Pergamon

European Journal of Cancer Vol. 31A, Nos 13/14, pp. 2239–2242, 1995
Elsevier Science Ltd
Printed in Great Britain
0959–8049/95 $9.50+0.00

0959-8049(95)00485-8

Original Paper

Alpha-fetoprotein–Concanavalin A Binding as a Marker to Discriminate Between Germ Cell Tumours and Liver Diseases

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In order to differentiate whether slight alpha-fetoprotein (AFP) increases observed in any patient are due to germ cell tumours (GCT) or to liver diseases (including hepatotoxicity of chemotherapy), we measured the binding ratio of the AFP to concanavalin A (ConA). A total of 218 serum samples were studied: 102 samples from 72 GCT patients and 116 from patients with liver diseases. Considering a cut-off value to be a ConA binding ratio of 15%, we distinguished AFP produced by GCT (>15%) from AFP produced by tumoral and non-tumoral liver diseases (≤15%) with a sensitivity of 98% and specificity of 100%. The difference between mean ConA binding ratios was statistically significant ($P < 0.0001$). We did not distinguish AFP produced by tumoral and non-tumoral liver diseases. ConA binding ratio may be a sensitive index to distinguish whether an increase of AFP concentration as low as 15 U/ml in a GCT patient during the follow-up is produced by the tumour or by liver dysfunction (including hepatotoxicity of chemotherapy).

Key words: alpha-fetoprotein, concanavalin A, germ cell tumours, non-tumoral liver diseases
Eur J Cancer, Vol. 31A, Nos 13/14, pp. 2239–2242, 1995

INTRODUCTION

ALPHA-FETOPROTEIN (AFP) is widely used in diagnosis, in monitoring therapy and in follow-up of patients with germ cell tumours (GCT) and hepatocellular carcinoma. As has been reported previously, an increase in AFP concentration in the follow-up of patients with GCT is difficult to interpret due to the fact that AFP may also rise in non-tumoral diseases of the liver [1–3].

Concanavalin A (ConA)-Sepharose is a lectin that allows the separation of AFP molecular variants according to their carbohydrate moiety. The yolk-sac type of AFP has an additional *N*-acetylglucosamine linked to the β-mannose that blocks the ConA binding site on AFP[4]. Therefore, if we measure the non-bound AFP fraction to ConA-Sepharose, the yolk-sac type of AFP shows a higher percentage than the liver type.

The aim of this study was to determine the ConA binding ratio in patients with GCT and in patients with liver diseases (tumoral and non-tumoral) in order to differentiate between AFP increases produced by malignancies and those due to non-tumoral liver activity, including hepatotoxicity of chemotherapy.

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Revised 11 Jul. 1995; accepted 17 Aug. 1995.

PATIENTS AND METHODS

Patients

We analysed a total of 218 serum samples with an AFP concentration above the reference range (≤9 U/ml). One hundred and two samples were obtained from 72 patients with non-seminomatous GCT, treated with cisplatin-based chemotherapy (66 testicular tumours, 3 mediastinal tumours, 1 retroperitoneal tumour and 2 ovarian tumours; Group 1). In 12 of these patients, we determined the AFP–ConA binding ratio serially during the course of treatment with a median of six determinations per patient (range = 2–7). Moreover, we analysed 66 patients with non-tumoral liver diseases (38 hepatocirrhosis, 23 chronic hepatitis, 3 acute viral hepatitis and 2 alcoholic hepatitis) (Group 2), 45 patients with hepatocellular carcinoma (Group 3) and 5 patients with non-germ cell hepatic metastases (Group 4).

Control material

Lyphocheck® (Bio-Rad, ECS Div., Anaheim, California, U.S.A.) was used as control sera in order to evaluate the reproducibility intra- and interserial of the AFP–ConA binding method.

ConA binding method

The AFP–ConA binding method has been previously described by Marrink *et al.* [5]. Polyethylene glycol (45% PEG 4000) was used to precipitate most of the proteins which, besides AFP, bind to ConA Sepharose. After protein precipitation, 0.5 ml of the serum samples were incubated with 1 ml ConA suspension (20%) overnight at 4°C with agitation. ConA binding ratio was calculated as the percentage of non-bound AFP:

$$\frac{\text{AFP in the supernatant after incubation} \times 100}{\text{AFP in serum sample before incubation}} \text{ [5]} .$$

AFP method

The method used to measure AFP concentration was an enzyme-chemiluminometric assay (Amerlite®, Kodak Diagnostics, Amerham, U.K.). Statistical analyses were performed by analysis of variance.

RESULTS

The results obtained for the determination of AFP concentration and ConA binding ratio in different groups of patients are shown in Table 1. There were no differences in the concentration of total AFP in the different groups of patients. Median concentrations (U/ml) obtained were: 67.7 in GCT, 79.8 in non-tumoral liver diseases, 163.6 in hepatocellular carcinomas and 18.4 in non-germ cell liver metastases. Differences were observed in the values of ConA binding ratio between the GCT group and all liver disease groups ($P < 0.0001$) but not among the different liver disease groups. Median values obtained were: 30.9% in GCT, 8.6% in non-tumoral liver diseases, 7.5% in hepatocellular carcinomas and 11.7% in non-germ cell liver metastases. In order to evaluate the reproducibility of the ConA method, we assessed the intra- and interserial imprecision of control material used ($n = 15$). For a median AFP concentration of 201.0 U/ml and a median ConA binding ratio of 2.6%, a total imprecision of 12.4% in the intraserial and 18.1% in the interserial study (15 month period) were obtained. Figure 1 is a graphical representation (Box & Whisker Plot) of AFP–ConA binding ratio in the four groups of patients studied. When we established a criterion of ConA binding ratio >15% in order to discriminate germinal AFP from that of liver origin, we obtained a sensitivity and specificity of 98% (Table 2). If the criterion was lowered to >10%, the sensitivity and specificity were 100 and 62%, respectively. The serial determination of ConA binding ratio in the group of patients with GCT during the course of treatment (median period studied: 1.5 months, range: 1–3 months) indicated a median intra-individual ConA binding ratio of 31.7% (range: 23.4–39.5%) with a mean intra-individual variability of 10.8% (range: 4.9–16.9%). Figure 2a,b shows serial determinations of AFP and their ConA binding ratio in 2 patients with non-seminomatous GCT. While the ConA binding ratio values were ≤15% in the patient in Figure 2a, indicating that AFP was not secreted by germ cells, in the patient in Figure 2b the ConA binding ratio values were >15%, suggesting that AFP was secreted by germ cells. Consequently, the determination of the ConA binding ratio may be useful in determining whether an increase of AFP concentration as low as 15 U/ml in a GCT patient during follow-up is produced by the tumour or by liver dysfunction.

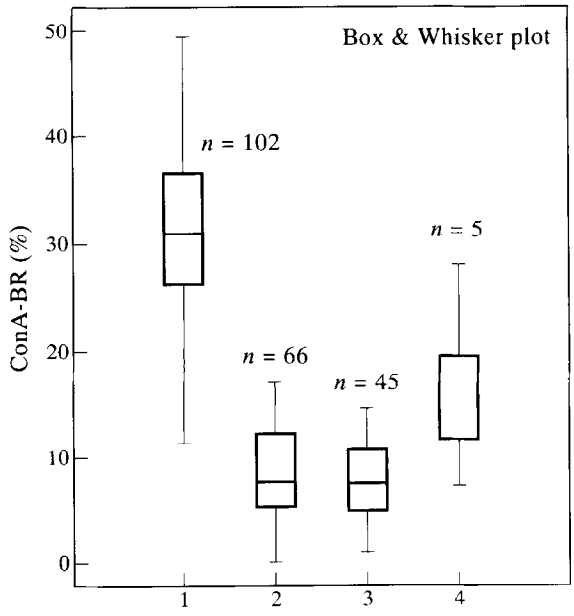

Figure 1. Concanavalin A binding ratio (median and range values) in the different groups of patients, expressed with the Box & Whisker Plot. Groups: 1 Germ cell tumours, 2 Non-tumoral liver disease, 3 Hepatocellular carcinomas, 4 Non-germ cell liver metastases. (ConA-BR: concanavalin A binding ratio).

Table 1. Total AFP concentrations (U/ml) and ConA binding ratios (%) in the different groups of patients

Disease	*n*	Total AFP*	ConA-BR†
Germ cell tumours	102	67.7 (10.7–20 500)	30.9 (11.4–49.3)
Non-tumoral liver diseases	66	79.8 (11.8–560)	8.6 (0.1–15)
Hepatocellular carcinomas	45	163.6 (12.0–10 000)	7.5 (1.3–14.6)
Non-germ cell liver metastases	5	18.4 (13.7–2440)	11.7 (7.3–28.1)

All values are expressed as median and range.

* No significant differences between groups.

† Significant differences ($P < 0.0001$) between germ cell tumour group and all liver disease groups together.

n, number of serum samples; AFP, alpha-fetoprotein; ConA-BR, concanavalin A binding ratio.

Table 2. Sensitivity and specificity of two different cut-off values of ConA binding ratio in order to discriminate germ cell AFP from that of hepatic origin

% ConA-BR	GCT	LD	NTLD	HC	LM	
>15	100	2	—	—	2	Sensitivity = 100/102 = 98%
≤15	2	114	66	45	3	Specificity = 114/116 = 98%
% ConA-BR	GCT	LD	NTLD	HC	LM	
>10	102	44	27	14	3	Sensitivity = 102/102 = 100%
≤10	—	72	39	31	2	Specificity = 72/116 = 62%

All liver diseases include non-tumoral liver diseases, hepatocellular carcinoma and non-germ cell liver metastases. ConA-BR, concanavalin A binding ratio; GCT, germ cell tumours; LD; all liver diseases; NTLD, non-tumoral liver diseases; HC, hepatocellular carcinoma; LM, non-germ cell liver metastases.

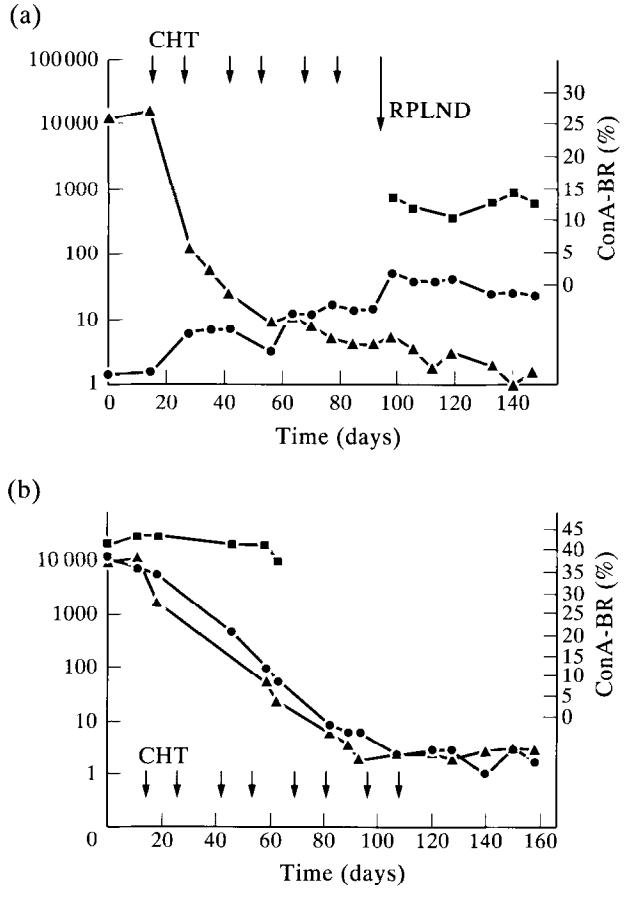

Figure 2. Determinations of alpha-fetoprotein (AFP) and concanavalin A (ConA) binding ratio. (a) 20 year old man with a non-seminomatous germ cell testicular tumour, who initially presented with a high level of beta-human chorionic gonadotrophin. Retroperitoneal lymph node dissection after chemotherapy showed fibrosis and necrosis with no evidence of tumour. All ConA binding ratio values were under 15% (10.8–14.7%) indicating that AFP was not secreted by germ cells. In this case, the AFP secretion was due to drug hepatotoxicity. (b) 16 year old man with a non-seminomatous germ cell testicular tumour, who initially presented with high levels of AFP and beta-human chorionic gonadotrophin. The elevated ConA-BR values over 15% (26.9–32.4%) indicate that AFP was secreted by germ cells. ● — ● Alpha-fetoprotein (U/ml). ■—■ Concanavalin A binding ratio. ▲—▲ Beta-human chorionic gonadotrophin (U/l). CHT, chemotherapy cycles; RPLND, retroperitoneal lymph node dissection; ConA-BR, concanavalin A binding ratio).

DISCUSSION

In our experience, the AFP–ConA binding ratio helps to differentiate the origin of any AFP increase. As has been previously reported, the affinity of AFP to ConA is independent of the total serum AFP concentration [6]. As is shown in Table 1, no significant differences in AFP concentrations were observed among groups, but there were highly significant differences ($P < 0.0001$) in the ConA binding ratio between the GCT group and liver disease groups (tumoral and non-tumoral), using the analysis of variance. These results agree with those reported previously which compare AFP affinity to ConA in GCT and liver diseases [4–8]. Unlike lentil lectin binding [6, 9–11], no difference was found for ConA binding between tumoral and non-tumoral liver diseases. Some authors [4, 8, 12] use ConA binding to differentiate primary tumours of the liver from liver metastases; in our experience, we also observed higher mean ConA binding ratio in the metastasic liver group than in the hepatocellular carcinoma group, but differences were not significant.

The fluctuation of the ConA ratios during the serial determinations of GCT patients was minimal. Any significant decrease of the ratio due to hepatic damage could therefore be reliably interpreted.

The total interserial imprecision of the AFP–ConA method obtained in the control sera group was higher than that obtained in the group of patients with GCT in the treatment period, probably because the interserial imprecision analysis was done throughout the study period (15 months) and the median ConA binding ratio in the control sera was in the lower range (2.6%) compared with the median obtained in the GCT group (31.7%).

In order to discriminate between AFP of germinal or liver origin, we established a criterion of AFP–ConA binding ratio >15% in GCT. Sensitivity was 98% and specificity was 98%. As in previously reported studies [5, 10], the method was unable to discriminate between a tumoral and non-tumoral origin of the AFP in the liver group. However, if the binding ratio criteria was lowered to 10%, as proposed by other authors [5], the sensitivity in the GCT group increased to 100%, but specificity decreased to 62%. As the aim of our study was to assess whether any AFP increase in patients with GCT, either receiving chemotherapy treatment or in complete remission (during the follow-up period), was due to hepatic diseases of any origin (viral hepatitis, HIV infection, toxic and drug-induced hepatitis, alcoholic liver disease), the binding ratio criteria of 15% fitted well.

In conclusion, the percentage AFP–ConA binding ratio

appears to be a highly sensitive index to discriminate between AFP of germinal or hepatic origin, but not between AFP of tumoral or non-tumoral liver diseases.

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