

A Non Parametric Approach for Comparison between Two Hepatocellular Carcinoma Markers: Alpha-Fetoprotein (AFP) and Insulin-like Growth Factor II (IGF-II)

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Abstract: Alpha-fetoprotein (AFP) is a useful tumor marker for diagnosis of hepatocellular carcinoma (HCC). There is over-expression of insulin-like growth factor (IGF)-II in HCC tissue. This study investigates the diagnostic application of IGF-II in HCC. Serum levels of IGF-II and AFP were determined in 97 patients with HCC, 97 patients with cirrhosis and 30 healthy controls. Both IGF-II and AFP levels in HCC were significantly higher than those in cirrhotic patients or controls, the IGF-II levels in cirrhotic patients were significantly lower than those in controls. The optimal cut-off values for diagnosing HCC were determined with Receiver Operating Characteristics (ROC) curve. The sensitivity, specificity and diagnostic accuracy values for AFP and IGF-II have been estimated. Determination of markers jointly used significantly increases the diagnostic accuracy and sensitivity, with a high specificity. So, serum IGF-II level can be used as complementary tumour marker to AFP for diagnosis of hepatocellular carcinoma.

Keywords: Insulin-like growth factor-II, Alpha-Fetoprotein, Hepatocellular Carcinoma, Diagnostic Tests, Receiver Operating Characteristic Curve.

1. Introduction

Hepatocellular carcinoma (HCC) is among the most common fatal solid tumours worldwide. The principal causes of the HCC development are the B and C hepatitis, particularly if they are responsible of the cirrhosis presence. In about 4-8% of the patients with cirrhosis the development of HCC determines a reduction of the life quality and, above all, of the survival expectations. For such reason it is recommended to B or C positive patients periodically have self-control themselves through the execution of the blood examinations, dosing of the Alpha-Fetoprotein (AFP); in fact the only examination of the correlated blood to the presence of the tumor is the AFP, that results elevated however only in about 50% of the cases.

Alpha-Fetoprotein, a molecule produced by the fetal liver in the embryo and in the fetus during their phase of development, has two principal applications: for the women in

pregnancy as examination of control to individualize some congenital malformations and as tumoral marker in adults and children for some types of tumor. It is noted as a marker of liver tumor but, as many other tumoral markers, is little sensitive and therefore it isn't reliable for the precocious diagnosis because its negative value doesn't allow to exclude that the liver tumor is equally present. Alpha-fetoprotein is not a useful tumor marker for diagnosis of small hepatocellular carcinoma (HCC). It is already as an alternative proceeds to check the course of a diagnosed liver cancer, in fact a reduction of the AFP levels can indicate a good effectiveness of a treatment and the absence of recidivists.

The Insulin-like Growth Factors (IGFs) are most important in the development and function of almost all the body organs; mainly they participate in the control of the embryological development. Such factors are a basic component of the system that checks the growth and the metabolism and they are for most produced by the cells of which the liver is composed (hepatocytes), under the stimulus of the hormone of the growth (GH) produced by the hypophysis. The family of the IGFs is composed by two proteins: the IGF1 and the IGF2. The IGF1 is a hormone of protein nature with a molecular structure similar to that of the insuline; it develops a main function in the growth regulation the after the birth but it also continues its anabolic effects in adult age. The IGF2 is a promoting factor of the growth, necessary during the embryonic development. Tsai et al., (2007) note that there is over-expression of insulin-like growth factor (IGF)-II in HCC tissue.

The present paper aims to show the diagnostic utility of IGF-II in hepatocellular carcinoma and to highlight it as a complementary tumour marker to AFP.

Moreover, we want to compare serum IGF-II and AFP levels among HCC patients, cirrhotic patients and healthy controls.

For this reason, serum levels of AFP and IGF-II were measured on 97 patients with HCC, 97 patients with cirrhosis and 30 healthy controls. This casuistry has been in relief from a specialized team of the Medical Clinic of University of Messina. In this context, we have to thank for Prof. Maria Antonietta Freni and Prof. Aldo Spadaro because their clinical competences and their help from a medical point of view have been essential and indispensable components into the realization of the present article.

2. The Methodology

In order to assess the existence of possible statistically significant differences among the three groups of subjects a non parametric inference based on permutation tests (or NPC Test) has been applied (Pesarin, 2001).

Receiver operating characteristic curves (ROC) and likelihood ratios were used to quantify and compare the diagnostic performance of AFP and IGF-II (Zou et al, 2007). ROC curves were constructed by calculating the sensitivity and specificity of IGF-II and AFP levels at several cut-off points. The cut-off value with the highest accuracy was selected as the diagnostic cut-off point.

Sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio and diagnostic accuracy were calculated for AFP and IGF-II, singly and jointly (Altman and Bland, 1994).

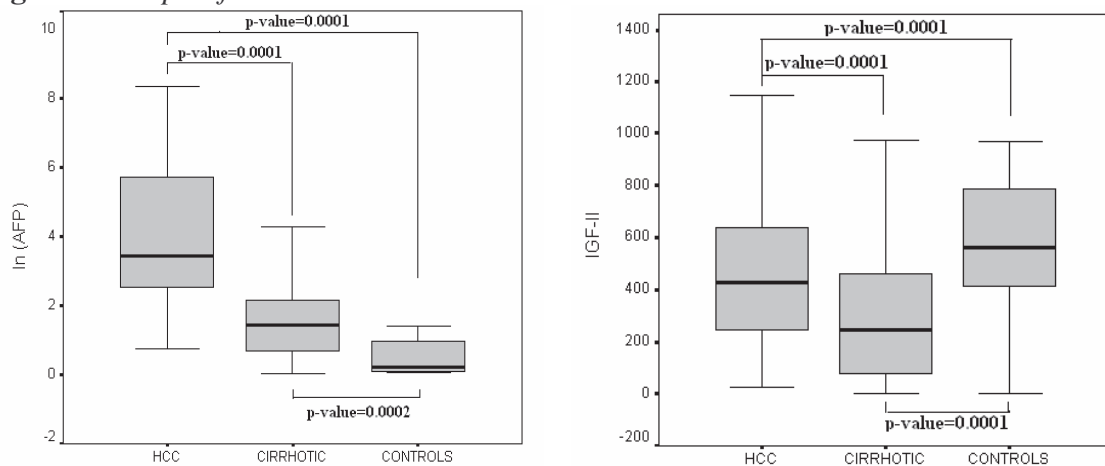
3. Data Analysis

The NPC test application has showed that in patients affected by HCC both AFP and IGF-II levels were significantly higher than in patients with cirrhosis alone ($p=0,0001$) or in healthy controls ($p=0,0001$). The serum AFP levels in patients with cirrhosis alone were also statistically higher than that of healthy controls ($p=0,0002$). However, serum IGF-II levels in patients with cirrhosis alone are lower when compared to healthy controls ($p=0,0001$) (Table 1 and Figure 1).

Table 1: Serum IGF-II and AFP mean levels, min and max value in cirrhotic patients with HCC, patients with cirrhosis alone and healthy controls

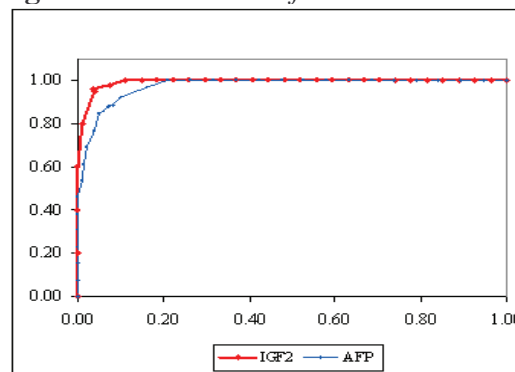
Groups	AFP (ng/ml)	IGF-II (ng/ml)
HCC	862.88 (2.10-9731)	515.04 (26-1436.60)
Cirrhotic	15.73 (1.02-154.80)	330.17 (1.83-975)
Controls	1.84 (1.06-4.10)	566.16 (1.10-969.70)

Figure 1: Boxplot for AFP e IGF-II serum levels



According to the ROC curve analysis, the optimal cut-off value for IGF-II was 796 ng/ml and for AFP was 132 ng/ml, with area under the ROC curve for AFP of 68.7% and for IGF-II of 54.7% (Figure 1).

Figure 1: ROC curves for AFP and IGF-II



Comparing the two markers, we can note that the AFP introduced a more elevated sensibility value than the IGF-II; with reference to the specificity; the difference between the two markers is lower, instead (Table 2). Evaluating the sensibility and specificity of AFP e IGF-II jointly used, we obtained a more elevated sensibility (in comparison to every marker singly used) even if the specificity is lower. This underlines the informative and diagnostic utility of the IGF-II.

Table 2: *Sensibility, specificity, VP+, VP-, likel.+ and likel.- of AFP e IGF-II*

Marker	Sensibility	Specificity	VP+	VP-	likel.+	likel.-
AFP	40.6%	96.9%	92.9%	62.0%	13.00	0,61
IGF-II	18.9%	90.6%	66.7%	52.7%	2.00	0,90
AFP and IGF-II	40.9%	87.7%	78.9%	62.2%	3.75	0.61

4. Final Remarks

Serum AFP is among the most intensively studied tumor markers for HCC. The test, when used with the conventional cut-off point of 400 ng/ml, has a sensitivity of about 48-63% and a specificity of 100% in detecting the presence of HCC in patients with compensated cirrhosis. In recent years various other serological markers have been developed for the diagnosis of HCC. However, most of these markers have been shown to be unsatisfactory in diagnosing small HCC owing to low sensitivity. For this reason and in consideration of the result illustrated by Tsai et al. (2007) concerning a sensibility value for IGF-II (44%), we focalized our interest toward IGF-II as HCC marker. Both IGF-II and AFP levels in HCC were significantly higher than those in cirrhotic patients and controls, the IGF-II levels in cirrhotic patients were significantly lower than those in controls. The optimal cut-off values for diagnosing HCC, determined with ROC curve, was 796 ng/ml for IGF-II and 132 ng/ml for AFP. The diagnostic utility of the joined action of AFP and IGF-II is assessed by the sensibility value, that is more elevated in comparison to every marker singly used.

References

- Altman DG, Bland JM.(1994), Diagnostic tests: sensitivity and specificity. *BMJ* 1994;308:1552
- Pesarin F. (2001) *Multivariate permutation tests with applications in biostatistics*. Wiley, Chichester.
- Tsai J. F., Jeng J. E., Chuang L. Y., You H. L., Wang L. Y., Hsieh M. Y., Chen S. C., Chuang W. L., Lin Z. Y., Yu M. L., Dai C. Y. (2007), Serum insulin-like growth factor-II as a serologic marker of small hepatocellular carcinoma, *Scandinavian Journal of Gastroenterology*, 40:1, 68 - 75
- Zou KH, O'Malley AJ, Mauri L (2007), Receiver-Operating Characteristic Analysis for Evaluating Diagnostic Tests and Predictive Models. *Circulation*. 2007;115:654-657.