J Gastroenterol 2007; 42:962–968
DOI 10.1007/s00535-007-2117-x

Journal of Gastroenterology



# *Lens culinaris* agglutinin-reactive α-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma

Hisamitsu Miyaaki[1,2], Osamu Nakashima[1], Mina Kurogi[1], Katsumi Eguchi[2], and Masamichi Kojiro[1]

[1]Department of Pathology and Research Center of Innovative Cancer Therapy of the 21st Century COE Program for Medical Science, Kurume University School of Medicine, Kurume, Japan
[2]First Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

***Background.*** We histopathologically examined *Lens culinaris* agglutinin-reactive α-fetoprotein (AFP-L3)-positive hepatocellular carcinoma (HCC) and protein induced by vitamin K absence (PIVKA) II-positive HCC to clarify the efficacy of these markers for predicting a poor prognosis. ***Methods.*** Serum AFP-L3 and PIVKA II was measured in 110 HCC patients. AFP-L3 was measured by lectin-affinity electrophoresis coupled with antibody-affinity blotting, and PIVKA II by using a high-sensitivity kit. The growth type, capsule formation, capsule infiltration, portal vein invasion, intrahepatic metastasis and histological tumor grade were evaluated pathologically. ***Results.*** Thirty-eight (35%) HCC patients were AFP-L3-positive, and 63 (57%) were PIVKA II-positive. In AFP-L3-positive HCC, the frequencies of an infiltrative growth type (positive : negative = 66% : 42%, $P = 0.027$) and a poorly differentiated type (positive : negative = 32% : 6%, $P < 0.001$) were significantly higher than in AFP-L3-negative HCC. In PIVKA II-positive HCC, the frequencies of an infiltrative growth type (positive : negative = 62% : 28%, $P < 0.001$), vascular invasion (positive : negative = 63% : 26%, $P < 0.001$), and intrahepatic metastasis (positive : negative = 38% : 4%, $P < 0.001$) were significantly higher than in PIVKA II-negative HCC. In both AFP-L3- and PIVKA II-positive HCC, the frequency of a poorly differentiated growth type was significantly higher than in HCC positive for either AFP-L3 or PIVKA II or HCC negative for both AFP-L3 and PIVKA II (both positive : either positive : both negative = 37% : 12% : 0%; $P = 0.014$, $P < 0.001$, respectively). ***Conclusions.*** AFP-L3 was related to progression from moderately differentiated to poorly differentiated HCC, whereas PIVKA II was more specific to vascular invasion. PIVKA II is therefore likely to be a useful indicator of vascular invasion.

**Key words:** hepatocellular carcinoma, pathological features, *Lens culinaris* agglutinin-reactive α-fetoprotein, protein induced by vitamin K absence II

## Introduction

α-Fetoprotein (AFP) is a well-known tumor marker of hepatocellular carcinoma (HCC), and its diagnostic efficacy has been confirmed. However, serum AFP levels also increase in liver diseases such as chronic hepatitis and cirrhosis. Therefore, the HCC specificity of AFP is sometimes questioned.[1,2] AFP has a structural variance in its chains, and the sugar chains obtained from HCC patients have high affinity to lectins such as *Lens culinaris* agglutinin and erythroagglutinating phytohemagglutinin.[3] The *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) has been suggested to be a significant marker of HCC.[4,5] In addition, it has been reported that an increase of AFP-L3 in HCC patients tends to be related to a poor prognosis.[6–13] Among HCC patients who receive nonsurgical treatment, the survival rate is lower in AFP-L3-positive patients than in AFP-L3-negative patients.[7] Pretreatment AFP-L3 positivity in patients who undergo transcatheter arterial embolization is also associated with a poorer prognosis.[10] However, few studies have investigated the relationship between pathological characteristics and AFP-L3 positivity in HCC patients. On the other hand, protein induced by vitamin K absence (PIVKA) II levels rarely increase in chronic hepatitis or cirrhosis, and it is highly specific to HCC.[14] In addition, PIVKA II has been reported to be a marker of a poor prognosis.[15] Therefore, this study examined the pathological characteristics of HCC patients in relation to AFP-L3 and PIVKA II.

Received: April 29, 2007 / Accepted: September 13, 2007
*Reprint requests to:* H. Miyaaki

## Methods

We examined HCC nodules with a nodular diameter of less than or equal to 5.0 cm that were surgically resected between 1998 and 2004 at Kurume University Hospital. AFP-L3 and PIVKA II were measured in 110 patients (84 male, 26 female) whose average age was 64.2 ± 10.3 years. Among them, 21 patients were hepatitis B virus surface antigen positive, 76 were hepatitis C virus antibody positive, and three were positive for both. The serum AFP level was determined by using commercially available kits. The AFP-L3 level was measured by lectin-affinity electrophoresis coupled with antibody-affinity blotting (AFP Differentiation Kit L, Wako Pure Chemical, Osaka, Japan). Serum AFP-L3 was finally expressed as a percentage of total AFP (AFP-L3/total AFP × 100%). The cutoff value used to determine positivity for AFP-L3 was 10%. The serum PIVKA II level was determined with a high-sensitivity kit (Eisai, Tokyo, Japan). Surgically resected liver specimens were fixed in 10% formalin, sliced into 4 μm-thick paraffin sections, and then step-sections were prepared for hematoxylin and eosin (HE) staining. The HCC specimens were classified into one of the following four gross types according to the Japanese Classification of Primary Cancer:[16] small nodular with indistinct margin, simple nodular, and two infiltrative growth types, simple nodular with extranodular growth and confluent multinodular types. Histological grade (i.e., well-differentiated, moderately differentiated, or poorly differentiated) was independently evaluated by two pathologists (HM, ON).

Groups were compared by using the $\chi$-squared test, and a value of $P < 0.05$ was considered to be statistically significant.

## Results

### *Patients' characteristics*

AFP-L3 was positive in 38 patients (35%) and negative in 72 (65%). PIVKA II was positive in 63 patients (57%) and negative in 47 (43%). Other clinical characteristics of these patients are shown in Table 1. Both AFP-L3 and PIVKA II were positive in 30 patients (27%), either AFP-L3 or PIVKA II, but not both, was positive in 41 patients (37%), and both were negative in 39 patients (35%). Other clinical characteristics of these patients are shown in Table 2.

### *Tumor diameter*

Among the 38 AFP-L3-positive cases, the tumor diameter was ≤20 mm in three (14%), 21–30 mm in 14 (30%),

**Table 1.** Clinical characteristics of the patients

| | AFP-L3 | | PIVKA II | |
|---|---|---|---|---|
| | Positive $n = 38$ | Negative $n = 72$ | Positive $n = 63$ | Negative $n = 47$ |
| Age (years) | 64.6 ± 14.2 | 64.3 ± 8.7 | 66.1 ± 10.1 | 61.2 ± 11.3 |
| Sex (M/F) | 25/13 | 59/13 | 45/18 | 9/47 |
| Virus (positive) | | | | |
| HBV | 8 | 13 | 9 | 12 |
| HCV | 27 | 49 | 47 | 29 |
| HBV & HCV | 1 | 2 | 2 | 1 |
| Nodular diameter (mm) | 32.8 ± 8.7 | 27.6 ± 10.2 | 33.1 ± 9.5 | 25.7 ± 9.5 |

AFP-L3, α-fetoprotein L3; PIVKA II, protein induced by vitamin K absence II; HBV, hepatitis B virus; HCV, hepatitis C virus

**Table 2.** Additional clinical characteristic of the patients

| | AFP-L3 and PIVKA II positive $n = 30$ | AFP-L3 or PIVKA II positive (but not both) $n = 41$ | AFP-L3 and PIVKA II negative $n = 39$ |
|---|---|---|---|
| Age (years) | 66.7 ± 11.7 | 57.8 ± 21.3 | 62.9 ± 9.1 |
| Sex (M/F) | 19/11 | 34/7 | 31/8 |
| Virus (positive) | | | |
| HBV | 6 | 5 | 10 |
| HCV | 22 | 30 | 24 |
| HBV & HCV | 2 | 0 | 1 |
| Nodular diameter | 33.4 ± 9.5 | 30.3 ± 4.6 | 24.7 ± 10.1 |

31–40 mm in 15 (54%), and 41–50 mm in six (39%; Table 3). Among the 63 PIVKA II-positive cases, the tumor diameter was ≤20 mm in seven (40%), 21–30 mm in 25 (51%), 31–40 mm in 18 (72%), and 41–50 mm in 13 (76%; Table 3). Among the 30 cases positive for both AFP-L3 and PIVKA II, the tumor diameter was ≤20 mm in three (14%), 21–30 mm in nine (20%), 31–40 mm in 12 (48%), and 41–50 mm in six (35%; Table 4). Among the 41 cases positive for either AFP-L3 or PIVKA II, the tumor diameter was ≤20 mm in four (18%), 21–30 mm in 21 (46%), 31–40 mm in nine (36%), and 41–50 mm in seven (41%; Table 4). Among the 39 cases negative for both AFP-L3 and PIVKA II, the tumor diameter was ≤20 mm in 15 (68%), 21–30 mm in 16 (35%), 31–40 mm in four (16%), and 41–50 mm in four (24%; Table 4).

### *Pathological findings*

The indistinct growth type of tumor was not found in patients positive for AFP-L3, PIVKA II, or both; all three tumors of this type were in patients negative for both AFP-L3 and PIVKA II (Tables 5, 6, and 7). The tumor was the simple nodular type in 13 AFP-L3-

**Table 3.** Tumor diameters between AFP-L3-positive and PIVKA II-positive cases

| Tumor diameter (cm) | AFP-L3 positive | PIVKA II positive |
|---|---|---|
| ≤2 | 3/22 (14%) | 7/22 (32%) |
| >2 and ≤3 | 14/46 (30%) | 25/46 (54%) |
| >3 and ≤4 | 15/25 (60%) | 18/25 (72%) |
| >4 and ≤5 | 6/17 (35%) | 13/17 (76%) |
| Total | 38/110 (35%) | 63/110 (57%) |

Values represent the ratios of positive cases to total number of cases examined for AFP-L3 and PIVKA II

**Table 4.** Tumor diameters among AFP-L3- and PIVKA II-positive and/or negative cases

| Tumor diameter | AFP-L3 and PIVKA II positive | AFP-L3 or PIVKA II positive (but not both) | AFP-L3 and PIVKA II negative |
|---|---|---|---|
| ≤2 | 3/22 (14%) | 4/22 (18%) | 15/22 (68%) |
| >2 and ≤3 | 9/46 (20%) | 21/46 (46%) | 16/46 (35%) |
| >3 and ≤4 | 12/25 (48%) | 9/25(36%) | 4/25 (16%) |
| >4 and ≤5 | 6/17 (35%) | 7/17 (41%) | 4/17 (24%) |
| Total | 30/110 (27%) | 41/110 (37%) | 39/110 (35%) |

**Table 5.** Pathological findings in HCC positive or negative for AFP-L3

| | AFP-L3 positive ($n$ =38) | AFP-L3 negative ($n$ = 72) | $P$ value |
|---|---|---|---|
| Growth type | | | |
| Indistinct nodular type | 0 (0%) | 3 (4%) | |
| Simple nodular type | 13 (34%) | 39 (54%) | |
| Infiltrative growth type | 25 (66%) | 30 (42%) | <0.027[a] |
| SN type with EG | 20 (53%) | 22 (31%) | |
| Confluent multinodular type | 5 (13%) | 8 (11%) | |
| Presence of | | | |
| Capsule formation | 32 (84%) | 55 (76%) | 0.337 |
| Capsule infiltration | 28 (74%) | 52 (72%) | 0.870 |
| Portal vein invasion | 20 (53%) | 32 (44%) | 0.413 |
| Intrahepatic metastasis | 11 (29%) | 15 (15%) | 0.341 |
| Histological grade | | | |
| Well-differentiated | 0 (0%) | 4 (6%) | |
| Moderately differentiated | 26 (68%) | 64 (88%) | |
| Poorly differentiated | 12 (32%) | 4 (6%) | <0.001 |

HCC, hepatocellular carcinoma; SN, simple nodular type; EG, extranodular growth
[a] vs. simple nodular type

**Table 6.** Pathological findings in HCC positive or negative for PIVKA II

| | PIVKA II positive ($n = 63$) | PIVKA II negative ($n = 47$) | $P$ value |
|---|---|---|---|
| Growth type | | | |
| Indistinct nodular type | 0 (0%) | 3 (6%) | |
| Simple nodular type | 24 (38%) | 31 (66%) | |
| Infiltrative growth type | 39 (62%) | 13 (28%) | <0.001[a] |
| SN type with EG | 30 (48%) | 9 (19%) | |
| Confluent multinodular type | 9 (14%) | 4 (9%) | |
| Presence of | | | |
| Capsule formation | 55 (87%) | 35 (74%) | 0.084 |
| Capsule infiltration | 48 (76%) | 32 (68%) | 0.345 |
| Portal invasion | 40 (63%) | 12 (26%) | <0.001 |
| Intrahepatic metastasis | 24 (38%) | 2 (4%) | <0.001 |
| Histological grade | | | |
| Well-differentiated | 0 (0%) | 4 (9%) | |
| Moderately differentiated | 50 (79%) | 40 (85%) | |
| Poorly differentiated | 13 (21%) | 3 (6%) | 0.07 |

[a] vs. simple nodular type

**Table 7.** Pathological findings in HCC positive for both AFP-L3 and PIVKA II, positive for either AFP-L3 or PIVKA II, or negative for both AFP-L3 and PIVKA II

| | AFP-L3 and PIVKA II positive ($n = 30$) | AFP-L3 or PIVKA II positive (but not both) ($n = 41$) | AFP-L3 and PIVKA II negative ($n = 39$) | $P$ value |
|---|---|---|---|---|
| Growth type | | | | |
| Indistinct nodular type | 0 (0%) | 0 (0%) | 3 (8%) | |
| Simple nodular type | 11 (36%) | 14 (34%) | 27 (69%) | |
| Infiltrative growth type | 19 (63%) | 27 (65%) | 9 (23%) | 0.826[a,c]<br><0.001[b,c] |
| SN type with EG | 15 (50%) | 21 (51%) | 6 (15%) | |
| Confluent multinodular | 4 (13%) | 6 (14%) | 3 (8%) | |
| Presence of | | | | |
| Capsular formation | 25 (83%) | 35 (85%) | 28 (72%) | 0.815[a]<br>0.260[b] |
| Capsular infiltration | 23 (76%) | 21 (51%) | 26 (66%) | 0.029[a]<br>0.118[b] |
| Portal vein invasion | 19 (63%) | 24 (58%) | 9(23%) | 0.878[a]<br><0.001[b] |
| Intrahepatic invasion | 10 (33%) | 15 (37%) | 2 (5%) | 0.777[a]<br>0.002[b] |
| Histological grade | | | | |
| Well differentiated | 0 (0%) | 0 (0%) | 4 (10%) | |
| Moderately differentiated | 18 (60%) | 37 (90%) | 35 (87%) | |
| Poorly differentiated | 12 (40%) | 4 (10%) | 0 (0%) | 0.002[a]<br><0.001[b] |

[a] Both AFP-L3 and PIVKA II positive vs. either AFP-L3 or PIVKA II positive
[b] Both AFP-L3 and PIVKA II positive vs. Both AFP-L3 and PIVKA II negative
[c] vs. simple nodular type

positive cases (34%), 39 AFP-L3-negative cases (54%), 24 PIVKA II-positive cases (38%), 31 PIVKA II-negative cases (66%), 11 cases positive for both AFP-L3 and PIVKA II (36%), 14 positive for either AFP-L3 or PIVKA II (34%), and 27 negative for both AFP-L3 and PIVKA II (69%).

The simple nodular type with extranodular growth was found in 20 AFP-L3-positive cases (45%), 22 AFP-L3-negative cases (31%), 30 PIVKA II-positive cases (48%), nine PIVKA II-negative cases (19%), 15 cases positive for both AFP-L3 and PIVKA II (50%), 21 positive for either AFP-L3 or PIVKA II (51%), and six negative for both AFP-L3 and PIVKA II (15%). The confluent multinodular type was found in five AFP-L3-positive cases (13%), eight AFP-L3-negative cases (11%), nine PIVKA II-positive cases (14%), four

PIVKA II-negative cases (9%), four cases positive for both (13%), six positive for either (14%), and three negative for both (8%). In the AFP-L3-positive and PIVKA II-positive patients and those positive for both, the frequency of the infiltrative growth type, which indicates a varying degree of invasive growth, was significantly higher than that of the simple nodular type (Tables 5, 6, and 7).

AFP-L3 positivity was not related to capsule formation, capsule infiltration, portal vein invasion, or intrahepatic metastasis (Table 5). PIVKA II positivity was not significantly related to capsule formation or capsule infiltration (Table 6). Positivity for both AFP-L3 and PIVKA II was not significantly related to capsule formation. The frequencies of portal vein invasion and intrahepatic metastasis were significantly higher in the PIVKA II-positive group than in the PIVKA II-negative group ($P < 0.001$). The frequencies of portal vein invasion and intrahepatic metastasis were also significantly higher in patients positive for both AFP-L3 and PIVKA II than in those negative for both.

*Histological grade*

AFP-L3 was negative in all four well-differentiated HCCs (0%), whereas it was positive in 26 moderately differentiated HCCs (68%) and 12 poorly differentiated HCCs (32%). The frequency of poorly differentiated HCC among AFP-L3-positive cases was significantly higher than that among AFP-L3-negative cases ($P < 0.001$, Table 5).

PIVKA II was negative in all five well-differentiated HCCs (0%), and positive in 50 moderately differentiated HCCs (79%) and 13 poorly differentiated HCCs (21%). Poorly differentiated HCC was significantly more frequent among PIVKA II-positive patients than among PIVKA II-negative cases ($P = 0.07$, Table 6).

Poorly differentiated HCC was significantly more frequent in cases positive for both AFP-L3 and PIVKA II than in those positive for only one or negative for both ($P = 0.002$, $P < 0.001$, respectively, Table 7).

## Discussion

AFP-L3 has been identified as a potential marker for the detection of small HCC, and it was recently reported to be a useful indicator of a poor prognosis in HCC patients.[6–13] However, few pathological studies have investigated poor prognoses in AFP-L3-positive patients. On the other hand, although PIVKA II is known to be a prognostic indicator for HCC, there are also few pathological studies on its relationship to a poor prognosis.[15] This study examined the pathological features of AFP-L3-positive HCC compared with PIVKA II-positive HCC.

Although AFP-L3 was developed as a tumor marker for the detection of early-stage HCC, the frequency of AFP-L3-positive HCC with a diameter of <2 cm in the study of Kuromatsu et al.[12] was low (36.3%). In the current study, only three AFP-L3-positive HCCs had a diameter of <2 cm (14%, Table 2), indicating that monitoring of AFP-L3 alone is not sufficient to detect early-stage HCC. On the other hand, among the 22 patients who were examined for PIVKA II, the tumor diameter was less than 2 cm in seven (31%). This rate is higher than that in AFP-L3-positive cases, indicating that PIVKA II is more specific to early-stage HCC than AFP-L3. However, 15 (68%) HCCs with a diameter of <2 cm were negative for both AFP-L3 and PIVKA II, showing that for detection of early-stage HCCs, AFP-L3 and PIVKA II should be used in combination with other appropriate tumor markers or imaging modalities.

Regarding growth type, there was no case of an indistinct nodular type tumor in HCC patients positive for both AFP-L3 and PIVKA II. An indistinct growth type is generally not associated with portal vein invasion or intrahepatic metastasis. Therefore, this type of tumor can be regarded as "carcinoma in situ" in the liver, and a complete cure can be expected after surgical resection.[17] These findings indicate that neither AFP-L3 nor PIVKA II are produced by indistinct type HCC.

The frequency of an infiltrative growth type, that is, the single nodular type with extra nodular growth or the confluent multinodular type, was significantly higher than the single nodular type in AFP-L3-positive cases ($P = 0.027$), PIVKA II-positive cases ($P < 0.001$), and cases positive for both ($P < 0.001$). In previous studies,[18,19] the frequencies of portal vein invasion and intrahepatic metastasis were significantly higher in the infiltrative growth type than in the single nodular type of tumor. These observations show that elevated AFP-L3 and PIVKA II levels indicate advanced HCC.

The frequency of HCC with capsule formation or capsule infiltration was not different between the positive and negative groups of either marker.

Portal vein invasion was found more frequently in the patients positive for either marker than in those negative for either, and more frequently in patients positive for both AFP-L3 and PIVKA II than in those negative for both. PIVKA II was more specific for detection of portal vein invasion than AFP-L3. Vascular invasion and intrahepatic metastasis have been reported to be associated with a poor prognosis of HCC patients.[20–22] A prospective study using imaging modalities reported that PIVKA II was the most useful predisposing parameter for the development of portal vein invasion.[23] Portal

vein invasion is confirmed microscopically and is undetectable by diagnostic imaging such as ultrasonography and computed tomography. Because intrahepatic metastasis of HCC occurs via the portal vein, metastasis is related to portal vein invasion.[24,25] Therefore, PIVKA II is thought to be more useful than AFP-L3 for the detection of portal vein invasion and intrahepatic metastasis. In addition, only nine HCCs with portal vein invasion and two with intrahepatic metastasis were found in patients negative for both AFP-L3 and PIVKA II, indicating that if both tumor markers are negative, then the possibility of HCC with vascular invasion and intrahepatic metastasis is low.

Regarding the histological grade, well-differentiated HCC was found only in patients negative for both AFP-L3 and PIVKA II, whereas the frequency of poorly differentiated HCC was significantly higher in AFP-L3-positive patients than in AFP-L3-negative patients (Tables 3 and 4). In addition, the frequency of poorly differentiated HCC was higher in AFP-L3-positive patients than in PIVKA II-positive patients. Several studies have reported that AFP-L3 levels are correlated with histological grade of HCC,[6,7,12,26] and in our current study, 12 of 16 poorly differentiated HCCs were AFP-L3 positive, and all 12 were also PIVKA II-positive. Because the level of fetal liver products, AFP (AFP-L3), decreases along with the growth of the human body,[27] AFP-L3 is produced by the immature HCC component.

In patients positive for both AFP-L3 and PIVKA II, poorly differentiated HCCs were significantly more frequent than in patients positive for either marker or negative for both. On the other hand, there were no poorly differentiated HCC cases among patients negative for both AFP-L3 and PIVKA II. These results show that if both tumor markers are negative, then the possibility of poorly differentiated HCC is quite low.

In conclusion, AFP-L3 positivity was high in poorly differentiated HCC in comparison with PIVKA II positivity, whereas PIVKA II positivity was high in HCCs with portal vein invasion or intrahepatic metastasis.

## References


1. Di Bisceglie AM, Hoofnagle JH. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. Cancer 1989;64:2117–20.
2. 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.
3. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. Hepatology 1990;12:1420–32.
4. 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.
5. 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
6. Yamashita F, Tanaka M, Satomura S, Tanikawa K. Prognostic significance of *Lens culinaris* agglutinin A-reactive alpha-fetoprotein in small hepatocellular carcinomas. Gastroenterology 1996;111:996–1001.
7. Hayashi K, Kumada T, Nakano S, Takeda I, Sugiyama K, Kiriyama S, et al. Usefulness of measurement of *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein as a marker of prognosis and recurrence of small hepatocellular carcinoma. Am J Gastroenterol 1999;94:3028–33.
8. Yamashiki N, Seki T, Wakabayashi M, Nakagawa T, Imamura M, Tamai T, et al. Usefulness of *Lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein (AFP-L3) as a marker of distant metastasis from hepatocellular carcinoma. Oncol Rep 1999;6: 1229–32.
9. Okuda K, Tanaka M, Kanazawa N, Nagashima J, Satomura S, Kinoshita H, et al. Evaluation of curability and prediction of prognosis after surgical treatment for hepatocellular carcinoma by *Lens culinaris* agglutinin-reactive alpha-fetoprotein. Int J Oncol 1999;14:265–71.
10. Song BC, Suh DJ, Yang SH, Lee HC, Chung YH, Sung KB, et al. *Lens culinaris* agglutinin-reactive alpha-fetoprotein as a prognostic marker in patients with hepatocellular carcinoma undergoing transcatheter arterial chemoembolization. J Clin Gastroenterol 2002;35:398–402.
11. Fukuda H. Tumor vascularity and *Lens culinaris* agglutinin reactive alpha-fetoprotein are predictors of long-term prognosis in patients with hepatocellular carcinoma after percutaneous ethanol injection therapy. Kurume Med J 1998;45:187–93.
12. Kuromatsu R, Tanaka M, Tanikawa K. Serum alpha-fetoprotein and *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. Liver 1993;13:177–82.
13. Takayama T, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, et al. Early hepatocellular carcinoma as an entity with a high rate of surgical cure. Hepatology 1998;28: 1241–6.
14. Motohara K, Kuroki Y, Kan H, Endo F, Matsuda I. Detection of vitamin K deficiency by use of an enzyme-linked immunosorbent assay for circulating abnormal prothrombin. Pediatr Res 1985; 19:354–7.
15. Nagaoka S, Yatsuhashi H, Hamada H, Yano K, Matsumoto T, Daikoku M, et al. The des-gamma-carboxy prothrombin index is a new prognostic indicator for hepatocellular carcinoma. Cancer 2003;98:2671–7.
16. Japan Liver Cancer Study Group. Classification of primary liver cancer. Tokyo: Kanehara; 1997.
17. Takayama T, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, et al. Early hepatocellular carcinoma as an entity with a high rate of surgical cure. Hepatology 1998;28: 1241–6.
18. Kanai T, Hirohashi S, Upton MP, Noguchi M, Kishi K, Makuuchi M, et al. Pathology of small hepatocellular carcinoma. A proposal for a new gross classification. Cancer 1987;60:810–9.
19. Hui AM, Takayama T, Sano K, Kubota K, Akahane M, Ohtomo K, et al. Predictive value of gross classification of hepatocellular carcinoma on recurrence and survival after hepatectomy. J Hepatol 2000;33:975–9.
20. Izumi R, Shimizu K, Ii T, Yagi M, Matsui O, Nonomura A, et al. Prognostic factors of hepatocellular carcinoma in patients undergoing hepatic resection. Gastroenterology 1994;106:720–7.
21. Liver Cancer Study Group of Japan. Predictive factors for long-term prognosis after partial hepatectomy for patients with hepatocellular carcinoma in Japan. The Liver Cancer Study Group of Japan. Cancer 1994;74:2772–80
22. Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence

after resection of hepatocellular carcinoma. Cancer 2000;89: 500–7.
23. 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.
24. Toyosaka A, Okamoto E, Mitsunobu M, Oriyama T, Nakao N, Miura K. Intrahepatic metastases in hepatocellular carcinoma: evidence for spread via the portal vein as an efferent vessel. Am J Gastroenterol 1996;91:1610–5.
25. Mitsunobu M, Toyosaka A, Oriyama T, Okamoto E, Nakao N. Intrahepatic metastases in hepatocellular carcinoma: the role of the portal vein as an efferent vessel. Clin Exp Metastasis 1996; 14:520–9.
26. 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
27. Yamashita K, Taketa K, Nishi S, Fukushima K, Ohkura T. Sugar chains of human cord serum alpha-fetoprotein: characteristics of *N*-linked sugar chains of glycoproteins produced in human liver and hepatocellular carcinomas. Cancer Res 1993;53: 2970–5.