Serum Alpha-fetoprotein and Its Lectin Reactivity in Liver Diseases: A Review

JAMES T. WU, PH.D.

Department of Pathology and ARUP, University of Utah School of Medicine, Salt Lake City, UT 84132

ABSTRACT

Increased serum concentration of alpha-fetoprotein (AFP) can be found in benign and malignant liver diseases, in yolk sac tumors, and in several nonhepatic neoplasms at advanced stage. The frequency and level of elevated serum AFP are highest in hepatocellular carcinoma (HCC) and yolk sac tumors. Most levels of serum AFP in HCC are greater than 500 ng per mL, whereas the serum AFP in most of the benign liver diseases is only moderately elevated and is transient in nature.

Determination of lectin reactivity of serum AFP is helpful for the differentiation of HCC from other diseases associated with elevated serum AFP. Determination of Len culinaris agglutinin (LCA) reactivity of serum AFP is useful for the differentiation of HCC from benign liver diseases, and for early detection of hepatoma. Determination of concanavalin A (Con A) nonreactive AFP variant is useful for the differentiation of HCC from yolk sac tumors and may also allow for the differentiation of HCC from nonhepatic neoplasms. However, reaction with several lectins may be required if differentiation among various nonhepatic neoplasms is needed.

Introduction

Alpha-fetoprotein (AFP), a major fetal serum protein, is produced mainly by fetal liver and yolk sac cells, and to a smaller extent by fetal gastrointestinal tract and kidney. The synthesis of AFP is almost completely absent in normal adult tissues but is reactivated in cancers, particularly in primary hepatoma and in yolk sac tumors.

Alpha-fetoprotein resembles albumin in many physicochemical properties. However, one major difference between human AFP and albumin is the carbohydrate content. While AFP contains three to four percent of carbohydrate, essentially no carbohydrate is found with albumin. The composition of the carbohydrate chain of human AFP is heterogeneous. The heterogeneity can be demonstrated by the reaction of AFP with lectins. In fact, human AFP can react with a variety of lectins of different specificities. They include Len culinaris agglutinin (LCA), concanavalin A (Con A), Ricinus communis agglutinin (RCA), Phaseolus vulgaris agglutinin E (PHA-E), Pisum sativum agglutinin (PSA) and Vicia faba agglutinin (VFA).
Hepatocellular Carcinoma (HCC)

Apparently, a certain type of alteration occurs in the control of gene expression for AFP and reactivates AFP synthesis in HCC and yolk sac tumors. It has been demonstrated that quantitative measurement of AFP is useful for the diagnosis of HCC and for monitoring patients with liver cancer during therapy.\(^3\)\(^,\)\(^14\)\(^,\)\(^17\)\(^,\)\(^33\) Measurement of AFP after chemotherapy or surgery also provides an early warning of recurrence for as much as six months prior to the appearance of other clinical signs.\(^14\)\(^,\)\(^33\) Up to 80 percent of patients with primary hepatoma have an elevated level of serum AFP. This percentage varies depending on the sensitivity of assay employed. The levels seen range from nanograms to milligrams per milliliter of serum,\(^3\) and most levels of serum AFP are above 500 ng per ml.\(^2\)

It is important to point out that a negative AFP test does not exclude a diagnosis of HCC but makes it much less likely, and vice versa. Persistent or increased AFP levels, after surgery or chemotherapy, indicate incomplete removal, spread of tumor, or metastases. In some cases, surgical intervention or chemotherapy may result in a decrease or even disappearance of AFP from the serum. This does not necessarily parallel improvement in the patient’s clinical state, and is difficult to explain. It could be owing to necrosis or an inhibitory effect of cytotoxic drugs on AFP production.\(^14\)

It appears from the immunohistological studies that not all tumor cells of primary hepatoma are AFP-positive. In fact, only a small proportion of the tumor cells (five to 20 percent) are involved in AFP synthesis.\(^24\) However, based on the in situ hybridization analysis of mRNA of AFP, Otsuru et al reported\(^20\) that the majority of tumor cells of HCC showed positive hybridization with AFP comple-
**FIGURE 1.** Comparison of the levels (1A) and frequencies (1B) of elevated serum AFP between benign liver diseases and primary hepatoma. Highest levels of serum alpha-fetoprotein in various diseases are used for plotting. Data used are from Bloomer et al.³

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extent of the preceding hepatic damage but also as a prognostic indicator of the extent and course of fulminant or subacute hepatitis¹⁰ or as a marker for recovery.¹² This is especially useful when serum enzymes have already returned to normal levels at the time the patient is seen for treatment. Concentrations of

**FIGURE 2.** Transient elevation of serum aspartate aminotransferase and serum alpha-fetoprotein in one patient with acute viral hepatitis
serum AFP are also frequently elevated in pediatric hepatic disorders such as Indian childhood cirrhosis, tyrosinemia, and neonatal hepatitis. It should be noted that the normal AFP range of both newborn babies and infants before eight months of age is much higher than that of adults and that the normal levels of serum AFP in infants are age dependent.

Nonhepatic Neoplasms

An increasing number of reports have indicated that elevated levels of serum AFP can be found in primary neoplasms other than hepatoma and yolk sac tumors. Although AFP elevations are found much less frequently in these nonhepatic neoplasms, the concentration of serum AFP in nonhepatic neoplasms may lie within the range for hepatocellular carcinoma. This is illustrated in figure 3. Most of these nonhepatic tumors are associated with the endoderm-derived gastrointestinal (GI) tract, however, tumors from mesoderm derived kidney and from urinary bladder, breast and ovary have also been reported. Usually the corresponding fetal cell of these AFP-producing, nonhepatic tumors is capable of synthesizing AFP. Apparently, reactivation of the AFP gene also occurs in these tumors and is responsible for the increased concentration of serum AFP. Listed in table

![Figure 3. Level (3A) and frequency (3B) of elevated serum alpha-fetoprotein (AFP) in nonhepatic neoplasms and primary hepatoma. Highest levels of serum AFP occurred in various nonhepatic neoplasms are used to plot in figure 3A. Data used are from McIntire et al.17](image-url)
TABLE I

Some Features Associated with Nonhepatic Neoplasms Producing Elevated Concentration of Serum Alpha-fetoprotein

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
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<td>Majority have regional or distant metastases.</td>
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<td>Liver may not be involved.</td>
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<td>Fetal cells are capable of synthesizing alpha-fetoprotein.</td>
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<td>Liver function tests are not necessarily abnormal.</td>
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<td>The level of serum alpha-fetoprotein responds to therapy.</td>
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<td>Compared to hepatoma, there is a higher percentage of Con A nonreactive serum alpha-fetoprotein.</td>
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<td>Only 20 to 30 percent have elevated concentration of serum alpha-fetoprotein.</td>
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I are several important features associated with these AFP-producing, nonhepatic tumors. The types of nonhepatic tumors capable of synthesizing AFP are listed in table II.

It is important to emphasize that even though most of these nonhepatic tumors have metastasized, liver metastasis is not required for the production of elevated serum AFP. Not only do many of these tumors not metastasize to the liver, but patients with nonentodermally derived neoplasms, (i.e., breast, kidney, and bladder) metastatic to the liver do not have elevated levels of AFP. It has also been observed that rapidly growing and poorly differentiated tumors generally have higher AFP concentration. As an Early Marker

It is well known that patients with liver cirrhosis and people with a history of positive for chronic hepatitis B surface antigen are at a much higher risk for the development of hepatoma. Since the level of serum AFP is usually much higher in primary liver carcinoma than that in benign liver diseases, serum AFP can therefore be used as a marker for the early detection of hepatoma in those high risk populations. However, only the Chinese screening program has been successful because of the high prevalence of liver cancer in China. In China, it was estimated that 89.9 percent of primary liver carcinoma is HCC and 89.6 percent of HCC has AFP level >20 ng per mL. Therefore, roughly 80 percent of primary liver cancers in China could be detected by a sensitive AFP assay. The AFP screening in China was most successful in detecting "subclinical HCC" and "small HCC". On the average, the Chinese program was able to detect HCC approximately 3.1 months before symptoms occurred. However, 20 to 50 percent of HCC still went undetected with the use of AFP screening alone.

TABLE II

Nonhepatic Neoplasms with Increased Concentration of Serum Alpha-fetoprotein

<table>
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<tr>
<th>Neoplasm</th>
<th>Concentration</th>
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<tr>
<td>Gallbladder carcinoma</td>
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<tr>
<td>Gastric carcinoma</td>
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<tr>
<td>Lung cancer</td>
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<tr>
<td>Melanotic neuroectodermal tumor of infancy</td>
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<td>Renal cell carcinoma</td>
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<td>Pulmonary atypical carcinoid tumor</td>
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<td>Pulmonary blastoma</td>
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<tr>
<td>Ovarian mucinous cystadenocarcinoma</td>
<td></td>
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<tr>
<td>Ovarian Sertoli-Leydig cell tumor</td>
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disease are transient; they will rise and then fall as have been discussed earlier in this article. Besides, the concentration of serum AFP in patients with benign liver diseases rarely exceeds 500 ng per mL (figure 1). In active liver diseases, the value of ALT is usually several times the normal.

It should be noted, however, that when differentiation is based on a fixed level of serum AFP, a significant number of patients with small HCC could be missed. The Chinese screening program found that if the diagnostic criterion for HCC was set at a level of AFP >500 ng per ml, at least 21.9 percent of small HCC would have gone undetected. As shown in figure 1, patients with benign liver diseases can have serum levels of AFP above 500 ng per mL, and patients with HCC can have serum levels less than 500 ng per mL. Consequently, differentiation of HCC from benign liver diseases will not be complete if differentiation is based on the measurement of serum concentration of AFP alone.

Differentiation Based on AFP Fucosylation

The carbohydrate composition of the sugar chain of a glycoprotein is dependent on a set of glycosylation enzymes present in the endoplasmic reticulum and in the Golgi apparatus of the cell. It is well known that certain glycosyltransferases may become elevated in some tumors and are useful as tumor markers. Consequently, the carbohydrate composition of many glycoproteins is expected to be different in benign and the malignant diseases because of the alteration of these enzymes.

Apparently, the composition of the carbohydrate chain of AFP changes following malignant transformation, and an increase of fucosylation of the carbohydrate chain of AFP is found in HCC. Since an increase of fucosylation enhances the lentil lectin (LCA) reactivity of serum AFP, determination of LCA reactivity of serum AFP allows a differentiation between HCC and benign liver diseases. A dramatic increase in fucosylation of serum AFP was also observed in five patients with a long history of hepatic cirrhosis when they started to develop HCC. Using affinity chromatography on a column containing lentil lectin bound Sepharose 4B, Buamah et al found that only seven to 15 percent of the serum AFP from patients with non-malignant liver diseases bound to the column, whereas an increased percentage of serum AFP, i.e., 25 to 83 percent, from patients with malignant liver diseases bound. Smith et al, using a similar affinity column chromatographic technique, found that most AFP in sera from patients with chronic liver diseases did not bind to LCA (86 percent), whereas only 37.8 to 45.7 percent of serum AFP from HCC patients were LCA-nonreactive.

Aoyagi et al employed the affinity electrophoretic procedure to determine the fucosylation index (F index, or the percentage of the LCA reactive AFP variant in total AFP) of serum AFP. They pointed out that the F index is especially useful for the distinction of HCC from benign liver diseases when the serum AFP is <1000 ng per mL. They found that the F index of AFP in all patients with HCC was 42 ± 31 percent and 68 percent of their 258 patients with HCC had an F index above 42 percent, whereas the F index of patients with benign liver diseases was much lower at 4 ± 7 percent. Since the F index is independent of the serum concentration of AFP, it may be useful for the discrimination of HCC from benign liver diseases at an early stage or for the early diagnosis of HCC in high risk patients.

A more sensitive technique of affinity electrophoresis was recently developed
to detect HCC-specific, or fucose-containing AFP variants. As little as 50 ng per ml of serum AFP could be detected by blotting the bands of AFP on a nitrocellulose membrane, precoated with the specific anti-AFP, and then staining with peroxidase-labeled anti-AFP antibody (lectin-immunoblotting). This more sensitive technique allowed detection of an HCC-specific AFP variant at a very early stage, in advance of any other evidence of HCC by clinical examination.

Differentiation Among Various Nonhepatic Neoplasms

Because glycosylation enzymes are different in different tissues, one would expect to find different carbohydrate chains of AFP and, hence, different lectin reactivities among various AFP-producing nonhepatic neoplasms. For example, measurement of Con A reactivity successfully differentiated yolk sac tumors from primary hepatoma, because the Con A nonreactive AFP variant is present at much higher concentrations in yolk sac tumors than in HCC. Con A reactivity of AFP also appears useful for the differentiation between primary hepatoma and the hepatic secondaries, and to distinguish HCC from other carcinomas. However, future studies are needed to determine whether metastasis to the liver has any effect on the lectin reactivity of the nonhepatic neoplasms.

At the present time, a single lectin reactivity is unlikely to differentiate among various AFP-producing, nonhepatic tumors. Instead, the pattern of reaction with several lectins may be required to distinguish one neoplasm from the others. A pattern of percent distribution of various AFP lectin variants may be identifiable to a specific tumor. Among all the lectins available, the combined use of Con A, LCH, PHA-E and Allo A holds most promise.

References


