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Short Communication

Lectin affinity electrophoresis of α-fetoprotein

Increased specificity and sensitivity as a marker of hepatocellular carcinoma

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ABSTRACT

α-Fetoprotein (AFP) is widely used as a marker of hepatocellular carcinoma (HCC) for assisting diagnosis and also for screening purposes, even though its sensitivity has been decreased slightly as a result of the earlier detection of HCC by ultrasonography. Using lectin-dependent fractionation of AFP, the diagnostic sensitivity as well as the specificity of AFP can be increased compared with measurement of total AFP. Furthermore, lectin-reactive forms of AFP, AFP-L3 and AFP-P4, have been shown to serve as preclinical markers of HCC. Accordingly, AFP is still the most reliable marker of HCC in screening and monitoring high-risk patients.

INTRODUCTION

Since the implementation of radioimmunoassay of α-fetoprotein (AFP), which was developed by Ruoslahti and Seppälä [1] and Nishi and Hirai [2] nearly twenty years ago, AFP has played an important role in screening for and diagnosing hepatocellular carcinoma (HCC) and other AFP-producing tumours. As a result of applying several types of imaging to screening high-risk patients with chronic hepatitis and liver cirrhosis, the AFP-positive rate of HCC has decreased significantly, *e.g.* for a cut-off level of 200 ng/ml the positive rate has decreased from 75 to 30–43% during this period (Table I). It is also evident that there is a wide overlap between the serum AFP level in benign and malignant liver diseases. In fact, the number of patients with chronic hepatitis and liver cirrhosis who have slightly increased serum levels of AFP, ranging from 11 to 200 ng/ml, is always higher than the number of patients with HCC. These patients obviously need further evaluation, preferably by analysing the elevated serum AFP, before considering invasive examinations such as angiography or biopsy of suspected hepatic lesions.

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TABLE I

SENSITIVITIES AND SPECIFICITIES OF AFP FOR DIFFERENT RANGES OF SERUM AFP LEVELS

CH = Chronic hepatitis; LC = liver cirrhosis; HCC = hepatocellular carcinoma. Numbers of patients are given. Values in parentheses are percentages.

Calendar year and disease	Serum AFP level (A) (ng/ml)		
	$A \leqslant 10$	$10 < A \leqslant 200$	$A > 200$
1971–1975			
CH	31 (44.3)	35 (50.0)	4 (5.7)
LC	23 (37.1)	38 (61.3)	1 (1.6)
HCC	2 (10.0)	3 (15.0)	15 (75.0)
April 1983–July 1985			
CH	71 (71.7)	20 (20.2)	8 (8.1)
LC	49 (43.0)	53 (46.5)	12 (10.5)
HCC	8 (19.0)	10 (23.8)	24 (57.1)
Aug. 1985–March 1987			
CH	106 (80.3)	24 (18.2)	2 (1.5)
LC	123 (64.0)	65 (33.9)	4 (2.1)
HCC	33 (34.4)	34 (35.4)	29 (30.2)
April 1987–March 1989			
CH	173 (72.4)	65 (27.2)	1 (0.4)
LC	147 (52.7)	131 (47.0)	1 (0.3)
HCC	10 (10.5)	44 (46.3)	41 (43.2)

A recently developed technique, affinity electrophoretic separation of AFP, appears to serve this purpose. Brębborowicz *et al.* [3] and Miyazaki *et al.* [4] have reported increased proportions of lentil lectin (LCA)-reactive AFP in patients with HCC and yolk sac tumours. They detected separated AFP components by crossed immunoelectrophoresis and protein staining. However, this system is not sensitive enough to be applied to low serum levels of AFP as described above. We have developed a highly sensitive method of antibody-affinity blotting for the detection of separated AFP bands [5,6]. Clinical evaluation of this method coupled with affinity electrophoresis of AFP is reported in this article.

Antibody-affinity blotting

This method is a blotting technique using nitrocellulose membranes which are precoated with purified horse antibodies to human AFP, followed by enzymatic amplification of transferred AFP with rabbit antibodies to human AFP and goat anti-rabbit IgG(H + L)-horseradish peroxidase conjugate (Bio-Rad Labs., Richmond, CA, USA). By this method, AFP bands of only a few per cent of applied AFP (4 μl of 200 ng/ml) can be quantitatively detected. The sensitivity of the detection system can be increased to 4 μl of 50 ng/ml by employing Vectastain ABC kit (Vector Labs., Burlingame, CA, USA) [7].

In this study, erythroagglutinating phytohaemagglutinin (E-PHA) was used in addition to LCA-A as affinity media by including these lectins in agarose gels at a concentration of 0.2 mg/ml for LCA-A and 0.5 mg/ml for E-PHA. Separated AFP bands were identified by the system of nomenclature proposed by Taketa and Hirai [6]. The major AFP bands are numbered consecutively from the anode so that the most anodal band is band 1, and the band numbers are suffixed by the capitalized initial letters of the lectins used: for example, AFP-L1, -L2 and -L3 for LCA-A and AFP-P1, -P2, -P3, -P4 and -P5 for E-PHA. Those bands may be readily identified, as shown in Fig. 1.

Altered lectin-reactive patterns of AFP in malignancies

Representative patterns of AFP bands separated by LCA-A and E-PHA affinity electrophoresis of sera from patients with benign and malignant diseases associated with AFP production are shown in Fig. 1. The proportion of AFP-L3 increases in HCC, while AFP-L2 increases in yolk sac tumours. Broad unresolved bands of AFP-L2 and AFP-L3 are frequently seen in gastric carcinomas. With E-PHA,

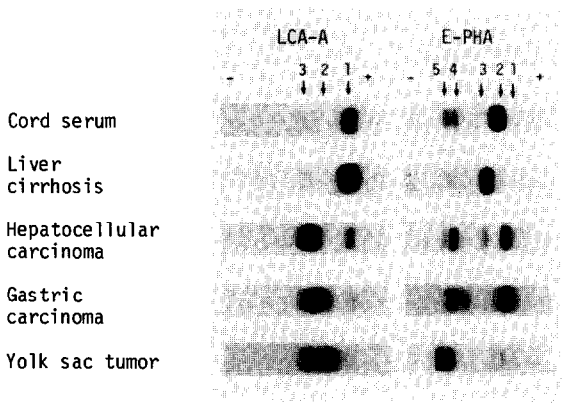

Fig. 1. Representative patterns of AFP bands separated by lectin affinity electrophoresis.

AFP separates into five bands, although only three bands are seen in cord serum AFP, namely AFP-P2 as a major band and AFP-P4 and AFP-P5 as minor ones. The proportions of AFP-P4 and AFP-P5 in liver cirrhosis are much less than those in cord serum, while the proportion of AFP-P4 is increased in HCC and that of AFP-P5 is increased in gastric carcinomas. In yolk sac tumours, AFP-P4 and AFP-P5 are major components. AFP-P3 is frequently present in patients with chronic hepatitis and liver cirrhosis with or without HCC. AFP-P1 is seen in most patients with extrahepatic tumours.

Accordingly, the AFP band pattern of HCC is characterized by increased proportions of AFP-L3 and AFP-P4. The degree of the increase varies from one case to another, reaching a value of over 90% for AFP-L3 and a value as high as 50% for AFP-P4. AFP-L3 and AFP-P4 increase independently, resulting in an increased sensitivity when these two markers are used in combination.

Clinical significance as early diagnostic markers

In an attempt to discriminate chronic hepatitic and cirrhotic patients without HCC from those with HCC, cut-off levels of AFP-L3 and AFP-P4 were determined using the values in those chronic liver disease patients who had no signs of HCC for at least one year after analysis of AFP-L3 and AFP-P4. Since the fractionation of AFP with lectin was carried out to increase the sensitivity of AFP as a diagnostic marker, not a screening marker, cut-off levels were set at the means plus three standard deviations of the values obtained for the benign liver diseases. From the cumulative frequency distribution of the values for a group of patients with chronic hepatitis and liver cirrhosis, the specificities for AFP-L3 and AFP-P4 may be calculated to be 99.9% for both. The actual cut-off levels and sensitivities of LCA-A- and E-PHA-dependent AFP bands are given in Table II.

Among the single bands of AFP used as markers of HCC of varying stages with serum AFP levels above 200 ng/ml, AFP-P4 has the highest sensitivity, 88%, followed by AFP-L3 with a sensitivity of 78% and AFP-P5 with a sensitivity of 57%. The addition of AFP-P4 and AFP-P5 gives a sensitivity of 91%, which is higher than either alone. Combined evaluation of AFP-L3 and AFP-P4, taking the result positive when either alone or both exceed the respective cut-off levels, gives the highest sensitivity of 97% at nearly the same specificity of 99.7%. Incidentally, the importance of AFP-C1, AFP-L2 and AFP-P5 as markers of gastrointestinal tumours and yolk sac tumours is also clearly shown in Table II.

In patients in whom serum AFP levels are below 200 ng/ml, a slightly reduced sensitivity of 68% is reported for AFP-L3 and 63% for AFP-P4, and 88% for AFP-L3 and/or AFP-P4 with a serum AFP level as low as 16 ng/ml [7]. When these results are taken together with the positive rate of total serum AFP level of HCC for the latest two-year period using a cut-off level of 10 ng/ml, the positive rate of AFP in HCC is calculated to be $46.3 \times 0.88 + 43.2 = 83.9\%$. This indicates that the overall positive rate of AFP assay, including AFP-L3 and AFP-P4, in the latest two years is greater than the initial positive rate of total serum AFP level alone in 1971–1975 shortly after the development of radioimmunoassay of AFP. Thus, AFP is still the most sensitive and specific marker of HCC notwithstanding the current prevalence of diagnostic imaging directed to HCC.

In our studies monitoring the variation in AFP-L3 and AFP-P4 content during the follow-up of

TABLE II

PERCENTAGE SENSITIVITIES OF AFP BANDS AT A SPECIFICITY OF 99.9%

CLD = Chronic liver disease (chronic hepatitis plus liver cirrhosis); GIT = gastrointestinal tumours; YST = yolk sac tumour. Numbers of cases studied were 43 for CLD, 58 for HCC, 21 for GIT and 7 for YST. Modified from ref. 7.

AFP band	Cut-off levels for CLD	HCC	GIT	YST
C1	9	34	95	100
L2	0	3	57[a]	100
L3	15	78	71[a]	71
L2 + L3 (or L2-3)	15	78	90	100
P1	1	22	57	86
P3	11	5	38	57
P4	12	88	90	86
P5	6	57	86	100
P4 + P5 (or P4-5)	16	91	100	100

[a] L3-2 is included when present.

TABLE III

TEMPORAL RELATIONSHIP BETWEEN ALTERED LECTIN REACTIVITY OF AFP AND DETECTION OF HCC BY IMAGING

HCC = Definite diagnosis of HCC; HCC(?) = HCC suspected; HCC(−) = HCC not demonstrated. Modified from ref. 8.

Date		AFP (g/l) (<20)	Scintigraphy	Ultrasonic examination	CT	Angiography	Lectin-reactive pattern of AFP
1981	Sept. 28	33.9					
	Oct. 7						LC
	Oct. 21		LC				
	Dec. 8						
1983	Jan. 19			LC			
	Jan. 24		LC				
	Aug. 31	47.5			HCC(?)		
	Oct. 18	40.4		HCC(?)			
1984	March 13						LC
	May 25	45.0					HCC
	Oct. 12	29.1		HCC(?)			
1985	Feb. 1	34.2		LC			
	March 19				HCC		
	March 27			HCC			
	July 11	41.4		HCC		HCC	
	Sept. 11				HCC		
1986	Feb. 14	747				HCC	
	March 29					HCC	
	Aug. 7	53, 100		HCC		HCC	
	Aug. 12			+			

patients with AFP-positive liver cirrhosis, as early as 10 months before localization of HCC, AFP-L3 and AFP-P4 were shown to become positive (Table III). The altered lectin-reactive pattern of AFP also precedes by 1 year the rise in total AFP level. In studies on another group of 35 cirrhotic patients we have demonstrated that AFP-L3 and AFP-P4 became positive at almost the same time in eight patients, AFP-L3 became positive first in three patients, and AFP-P4 became positive first in six patients 1–25 months before detection of HCC by diagnostic imaging [9]. The results suggest that the AFP-L3 and AFP-P4 serve as preclinical markers of HCC. Whether the altered lectin-reactive patterns of AFP represent the presence of HCC cells or of merely a preneoplastic state of hepatocytes remains to be solved in future studies.

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It is an honour for us to dedicate this paper to Professor J. Porath in recognition of his 70th birthday and as a reflection of our ever-lasting friendship.

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