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Alpha-fetoprotein–Lectin Binding as a Marker of Tumour Activity or Liver Damage

Jan Marrink, Dirk Th. Sleijfer, Elisabeth G.E. de Vries and Heimen Schraffordt Koops

To establish whether alpha-fetoprotein (AFP) produced in the early post-treatment phase of a patient with a germ cell tumour of the testis or the ovary originates from the tumour or is due to an underlying disturbance in liver function, the binding of AFP to concanavalin A (Con A) was investigated as a discriminative variable. A two-step assay is described that can distinguish the type of AFP produced at levels as low as 10 ng/ml. A Con A-binding ratio of 12–43% was found in the patients with disseminated germ cell tumours and in patients with AFP-positive gastrointestinal carcinomas. AFP from the liver gives ratios below 10%.
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INTRODUCTION

RAISED SERUM levels of alpha-fetoprotein (AFP) are generally associated with tumours of tissues of endodermal origin. Therefore this marker is widely used in the diagnosis and monitoring of patients with hepato-cellular carcinoma and germ cell tumours. To a lesser extent, AFP is found in the serum of patients with gastrointestinal carcinomas. The interpretation of any increase in AFP levels in follow-up is hampered by the fact that the serum concentration also increases in non-malignant diseases in which the liver is involved. Moreover, reversible increases in AFP might be due to the hepatotoxic effect of chemotherapy [1–3]. Thus a method to distinguish AFP produced by maligancies from that due to benign liver activity would be useful, especially in the early phase of a relapse when marker levels are low.

Variations in the carbohydrate moiety of AFP result in different reactivities with lectins [4]. Different patterns of

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AFP–lectin binding can facilitate differential diagnosis of primary hepatomas and germ cell tumours and may be of use in discriminating increases due to malignant deterioration of a germ cell tumour vs. a rise caused by an underlying liver disease. Concanavalin A (Con A) binding has been measured by affinity electrophoresis [5,6], affinity-crossed-immuno-electrophoresis [7,8] and affinity chromatographic separation on minicolumns [9–13]. These methods, however, are laborious and not useful at low AFP levels (i.e. under 100 ng/ml). We have developed a quantitative two-step procedure for the analysis of the AFP–con A binding ratio that is simple and sensitive.

PATIENTS AND METHODS

Patients

Sera were taken from the following patients. Group I: 130 patients with non-seminomatous germ cell tumours of the testis or ovary. Group II: 30 patients with liver disease (including 14 with primary liver cancer). Group III: 9 patients with elevated levels of AFP out of a group of 66 patients with carcinoma of the gastrointestinal tract (Table 1). The only eligibility criterion was a positive serum AFP level (i.e. above 10 ng/ml). Sera were frozen at −20°C until use.

AFP–Con A binding ratio

Con A Sepharose (Pharmacia) was washed on a glass filter with Con A buffer 0.5 mmol/l NaCl, 1 mmol/l $MnCl_2$, 1 mmol/l $MgCl_2$, 1 mmol/l $CaCl_2$ and 0.1 mmol/l sodium acetate buffer, pH=6.5. The Con A was subsequently dried in air. Con A buffer was added to 2 g of dry Con A Sepharose up to a volume of 10 ml. 1 ml of this suspension was mixed in a small tube with 1 ml Con A buffer. After centrifugation for 10 min at 1000 *g*, the supernatant was decanted; filter paper was used to remove the last drops. The tube with the Con A precipitate thus prepared is the Con A tube.

Sera with an AFP concentration above 300 ng/ml were diluted with normal human serum to an AFP concentration of approximately 300 ng/ml. Subsequently the sample was diluted 2.5 times: to 720 µl of the eventually prediluted serum sample, 480 µl of 2.5 times concentrated con A buffer was added; then, dropwise, 600 µl 45% PEG 4000 (poly-ethylene glycol, Merck) was added with continuous stirring. After centrifugation for 10 min at 1000 *g*, the supernatant was kept as the serum sample (i.e. before incubation with Con A).

PEG 4000 was used to precipitate most of the proteins which, besides AFP, bind to Con A, thus reducing the amount of Con A Sepharose needed.

In the first step of our assay 500 µl serum sample was pipetted into a Con A tube and incubated overnight at 4°C with an overhead rotator and subsequently centrifuged for 10 min at 1000 *g* at 4°C. The supernatant was the Con A sample (i.e. after incubation with Con A). In the second step the AFP concentrations were measured in the two samples (Abbott enzyme immunoassay). The ratio is defined as the percentage of non-bound AFP: (AFP in Con A-sample) × 100 ÷ (AFP in serum sample).

Table 1. Serum AFP elevations in patients with gastrointestinal cancers

Primary tumour	No. of patients	No. elevated
Oesophagus	3	0
Stomach	28	7 (25%)
Ileum	1	0
Colon	28	1 (3%)
Rectum	5	0
Pancreas	1	1
Total	66	9 (14%)

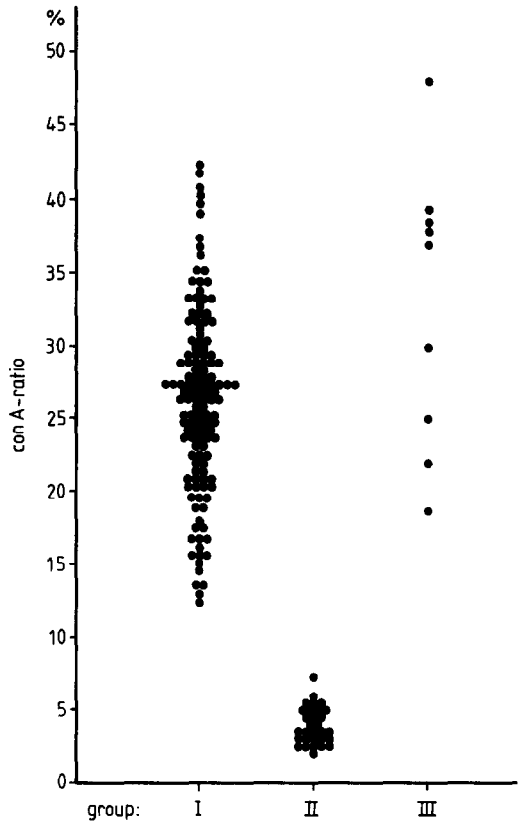

Fig. 1. Con A-ratios in non-seminomatous germ cell tumour patients (group I, *n* = 130), patients with liver disease (group II, *n* = 30) and patients with gastric carcinoma (group III, *n* = 9).

RESULTS

AFP levels

In group I, the patients with non-seminoma germ cell tumours, serum AFP ranged from 30 to 120 000 ng/ml. In group II, the patients with liver disease the range was 30–299 000 ng/ml. In group III (gastrointestinal cancer), the range was 14–78 000 ng/ml. In group III, 14% of the original 66 patients screened were AFP positive, depending on the primary site of the tumour (Table 1).

AFP–Con A ratio

The germ cell tumour patients revealed non-bound AFP–Con A ratios of 12–43%, whereas the liver group (primary liver cancer and benign liver diseases) had values below 10% (Fig. 1). The gastrointestinal carcinoma patients had ratios in the same range as the germ cell tumour group.

Follow-up

Figures 2–5 show AFP levels and AFP–Con A ratios in representative patients from each group.

In the patient with a non-seminoma germ cell tumour (Fig. 2) the initial decline of the AFP curve was followed by increasing

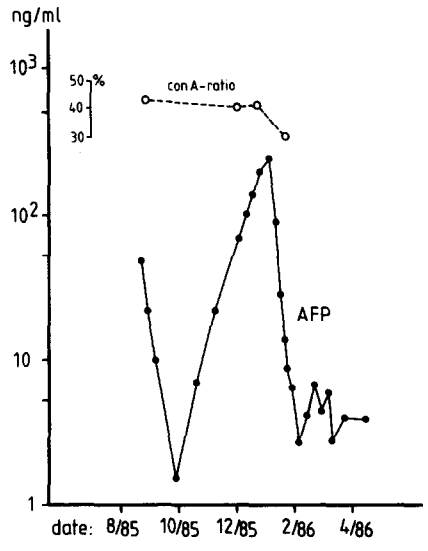

Fig. 2. Patient with non-seminatous germ cell tumour who relapsed.

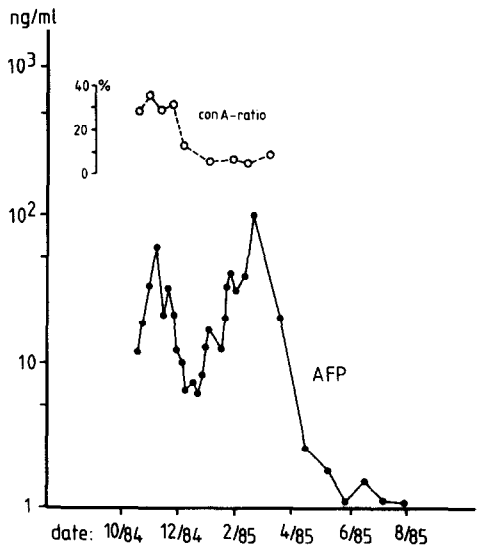

Fig. 4. Patient with non-seminatous germ cell tumour who had HB_sAg positive hepatitis in early 1985.

levels, warning of possible relapse. The Con A-ratio remained high (around 40%), indicative from a laboratory point of view of release of tumour AFP. The renewed growth of tumour was confirmed histologically and treated successfully with chemotherapy.

Patients with liver disease had low Con A ratios (Fig. 3). After the initial fall of AFP in this hepatoma patient, demonstrating the effect of the liver transplantation, the marker rose exponentially, a biochemical sign of malignant change. The Con A-ratio (3–5%) remained stable during the whole period, which is in line with the liver type of AFP produced during both periods.

Figure 4 shows differences in AFP types in relation to Con A-binding ratio within the same patient. In this patient with a non-seminomatous germ cell tumour, the initial fall of AFP after institution of chemotherapy was followed by an increase up to about 100 ng/ml. That this increase was not due to renewed production of tumour AFP but to liver activity could be concluded from the behaviour of the Con A-ratio curve. The initial value of around 30% (i.e. AFP of germ cell tumour origin) dramatically dropped to values below 8% at the top of the second AFP peak. Other laboratory indices (such as transaminases) confirmed the existence of liver function defects, due to a HbsAg-positive hepatitis which became manifest at this time.

In a patient with a carcinoma of the stomach, high levels of AFP, rose during follow-up, all of which exhibit a high Con A-ratio (Fig. 5).

DISCUSSION

AFP is a valuable marker in the diagnosis and monitoring of cancer patients, especially those with germ cell tumours and primary liver carcinomas [14–16]. In gastric cancer, about 15% of patients are positive [17,18], a value which we confirmed (14%). In benign liver diseases, such as hepatitis and cirrhosis,

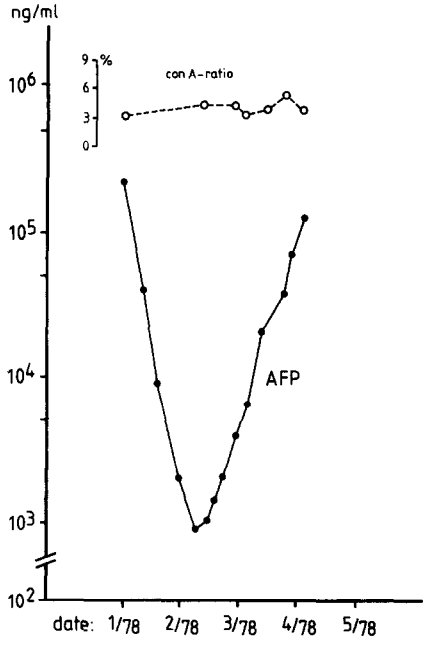

Fig. 3. Patient with hepatocellular carcinoma who had liver transplantation and subsequent relapse.

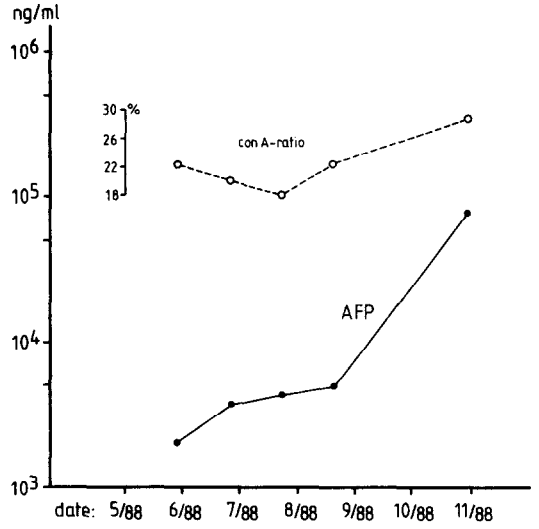

Fig. 5. Patient with carcinoma of the stomach who had high levels of tumour-AFP.

there might also be moderate production of AFP, generally below 500 ng/ml [16].

The prognosis of patients with non-seminomatous germ cell tumours has been greatly improved by cisplatin-containing chemotherapy [19–24]. Regular AFP measurements in the sera of these patients provide valuable information about the effect of therapy. The clinician should be certain about the relation between any increase in AFP and recurrence. This means that even at low levels of AFP production—i.e. above the upper normal value—the question whether the AFP is produced by the germ cell tumour or is due to liver dysfunction needs to be answered.

Our two-step assay can establish Con A-binding ratios at AFP levels as low as 10 ng/ml. Ratios for the non-bound fraction were 12–43% in the group with germ cell tumours and below 10% for patients with primary liver cancer or benign liver disease. These values agree with those reported previously [6–13]. During follow-up of our patients the Con A-ratio remained within certain limits around a fixed value for a particular patient, depending on the source of the AFP.

The practical use of our assay was demonstrated in those patients who had a secondary AFP increase. In some it was a true sign of relapse, in others the AFP rise was a false positive for renewed tumour activity. In such cases AFP production could be attributed to liver damage or regenerative processes within the liver. Hepatic dysfunction is often seen as a result of the hepatotoxic chemotherapy [1–3], hepatic synthesis of AFP is responsible for the rising level of the marker. Our assay enables discrimination at AFP levels down to 10 ng/ml.

Our findings shed light on the incidentally reported false-positive AFP increment during follow-up of germ cell tumour patients without clinical signs of relapse [1,25], and also in so-called AFP-positive seminoma patients. It is very important for treatment to distinguish a pure seminoma from the non-seminoma or mixed group. Since pure seminomas are negative for AFP any serum AFP should be taken as a sign that the marker is produced by non-seminomatous elements (which apparently were overlooked) or that the AFP is of liver origin. When serum AFP is enhanced in a germ cell tumour patient, a gastrointestinal carcinoma might be the cause since Con A-ratios could fall within the same range.

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