

# Serological markers for hepatocellular carcinoma – modern trends

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## Abstract

*Imaging techniques [ultrasonography (USG), computer tomography (CT) and nuclear magnetic resonance (NMR)] are still the most important element for surveillance of liver cirrhosis patients in the detection of primary liver cancer. However, in order to improve the sensitivity, specificity and predictive value of the diagnostic process, the determination of several serological markers of cancers must be included. For example, the sensitivity of a combined USG examination and AFP determination exceeds 92%.*

*The serological tests proposed by us for the detection of hepatocellular carcinoma (HCC) include alpha-fetoprotein (AFP), alpha-L-fucosidase (AFU), and anti-p53 antibody tests. Their value depends mainly on the sensitivity and specificity of AFU and AFP tests. It should be underlined that the AFU method achieves better parameters for the detection of HCC than the AFP test. The increased detection rate of anti-p53 antibodies is found not only in patients with primary liver cancer, but also in carcinomas of the other digestive tract organs; thus, this test lacks specificity for HCC. Finally, the costs of surveillance of patients with LC for HCC are rather high, and limit the broader use of surveillance.*

**Key words:** hepatocellular carcinoma (HCC), alpha-fetoprotein, alpha-fucosidase, anti-p53 antibodies, costs of HCC surveillance

(*Centr Eur J Immunol* 2005; 30 (1-2): 32-36)

## Introduction

Hepatocellular carcinoma (HCC) has become increasingly frequent throughout the world, and is listed as the fifth most common human cancer [1]. The main etiological factors are: infection with hepatotropic viruses, hepatitis C, and hepatitis B, (HCV and HBV); and alcohol-related diseases. In the meantime, the planning and execution of a widespread programme of vaccination against hepatitis B in at least 151 countries has reduced the participation of this virus in liver carcinogenesis. On the other hand, HCV has become an increasing cause of chronic hepatitis, liver cirrhosis, and HCC, because a prophylactic vaccine has not been developed [2]. Currently, it is recognised that chronic hepatitis C causes 60-70% of HCC cases, alcohol-related disease 20%, and chronic hepatitis B 15%.

On a world scale, chronic liver diseases cause 1,400,000 deaths/annually. Within this number, deaths from HCC

amount to 618,000 deaths (44%) [3]. However, the HCC morbidity in certain regions of the world is very diversified. The highest morbidity, 50-120/100,000 inhabitants, is found in Far Eastern countries (China, Taiwan and Japan), and sub-Saharan Africa [4]. In Central Europe, the morbidity is at least 10x lower, eg. in Poland it was 4/100,000 males and 3.5/100,000 females, in 2002 [5].

There are 600,000 persons infected with HCV in Poland, according to epidemiological estimates from the National Institute of Hygiene [6]. It is difficult to determine the moment of infection, and the estimation of the time required from the entrance of the virus to the organism until the development of HCC, in most cases. This is caused by the mainly symptomless course of HCV infection. Help in this respect came from studies on post-transfusion hepatitis C, where evolution from the infection to cirrhosis was 20 yrs, and to HCC development, 28 years. According to estimates performed on 384 patients,

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persons infected with HCV have a 1-4% chance of developing HCC during the one year [7]. The attached figures demonstrate the evolution of HCV expression in liver tissue – from a moderate in chronic hepatitis C (fig. 1) to a very intensive in hepatocellular carcinoma cells (fig. 2).

In order to diminish mortality resulting from HCC development, a surveillance of patients with cirrhosis was proposed [8]. It is estimated that patients with cirrhosis develop HCC at a rate of 11% yearly. The surveillance consists of ultrasonographic (also CT and NMR) examination of the liver and investigation for serological markers of cancer [8, 9]. We will now describe the value of examination of serological markers of cancer; a broad review has been given in Szymendera et al. [10].

The main properties of serological cancer markers are as follows:

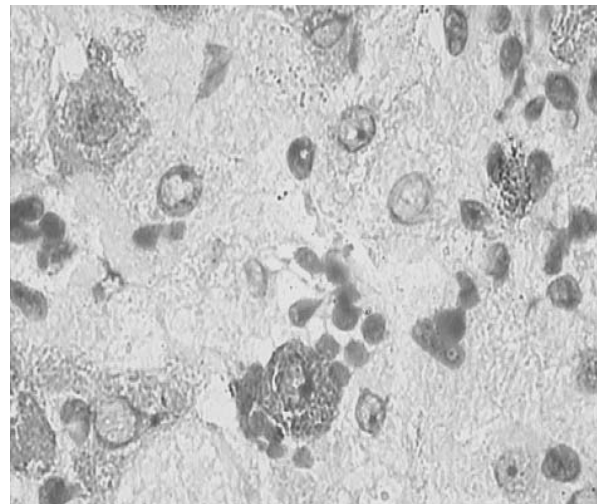
- 1) an increased level of the marker should be encountered significantly more frequently in persons suffering from cancer than in the healthy subjects,
- 2) the concentration of a marker in serum should be proportional to the number of cells able to synthesise it, i.e. neoplastic cells,
- 3) each procedure (operation) leading to a decrease in the number of neoplastic cells in the organism should cause a decrease in the marker's concentration during a time-period corresponding to the half-life [11].

### Alpha-fetoprotein

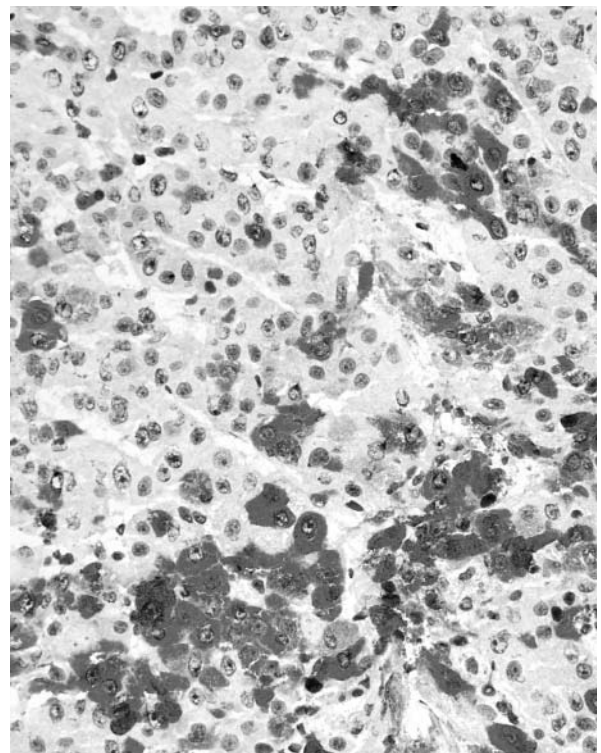
How were the first cancer markers discovered? One of the first markers connected with HCC was alfa-fetoprotein. It was detected as a fetal protein by Bergstrand & Czar [12]. However, the first scientists, who discovered a clear increase in this protein in cases of experimental HCC were Abelev in 1963 and Tatarinov in 1964. Animals possessing tumours other than HCC did not show the increase of AFP in serum. This work was performed by the rather insensitive methods of double immunodiffusion and immunoelectrophoresis. Similar studies were performed in humans, detecting AFP in most patients with HCC and in several patients having both HCC and cholangiocarcinoma [13]. After further studies, the authors predicted that 'negative results of AFP in patients with metastatic tumours to the liver will be very useful in the future differential diagnostics of liver tumours', which is very true.

The next studies were carried out on a rare cancer of children, hepatoblastoma. An increased level of AFP appeared in 84% of the children [14]. This was expected, because hepatoblasts are precursors of hepatocytes. The long-time value of Tatarinov's and Abelev's discovery is the fact of publication of Michael Kew's article which appeared in the series 'Milestones of liver disease', on the occasion of the 25th anniversary of the original Abelev article in 1967 [15].

The basic parameters of AFP as a marker for HCC detection were established after the introduction of high sensitivity tests, i.e. radioimmunoassay and ELISA.



**Fig. 1.** Identification of HCV antigens in hepatocytes of the patient with chronic hepatitis C. Envision detection system, 200x



**Fig. 2.** Intensive staining for HCV antigens in the cytoplasm of hepatocytes in a case of hepatocellular carcinoma. Envision detection system, 100x

However, particular authors took into consideration different upper limits of normal values ('cut-off' values). It appears that at different 'cut-off' values [e.g.: 16, 18 (17.8) and 20 mg/ml] the indices from AFP tests in detection of HCC in patients with liver cirrhosis (LC) were as follows: sensitivity 30-64%, specificity 76-91%, predictive values: 9-37% [16-20].

The predictive value of ultrasonographic examination is much higher, at 75% [18]. Already in 2000 the European Association for the Study of the Liver approved the surveillance method of the patients with LC of different etiology; it consists of imaging of liver and determination of serum AFP each 6 months [8]. Imaging of the liver may be performed by ultrasonographic examination, spiral computer tomography, or magnetic resonance. The predictive value of both the USG examination and AFP testing in liver cirrhosis patients is evaluated for >92% [21].

Despite this high specificity and predictive value, there are HCC cases which do not give a satisfactory echo in USG, and also do not secrete significant amounts of AFP [22]. In order to increase the sensitivity of AFP determination, two modifications were proposed. The first is the detection of AFP mRNA by the nested RT-PCR method in peripheral blood mononuclears [23]. The second is the detection of AFP fraction reacting with the *Lens culinaris* agglutinin, AFP-L3 [24]. It was found that both these modifications have higher sensitivity and correlate with the course of HCC. Two of us (MWS, ABK) investigated the diagnostic efficacy of AFP-L3 and AFP tests in discrimination between liver cirrhosis and HCC of viral origin. In these studies, higher sensitivity of AFP-L3 in comparison to AFP (88.9 vs 61.1%) was shown, while specificity (83.3 vs 88.8%) and positive predictive value (84.2 vs 84.6%) were at the same level [25]. There are still no commercial tests for AFP-L3. The above mentioned authors suggest that several serological markers are needed for the early detection of HCC. These are: PIVKA (protein induced by vitamin K absence), rGT (r-glutamyl transpeptidase), TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ), PAP (pancreatitis-associated protein), STK (serine-threonine kinase 15), and PGCP (plasma glutamate carboxypeptidase) [24, 26].

However, the limiting factor may be the economic cost of surveillance (see below).

### Alpha-L-fucosidase

Just 20 years after the discovery of the value of AFP in the detection of HCC, the finding of alfa-L-fucosidase, as a similar marker for HCC detection, was described [27]. Alfa-L-fucosidase (AFU) is a lysosomal enzyme present in all mammalian cells; its natural substrates are sugar residues, containing L-fucose. A physiological increase in AFU activity was observed in neonates until 15 days of life, then it decreases until 1 yr of life, when it achieves an adult level. AFU activity increases during pregnancy, but returns to normal values after delivery [28]. The diagnostic utility of AFU determination has been observed in the following diseases: lysosomal storage diseases, diabetes, myocardial infarct, acute pancreatitis, acute hepatitis, HCC, and also gastric, breast and ovary cancers [28]. Since the important study of Giardina et al. [16], several investigations were devoted to the value of AFU activity determination in the detection of HCC.

AFU activity determines the enzymatic reaction. Thus, the test should be done within 30 days of the freezing of sample; the results are given in nmol/ml/h. The cut-off values estimated from the results in reference groups of healthy blood donors ( $x+3SD$ ) are the following: 433 nmol/ml/h [29], 443 nmol/ml/h [16, 26], 516 nmol/ml/h [30], 621 nmol/ml/h [31], 700 nmol/ml/h [32]. According to these authors, the sensitivity of an AFU test in the detection of HCC is 71-85%, and specificity ~91%. A very important practical observation was the increase of AFU activity 6 to 9 months before the finding of USG changes typical of HCC: in 7/19 patients [26] and in 23/27 patients with liver cirrhosis [32]. These authors conclude that patients with cirrhosis, in whom increased AFU activity was detected, should have more frequent examination of the liver by USG; i.e. every 3 months. Here we should add that AFP and AFU are synthesized by different cells, and thus tests which determine the 2 markers may supplement each other in the surveillance of patients with cirrhosis. It appears that these 2 markers have the highest specificity for the development of HCC.

### Anti-p53 antibodies

The subsequent marker of HCC detection may be the determination of anti-p53 antibodies. The p53 gene is recognized as a 'genome guardian', or as a cancer suppressor gene. Mutations of this gene are the most frequent changes found in persons with cancer [33-35]. Furthermore, a high percentage of codon 249 point mutations of the p53 gene in patients with HCC from China and from South Africa were found [36]. Mutated p53 proteins have a prolonged half-time in comparison to 'wild-type' protein, and cause an increased concentration of this protein in neoplastic cells. These two phenomena, i.e. the structural mutations and prolonged survival time, provoke the formation of anti-p53 autoantibodies in patients with cancer [35]. Because of the big interest in the use of this determinations in cancer, commercial tests for the detection of anti-p53 in serum are available [e.g. Dianova, Hamburg, Germany].

Anti-p53 antibodies were very seldom found by this test in healthy blood donors, scoring only 0.5%. However, increased frequencies were found in patients with primary liver cancer, at 20-25% [37, 38]. On the other hand, anti-p53 antibodies were detected in 35% of patients with gastric carcinoma, in 60% of patients with esophageal cancer, in 70% of patients with colon carcinoma, in 75% of patients with pancreatic cancer, and in 100% of patients with cholangiocarcinoma [24]. Thus, the anti-p53 test does not show specificity for detection of HCC, but may be used for differential diagnosis.

### Cost of HCC surveillance

We should also mention the estimated cost of surveillance, although this is not the main topic of this review.

We will give examples of two studies; in the first the surveillance was performed according to EASL criteria, i.e. USG examination and serum AFP determination every 6 months in patients with LC. Patients with class C changes according to Child-Pugh, aged >60 yrs, with focal changes of the liver already detected by USG, and with an initial AFP value >200 ng/ml, were excluded. Altogether, 313 patients were analysed over 2.5 years. The cost of detection of curable HCC was 17,934 USD, but the cost of surveillance for a year of patient survival was estimated as >112,000 USD [9].

The second study introduced the modified surveillance programme, of USG examination, AFP concentration, and AFU activity every 6 months, in HCV infected patients. Patients with class C changes on the Child-Pugh scale were excluded. Altogether, 145 persons were qualified, i.e. 101 with chronic hepatitis and 44 with liver cirrhosis; the observation period was close to 4 yrs. The cost of surveillance for the detection of one HCC case was 32,667 PLN [31]. We conclude from these studies that the costs of surveillance of patients with LC are very high.

#### Acknowledgement

This work was supported by grant: 3 P05E 14225 from the Committee for Scientific Research, Warsaw. The paper was presented as a lecture at the Fifth Conference 'Progress of Immunopathology in Clinical Diagnostics', Poznan, 23-24.09.2005; Chairman: Prof. dr Jan Zeromski

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