Markedly Increased Alpha-Fetoprotein Concentration in Serum in Alcoholic Liver Disease: Malignant Tumor or Nonneoplastic Changes?

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We present a patient with a history of alcohol abuse who was admitted to a hospital in poor condition. His serum concentration of alpha-fetoprotein was markedly increased [initially 1400 kilo-int. units/L, after four months 6600 kilo-int. units/L (~1700 and 7900 μg/L, respectively)]. This finding led us to suspect the presence of a liver tumor, which was supported by ultrasonographic examination. However, further evaluation did not confirm the contention of hepatic malignancy at that time.

We discuss possible mechanisms for an increase in serum alpha-fetoprotein concentration in nonneoplastic liver disorders and suggest investigations that may lead to a better understanding of such cases.

Case History

The patient was admitted to a hospital in 1987 at age 64 years. He had a two-week history of deterioration, malaise, and jaundice. He had abused alcohol periodically for many years. Before admission he had been drinking heavily for a couple of months but had not taken any drugs known to cause liver damage nor been exposed to any occupational chemical hazard. He had not received any blood transfusions, nor had he been known to use drugs intravenously. He had been treated with diet alone for diabetes mellitus type II since the age of 50 years and with metoprolol, hydralazine, and spironolactone for arterial hypertension.

Physical examination showed jaundice, vascular epidermal spiders, and an enlarged and lobulated liver, which was palpable 4 cm below the right costal margin. The patient was weak, tremulous, and somewhat confused. Hepatic ultrasonography disclosed a large tumor, 8 cm in diameter, in the right hepatic lobe and also showed a mixed echographic pattern typical of cirrhosis. A moderate amount of ascites was detected with ultrasonography, although not by clinical examination.

Biochemical evaluation of the liver function showed increased serum activities of aspartate and alanine aminotransferases (Figure 1) and of alkaline phosphatase (6.7 μkat/L, upper normal reference limit 5.0 μkat/L). The concentration of serum bilirubin was markedly increased, and the plasma activity of the prothrombin complex was low (Figure 1). Other findings were a decreased serum albumin concentration (25 g/L; lower normal reference limit 33 g/L) and increased serum concentrations of immunoglobulins A (8.9 g/L; normal reference limit <3.0 g/L), G (23 g/L with polyclonal distribution; normal range 7–14 g/L), and M (2.1 g/L; normal reference limit <1.5 g/L). There was no serological evidence of infection with hepatitis B virus. We did not judge the amount of ascites fluid to be large enough to indicate diagnostic paracenthes.

The serum concentration of alpha-fetoprotein was 1400 kilo-int. units/L at admission (upper normal reference limit 15 kilo-int. units/L). This protein was determined by a double-antibody solid-phase radioimmunoassay (competitive type, labeled analyte immunoassay, RIA-gnost® AFP-Tachist®; Behringwerke AG, Marburg, F.R.G.) calibrated against the World Health Organization human cord serum standard 72/225. According to an international collaborative study (1), 1 int. unit corresponds to 1.2 ng (90% confidence interval 1.0–1.4). It has been reported that falsely high values for alpha-fetoprotein in serum may occur as a result of interfering heterophilic antibodies, which is an interference of particular concern with immunometric assays (2, 3). No analytical interference was responsible for the high values in our case though, judging from the results from multiple dilutions.

From the history of long-standing alcohol abuse, the ultrasonographic findings, an aspartate aminotransferase/alanine aminotransferase activity ratio well above 1.0, and increased immunoglobulin concentrations, in particular A and G classes, we concluded that the patient suffered from liver cirrhosis; i.e., he was at risk for developing hepatocellular carcinoma. Because we detected a tumor by ultrasonography and because the concentration of alpha-fetoprotein in serum was increased, we concluded that the patient did have this malignancy even though the diagnosis had not been confirmed by liver biopsy. No therapy directed against the presumptive carcinoma was offered, in view of the patient's poor condition. He was treated symptomatically with bed rest and lactulose. Because it seemed probable that the blood pressure could be controlled by only metoprolol, both hydralazine and spironolactone were initially withdrawn. However, because of the ultrasonographic finding of ascites 12 days after admission, we restarted treatment with spironolactone. There was no need for other diuretics. The patient's condition
improved gradually, and he was discharged three weeks after admission, mentally alert and in good physical condition. The patient abstained totally from alcohol after discharge from the hospital.

During the next 13 weeks there was a marked increase of the alpha-fetoprotein concentration, which reached a maximal value of 6600 kilo-int. units/L (Figure 1). There was also a mild increase of the aminotransferase activities. However, the other results from the biochemical evaluation of the liver tended slowly to normalize. We then performed a liver biopsy because of the patient's improved condition and the absence of a coherent picture from the biochemical evaluation. Guided by ultrasonography, we obtained a fine-needle aspiration specimen 17 weeks after the first admission. The light-microscopic cytological examination revealed necrosis and cellular changes, including degenerative as well as regenerative processes, in different areas of the liver. No malignant cells were found. Considering the diagnostic difficulties with liver carcinoma, we felt that this diagnosis was not excluded, and the patient was scheduled for further clinical and biochemical follow-up.

After one year, the activities of aminotransferases, alkaline phosphatases, and plasma prothrombin complex as well as the concentration of serum bilirubin were almost normal (Figure 1). The concentration changes of alpha-fetoprotein (logarithmic scale in Figure 1) were essentially synchronous with those of the aminotransferases, albeit more marked, and values ≥15 kilo-int. units/L were observed after one year. Neither careful examination of records nor interview gave any information to explain the transient increases in alpha-fetoprotein and aminotransferase concentration during the fourth month after admission.

Reevaluation was done after two years, when the patient was in good condition and without ascites, as evidenced by computed tomography of the abdomen. His liver was still enlarged and lobulated, and hepatic sonography showed an unchanged picture. A liver biopsy by the Menghini technique revealed cirrhosis without any evidence for hepatic malignancy.

Efforts to demonstrate a metabolic liver disease were done initially and after 2.5 years. However, no abnormality could be found after determining serum α₁-antitrypsin, α₁-antichymotrypsin, ceruloplasmin, and ferritin or urinary porphyrins. Also, amino acid concentrations in serum and urine were within the normal reference intervals. Normal concentrations were found for chorionic gonadotropin and its beta-peptide.

The patient was followed for more than three years through regular outpatient visits. He was now a teetotaller and was still in good condition. Normal results were obtained for the liver tests referred to above except for high concentrations of immunoglobulins (8.5, 19, and 1.7 g/L, respectively, for immunoglobulins A, G, and M). The concentration of serum alpha-fetoprotein was <15 kilo-int. units/L after one year and remained so during the next two years; in November 1990 it was 7 kilo-int. units/L.

In March 1991, three years and four months after the initial admission, the patient was admitted again because of a three-week history of deterioration, malaise, and jaundice. Physical examination showed jaundice and an enlarged liver. Hepatic ultrasonography disclosed a sonographic pattern typical of advanced malig-
nancy in both hepatic lobes. The cytologic examination indicated hepatocellular carcinoma. Biochemical evaluation of the liver function showed increased serum activities of aspartate and alanine aminotransferases (3.2 and 1.9 μkat/L, respectively) and of alkaline phosphatase (12 μkat/L). The concentration of serum bilirubin was markedly increased (330 μmol/L), and the plasma activity of the prothrombin complex was slightly decreased (60%). There was no serological evidence of infection with hepatitis C virus.

The concentration of alpha-fetoprotein was now only slightly increased, 17 kilo-int. units/L. At this time, determination was done with an immunoradiometric assay (RIA-gnost AFP; coated tube). This assay, using the same calibrator as the previous assay, has an upper normal reference limit of 5 kilo-int. units/L.

The patient deteriorated rapidly and died three weeks after admission. The autopsy, which included histopathological examination of the liver, revealed widespread hepatocellular carcinoma in both liver lobes as well as liver cirrhosis. There was no evidence for metastatic disease outside the liver. The peritoneum was normal and no ascites was present.

Discussion

Several diagnostic alternatives may be considered for our patient. One is that he initially did have a hepatocellular (or other) carcinoma, which regressed spontaneously and (or) changed its secretion of alpha-fetoprotein with a concomitant normalization of hepatic function. Another is that the increased alpha-fetoprotein concentrations at first admission resulted from nonneoplastic changes.

Alpha-Fetoprotein as a Marker of Hepatocellular Carcinoma

Alpha-fetoprotein was first shown to be a serum component by the Swedish investigators Bergstrand and Czar (4), who studied fetal serum with paper electrophoresis. Alpha-fetoprotein as a marker for hepatocellular carcinoma originates from the studies by the Russian investigators Abelev et al. (5), working with a transplantable mouse hepatoma, and Tatarinov (6), who reported increased serum concentrations in a patient with hepatic carcinoma. Many analytes have been proposed as biochemical markers of such tumors, but alpha-fetoprotein remains the one most commonly used (7). Increased serum concentrations, i.e., >10–15 μg/L, may also be encountered in patients with other neoplasms (8–10). In patients with liver cirrhosis where extrahepatic malignancy is not suspected, a serum concentration >500 μg/L is considered to be highly specific for diagnosing hepatocellular carcinoma (7, 11–13), but massive hepatic necrosis, e.g., due to viral hepatitis, may also cause increased values.

Since patients at risk for hepatocellular carcinoma began to be examined by ultrasonography or roentgenographic methods, there has been a steady decrease in reports of sensitivity of serum alpha-fetoprotein used diagnostically for that neoplasm (14). Attempts have been made to increase the diagnostic efficiency of the alpha-fetoprotein determination by utilizing the microheterogeneity caused by different carbohydrate moieties (14), but such methods are not in general use.

Survival time has been reported to be inversely related to the alpha-fetoprotein concentration (15, 16), patients with values >1000 μg/L having a median time of less than six months (16). Serum alpha-fetoprotein concentrations correlate to the size of the tumor, but there are large interindividual differences, with tumor differentiation apparently playing some role (14, 17). Furthermore, a spontaneous decrease in concentration without corresponding change in tumor size was reported in several studies, complete normalization being reported in single cases (17, 18). However, a return to previous high values usually occurs within a few months (18). In a few other cases, parallel decreases of alpha-fetoprotein concentration and tumor size were observed (19).

Alpha-Fetoprotein in Nonneoplastic Liver Disorders

Increases in serum alpha-fetoprotein concentration may be found in many nonneoplastic hepatic disorders, e.g., viral hepatitis, drug hepatitis, chronic active hepatitis, cirrhosis of various etiologies, alcoholic liver disease, and extrahepatic biliary disease (20–26). The finding of an increased concentration of alpha-fetoprotein in patients with chronic viral hepatitis was reported to indicate decreased survival (27). The alpha-fetoprotein concentration is usually only moderately increased, but values >500 μg/L have been occasionally found. This is particularly true in disorders in which there is substantial hepatocellular necrosis, e.g., acute exacerbations of chronic active hepatitis, fulminant viral hepatitis, halothane hepatitis, and hepatic ischemia (8, 22–24, 26). The highest values reported are 6000 μg/L in acute viral hepatitis (20), 8650 μg/L in chronic active hepatitis (28), and 7190 μg/L in active liver cirrhosis (29), which is the same order of magnitude as in our case.

Moderately increased concentration of serum alpha-fetoprotein has been documented in patients with alcoholic liver disease, but it is by no means a regular finding. Different reports give widely different figures for the prevalence of increased alpha-fetoprotein concentration (Table 1). For instance, although 13 of 75 patients (17%) with postalcoholic liver cirrhosis were reported to have concentrations >50 μg/L/L (30), none of 113 patients with cirrhosis in another study (34) had an alpha-fetoprotein concentration above that limit. In a study of 77 patients (28) only patients with alcoholic hepatitis had increased values. The differences between the results from different studies cannot be readily explained; reasons for these differences may be the nature of the study population, the degree and extent of hepatic cell necrosis resulting from intoxication, the timing of blood sampling for assay in relation to the period of alcohol abuse [ethanol depresses serum alpha-fetoprotein concentrations in rats, with a rebound increase after alcohol withdrawal (36)], previous or present hepatitis virus infection, metabolic liver disease,
Table 1. Prevalence of Increased Serum Concentration of Alpha-Fetoprotein in Patients with Alcoholic Liver Disease: Reports since 1974

<table>
<thead>
<tr>
<th>Publication year (ref.)</th>
<th>No. of cases</th>
<th>Decision limit, µg/L</th>
<th>Increased concn, %</th>
<th>Maximum concn, µg/L</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>1975 (30)</td>
<td>75</td>
<td>50</td>
<td>17</td>
<td>440</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td>1975 (32)</td>
<td>77</td>
<td>40</td>
<td>8</td>
<td>118</td>
<td>Increased values only in alcoholic hepatitis (n = 40)</td>
</tr>
<tr>
<td>1978 (31)</td>
<td>43</td>
<td>30</td>
<td>14</td>
<td></td>
<td></td>
</tr>
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<td>32</td>
<td>20</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1982 (33)</td>
<td>33</td>
<td>40</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1982 (33)</td>
<td>110</td>
<td>20</td>
<td>9</td>
<td>100</td>
<td>Fatty liver, cirrhosis, alcoholic hepatitis</td>
</tr>
<tr>
<td>1986 (34)</td>
<td>134</td>
<td>20</td>
<td>6</td>
<td>39</td>
<td>Cirrhosis (n = 113)</td>
</tr>
<tr>
<td>1988 (35)</td>
<td>30</td>
<td>10</td>
<td>27</td>
<td>600</td>
<td></td>
</tr>
</tbody>
</table>

Increased alpha-fetoprotein concentration indicates a poor prognosis; in one study of a large group of patients with liver cirrhosis, 27% of the patients with a concentration >50 µg/L had developed hepatocellular carcinoma, in contrast to 1% of those with a concentration <50 µg/L (37). Although that study did not differentiate between postalcoholic, posthepatitic, and cryptogenic cirrhosis, earlier studies from these authors had shown approximately equal risk of carcinoma developing in these groups.

Two case reports highlight the diagnostic difficulties in these cases. One patient had postalcoholic active liver cirrhosis with a transient rise in serum alpha-fetoprotein concentration of the magnitude observed here (29). In another patient, who had alcoholic hepatitis, a transient increase of serum alpha-fetoprotein concentration was found with counterimmunoelectrophoresis (38). That patient resumed heavy alcohol consumption; the alpha-fetoprotein concentration remained low during one year of follow-up.

Thus, patients with alcoholic liver disease may display increased serum concentrations of alpha-fetoprotein, the reported prevalence of increased values differing widely between studies. Although some of the cases with increased concentrations later were reported to have hepatocellular carcinoma, and therefore were presumed to have already had this cause for the alphafetoprotein increase when it was first registered, other patients were reported to have a transitory increase without a demonstrable relationship to carcinoma.

Course of Hepatocellular Carcinoma

Patients with liver cirrhosis are at risk for developing primary liver cancer (39). Hepatocellular carcinoma may develop uncentrally or multicentrically (40). Usually the prognosis is poor, but the rate of progression may vary in each patient (17). There are even reports of spontaneous regression (41-44): In one patient with a 10 × 10 cm tumor and serum alpha-fetoprotein concentration of 2680 µg/L, spontaneous regression could be confirmed by autopsy two years after diagnosis (41). One patient with hepatocellular carcinoma, bone metastasis, and an alpha-fetoprotein concentration of 26200 µg/L (which regressed spontaneously) was monitored for five years and was apparently healthy after that time (42). Another patient with alcoholic cirrhosis and serological evidence of previous hepatitis B infection, in which alpha-fetoprotein could not be demonstrated by counterimmunoelectrophoresis, had disseminated intrahepatic hepatocellular carcinoma, which regressed spontaneously; the patient was apparently healthy after four years (43). A fourth case of hepatocellular carcinoma, for which there is no information on serum alpha-fetoprotein concentrations, regressed spontaneously and had a disease-free period of ≥13 years (44). We have also observed, as part of a comprehensive study of primary liver cancer, a case of histologically confirmed hepatocellular cancer with spontaneous regression within two years, as evidenced by celiac angiography as well as by exploratory laparotomy after another two years. This patient survived for 15 years (unpublished data). Thus, spontaneous regression of hepatocellular carcinoma has been reported, but, judging from the number of cases described, it seems to be a very rare occurrence.

Diagnostic Considerations

We conclude that the patient's poor clinical condition at admission and the abnormal laboratory findings were due to alcoholic hepatitis in a cirrhotic liver. We assume that widespread necrosis occurred during the weeks preceding admission. After admission, test results indicated recovery of hepatic cell integrity (as evidenced by the aminotransferases) and function (bilirubin and prothrombin complex). Evaluating the hepatic ultrasonographic pattern is notoriously difficult in patients with chronic disease of the liver with regions of regeneration (45). The "tumor" as well as the lobulation of the liver found at hepatic sonography probably represented macronodular cirrhosis, in view of the absence of change during two years and the absence of light-microscopic evidence of malignancy. Such an examination of ultrasound-guided fine-needle liver biopsy reportedly has a diagnostic sensitivity of 95% (46).

We explored the possibility that not only alcoholic liver disease, but also a toxic or viral agent or metabolic liver disease, was responsible for the biochemical abnormalities at admission. However, no occupational chemical hazard or toxic agent other than ethanol could be identified. Regarding viral infections, acute hepatitis A is unlikely in view of the absence of marked aminotransferase increases. There was no evidence of hep-
atiitis B or C infection or of metabolic liver disease, such as hemochromatosis, Wilson disease, porphyria, abnor-
mal protease synthesis (α1-antitrypsin or α1-antichy-
motrypsin), immune disorder, or abnormal amino acid
metabolism (e.g., tyrosinemia or cystathioninuria).

The initially increased serum alpha-fetoprotein con-
centrations and the increase months after discharge (Figure 1) are enigmatic. The possibility of release from necrotic hepatocellular carcinoma cannot be excluded; cases with spontaneous regression have been described. In that case, we would have to assume that necrosis of tumor tissue occurred not only when the patient was admitted, but also after four months when he was seemingly healthy. For several reasons, therefore, we do not consider hepatic malignancy as a likely cause of the initial alpha-fetoprotein changes; the patient developed hepatocellular carcinoma more than three years after the initial admission, when the serum concentration of alpha-fetoprotein was only slightly increased.

It is possible that the late increase in serum alpha-
fetoprotein concentration, i.e., during the months after initial admission, was due to reabsorption of alpha-
fetoprotein from the peritoneal cavity when the ascites fluid was being eliminated by diuretic treatment. Of course, this does not explain the primary cause of the serum alpha-fetoprotein increase.

The similarity of the concentration changes for ami-
notransferases and alpha-fetoprotein suggests cellular release of these components. However, leakage across liver cell membranes cannot alone explain the increased serum concentrations of alpha-fetoprotein, because these were far greater than the modest increases in aminotransferase concentrations; thus, increased alpha-
fetoprotein synthesis is likely. One possibility is that an immunological reaction to material released from the necrotic tissue played a role, because morphological changes in alcoholic liver disease may partly be due to autoimmune phenomena (47, 48). Another possibility is increased synthesis because of liver regeneration.

Possible Mechanisms for Increased Alpha-Fetoprotein Synthesis in Nonneoplastic Liver Disorders

Activation of alpha-fetoprotein synthesis in hepatocyes. Results from experiments with hepatotoxic agents led Taketa (14) to suggest that dedifferentiation of hepatocytes, leading to increased capacity for alpha-
fetoprotein production, occurs after widespread liver injury. Intercellular interactions may be important for the regulation of alpha-fetoprotein synthesis in liver cells (49). Free hepatocytes may produce alpha-fetoprotein, e.g., in liver cell culture and when the normal architecture is broken as a result of tissue necrosis, whereas hepatocytes in the strict network characteristic of lobular cells lose this capacity. There is evidence for a growth factor regulation of alpha-fetoprotein synthesis under cell-culture conditions (50).

Proliferation of immature liver cells. Serial measure-
ments in patients with viral hepatitis showed increased serum alpha-fetoprotein concentration while hepatic necrosis subsided, suggesting that increased alpha-feto-
protein concentration reflects hepatic regenerative ac-
tivity (20, 22, 23). However, the absence of changes in serum alpha-fetoprotein concentration after partial hepa-
tectomy in adult humans has been taken as evidence against a relationship to liver regeneration (51, 52). Nevertheless, Abelev (53) found that liver regeneration in rats leads to alpha-fetoprotein synthesis in partially hepatectomized animals, but only when resection is performed within five weeks after birth. Furthermore, liver regeneration after any injury results in increased alpha-fetoprotein concentrations in human newborns but not in older children (51).

We suggest, as a plausible explanation for these seemingly contradictory findings, that liver regeneration after widespread necrosis in adults, as well as after any injury in newborns, occurs mainly through prolif-
eration of immature liver cells, whereas regeneration in adults after heptectomy occurs through multiplication of mature hepatocytes (54). The basis for our hypothesis is as follows:

- The change from large concentrations of alpha-feto-
protein during fetal life to the small concentrations some months after birth (55) results from maturation of liver cells, causing a decreased ability to produce alpha-
fetoprotein.

- Immature cells also exist in the adult liver (56, 57). Studies of experimental carcinogenesis in rats showed so-called oval cells appearing in the periphery of the hepatic lobules. These cells may differentiate into hepa-
tocytes or bile-duct cells and may cause the alterations of the isoenzyme patterns of some intracellular liver enzymes toward a fetal pattern observed early after carcinogen administration, as well as increased concentra-
tion of circulating alpha-fetoprotein (57, 58). Immuno-
fluorescence studies in humans recovering from acute hepatitis showed that alpha-fetoprotein is produced mainly in oval cells and in cells that appear to be a transition to hepatocytes (59).

- Livers of fetuses and newborn infants of mothers who are carriers of hepatitis B virus are resistant to infection with this agent until several weeks postpartum, as evidenced by the late appearance of circulating virus in the blood (60, 61). This observation is explained by immature cells being less susceptible to infection than are mature cells (61).

Thus, adults will probably have a relative enrichment of resistant immature cells able to produce alpha-feto-
protein after liver necrosis, because of viral infection. This may also be the case in alcohol-induced necrosis.

A better understanding of the etiology of the changes in serum alpha-fetoprotein concentration in alcoholic liver disease requires longitudinal studies comprising both monitoring of biochemical variables and studies of liver cell morphology and function that identify the cells involved in the release of alpha-fetoprotein to the circu-
lation. Proliferation of undifferentiated cells may be clinically important because such cells have been implicated in the pathogenesis of liver carcinoma (62). In a particular patient, therefore, even if an increased alpha-
fetoprotein concentration does not indicate a carcinoma,
it may indicate risk of later development of such carcinoma. Determinations of growth factors implicated in hepatocyte regeneration (63, 64) in conjunction with alpha-fetoprotein determinations may provide a better understanding of the degree of hepatocyte destruction and recruitment of different cell types in hepatic regeneration.

We speculate that hepatic regeneration after widespread hepatic necrosis induced by alcohol abuse involves proliferation of undifferentiated liver cells. Such cells, which have been shown to produce alpha-fetoprotein, have been implicated in the development of hepatic carcinoma. There is a need for a better understanding of the mechanisms involved in alpha-fetoprotein synthesis and release in this group of patients, and of the clinical importance of an increased serum concentration of alpha-fetoprotein.

References
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