Review on the clinical roles and limitations of AFP as marker for HCC and other diseases

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Abstract:
The tumor marker for hepatocellular carcinoma, alpha-fetoprotein (AFP), has been utilized in
association with ultrasound and other imaging methods (HCC). Its utility is constrained by
low sensitivity and specificity, as well as differences between the various measurement
methods. Also, depending on the patient's characteristics and the used AFP cut-off values, its
accuracy fluctuates. Des-gamma-carboxyprothrombin, a new biomarker, was combined with
AFP to significantly increase its ability to identify HCC. Elevated AFP levels are usually
seen in patients with acute and chronic liver diseases, as well as cirrhosis, and could indicate
the severity of hepatic damage and subsequent regeneration. AFP increases can also result
from hereditary conditions and other non-hepatic diseases.

KEY WORDS: Alpha-fetoprotein; Hepatocellular carcinoma; Alpha-; Cirrhosis; Tumor
markers.
Introduction:

Serum AFP is a protein produced primarily by fetal liver and the portion of a developing embryo that is similar to the yolk cavity in bird eggs (yolk sac tissues). Its concentration is typically elevated when a baby is born and decline rapidly. In healthy children and non-pregnant adults, AFP is normally only detectable at very low levels. Liver damage and certain cancers such as testicular and ovarian cancers can increase AFP concentrations significantly. It is also produced whenever liver cells are regenerating such as with chronic liver diseases and tumours. Generally, the normal range of AFP is < 10 ng/mL. Moderate levels of AFP (> 500 ng/ml) can be seen in patients with chronic hepatitis. Moreover, Levels of AFP can be mildly or even moderately elevated in many patients with different types of acute and chronic liver diseases without liver cancer being detected in the body. Because serum AFP is above the normal range in 60.0% to 70% of primary liver cancer cases, it is utilized for screening and diagnosis of liver cancer. Unfortunately, AFP levels are normal in 30.0% to 40.0% of all liver cancer, in which case cancer can only be detected by finding a mass on ultrasound or computed tomography (CT) scan[1, 2]. It was first proposed in the 1960s to use AFP as a tumor marker for hepatocellular carcinoma (HCC). Due to its poor sensitivity and specificity, AFP has been criticized for its efficacy in surveillance and diagnostic testing. Nonetheless, in addition to ultrasonography (US) and other imaging modalities, AFP still plays a crucial role in HCC surveillance[3-6]. The clinical functions and implications of AFP were the main topics of this review. Furthermore reviewed were the patterns of AFP elevation in various non-HCC etiologies.

The structure and biochemistry of AFP:

AFP is a single-chain sialylated glycoprotein composed of approximately 580 amino acid residues and 3-4% carbohydrate [7]. The molecular weight of AFP has been determined to be about 67,000 Daltons by SDS gel electrophoresis. The isoelectric point of the negatively charged protein AFP is between pH 4.57 and 5.08. These variations in charge, due only in part to sialic acid content, do not affect the antigenic properties of this protein. By using crossover immunoelectrophoresis and extended agarose gel electrophoresis, at least three different species of AFP have been isolated. There is a correlation between the relative amounts of the most negatively charged species and the inhibitory effect of AFP on in vitro
transformation of lymphocytes[7]. Treatment with neuraminidase to remove completely the sialic acid residues does not alter the biologic potency. Thus differences in sialic acid content are only partly responsible for the microheterogeneity of AFP and variability of other charged regions is also present [8]. In addition to the monomeric forms, polymers with dimeric and trimeric forms that dissociate into the monomer when exposed to disulfide reducing agents have also been identified, implying that their formation is dependent upon intermolecular sulfide bonds. The chemical and antigenic structures of AFP's isolated from various mammals are closely related. In addition, no antigenic differences have been identified between AFP's isolated from tumor bearing adults and from fetal sources[7,9]. Immunization of an animal with its own AFP does not lead to antibody formation because of tolerance. Tolerance can be broken by altering AFP or by immunization with a cross-reacting AFP from another species. These antibodies can then eliminate the animal's normal serum AFP [9].

Another interesting and not yet fully explored property of AFP is the high affinity of murine AFP for certain estrogens by means of which APP could influence cell growth. Human AFP has a much lower estrogen binding ability[10].

**AFP biosynthesis and serum levels:**

As early as 29 days after conception, human embryos can be identified synthesizing AFP [7]. At 11–12 weeks of gestation, the yolk sac is atretic. AFP synthesis occurs predominantly in fetal hepatocytes although a small amount may be produced by the fetal gut. AFP levels in fetal serum reach a maximum of 3,000 J.Lg per ml at about 14th week of gestation, exceeding in its concentration all other fetal plasma proteins including albumin. Serum concentrations decrease thereafter to 200 to 300 J.Lg per ml at age 32 weeks of gestation, to 20 to 120 J.Lg per ml at term and then drop sharply after birth. During the first 2 mo of life serum levels are about 400 ng per ml, fall to 30 ng per ml by 6 mo of age and to less than 15 ng per ml by 1 to 2 yr. Thereafter, the levels of AFP serum are maintained between 3 and 15 ng per ml due to synthesis of AFP which has a biological half life of about 6 days. During adult life serum levels can rise again in the event of occurrence of regenerative or neoplastic proliferation of hepatocytes and of a number of extrahepatic tumors[7]. The dynamics of AFP and albumin synthesis have been studied by a number of investigators[11,12]. The serum levels seem to correlate with the number of hepatocytes which produce this protein. In experiments with rat
hepatoma lines, it has been shown that some clones produce high levels of AFP and low levels of albumin and others the reverse. Moreover AFP and albumin are produced during different phases of cell cycle. AFP is synthesized during Gland S phase while albumin is produced from mid-S through G2[9,13]. Immunofluorescence studies have shown that AFP and albumin are probably synthesized in different hepatic cells. At the level of m-RNA, AFP synthesis in the developing liver is controlled. In adult liver, there is a significant reduction in the level of AFP mRNA.[14]. The synthesis of serum AFP was studied in 16 human embryos and fetuses between 4.2 and 18 weeks of gestation by incubation of selected tissues with 14C-labeled amino acids followed by immunoelectrophoresis of the culture fluids and radioautography. Both cultures of the developing yolk sac and each liver produced rather high levels of radioactive AFP. Almost all of the gastrointestinal tract cultures produced smaller concentrations of labelled AFP. Only 1 of the 14 cultured placentas and 1 of 9 conceptuses' kidneys produced labeled AFP. Lung, thymus, pancreas, skeletal muscle, amnion, or chorion cultures didn't yield any AFP that could be detected. The immunological techniques for the quantitation of AFP vary greatly in their sensitivity. High AFP levels can be determined by single radial immunodiffusion or rocket immunoelectrophoresis. Smaller amounts (5 to 500 ng per ml) are commonly quantitated by radioimmunoassay[7].

How is AFP determined?

Immunoelectrophoresis was initially used to test AFP, but it was not a very sensitive technique. New methods, including enzyme immunoassays and radioimmunoassays, were applied in the 1970s and 1980s. The earlier clinical assays were then modified and developed upon by a quantitative automated chemiluminescent enzyme immunoassay[15,16]. In this procedure, blood is put on a magnetic plate that already has an anti-AFP antibody attached to it. The excess AFP is then bound to by a second chemiluminescent detecting antibody that is added to the same magnetic plate. After washing away all of the unbound detecting antibody, a developer, an organic substrate that emits light and becomes luminous, is applied. The antibody is found using a chemiluminometer, the antibody is detected using a chemiluminometer, and the results are quantified using established AFP standards. Measurement interference, however, is possible. Interfering antibodies occasionally attach to both the capture and detect antibodies in an one step method of AFP detection, producing a
false positive result (Figure 1B). In contrast, interference antibodies may keep reagents invisible and avoid the AFP from properly interacting with particular anti-AFP antibodies [17].

THE CLINICAL SIGNIFICANCE OF AFP

The AFP in Primary Hepatocellular Cancer:
The value of AFP as an HCC screening test is now increasingly being questioned. Marrero et al. showed that AFP had a sensitivity of 66% and specificity of 81%, at a cut off value 10.9 ng/ml.12.

They found AFP most useful for early stage liver cancers. They also compared AFP with other new biomarkers like des-gamma carboxyprothrombin (DCP) and lectin-bound alpha fetoprotein (AFP-L3) and concluded that AFP is still a better serum biomarker [18]. Stefaniuk et al. observed that AFP has high specificity but with cut off value > 100 ng/ml, sensitivity is only 20 – 30% which means 70 – 80% patients with HCC will not receive treatment when it was needed[19]. In another study, it was observed that AFP at cut off value 20 ng/ml, has 60% sensitivity and positive predictive value range from 9 – 50%, depending on etiology of disease [20]. Pervaiz et al. found AFP 72% sensitive and 89% specific for diagnosis of HCC [21]. This limitation of AFP is most likely due to the fact that a number of small tumours may not secrete AFP resulting in normal levels despite the presence of HCC [22]. Gupta concluded in his review that AFP has limited utility in identifying hepatocellular carcinoma in patients with hepatitis C[23].

Sub-optimal performance of AFP has lead to introduction of number of new markers for diagnosis in the form of DCP, AFP-L3, alpha fucosidase, squamous cell carcinoma antigen (SCCA) and glypican 3 (GCP-3). All these new markers also have their own limitations for diagnosing HCC and particularly have low predictive value for small size tumours [19]. Availability of these markers in cost effective manner to patients at large, is another limiting factor.

Limitations in the sensitivity of AFP in surveillance of high-risk populations have led to the use of ultrasonography (US) as an additional method for the detection of HCC. US has 78 – 90% sensitivity and 93% specific for detection of HCC, making it a useful adjunct to AFP for diagnosis[24]. As per EASL guidelines 2012, use of serum AFP in addition to ultrasound
adds 6 – 8% to sensitivity for HCC determination but also increases the cost of screening[25]. Economic analysis by Coon et al. has found use of both AFP and abdominal ultrasound for screening in patients of cirrhosis most cost effective[26]. Baig et al. have found AFP a useful and cost effective tool for screening of patients with HCC in patients of the region[27]. According to latest APASL guidelines, both ultrasonography and serum alpha fetoprotein should be used for screening of HCC[28]. EASL guidelines have suggested that persistently raised AFP levels are a risk factor for HCC development and can be used to help define at-risk populations[25].

Finally, despite sub-optimal sensitivity, AFP is still the best available screening serological test for HCC and can be used along with ultrasonography for early diagnosis of liver cancer.

**other HCC biomarkers:**

**Glypican-3 (GPC3):**

Glypican-3 (GPC3); among the members of heparan sulfate proteoglycans. However it is highly expressed during embryogenesis and is involved in organogenesis, its exact biological function remains unknown. It binds to the cell membrane through the glycosylphosphatidylinositol anchors. GPC3 is able to interact with several growth factors that either stimulates or inhibits the growth GPC3 mRNA and protein are expressed in more than 80% of human HCC but not in normal tissues with the exception of placenta and fetal liver[30-29]. It was found in the sera from 40–50% of HCC patients, while it was not detectable in sera from patients with chronic hepatitis or liver cirrhosis or in sera from healthy people[31].

**Alpha-l-Fucosidase (AFU):**

Alpha-l-Fucosidase (AFU) is a glycosidase primarily found in lysosome and involved in the degradation of a variety of fucose-containing fucoglycoconjugates[32]. The alterations of AFU catalytic activity in human cells, tissues and body fluids can be used to diagnose human malignancies, like primary HCC[33,34,35,36]. Early identification of HCC is facilitated by the sustained high AFU level in the serum of liver disease patients[32,36]. The diagnostic ability of this marker is still unknown, and there are currently no quantitative serum assays available.
Des-γ-carboxy Prothrombin:
Malignant hepatocytes seem to lack the ability to carboxylate glutamic acid to form γ carboxyglutamic acid. The resulting abnormal prothrombin has been referred to as des-γ carboxyprothrombin (DCP). Because this is the same prothrombin formed by vitamin K absence or antagonism, DCP is also known as PIVKA-II. Although DCP has demonstrated a greater specificity than AFP, it still lacks sensitivity, especially for HCC tumors less than 3 cm in diameter, with sensitivity ranging from 19 to 48%. Compared with AFP, DCP levels had higher sensitivity and specificity in differentiating HCC from nonmalignant chronic liver disease. One prospective study screening cirrhotic patients for HCC, using cutoff values of 40 ng/mL for AFP and 80 mAU/mL for DCP, showed 65% sensitivity and 85% specificity when both markers were combined[37].

Other markers of HCC that have been studied include tumor-associated isoenzymes of γ-glutamyl transpeptidase, urinary TGF-β-1, serum levels of circulating intercellular adhesion molecule (ICAM) -1, Insulin-like growth factor-II (IGF-II), Insulin-like growth factor-binding protein-2 (IGFBP-2), Human cervical cancer oncogene (HCCR), Golgi protein73. Hepatocytes growth factor (HGF), KL-6, serum proteomics and HCC-specific auto-antibodies. None of these diagnostic tests have demonstrated superior accuracy compared with serum AFP. Two tumor markers, abnormal vitamin B12-binding protein and neurotensin have been linked specifically to the fibrolamellar variant of HCC.

AFP elevation in liver disease and cirrhosis:
The results of AFP levels should be evaluated carefully since an increase in AFP has been detected in a variety of chronic liver diseases without HCC and other cancers [38]. It is well recognized that AFP is implicated in liver fibrosis, inflammation, and liver regeneration 15%-58% of chronic hepatitis cases and 11%-47% of cirrhosis cases have been observed to have less pronounced AFP elevation > 10 ng/mL,[39]. The standard cutoff for identifying benign from malignant liver diseases was 500 ng/mL, although this can vary [40].Studies have indicated that greater histological severity, such as inflammation, cirrhosis, and HCC, is correlated with a gradual increase in serum AFP levels[41].
Acute Hepatitis:
The severity of hepatic destruction in acute hepatitis is correlated with the degree of AFP elevation. [38,42] There have been reports of levels as high as 3000 to 7190 ng/mL on occasion. The levels range from 10 ng/mL to 1000 ng/mL. Within 1 week of the onset of clinical hepatitis in children with acute hepatitis B, AFP was detected, and by the time they recovered and lost their HbsAg, it had returned to normal [43]. Acute phase response to liver injury, hepatocyte regeneration, virus control, or mediated AFP production are some of the potential mechanisms of AFP increase. [44] There is typically a latent phase of 5 to 16 days following significant alanine aminotransferase (ALT) elevation for AFP elevation, which is most likely caused by liver regeneration[45]. AFP often peaks when liver damage is reducing and hepatic remodeling is starting. [45]. Hence, the highest AFP level in acute hepatitis typically occurs during the illness' recovery phase and is an indication of the liver's regenerating process[46].

Chronic Hepatitis B:
According to Liu et al. [47], the degree of liver damage in CHB patients had a significant impact on AFP serum levels. 2 Between AFP and the phases of fibrosis and inflammation, there were both moderate and significant positive correlations. A significant weak to moderate association between AFP and the values of AST, ALT, and GGT was also observed (Spearman's correlation coefficients, 0.22, 0.331, and 0.445, respectively; all p 0.001). Overall, the serum AFP levels increased along with the abnormal levels of inflammation and fibrosis among CHB patients. In this study [48], the effects of gender and age on variation in serum AFP levels were insignificant.

The METAVIR semiquantitative scoring system [50] is similar to the specific program of prevention and cure for viral hepatitis that is typically used in China[49] and classifies fibrosis into five stages: F0 (no fibrosis), F1 (mild fibrosis without septa), F2 (moderate fibrosis with few septa), F3 (severe fibrosis with numerous septa without cirrhosis), and F4 (cirrhosis). Antiviral treatment is administered to CHB patients with severe fibrosis (METAVIR F > 2). In the study by Liu et al., patients in stages S3 and S4 had significantly higher AFP levels than those at stages S0, S1, and S2, which were all normal. The correlation between AFP and the phases of fibrosis was 0.404. In CHB patients, AFP levels also shown a
moderately positive trend in correlation to fibrosis stages. Similar findings were reported when transient elastography was used to the correlation between liver and AFP levels and liver stiffness (the correlation coefficient between AFP and fibrosis stages was 0.317) [51]. One critical find was that blood AFP levels might diagnose also the degree of fibrosis and inflammation in patients with "normal" serum AFP levels. Regardless of the association between AFP and liver regeneration, low AFP values in some adult individuals still indicated a "severe" disease.

Using a METAVIR-like scoring system, the study [50] compared the predictive value of liver biopsy to that of AFP. As fibrosis and inflammation were directly correlated with AFP, this suggested that AFP had predictive value. The most important criterion for the approach's application in clinical practice is the number of patients correctly identified by the procedure for a specific end-point based on the method's reference standard.[52]. Based on these parameters, AFP is a promising biomarker to evaluate liver pathology.

Finally, the subclinical importance of serum AFP levels can be established by examining the association between different stages of fibrosis and inflammation and serum AFP. In CHB patients, the progression of fibrosis and inflammation was accompanied by an increase in serum AFP levels. Since no single laboratory parameter can now accurately predict the prognosis of liver pathology alone, it is an important future goal to develop a mathematical model that predicts the prognosis of liver pathology using a combination of multiple biomarkers.

**Clinical Implications of Alpha-Fetoprotein in Chronic Hepatitis C:**

A clinical serum marker for the diagnosis of HCC is AFP. Patients with viral hepatitis without HCC are also observed to have elevated AFP levels, particularly those with CHC and liver cirrhosis. In Western countries, between 10% and 43% of people with CHC had increased AFP levels[53-54-55]. Different patient populations, sample sizes, and definitions of serum AFP elevation could allow for the wide variation in the incidence of elevated AFP levels. The prevalence of high serum AFP levels may have been affected by the inclusion of patients at various stages of CHC.

Patients with chronic hepatitis B have been shown to have elevated serum AFP levels that associated with elevated serum ALT levels, especially if histology shows bridging necrosis [
It has been shown previously that the levels of ALT in CHC patients are mildly elevated[57] and elevated serum AFP levels were correlated positively with serum ALT. W.C. Tai, et al showed that elevated levels of AFP serum were correlated with a higher HAI inflammation score (p=0.002)[58]

Thrombocytopenia is usually observed in CHC patients with liver cirrhosis, especially in combination with splenomegaly. Thrombocytopenia, defined as a platelet count <150×10^9 cells/L, has been reported to be an alternative marker for diagnosing liver cirrhosis[59].

Recently, Peng et al reported that liver fibrosis (fibrosis stage F4) is associated with hepatic progenitor cell activation.

Furthermore, AFP gene activation is associated with hepatic progenitor cell activation and results in increased AFP production in patients with advanced fibrosis. In the studies by Bayati et al [60] and Hu et al [61], the AFP level was used to predict liver fibrosis with a sensitivity of 22.8–35% and a specificity of 94.5–98.6%. When we set an AFP level >6ng/mL as the cut-off value, the sensitivity and specificity for predicting advanced fibrosis was 74.3% and 68.4%, respectively. However, the predictive value of serum AFP needs to be validated with further independent cohorts with a larger sample size. In conclusion, CHC or liver cirrhosis with elevated serum AFP levels in relation to old age, high HAI inflammation scores, high ALT levels and low platelet counts. Advanced fibrosis in relation to elevated AFP and AST levels, low platelet count, and HCV genotype 1b.

According to Fouad et al. [62], A prolonged virological response with direct-acting antiviral agents (DAA) therapy was associated with a significant decrease in serum AFP and may be an indicator of a treatment’s effectiveness.] 62 [In a different trial, DAA treatment resulted in a normal AFP in 60% of patients, compared to only 23% of control subjects. [63] Moreover, therapeutic phlebotomy has been shown to lower AFP levels in CHC [64]. Iron depletion decreases oxidative stress, which in turn indirectly lowers AFP. It has been hypothesized that iron-mediated oxidative stress is related to hepatic damage[65].

**HPAFP (hereditary persistence of AFP):**

The last several decades of research on AFP have shown that autosomal dominant inherited gene mutation is responsible for the persistently elevated levels of AFP in adults
In such a case, constantly elevated levels of AFP, in the range of 0.009 – 3.564 µg/mL, are observed in the serum. From 1983 to 2010, HPAFP was recorded in 19 families. Two-point mutations on chromosome 4 were found (a-55 C>A and a-119 G>A) in the site of binding of HNF-1 (hepatocyte nuclear factor-1) to the AFP gene promoter. HNF-1 (responsible for stimulation) and NF-1 (mainly responsible for suppression) are 2 important transcription factors of the AFP gene. Genetic mutations increase the affinity of HNF-1 to the AFP promoter and cause elevated AFP transcription. In addition, elevated binding of HNF-1 to the promoter results in decreased binding of NF-1 (due to the partial overlap of HNF-1- and NF-1-binding sites), which further stimulates transcription [66].

**other tumour:**

For preoperative assessment during initial management, early recurrence, or metastases detection upon follow-up, AFP is an onco-fetal antigen that is therapeutically advantageous when used in combination with ultrasonography and cross-sectional imaging with MRI or CT of the chest, abdomen, and pelvis. 90% of cases of yolk sac tumors, a kind of prepubertal testicular tumor, are reported to have AFP. Serum AFP levels are high in newborns (up to 50,000 ng/ml), reduce to normal levels by 12 months of age, and then continue to decrease with age. After age one, the serum AFP normal value is less than 15 ng/ml. AFP has a half-life of 5 to 7 days. Due to the fact that these tumor markers are produced by NSGCTs but not by pure seminoma, any increase in the serum level in an adult may indicate occult NSGCTs. Men's elevated serum AFP levels over 10,000 mg/l may also be associated with disease of the liver and gallbladder pathology.[67]
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