolization with and without embolization. *Hepatol Res* 2002; 23:202–210
19. Ikeda M, Maeda S, Shibata J, et al. Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. *Oncology* 2004; 66:24–31
20. Takayasu K, Arii S, Ikai I, et al. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006; 131:461–469
21. Takayasu K, Furukawa H, Wakao F, et al. CT diagnosis of early hepatocellular carcinoma: sensitivity, findings, and CT-pathologic correlation. *AJR* 1995; 164:885–890
22. Baron RL, Oliver JH 3rd, Dodd GD 3rd, Nalesnik M, Holbert BL, Carr B. Hepatocellular carcinoma: evaluation with biphasic, contrast-enhanced, helical CT. *Radiology* 1996; 199:505–511
23. The Liver Cancer Study Group of Japan. *The general rules for the clinical and pathological study of primary liver cancer.* Tokyo, Japan: Kanehara, 2003
24. Nanashima A, Sumida Y, Morino S, et al. The Japanese integrated staging score using liver damage grade for hepatocellular carcinoma in patients after hepatectomy. *Eur J Surg Oncol* 2004; 30: 765–770
25. Makuuchi M, Belghiti J, Belli G, et al. IHPBA concordant classification of primary liver cancer: working group report. *J Hepatobiliary Pancreat Surg* 2003; 10:26–30
26. Murakami T. *Local therapy: radiological intervention (transcatheter arterial embolization).* Tokyo: Arcmedia, 2004:182–192
27. Rosenbaum PR, Rubin DB. The central role of the propensity score in observational studies for causal effects. *Biometrika* 1983; 70:41–55
28. Gum PA, Thamilarasan M, Watanabe J, Blackstone EH, Lauer MS. Aspirin use and all-cause mortality among patients being evaluated for known or suspected coronary artery disease: a propensity analysis. *JAMA* 2001; 286:1187–1194
29. Lindenauer PK, Pekow P, Wang K, Gutierrez B, Benjamin EM. Lipid-lowering therapy and in-

hospital mortality following major noncardiac surgery. *JAMA* 2004; 291:2092–2099

30. Takayasu K, Shima Y, Muramatsu Y, et al. Hepatocellular carcinoma: treatment with intraarterial iodized oil with and without chemotherapeutic agents. *Radiology* 1987; 163:345–351
31. Okayasu I, Hatakeyama S, Yoshida T, et al. Selective and persistent deposition and gradual drainage of iodized oil, lipiodol, in the hepatocellular carcinoma after injection into the feeding hepatic artery. *Am J Clin Pathol* 1988; 90:536–544
32. Kan Z, Sato M, Ivancev K, et al. Distribution and effect of iodized poppyseed oil in the liver after hepatic artery embolization: experimental study in several animal species. *Radiology* 1993; 186: 861–866
33. Okusaka T, Okada S, Ueno H, et al. Transcatheter arterial embolization with zinostatin stimalamer for hepatocellular carcinoma. *Oncology* 2002; 62:228–233
34. Okusaka T, Okada S, Ishii H, et al. Transarterial chemotherapy with zinostatin stimalamer for hepatocellular carcinoma. *Oncology* 1998; 55:276–283
35. Madden MV, Krige JE, Bailey S, et al. Randomised trial of targeted chemotherapy with lipiodol and 5-epidoxorubicin compared with symptomatic treatment for hepatoma. *Gut* 1993; 34: 1598–1600
36. Caturelli E, Siena DA, Fusilli S, et al. Transcatheter arterial chemoembolization for hepatocellular carcinoma in patients with cirrhosis: evaluation of damage to nontumorous liver tissue—long-term prospective study. *Radiology* 2000; 215:123–128
37. Varela M, Real MI, Burrel M, et al. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007; 46:474–481
38. Kulik LM, Carr BI, Mulcahy MF, et al. Safety and efficacy of ^{90}Y radiotherapy for hepatocellular carcinoma with and without portal vein thrombosis. *Hepatology* 2008; 47:71–81
39. Maluccio MA, Covey AM, Porat LB, et al. Transcatheter arterial embolization with only particles for the treatment of unresectable hepatocellular carcinoma. *J Vasc Interv Radiol* 2008; 19:862–869

J Gastroenterol (2010) 45:1272–1282
DOI 10.1007/s00535-010-0278-5

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

AFP, AFP-L3, DCP, and GP73 as markers for monitoring treatment response and recurrence and as surrogate markers of clinicopathological variables of HCC

Kentaroh Yamamoto · Hiroshi Imamura · Yutaka Matsuyama · Yukio Kume · Hitoshi Ikeda · Gary L. Norman · Zakera Shums · Taku Aoki · Kiyoshi Hasegawa · Yoshifumi Beck · Yasuhiko Sugawara · Norihiro Kokudo

Received: 12 April 2010 / Accepted: 14 June 2010 / Published online: 13 July 2010
© Springer 2010

Abstract

Background Alpha-fetoprotein (AFP), lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), des-γ-carboxy prothrombin (DCP), and Golgi protein-73 (GP73) have been used or proposed as tumor markers for hepatocellular carcinoma (HCC).

Methods They were measured in 96 patients undergoing hepatectomy for HCC to investigate their treatment response and association with variables linked with tumor invasiveness and/or prognosis. Values at 1 month post-surgery in the 77 patients without recurrence within 6 postoperative months were adopted as those after surgery.

Results GP73 levels did not change after hepatectomy, but levels of other markers decreased and areas under receiver operating characteristic curves (95% CI) were: 0.64 (0.56–0.72), 0.63 (0.55–0.71), 0.79 (0.73–0.86), and 0.63 (0.55–0.71) for AFP, AFP-L3, DCP, and combination of AFP and AFP-L3, respectively. Cutoff points giving specificities of 96.1% (sensitivities at these points) were: 124 ng/mL (28.1%), 10% (21.9%), and 60 mAU/mL (52.1%), for AFP, AFP-L3, and DCP, respectively. The combination of AFP and AFP-L3 provided a sensitivity of 26.0% at a specificity of 96.1%. The increased DCP value was, or tended to be, associated with a larger tumor, vascular invasion, intrahepatic metastases, and a lower grade of tumor cell differentiation. Although similar associations were found between AFP and vascular invasion as well as a lower grade of tumor cell differentiation, no such relationship was found with AFP-L3.

Conclusions DCP is a more effective tumor marker than AFP and AFP-L3. AFP-L3 showed comparable accuracy to AFP but no benefit was found in their combination. GP73 did not play a significant role in this context. Indices of tumor invasiveness were most closely associated with DCP.

Keywords AFP · AFP-L3 · DCP · GP73 · HCC

Abbreviations

AFP	Alpha-fetoprotein
AFP-L3	Lens culinaris agglutinin-reactive fraction of AFP
AUROC	Areas under ROC curve
CI	Confidence interval
DCP	Des-γ-carboxy prothrombin
GP73	Golgi protein-73
HCC	Hepatocellular carcinoma
ROC curve	Receiver operating characteristic curve

K. Yamamoto · H. Imamura · T. Aoki · K. Hasegawa · Y. Beck · Y. Sugawara · N. Kokudo
Hepato-Biliary-Pancreatic Surgery Division, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Y. Matsuyama
Department of Biostatistics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Y. Kume · H. Ikeda
Department of Clinical Laboratory Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

G. L. Norman · Z. Shums
INOVA Diagnostics, Inc., San Diego, CA, USA

H. Imamura (✉)
Department of Hepatobiliary-Pancreatic Surgery, Juntendo School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
e-mail: himamura-tky@umin.ac.jp

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of death from cancer worldwide and its incidence has been increasing in countries where the incidence of HCV infection is increasing [1]. Serum alpha-fetoprotein (AFP) has been used as a de facto standard biological tumor marker of HCC since the 1970s. However, AFP can be elevated in patients with chronic hepatitis and/or cirrhosis in the absence of HCC, leading to an unreliable role of AFP in surveillance [2].

To date, several other tumor markers have been investigated as complements for AFP. Plasma des-γ-carboxy prothrombin (DCP), also known as protein induced by vitamin K deficiency or antagonist-II (PIVKA-II), was first reported in 1984 [3]; and it has been widely used for two decades in Japan, especially since 1997 when DCP could be measured with a tenfold higher sensitivity [4]. The lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) has also been proposed as a marker for HCC, and has been commonly used in Japan since the 1990s. AFP-L3 is a fucosylated variant of AFP and the percentage of AFP-L3 over total AFP levels is used as an index of HCC [5]. AFP-L3 is reportedly more specific to HCC than AFP, representing its malignant potential [6]. In the meantime, Golgi protein-73 (GP73) has recently been shown to have a superior diagnostic ability to AFP [7, 8].

In addition to their use as diagnostic tools for surveillance, biological tumor markers play several important roles in the following aspects: monitoring treatment response, as indices of specific clinicopathological variables that provide prognostic information, and detecting disease relapse after curative treatment [9]. However, these aspects have never before been investigated in a comprehensive manner.

In the present study, we investigated the roles of AFP, DCP, AFP-L3, and GP73 as HCC tumor markers, paying particular attention to these unaddressed issues in patients with HCC undergoing liver resection.

Materials and methods

Patients

Between June 2007 and August 2008, 116 consecutive patients who were scheduled to undergo liver resection for suspected HCC were enrolled and followed prospectively at the Hepato-Biliary-Pancreatic Surgery Division of Tokyo University Hospital, Tokyo, Japan (Fig. 1). The

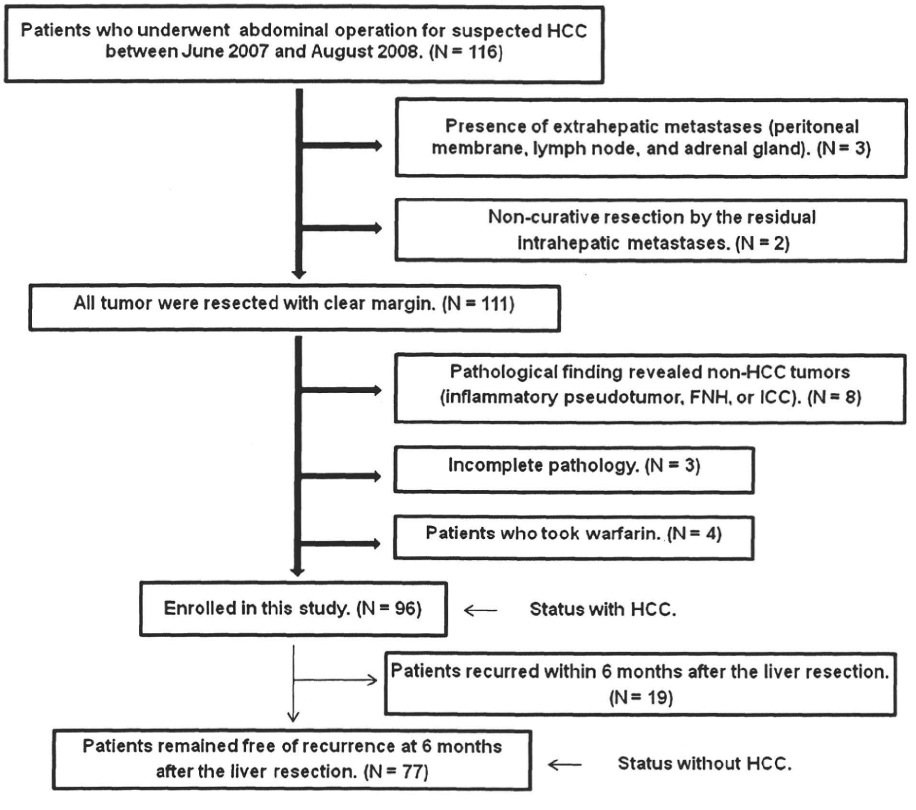

Fig. 1 Schematic flowchart of enrolled patients. *HCC* hepatocellular carcinoma, *FNH* focal nodular hyperplasia, *ICC* intrahepatic cholangiocarcinoma

study protocol was approved by the institutional ethics board and written informed consent was obtained from each subject before treatment. Preoperative diagnosis of HCC was made by using abdominal ultrasonography and dynamic computed tomography (CT) scanning. Other imaging modalities were added if necessary. The indication of liver resection was determined according to previously described criteria [10].

Curative resection was defined as the removal of all recognizable tumors with a clear margin. HCC diagnosis was finally confirmed by pathological examination of the resected specimens in all cases.

From these 116 patients, 96 were finally included. Twenty patients were excluded for the following reasons: three by the presence of extrahepatic metastases found intraoperatively, two due to non-curative liver resection, eight in whom pathological examination revealed non-HCC tumors (intrahepatic cholangiocarcinoma in five, inflammatory pseudotumors in two, focal nodular hyperplasia in one), three due to the incomplete pathological examination as a result of total necrosis of the tumor, and four by the prescription of warfarin, a DCP-inducing agent (Fig. 1). Patients' background characteristics and tumor characteristics are presented in Tables 1 and 2, respectively.

Table 1 Patient background characteristics ($N = 96$)

Variables	*N*
Age (years)[a]	67.0 (21.0–84.0)
Gender	
Male	80 (83.3%)
Female	16 (16.7%)
Hepatitis B virus infection[b]	
No	70 (72.9%)
Yes	26 (27.1%)
Hepatitis C virus infection[b]	
No	50 (52.1%)
Yes	46 (47.9%)
Indocyanine green retention rate at 15 min (ICG R15)[c]	
<10%	36 (37.5%)
10–19%	46 (47.9%)
20–29%	9 (9.4%)
30–39%	5 (5.2%)
Child–Turcotte–Pugh grade[d]	
A	80 (83.3%)
B	16 (16.7%)
Background liver status[e]	
Normal liver	5 (5.2%)
Chronic hepatitis	57 (59.4%)
Cirrhosis	34 (35.4%)

24 patients were negative for both hepatitis B and C virus infection

[a] Expressed as median with range

[b] No patients were positive for both hepatitis B and C virus infection

[c] No patient was ICG R15 ≥40%

[d] No patient was Child–Turcotte–Pugh grade C

[e] Pathological findings assessed in the resected specimen

Follow-up after hepatectomy

Monthly follow-up was conducted by assessment of tumor markers (AFP, DCP, and AFP-L3) and ultrasound. Dynamic CT scan was conducted at 3 and 6 months post-surgery. We defined recurrence as the appearance of new lesions with radiological features typical of HCC, as confirmed by at least two imaging methods [11].

Tumor marker measurement

Blood samples for tumor markers were taken both 7 days prior to and 1 month after liver resection. Serum AFP levels were measured by an immunometric assay (ST AIA-PACK AFP, Tosoh, Tokyo, Japan). Serum AFP-L3 levels were measured by lectin-affinity electrophoresis coupled with antibody-affinity blotting (LBA AFP-L3, Wako Pure Chemical Industries, Osaka, Japan), and were expressed by the ratio of AFP-L3 to total AFP (%) [5, 12]. AFP-L3 levels were not detected when AFP concentrations were <10 ng/mL, thus AFP-L3 values were defined as 0% in this range [13]. Plasma DCP levels were measured by the two-step enzyme immunoassay (Picolumi PIVKA-II, Eizai, Tokyo, Japan) [4]. Serum GP73 autoantigen and GP73 autoantibody levels were measured by prototype enzyme-linked immunosorbent assays (Quanta Lite™ GP73 Antigen ELISA and Quanta Lite™ GP73 Antibody ELISA, INOVA Diagnostics Inc., San Diego, USA). Assay results were assessed spectrophotometrically and expressed as optical densities (OD).

Relationship between tumor markers

In the measurement of multiple tumor markers, marker values should ideally be independent to each other. With this in mind, we assessed the relationship between each tumor marker before liver resection.

Ability of tumor markers to assess therapeutic response

The marker values of 96 patients before liver resection were defined as those with HCC. Of these, 77 patients remained free of recurrence 6 months after liver resection. Marker values of these 77 patients 1 month post-surgery were defined as values at complete tumor remission. To assess the ability of tumor markers to reflect the therapeutic response after curative resection, we constructed receiver operating characteristic (ROC) curves, and calculated the

Table 2 Characteristics of tumor-related variables and correlations with tumor marker values ($N = 96$)

Variables	N (%)	AFP (ng/mL)[a]	AFP-L3 (%)[a]	DCP (mAU/mL)[a]
Tumor size (mm)				
≤20	33 (34.4)	5.0 (3.0–32.0)	0.0 (0.0–1.0)	20.0 (16.0–29.0)
20–50	39 (40.6)	7.0 (4.0–384.0)	0.0 (0.0–9.4)	74.0 (22.0–203.0)
>50	24 (25.0)	17.5 (5.3–811.3)	0.5 (0.0–14.0)	924.0 (220.5–10286.0)
		$r_S = 0.25$, $P < 0.05$	$r_S = 0.14$, $P = 0.17$	$r_S = 0.66$, $P < 0.0001$
Tumor number				
1	64 (66.7)	7.5 (4.0–142.8)	0.0 (0.0–2.3)	70.0 (19.0–414.8)
2	23 (24.0)	10.0 (4.0–126.0)	0.3 (0.0–9.4)	36.0 (23.0–288.0)
≥3	9 (9.3)	37.0 (3.0–1998.0)	0.5 (0.0–7.5)	164.0 (17.0–459.0)
		$P = 0.88$	$P = 0.76$	$P = 0.93$
Capsular formation				
No	7 (7.3)	11.0 (4.0–53.0)	0.0 (0.0–0.5)	22.0 (11.0–288.0)
Yes	89 (92.7)	8.0 (4.0–152.0)	0.0 (0.0–6.8)	69.0 (20.5–384.0)
		$P = 0.85$	$P = 0.62$	$P = 0.19$
Capsular infiltration[b]				
No	21 (23.6)	6.0 (3.0–114.0)	0.0 (0.0–2.3)	27.0 (17.5–173.5)
Yes	68 (76.4)	9.5 (4.0–357.3)	0.0 (0.0–10.0)	80.0 (21.5–414.8)
		$P = 0.26$	$P = 0.70$	$P = 0.14$
Vascular invasion				
No	58 (60.4)	6.0 (3.0–53.5)	0.0 (0.0–3.5)	31.5 (18.5–224.3)
Yes	38 (39.6)	17.5 (5.0–795.8)	0.5 (0.0–10.4)	132.5 (27.3–789.8)
		$P < 0.05$	$P = 0.23$	$P < 0.05$
Intrahepatic metastases				
No	84 (87.5)	7.5 (4.0–119.5)	0.0 (0.0–6.1)	45.5 (20.0–200.5)
Yes	12 (12.5)	78.5 (4.0–687.0)	0.3 (0.0–3.2)	377.0 (58.3–12143.0)
		$P = 0.38$	$P = 0.92$	$P = 0.06$
Tumor differentiation				
Well	11 (11.5)	4.0 (2.0–14.0)	0.0 (0.0–0.5)	22.0 (16.0–108.0)
Moderate	70 (72.9)	7.0 (4.0–143.5)	0.0 (0.0–5.3)	67.5 (19.8–312.0)
Poor	15 (15.6)	34.0 (7.0–1072.0)	0.5 (0.0–21.8)	173.0 (32.0–9888.0)
		$P = 0.10$	$P = 0.10$	$P < 0.05$

AFP alpha-fetoprotein, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *DCP* des-γ-carboxy prothrombin

[a] Median with inter-quartile range

[b] We assessed 89 patients with tumor capsular formation

areas under ROC curves (AUROCs). The sensitivity/specificity at several cutoff points which were conventionally used and of specific interest in the present study were also calculated.

AFP-L3 is always measured simultaneously with AFP and its significance depends on that of AFP [6]. Similarly, AFP-L3 is thought to play a role in patients with intermediately elevated AFP values, because of its high specificity [6, 14]. With this in mind, the significance of the AFP-L3 measurement in addition to AFP was investigated through the ROC curve constructed by combining the two assays. In the combination assays, three different cutoff ranges were set as follows: in the low (AFP value <20 ng/mL) and the high (AFP value ≥400 ng/mL) cutoff ranges, cutoff points were varied according to AFP values; whereas in the intermediate cutoff range (20 ng/mL ≤ AFP value < 400 ng/mL), cutoff points were varied according to AFP-L3 values where AFP values <20 ng/mL were always classified into marker negative while AFP values ≥400 ng/mL were classified into marker positive. For example, when the AFP-L3 value of 15% was adopted as the cutoff value in the intermediate cutoff range, a patient with AFP of 800 ng/mL was classified as marker positive even when the AFP-L3 value was 5%. Here, the transition point of AFP at 400 ng/mL was adopted according to the EASL 2000 criteria [15].

Association of tumor marker values with clinicopathological variables representative of tumor invasiveness and prognosis

We assessed the association of respective marker values with clinicopathological variables that have been reported as being representative of tumor invasiveness and/or poor prognosis. Variables were assessed pathologically on the resected specimens (Table 2). Vascular invasion was defined as the presence of portal vein invasion, venous invasion, or biliary invasion. Multiple primary tumor nodules and intrahepatic metastases were differentiated by using the guidelines proposed by the Liver Cancer Study Group of Japan [16].

Alteration of marker positive/negative status through hepatectomy and postoperative marker positive status as an early indicator of tumor recurrence

We examined the alteration in the marker positive/negative status through treatment in a patient-by-patient manner. Then, we assessed the association between marker positive/negative status and tumor recurrence during the early postoperative phase, recurrence within 6 months of liver resection.

Statistical analysis

Marker values were expressed as medians with inter-quartile ranges. Correlations between marker values were analyzed by Spearman's rank correlation (r_S). AUROCs for markers were compared by Wilcoxon's rank sum test [17]. Associations between marker values and clinicopathological variables were analyzed by Wilcoxon's rank sum test or by the Kruskal–Wallis test, as appropriate. P values <0.05 were accepted as statistically significant. All statistical analyses were performed using the GraphPad Prism® computer software, version 5 (GraphPad Software Inc., San Diego, CA, USA).

Results

Relationship between tumor markers

The values of AFP and AFP-L3 showed a close association ($r_S = 0.83$), and those of GP73 autoantigen and GP73 autoantibody were moderately related ($r_S = 0.48$). No significant correlation was found in any of the other combinations of tumor marker values (Table 3).

Ability of tumor markers to assess therapeutic response

Tumor marker values of 96 patients before liver resection and those of 77 patients 1 month post-surgery in whom no recurrence was detected until 6 months post-surgery are depicted in Fig. 2.

Since GP73 did not appear to be a tumor marker representing tumor status, the following analyses exclude GP73 autoantigen and GP73 autoantibody results. The overall abilities of these tumor markers and the combination of AFP and AFP-L3 to reflect the therapeutic response after curative resection are depicted in Fig. 3.

Sensitivity/specificity at various cutoff points for these tumor markers is demonstrated in Table 4. The sensitivity of DCP was higher than those of other markers at a specificity of 96.1%, while sensitivities of other markers were of a similar extent. At a specificity of 97.4%, sensitivities of AFP-L3 and the combination of AFP and AFP-L3 were 16.7 and 26.0%, respectively ($P = 0.12$).

Association of tumor marker values with clinicopathological variables representative of tumor invasiveness and prognosis

Correlations between AFP, AFP-L3, and DCP values and clinicopathological variables are shown in Table 2. Increased DCP value was associated with the indices representing tumor growth and invasiveness such as tumor size, presence of vascular invasion, and lower grade of tumor cell differentiation. Although similar, albeit moderate, tendency was observed in the relationship between AFP value and these variables, no apparent association was found between AFP-L3 value and these indices.

Alteration of marker positive/negative status through hepatectomy and postoperative marker positive status as an early indicator of tumor recurrence

In this analysis, the cutoff points for various marker values were set at those which gave the equivalent specificities

Table 3 Correlation (r_S) between the levels of respective tumor marker values in 96 patients with HCC before liver resection

	AFP (ng/mL)	AFP-L3 (%)	DCP (mAU/mL)	GP73 autoantigen (OD)	GP73 autoantibody (OD)
AFP (ng/mL)	–	0.83	0.14	0.07	0.05
AFP-L3 (%)	–	–	0.11	0.05	0.04
DCP (mAU/mL)	–	–	–	−0.18	−0.17
GP73 autoantigen (OD)	–	–	–	–	0.48

AFP alpha-fetoprotein, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *DCP* des-γ-carboxy prothrombin, *GP73* Golgi protein-73

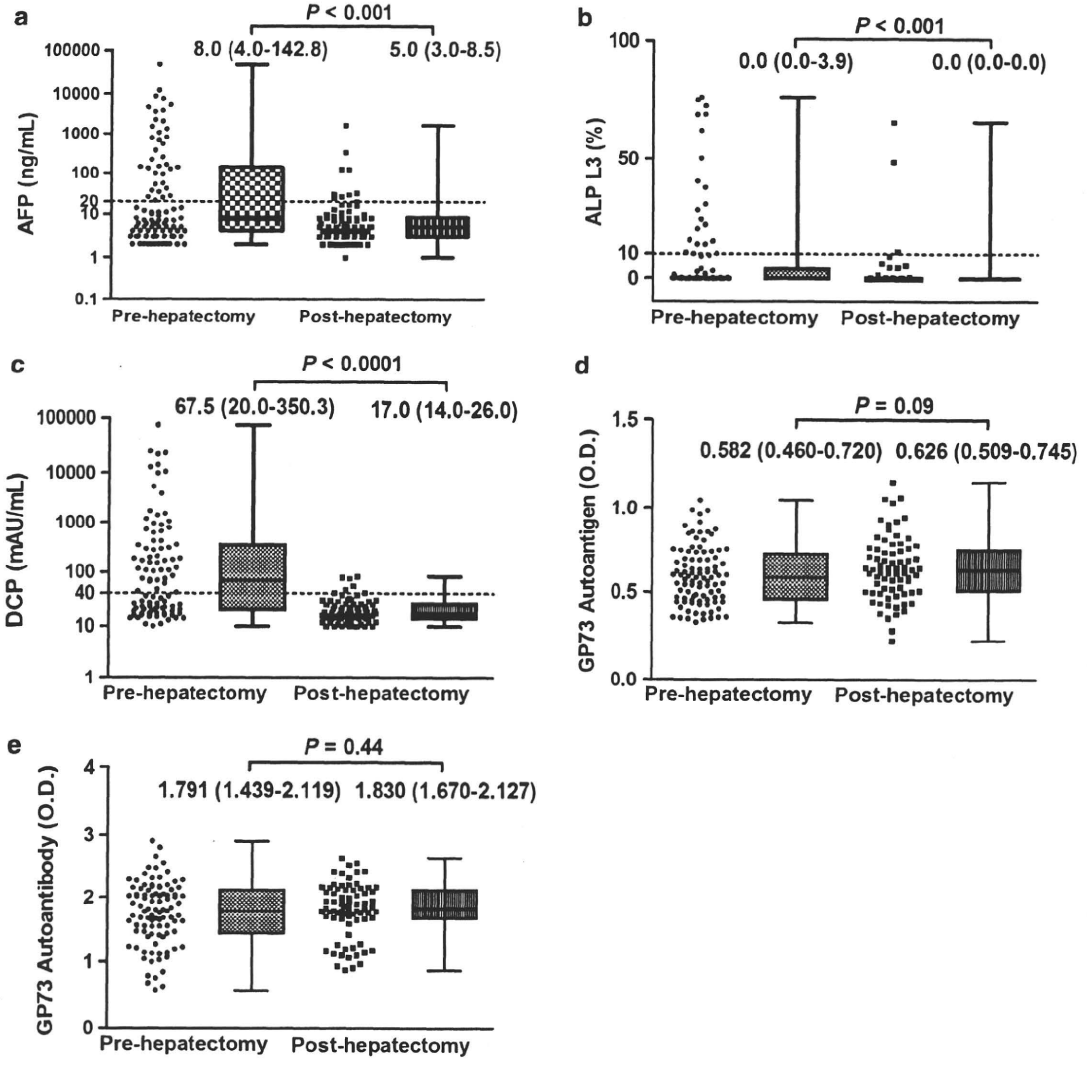

Fig. 2 Tumor marker values before hepatectomy ($N = 96$) and 1 month after hepatectomy ($N = 77$). Values after hepatectomy exclude the 19 patients whose tumor recurred within six postoperative months. **a** AFP, **b** AFP-L3, **c** DCP, **d** GP73 autoantigen, **e** GP73 autoantibody. Marker distributions are expressed by *scatter dot plots* and *box* and *whiskers*. *Top* and *bottom of boxes* are first and third quartiles, respectively. *Length of box* represents inter-quartile range within which 50% values were located. *Line through middle of each box* represents median. *Error bars* show minimum and maximum values (range). *Figures above box* and *whiskers* represent medians (inter-quartile ranges). *Dashed lines* represent cutoff values most frequently used in clinical settings and as follows: AFP, 20 ng/mL; AFP-L3, 10%; DCP, 40 mAU/mL

taking into account conventionally used values [18]. They were 200 ng/mL for AFP, 10% for AFP-L3, and 60 mAU/mL for DCP (Table 4). Specificities at these points were 97.4% for AFP, 96.1% for AFP-L3, 96.1% for DCP, and 96.1% for the combination of AFP and AFP-L3. Analysis was conducted in patients with positive preoperative marker status. The rates of patients who still had marker positive status 1 month post-surgery were as follows: 6/21 (28.6%) for AFP, 6/21 (28.6%) for AFP-L3, 4/50 (8.0%) for DCP, and 8/25 (32.0%) for the combination of AFP and AFP-L3. In these patients, imaging-proven recurrences within six postoperative months were detected with the following incidences: 4/6 (66.7%), 4/6 (66.7%), 2/4 (50.0%), and 5/8 (62.5%) for AFP, AFP-L3, DCP, and the combination of AFP and AFP-L3, respectively. Whereas, recurrence rates within six postoperative months for those patients whose preoperative positive marker status turned negative 1 month post-surgery were as follows: 4/15 (26.7%), 2/15 (13.3%), 11/46 (23.9%), and 3/17 (17.7%) for AFP, AFP-L3, DCP, and the combination of AFP and AFP-L3, respectively. Therefore, the risk of developing early postoperative recurrence in patients whose marker status remained positive 1 month post-surgery was higher than in patients whose marker values changed to negative

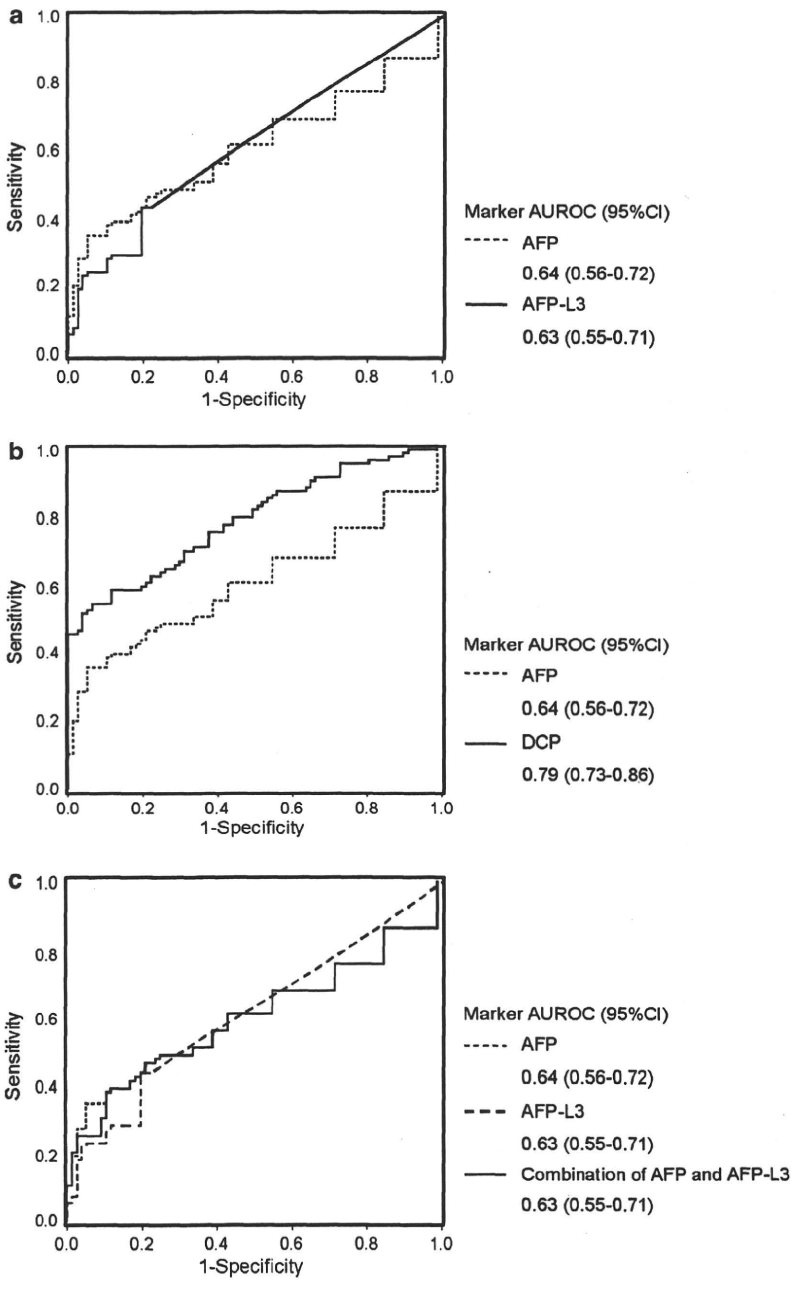

Fig. 3 Receiver operating characteristic (ROC) curves comparing tumor markers and corresponding area under ROCs (AUROCs) (95% CI). **a** AFP versus AFP-L3. $P = 0.73$. **b** AFP versus DCP. $P < 0.005$. **c** AFP or AFP-L3 versus combination of AFP and AFL-L3. $P = 0.16$ (AFP vs. combination of AFP and AFP-L3) and $P = 0.27$ (AFP-L3 vs. combination of AFP and AFP-L3), respectively

status. This was expressed as the risk ratio (95% CI) in Table 5: 2.5 (0.9–6.9), 5.0 (1.2–20.5), 2.1 (0.4–25.3), 3.5 (1.1–11.3) for AFP, AFP-L3, DCP, and the combination of AFP and AFP-L3, respectively.

Discussion

AFP, AFP-L3, and DCP have been widely used in Japan for screening and monitoring treatment response and/or relapse [19, 20]. Ideally, levels of tumor markers should fall to within a normal range after effective treatment and rise before the tumor relapse is detected by imaging studies. This aspect is especially important in the case of transcatheter arterial embolization and chemotherapy, because radiological findings do not necessarily reflect the degree of biological remission achieved by necrosis or fibrosis [21].

HCC biomarkers have also been reported to substitute as markers of specific clinicopathological variables representing the malignant potential of the tumor. In cases of

Table 4 Sensitivity/specificity at various cutoff points for tumor markers

AFP (ng/mL)	15	20	124[‡]	200
Sensitivity (%)	39.6	39.0	28.1	21.9
Specificity (%)	83.1	87.0	96.1	97.4
AFP-L3 (%)	5	10[‡]	15[§]	20
Sensitivity (%)	24.0	21.9	16.7	14.6
Specificity (%)	92.2	96.1	97.4	97.4
DCP (mAU/mL)	20	30	40	60[‡]
Sensitivity (%)	77.1	59.4	55.2	52.1
Specificity (%)	58.4	80.5	90.9	96.1
Combination of AFP (ng/mL) and AFP-L3 (%)	20[a] (AFP)	10[b,‡] (AFP-L3)	15[b,§] (AFP-L3)	400[a] (AFP)
Sensitivity (%)	39.6	26.0	26.0	17.7
Specificity (%)	87	96.1	97.4	98.7
Combination of AFP (ng/mL) or DCP (mAU/mL)	20 (AFP) or 40 (DCP)	20 (AFP) or 60 (DCP)	400 (AFP) or 40 (DCP)	400 (AFP) or 60 (DCP)
Sensitivity (%)	68.8	67.7	59.4	57.3
Specificity (%)	79.2	83.1	89.6	94.8

AFP alpha-fetoprotein, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *DCP* des-γ-carboxy prothrombin, *CI* confidence interval

[a] AFP values of 20 or 400 ng/mL was adopted as cutoff value irrespective of AFP-L3 value

[b] Patients with AFP value <20 ng/mL classified as marker negative; AFP value ≥400 ng/mL as marker positive. For patients with 20 ng/mL ≤ AFP < 400 ng/mL, AFP-L3 values of 10 or 15% adopted as cutoff value

[‡] Comparisons of sensitivities: AFP versus AFP-L3, $P = 0.33$ (95% CI −0.06 to 0.18); AFP versus DCP, $P < 0.001$ (95% CI −0.37 to −0.11); AFP versus combination of AFP and AFP-L3, $P = 0.73$ (95% CI −0.10 to 0.14); AFP-L3 versus DCP, $P < 0.001$ (95% CI −0.43 to −0.17); AFP-L3 versus combination of AFP and AFP-L3, $P = 0.51$ (95% CI −0.16 to 0.07)

[§] AFP-L3 versus combination of AFP and AFP-L3, $P = 0.12$ (95% CI −0.20 to 0.02)

non-surgical therapy, markers could therefore provide prognostic data when pathological information are unobtainable; and in cases of liver resection and transplantation, they may do so prior to the treatment [22]. In the present investigation, we evaluated these aspects of AFP, AFP-L3, and DCP as well as GP73.

GP73 antigen expression is barely detectable in normal subjects, but is strongly upregulated in the hepatocytes of patients with acute hepatitis, cirrhosis, and during the progression of chronic liver disease. GP73 was also a promising serum marker for HCC in preliminary studies [7, 8]. In the present study, however, neither GP73 autoantibody nor GP73 autoantigen levels appear to reflect the tumor status (Fig. 2). Other studies also reported the insufficiency of serum GP73 as an HCC-specific marker, although they confirmed that it may be a marker for chronic liver diseases or hepatitis C virus-related HCC [23, 24]. It should be noted that GP73 was increased in patients with liver disease, in particular, with the advancement of disease; HCC usually develops in the later stages of hepatitis C virus infection; and all previous studies suggesting the significance of GP73 as an HCC tumor marker were cross-sectional. Hence, it is more appropriate to consider that high levels of GP73 in patients with HCC reflect the fact that HCC develops at the advanced stage of chronic liver diseases.

To date, several studies have assessed the diagnostic accuracies of AFP-L3 and/or DCP in comparison with that of AFP through ROC curves. Two studies examined the significance of AFP-L3 and two of them reported that it was comparable to that of AFP [25, 26]. Five studies evaluated DCP [27–31], and four of these reported the superiority of DCP over AFP [27, 28, 30, 31]. Two studies examined the accuracies of AFP-L3 and DCP simultaneously in comparison with AFP [14, 32]. The former appeared to rank diagnostic accuracies in the decreasing order of DCP, AFP, and AFP-L3 [14], although no statistical comparison was done. The latter reported that AFP,

Table 5 Alteration of marker positive/negative status through hepatectomy and association with early phase of recurrence

Pre-hepatectomy		Post-hepatectomy			
Marker status	No. patients (rate)	Marker status	No. patients (rate)	No. patients with tumor recurrence ≤6 months	Risk ratio (95% CI)
Cutoff values set at 200 ng/mL					
AFP (−)	75/96 (78.1%)	AFP (−)	75/75 (100%)	11/75 (14.7%)	
		AFP (+)	0/75 (0.0%)	0/0 (0.0%)	
AFP (+)	21/96 (21.9%)	AFP (−)	15/21 (71.4%)	4/15 (26.7%)	2.50 (0.91–6.88)
		AFP (+)	6/21 (28.6%)	4/6 (66.7%)	
Cutoff values set at 10%					
AFP-L3 (−)	75/96 (78.1%)	AFP-L3 (−)	74/75 (98.7%)	13/74 (17.6%)	
		AFP-L3 (+)	1/75 (1.3%)	0/1 (0.0%)	
AFP-L3 (+)	21/96 (21.9%)	AFP-L3 (−)	15/21 (71.4%)	2/15 (13.3%)	5.00 (1.22–20.46)
		AFP-L3 (+)	6/21 (28.6%)	4/6 (66.7%)	
Cutoff values set at 60 mAU/mL					
DCP (−)	46/96 (47.9%)	DCP (−)	44/46 (95.7%)	5/44 (11.4%)	
		DCP (+)	2/46 (4.3%)	1/2 (50.0%)	
DCP (+)	50/96 (52.1%)	DCP (−)	46/50 (92.0%)	11/46 (23.9%)	2.09 (0.40–25.32)
		DCP (+)	4/50 (8.0%)	2/4 (50.0%)	
Cutoff values set accordingly[a]					
Combination (−)	71/96 (74.0%)	Combination (−)	70/71 (98.6%)	11/70 (15.7%)	
		Combination (+)	1/71 (1.4%)	0/1 (0.0%)	
Combination (+)	25/96 (26.0%)	Combination (−)	17/25 (68.0%)	3/17 (17.7%)	3.54 (1.11–11.29)
		Combination (+)	8/25 (32.0%)	5/8 (62.5%)	

AFP alpha-fetoprotein, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *DCP* des-γ-carboxy prothrombin, *CI* confidence interval

[a] Patients with AFP value <20 ng/mL classified as marker negative; AFP value ≥400 ng/mL as marker positive. For patients with 20 ng/mL ≤ AFP < 400 ng/mL, AFP-L3 values of 10% were adopted as cutoff value

which showed similar accuracy to DCP, was superior to AFP-L3 [32].

The present analyses revealed that DCP was superior to AFP while AFP-L3 was comparable to AFP (Fig. 3; Table 4). This finding agrees with the general conclusion from previous studies. We must bear in mind, however, that although AFP is inferior to DCP as a single tumor marker, they are independent markers and thus thought to be complementary to each other (Table 3). Furthermore, we sought the utility of additional measurements of AFP-L3 given known AFP values based on the considerations described earlier. It is of interest that the diagnostic accuracy of the combination of AFP with AFP-L3 was equivalent to AFP alone but superior to AFP-L3 alone (Fig. 3; Table 4). These results argue that the additional measurement of AFP-L3 to AFP is not mandatory, and that to the contrary, AFP-L3 data should always be interpreted in reference to AFP.

A high specificity has been reported as a feature of AFP-L3 [6]. The apparent discrepancy of the present results from reported characteristics can not be explained straightforwardly. One possible explanation may be related to the limitation of this study, that is, the present cohort comprised those undergoing hepatectomy. The majority of patients had one or two HCC nodules and the tumor diameter was relatively larger than that of the non-surgical cohort. The significance of AFP-L3 may be more marked in patients with multiple and/or small HCC nodules. Recently, a newly developed micro-total analysis system (μ-TAS) was reported not only to have higher analytical sensitivity than current methods in the determination of AFP-L3 but also to have the ability to measure AFP-L3 at a lower total AFP concentration [33]. Further studies by use of the μ-TAS system are expected to answer the question unaddressed in the present study.

Although the association of tumor markers with clinicopathological variables has been evaluated in many studies, the majority of these works only assessed associations with variables of interest and/or exclusively for AFP, AFP-L3, or DCP. Variables were also usually assessed by radiological findings or in specimens obtained by biopsy. In the present study, we investigated these associations in a comprehensive manner using pathological findings of resected specimens (Table 2). An elevated DCP value was broadly associated with variables representing tumor invasiveness and/or poor prognosis (Table 2). A similar, moderate association was confirmed for AFP but not

AFP-L3, although a similar trend was observed. This agrees in part with previous studies, which showed that respective tumor markers stood for specific pathological indices, for example, AFP for poor degree of tumor cell differentiation [34, 35], AFP-L3 for poor degree of tumor cell differentiation and presence of vascular invasion [36–38], and DCP for the presence of vascular invasion and/or intrahepatic metastasis [34, 39, 40].

One of the unique features of the present study is that we were able to follow the alterations in tumor marker values before and after the hepatectomy in a patient-by-patient manner. At cutoff points giving fairly high specificities (96.1–97.4%), almost all patients who had been negative for respective tumor markers before the hepatectomy also remained marker negative after the operation. By contrast, a considerable proportion of patients who had been marker positive did not attain marker negative status even after the curative liver resection (Table 5). Later follow-up revealed that this had been the unidentified sign of recurrence. This prediction ability appeared to be most prominent in AFP-L3 compared with AFP or DCP. This observation is in line with previous reports that suggested the significance of AFP-L3 lies in the early recognition of HCC in the follow-up of patients with cirrhosis [6].

In conclusion, DCP was shown to be a better tumor marker than AFP and AFP-L3 in monitoring the treatment response in patients with HCC, but AFP was a useful complementary marker to DCP. The accuracy of AFP-L3 was comparable to that of AFP, but no benefit was found in the additional measurement of AFP-L3 to AFP. Conversely, AFP-L3 values should be interpreted in reference of those of AFP. GP73 was not an HCC marker under the present clinical conditions. Correlation of tumor marker values with clinicopathological variables representing the malignant potential of HCC and/or poor prognosis was strongest for DCP, followed by AFP and AFP-L3 in this order. The significance of the AFP-L3 measurement may lie in the early recognition of tumor recurrence after treatment.

Acknowledgments This work was supported by Grant-in-Aid for Scientific Research (B) (20390352) and Mitsui Life Social Welfare Foundation.

References

1. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology. 2004;127:S27–34.
2. Bruix J, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208–36.
3. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Eng J Med. 1984;310:1427–31.
4. Nomura F, Ishijima M, Kuwa K, Tanaka N, Nakai T, Ohnishi K. Serum des-gamma-carboxy prothrombin levels determined by a new generation of sensitive immunoassays in patients with small-sized hepatocellular carcinoma. Am J Gastroenterol. 1999;94:650–4.
5. Aoyagi Y, Isemura M, Suzuki Y, Sekine C, Soga K, Ozaki T, et al. Fucosylated alpha-fetoprotein as marker of early hepatocellular carcinoma. Lancet. 1985;2:1353–4.
6. Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, et al. A collaborative study for the evaluation of lectin-reactive alpha-fetoprotein in early detection of hepatocellular carcinoma. Cancer Res. 1993;53:5419–23.
7. Marrero JA, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, et al. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol. 2005;43:1007–12.
8. Hu JS, Wu DW, Liang S, Miao XY. GP73, a resident Golgi glycoprotein, is sensibility and specificity for hepatocellular carcinoma of diagnosis in a hepatitis B-endemic Asian population. Med Oncol. 2009;27:339–45.
9. Yuen MF, Lai CL. Serological markers of liver cancer. Best Pract Res Clin Gastroenterol. 2005;19:91–9.
10. Makuuchi M, Kosuge T, Takayama T, Yamazaki S, Kakazu T, Miyagawa S, et al. Surgery for small liver cancers. Semin Surg Oncol. 1993;9:298–304.
11. Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, et al. Adoptive immunotherapy to lower postsurgical recurrence rate of hepatocellular carcinoma: a randomized trial. Lancet. 2000;356:802–7.
12. Shimizu K, Taniichi T, Satomura S, Matsuura S, Taga H, Taketa K. Establishment of assay kits for the determination of microheterogeneities of alpha-fetoprotein using lectin-affinity electrophoresis. Clin Chim Acta. 1993;214:3–12.
13. Toyoda H, Kumada T, Kaneoka Y, Osaki Y, Kimura T, Arimoto A, et al. Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. J Hepatol. 2008;49:223–32.
14. Durazo FA, Blatt LM, Corey WG, Lin JH, Han S, Saab S, et al. Des-gamma-carboxyprothrombin, alpha-fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol. 2008;23:1541–8.
15. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol. 2001;35:421–30.
16. Liver Cancer Study Group In Japan. The general rules for the clinical and pathological study of primary liver cancer. 5th ed. Tokyo: Kanehara; 2008.
17. Metz CE. Basic principles of ROC analysis. Semin Nucl Med. 1978;8:283–98.
18. Tateishi R, Yoshida H, Matsuyama Y, Mine N, Kondo Y, Omata M. Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. Hepatol Int. 2008;2:17–30.
19. Tateishi R, Shiina S, Yoshida H, Teratani T, Obi S, Yamashiki N, et al. Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. Hepatology. 2006;44:1518–27.
20. Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res. 2008;38:37–51.
21. Johnson PJ. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liver Dis. 2001;5:145–59.
22. Hasegawa K, Imamura H, Ijichi M, Matsuyama Y, Sano K, Sugawara Y, et al. Inclusion of tumor markers improves the

correlation of the Milan criteria with vascular invasion and tumor cell differentiation in patients with hepatocellular carcinoma undergoing liver resection (#JGSU-D-07–00462). J Gastrointest Surg. 2008;12:858–66.
23. Gu Y, Chen W, Zhao Y, Chen L, Peng T. Quantitative analysis of elevated serum Golgi protein-73 expression in patients with liver diseases. Ann Clin Biochem. 2009;46:38–43.
24. Riener MO, Stenner F, Liewen H, Soll C, Breitenstein S, Pestalozzi BC, et al. Golgi phosphoprotein 2 (GOLPH2) expression in liver tumors and its values as a serum marker in hepatocellular carcinomas. Hepatology. 2009;49:1602–9.
25. Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, et al. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. Clin Gastroenterol Hepatol. 2007;5:394–402.
26. Sterling RK, Jeffers L, Gordon F, Sherman M, Venook AP, Reddy KR, et al. Clinical utility of AFP-L3% measurement in North American patients with HCV-related cirrhosis. Am J Gastroenterol. 2007;102:2196–205.
27. Marrero JA, Su GL, Wei W, Sherman M, Venook AP, Reddy KR, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in American patients. Hepatology. 2003;37:1114–21.
28. Wang CS, Lin CL, Lee HC, Chen KY, Chiang MF, Chen HS, et al. Usefulness of serum des-gamma-carboxy prothrombin in detection of hepatocellular carcinoma. World J Gastroenterol. 2005;11:6115–9.
29. Nakamura S, Nouso K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, et al. Sensitivity and specificity of des-gammma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinoma varies according to tumor size. Am J Gastroenterol. 2006;101:2038–43.
30. Kim do Y, Paik YH, Ahn SH, Youn YJ, Choi JW, Kim JK, et al. PIVKA-II is a useful tumor marker for recurrent hepatocellular carcinoma after surgical resection. Oncology. 2007;72:52–7.
31. Yamamoto K, Imamura H, Matsuyama Y, Hasegawa K, Beck Y, Sugawara Y, et al. Significance of alpha-fetoprotein and des-γ-carboxy prothrombin in patients with hepatocellular carcinoma undergoing hepatectomy. Ann Surg Oncol. 2009;16:1795–804.
32. Marrero JA, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, et al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. Gastroenterology. 2009;137:110–8.
33. Kagebayashi C, Yamaguchi I, Akinaga A, et al. Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. Anal Biochem. 2009;388:306–11.
34. Imamura H, Matsuyama Y, Miyagawa Y, Ishida K, Shimada R, Miyagawa S, et al. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. Br J Surg. 1999;86:1032–8.
35. Oishi K, Itamoto T, Amano H, Fukuda S, Ohdan H, Tashiro H, et al. Clinicopathologic features of poorly differentiated hepatocellular carcinoma. J Surg Oncol. 2007;95:311–6.
36. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Yamamoto M, et al. Clinicopathologic features of patients with hepatocellular carcinoma seropositive for alpha-fetoprotein-L3 and seronegative for des-gamma-carboxy prothrombin in comparison with those seropositive for des-gamma-carboxy prothrombin alone. J Gastroenterol Hepatol. 2002;17:772–8.
37. Tada T, Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, et al. Relationship between Lens culinaris agglutinin-reactive alpha-fetoprotein and pathologic features of hepatocellular carcinoma. Liver Int. 2005;25:848–53.
38. Miyaaki H, Nakashima O, Kurogi M, Eguchi K, Kojiro M. Lens cullinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. J Gastroenterol. 2007;42:962–8.
39. Inoue S, Nakao A, Harada A, Nonami T, Takagi H. Clinical significance of abnormal prothrombin (DCP) in relation to postoperative survival and prognosis in patients with hepatocellular carcinoma. Am J Gastroenterol. 1994;89:2222–6.
40. Suehiro T, Sugimachi K, Matsumata T, Itasaka H, Taketomi A, Maeda T. Protein induced by vitamin K absence or antagonist II as a prognostic marker in hepatocellular carcinoma. Comparison with alpha-fetoprotein. Cancer. 1994;73:2464–71.

ORIGINAL RESEARCH ARTICLE

Clin Pharmacokinet 2010; 49 (8): 549-558
0312-5963/10/0008-0549/$49.95/0

© 2010 Adis Data Information BV. All rights reserved.

Population Pharmacokinetics of Gemcitabine and Its Metabolite in Japanese Cancer Patients

Impact of Genetic Polymorphisms

Emiko Sugiyama,[1,2] *Nahoko Kaniwa,*[1,2] *Su-Ryang Kim,*[1] *Ryuichi Hasegawa,*[2] *Yoshiro Saito,*[1,2] *Hideki Ueno,*[3] *Takuji Okusaka,*[3] *Masafumi Ikeda,*[3] *Chigusa Morizane,*[3] *Shunsuke Kondo,*[3] *Noboru Yamamoto,*[4] *Tomohide Tamura,*[4] *Junji Furuse,*[5] *Hiroshi Ishii,*[5] *Teruhiko Yoshida,*[6] *Nagahiro Saijo*[7] and *Jun-Ichi Sawada*[8]

1 Project Team for Pharmacogenetics, National Institute of Health Sciences, Tokyo, Japan
2 Division of Medicinal Safety Sciences, National Institute of Health Sciences, Tokyo, Japan
3 Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan
4 Thoracic Oncology Division, National Cancer Center Hospital, Tokyo, Japan
5 Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital East, Kashiwa, Japan
6 Genetics Division, National Cancer Center Research Institute, Tokyo, Japan
7 National Cancer Center Hospital East, Chiba, Japan
8 Pharmaceuticals and Medical Devices Agency, Tokyo, Japan

Abstract

Background and Objective: Gemcitabine (2′,2′-difluorodeoxycytidine) is an anticancer drug, which is effective against solid tumours, including non-small-cell lung cancer and pancreatic cancer. After gemcitabine is transported into cells by equilibrative and concentrative nucleoside transporters, it is phosphorylated by deoxycytidine kinase (DCK) and further phosphorylated to its active diphosphorylated and triphosphorylated forms. Gemcitabine is rapidly metabolized by cytidine deaminase (CDA) to an inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU), which is excreted into the urine. Toxicities of gemcitabine are generally mild, but unpredictable severe toxicities such as myelosuppression and interstitial pneumonia are occasionally encountered. The aim of this study was to determine the factors, including genetic polymorphisms of *CDA*, *DCK* and solute carrier family 29A1 (*SLC29A1* [*hENT1*]), that alter the pharmacokinetics of gemcitabine in Japanese cancer patients.

Patients and Methods: 250 Japanese cancer patients who received 30-minute intravenous infusions of gemcitabine at 800 or 1000 mg/m^2 in the period between September 2002 and July 2004 were recruited for this study. However, four patients were excluded from the final model built in this study because they showed bimodal concentration-time curves. Two patients who experienced gemcitabine-derived life-threatening toxicities in October 2006 and January 2008 were added to this analysis. One of these patients received 30-minute intravenous infusions of gemcitabine at 454 mg/m^2 instead of the usual dose (1000 mg/m^2).

Plasma concentrations of gemcitabine and dFdU were measured by high-performance liquid chromatography-photodiode array/mass spectrometry. In total, 1973 and 1975 plasma concentrations of gemcitabine and dFdU, respectively, were used to build population pharmacokinetic models using nonlinear mixed-effects modelling software (NONMEM® version V level 1.1).

Results and Discussion: Two-compartment models fitted well to plasma concentration-time curves for both gemcitabine and dFdU. Major contributing factors for gemcitabine clearance were genetic polymorphisms of *CDA*, including homozygous *CDA**3 [208G>A (Ala70Thr)] (64% decrease), heterozygous *3 (17% decrease) and *CDA* –31delC (an approximate 7% increase per deletion), which has a strong association with *CDA**2 [79A>C (Lys27Gln)], and coadministered S-1, an oral, multicomponent anti-cancer drug mixture consisting of tegafur, gimeracil and oteracil (an approximate 19% increase). The estimated contribution of homozygous *CDA**3 to gemcitabine clearance provides an explanation for the life-threatening severe adverse reactions, including grade 4 neutropenia observed in three Japanese patients with homozygous

*CDA**3. Genetic polymorphisms of *DCK* and *SLC29A1* (*hENT1*) had no significant correlation with gemcitabine pharmacokinetic parameters. Aging and increased serum creatinine levels correlated with decreased dFdU clearance.

Conclusion: A population pharmacokinetic model that included *CDA* genotypes as a covariate for gemcitabine and dFdU in Japanese cancer patients was successfully constructed. The model confirms the clinical importance of the *CDA**3 genotype.

Background

Gemcitabine (2′,2′-difluorodeoxycytidine, dFdC) is an anticancer drug, which is effective against solid tumours, including non-small-cell lung cancer and pancreatic cancer.[1] After gemcitabine is transported into cells by equilibrative and concentrative nucleoside transporters (ENTs and CNTs),[2,3] it is initially phosphorylated by deoxycytidine kinase (DCK) to 2′,2′-difluorodeoxycytidine monophosphate and then is further phosphorylated to its active diphosphorylated and triphosphorylated forms, dFdCDP and dFdCTP.[4,5] Gemcitabine is rapidly metabolized by cytidine deaminase (CDA) to an inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU), which is excreted into the urine.[6]

Toxicities of gemcitabine are generally mild,[7,8] but unpredictable severe toxicities such as myelosuppression and interstitial pneumonia are occasionally encountered.[9-11] In a previous paper, we reported that a single nucleotide polymorphism (SNP) of the *CDA* gene, *CDA* 208G>A (Ala70Thr), designated as *CDA**3, caused reduced CDA activity and led to increased incidences of severe neutropenia in patients receiving gemcitabine-based combination chemotherapy.[12] Moreover, all three patients who were homozygous for *CDA**3 encountered life-threatening gemcitabine-mediated toxicities, including grade 4 neutropenia.[13,14] Pharmacokinetic data available from two of these patients revealed a gemcitabine clearance of about 20% of the median clearance rate.[12,14]

In this study, we performed a population pharmacokinetic analysis of gemcitabine in Japanese cancer patients to determine which factors, including genetic factors, affect the pharmacokinetics of gemcitabine and to facilitate individualized gemcitabine-based chemotherapies.

Methods

Patients

The ethics committees of the National Cancer Center (Tokyo, Japan) and the National Institute of Health Sciences (Tokyo, Japan) approved this study. Written informed consent was obtained from all participants. 250 patients, who received 30-minute intravenous infusions of gemcitabine 800 or 1000 mg/m^2 from September 2002 to July 2004, participated in this study. Two patients[14] who experienced gemcitabine-mediated life-threatening toxicities in October 2006 and January 2008 were added to the study. One of these patients, who carried a homozygous *CDA**3 gene, received 30-minute intravenous infusions of gemcitabine 454 mg/m^2 instead of the usual dose (1000 mg/m^2). All patients in this study have been previously reported,[12,14] and their characteristics are summarized in table I.

Table I. Demographic and clinical profiles of the gemcitabine (dFdC)-treated population

Variable	Value
Sex (n; male/female)	162/86
Dose (n; mg/m^2)	
1000	243
800	4
454	1
Pancreatic cancer (n)	207
Lung cancer (n)	35
Methothelium cancer (n)	6
Monotherapy (n)	182
Combination therapy (n)	66
cisplatin	26
carboplatin	16
fluorouracil	4
S-1	10
vinorelbine	10
Age (y)[a]	62.67±9.04 [35–80]
BSA (m^2)[a]	1.56±0.17 [1.14–1.97]
Bodyweight (kg)[a]	54.56±9.76 [30–80.3]
Serum creatinine (mg/dL)[a]	0.70±0.19 [0.4–1.5]

a The values are expressed as mean ± SD [range].

BSA = body surface area; **S-1** = an oral product of tegafur with gimeracil and oteracil.

© 2010 Adis Data Information BV. All rights reserved.

Table II. Allele frequencies of the genetic polymorphisms of *CDA*, *DCK* and *SLC29A1* investigated in this study

Gene	Single nucleotide polymorphism ID	Location	Nucleotide change	Amino acid change	Subjects (n)			Allele frequency
					wild-type	heterozygous	homozygous	
CDA	MPJ6_CDA007	Exon1 (5′-UTR)	–31delC		83	110	55	0.444
	MPJ6_CDA009	Exon1	79A>C	Lys27Gln	160	73	15	0.208
	MPJ6_CDA010	Intron1	IVS1+37G>A		175	59	14	0.175
	MPJ6_CDA011	Exon2	208G>A	Ala70Thr	230	16	2	0.040
DCK	MPJ6_DCK008	5′-Flanking	–360C>G		187	58	3	0.129
	MPJ6_DCK016	Exon3	364C>T	Pro122Ser	219	28	1	0.060
SLC29A1	MPJ6_ET1005	5′-Flanking	–5851G>A		215	31	0	0.063
	MPJ6_ET1008	5′-Flanking	–3797A>G		84	128	34	0.398
	MPJ6_ET1011	5′-Flanking	–3268_–3249del AGGCTCGCGAGCGGAGGTGC		15	114	117	0.707
	MPJ6_ET1026	Intron8	IVS8+169G>A		222	23	1	0.051
	MPJ6_ET1029	Intron10	IVS10+160A>C		154	73	19	0.226
	MPJ6_ET1036	Exon12 (3′-UTR)	1840(*469)C>A		161	69	16	0.205
	MPJ6_ET1039	3′-Flanking	1984+69 (*682)A>C		139	83	24	0.266

CDA = cytidine deaminase; **DCK** = deoxycytidine kinase; **SLC** = solute carrier family; **UTR** = upper translational region.

Plasma Gemcitabine and dFdU Concentrations and Genotypes

Plasma concentrations of gemcitabine and dFdU and genotypes of *CDA*, *DCK* and solute carrier family 29A1 (*SLC29A1* [coding *hENT1*]) have been previously reported.[12,15,16] Blood samples for determining the plasma concentrations were collected before and at timepoints 0, 15, 30, 60, 90, 120 and 240 minutes after completion of the infusion. Table II summarizes the allele frequencies of *CDA*, *DCK* and *hENT1* genotypes for which effects on the pharmacokinetic parameters of gemcitabine were investigated in this study.

Population Pharmacokinetic Model Development

Population pharmacokinetics of gemcitabine and dFdU were analysed using nonlinear mixed-effects modelling software (NONMEM® version V level 1.1; ICON Plc, Dublin, Ireland). The first-order method was applied during the building of population pharmacokinetic models, and the first-order conditional estimation (FOCE) method was applied to obtain estimations in the final model. Selection of covariates was carried out by the forward stepwise inclusion and deletion method at a threshold p-value of 0.001 by a χ^2 test.

De Pas et al.[17] reported linear pharmacokinetics of gemcitabine up to 1500 mg/m^2, after which nonlinear pharmacokinetic behaviour and higher interpatient variability in the maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve (AUC) were reported. Since all patients received gemcitabine at doses ≤1000 mg/m^2, linear compartment models were selected as gemcitabine population pharmacokinetic models.

To develop a basic population pharmacokinetic model for gemcitabine (selection of a compartment model and description of interindividual and residual error variability), we fitted a one-compartment or two-compartment linear model to plasma concentrations of gemcitabine. The estimated population parameters for a one-compartment model were the volume of distribution (V_d) and clearance (CL), and those for a two-compartment model were the volume of distribution in the central compartment (V_1), clearance (CL_1), the volume of distribution in the peripheral compartment (V_2) and intercompartmental clearance (Q) [step 1]. As previously reported, two patients carrying homozygous *CDA**3 showed unexpectedly high plasma concentrations of gemcitabine;[12,14] therefore, these patients were excluded from this step. Four patients who showed bimodal concentration-time curves for gemcitabine were also excluded from the analysis because an extraordinarily large apparent V_1 for gemcitabine was estimated when they were included. Next, after the data obtained from the two *CDA**3 homozygous patients were added, the contribution of *CDA**3 to the population pharmacokinetics of

© 2010 Adis Data Information BV. All rights reserved.

gemcitabine was determined (step 2). Similarly, additional candidate covariates, most of which had previously been shown to have univariate correlations with model-independent pharmacokinetic parameters,[12] were examined for their contributions; they included other genetic polymorphisms of *CDA*,[12] *DCK*[15] and *SLC29A1* (*hENT1*),[16] regimens of chemotherapies and patients' characteristics (step 3).

In order to consider the metabolic pathway from gemcitabine to dFdU and dFdU pharmacokinetics, we used subroutines provided by NONMEM® (ADVAN5 and MODEL); the former is prepared for general linear models and the latter defines compartment attributes and rate constants. We examined whether dFdU followed a one-compartment or two-compartment model. The estimated population parameters of dFdU for a one-compartment model were total dFdU clearance (CL_m) and the dFdU volume of distribution (V_m), and those for a two-compartment model were CL_m, the dFdU volume of distribution in the central compartment (V_{m1}), the dFdU volume of distribution in the peripheral compartment (V_{m2}) and intercompartmental dFdU clearance (Q_m). Since only the subroutine TRANS1 (in which micropharmacokinetic parameters such as intercompartmental rate constants [k_{12} or k_{21}] are used) is provided by NONMEM® for ADVAN5, we adequately transformed micropharmacokinetic parameters to macropharmacokinetic ones such as the V_1, V_2, CL and Q. The metabolic fraction of dFdU (f_m) was assumed to be 1 because more than 90% of the administered gemcitabine was recovered as dFdU in the urine.[6] Compartment and error models for dFdU were determined (step 4). Subsequently, selection of candidate covariates for dFdU, which had previously been reported to have correlations with model-independent pharmacokinetic parameters of dFdU,[12] was carried out (step 5). Finally, pharmacokinetic parameters of the model constructed in step 5 were re-estimated using the FOCE method. The final model was evaluated by plots of the observed and predicted individual plasma concentrations of gemcitabine and dFdU, and by plots of the observed plasma concentrations and population conditional weighted residuals, which were calculated using Xpose 4.0 software (Uppsala University, Uppsala, Sweden).[18]

Results

Development of a Population Pharmacokinetic Model for Gemcitabine

We used 1973 plasma concentrations of gemcitabine obtained from 248 patients (figure 1a) for the following analysis. As described in the Methods, the two patients with homozygous *CDA**3 (*CDA* 208G>A) were excluded from the first step analysis because of their extraordinarily different pharmacokinetic profiles (figure 1). Pharmacokinetics of gemcitabine in Japanese cancer patients, excluding the *CDA**3-homozygous patients, were estimated using a two-compartment model. The objective function value (OFV) was at a minimum when interindividual variabilities of pharmacokinetic parameters were assumed to distribute log-normally, and when the variation in the residual errors was assumed to have both proportional and additive components (model 1).

In order to build a model taking into account the *3/*3 patients with extremely low clearance,[12] we added a

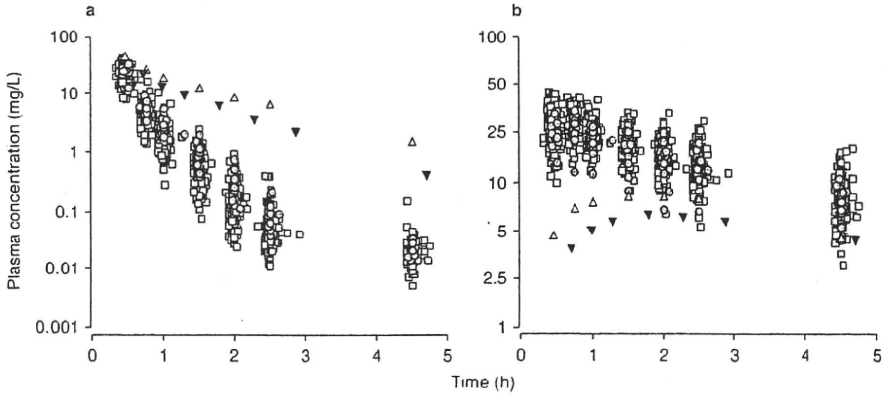

Fig. 1. Plasma concentration-time plots of (a) gemcitabine (dFdC) and (b) 2′,2′-difluorodeoxyuridine (dFdU). The light grey squares represent patients without *CDA**3 and the dark grey circles represent patients with heterozygous *CDA**3. The white triangles and black inverted triangles represent patients with homozygous *CDA**3 receiving 1000 and 454 mg/m^2 of gemcitabine, respectively.

© 2010 Adis Data Information BV. All rights reserved.

Table III. Building of covariate population pharmacokinetic models for gemcitabine (dFdC) and 2′,2′-difluorodeoxyuridine (dFdU)

Model name	Parameter	Factor[a]	OFV	Model used for comparison	ΔOFV	p-Value
Gemcitabine						
1		Basic model	−532.19			
	CL_1	*CDA**3 (linear)	−2206.79	1	−1674.60	<0.000001
2	CL_1	***CDA**3 homozygous**	−2260.86	1	−1728.68	<0.000001
3	CL_1	***CDA**3 heterozygous**	−2276.14	2	−15.28	9.28E−05
4	CL_1	BSA	−2405.26	3	−129.12	
	V_1	BSA	−2204.71	3	71.43	
	CL_1	Bodyweight	−2276.05	3	0.094	
	V_1	Bodyweight	−2176.05	3	100.09	
	CL_1	Age	−2409.77	4	−4.51	0.034
	V_1	Age	−2410.98	4	−5.72	0.017
	CL_1	Sex	−2406.40	4	−1.14	0.29
	V_1	Sex	−2406.52	4	−1.26	0.26
	CL_1	Cisplatin	−2407.248	4	−1.99	0.16
5	CL_1	**S-1**	−2427.66	4	−22.40	2.21E−06
6	CL_1	***CDA*−31delC**	−2464.89	5	−37.23	1.05E−09
	CL_1	*CDA**2	−2441.21	5	−13.55	0.00023
	CL_1	*CDA* IVS1+37G>A	−2441.84	5	−14.18	0.00017
dFdU						
7		Basic model	91.694			
8	CL_m	**BSA**	45.958	7	−45.736	
	V_{m1}	BSA	10.795	7	−80.899	
9	V_{m1}	**BSA**	−31.64	8	−77.598	
	CL_m	Bodyweight	163.251	7	71.557	
	V_{m1}	Bodyweight	2.496	7	−89.198	
10	CL_m	**Creatinine**	−166.798	9	−135.158	3.05E−31
11	CL_m	**Age**	−197.342	10	−30.544	3.26E−08
12	V_{m1}	**Age**	−212.069	11	−14.73	0.000124
13	V_{m1}	**Sex**	−243.914	12	−31.845	1.67E−08
	CL_m	Sex	−253.677	13	−9.763	0.00178

a The factors indicated in bold type were selected as covariates for the final model.

BSA=body surface area; **CL_1**=clearance of gemcitabine; **CL_m**=clearance of the metabolite dFdU; **OFV**=objective function value; **S-1**=an oral product of tegafur with gimeracil and oteracil; **V_1**=apparent volume of distribution of the central compartment of gemcitabine; **V_{m1}**=apparent volume of distribution of the central compartment of dFdU.

covariate to the basic model to account for the effect of homozygosity of *3 (θ_{*3homo}) on the clearance of gemcitabine (equation 1):

$$CL = \theta_1 \times (1 - \theta_{*3homo} \times CDA*3homo) \quad \text{(Eq. 1)}$$

where CL is total gemcitabine clearance in a patient of interest; θ_1 is gemcitabine clearance in patients without *3/*3; and *CDA**3homo is 1 for *3/*3 and 0 for other patients (*3/non-*3 or non-*3/non-*3). This modification significantly reduced the OFV, as shown in table III (model 2).

Next, the effect of heterozygous *3 on gemcitabine clearance was examined by comparing equations 2 and 3:

$$CL = \theta_2 \times (1 - \theta_{*3homo} \times CDA*3) \quad \text{(Eq. 2)}$$

$$CL = \theta_2 \times (1 - \theta_{*3hetero} \times CDA*3hetero) \times (1 - \theta_{*3homo} \times CDA*3homo) \quad \text{(Eq. 3)}$$

© 2010 Adis Data Information BV. All rights reserved.