liver failure can be the direct cause of death even in patients without HCC recurrence. Thus, preservation of liver function is essential for improving prognosis of HBV-related HCC patients.

Lamivudine (LAM), 2′3′-dideoxy-3′-thiacytidine, is a potent reverse transcriptase inhibitor and has been used for the treatment of chronic hepatitis B as well as HIV infection [11]. LAM has been shown to be safe and well tolerated in patients with HBV infection, including those with severely decompensated cirrhosis [12–14]. With the inhibition of HBV replication, inflammatory reaction in the liver subsides and the liver can be protected from further deterioration in function. The antiviral therapy with LAM appears to be suitable also for patients with HBV-related HCC after cancer treatment but there have been few reports. The objective of this study is to elucidate the safety and efficacy of LAM treatment in HBV-related HCC patients treated with RFA.

Patients and methods

Patients

This is a retrospective study on clinical experience in a single center. Between January 2000 and December 2005, a total of 1050 patients were admitted to our hospital for RFA treatment of HCC. HBs antigen was positive in 110 patients and 104 of them received curative RFA therapy, as judged by subsequent imaging studies (Fig. 1). In this study, the medical records of these 104 patients were reviewed.

RFA was performed percutaneously, using monopolar radiofrequency generator (CC-1 Cosman Coagulator, Radionics, Burlington, MA, USA) and internally cooled-tip radiofrequency electrode (Radionics) as described elsewhere [4, 10]. The effectiveness of ablation was evaluated with contrast-enhanced computed tomography in each patient. At the time of RFA, baseline characters of enrolled patients, including age, sex, and biochemical tests, were recorded. Serum level of HBV-DNA was determined by the transcription-mediated-amplification (TMA) method.

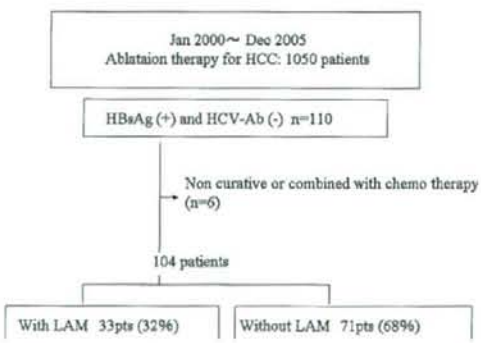

Fig. 1 Patient enrollment flow

Lamivudine treatment

The decision to prescribe LAM, which became available in Japan from November 2000, after RFA treatment was at the discretion of each patient and the physician in charge on discussing merits and demerits of the therapy. In particular, the possibility of emergence of LAM-resistant HBV mutants remained a major concern since no other anti-HBV agents were available at that time. This situation practically precluded studies with randomized assignment.

When indicated, LAM was given at a dose of 100 mg per day orally after obtaining written informed consent. The serum level of HBV-DNA was monitored with TMA method every month, together with biochemical tests for liver function such as alanine aminotransferase (ALT), albumin (ALB), and total bilirubin (TBIL). Recurrence of HCC was monitored with ultrasonography and computed tomography every 3–4 months, and RFA was repeated when necessary. Blood tests and imagings were also applied to those patients who did not choose to receive LAM.

Effects of lamivudine

Although the indication of LAM was not based on rigid criteria, certain factors concerning liver function and HCC status were likely to have affected the decision. Retrospectively, we calculated the propensity score, or "probability," of receiving LAM for each patient by using unconditional logistic regression. Then, a matched control was selected from the patients who did not receive LAM for each patient who did, by using the propensity score thus derived as the matching factor.

Changes in liver function indices were compared in three distinct manners. First, sequential changes in the average values were compared among overall patients. Second, subset analysis was performed on the basis of the initial liver function classified by Child-Pugh score. Then, pair-wise comparison was performed between each patient in the LAM group and a matched control selected as described in the following section.

Statistic procedures

The propensity score for LAM administration was calculated by using a logistic regression model with LAM

administration as the dependent variable and sex, age, liver function (represented by Child-Pugh score), and HCC stage as the independent variables. Since all patients who subsequently received LAM were positive for serum HBV-DNA before administration, controls were included only when they were also positive. A control was selected for each patient who received LAM by using the propensity score as the matching variable, the maximum distance of which was set at 0.1, with an SAS macro, gmatch (http://www.mayoresearch.mayo.edu). The changes in serum ALT, ALB, and TBIL levels in the first year were compared between the cases and controls with Wilcoxon signed rank test. Survival rates were calculated with the Kaplan–Meier method and assessed with the log-rank test. P values less than 0.05 were regarded as statistically significant. All analyses were performed with SAS software for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Patients

Of the 104 patients enrolled, 33 patients received LAM after ablation therapy. Serum HBV-DNA was positive in all these patients before LAM administration. In the remaining 71 patients, serum HBV-DNA was positive in 45 and was below the detection threshold in the remaining 26 patients. The propensity score for LAM administration was calculated among 78 patients positive for HBV-DNA: 33 who received LAM and 45 who did not. In logistic regression, patients in Child-Pugh classes B and C were more likely to have received LAM than those in class A, although the difference was not statistically significant (odds ratio: 2.04, $P = 0.2030$). Other factors, namely, sex, age, and tumor stage, were not significant either (Table 1). However, by using the estimated coefficients for those four independent variables, the probability, or propensity score, of each patient receiving LAM could be calculated. The propensity score showed a concordance index of 0.652 for the prediction of LAM administration. On the basis of the propensity score, one matched control was selected for each patient who received LAM, allowing the maximum difference of 0.1 in the score. Thus, 28 one-to-one matched pairs were created.

Antiviral efficacy of lamivudine

Serum HBV-DNA became negative by TMA assay in 1–9 months of administration in 24 patients (73%) who received LAM. Among those patients, serum HBV-DNA became detectable again in one (10%), six (35%), and seven (58%) patients at 1, 2, and 3 years, respectively. Serum HBV-DNA remained positive but decreased within the detection range in the remaining 9 patients. ALT level was decreased into the normal range in six patients and remained above the normal range in three patients. Reactivated hepatitis, defined as redetection of HBV-DNA and elevation of ALT level higher than 2× the upper normal limit, was seen in four. Two of them received adefovir treatment, which was effective in achieving negative HBV-DNA and normal ALT. The other two died of recurrent HCC before administration of adefovir. Serum ALT levels were normalized in a total of 25 patients (76%): serum HBV-DNA was negative in 19 and positive in 6. Serum ALT remained abnormal in the remaining 8 patients, and 5 of them were negative for serum HBV-DNA. No adverse effects attributable to LAM were recorded.

Changes in liver function

Changes in overall liver function indices among the 104 patients are shown in Fig. 2A–C, where serum ALT, ALB, and TBIL levels during 4 years were compared between the LAM group and the nontreatment group. Although the baseline levels of ALT and bilirubin were significantly higher, and that of ALB significantly lower, in the LAM group the difference lost significance after 1 year.

Because of the different baseline characteristics between the two groups (Table 1), we also conducted subset analysis including only those patients positive for baseline HBV-DNA and stratified them on the basis of baseline liver function (Child-Pugh A vs. Child-Pugh B). The changes in the levels of ALT, ALB, and TBIL in the first year were compared between the LAM group and the nontreatment group in an unpaired manner with Mann–Whitney U test. The Child-Pugh A subset included 20 patients in the LAM group and 32 patients in the nontreatment group. The differences between the two groups in the first-year change in ALT, ALB, and TBIL were significant ($P = 0.0014$, 0.0036, and 0.0092, respectively) (Table 2). In the Child-Pugh B subset (12 in the LAM group and 11 in the nontreatment group), similar trends were observed but none were statistically significant (Table 2).

In the above subset analysis based on liver function, similarity of factors other than liver function was not guaranteed in each subset, and there was a possibility of systemic bias. Thus, we sought to confirm the results by paired statistics, selecting a matched control for each patient in the LAM group on the basis of the propensity score. A total of 28 pairs were provided, and the differences in the first-year changes in ALT, ALB, and TBIL levels were compared with Wilcoxon signed rank test. The

Table 1 Baseline characteristics at HCC treatment

n	LAM(+) 33	LAM(–) 71	*P* value
Age[a]	57 ± 6	59 ± 10	0.3306[c]
Sex (male/female)	23:10	55:16	0.3934[d]
Follow-up duration (months)[a]	33 ± 20	47 ± 22	
HBcAg (+)	8 (24%)	11 (15%)	0.2870[d]
HBV-DNA ≥3.7 LGE/ml	33 (100%)	45 (63%)	<0.0001[d]
ALT (IU/l)[b]	54 (19–273)	36 (12–159)	<0.0001[e]
Albumin (g/dl)[a]	3.4 ± 0.6	3.8 ± 0.4	0.0003[c]
Bilirubin (mg/dl)[a]	1.5 ± 0.7	0.9 ± 0.4	<0.0001[c]
Child class A/B+C	20/13	51/20	0.2565[d]
Number of tumor[a]	1 (1–4)	1 (1–4)	0.8834[e]
Size of largest tumor (mm)	26 ± 9	28 ± 13	0.2981[c]
T stage 1+2/3+4	19/14	52/19	0.1123[d]
AFP (mg/dl)[b]	16 (2–16709)	12 (1–4391)	0.7481[e]

Data are shown as [a] mean ± SD or [b] median (range)
P values by [c] Student's *t* test, [d] χ^2 test, or [e] Mann–Whitney *U* test

difference in changes in each index was statistically significant between the two groups, showing a tendency toward improvement in the LAM group (Fig. 3). Five cases in the LAM group were not matched and excluded from this subanalysis. Changes in the first year in ALT, ALB, and TBIL among those cases were −18.2 + 60.7 IU/l, −0.10 + 0.71 g/dl, and 0.0 + 1.07 mg/dl, respectively.

Survival

During the observation period, 8 (24.2%) of 33 patients in the LAM group and 32 (45.1%) of 71 patients in the nontreatment group died. Direct consequences of HCC were the cause of death in 7 in the former and 19 in the latter group, whereas liver failure caused death in none in the former and 7 in the latter group. Overall survival did not differ statistically between the two groups (Fig. 4). There was no difference in recurrence-free survival between the two groups. Five patients on LAM developed recurrent HCC during sustained HBV-DNA negativity (median: 9 months, range: 6–22 months).

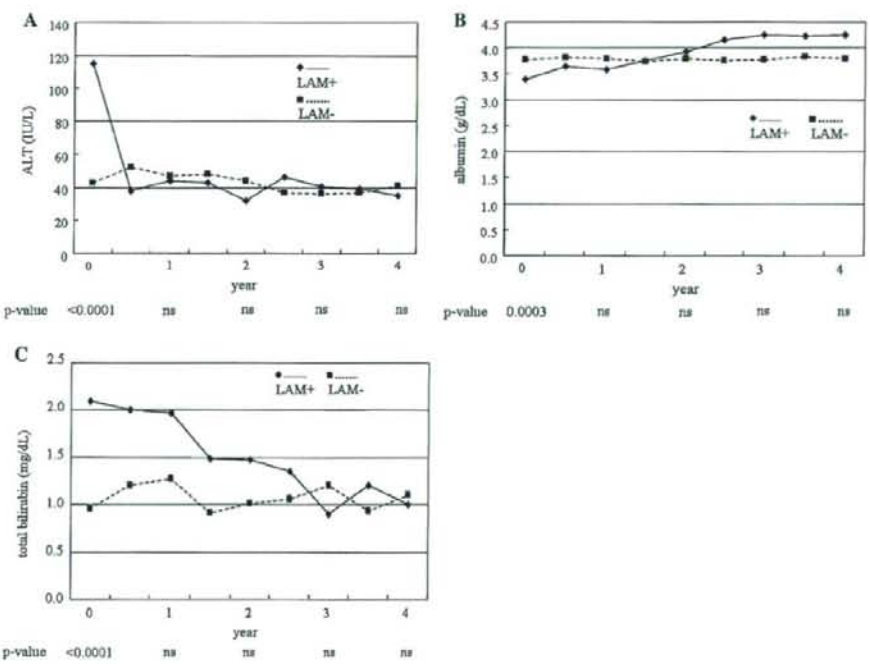

Fig. 2 Changes in liver function indices with and without lamivudine. (**A**) ALT, (**B**) ALB concentration, and (**C**) TBIL concentration. LAM group: $n = 33$, nontreatment group: $n = 71$. Abbreviation: ns, nonsignificant

Table 2 Changes in liver function indices

	Baseline	1 year	Δ	*P* value
Child-Pugh Class A LAM(+) n = 20, LAM(–) n = 32				
ALT (IU/l)				
LAM(+)	62 (52–273)	34 (11–92)	–27 (–237 to +40)	0.0014
LAM(–)	37 (12–118)	42 (10–170)	+5 (–61 to +127)	
Albumin (g/dl)				
LAM(+)	3.6 (2.7–4.8)	4.0 (3–4.7)	+0.3 (–0.7 to +1.2)	0.0036
LAM(–)	4.0 (3.0–4.6)	4.0 (2.8–4.4)	±0.0 (–0.7 to +0.7)	
Bilirubin (mg/dl)				
LAM(+)	1.2 (0.5–1.7)	0.8 (0.5–1.9)	–0.3 (–1.0 to +0.5)	0.0092
LAM(–)	0.7 (0.4–1.6)	0.9 (0.3–2.0)	+0.2 (–0.4 to +0.9)	
Child-Pugh Class B LAM(+) n = 12, LAM(–) n = 11				
ALT (IU/l)				
LAM(+)	49 (30–249)	37 (14–82)	–8.5 (–192 to +30)	0.2864
LAM(–)	46 (14–107)	49 (14–103)	+3 (–37 to +22)	
Albumin (g/dl)				
LAM(+)	3.0 (2.6–4.0)	3.2 (2.4–4.1)	+0.1 (–0.9 to +0.7)	>0.9999
LAM(–)	3.3 (2.7–3.9)	3.3 (2.4–3.8)	+0.1 (–0.3 to +0.4)	
Bilirubin (mg/dl)				
LAM(+)	2.1 (1.1–3.4)	1.7 (0.4–4.3)	–0.3 (–2 to +1.2)	0.0507
LAM(–)	1.1 (0.5–1.3)	1.2 (0.5–2.0)	+0.4 (–0.3 to +0.8)	

Data shown as median (range)
Δ, changes in the first year. *P* values by Mann–Whitney *U* test

Discussion

Today, not only LAM but also other new drugs, such as adefovir and entecavir, are vigorously used for chronic hepatitis B [12–16]. LAM, the first agent of this category, was reported to decrease the incidence of HBV-related HCC [14, 17, 18]. However, the effect of LAM on the prognosis of HCC patients has not been well documented. Preservation of liver function, which may be already deteriorated, is a prerequisite for the improvement of prognosis of HCC patients. Interferon therapy to HCV-related HCC patients after cancer treatment seems effective in improving survival [19, 20]. Similar strategies may be applied to HBV-related HCC. We have shown that LAM in HCC patients treated with RFA resulted in significant decreases in serum ALT and bilirubin levels and increases in ALB level. No particular adverse effects were noted, and the emergence of resistant virus was well controlled. The improvement in liver function may be beneficial not only for preventing liver failure but also for broadening treatment options for recurrent HCC.

In the current retrospective study, the indication for LAM administration was not decided systematically except that all patients given LAM were initially HBV-DNA positive. No single factor was a significant determinant of LAM administration, as revealed by logistic regression. Nevertheless, the decision was not completely arbitrary since the determinant used in propensity score adequately predicted LAM administration. In this setting, we performed pair-wise analysis and found significant improvement in liver function with LAM treatment.

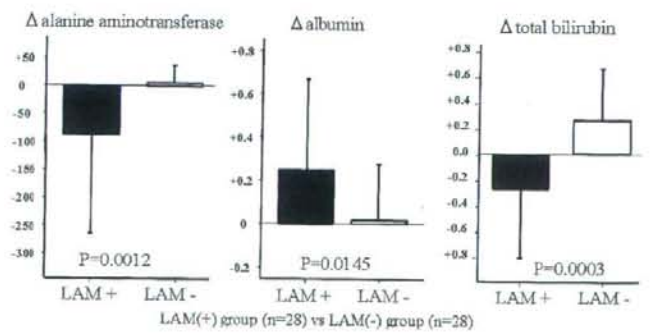

Fig. 3 Changes in liver function indices in paired comparison. The first-year changes in ALT level, ALB concentration, and TBIL concentration (shown as ΔALT, ΔALB, and ΔTBIL, respectively) were compared between each patient in LAM group and corresponding paired control matched by propensity score (total 28 pairs). *P* values were determined by Wilcoxon signed rank test

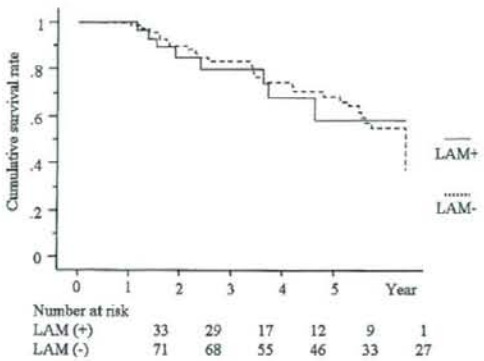

Fig. 4 Kaplan–Meier curves comparing the cumulative survival rates in LAM-treated and control group. Statistical analysis was done with log-rank test

We observed no significant differences in the overall and recurrence-free survival between LAM and nontreatment groups. The baseline liver function was poorer and AFP, a strong predictor of recurrence [21], was higher in the LAM group. Noninferior survival in the LAM group may suggest some beneficial effects of LAM administration. However, we did not find significant effects of LAM in multivariate analysis, possibly due to the small number of events (40 deaths). To conclude the efficacy of LAM treatment on recurrence-free or overall survival, either much larger cohort studies or well-designed randomized control trials will be required. The current study demonstrated the safety of LAM administration in HCC patients after HCC treatment, including the data on LAM resistance similar to a previous report [22].

In conclusion, LAM treatment after ablation therapy for HBV-related HCC improved liver function without any particular untoward effects. Its effects on survival remain to be studied in future studies.

References

1. Shiratori Y, Shiina S, Imamura M, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C-viral infection in Japan. Hepatology 1995;22:1027–33
2. Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B, C: a prospective study of 251 patients. Hepatology 1995;21:650–5
3. Ikeda K, Saitoh S, Suzuki Y, et al. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. J Hepatol 1998;28:930–8
4. Tateishi R, Shiina S, Teratani T, et al. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer 2005;103:1201–9
5. Rossi S, Di Stasi M, Buscarini E, et al. Percutaneous radiofrequency interstitial thermal ablation in the treatment of small hepatocellular carcinoma. Cancer J Sci Am 1995;1:73
6. Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS. Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. Radiology 1999;210:655–61
7. Shiina S, Teratani T, Obi S, Hamamura K, Koike Y, Omata M. Nonsurgical treatment of hepatocellular carcinoma: from percutaneous ethanol injection therapy and percutaneous microwave coagulation therapy to radiofrequency ablation. Oncology 2002;62 Suppl 1:64–8
8. Lencioni RA, Allgaier HP, Cioni D, et al. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. Radiology 2003;228:235–40
9. Shiina S, Teratani T, Obi S, et al. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology 2005;129:122–30
10. Omata M, Tateishi R, Yoshida H, Shiina S. Treatment of hepatocellular carcinoma by percutaneous tumor ablation methods: ethanol injection therapy and radiofrequency ablation. Gastroenterology 2004;127:S159–66
11. Omata M. Treatment of chronic hepatitis B infection. N Engl J Med 1998;339:114–5
12. Villeneuve JP, Condreay LD, Willems B, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. Hepatology 2000;31:207–10
13. Yao FY, Terrault NA, Freise C, Maslow L, Bass NM. Lamivudine treatment is beneficial in patients with severely decompensated cirrhosis and actively replicating hepatitis B infection awaiting liver transplantation: a comparative study using a matched, untreated cohort. Hepatology 2001;34:411–6
14. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521–31
15. Marcellin P, Chang TT, Lim SG, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348:808–16
16. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med 2003;348:800–7
17. Yoshida H, Shiratori Y, Moriyama M, et al., for the IHIT (Inhibition of Hepatocarcinogenesis by Interferon Therapy) Study Group. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. Ann Intern Med 1999;131:174–81
18. Nishiguchi S, Kuroki T, Nakatani S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;346:1051–55
19. Shiratori Y, Shiina S, Teratani T, et al. Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. Ann Intern Med 2003;138:299–306
20. Kubo S, Nishiguchi S, Hirohashi K, et al. Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. Ann Intern Med 2001;134:963–7
21. Tateishi R, Shiina S, Yoshida H, et al. Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. Hepatology 2006;44:1518–27
22. Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527–32

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ORIGINAL ARTICLE

Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review

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Abstract *Background and aims* The role of alphafetoprotein (AFP) in the diagnosis and surveillance of hepatocellular carcinoma (HCC) is getting smaller owing to the advances in imaging modalities. The aims of this study were to assess the diagnostic accuracy of tumor markers in small HCC and to find the optimal cutoff value of each tumor marker for efficient surveillance. *Methods* Studies in all languages were identified by searching MEDLINE from 1982 to 2002. Studies were included when they showed sensitivity and specificity for HCCs 5 cm or smaller and recruited only patients with chronic hepatitis or liver cirrhosis as control. We assessed diagnostic odds ratios (DORs) for the evaluation of diagnostic accuracy of tumor markers and positive likelihood ratios (LRs+) to find the optimal cutoff value. DORs and LRs+ were combined according to the random effect model. The summary receiver operating characteristics (ROC) curve was also assessed. *Results* Seventeen articles on three tumor markers—AFP, des-gamma-carboxyprothrombin (DCP), and *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3)—were enrolled after full-text evaluation. AFP was inferior to DCP and AFP-L3 in both DOR (4.50 vs. 8.16 and 10.50) and area under the ROC curve (0.647 vs. 0.688 and 0.695). Optimal cutoff values that provide the best LR+ were 200 ng/ml for AFP, 40 mAU/ml for DCP, and 15% for AFP-L3. *Conclusions* Diagnostic accuracy of AFP in small HCC was substantially limited. Surveillance including other tumor markers with optimal cutoff value should be conducted to confirm the efficacy of the policy.

Keywords Hepatocellular carcinoma · Alphafetoprotein · Des-gamma-carboxyprothrombin · *Lens culinaris* agglutinin-reactive fraction of alphafetoprotein · Metaanalysis

Abbreviations

AFP	Alphafetoprotein
AFP-L3	*Lens culinaris* agglutinin-reactive fraction of AFP
AUC	Area under the curve
CI	Confidence interval
DCP	Des-gamma-carboxyprothrombin
DOR	Diagnostic odds ratio
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
LR+	Positive likelihood ratio
ROC	Receiver operating characteristics
SE	Standard error

Introduction

Hepatocellular carcinoma (HCC) is a common worldwide malignancy, with the United States showing an increasing incidence rate [1, 2]. Approximately, 75–80% of primary liver cancers are attributable to persistent viral infections

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with either hepatitis B virus (HBV) or hepatitis C virus (HCV) [3–6], and the annual incidence of HCC with cirrhosis is 1–7% [7–11].

In the ordinary diagnostic process of HCC, a space-occupying lesion in the liver is first detected by imaging modalities such as ultrasonography and then confirmed by dynamic CT or MRI with contrast media. Typical HCC shows hypervascularity in the arterial phase and washout of contrast media in the portal-venous phase [12, 13]. The final diagnosis was made pathologically when a patient receives percutaneous biopsy, hepatic resection, or liver transplantation.

Alphafetoprotein (AFP) has served as a diagnostic test for HCC since the 1970s, when most patients with HCC were diagnosed at an advanced stage with clinical symptoms [14]. Concentrations higher than 500 ng/ml can be confirmatory in that situation. Nowadays quite a few small HCCs (e.g., 3 cm or smaller) can be detected owing to advances in imaging modalities, and it is known that significant numbers of small HCCs do not secrete a diagnostic level of AFP [15]. Furthermore, AFP levels are elevated both in patients with HCC and in those with chronic liver diseases, and there is a wide overlap between the two groups [16, 17]. Thus, the role of AFP as a diagnostic test is getting smaller.

Another use of AFP is in detecting asymptomatic HCC via periodic screening for high-risk patients. High positive predictive value with an appropriate cutoff value is mandatory for an effective surveillance program.

To date, many tumor markers have been proposed as a complement or substitute for AFP in the diagnosis of HCC, such as des-gamma-carboxyprothrombin (DCP) [18, 19], *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) [20, 21], and various cytokines [22–24]. The aim of this study is to compare the diagnostic accuracy of AFP in small HCC with other biomarkers and to find the optimal cutoff value of each tumor marker for efficient surveillance.

Methods

We conducted this study as part of a project to establish evidence-based guidelines for the diagnosis and treatment of HCC. This project is supported by the Ministry of Health, Labour and Welfare of Japan.

Study search protocol

First, we settled upon two research questions: (1) How accurate is each tumor marker at diagnosing small HCC? (2) What is the optimal cutoff value of each tumor marker for an effective screening strategy?

To identify relevant articles, we searched MEDLINE (from January 1982 to December 2002), using *liver neoplasms* and *tumor markers* as Medical Subject Headings terms (Appendix 1). Subsequently, we reran searches using more specific terms (Appendix 2). A search algorithm was constructed by experts at the International Medical Information Center (Shinanomachi, Shinjuku, Japan). We supplemented these sources by searching the Cochrane Collaboration Library and hand-searching bibliographies of systematic reviews and relevant original articles. Bibliographies were downloaded into a specially designed database made by FileMaker Ver. 5 (FileMaker, Inc., Santa Clara, CA, USA) for the project.

Inclusion criteria

Studies were included when they met all the following criteria: (1) the sensitivity and specificity were described or could be calculated from tables or figures, (2) the maximum size of nodules was 5 cm or smaller, or sensitivity of nodules smaller than 5 cm could be calculated, and (3) only patients with chronic hepatitis or cirrhosis were recruited as control. We then selected tumor markers for which at least three articles were available. We excluded any meeting abstracts not accompanied by full articles, and other incomplete reports.

Two authors independently reviewed the article titles and abstracts identified by the search, evaluated each study for inclusion, and retrieved potentially eligible articles for full-text evaluation. Any discrepancies were settled by a third author.

Data extraction

Two authors independently extracted data from each eligible article. A 2×2 table was reconstructed for every tumor marker in the article. When tables could be reconstructed for 2 or more upper limits of tumor size (e.g., 3 and 5 cm), we adopted the larger size up to 5 cm. When articles provided 2 or more tables for 2 or more cutoff values (e.g., 20 and 100 ng/ml for AFP), all available tables were reconstructed separately.

Potential confounders and quality assessment

We considered eight variables to be potential confounders for explaining heterogeneity and interstudy variability: (1) proportion of chronic hepatitis and cirrhosis patients; (2) proportion of patients with hepatitis B and C; (3) year of publication; (4) study design: cohort or case–control study;

(5) proportion of histologically proven HCC patients; (6) blinding: whether final diagnosis of HCC was performed independently from the test result; (7) consecutive recruitment of patients; and (8) existence of verification bias: whether only patients with positive test results received the reference standard. The latter four variables were introduced to assess the quality of articles according to the guidelines of Irwig et al. [25]. All these variables were defined a priori; two authors independently evaluated the articles and a third author independently settled discrepancies.

All data were input to a standardized form for assessing the characteristics of enrolled articles.

Statistical analysis

Diagnostic odds ratio

To answer the first research question (i.e., how is the accuracy of each tumor marker at diagnosing small HCC), we assessed the diagnostic odds ratio (DOR) of each tumor marker that represents the comprehensive ability of a diagnostic test according to the following formula. Since there is an inverse association between sensitivity and specificity that differs according to test thresholds, it is inappropriate to estimate their means separately [26].

$$\mathrm{DOR} = \frac{\text{sensitivity}}{1-\text{specificity}} \Big/ \frac{1-\text{sensitivity}}{\text{specificity}}$$

Assessment for potential confounders

To examine the factors associated with variation in the DOR, a regression model was formulated and tested. We applied linear regression analysis with log DOR as a dependent variable and the previously described eight factors as independent variables. The P value threshold for statistical significance was set at 0.05.

Summary receiver operating characteristic curve

In addition, we applied another approach to combine the results of primary studies, that is, to draw a summary receiver operating characteristic (SROC) curve according to Moses et al. [27]. This model hypothesizes that there is a linear relationship between

$$D = \log\left(\frac{\text{sensitivity}}{1-\text{sensitivity}}\right) - \log\left(\frac{1-\text{specificity}}{\text{specificity}}\right) = \log \mathrm{DOR}$$

and

$$S = \log\left(\frac{\text{sensitivity}}{1-\text{sensitivity}}\right) + \log\left(\frac{1-\text{specificity}}{\text{specificity}}\right).$$

We applied weighted linear regression analysis for each tumor marker and drew SROC curves. We also calculated the area under the curve (AUC) and its standard error (SE) according to the method of Walter [28].

Assessment for publication bias

To assess the presence of publication bias, we created funnel plots for each diagnostic test. We plotted the inverse of the standard error of the natural logarithm of the DOR against the natural logarithm of the DOR. Its asymmetry was tested by significance test using the linear regression method suggested by Egger et al. [29]. In the regression, the standardized effect, defined as the effect divided by its standard error, is regressed against the precision of the effect, defined as the inverse of the standard error:

$$\frac{\log \mathrm{DOR}}{\mathrm{SE}} = \alpha + \beta \frac{1}{\mathrm{SE}}$$

The intercept α provides a quantitative measure of the asymmetry and is of major interest. The more the intercept deviates from zero, the more pronounced the asymmetry. Negative values of α will indicate that less precise studies have a more pronounced effect than more precise studies, suggesting publication bias. On the other hand, positive intercept does not suggest selection bias rather than heterogeneity of included studies. A P value less than 0.1 of zero intercept is considered statistically significant.

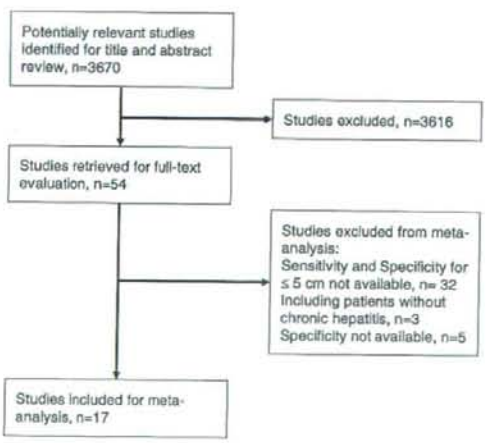


Fig. 1 Study inclusion flow diagram

Table 1 Characteristics of included studies

Author (reference)	Diagnostic test	Study design	Country	Patients with HCC				Control		
				n	Etiology	Characteristics of HCC	Modalities of diagnosis	*n*	Etiology	Characteristics of patients
Cottone et al. [68]	AFP (RIA)	Co	Italy	30	7% with HBV, 73% with HCV	100% ≤5 cm	100% by pathology	147	12% with HBV, 71% with HCV	100% with LC
Fujiyama et al. [69]	AFP (RIA), DCP (EIA)	CC	Japan	120	NR with HBV and HCV	34% ≤5 cm, 10% ≤2 cm	NR	334	NR with HBV and HCV	41% with LC, 59% with CH
Ikoma et al. [70]	AFP (EIA), DCP (EIA)	CC	Japan	63	14% with HBV, 70% with HCV	56% ≤3 cm, 20% ≤2 cm	33% by pathology, 67% by imaging	188	22% with HBV, 84% with HCV	24% with LC, 76% with CH
Kasahara et al. [71]	AFP (EIA), DCP (EIA)	CC	Japan	112	NR with HBV and HCV	15% ≤5 cm	100% by imaging	403	NR with HBV and HCV	28% with LC, 72% with CH
Maringhin et al. [72]	AFP (RIA)	Co	Italy	146	80% with HBV	38% ≤5 cm	50% by pathology	217	65% with HBV	100% with LC
Mita et al. [73]	AFP (EIA), DCP (sEIA), AFP-L3 (EP)	CC	Japan	91	17% with HBV, 70% with HCV	47% ≤2 cm	100% by pathology or imaging[e]	57	32% with HBV, 40% with HCV	100% with LC
Nomura et al. [74]	AFP (EIA), DCP (EIA)	CC	Japan	27	7% with HBV, 89% with HCV	100% ≤3 cm	100% by pathology	101	NR with HBV and HCV	68% with LC, 32% with CH
Nomura et al. [75]	AFP (EIA), DCP (EIA, ECLIA, IRMA), AFP-L3 (EP)	CC	Japan	36	NR with HBV and HCV	100% ≤3 cm, 53% ≤2 cm	100% by pathology	49	NR with HBV and HCV	100% with LC
Oka et al. [76]	AFP (various[b]), AFP-L3 (EP)	CC	Japan	388	9% with HBV, 78% with HCV	32% ≤2 cm	26% by pathology, 74% by imaging	212	100% with HBV or HCV	100% with CH or LC
Saitoh et al. [78]	AFP (RIA), DCP (ABC)	CC	Japan	115	17% with HBV, 80% with HCV	100% ≤2 cm	72% by pathology, 28% by imaging		NR with HBV and HCV	45% with LC, 55% with CH
Sassa et al. [77]	AFP (RIA), DCP (EIA), AFP-L3 (EP)	CC	Japan	61	15% with HBV, 84% with HCV	100% ≤2 cm	92% by pathology	134	17% with HBV, 79% with HCV	56% with LC, 44% with CH
Shimauchi et al. [79][b]	AFP (RIA), DCP (sEIA), AFP-L3 (EP)	Co	Japan	21	14% with HBV, 76% with HCV	95% ≤3 cm, 67% ≤2 cm	100% by pathology	57	14% with HBV, 79% with HCV	100% with LC
Shiraki et al. [80]	AFP (RIA), DCP (EIA), AFP-L3 (EP)	Co	Japan	51	20% with HBV, 80% with HCV	80% ≤3 cm, 57% ≤2 cm	88% by pathology, 12% by imaging	21	24% with HBV, 76% with HCV	100% with LC
Suehiro et al. [81]	AFP (NR), DCP (EIA)	CC	Japan	185	NR with HBV and HCV	39% ≤6 cm, 21% ≤3 cm	49% by pathology[f]	85	NR with HBV and HCV	100% with LC
Taketa et al. [82][c]	AFP (RIA/EIA), AFP-L3 (EP)	CC	Japan	167	21% with HBV, 50% with HCV	87% ≤5 cm, 68% ≤3 cm	51% by pathology, 49% by imaging	181	33% with HBV, 44% with HCV	69% with LC, 31% with CH

Table 1 continued

Author (reference)	Diagnostic test	Study design	Country	Patients with HCC			Control		
				n	Etiology	Characteristics of HCC	Modalities of diagnosis	*n*	Etiology	Characteristics of patients
Tanabe et al. [83][d]	AFP (RIA), DCP (EIA)	CC	Japan	571	NR with HBV and HCV	32% ≤5 cm, 8% ≤2 cm	100% by pathology or imaging	28	NR with HBV and HCV	100% with LC
Tsai et al. [84]	AFP (RIA), DCP (EIA, SC)	CC	Taiwan	39[d]	62% with HBV, NR with HCV	100% ≤5 cm, 54% ≤2 cm	100% by pathology or imaging	54	NR with HBV and HCV	52% with LC, 48% with CH

[a] According to each hospital's available kit

[b] Specificity for AFP was not reported

[c] Sensitivity was only reported on AFP-L3

[d] DCP was reported in only 31 patients with HCC

[e] Exact percentage was not reported

[f] 100% pathologically proven for HCCs smaller than 3 cm in diameter

Abbreviations: ABC, avidin–biotin complex method; AFP, alphafetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; CC, case–control study; CH, chronic hepatitis; Co, cohort study; DCP, des-gamma-carboxyprothrombin; ECLIA, electrochemiluminescence assay; EIA, enzyme immunoassay; sEIA, sensitive enzyme immunoassay; EP, electrophoresis; HBV, hepatitis B virus; HCV, hepatitis C virus; LC, liver cirrhosis; NR, not reported; RIA, radioimmunoassay

Positive likelihood ratio

To answer the second question (i.e., what is the optimal cutoff value), we assessed positive likelihood ratio (LR+) on the assumption that a positive result may lead to a confirming test (e.g., dynamic CT). LR+, which is always discussed with negative likelihood ratios, was calculated on the following formula and express how much more frequent the positive results is among subjects with disease than among subjects without disease, independent from pretest probability or disease prevalence.

$$\mathrm{LR+} = \frac{\text{sensitivity}}{1 - \text{specificity}}$$

LRs+ above 10 are considered to provide a strong evidence to rule in diagnoses respectively in most circumstances [30]. Positive predictive values (= posttest probabilities) can be calculated on the following formula.

$$\text{Post test odds} = \text{pretest odds} \times \mathrm{LR+}$$

$$\text{Post test probability} = \frac{\text{posttestodds}}{\text{posttest odds} + 1}$$

When the sensitivity or specificity equaled zero, we added 0.5 to each cell of the original 2 × 2 table to avoid dividing by zero. We calculated the pooled DORs and LRs and their 95% confidence intervals (CIs) for random effect models on the basis of DerSimonian and Laird [31]. We assessed the heterogeneity of the odds ratio by a homogeneity chi-square test.

We used MetaDiSc statistical software [32] and Comprehensive Meta Analysis Ver. 1.0 (Biostat, Englewood, NJ, USA) for the analysis of LRs and DOR, and S-PLUS 2000 (MathSoft Inc., Seattle, WA, USA) for regression analysis and drawing SROC curves.

Role of the funding sources

The funding sources had no role in the choice of topic; the collection, analysis, or interpretation of the data; or the decision to submit the manuscript for publication.

Results

The primary MEDLINE search identified 2,685 articles potentially relevant to the topic. After reviewing titles and abstracts of these articles, the following three tumor markers were found in at least three articles that provided sensitivity and specificity: AFP, DCP, and AFP-L3. We ran another MEDLINE search using more specific terms (Appendix 2) and identified 985 additional articles to be reviewed.

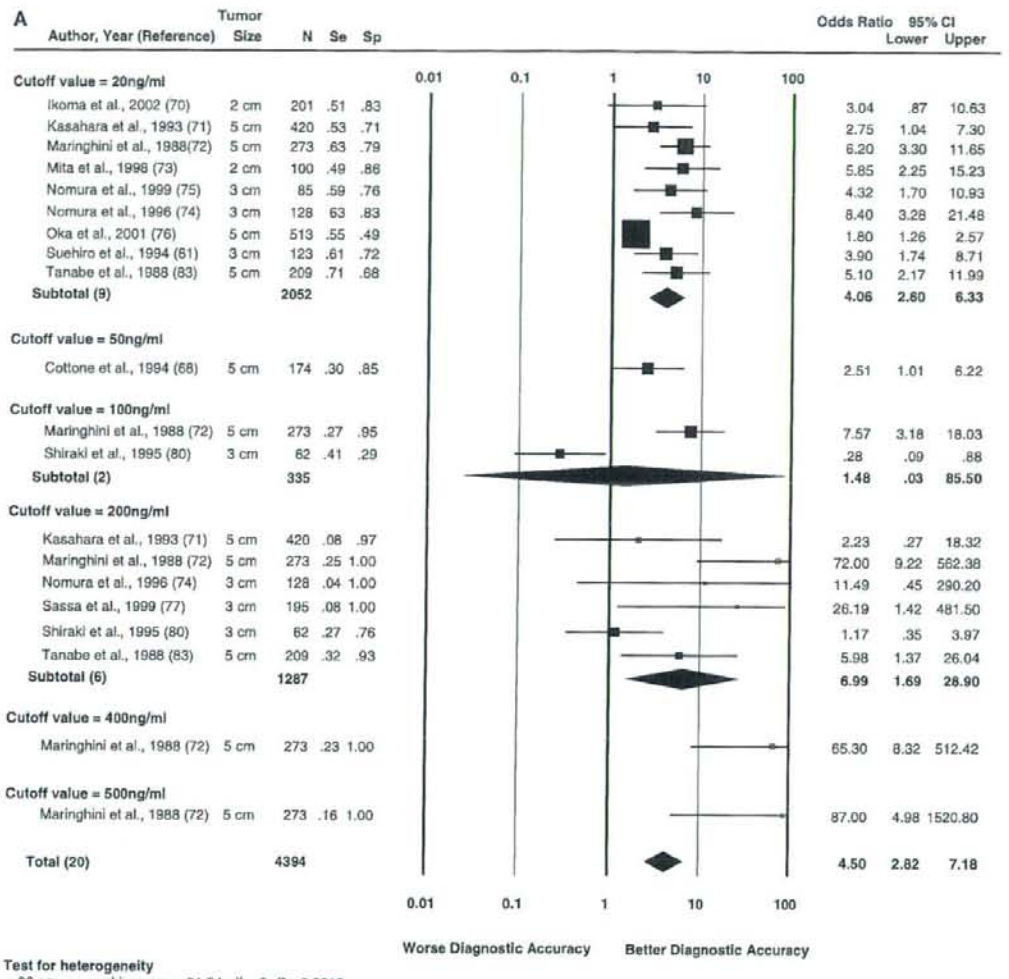
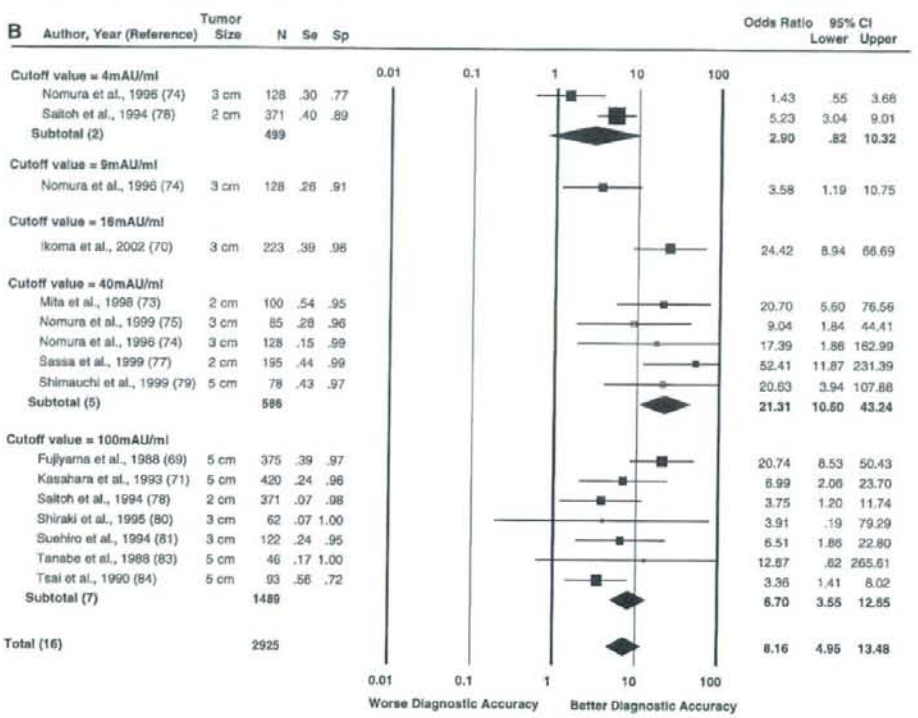


Fig. 2 Forest plots of DOR of AFP (**a**), DCP (**b**), and AFP-L3 (**c**). Diagnostic odds ratios were combined using cutoff value as stratification factor. AFP, alphafetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; DCP, des-gamma-carboxyprothrombin

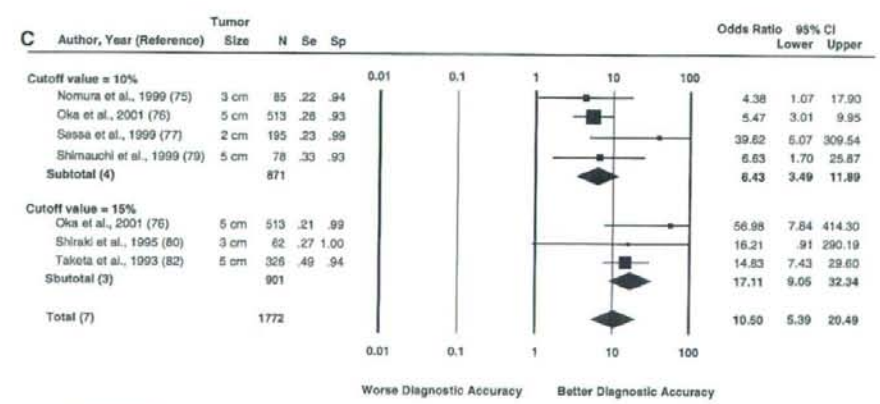
We also identified two systematic reviews on detecting HCC in patients with chronic hepatitis C [33, 34] and checked their bibliographies.

After reviewing titles and abstracts, 54 articles were retrieved for full-text evaluation. Thirty-seven of them were excluded: 32 did not provide sensitivity for tumors less than 5 cm in diameter [15, 19, 21, 22, 35–62], 3 recruited patients without chronic hepatitis [46, 52, 63], and 5 articles did not provide specificity [56, 64–67]. Finally, 17 articles were included in our study (Fig. 1, Table 1) [68–84]. Eleven studies included data on more than one tumor marker [70, 71, 73–77, 79–81, 83], and 4 of them provided sensitivity and specificity of a combination of two tumor markers [70, 74, 77, 79]. As 8 studies provided sensitivity and specificity with more than one cutoff value [68, 70, 71, 74, 76, 80, 83, 85], we could evaluate 50 DORs in total.



Test for heterogeneity

4 mAU/ml chi-square = 5.44; df = 1; $P = 0.20$
40 mAU/ml chi-square = 2.58; df = 4; $P = 0.63$
100 mAU/ml chi-square = 10.14; df = 6; $P = 0.12$
combined chi-square = 41.29; df = 15; $P = 0.0003$



Test for heterogeneity

10% chi-square = 3.59; df = 3; $P = 0.31$
15% chi-square = 1.98; df = 2; $P = 0.37$
combined chi-square = 12.1; df = 6; $P = 0.061$

Fig. 2 continued

Table 2 Diagnostic odds ratio of the combination of two diagnostic tests

Author (reference)	Test	Cutoff value	Sensitivity	Specificity	DOR (95% CI)
Ikoma et al. [70]	AFP + DCP	20 ng/ml, 16 mAU/ml	0.83	0.84	25.46 (9.73–66.6)
Sassa et al. [77]	AFP + DCP	200 ng/ml, 40 mAU/ml	0.48	0.99	59.81 (13.56–263.8)
Nomura et al. [74]	AFP-L3 + DCP	10%, 40 mAU/ml	0.42	0.90	6.29 (2.02–19.6)
Shimauchi et al. [79]	AFP-L3 + DCP	10%, 40 mAU/ml	0.67	0.90	17.0 (4.92–58.8)
Sassa et al. [77]	AFP + AFP-L3	200 ng/ml, 10%	0.25	0.99	43.4 (5.57–337.5)

Abbreviations: AFP, alphafetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; DCP, des-gamma-carboxyprothrombin

Sensitivity, specificity, and DOR

Diagnostic odds ratios calculated for each tumor marker were stratified by cutoff values and combined (Fig. 2). Combined odds ratios (95% CI) for AFP, DCP, and AFP-L3 according to the random effects model were 4.50 (2.82–7.18), 8.16 (4.95–13.48), and 10.50 (5.39–20.49), respectively. There was significant heterogeneity among studies in AFP and DCP. The DORs of the combinations of two markers are shown in Table 2.

Assessment for potential confounders

Table 3 shows the results of regression analysis for potential confounders. We excluded blinding in the reference standard from the analysis because none of the included studies provided sufficient information regarding blinding. A significant decrease in the odds ratio of DCP (slope = −0.551, $P = 0.02$) was also observed when all patients had pathologically proven HCC. The diagnostic accuracy of AFP significantly deteriorates when the majority of patients were HCV-positive (slope = −1.038, $P = 0.03$).

SROC analysis

Figure 3 shows the scatterplot of (1—specificity) against sensitivity in each study for the diagnostic test and SROC curves. AFP-L3 and DCP showed higher diagnostic accuracy than AFP (Table 4). We also plotted the combined sensitivity and specificity of AFP + DCP, AFP-L3 + DCP, and AFP + AFP-L3 provided in four studies [70, 74, 77, 79].

Assessment for publication bias

Funnel plots of three tumor markers showed asymmetry (Appendix Fig. A1). Intercepts and P values of AFP, DCP,

Table 3 Results of regression analysis for potential confounders

Variable	AFP		DCP		AFP-L3	
	Slope	P	Slope	P	Slope	P
Liver function[a]	0.382	0.89	−0.183	0.70	NA	NA
Etiology[b]	**−1.038**	**0.03**	NA	NA	0.044	0.94
Publication year[c]	−0.573	0.07	0.147	0.57	−0.404	0.38
Study design[d]	0.194	0.56	0.009	0.98	−0.190	0.69
Diagnosis[e]	−0.618	0.07	**−0.551**	**0.02**	−0.464	0.25
Consecutive recruitment[f]	0.160	0.63	−0.093	0.75	−0.190	0.69
Verification bias[g]	0.845	0.19	NA	NA	NA	NA

[a] 0, less than 50% of patients had liver cirrhosis; 1, more than 50% of patients had liver cirrhosis

[b] 0, less than 50% of patients were HCV-positive; 1, more than 50% of patients were HCV-positive

[c] 0, year of publication 1994 or earlier; 1, year of publication 1994 or later

[d] 0, case–control study; 1, cohort study

[e] 0, not all patients were diagnosed by pathology; 1, all patients were diagnosed by pathology

[f] 0, nonconsecutive recruitment of patients; 1, consecutive recruitment of patients

[g] 0, present; 1, absent

Values in boldface have statistical significance

Abbreviations: AFP, alphafetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; DCP, des-gamma-carboxyprothrombin; NA, not applicable because all studies were categorized into a single group

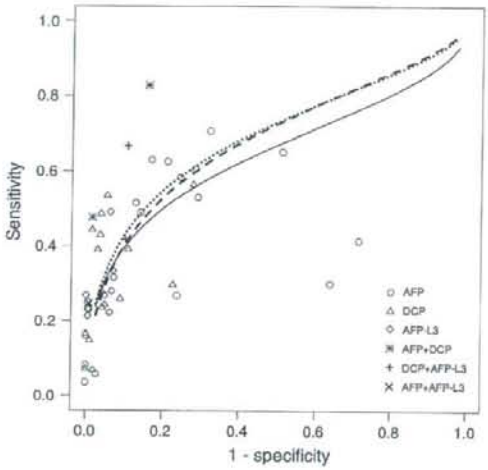


Fig. 3 SROC curves for 3 diagnostic tests for HCC. Solid line and circles, AFP; dashed line and triangles, DCP; dotted line and diamonds, AFP-L3; asterisks, AFP + DCP; pluses, DCP + AFP-L3; cross, AFP + AFP-L3. AFP, alphafetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; DCP, des-gamma-carboxyprothrombin; HCC, hepatocellular carcinoma; SROC, summary receiver operating characteristics

and AFP-L3 were 3.59 ($P = 0.0003$), 5.30 ($P = 0.0001$), and 6.78 ($P = 0.0047$), respectively. As previously described, a positive intercept does not suggest publication bias but rather heterogeneity of included studies.

Positive likelihood ratio

LRs+ of each tumor marker stratified by cutoff values were calculated and combined (Fig. 4). To assess the optimal cutoff value, we compared LRs+ with the following cutoff values since there were three or fewer studies with other cutoff values: 20 and 200 ng/ml of AFP, 40 and 100 mAU/ml of DCP, and 10% and 15% of AFP-L3. LRs+ with a cutoff value of 200 ng/ml of AFP (5.85, 95% CI: 1.49–22.93), 40 mAU/ml of DCP (12.60, 6.65–23.87), and 15% of AFP-L3 (13.10, 3.89–44.97) were better than those with, respectively, 20 ng/ml of AFP (2.45, 1.74–3.45), 100 mAU/ml of DCP (4.91, 2.43–9.91), and 10% of AFP-L3 (4.89, 2.77–8.61).

Discussion

Our literature search identified two potential candidates for substituting or complementing AFP in the diagnosis of HCC, namely, DCP and AFP-L3. DCP, also known as prothrombin induced by vitamin K absence-II (PIVKA-II), is an abnormal prothrombin protein that is present at higher levels in the serum of HCC patients. Since the report by Liebman et al. [18], DCP has been recognized as not only a highly specific marker for HCC but also a predictor of prognosis of HCC patients [86, 87]. AFP-L3 is a fucosylated variant of AFP that reacts with *Lens culinaris* agglutinin A and can differentiate an increase of AFP due to HCC from that due to benign liver disease [20, 21, 82].

Table 4 Results of SROC analyses for three diagnostic tests for HCC

Test	Intercept	Slope	AUC	SE (AUC)
AFP	0.940	−0.321	0.647	0.027
DCP	1.207	−0.230	0.688	0.083
AFP-L3	1.266	−0.262	0.695	0.166

Abbreviations: AFP, alphafetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; AUC, area under the curve; DCP, des-gamma-carboxyprothrombin; HCC, hepatocellular carcinoma; SE, standard error; SROC, summary receiver operating characteristics curve

In this study, the diagnostic accuracy of AFP was found to be inferior to that of DCP and AFP-L3 in both meta-analysis with random effects model and SROC analysis. It is apparent that the inferiority of AFP in DORs is due to its lack of specificity as compared with the other two markers. The false-positive rate of AFP with a cutoff value of 20 ng/ml was 0.14–0.32. It should be stated that the role of AFP as a diagnostic test is almost over in the era of advanced imaging technologies. Instead, the other two markers, which showed superior specificity around 0.95, will play a role as a confirmatory test.

During the literature-searching process, we identified two systematic reviews of the diagnostic accuracy of AFP [33, 34]. Both of them focused on HCV-positive patients based on the concept that the natural history of hepatitis C is different from that of hepatitis B [6, 40]. Elevated AFP has been observed more frequently in patients with chronic hepatitis B than C [46, 57], and the present results of meta-regression on the etiology of liver disease may support this finding. Diagnostic accuracy deteriorated when the majority of patients were HCV positive. In contrast, we did not encounter this phenomenon with AFP-L3.

We also assessed potential confounders other than liver disease etiology. The presence or absence of liver cirrhosis, which strongly influences the risk of HCC in HCV-positive patients, did not affect the DOR. The year of publication, which is associated with improvement in the diagnostic accuracy of the reference standard (e.g., dynamic CT), also did not show significant influence on the DOR.

Research on randomized trials and observational studies has suggested that the major reason for not publishing is that investigators do not submit studies with negative results for

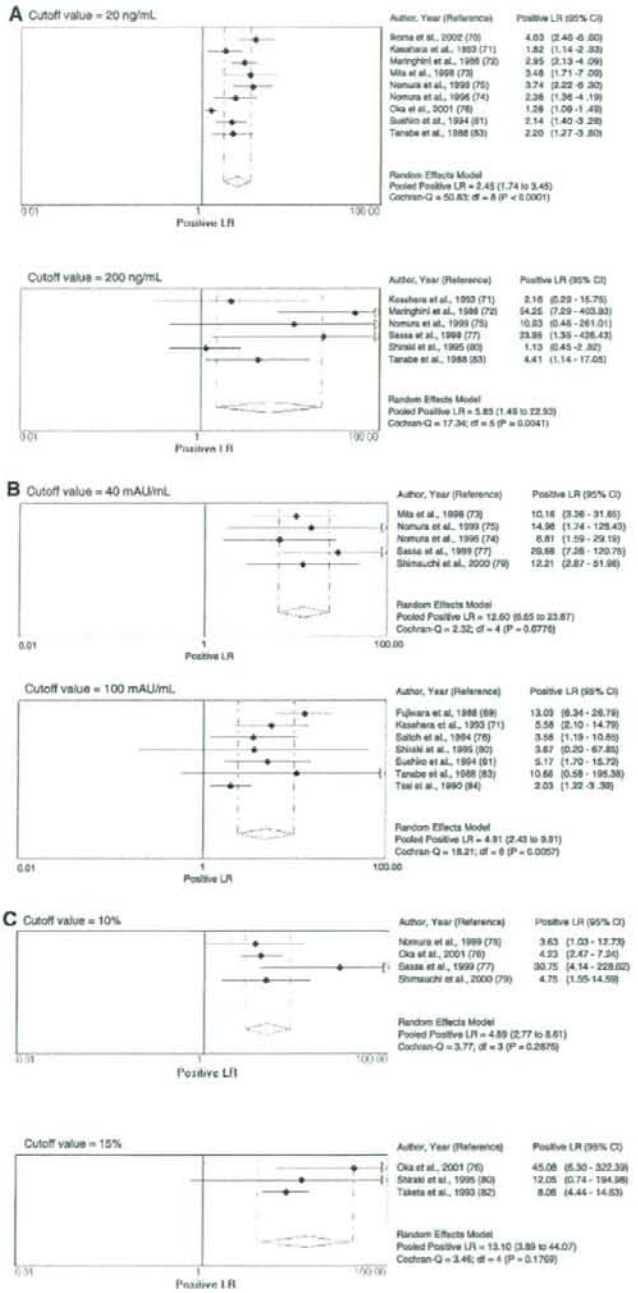


Fig. 4 Forest plots of positive likelihood ratio of AFP (**a**), DCP (**b**), and AFP-L3 (**c**). Likelihood ratios were combined on a cutoff value basis. AFP, alphafetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; DCP, des-gamma-carboxyprothrombin; LR, likelihood ratio

publication [88]. Publication bias is more serious in observational studies than in interventional studies. Most of the studies on DCP and AFP-L3 included in the current analysis assessed the diagnostic accuracy in comparison with AFP. Thus, there might also be publication bias when true results suggesting inferiority of DCP or AFP-L3 to AFP were possibly left unpublished, but funnel plot analysis could obviously not reveal the existence of such publication bias.

Study design features significantly affect the entire results of a study on diagnostic tests [89–91]. In a case–control study, to prevent the possibility of selection bias, patients should be recruited consecutively from a relevant clinical population covering the spectrum of disease that is likely to be encountered in the current or future use of the test. In surveillance as a cohort study consisting of periodical tests, verification bias exists when the decision to perform the reference test is based on the result of the test under examination. The results of regression analysis showed no significant difference between well-designed studies and others. This might be because the number of quality studies was too small.

As it is inappropriate to conduct surveillance for HCC without ultrasound in a high-risk population [15, 92], the major issue is whether to combine ultrasound and tumor markers in the surveillance. The fact that DCP and AFP-L3 showed LRs+ of larger than 10, which are interpreted as conclusive, make it quite reasonable to consider inclusion of those markers in surveillance. In fact, DCP with a cutoff value of 40 mAU and AFP-L3 with a cutoff value of 15% were adopted in the Japanese guideline for the diagnosis and treatment of HCC [93]. The higher diagnostic accuracy shown in the combination of two tumor markers, rather than by AFP alone, suggests that inclusion of two or three markers may improve the efficiency of surveillance programs.

The aim of this study is not to directly assess the effectiveness of surveillance programs for HCC but to find potential candidates to be included in the surveillance. The effectiveness of surveillance depends on various factors—disease prevalence, identification of high-risk populations, interval of diagnostic tests, type of confirmation tests, and treatment modality when disease is confirmed—and should also finally be assessed in terms of cost-effectiveness. Inclusion of new diagnostic tests in surveillance programs may make it possible to detect additional small HCCs. However, it should be verified whether improvement of effectiveness is always justified by increased cost. In conclusion, surveillance of HCC in high-risk populations that includes DCP and/or AFP-L3 should be conducted and assessed on the basis of acceptable cost-effectiveness.

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Appendix

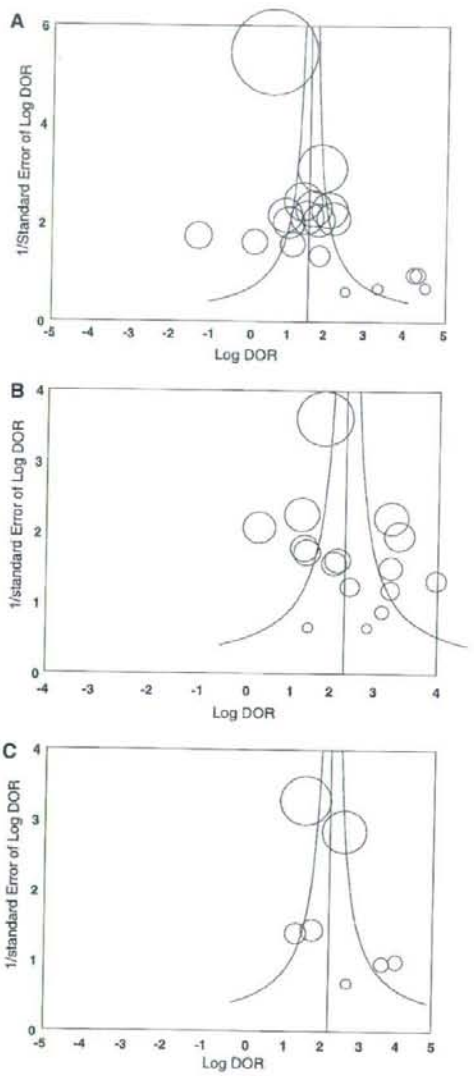

Fig. A1 Funnel plots of DOR of AFP (**a**), DCP (**b**), and AFP-L3 (**c**). Inverse of standard error of natural logarithm of the odds ratio against natural logarithm of the DOR is plotted. The area of the circle is proportional to the inverse of standard error of natural logarithm of the odds ratio. AFP, alphafetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; DCP, des-gamma-carboxyprothrombin; DOR, diagnostic odds ratio

Appendix 1 Primary research

	Key words	No. of articles
1	Liver Neoplasms [MAJR] AND 1982:2002 [DP]	36,650
2	1 AND (Human [MeSH] OR hominidae [MeSH])	28,685
3	2 AND Tumor Markers, Biological [MeSH]	2,685

Appendix 2 Secondary research

	Key words	No. of articles
1	Liver Neoplasms [MAJR] AND 1982:2002 [DP]	36,650
2	1 AND (Human [MeSH] OR hominidae [MeSH])	28,685
4	2 AND (alpha-Fetoproteins [MeSH] OR alpha-fetoprotein [TW] OR alphafetoprotein [TW] OR AFP [TW])	2,587
5	2 AND (prothrombin [MeSH] OR des-gamma-carboxyprothrombin [TW] OR des-gamma-carboxy-prothrombin [TW] OR desgammacarboxyprothrombin [TW] OR DCP [TW] OR PIVKA [TW])	243
6	4 OR 5	2,650
7	3 OR 6	3,670

References

1. Di Bisceglie AM, Rustgi VK, Hoofnagle JH, Dusheiko GM, Lotze MT. NIH conference. Hepatocellular carcinoma. Ann Intern Med 1988;108:390–401.
2. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–50.
3. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004;127:S5–S16.
4. Blumberg BS, Larouze B, London WT, Werner B, Hesser JE, Millman I, et al. The relation of infection with the hepatitis B agent to primary hepatic carcinoma. Am J Pathol 1975;81:669–82.
5. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797–801.
6. Shiratori Y, Shiina S, Imamura M, Kato N, Kanai F, Okudaira T, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. Hepatology 1995;22:1027–33.
7. Fattovich G, Giustina G, Schalm SW, Hadziyannis S, Sanchez-Tapias J, Almasio P, et al., for the EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. Hepatology 1995;21:77–82.
8. Kato Y, Nakata K, Omagari K, Furukawa R, Kusumoto Y, Mori I, et al. Risk of hepatocellular carcinoma in patients with cirrhosis in Japan. Analysis of infectious hepatitis viruses. Cancer 1994;74:2234–8.
9. Cottone M, Turri M, Caltagirone M, Parisi P, Orlando A, Fiorentino G, et al. Screening for hepatocellular carcinoma in patients with Child's A cirrhosis: an 8-year prospective study by ultrasound and alphafetoprotein. J Hepatol 1994;21:1029–34.
10. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al., for the IHIT (Inhibition of Hepatocarcinogenesis by Interferon Therapy) Study Group. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. Ann Intern Med 1999;131:174–81.
11. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology 1997;112:463–72.
12. Torzilli G, Minagawa M, Takayama T, Inoue K, Hui AM, Kubota K, et al. Accurate preoperative evaluation of liver mass lesions without fine-needle biopsy. Hepatology 1999;30:889–93.
13. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005;42:1208–36.
14. Kew MC. Alpha-fetoprotein. In: Read AE, editor. Modern trends in gastroenterology. London: Butterworths; 1975. p. 91.
15. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. Hepatology 1995;22:432–8.
16. Bloomer JR, Waldmann TA, McIntire KR, Klatskin G. Alpha-fetoprotein in noneoplastic hepatic disorders. JAMA 1975; 233:38–41.
17. Alpert E, Feller ER. Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. Gastroenterology 1978;74:856–8.
18. 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 Engl J Med 1984;310:1427–31.
19. Okuda H, Obata H, Nakanishi T, Furukawa R, Hashimoto E. Production of abnormal prothrombin (des-gamma-carboxy prothrombin) by hepatocellular carcinoma. A clinical and experimental study. J Hepatol 1987;4:357–63.
20. Aoyagi Y, Isemura M, Yosizawa Z, Suzuki Y, Sekine C, Ono T, et al. Fucosylation of serum alpha-fetoprotein in patients with primary hepatocellular carcinoma. Biochim Biophys Acta 1985;830:217–23.
21. Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med 1993;328:1802–6.
22. Izzo F, Cremona F, Delrio P, Leonardi E, Castello G, Pignata S, et al. Soluble interleukin-2 receptor levels in hepatocellular cancer: a more sensitive marker than alfa fetoprotein. Ann Surg Oncol 1999;6:178–85.
23. Sacco R, Leuci D, Tortorella C, Fiore G, Marinosci F, Schiraldi O, et al. Transforming growth factor beta1 and soluble Fas serum levels in hepatocellular carcinoma. Cytokine 2000;12:811–4.
24. Kakumu S, Ito S, Ishikawa T, Mita Y, Tagaya T, Fukuzawa Y, et al. Decreased function of peripheral blood dendritic cells in patients with hepatocellular carcinoma with hepatitis B and C virus infection. J Gastroenterol Hepatol 2000;15:431–6.
25. Irwig L, Tosteson AN, Gatsonis C, Lau J, Colditz G, Chalmers TC, et al. Guidelines for meta-analyses evaluating diagnostic tests. Ann Intern Med 1994;120:667–76.
26. Irwig L, Macaskill P, Glasziou P, Fahey M. Meta-analytic methods for diagnostic test accuracy. J Clin Epidemiol 1995; 48:119–30; discussion 31–2.
27. Moses LE, Shapiro D, Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: data-analytic approaches and some additional considerations. Stat Med 1993;12:1293–316.
28. Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. Stat Med 2002;21:1237–56.

29. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315: 629–34.
30. Jaeschke R, Guyatt G, Lijimer J. Diagnostic tests. In: Guyatt G, Rennie D, editors. User's guide to the medical literature. Chicago: AMA Press; 2002. p. 121–40.
31. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
32. Zamora J, Abraira V, Muriel A, Khan KS, Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. BMC Med Res Methodol 2006;6:31
33. Gebo KA, Chander G, Jenckes MW, Ghanem KG, Herlong HF, Torbenson MS, et al. Screening tests for hepatocellular carcinoma in patients with chronic hepatitis C: a systematic review. Hepatology 2002;36:S84–92.
34. Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. Ann Intern Med 2003;139:46–50.
35. Fujiyama S, Izuno K, Yamasaki K, Sato T, Taketa K. Determination of optimum cutoff levels of plasma des-gamma-carboxy prothrombin and serum alpha-fetoprotein for the diagnosis of hepatocellular carcinoma using receiver operating characteristic curves. Tumour Biol 1992;13:316–23.
36. Tong MJ, Blatt LM, Kao VW. Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. J Gastroenterol Hepatol 2001;16:553–9.
37. Pateron D, Ganne N, Trinchet JC, Aurousseau MH, Mal F, Meicler C, et al. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. J Hepatol 1994; 20:65–71.
38. Piantino P, Arrigoni A, Brunetto MR, Gindro T. Alpha-fetoprotein in hepatic pathology and hepatocarcinoma. J Nucl Med Allied Sci 1989;33:34–8.
39. Khien VV, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV, et al. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. Int J Biol Markers 2001;16:105–11.
40. Trevisani F, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J Hepatol 2001;34:570–5.
41. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology 2000;32:842–6.
42. Peng YC, Chan CS, Chen GH. The effectiveness of serum alpha-fetoprotein level in anti-HCV positive patients for screening hepatocellular carcinoma. Hepatogastroenterology 1999;46: 3208–11.
43. Kuromatsu R, Tanaka M, Shimauchi Y, Shimada M, Tanikawa K, Watanabe K, et al. Usefulness of ED036 kit for measuring serum PIVKA-II levels in small hepatocellular carcinoma. J Gastroenterol 1997;32:507–12.
44. Furui J, Furukawa M, Kanematsu T. The low positive rate of serum alpha-fetoprotein levels in hepatitis C virus antibody-positive patients with hepatocellular carcinoma. Hepatogastroenterology 1995;42:445–9.
45. Grazi GL, Mazziotti A, Legnani C, Jovine E, Miniero R, Gallucci A, et al. The role of tumor markers in the diagnosis of hepatocellular carcinoma, with special reference to the des-gamma-carboxy prothrombin. Liver Transpl Surg 1995;1:249–55.
46. Tsai JF, Chang WY, Jeng JE, Ho MS, Lin ZY, Tsai JH. Frequency of raised alpha-fetoprotein level among Chinese patients with hepatocellular carcinoma related to hepatitis B and C. Br J Cancer 1994;69:1157–9.
47. Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. Hepatology 1994;19:61–6.
48. Tu Z, Yin Z, Wu M. Prospective study on the diagnosis of hepatocellular carcinoma by using alpha-fetoprotein reactive to lentil lectin. Chin Med Sci J 1992;7:191–6.
49. Taketa K, Sekiya C, Namiki M, Akamatsu K, Ohta Y, Endo Y, et al. Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. Gastroenterology 1990;99:508–18.
50. Deyashiki Y, Nishioka Y, Takahashi K, Kosaka Y, Suzuki K. Evaluation of des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma. Cancer 1989;64:2546–51.
51. King MA, Kew MC, Kuyl JM, Atkinson PM. A comparison between des-gamma-carboxy prothrombin and alpha-fetoprotein as markers of hepatocellular carcinoma in southern African blacks. J Gastroenterol Hepatol 1989;4:17–24.
52. Aoyagi Y, Suzuki Y, Isemura M, Nomoto M, Sekine C, Igarashi K, et al. The fucosylation index of alpha-fetoprotein and its usefulness in the early diagnosis of hepatocellular carcinoma. Cancer 1988;61:769–74.
53. Chan DW, Kelsten M, Rock R, Bruzek D. Evaluation of a monoclonal immunoenzymometric assay for alpha-fetoprotein. Clin Chem 1986;32:1318–22.
54. Saitoh S, Ikeda K, Koida I, Suzuki Y, Kobayashi M, Tsubota A, et al. Diagnosis of hepatocellular carcinoma by concanavalin A affinity electrophoresis of serum alpha-fetoprotein. Cancer 1995;76:1139–44.
55. Chalasani N, Horlander JC Sr, Said A, Hoen H, Kopecky KK, Stockberger SM Jr, et al. Screening for hepatocellular carcinoma in patients with advanced cirrhosis. Am J Gastroenterol 1999;94:2988–93.
56. Izzo F, Cremona F, Ruffolo F, Palaia R, Parisi V, Curley SA. Outcome of 67 patients with hepatocellular cancer detected during screening of 1125 patients with chronic hepatitis. Ann Surg 1998;227:513–8.
57. Cedrone A, Covino M, Caturelli E, Pompili M, Lorenzelli G, Villani MR, et al. Utility of alpha-fetoprotein (AFP) in the screening of patients with virus-related chronic liver disease: does different viral etiology influence AFP levels in HCC? A study in 350 western patients. Hepatogastroenterology 2000;47:1654–8.
58. Nguyen MH, Garcia RT, Simpson PW, Wright TL, Keeffe EB. Racial differences in effectiveness of alpha-fetoprotein for diagnosis of hepatocellular carcinoma in hepatitis C virus cirrhosis. Hepatology 2002;36:410–7.
59. Larcos G, Sorokopud H, Berry G, Farrell GC. Sonographic screening for hepatocellular carcinoma in patients with chronic hepatitis or cirrhosis: an evaluation. Am J Roentgenol 1998;171:433–5.
60. Tsai JF, Jeng JE, Ho MS, Chang WY, Lin ZY, Tsai JH. Clinical evaluation of serum alpha-fetoprotein and circulating immune complexes as tumour markers of hepatocellular carcinoma. Br J Cancer 1995;72:442–6.
61. Izzo F, Cremona F, Ruffolo F, Palaia R, Parisi V, Curley S. Detection of hepatocellular cancer during screening of 1125 patients with chronic hepatitis virus infection. J Chemother 1997;9:151–2.
62. Curley SA, Izzo F, Gallipoli A, de Bellis M, Cremona F, Parisi V. Identification and screening of 416 patients with chronic hepatitis at high risk to develop hepatocellular cancer. Ann Surg 1995;222:375–80; discussion 80–3
63. Gambarin-Gelwan M, Wolf DC, Shapiro R, Schwartz ME, Min AD. Sensitivity of commonly available screening tests in

detecting hepatocellular carcinoma in cirrhotic patients undergoing liver transplantation. Am J Gastroenterol 2000;95:1535–8.
64. Izuno K, Fujiyama S, Yamasaki K, Sato M, Sato T. Early detection of hepatocellular carcinoma associated with cirrhosis by combined assay of des-gamma-carboxy prothrombin and alpha-fetoprotein: a prospective study. Hepatogastroenterology 1995;42:387–93.
65. Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology 1993;18:47–53.
66. Tradati F, Colombo M, Mannucci PM, Rumi MG, De Fazio C, Gamba G, et al., for the Study Group of the Association of Italian Hemophilia Centers. A prospective multicenter study of hepatocellular carcinoma in Italian hemophiliacs with chronic hepatitis C. Blood 1998;91:1173–7.
67. Solmi L, Primerano AM, Gandolfi L. Ultrasound follow-up of patients at risk for hepatocellular carcinoma: results of a prospective study on 360 cases. Am J Gastroenterol 1996;91: 1189–94.
68. Cottone M, Turri M, Caltagirone M, Parisi P, Orlando A, Fiorentino G, et al. Screening for hepatocellular carcinoma in patients with Child's A cirrhosis: an 8-year prospective study by ultrasound and alphafetoprotein. J Hepatol 1994;21:1029–34.
69. Fujiyama S, Morishita T, Hashiguchi O, Sato T. Plasma abnormal prothrombin (des-gamma-carboxy prothrombin) as a marker of hepatocellular carcinoma. Cancer 1988;61:1621–8.
70. Ikoma J, Kaito M, Ishihara T, Nakagawa N, Kamei A, Fujita N, et al. Early diagnosis of hepatocellular carcinoma using a sensitive assay for serum des-gamma-carboxy prothrombin: a prospective study. Hepatogastroenterology 2002;49:235–8.
71. Kasahara A, Hayashi N, Fusamoto H, Kawada Y, Imai Y, Yamamoto H, et al. Clinical evaluation of plasma des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma in patients with tumors of various sizes. Dig Dis Sci 1993;38:2170–6.
72. Maringhini A, Cottone M, Sciarrino E, Marceno MP, La Seta F, Fusco G, et al. Ultrasonography and alpha-fetoprotein in diagnosis of hepatocellular carcinoma in cirrhosis. Dig Dis Sci 1988;33:47–51.
73. Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. Cancer 1998;82:1643–8.
74. Nomura F, Ishijima M, Horikoshi A, Nakai T, Ohnishi K. Determination of serum des-gamma-carboxy prothrombin levels in patients with small-sized hepatocellular carcinoma: comparison of the conventional enzyme immunoassay and two modified methods. Am J Gastroenterol 1996;91:1380–3.
75. 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.
76. Oka H, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, et al. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of lens culinaris agglutinin-reactive alpha-fetoprotein. J Gastroenterol Hepatol 2001;16:1378–83.
77. Sassa T, Kumada T, Nakano S, Uematsu T. Clinical utility of simultaneous measurement of serum high-sensitivity des-gamma-carboxy prothrombin and lens culinaris agglutinin A-reactive alpha-fetoprotein in patients with small hepatocellular carcinoma. Eur J Gastroenterol Hepatol 1999;11:1387–92.
78. Saitoh S, Ikeda K, Koida I, Tsubota A, Arase Y, Chayama K, et al. Serum des-gamma-carboxyprothrombin concentration determined by the avidin–biotin complex method in small hepatocellular carcinomas. Cancer 1994;74:2918–23.
79. Shimauchi Y, Tanaka M, Kuromatsu R, Ogata R, Tateishi Y, Itano S, et al. A simultaneous monitoring of lens culinaris agglutinin A-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin as an early diagnosis of hepatocellular carcinoma in the follow-up of cirrhotic patients. Oncol Rep 2000;7:249–56.
80. Shiraki K, Takase K, Tameda Y, Hamada M, Kosaka Y, Nakano T. A clinical study of lectin-reactive alpha-fetoprotein as an early indicator of hepatocellular carcinoma in the follow-up of cirrhotic patients. Hepatology 1995;22:802–7.
81. 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.
82. Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, et al. A collaborative study for the evaluation of lectin-reactive alpha-fetoproteins in early detection of hepatocellular carcinoma. Cancer Res 1993;53:5419–23.
83. Tanabe Y, Ohnishi K, Nomura F, Iida S. Plasma abnormal prothrombin levels in patients with small hepatocellular carcinoma. Am J Gastroenterol 1988;83:1386–9.
84. Tsai SL, Huang GT, Yang PM, Sheu JC, Sung JL, Chen DS. Plasma des-gamma-carboxyprothrombin in the early stage of hepatocellular carcinoma. Hepatology 1990;11:481–8.
85. Giannini E, Arzani L, Borro P, Botta F, Fasoli A, Risso D, et al. Does surveillance for hepatocellular carcinoma in HCV cirrhotic patients improve treatment outcome mainly due to better clinical status at diagnosis? Hepatogastroenterology 2000;47:1395–8.
86. Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, et al. Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. Cancer 2001;91:561–9.
87. 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.
88. Begg CB, Berlin JA. Publication bias and dissemination of clinical research. J Natl Cancer Inst 1989;81:107–15.
89. Lijmer JG, Mol BW, Heisterkamp S, Bonsel GJ, Prins MH, van der Meulen JH, et al. Empirical evidence of design-related bias in studies of diagnostic tests. JAMA 1999;282:1061–6.
90. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. Ann Intern Med 2003;138:40–4.
91. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med 2003;138:W1–12.
92. Lok AS, Lai CL. Alpha-fetoprotein monitoring in Chinese patients with chronic hepatitis B virus infection: role in the early detection of hepatocellular carcinoma. Hepatology 1989;9:110–5.
93. The Study Group for Evidence Based Guideline for the Diagnosis and Treatment of Hepatocellular Carcinoma. Evidence based guideline for the diagnosis and treatment of hepatocellular carcinoma. Tokyo: Kanehara & Co; 2005.

Obesity Is an Independent Risk Factor for Hepatocellular Carcinoma Development in Chronic Hepatitis C Patients

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See Loomba R et al on page 953 for companion article in the April 2008 issue of *Gastroenterology*.

Background & Aims: **It is not fully elucidated whether obesity enhances hepatocarcinogenesis in patients with chronic hepatitis C. The aim of this study was to investigate the relationship between body weight and risk of hepatocarcinogenesis in chronic hepatitis C patients.** ***Methods:*** **We enrolled 1431 patients with chronic hepatitis C who visited our liver clinic between 1994 and 2004, excluding those with hepatocellular carcinoma (HCC) at their visit or with a previous history of HCC. They were divided into 4 groups according to body mass index (BMI): underweight (≤18.5 kg/m², N = 112); normal (18.5 to less than 25 kg/m², N = 1023); overweight (25 to less than 30 kg/m², N = 265); and obese (>30 kg/m², N = 31). We assessed the impact of obesity on the hepatocarcinogenesis adjusted by multivariate Cox proportional hazard regression with other risk factors found significant in univariate analysis.** ***Results:*** **During the follow-up period (mean, 6.1 y), HCC developed in 340 patients, showing cumulative incidence rates of 10.5%, 19.7%, and 36.8% at 3, 5, and 10 years, respectively. The incidence differed significantly among the BMI groups ($P = .007$). Adjusting for other significant factors, overweight and obesity were shown to be an independent risk factor of HCC, with a hazard ratio of 1.86 (95% confidence interval, 1.09–3.16; $P = .022$) and 3.10 (95% confidence interval, 1.41–6.81; $P = .005$) as compared with the underweight patients.** ***Conclusions:*** **The risk of HCC in patients with chronic hepatitis C increases in proportion to BMI in a wide range of its values, from underweight to obese.**

Chronic hepatitis C virus (HCV) infection affects more than 170 million people worldwide currently, posing a major health care problem. The prevalence is between 1% and 3% in the United States,[1] Southern European countries,[2,3] and Japan.[4] Although acute HCV infection usually is asymptomatic or accompanied by only mild nonspecific symptoms, chronic infection follows in as much as 80% of cases.[2] Once chronicity has been established, spontaneous clearance of viremia is rare, and cirrhosis may develop in 20 to 30 years.[2] According to a previous report, about one third of patients showed progression to cirrhosis in 20 years or less, whereas no progression in fibrosis was noted in another third for 30 years or longer.[5] Factors reported to accelerate fibrosis include old age, male sex, heavy alcohol intake, and immunosuppressive states such as co-infection with human immunodeficiency virus.[3–9] Once cirrhosis is established, the risk of hepatocellular carcinoma (HCC) development is increased to 1% to 4% per year.[2,10] Much higher incidence rates, 5% to 8% per year, are reported from Japan.[11–13] Thus, the risk factors that accelerate fibrosis are also risk factors for HCC development.

Recently, various epidemiologic and other studies extensively have investigated possible risk factors of liver cancer caused by chronic hepatic diseases, identifying sex, age, severity of hepatic inflammation and fibrosis, and race.[4,14,15] In addition, interest in nonalcoholic fatty liver disease has prompted investigation of additional possible risk factors including comorbidity with diabetes mellitus,[4,15,16] obesity as indicated by body mass index (BMI),[14,17,18] and hyperinsulinemia.[19,20] Muto et al[21] and Ioannou et al[22] also reported that a BMI higher than 25 kg/m² was a risk factor for hepatocarcinogenesis. Although those previous reports did not evaluate hepatocarcinogenesis among HCV-positive patients in particular, it can be speculated that a higher BMI is an independent risk factor for hepatocarcinogenesis in chronic hepatitis C patients. Thus, we conducted this retrospective, follow-up study with consecutive patients to assess the potential effects of obesity on hepatocarcinogenesis among chronic hepatitis C patients.

Patients and Methods

Patients

Between January 1994 and December 2004, a total of 1954 HCV RNA–positive patients, excluding those with HCC or a past history of it, visited the liver clinic of the Department of Gastroenterology at the University of Tokyo Hospital. We analyzed 1431 of these patients, excluding 87 patients with concomitant hepatitis B virus surface antigen positivity and 423 patients who visited only for consultation purposes. Thirteen patients with intractable ascites also were excluded because this study was focused on the relationship between obesity and hepatocarcinogenesis and the amount of ascites affects body weight in these patients. These patients were all Japanese. All

Abbreviations used in this paper: AFP, α-fetoprotein; ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; SVR, sustained virologic response.

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