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Review Article

# Persistently Rising Alpha-fetoprotein in the Diagnosis of Hepatocellular Carcinoma: A Review

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## Abstract

Hepatocellular carcinoma (HCC), one of the most common malignant tumors worldwide, is known for its grim prognosis, with untreated life expectancy being only a matter of months after the diagnosis. The difficulty in making a diagnosis early is one of the main contributing factors to the poor prognosis. Alpha-fetoprotein (AFP) had long been used as a surveillance tool, but suboptimal specificity and sensitivity has prompted liver societies to abandon the recommendation for its universal use, even in combination with ultrasonography. Most studies have shown no obvious correlation between serum AFP level and HCC tumor size, stage, or survival post-diagnosis. However, some studies concluded that a gradual rise or persistent elevation in AFP were positive predictors for tumor development. Other studies reported a fall in AFP followed by a rise in patients with HCC as well as persistently rising AFP levels without development of HCC on follow up. Our calculation of the sensitivity and specificity of persistently rising AFP for HCC were both low, at 60% and 35.8%, respectively, indicating that the presence of persistently rising AFP per se did not offer diagnostic benefit. In addition, our calculated mean slopes of persistently rising AFP levels in HCC and non-HCC patients were numerically very different, but the difference was not statistically significant. We conclude that the published data do not support a role for rising AFP levels per se in the diagnosis of HCC.

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## Introduction

Hepatocellular carcinoma (HCC) most commonly develops in patients with chronic liver disease and cirrhosis.[1,2] However, it often causes few symptoms until it becomes very large, and at that stage is frequently associated with poor long-term outcome.[3] Therefore, much research has been directed at early detection through screening.[3] After the discovery that alpha-fetoprotein (AFP) was secreted by HCC, screening for serum AFP was recommended as surveillance for HCC generally and in combination with abdominal ultrasound in high-risk populations.[4] This strategy was attractive because it is inexpensive and readily available, but many studies have subsequently reported poor specificity and sensitivity when AFP is used alone.[5] Serum AFP levels are normal in 30–40% of patients with HCC.[6] The American Association for the Study of Liver Diseases made AFP optional in its recommendation for screening with ultrasonography, while the European Association for the Study of the Liver abandoned AFP altogether in recommending ultrasonography alone.[7,8] Part of the problem in terms of specificity lies in the fact that AFP is also released by normal liver in the presence of inflammation and hepatocyte damage. Because most HCCs develop in cases of chronic liver disease with waxing and waning inflammatory changes, in order to distinguish HCC from background hepatitis, high diagnostic cut-off levels of AFP were required, generally in the 400–500 ng/mL range. Accordingly, many HCCs did not achieve these diagnostic levels until late stages, if at all, and this has contributed to the reported lack of sensitivity.

## General review

AFP is an oncofetal glycoprotein synthesized in high concentrations during fetal development.[9] While its production decreases markedly after birth, small amounts are still produced in the adult.[10] AFP function is unclear, but some studies have suggested immunoregulatory properties and effects on cellular growth.[10] AFP concentrations may increase with hepatocyte regeneration and proliferation during progression to cirrhosis, flare-up of hepatitis or during the recovery phase.[9] Increase in AFP levels has also been observed in pregnancy, hepatomas, teratomas, and malignancies of primitive gut origin.[6] Elevated AFP in maternal serum or amniotic fluid during pregnancy is used as a diagnostic tool for detection of fetal abnormalities, such as neural-tube defects.[10] Additionally, AFP has been used as a marker for HCC and germ cell tumors.[9] Markedly elevated AFP is usually associated with HCC, while moderate elevation of AFP to less than 150 ng/mL usually signifies acute or chronic viral hepatitis and cirrhosis.[11]

**Keywords:** Alpha-fetoprotein, AFP; Hepatocellular carcinoma, HCC; Cancer screening.
**Abbreviations:** AFP, alpha-fetoprotein; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma.
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The cause of elevated AFP in hepatitis in unclear, but current theories include injury sequelae, change in hepatocyte structure and stromal interaction, hepatocyte necrosis, and regeneration. On the other hand, increases in AFP levels with HCC are likely secondary to a greater cell turnover, regeneration, tumor necrosis, and cell ischemia. A meta-analysis of 29,898 articles on AFP diagnostic cutoff for HCC conducted in 2020 revealed that the threshold of 400 ng/mL was superior to 200 ng/mL in terms of sensitivity and specificity, both when used alone and with ultrasonography.[12] A lower threshold level of 10 ng/mL to detect HCC was also investigated. This resulted in calculations of a sensitivity and specificity of 82.6 and 71.2%, respectively. But, the study was performed in Indonesia and may not be applicable to other regions.[13]

Using PubMed, we found several reports on the use of repeated measurements of AFP to calculate trends of AFP levels as an HCC diagnostic tool. The aim of the present review was to examine the reported incidence of persistently rising AFP and determine its value in the diagnosis of HCC. To avoid confusion, we have used the term "persistently rising" to indicate consecutively increasing levels without an interval decrease. For AFP levels considered to be diagnostic, we use the descriptor "above the diagnostic cutoff". For levels that are above the stated normal laboratory value, but below a diagnostic cutoff, we use the descriptor "above the upper limit of normal". Wherever possible, we have provided the stated reference values.

## Studies on persistently rising AFP in the diagnosis of HCC

AFP has been reported to be diagnostically elevated with cutoff level of 400 ng/mL in the serum of between 28% to 87% of patients with primary HCC.[14] The highest incidence rates were found in South Africa (78%), Senegal (72%), and Taiwan (73%) compared to 29% in Great Britain and 28–50% in the USA.[14] The factors associated with a higher chance of AFP elevation are male sex, older age, and presence of cirrhosis.[15] In some studies, AFP went up following a rise in alanine aminotransferase (ALT), suggesting a correlation to liver regeneration.[16] Other studies showed a parallel increase in AFP and ALT, indicating a correlation between hepatocyte damage rather than liver regeneration.[17] Thus far, most studies have shown no obvious correlation between serum AFP levels in HCC and disease parameters such as tumor size and stage or survival time after diagnosis.[18] Rather than studying the value of single AFP values, several studies have suggested the importance of the dynamics of the serum AFP for the diagnosis of HCC.[14]

Chen *et al*.[19] conducted a study on 17 patients with HCC of less than 3 cm, to determine AFP response in the early stage of HCC. The AFP levels were normal in six patients (35%) and were elevated from 645 to 1,140 μg/L in the remaining patients. Ten of the patients had surgical resection, while the other seven did not. In those seven patients, the AFP levels and tumor sizes were observed over the course of 3 to 26 months. In one patient, the AFP levels were consistently normal, in two patients AFP levels were persistently rising (defined as increases over time with no interval decreases), while in the other four, AFP levels had an elevation, followed by a fall and subsequent rise in levels. All seven patients had AFP below the 400 ng/mL cutoff level on presentation. Only two (29%) out of the seven were noted to have persistently rising AFP levels. This study concluded that the serum AFP levels are frequently normal in early-stage HCC and are not a reliable marker for its early detection.[19] Chen *et al*.[19] also concluded that a spontaneous fall in the serum AFP levels is not uncommon and cannot be used to rule out the disease. The strength of the study was a long follow-up period, which allowed for detection of more HCC growth. The primary weaknesses of the study were low statistical power (with only 17 patients included) and lack of a clear diagnostic cutoff level established.

Lok *et al*.[15] conducted a study of 290 patients aged 7 to 74 years with chronic hepatitis B infection, who were followed from 1 to 4 years to establish the value of AFP monitoring in the early detection of HCC. This study identified six patients with asymptomatic HCC with only three out of the six having resectable lesions. The low resectability rate may be due to the time lag between the time of tumor diagnosis and the onset of AFP elevation. Of the 290 patients, 260 (92%) had normal AFP levels at the time of presentation. Twenty of these developed elevated AFP levels, with 20 ng/mL being the upper limit of normal. Three patients had persistently rising AFP levels measured every 3 months over course of 13–32 months. Twenty-four of the two-hundred and ninety patients had elevated AFP levels with an upper limit of normal of 20 ng/mL on presentation. Seven of them had elevated AFP levels throughout the follow-up period, with three patients developing HCC after 17–36 months of follow up. Interestingly, of the patients with normal AFP on presentation, only those with persistently rising AFP went on to develop HCC, while the ones with a single episode or recurrent episodes of the AFP rise did not. Similarly, of the patients with elevated AFP on presentation, only those with persistent elevation in AFP went on to develop HCC, while the ones with recurrent episodes of AFP rise did not. Ninety-two percent of the total patient pool had normal AFP levels at presentation, with only 1% of them demonstrating persistently rising AFP levels and subsequent development of HCC. Eight percent of the total patient pool had AFP of greater than 20 ng/mL on presentation, with twelve and a half percent of them demonstrating continuous elevation in AFP and subsequently developing HCC. A persistent rise in AFP and continuous elevation in its levels both were found to be positive predictors of tumor development, while a single or recurrent elevations may not have been. A strength of the study is a long follow-up period, which allowed for detection of more HCC and involved a broad range of age groups. The minor weakness of the study is that the patient population was predominantly male.

Taketa *et al*.[20] separated lens culinaris agglutinin-A-reactive AFP (AFP-L3) and erythroagglutinating phytohemagglutinin-reactive AFP (AFP-P4+P5) serum AFP forms in an attempt to study differences in sensitivities and specificities in early detection of HCC. They collected data from 424 patients, of whom eight patients had acute hepatitis, 56 patients had chronic hepatitis, 125 patients had liver cirrhosis, 219 patients had HCC, 7 patients had yolk sac tumor, and 9 patients had gastrointestinal tumors. The investigators followed 48 patients with benign liver disease (10 with chronic hepatitis and 38 with liver cirrhosis) for 39 months, at an average interval of 4 months, for trends in AFP-L3 and AFP-P4+P5. Of 48 patients, 27 subsequently developed HCC, of which 13 (48%) showed a persistent rise in AFP-L3 prior to tumor detection and 10 (33%) showed a persistent rise in AFP-P4+P5. AFP-L3 exceeded the cutoff of 15% on average 4.0±4.9 months, and AFP-P4+P5 on average 5.9±3.7 months prior to HCC detection. In 17 out of 27 patients who went on to develop HCC, AFP-L3 and AFP-P4+P5 remained below the cutoff level of a 15% increase before HCC detection. Interestingly, 52% of 21 patients who did not develop HCC had fluctuating levels of both AFP-L3 and AFP-P4+P5. As a result, the authors concluded that the sensitivity and specificity of preclinical rise in percent of AFP-L3 were 48 and 81, respectively, and for AFP-P4+P5 were 41 and 33, respectively. Taketa *et al*.[20] also suggested the possible use of AFP in early diagnosis of HCC by trend analysis but proposed to use the fractions AFP-L3 and AFP-P4+P5 to in-

**Table 1. Sensitivity and specificity data for persistently rising AFP levels* in the diagnosis of HCC**

| | True | False |
|---|---|---|
| Positive | 227 | 272 |
| Negative | 19.1 | 147 |

*Data on the presence or absence of persistently rising AFP levels, and the presence or absence of HCC based on cases in the literature.[11,15,19–24] Persistently rising AFP level criteria varied, as defined by the authors of each study. AFP, alpha-fetoprotein.

crease specificity for HCC. Thirty-seven percent of patients who went on to develop HCC had AFP above the cutoff level, and 81% of patients who developed HCC had persistently rising AFP-P4+5 or AFP-L3 levels. The study had a good statistical power, with 424 patients included, but how the cutoff level was established was not mentioned. As fractionation of AFP is not commercially available, it will be challenging to compare this study to others and apply the results clinically.

Liaw *et al*.[21] studied 432 hepatitis B surface antigen (HBsAg)-positive and 105 HBsAg-negative patients with clinically proven chronic hepatitis for a period of 6–85 months, with intervals of 3–6 months. The authors suggested that unlike in acute viral hepatitis, elevation of AFP levels in patients with chronic liver disease are concerning for underlying malignancy. They found that rises in AFP levels of greater than 100 ng/mL without concurrent elevation in ALT could predict HCC with specificity of 98.7% and sensitivity of 66.7%. On the other hand, moderate elevation in ALT along with rise in AFP almost always excluded HCC. Liaw *et al*.[21] emphasized, however, that slowly rising levels of AFP are concerning for HCC with or without the elevation in ALT. Their upper limit of normal was 20 ng/mL. Over the course of follow up of 6–85 months, 45.6% of patients had AFP levels above the upper limit of normal. Out of the 423 patients included in the study, only 9 (2%) were found to have asymptomatic HCC. Of those 9 patients, 7 (78%) had persistently rising AFP levels on follow up. The study had a good statistical power, with 432 patients included, and a long follow-up period of 6–85 months. However, the study did not discuss the number of patients who subsequently developed symptomatic HCC.

Goldstein *et al*.[11] studied 81 patients with chronic hepatitis C and the relationship of serum AFP and ALT values. The AFP levels were significantly greater in patients with fibrosis, while ALT levels were markedly elevated in the fibrosis-cirrhosis group. The authors concluded that among all patients, increasing serum AFP values significantly correlated with increasing ALT values. The study had good statistical power, with 81 patients included. The minor weakness of the study is that it was limited to patients with hepatitis C. Of more consequence is that there was no clear follow-up period.

Aoyagi *et al*.[22] conducted a study on 372 patients, 258 of whom had a diagnosis of HCC, while the other 114 patients had benign liver diseases. Individual patterns from longitudinal follow up in patients with HCC showed a persistent rise in AFP levels, while in patients with benign liver disease, AFP fluctuations were observed without an obvious uptrend. In another study, Aoyagi *et al*.[23] reported five patients with cirrhosis and persistently rising AFP levels in whom no HCC developed in follow up from 3–9 years. In both studies, the fucosylation index of AFP was used to increase sensitivity and specificity of early HCC detection.[22,23] The sensitivity and specificity of persistently rising AFP was found to be 69% and 96%, respectively. The study had a good statistical power, with 258 patients included and was stratified patients by tumor size. However, measurement of the fucosylation index of AFP is not widely available, which makes this study challenging to compare to others.

**Table 2. Slope values for persistently rising, non-diagnostic AFP levels* in patients with a diagnosis of HCC**

| Slope | Reference |
|---|---|
| 1.50 | 15 |
| 149 | 15 |
| 903 | 15 |
| 51.4 | 15 |
| 520 | 15 |
| 664 | 19 |
| 12,000 | 19 |
| 13.6 | 22 |
| 16.2 | 23 |

*Persistently rising AFP levels, as defined by the author of each study. AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

Okuda *et al*.[24] reported five cases of small HCC detected during a routine clinical follow up. Two of the patients had a steep rise in AFP, which prompted more imaging and led to a subsequent diagnosis of HCC. In the other three, AFP was slightly but persistently rising, which also prompted more imaging to be done. Their finding brought to attention the fact that patients in whom AFP levels were below the diagnostic cutoff but continued to persistently rise suggested the presence of a small HCC and the need for a more thorough work up. In that study, 60% of patients who developed HCC had persistently rising AFP levels. However, the study was small, with only five cases reported. The authors also did not mention what diagnostic cutoff was used. The study was performed in Japan, with only Japanese patients included, making it likely less applicable in patients of other ethnic origins.

Although the aim of this review was simply to critically assess the literature in terms of the value of rising AFP in HCC diagnosis, we wondered whether the existing published data could be combined to determine any predictive value in AFP level trends. We included data from patients who had persistently rising AFP levels as defined by the individual authors of each of the papers. Based on those reported data, we calculated true positive value for persistently rising AFP to be 227.5, while the false positive value was found to be 272.5. Similarly, the false negative rate was 147.8 and the true negative rate was 19.1. Therefore, the sensitivity and specificity of persistently rising AFP levels in diagnosis of HCC were found to be 60.6% and 35.8%, respectively (Table 1), indicating that the presence of persistently rising AFP per se did not offer diagnostic benefit. However, perhaps differences in the rates of rise of AFP levels were statistically significant. Therefore, to address this issue, using data on persistently rising AFP levels from the literature, we calculated the mean AFP slope (ng/mL as a function of time of surveillance in months) for patients with diagnosis of HCC (Table 2) compared to the mean AFP slope in patients diagnosed not to have HCC (Table 3). Studies that lacked individual data points to permit such calculations were excluded. All studies used 20 ng/mL as the upper limit of normal. The mean slope for persistently rising non-diagnostic AFP levels in patients diagnosed with HCC was calculated to be 1,590, while the mean slope for persistently rising AFP levels in patients diagnosed not to have HCC was calculated to be 46.1. Using a Wilcoxon rank sum test and MathWorks software to compare the two distributions, the calculated *p*-value was 0.074. Although numerically very different, because of the high variance, the calculations indicate that the

**Table 3. Slope values for persistently rising AFP levels* in patients diagnosed not to have HCC**

| Slope | Reference |
|---|---|
| 107 | 15 |
| 210 | 21 |
| 33.3 | 22 |
| 2.27 | 23 |
| 4.94 | 23 |
| 4.55 | 23 |
| 1.50 | 23 |
| 5.27 | 23 |

*Persistently rising AFP levels, as defined by the author of each study. AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

differences in slopes of AFP levels between HCC and non-HCC patients were not statistically significant.

## Discussion

The AFP cutoff level diagnostic for HCC has long been a subject of debate. As discussed above, a meta-analysis from 2020 suggested that a cutoff level of 400 ng/mL was superior to 200 ng/mL in terms of sensitivity and specificity both when used alone or with ultrasonography.[12] But, some of the individual studies reviewed here provided no diagnostic cutoff values. Taketa *et al*.,[20] on the other hand, used a percentage (15%) as their diagnostic cutoff for diagnosis of HCC. This variability in threshold for AFP elevation and diagnosis of HCC contribute to the difficulty in comparing studies. However, it does seem clear that using a single value diagnostic cutoff is not of value in surveillance of the US population.

A major contributor to the lack of sensitivity and specificity is the fact that AFP is also released in response to inflammation or hepatocyte damage.[25] Hepatocyte proliferation during regeneration has been shown to be associated with elevated AFP levels.[25] For this reason, some reports have advocated the use of aminotransferases to compensate for this contribution.[11,21,26] As discussed previously, Liaw *et al*.[21] studied the correlation of AFP and ALT elevation, concluding that rises in AFP of greater than 100 ng/mL without concurrent rises in ALT as well as persistently rising AFP levels, regardless of ALT trends, were both predictive of HCC. The limitation of the study is that they only studied cases of hepatitis B, and excluded other etiologies, such as alcoholic hepatitis, drug-induced hepatitis, steatohepatitis, etc. Goldstein *et al*.[11] performed a similar study on hepatitis C-infected patients. A more diverse patient population may make the results of studies investigating the AFP to ALT correlation more applicable to clinical use.

The notion of the potential value of persistently rising levels of AFP instead of single cutoff values as a diagnostic for HCC is not novel. Indeed, the British Society of Gastroenterology guidelines from 2003 stated that a rising AFP over time, even if the level does not reach 400 ng/mL, is virtually diagnostic of HCC.[27] However, the data are not easy to compare between studies because there is no standard definition of persistently rising levels or of measurement intervals. Chen *et al*.,[19] Lok *et al*.,[15] and Taketa *et al*.[20] followed their patients for 3–26 months, 13–36 months, and 39 months, respectively. Both Chen *et al*.[19] and Lok *et al*.[15] had 6-month intervals between the data collection. Taketa *et al*.[20] had 4-month intervals. Liaw *et al*.[21] had the most frequent follow-up interval of 3 months, which likely helped avoid missing any fluctuation in the AFP levels that would have otherwise been overlooked. Both Liaw *et al*.[21] and Ayoagi *et al*.[23] had longer follow-up periods, of 6–85 months and 3–9 years, respectively. Longer follow up likely contributed to more HCC being detected when compared to studies that had a shorter follow-up period. Okuda *et al*.[24] had no documentation of follow-up time used, which makes it more challenging to compare this study to others.

So, out of seven studies reviewed and discussed, five had a range of 60% to 81% of patients who subsequently developed HCC and demonstrated persistently rising AFP levels. Of note, three out of these studies used the fucosylation index or fractions of AFP, which improved sensitivity and specificity, but may not be applicable or compared to studies that used the whole AFP. Two other studies reported that only 1% to 29% of patients who subsequently developed HCC demonstrated persistently rising AFP levels, which would argue against the use of this trend.

The current review covered data from several studies in which rising levels were recorded, but were not necessarily the focus of the studies. However, the number of studies was relatively small. This together with the differences in criteria, follow-up variables, and lack of meta-analysis are weaknesses. Our calculated values of the specificity and sensitivity of persistently rising AFP for HCC were both low. A comparison of mean slopes of AFP levels has not been reported previously. The rationale for using slopes is based on the assumption that HCC is more likely to increase progressively in size, and the persistently increasing tumor mass is more likely to be associated with persistently increasing levels of AFP. Our comparison of slopes indicated no statistical significance in the rate of rise of persistently elevated AFP levels of HCC cases compared to non-HCC cases. However, that does not necessarily mean that rising AFP levels are of no HCC diagnostic value since the sample size was small, and lacked uniformity in the intervals between measurements and the duration of observation, any of which could have biased the results.

## Conclusions

Thus far, there has been no evidence that serum AFP levels correlate with tumor size, stage, or survival post-diagnosis. For this reason, societies have largely abandoned the use of single AFP level cutoffs for HCC surveillance. The data in the literature on persistently rising AFP for HCC are sparce and conclusions inconsistent. Using AFP level data in the literature, our calculated figures on specificity and sensitivity of persistently rising AFP for HCC were both low, indicating that this measurement was not superior to a single value cutoff. Furthermore, a statistically significant difference in persistently rising AFP levels between HCC and non-HCC patients was not confirmed by comparison of mean slopes, although the *p*-value was close to being significant. Our review was limited by the heterogeneity, scarcity of the data, and lack of uniformly applied definition of persistently rising levels of AFP. Therefore, large prospective studies with universal definitions and criteria are needed to settle the question of whether persistently rising AFP levels can be of value in the diagnosis of early-stage HCC.

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## Conflict of interest

GYW has been the editor-in-chief of *Journal of Clinical and Translational Hepatology* since 2012. AT has no conflict of interests related to this publication.

## Author contributions

Proposed concept for review and revised the manuscript with critical revisions (GYW), drafted the manuscript (AT).

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