Original article

Preoperative serum α-fetoprotein and prognosis after hepatectomy for hepatocellular carcinoma

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Background: **While the majority of studies report that a raised serum α-fetoprotein (AFP) level before operation is associated with a high risk of recurrence and death in patients who undergo hepatectomy for hepatocellular carcinoma (HCC), results are conflicting. The aim of this study was to assess the prognostic value of AFP.**

Methods: **Serum AFP levels were measured in patients with hepatitis-associated HCC who underwent hepatectomy between 1995 and 2012. Kaplan–Meier and multivariable analyses were performed to identify risk factors for overall and disease-free survival. Univariable and multivariable Cox proportional hazards regression was used to evaluate the predictive value of AFP. Receiver operating characteristic (ROC) curves were generated to identify the AFP level that had the highest accuracy in discriminating between survivors and non-survivors.**

Results: **Some 376 patients with hepatitis B virus (HBV)-associated HCC were included in the study. The overall survival rate was 58·8 per cent in patients with an AFP level of 400 ng/ml or less compared with 40·4 per cent for those with a level exceeding 400 ng/ml ($P = 0·001$). AFP concentration above 400 ng/ml was an independent risk factor for shorter disease-free and overall survival after surgery. ROC analysis indicated that the optimal cut-off values for AFP varied for different subtypes of HCC. The sensitivity and specificity were lower with areas under the ROC curve of less than 0·600. An AFP level greater than 400 ng/ml was not sensitive enough to predict the prognosis in patients with an HCC diameter smaller than 3 cm.**

Conclusion: **A serum AFP level above 400 ng/ml predicts poor overall and recurrence-free survival after hepatectomy in patients with HBV-associated HCC. AFP is not a strong prognostic marker given its poor discriminatory power, with low sensitivity and specificity.**

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Introduction

Primary liver cancer including hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality[1]. Hepatitis B (HBV) or C (HCV) virus is the major risk factor for HCC[1]. Hence, surveillance for HCC in carriers of HBV or HCV is nowadays performed with the aim of detecting HCC at an early stage. α-Fetoprotein (AFP) is a biomarker for HCC[2]. AFP is a protein produced by fetal hepatocytes, yolk sac cells, and gastrointestinal cells immediately after birth[3]. Serum AFP levels gradually decrease to less than 10 ng/ml within 300 days after birth[3]. In healthy adults, the serum AFP concentration is lower than 20 ng/ml[4]. AFP plays an important role in regulation of oncogenic and ontogenetic growth[2]. Although an early study[5] indicated that AFP and its peptide fragments inhibit oncogenic growth, recent studies[6,7] have shown that AFP promotes HCC cell growth. AFP-positive HCC has a higher cell proliferative rate than AFP-negative HCC[6], and downregulation of AFP suppresses HCC cell proliferation[7]. The use of AFP as a prognostic indicator of overall and disease-free survival following liver resection or other oncological therapy has been proposed[8–10]. Other studies[11–13] have reported that serum AFP is not a good prognostic indicator for HCC.

HBV-associated HCC is more tightly associated with a raised serum level of AFP than HCV-associated HCC

and non-virus-related liver cancer[14]. HBV-associated HCC can be further classified according to the degree of underlying liver fibrosis and cirrhosis. Therefore, to study the association between serum AFP and HCC prognosis, the different subtypes of HCC and varying underlying liver diseases should be taken into account. Another issue raised with respect to the use of serum AFP as a prognostic marker is the optimal cut-off level. Previous studies[9,11,15–17] have used different cut-off values ranging from 10 to 1000 ng/ml. The aim of this study was to assess the relationship between preoperative serum AFP level and tumour characteristics and survival in patients with HBV-associated HCC.

Methods

The design of this study followed the Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK)[18] and the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) Statement[19].

Patients

Patients who underwent hepatectomy with curative intent for HBV-associated HCC at the Prince of Wales Hospital, Hong Kong, from November 1995 to December 2012 were identified from an institutional database. Inclusion criteria were: liver function tests showing evidence of Child–Pugh grade A liver cirrhosis and clearance of indocyanine green at 15 min of less than 15 per cent; no distant metastasis; and hepatectomy with tumour-negative resection margins. Excluded were: patients with autoimmune liver disease, or serious heart, lung, kidney or blood diseases; patients with other malignant tumours; those without preoperative AFP, HBV surface antigen (HBsAg) or anti-HCV serum levels; and patients who were lost to follow-up. Patients negative for HBsAg or positive for anti-HCV, and those with portal hypertension (presence of ascites, dilated veins or varices seen during physical examination of the abdomen or anus; perioesophageal or perisplenic varices on axial imaging; platelet count below 100×10^9/l) were also excluded. At the time of surgery, all patients or their legal representatives gave written informed consent for the diagnostic evaluation, treatment and follow-up of HCC. This study was approved by the joint Chinese University of Hong Kong–New Territories East Cluster clinical research ethics committee.

Before surgery, patients underwent three-phase CT with intravenous contrast or multiphase MRI with intravenous contrast. Serum AFP levels were measured by electrochemiluminescence immunoassay (E170 Analytics; Roche Diagnostics, Indianapolis, Indiana, USA)[20]. The AFP data included in this analysis were measurements obtained before surgery. The data on other biochemical markers, including albumin, alanine aminotransferase (ALT) and bilirubin, were retrieved from the patients' medical records. Following resection, all liver specimens were examined by a single dedicated liver pathologist who was unaware of the AFP levels and clinical outcome.

Follow-up

Patients were seen in the outpatient clinic every 3 months in the first year after surgery, every 4 months in the second year, and every 6 months thereafter; all patients were followed until 8 November 2014 unless they died earlier. Serum levels of AFP were measured serially at least every 2 months. CT or MRI images were obtained at least every 3 months during the postoperative follow-up. Tumour recurrence was diagnosed by contrast-enhanced CT or MRI. Information on deaths was obtained from the social security death index, medical records or notifications from family members. The primary endpoint was death or tumour recurrence.

Statistical analysis

Continuous data are expressed as median (range). For evaluation of basic characteristics according to AFP level, categorical variables were analysed by means of χ^2 test (or Fisher's exact test if any expected frequency was less than 1, or 20 per cent of expected frequencies were 5 or less), and continuous variables by non-parametric Wilcoxon rank-sum test. Linear associations were evaluated using Spearman's or Pearson's correlation coefficients.

Overall survival was defined as time from operation to death or until 8 November 2014, the latest date of follow-up. Disease-free survival was defined as the time from curative hepatectomy to the first recurrence of disease, either locoregional recurrence and/or distant metastases. The Kaplan–Meier method was used to calculate overall and disease-free survival, and the log rank test to assess differences between survival curves. Based on recommendations from the literature[21], AFP cut-off values of 20, 200 and 400 ng/ml were used to assess the relationship between AFP levels and survival and recurrence. Univariable and multivariable Cox proportional hazards regression was performed to evaluate the predictive value of AFP and clinicopathological features for survival, including age, sex, tumour diameter, number of tumour lesions, presence of liver cirrhosis, preoperative serum ALT, bilirubin and albumin levels, histological differentiation and macroscopic vascular invasion; variables in the final model were

Table 1 Association of serum α-fetoprotein level with main demographic and clinical characteristics, survival and recurrence

		AFP level		
	All patients ($n = 376$)	≤ 400 ng/ml ($n = 266$)	> 400 ng/ml ($n = 110$)	P§
Age (years)*	55 (27–84)	57 (34–84)	54 (27–81)	0·121¶
Sex ratio (M : F)	321 : 55	228 : 38	93 : 17	0·770
HCC diameter (cm)*	3·7 (1–15)	3·3 (1·1–15)	5·2 (1–15)	0·137
No. of lesions				0·559
Single	301 (80·1)	215 (80·8)	86 (78·2)	
Multiple	75 (19·9)	51 (19·2)	24 (21·8)	
Alanine aminotransferase (units/l)*	64·2 (11–227)	61·0 (15–224)	66·6 (11–227)	0·495¶
Bilirubin (μmol/l)*	10 (3–27)	10 (4–27)	11 (3–27)	0·932¶
Albumin (g/dl)*	40 (28–49)	40 (28–49)	40 (28–49)	0·844¶
HBV DNA (copies/ml)†				0·630
$\leq 10^5$	77 of 159 (48·4)	58 of 117 (49·6)	19 of 42 (45)	
$> 10^5$	82 of 159 (51·6)	59 of 117 (50·4)	23 of 42 (55)	
Histological differentiation				0·050
Well differentiated	52 (13·8)	43 (16·2)	9 (8·2)	
Moderately differentiated	267 (71·0)	188 (70·7)	79 (71·8)	
Poorly differentiated	57 (15·2)	35 (13·1)	22 (20·0)	
Macroscopic vascular invasion				0·010
No	301 (80·1)	222 (83·5)	79 (71·8)	
Yes	75 (19·9)	44 (16·5)	31 (28·2)	
Died within 5 years of resection‡	109 of 239 (45·6)	69 of 171 (40·4)	40 of 68 (59)	0·010
Recurrence within 5 years of resection‡	85 of 239 (35·6)	54 of 171 (31·6)	31 of 68 (46)	0·041

Values in parentheses are percentages unless indicated otherwise; *values are median (range). †Data missing for 217 patients. ‡Only 239 patients were followed up for more than 5 years. AFP, α-fetoprotein; HCC, hepatocellular carcinoma; HBV, hepatitis B virus. §χ^2 test, except ¶Wilcoxon rank-sum test.

selected by using the least absolute shrinkage and selection operator (LASSO) method[22]. Receiver operating characteristic (ROC) curves were generated to identify the AFP value that had the highest accuracy in discriminating between survivors and non-survivors. The optimal cut-off for AFP was the value that provided the highest sensitivity and specificity[23]. This value might vary for different types of HCC, so the significance of serum AFP levels was also studied in different subgroups. The cut-off values for serum ALT, albumin, bilirubin and viral load were 80 units/l, 35 g/l, 20 μmol/l and 10^5 copies/ml respectively, based on previous studies[24,25]. Statistical analysis was performed using SPSS® version 16.0 (IBM, Armonk, New York, USA), R version 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria) and MedCalc® (MedCalc Software, Mariakerke, Belgium). Two-tailed $P < 0·050$ was considered statistically significant. Multiple imputation was used to impute missing values. Iterative rounds of imputation ($n = 25$) were performed using the mi command in Stata® version 12.0 (StataCorp, College Station, Texas, USA).

Results

Some 571 patients underwent hepatectomy for HCC. Excluded were 99 patients who were HBsAg-negative or anti-HCV-positive, and 77 patients with missing data on AFP levels, HBsAg or lost to follow-up. Some 14 patients were excluded as they had Child–Pugh grade B or C liver cirrhosis, as were five other patients with portal vein hypertension. *Table 1* summarizes the characteristics of 376 patients included in the study. The median follow-up time was 106·3 (24–215) months. At the last date of follow-up, 194 patients (51·6 per cent) had died and 191 (50·8 per cent) had recurrence of disease.

The distribution of AFP levels was skewed, ranging from 1 to 699 800·0 (mean 7372·9, median 52·0) ng/ml, and levels were not normally distributed after repeated logarithmic transformation (*Fig. 1*). The AFP level was increased in tumours that had vascular invasion compared with lesions with no vascular invasion. There was no association between AFP and tumour size (3 cm or less *versus* more than 3 cm) or presence of liver cirrhosis (*Fig. 1*).

Overall and disease-free survival curves according to predefined AFP levels (cut-off values 20, 200 and 400 ng/ml) are shown in *Fig. 2*. Overall survival at 5 years was 58·8 per cent for patients with an AFP concentration of 400 ng/ml or less *versus* 40·4 per cent for those with a level over 400 ng/ml ($P = 0·001$). Patient and tumour characteristics according to AFP level (400 ng/ml or less *versus* over 400 ng/ml) are shown in *Table 1*.

Univariable analyses showed that a serum AFP level above 400 ng/ml was associated with an increased risk

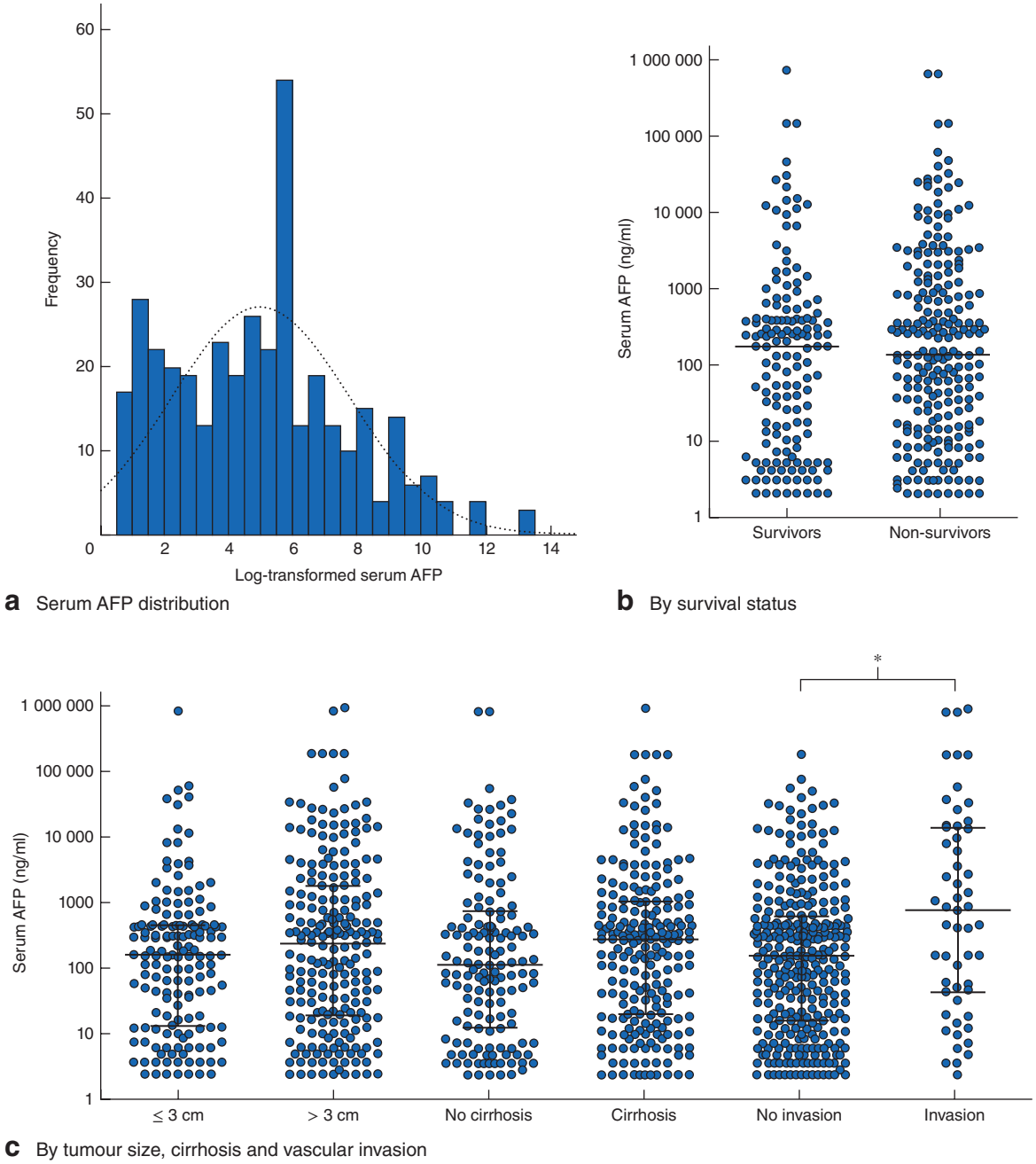
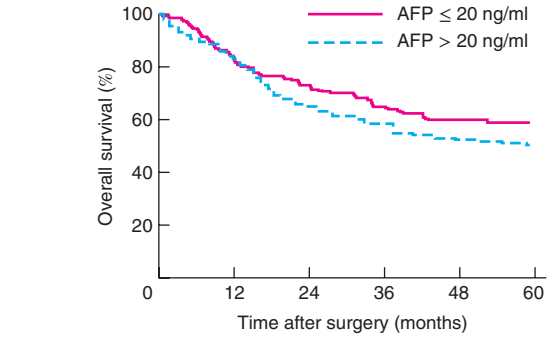


Fig. 1 **a** Log-transformed serum α-fetoprotein (AFP) levels in the study cohort (376 patients), and serum AFP levels (not transformed) subdivided according to **b** survival status and **c** tumour size, presence of cirrhosis and presence of vascular invasion. Data for individual patients and median (range) values are shown in **b** and **c**. *$P = 0{\cdot}001$ (Wilcoxon rank-sum test)

of death and recurrence (*Tables S1* and *S2*, supporting information). In the multivariable analysis, AFP remained significantly associated with overall (hazard ratio 1·79, 95 per cent c.i. 1·24 to 2·56) and recurrence-free (hazard ratio 1·67, 1·19 to 2·37) survival.

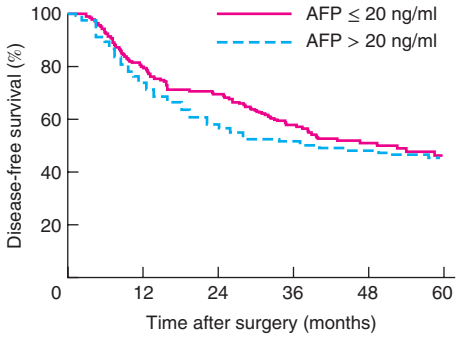
Among the 155 patients with HCC and a tumour diameter of 3 cm or less, there was no difference in overall survival between those with an AFP level greater than 400 ng/ml and patients with a lower AFP level ($P = 0{\cdot}591$) (*Fig. 1a*, supporting information). Among the 221 patients



No. at risk

≤ 20 ng/ml	168	136	121	85	59	43
> 20 ng/ml	208	170	132	114	95	82

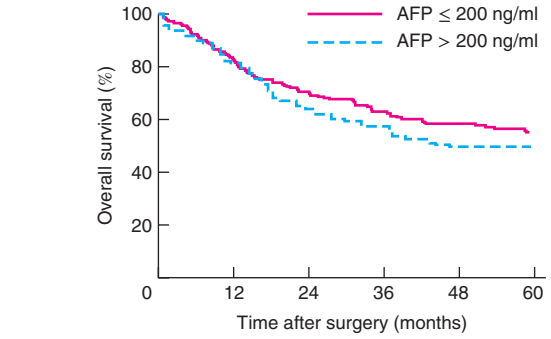
a Overall survival, AFP cut-off 20 ng/ml



No. at risk

≤ 20 ng/ml	168	126	110	76	50	31
> 20 ng/ml	208	137	104	88	73	61

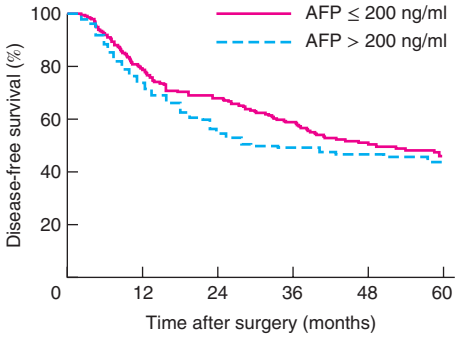
b Disease-free survival, AFP cut-off 20 ng/ml



No. at risk

≤ 200 ng/ml	226	184	158	119	91	71
> 200 ng/ml	150	122	95	80	63	54

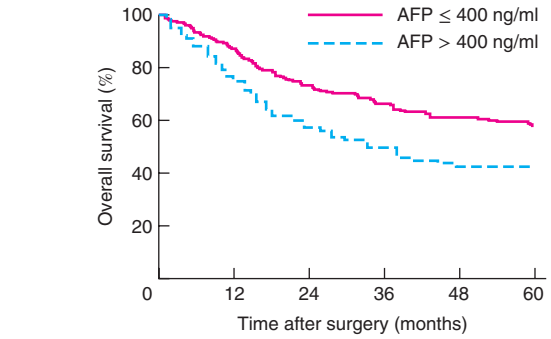
c Overall survival, AFP cut-off 200 ng/ml



No. at risk

≤ 200 ng/ml	226	164	140	104	74	53
> 200 ng/ml	150	99	74	60	49	39

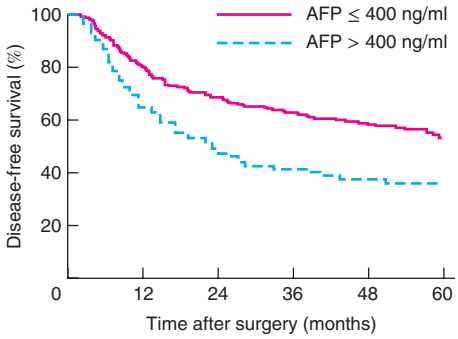
d Disease-free survival, AFP cut-off 200 ng/ml



No. at risk

≤ 400 ng/ml	226	225	192	151	119	97
> 400 ng/ml	110	81	61	48	35	28

e Overall survival, AFP cut-off 400 ng/ml

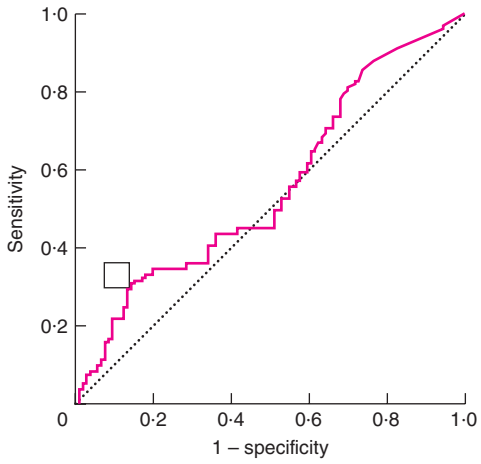


No. at risk

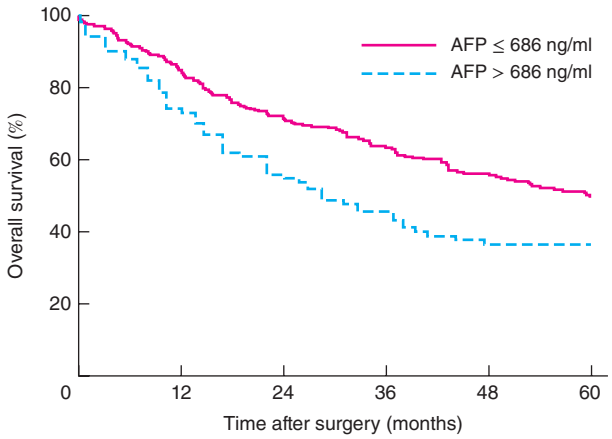
≤ 400 ng/ml	226	199	168	130	98	75
> 400 ng/ml	150	64	46	34	25	17

f Disease-free survival, AFP cut-off 400 ng/ml

Fig. 2 Kaplan–Meier analysis of **a**,**c**,**e** overall and **b**,**d**,**f** disease-free survival in 376 patients stratified according to serum α-fetoprotein (AFP) level, with a cut-off value of **a**,**b** 20 ng/ml, **c**,**d** 200 ng/ml and **e**,**f** 400 ng/ml. **a** $P = 0{\cdot}085$, **b** $P = 0{\cdot}301$, **c** $P = 0{\cdot}161$, **d** $P = 0{\cdot}252$, **e** $P = 0{\cdot}001$, **f** $P = 0{\cdot}001$ (log rank test)



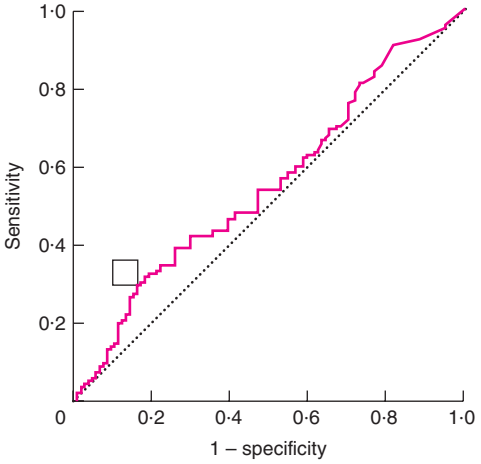
a ROC curve, overall survival



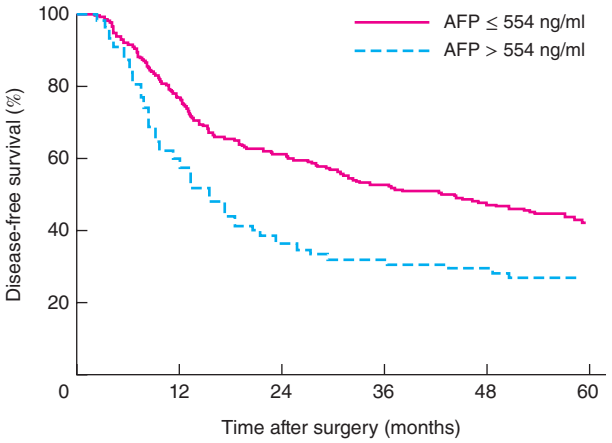
No. at risk

≤ 686 ng/ml	227	234	199	158	126	104
> 686 ng/ml	99	73	55	41	28	20

c Overall survival curves



b ROC curve, disease-free survival



No. at risk

≤ 554 ng/ml	266	187	145	110	90	75
> 554 ng/ml	110	57	35	26	22	17

d Disease-free survival curves

Fig. 3 Receiver operating characteristic (ROC) curves showing the accuracy of serum α-fetoprotein (AFP) level for discriminating between **a** survivors and non-survivors and **b** patients with and without recurrence. The open square represents the best cut-off value of serum AFP (686 ng/ml for overall survival, 554 ng/ml for disease-free survival). **c**,**d** Kaplan–Meier analysis of **c** overall survival and **d** disease-free survival according to appropriate AFP cut-off level. **c** $P = 0{\cdot}002$, **d** $P = 0{\cdot}001$ (log rank test)

with tumours larger than 3 cm, an AFP level exceeding 400 ng/ml was significantly associated with poor overall survival ($P = 0{\cdot}001$) (*Fig. 1b*, supporting information).

ROC curves were generated from the whole cohort to identify the AFP value with highest accuracy in discriminating survivors and non-survivors, and patients with and without recurrence. The area under the ROC curve (AUROC) for serum AFP predicting survival and recurrence was 0·558 (95 per cent c.i. 0·484 to 0·631) (*Fig. 3a*). An AFP cut-off level of 686 ng/ml identified by the ROC curve had good specificity (87·3 (95 per cent c.i. 77·4 to 92·2) per cent) but low sensitivity (30·9 (23·0 to 38·8) per cent). The AUROC for AFP predicting recurrence was 0·556 (0·483 to 0·628) (*Fig. 3b*) and a cut-off

of 554 ng/ml was identified. This also had good specificity (81·4 (73·3 to 89·2) per cent) but low sensitivity (34·1 (26·2 to 43·3) per cent). Serum AFP levels greater than 686 and 554 ng/ml were significantly associated with worse overall and disease-free survival respectively (*Fig. 3c*, *d*). The optimal cut-off points for AFP varied among different subgroups, ranging from 4 to 7720 ng/ml, and the AUROC ranged from 0·503 to 0·704 (*Table S3*, supporting information).

The AUROC, sensitivity and specificity of AFP for predicting survival and recurrence were significantly lower than those of macroscopic vascular invasion and number of tumour lesions (*Table S4*, supporting information). Unlike AFP level, macroscopic vascular invasion and number of tumour lesions were related to the prognosis not only of patients in the whole cohort but also of those in different subgroups.

Discussion

The identification of accurate predictors of prognosis is of clinical importance for defining treatment strategies in patients with HCC. Measurement of serum AFP levels is simple, and has been used widely for surveillance and diagnosis of HCC[26]. It has even been proposed that preoperative serum AFP level should be included in the Barcelona Clinic Liver Centre staging guidelines for clinical treatment of HCC[10]. In present study, the relationship between preoperative serum AFP level and prognosis of HCC in a cohort of patients with HBV-related HCC was examined. Overall and disease-free survival was compared between patient groups using different cut-off values of AFP, including 20, 200 and 400 ng/ml, but only a value of 400 ng/ml discriminated in overall and recurrence-free survival. Patients with an AFP level over 400 ng/ml had tumours with a higher risk of vascular invasion. Multivariable Cox regression analyses identified an AFP level exceeding 400 ng/ml as an independent prognostic predictor for overall and disease-free survival, consistent with the findings of Wang and colleagues[27]. However, an AFP concentration above 400 ng/ml was not a powerful marker for predicting overall survival in patients with small HCCs. The optimal cut-off value of AFP identified by ROC analysis was 9 ng/ml for small HCC. The reasons for the discrepancies regarding cut-off values of AFP for the prediction of different subtypes of HCC remain complex and unexplained.

The prognosis of patients with HCC was associated with many variables, including tumour size, grade of differentiation and co-existing liver disease; therefore, the optimal cut-off value might vary for different subtypes of HCC. The present study showed by ROC curve analysis that there were differences in the optimal cut-off levels of AFP for subgroups, ranging from 4 to 7720 ng/ml. Most of the AUROC values were less than 0·600, indicating poor discriminatory ability, and the sensitivity and specificity of the tests were low. The finding that AFP is a weak prognostic indicator for patients with HCC has already been reported by Huo and co-workers[28]. In that study, among patients undergoing surgery for HCC, a preoperative serum AFP level exceeding 400 ng/ml was predictor of recurrence and survival, independent of tumour size. However, there was no association between AFP level and survival in patients who did not have surgical treatment of HCC. Tangkijvanich and colleagues[29] also reported that AFP should not be used as a sensitive tumour marker, particularly for early-stage HBsAg-negative HCC.

This study has some limitations. The selection criteria for enrolment in the study were very strict. Hence, only 376 patients with HBV-related HCC were included, which makes the sample size relatively small for subgroup analyses. At the same time, a homogeneous group of patients with complete data was generated, which improves the internal validity of the present findings. This study suggests that serum AFP is not a powerful prognostic predictor for patients with HBV-associated HCC; however, preoperative serum AFP is easily measured and, when used in combination with pathology, imaging studies or other serum markers, may help in the assessment of prognosis after surgery.

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Supporting information

Additional supporting information may be found in the online version of this article:

Fig. S1 Kaplan–Meier analysis of overall and disease-free survival in patients stratified by tumour size (3 cm or less, more than 3 cm) and by serum α-fetoprotein level (400 ng/ml or less, more than 400 ng/ml) (Word document)

Table S1 Cox proportional hazard regression analysis of overall survival (Word document)

Table S2 Cox proportional hazard regression analysis of disease-free survival (Word document)

Table S3 Receiver operating characteristic (ROC) curve analysis for the performance of serum α-fetoprotein levels in subgroups of patients with hepatocellular carcinoma (Word document)

Table S4 Predictive value of α-fetoprotein levels, number of lesions and macroscopic vascular invasion for survival in patients with hepatocellular carcinoma (Word document)