Original Article

The Use of Alpha-fetoprotein for Detecting Hepatocellular Carcinoma May Potentially be More Beneficial for Patients with Aggressive Hepatocellular Carcinomas and High Alpha-fetoprotein Levels

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Objective: There is some controversy as to whether alpha-fetoprotein [AFP] should be used in screening for hepatocellular carcinoma [HCC] or not.

Materials and Methods: Data from our hospital HCC registry 2010 to 2014 were retrospectively analyzed. Asymptomatic patients with HCC who were diagnosed by AFP alone (where ultrasound [US] did not detect a lesion or trigger a diagnostic test) were compared with patients detected by US.

Results: Out of 314 patients in the registry, 43 patients were diagnosed without symptoms. US detected 33 patients with HCC, while 10 patients (equal to 23.3% of the total or 30% additional patients) were detected by AFP alone. These patients were younger, had higher median AFP level and were at a better BCLC stage than those detected by US. The treatment outcomes for these patients were no different from the US detected patients, whether in terms of death, local tumor control or recurrence.

Conclusion: The use of AFP in addition to US allowed earlier detection in 30% more patients than if US was used alone. These patients had higher AFP but achieved the same outcomes as those in the US detected group who had lower AFP. The use of AFP in screening may therefore be of particular benefit in terms of early detection and treatment for those patients with the more aggressive HCC with high AFP.

Keywords: Alpha-fetoprotein, Hepatocellular carcinoma, Screening, Survival, Tumor marker

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Hepatocellular carcinoma [HCC] is a common cause of cancer death in hepatitis B [HBV] endemic areas such as China, Thailand and much of Asia. Patients often present late with advanced cancer and generally do not have long survival. Surveillance for HCC appears to reduce mortality(1) but the optimal strategy for surveillance is still uncertain. Previous screening recommendations suggested using both ultrasound US) and alpha-fetoprotein [AFP] levels for screening HCC. However, the most recent guideline of the European Association for the Study of the Liver [EASL](2) and the 2011 guideline from the American Association for the Study of Liver Diseases [AASLD](3) discarded using AFP test for HCC screening due to poor sensitivity and specificity of the AFP test. When combined with US, AFP levels were said to able to provide an additional detection in only 6% to 8% of cases not previously identified by US(4,5). More recently,

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the 2017 AASLD guideline has re-introduced AFP as a screening test to be used optionally in combination with US[6].

Many other studies have supported the complimentary use of AFP with US in improving the effectiveness of HCC surveillance[6,7]. A retrospective study of 111 HCC cases in UK reported that a total of 7 out of 24 (29.1%) cases of HCC would have had their diagnosis delayed if AFP had not been used in their surveillance strategy[7]. In addition, US can overlook small HCCs, particularly in nodular and cirrhotic livers. Meta-analyses have reported a sensitivity of 60% or less for US screening suggesting that many early HCCs may be missed or have delayed diagnosis[4,8]. Moreover, AFP testing might more accurately detect HCC in different subgroups of patients and if different cut-off values are used[9,10]. These data suggest that AFP might still play an important role in HCC screening in certain sub-populations of patients with cirrhosis, if thoughtfully implemented. Another group which may particularly benefit from AFP screening is actually those with HCCs with high AFP levels. AFP has been shown to act as a prognostic factor. Many studies have documented that higher AFP levels are associated with worse post-operative outcomes, with increased and earlier recurrence[11,12], and that this may be related to a more aggressive HCC phenotype-genotype[13]. The use of AFP in screening may benefit this subgroup more than others because it would allow for earlier diagnosis and treatment in this high-risk group than if ultrasound were done on its own. In addition, other methods of using AFP as part of screening, for example using the change in the AFP levels, as a trigger for further investigations have been reported[14-17]. These new methods may improve the cost effectiveness of AFP in HCC screening.

From all of the above points, AFP still remains on some guidelines, such as the Asia-Pacific Association for the Study of the Liver [APASL] guidelines for both HCC[18] and Hepatitis B[19] and the most recent 2017 AASLD guideline[6]. Our study aimed to explore the role of AFP in HCC screening in real-life clinical practice in a HBV endemic area. We investigated to see if screening with AFP increased early detection and what the outcomes were for the group of patients whose tumors were first detected by AFP alone.

Materials and Methods

Study patients

Patients were identified from our hospital HCC registry data, during the period 2010 to 2014. The registry was started in 2010 and consecutive patients who were diagnosed with HCC and seen in the internal medicine, hepatobiliary surgery and liver clinics in Ramathibodi Hospital were prospectively entered into the registry. The clinical details, treatments and the outcomes of these patients were documented for use in research and auditing. No specific screening strategy was set for the patients recorded in the registry as patients were only entered into the registry after diagnosis. Screening, when performed, was done at the attending physicians' discretion. Ramathibodi Hospital is a tertiary referral center that receives patients from all over Thailand for treatment of their HCC. In this study we included only the HCC patients who had no symptoms, whether they were identified by regular surveillance or not. The HCC cases identified by surveillance were defined as the patients who received at least one planned 6-monthly US and/or AFP before the HCC diagnosis. Patients identified outside of surveillance included patients who had a screening test as part of a random or annual check-up unrelated to chronic liver disease or hepatology clinic.

The HCC diagnosis was made according to the AASLD guideline, with the radiological hallmark finding of arterial enhancement and venous wash-out seen in a triple phase contrast-enhanced computed tomography [CT] or magnetic resonance imaging [MRI], or both; or by histopathological diagnosis. According to our local laboratory, serum AFP was considered abnormal when >7.2 ng/mL. An abnormal US was defined as the new nodule or growth of a previous nodule from US findings.

The data from the registry was analyzed retrospectively. The definitions of the 'detection methods' were based on the test that triggered the confirmatory test for HCC diagnosis; 'AFP only'was defined as the cases who were detected by an abnormal AFP level but had a normal US; 'US only' as defined as the cases who were detected by an abnormal US but normal AFP level, and 'US and AFP' as defined as the cases who were detected by both abnormal US and AFP level at approximately the same time period.

The definition of 'local control' of the tumor was that after the detected lesion was treated, there was no viable tumor seen in the liver over a period of at least 6 months. Any HCC detected after that was deemed as recurrence.

The demographic data, etiology of liver disease, severity of liver disease (cirrhosis, Child Pugh classification), HCC detection method (US, AFP or both), diagnosis method, Barcelona liver clinic

[BCLC] staging, treatment modality and outcome were reviewed.

As we were interested in the role of AFP in HCC screening we classified the patients according to their detection method; 'AFP only', 'US only' and 'US and AFP'. We analyze the descriptive data and identified the patients whose HCC diagnosis were triggered solely by abnormal AFP (AFP only) to assess the factors that may affect the usefulness of the AFP test.

The study received ethical clearance from the local hospital Ethical committee.

Statistical analysis

Statistical analysis was performed using STATA version 14. Results were expressed as frequency, median and range, mean ± standard deviation (SD) as appropriate. Categorical variables were compared using Pearson Chi-square or Fisher's exact test. Continuous variables were compared using the two-tailed student t-test. The cumulative probability of mortality was calculated using the Kaplan-Meier method. Statistical significance was assumed when *p*-value <0.05.

Results

During 2010 to 2014, there were 314 patients in the registry, of these, 43 patients were diagnosed with HCC without symptoms. Thirty-seven (86%) were identified from regular surveillance, whilst the rest were identified at random or yearly check-up. There were 10 (23.3%) patients whose HCC diagnosis was triggered solely by an abnormal AFP, and the numbers of HCC patients according to detection methods are shown in Table 1. The mean duration of follow-up of the patients in the study was 4.5±2.3 years.

The baseline characteristics of HCC patients who were detected by AFP only, versus US with or without AFP (US ± AFP) are shown in Table 2. The major etiology of liver disease was HBV infection, which was found in 60% in the AFP only group vs. 51.5% in the US ± AFP group. The mean age of patients in the AFP only group was significantly less than the US ± AFP group (58.9 years vs. 66.6 years). In the AFP only group, the HCCs were also detected in an earlier Barcelona Clinic Liver Cancer [BCLC] tumor classification stage, with 60% in stage 0 and 40% in stage A for the AFP only group vs. 12% and 66.7% in the US ± AFP group, respectively. Patients in the AFP only group were also all within the Milan criteria at the time of diagnosis, compared to 79% in the US ± AFP group, but this did not reach statistical significance. The mortality and local control of the HCC between two groups were not different. Although recurrence appeared to occur more frequently in the US ± AFP groups (37.5% vs. 56%), this did not reach statistical significance.

One patient was classified as Child-Pugh (C-P) class C, although at the time of the initial detection of the liver tumor he was calculated to be in C-P class B. His liver disease had progressed by the time the diagnosis was confirmed to be HCC. One patient was found to have an 11 cm HCC at annual check-up. Another patient was diagnosed with an 8 cm tumor after it had been missed in a prior CT colonography and screening was restarted after an interval of 1 year, at which point the tumor was detected by both US and AFP. Two patients were screened but ended having an 18 and 19 month interval between tests and were found to each have a 5 cm and 4.8 cm HCC, respectively. Another patient was found to have a 4.9 cm HCC, but the interval between the screening was 6 months.

The method of diagnosis was histological in 23 patients, 13 of the remaining patients had HCC larger than 2 cm and typical contrast-enhanced imaging characteristics with arterial enhancement and venous wash out on a background of liver disease, and the last remaining 7 patients had HCC size between 1 to 2 cm, with typical enhancement patterns on CT or MRI. Follow-up of these 7 patients showed that 4 patients had recurrence and 3 patients remained recurrence-free at the end of the study period. In two of these 3 patients who were recurrence-free at the end of the study, there were new small HCCs arising within the study period, but these were fully controlled with radiofrequency ablation during the study period.

Seven patients (70%) who were in the AFP only group achieved local tumor control after treatment. Three patients subsequently developed recurrence in the liver and two patients (20%) died by the end of the study. For the group whose HCCs were detected by US, 25 (75.8%) achieved local tumor control after treatment, 14 (56%) had recurrence and 11 patients (33%) had died by the end of the study. The comparison of the survival probabilities between the two groups is shown in Figure 1. No significant difference was seen between the two groups in terms of survival.

Discussion

In Thailand, and in many other countries where HBV is endemic, HCC is detected at a late and often at an untreatable stage[20-22]. Screening is therefore

important in detecting patients early when the tumor is more amenable to treatment. However, it remains unclear as to how best to screen for HCC in patients with chronic liver disease. At one stage some Western guidelines have suggested that AFP testing should not be used as it is not accurate enough and adds little over the detection by ultrasound alone[2,3]. More recently, the American guideline has re-introduced AFP as an optional supplementary screening test to be performed with ultrasound[6]. In this study, we did not focus on calculating the cost-effectiveness of the test but investigated the proportion of patients found by AFP alone, looking at their clinical characteristics and survival. We looked at the performance of AFP testing in the real-world where the definition of an abnormal test may not be the absolute value and the follow-up of a screening test do not always follow the guidelines perfectly.

In our study, patients who were detected by AFP alone accounted for 23% of the asymptomatic HCCs, or an additional 30% above the number who were detected by US. This is similar to the 29% of those under surveillance who were detected by AFP alone in a recent UK study[7]. These results which show a significant detection add-on by AFP may reflect the lower sensitivity the US has in the real-life busy clinical practice, where time pressures and lack of resources may impinge on the test's accuracy. The tumor size at detection was not statistically different between the groups but patients with HCC detected by AFP alone were younger and were found at an earlier BCLC stage (p-value <0.05). All the patients detected by AFP alone were also found to have tumors within the Milan criteria.

HCC with higher AFP are thought to be more aggressive and have been reported to have a poorer prognosis after surgery[23,24]. This is thought to be related to the genotypic difference in HCC with high AFP levels. Although historically HCC with high AFP levels have been shown to have worse outcomes, independent of size and number of tumors, and to be associated with poorly differentiated tumors[25], only recently has this been reported to be related to certain genetic changes. Molecular classification of HCC has shown that high AFP levels correlate with a S2 or hepatoblastoma phenotype with changes in the activation of the MYC, AKT and IGF2 genes, and downregulation of IFN-related genes[13], as well as having more chromosomal alterations in 1q, 8p, 13q, 16q, 17p and 17q[26]. More recently AFP has been associated with the expression of migration and metastasis-enhancing proteins, including CXC motif chemokine receptor 4 [CXCR4], keratin 19 [K19], epithelial cell adhesion molecule [EpCAM] and matrix metalloproteinase 2/9 [MMP2/9][27]. AFP has also been shown to be more prevalent in HBV associated HCCs, and together with the AFP receptor, may be up-regulated by HBV x protein[28].

The median AFP level in the AFP only group was also higher than those detected by US, whilst the tumor size was the same if not smaller in the AFP only group. This suggests that the AFP only group may have been comprised with patients who would have progressed on to have more aggressive HCC with higher AFP and consequently have poorer outcomes even after surgery. However, in contrast to previous studies showing worse outcomes for patients with higher AFP levels, the final outcomes of the patients detected by AFP only in our study were not worse than those detected by US, as determined by local control, recurrence or mortality, and in fact, they were slightly better, although this did not reach statistical significance.

A previous study had suggested that in contrast to large HCC, HCCs less than 3 cm in size with high AFP levels do not seem to have a worse prognosis when treated early, when compared to HCCs with low

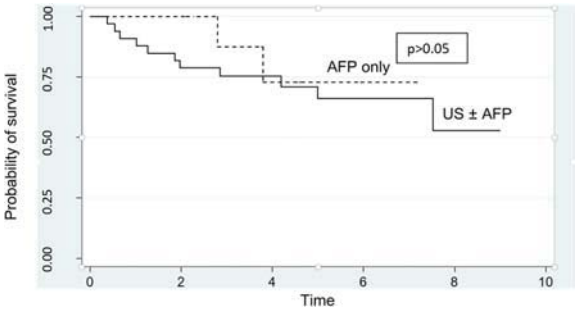

Figure 1. Survival probabilities of patients detected by ultrasound (US, solid line) vs. alpha-fetoprotein [AFP] only (dashed line). The survival was indifferent (p>0.05).

Table 1. Number and detection methods of 43 asymptomatic hepatocellular carcinoma patients

Detection methods	Number (%)
AFP only	10 (23.3)
Ultrasound only	16 (37.2)
Ultrasound and AFP	17 (39.5)

AFP = alpha-fetoprotein

Table 2. Patient characteristics of hepatocellular carcinoma cases who were detected by alpha-fetoprotein (AFP) only versus ultrasonography with/without AFP

	AFP only (n = 10)	US ± AFP (n = 33)	*p*-value
Male, n (%)	7 (70)	24 (72.7)	1.000
Age, mean ± SD	58.9±9.4	66.6±7.8	0.013
BMI, mean ± SD	23.4±3.6	25.1±3.5	0.209
Causes of liver disease, n (%)			
HBV	6 (60)	17 (51.5)	
HCV	4 (40)	8 (24.2)	
HBC/HCV coinfection	0	2 (6.1)	0.937
NBNC	0	3 (9.1)	
Alcohol	0	3 (9.1)	
Cirrhosis, n (%)	9 (90)	29 (87.9)	1.000
Child-Pugh Class, n (%)			
A	9 (100)	23 (79.3)	
B	0	5 (17.2)	0.471
C	0	1 (3.5)	
AFP at detection (ng/dL), median (range)	54.7 (12 to 9,029)	8.01 (1.27 to 23,177)	0.024
Total bilirubin (mg/dL), median (range)	1.1 (0.4 to 1.3)	0.7 (0.3 to 3.4)	0.181
Albumin (g/dL), mean ± SD	3.8±0.4	3.6±0.7	0.535
Platelet (x10^3), median (range)	131.5 (65 to 220)	127 (45 to 364)	0.752
Maximum tumor size (cm), median (range)	1.7 (0.6 to 4.8)	2.75 (0.9 to 11.3)	0.159
BCLC stage, n (%)			
0	6 (60)	4 (12.1)	
A	4 (40)	22 (66.7)	
B	0	3 (9.1)	0.046
C	0	3 (9.1)	
D	0	1 (3)	
Within Milan criteria, n (%)	10 (100)	26 (78.8)	0.172
Death, n (%)	2 (20)	11 (33.3)	0.696
Local tumor control, n (%)	7 (70)	25 (75.8)	0.698
Recurrent tumor, n (%)	3 (37.5)	14 (56)	0.438

AFP = alpha-fetoprotein; BCLC = Barcelona Clinic Liver Cancer staging; BMI = body mass index; HBV = hepatitis B virus; HCV = hepatitis C virus; NBNC = non-B non C hepatitis; US = ultrasound

AFP levels[29]. However in contrast to our study, this study had patients whose etiology were predominantly HCV. If we believe that HCCs which produces high levels of AFP when small grow to be more aggressive tumors, as suggested by the molecular evidence described above, then our results would seem to suggest that the use of AFP in screening not only detects a significant proportion of HCC earlier, but that this group of HCCs are also those at risk of having worse outcomes if the HCCs are allowed to grow to a larger size. This difference in outcome between high and low AFP has been reported even in patients who have surgery[10] and even if the tumors only grow as large as 5 cm[30].

It is interesting to note that in our study, 6 patients who had the highest AFP levels were patients with HCV and not HBV. This was despite the largest tumor in this group being only 4.6 cm while the other five were 3.1 cm or smaller. Of the 6 patients, 2 patients had 2 tumors at the time of diagnosis, and 4 had single tumors. It is not clear why HCV patients in our cohort had higher levels of AFP than patients with HBV. Although studies have shown that AFP is a prognostic marker for HCC in patients with hepatitis C[31] similar to in HBV, AFP has also been demonstrated to be associated with aldo-keto reductase family 1 member B10 [AKR1B10] over-expression which is an early event in the hepatocarcinogenesis in HCV related HCC[32,33]. The exact mechanism for the association between AFP and AKR1B10 is not clear, but AKR1B10 is an oxidoreductase and has previously been detected in other cancers. It is thought to inhibit retinoic acid

signaling which regulates cell differentiation and therefore its over-expression promotes premature and neoplastic cells[34].

The standard cut-off level for AFP to diagnose HCC is 200 ng/mL. However the level used in screening HCC varies, and the optimum level has been suggested to be 20 ng/mL and 59 ng/mL in non-HCV and HCV cases, respectively[9]. However more recently, it has been suggested that the absolute level of AFP may not be the most effective marker to use but the change in AFP level should be used instead to trigger the diagnostic testing[14-16]. Unfortunately in our study it was not clear what stimulated the attending doctors to send the patients for the diagnostic tests, but it can be seen that some of the patients had levels below 20 ng/mL when they were tested, suggesting that in real-life the change in AFP level may already be used in screening. This would suggest that previous cost-effective analyses performed using absolute levels of AFP to say whether HCC would be detected or not, may need to be revisited.

Our study had patients whose HCCs were larger than that would be expected by screening. Due to the inclusion of patients whose HCCs were detected at annual check-up as well as real-life surveillance patients, our patients had a wide range of HCC sizes. Symptoms from HCC may not occur until the tumor is large and this explained the presence of an 11 cm tumor which was detected at annual check-up. As HCC is a common cause of cancer death in Thailand, AFP and US are sometimes performed in the private clinics as part of a check-up package. In addition to check-ups, the other reason for finding large HCC is that surveillance in real-life is often not as frequently or strictly performed as recommended by the international guidelines[35,36]. This also occurred in our practice and may have allowed some patients to develop larger tumors than expected.

Another problem in Thailand, and in many other resource-limited countries, is that only a small proportion of the at-risk patients undergo screening with US. This is similar to other more developed countries where the uptake in screening is unsatisfactory[37]. The reasons for this are multiple, but include patients' lack the awareness of their own risk, as well as the cost and resources needed for performing an ultrasound test. This then results in the low numbers of patients who undergo curative treatment, as seen in our registry or in other reports in the literature[20,22]. The cost of an AFP test is 200 to 270 baht (equivalent to approximately 6 to 8 USD) compared to an ultrasound test which costs 800 to 1,200 baht (equivalent to 23 to 35 USD) as well as the need for trained personnel to operate the US. So in practical terms there may be a group of at-risk people in HBV endemic countries who can access and afford AFP testing whilst they cannot with regular US (where the tests are not reimbursed by insurance).

Our study is limited by the low number of patients and the retrospective nature of the study using data from a tertiary hospital HCC registry. These limitations may make it difficult to generalize the findings of this study on AFP in general. As such, the concept that AFP use in screening may be of particularly benefit to those patients with aggressive HCCs with high AFP levels may need to be confirmed with a larger cohort in a HBV endemic area in a prospective manner. Nevertheless, this aspect of the benefit of using AFP in screening, namely that it may particularly useful for those patients with the aggressive high AFP phenotype,who would do worse when detected later with US, has not been fully investigated previously.

The results of our study suggested that AFP testing helps to detect an additional 30% of patients at an earlier stage than if US was used alone.These patients have a higher AFP level than those who were detected by US (where the latter group also included those who had elevated AFP levels). These patients who were detected by AFP alone, with potentially more aggressive HCC, had equal if not better outcomes after treatment, compared to those detected with US, and therefore this is one more reason to support the continued use of AFP testing for screening in HBV endemic countries.

Conclusion

The addition of AFP to US for screening HCC detected an additional 30% of patients at an earlier stage when compared to US detection.The use of AFP in screening may be of particular benefit for those with high-risk tumors with elevated AFP because it allows for earlier detection and treatment, achieving outcomes comparable to HCC with lower AFP levels.

What is already known on this topic?

HCC is a common cause of cancer death worldwide. This is because it is often detected late. Screening has been suggested for early diagnosis and to improve outcomes. Currently there is a debate whether to include AFP testing as part of the screening process. Not all HCC produce AFP. The subgroup of

HCC that produce high AFP levels have worse outcomes whether they have curative resection or palliative treatments.

What this study adds?

This study shows that using AFP as part of screening detects an extra 30% of HCC patients at an early stage. At the time of detection this group who were detected by AFP alone had a higher median AFP level and were younger with no other difference in tumor stage compared to those detected by US. However the treatment outcomes, whether determined by local disease control, recurrence or survival were no different to the other group that had the lower AFP level. Therefore using AFP as part of screening may be of particular benefit for patients with aggressive HCC and high AFP production, who may have poorer outcomes if they are detected at a later time using US alone.

Potential conflicts of interest

None.

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