

## Comparison of alpha-fetoprotein with some other tumour markers in Malaysians with hepatocellular carcinoma

JB LOPEZ MAACB, MSc, \*M BALASEGARAM FRCS, Julai TIMOR and \*\*V THAMBYRAJAH, PhD

Division of Endocrinology, Institute for Medical Research, \*Bala Surgical Specialist Clinic, and \*\*Department of Biochemistry, University of Malaya, Kuala Lumpur.

### Abstract:

Although alpha-fetoprotein (AFP) is regarded as the reference marker for hepatocellular carcinoma (HCC), it sometimes produces false results. The objective of this study was to see if some of the readily available laboratory markers could complement **AFP** to improve the laboratory diagnosis of HCC. The markers tested and their sensitivities were: CA 125, 92%; femtin, 71.3%; CA 19-9, 69.8%; beta-2-microglobulin (**B2M**), 53.3%; CA 72-4, 13.6%; and carcinoembryonic antigen (CEA), 10.6%. In comparison, AFP had a sensitivity of 58.8%. CA 72-4 and CEA (at the "tumour" cut-off level of 20 ng/ml) had specificities of **100%**, and **AFP**, 97.4%. The specificities of the other markers were less impressive: CEA, 77.8% (at the cut-off level of 5 ng/ml); **ferritin**, 48.6%; CA 125, 48.5%; **B2M**, 39.6%; and CA 19-9, **37.3%**. The **efficiencies** of the markers for HCC, which are based on the consideration of sensitivity and specificity together, were as follows: AFP, 77.6%; CA 125, 71.3%; femtin, 60.5%; CA 19-9, 55.3; **B2M**, 46.9%; CEA, 40.8%; and CA 72-4, 34.5%. The receiver-operating characteristic plots confirmed **AFP** to be the most efficient marker for HCC. Nevertheless, it is proposed that CA 125 be combined with **AFP** for HCC screening because of their excellent sensitivity and specificity, respectively: a negative result for both, or even just CA 125 alone, would indicate that the disease is unlikely while a positive **AFP** (which would likely occur with a positive CA 125) would make its presence highly probable. A positive CA 125 and negative **AFP** would be equivocal for HCC. Other markers in combination with **AFP** are less useful.

**Key words:** hepatocellular carcinoma, tumour markers, sensitivity, specificity.

### INTRODUCTION

Hepatocellular carcinoma (HCC) has been estimated to be the sixth most common tumour among males, worldwide.<sup>1</sup> It has an insidious onset and silent course which renders early diagnosis **difficult**.<sup>2,3</sup> Furthermore, it does not give a **specific** picture with any of the imaging **modalities** used for the **liver**.<sup>2</sup> Although serum alpha-fetoprotein (**AFP**) has been regarded as the reference diagnostic test for HCC, it is responsible for a number of false (positive and negative) results; indeed, normal levels may be encountered in about 10-50% of symptomatic **HCC**.<sup>3</sup> Among the approaches attempted to improve the laboratory diagnosis of HCC have been the testing for various other serum **markers**.<sup>2-4</sup>

The objective of this study was to assess the performance of the following serum markers in conjunction with **AFP**: CA 125, femtin, CA 19-9, carcinoembryonic antigen (CEA), CA 72-4 and beta-2-microglobulin (**B2M**). CA 125 is an

established tumour marker for ovarian cancer while femtin is an iron-storage protein. Despite being better known for their application in other diseases, both of these<sup>2,4</sup> and the other markers have been shown to be also raised in liver disease. Raised CA 19-9 has been found in patients with a variety of gastrointestinal tumours, particularly pancreatic carcinoma. Like the pancreas, the liver is a **foregut derivative**.<sup>8</sup> CEA is a useful marker of digestive tract **malignancies**.<sup>2</sup> Increased serum B2M values have been previously demonstrated in patients with liver cancer? other solid tumours, and, renal and inflammatory **disease**.<sup>10</sup> CA 72-4 is expressed by a wide range of human adenocarcinomas and has been introduced as a marker for gastric and gastrointestinal carcinoma **patients**.<sup>11-13</sup>

### SUBJECTS, METHODS AND MATERIALS

#### Subjects

Blood samples from 80 HCC, 76 benign liver diseases (BLD) and 70 apparently healthy adults

Address for correspondence and reprint requests: JB Lopez, Division of Endocrinology, Institute for Medical Research, Jalan Pahang, 50588 K—Lumpur, —

(HA) subjects were used in this study. The HCC and BLD patients were, in the main, from the Kuala Lumpur Hospital (KLH) and a private clinic specialising in liver diseases. HCC was confirmed by the histopathological examination of biopsy samples in all but 2 cases where **confirmation** was achieved by the cytological examination of ascitic fluid. The types and numbers of the BLD patients were as follows: 32 patients had cirrhosis, 24 hepatitis, 10 obstructive jaundice due to gallstones, 4 acute cholecystitis, 2 amoebic liver abscess, and 4 others miscellaneous liver diseases. The subjects were randomly selected for the assay of markers since it was not possible to test every subject for each marker.

#### Methods and materials

The sera were stored in small aliquots at  $-60^{\circ}\text{C}$  until use. AFP, CA 125, CA 19-9, CEA and B2M were measured on an automated immunoassay analyser, the Abbott IMx (Abbott Diagnostics, N. Chicago, USA), with **Microparticle Enzyme Immunoassay** kits from the same source. Serum femtin was measured by radioimmunoassay kits from **Amersham International** (Amersham, UK), and CA 72-4 by immunoradiometric assay kits from Centocor (Malvern, PA, USA).

#### Statistics

The predictive value parameters 'sensitivity', 'specificity' and 'efficiency' as described by Galen and **Gambino**<sup>14</sup> were used to **assess** the performance of the markers. The BLD subjects served as controls for the calculation of specificity and efficiency.

## RESULTS AND DISCUSSION

#### Age, sex and race of HCC patients

The ages of 76 patients which were recorded ranged from 12-84 years, of whom 62 (82%) were 241 years of age. The observation that the majority of our patients were above 40 years of age is similar to the findings for other countries of this **region**.<sup>4, 15-17</sup>

Of the 79 patients whose sex were recorded, 64 were males and 15 females, giving a **male:female** ratio of **4.3:1**. The male sex is a known risk factor for developing **HCC**<sup>4</sup> and this ratio appears to conform to that of populations with an intermediate risk for the disease.<sup>11</sup>

Seventy-one percent (57180) of our HCC cases were of ethnic Chinese origin, while

Malays constituted 20% (16180) and Indians 6.3% (5180). **Kew**<sup>15</sup> has stated that Chinese and Africans from sub-Saharan Africa are at the greatest risk for developing HCC and comprise the vast majority of patients with this tumour.

#### Cut-off values

The cut-off values of markers used in this study are given in Table 1. The AFP values of the 53 HA were all **<10 ng/ml** and ranged from 0.9-6.1 **ng/ml** with a median of 2.3 **ng/ml**. However, the 200 **ng/ml** cut-off level was selected since it provided a clear distinction of HCC from **BLD**.<sup>18</sup>

The cut-off values for CA 125 were based on 95th percentile for 31 females and 31 males. For **ferritin**, the cut-off level of 400 **ng/ml** had been previously used by **us**.<sup>7</sup> The **97.5th** percentile values of 25 HA for CA 19-9 was 31.1 **U/ml** while the **mean+2sd** was 36.7 **U/ml**; we based our cut-off for this marker on the latter value. The 95th percentile value for the CEA determinations of 25 HA was 4.1 **ng/ml** while the **mean+2sd** was 4.0 **ng/ml**; The 5.0 **ng/ml** cut-off, however, appeared to better distinguish HA from HCC patients. The cut-off value of B2M was 95th percentile value from the determination of 20 HA results. For CA 72-4, we used the published cut-off value of 4.0 **U/ml**.<sup>13</sup>

#### Tumour marker assays

The proportions of patients with positive tumour markers are given in Table 1 and the sensitivity, specificity and efficiency (relative to BLD) derived from this for each marker are given in Table 2.

Although the sensitivity of AFP at the cut-off level of 200 **ng/ml** was **58.8%**, it improved to 76.3% at the 10 **ng/ml** cut-off.<sup>18</sup> CA 125, however, was the most sensitive marker for HCC. Its sensitivity of 92% was similar to the figure of 90.4% (1041115) reported by Elias and **Kew**.<sup>5</sup> Femtin and CA 19-9 were also more sensitive than AFP. Sensitivities ranging from 38% to 90% have been reported for femtin in HCC (see references cited by Lopez et al?). While the sensitivity of CA 19-9 obtained in this study (70%) was higher than the 51.3% previously **reported**,<sup>8</sup> that for B2M was slightly lower (**53%**, as against **59%**)<sup>9</sup>. When AFP was individually combined with CA 125, CA 19-9, femtin and **B2M**, the sensitivities improved as indicated in Table 3.

Although CEA has been reported to be elevated in 32-79% of **HCC**,<sup>2</sup> only 11% (7166)

**TABLE 1: Cut-off values and proportions of positive results among HCC and BLD patients for various tumour markers**

Tumour Marker	Cut-off	HCC	BLD
AFP <sup>18</sup>	200 ng/ml	47/80	2/76
CA 125 <sup>6</sup>	M = 12 U/ml F = 55 U/ml	69/75	35/68
Femtin <sup>7</sup>	400 ng/ml	57/80	37/72
CA 19-9	37 U/ml	44/63	32/51
B2M	2.1 mg/ml	32/60	32/53
CEA	5.0 ng/ml	7/66	12/54
CA 72-4	4.0 U/ml	3/22	0/7

M = male; F = female

of our patients had raised values above the cut-off level of 5.0 ng/ml. However, 6 of these patients (6/66 or 9%) with raised CEA had levels within the "tumour" range of >20 ng/ml.<sup>3</sup> Our results for the sensitivity of CA 72-4 in HCC are consistent with the report of Wu and Carlisle<sup>12</sup> that the marker is present in low frequency of elevation and concentration in serum for many tumours.

The specificity of 100% was achieved by CA 72-4 since none of the 7 cases of cirrhosis tested exceeded the cut-off value (Table 2). Although the specificity of CEA was 77.8% at the cut-off

level of 5.0 ng/ml, it improved to 100% at the 20 ng/ml cut-off. With the possible exceptions of CA 72-4 and CEA, AFP was, by far, the most specific marker for HCC. A positive result for AFP, therefore, indicates a high likelihood for the disease. CA 125, ferritin, CA 19-9 and B2M all had specificities of <50%.

The parameter, **efficiency**, takes into consideration both sensitivity and specificity. AFP was the single most efficient marker for the diagnosis of HCC followed by CA 125 (Table 2). This was **confirmed** by the receiver-operating characteristic plots in Fig. 1 which clearly show

**TABLE 2: Sensitivity, specificity and efficiency (relative to BLD), in percent, of tumour markers in HCC**

Tumour Marker	Sensitivity*	Specificity**	Efficiency***
AFP <sup>18</sup>	58.8	97.4	77.6
CA 125 <sup>6</sup>	92.0	48.5	71.3
Femtin <sup>7</sup>	71.3	48.6	60.5
CA 19-9	69.8	37.3	55.3
B2M	53.3	39.6	46.9
CEA	10.6	77.8	40.8
CA 72-4	13.6	100.0	34.5

\* Sensitivity =  $TP/(TP+FN)$ , where TP = true positive, FN = false negative

\*\* Specificity =  $TN/(TN+FP)$ , where TN = true negative, FP = false positive

\*\*\* Efficiency =  $(TP+TN)/(TP+FP+TN+FN)$

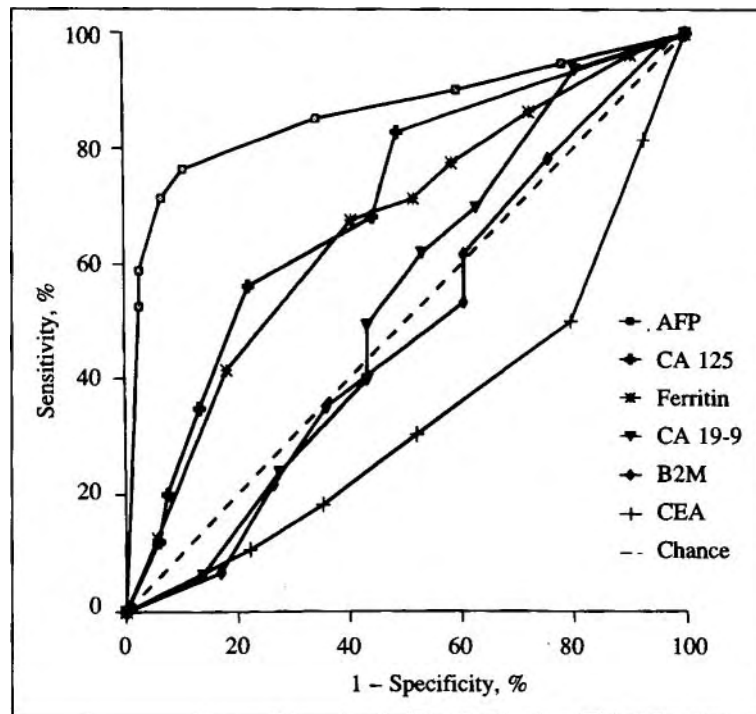


FIG.1: Receiver-operating characteristic curves for the various tumour markers in HCC, when compared with benign liver diseases. The cut-off values for the points of the graphs (from left to right and excluding those of 0 and 100%) are given in the table below:

AFP (ng/ml)	CA 125 (U/ml)	Ferritin (ng/ml)	CA 19-9 (U/ml)	B2M (ng/ml)	CEA (ng/ml)
500	300	2000	500	4.0	5.0
200	200	1000	200	3.0	4.0
20	100	500	100	2.5	3.0
10	50	400	80	2.1	2.0
5	30	300	50	2.0	1.0
3	20	200	37	1.5	
2		50	10	1.0	

that that for AFP has the largest area under the curve.

**CONCLUSION**

The choice of a marker for a disease is based on a compromise between sensitivity and specificity. The poor specificities of ferritin, CA 19-9 and B2M limited their use as routine markers for HCC; B2M, in addition, displayed unsatisfactory sensitivity. While the specificities of CEA (especially at the 20 ng/ml cut-off) and CA 72-4 were excellent, their poor sensitivities precluded their use in HCC.

An important goal for the diagnosis of HCC in individuals at risk (e.g. hepatitis B antigen positive subjects) is the early detection of the tumour at a stage where it is potentially resectable.<sup>3,4</sup> Our data suggests that AFP alone is a relatively insensitive marker for HCC screening. However, it would appear that a combination of CA 125 and AFP, chosen for their excellent sensitivity and specificity, respectively, would better fulfill this role. This is borne out by the data in Table 3 which shows the sensitivity and efficiency of AFP in combination with the other markers. The AFP-

TABLE 3: Sensitivity and efficiency, in percent, of AFP in combination with some tumour markers in HCC

Tumour Marker Pair: AFP with	Sensitivity	Efficiency
CA 125 <sup>6</sup>	96.0	73.4
Ferritin <sup>7</sup>	88.8	70.1
CA 19-9	90.5	66.7
B2M	76.7	59.3

*NB: values for AFP in combination with CEA and CA 72-4 were not calculated since the individual sensitivities of the latter markers were very low (see TABLE 2); any improvement in the sensitivity of the combined markers would be, in the main, due to the better sensitivity of AFP.*

CA 125 combination had the highest sensitivity and was able to correctly diagnose 96% of the HCC patients; furthermore, it had the highest efficiency among the pairs considered. Thus, a negative result for AFP and CA 125, or even just CA 125 alone, would indicate that the disease is unlikely, while a positive AFP, which would very likely occur with a positive CA 125, would make its presence highly probable. A positive CA 125 and negative AFP would be equivocal for HCC.<sup>6</sup> Other markers in combination with AFP are less useful.

#### ACKNOWLEDGEMENTS

We are indebted to the doctors of the Surgical Department and Dr. G Doraisamy, of the KLH, for their contributions. The authors thank the Director, IMR, for permission to publish this paper. This work was partially funded by grants from the University to VT and the National Council for Scientific Research and Development, Malaysia to JBL.

#### REFERENCES

- Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer* 1993; 54: 594-606.
- Kew MC. Tumour markers of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1989; 4: 373-84.
- Dusheiko GM. Hepatocellular carcinoma associated with chronic viral hepatitis - aetiology, diagnosis and treatment. *Br Med Bull* 1990; 46: 492-511.
- Di Bisceglie AM, Rustgi VK, Hoofnagle JH, Dusheiko GM, Lotze MT. Hepatocellular carcinoma. *Ann Intern Med* 1988; 108: 390-401.
- Elias J, Kew MC. Evaluation of CA 125 as a serum marker of hepatocellular carcinoma. *Int J Cancer* 1990; 46: 805-7.
- Lopez JB, Balasegaram M, Thambyrajah V. Serum CA 125 as a marker of hepatocellular carcinoma. *Int J Biol Markers* 1996; 11: 178-82.
- Lopez JB, Thambyrajah V, Balasegaram M, Satgunasingam N. Serum ferritin in hepatocellular carcinoma and benign liver diseases. *Diagn Oncol* 1994-5; 4: 143-7.
- Kew MC, Berger EL, Koprowski H. Carbohydrate antigen 19-9 as a serum marker of hepatocellular carcinoma: comparison with alpha-fetoprotein. *Br J Cancer* 1987; 56: 86-8.
- Kew MC, Vincent C, Rossel M, Revillard J-P. High serum levels of secretory component in hepatocellular carcinoma. *Am J Med* 1988; 85: 327-30.
- Karlsson FA, Wibell L, Evrin PE.  $\beta_2$ -microglobulin in clinical medicine. *Scand J Clin Lab Invest* 1980; 40 (Suppl 154): 27-37.
- Byrne DJ, Browning MCK, Cuschieri A. CA 72-4: a new tumour marker for gastric cancer. *Br J Surg* 1990; 77: 1010-3.
- Wu JT, Carlisle P. Low frequency and low level of elevation of serum CA 72-4 in human carcinomas in comparison with established tumor markers. *J Clin Lab Anal* 1992; 6: 59-64.
- Ohuchi N, Takahashi K, Matoba N, Sato T, Taira Y, Sakai N, Masuda M, Mori S. Comparison of serum assays for TAG-72, CA 19-9 and CEA in gastrointestinal carcinoma patients. *Jpn J Clin Oncol* 1989; 19: 242-8.
- Galen RS, Gambino SR. Beyond normality: the predictive value and efficiency of medical diagnosis. John Wiley and Sons, New York, 1975.
- Kew MC. The development of hepatocellular cancer in humans. *Cancer Surv* 1986; 5: 719-39.
- Lingao AL. The relationship of hepatocellular carcinoma and liver cirrhosis to hepatitis B virus infection in the Philippines. *Gastroenterol Jpn* 1989;

- 24: 425-33.
17. Sulaiman HA. Hepatitis B virus infection in liver cirrhosis and hepatocellular carcinoma in Jakarta, Indonesia. *Gastroenterol Jpn* 1989; 24: 434-41.
  18. Lopez JB, Thambyrajah V, Balasegaram M, Julai Timor. Appropriate cut-off levels for serum alpha-fetoprotein in hepatocellular carcinoma. *Diagn Oncol* 1994-95; 4: 287-91.