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FAST TRACK

Progressive increase of SCCA-IgM immune complexes in cirrhotic patients is associated with development of hepatocellular carcinoma

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About 3–4% of cirrhotic patients develop primary liver cancer every year. Specific serologic markers have not yet been identified for screening of high risk patients. The serpin squamous cell carcinoma antigen (SCCA) is overexpressed in liver cancer and circulating SCCA-IgM complexes have been described in patients with hepatocellular carcinoma (HCC). The aim of the present study was to assess the behavior of SCCA-IgM in relation to HCC development in patients with cirrhosis. A retrospective, longitudinal study was conducted in a cohort of prospectively followed cirrhotic patients. Two groups with similar clinical profile at presentation were studied : group A included 16 patients who developed HCC during a median follow up of 4 years; group B included 17 patients who did not develop HCC during the same time interval. Circulating SCCA-IgM immune complexes were determined using a recently standardized ELISA assay. At presentation similar levels of SCCA-IgM complexes [mean ± SD: 267.40 ± 382.25 U/ml *vs.* 249.10 ± 446.90 U/ml, $p = 0.9006$] and of alpha-fetoprotein [AFP; 24.11 ± 59.04 IU/ml *vs.* 10.91 ± 23.34 IU/ml, $p = 0.3995$] were detected in group A and in group B. The increase over time (ϕ) of SCCA-IgM, assessed within at least one year before clinical diagnosis of HCC, was remarkably higher in group A than in group B (mean ± SD = 280.05 ± 606.71 (U/ml)/year *vs.* −37.92 ± 95.94 (U/ml)/year, $p = 0.0408$), while AFP increase was not significantly different (11.89 ± 23.27 (IU/ml)/year *vs.* 3.67 ± 11.46 (IU/ml)/year, $p = 0.2179$). Receiver operating characteristic (ROC) curves were plotted for the rate of change in the levels of both markers and the diagnostic accuracy measured as AUROC was higher for SCCA-IgM ϕ (0.821) than for AFP ϕ (0.654). In conclusion, the progressive increase of SCCA-IgM over time was associated with liver tumor development, suggesting that monitoring the behavior of SCCA-IgM might become useful to identify cirrhotic patients at higher risk of HCC development.

Key words: SCCA-IgM; hepatocellular carcinoma; serologic prognostic marker; cirrhosis

Hepatocellular carcinoma (HCC) is one of the major health problems worldwide, due to its high incidence and severe prognosis. Figures depicting half a million new cases per year have been reported, and projection studies have estimated an increase of tumor development within the next decade in developed countries.[1] The reasons advocated to explain this phenomenon are the increased rate of HCV infection and an improvement in clinical management of liver cirrhosis, identified as the major risk factor for HCC development.[2] About 3–4% of cirrhotic patients develop primary liver cancer every year and this justifies surveillance programs to detect HCC at an early stage.[3] The prognosis of the patients depends mainly on the evolutionary stage of the neoplasm, ranging from 5-years survival higher than 70% in surgical patients to less than 3 months in very advanced tumors.[4] Tumor size is one of the main factors influencing the possibility of both surgical or ablative interventions, and small tumors have a better chance to be cured. The best strategy to monitor cirrhotic patients is echographic follow-up, which is able to reveal hepatic lesions of about 1 cm size, whereas alpha-fetoprotein (AFP) is currently used in clinical practice but its poor specificity and sensitivity arise concerns on its use as a screening tool.[5] Till date, no specific serologic markers have been identified to predict tumor development in cirrhotic patients to better focus surveillance programs and address health related resources. Recent findings have identified the occurrence of the serine protease inhibitor *squamous cell carcinoma antigen* (SCCA) hyper-expression in liver cancer tissue[6,7] and a new serologic assay has been developed to detect circulating SCCA.[8] The best results were obtained when SCCA complexed with IgM was determined, and significantly higher values were observed in HCC, compared to patients with chronic liver disease and cirrhosis in a cross-sectional study.[8] The aim of the present study was to assess the behavior of SCCA-IgM immune complexes in relation to HCC development in patients with cirrhosis.

A retrospective, longitudinal study was conducted in a cohort of cirrhotic patients with HCV infection, regularly followed up in our institution with serum alpha-fetoprotein (AFP) testing and hepatic ultrasonography every 6 months. The patients were divided into the following groups: group A included 16 cirrhotic patients who developed HCC during a median follow up of 4 years (range 2–8 years). The diagnosis of liver cancer was formulated on the basis of ultrasound results, confirmed by CT scan, by magnetic resonance when indicated and by ultrasound-guided fine needle biopsy. Group B included 17 control patients with cirrhosis, who did not develop HCC during the same time interval. Both groups had similar clinical profile at presentation (Table I) and none of the patients received antiviral therapy during the previous 8–10 years before and at the time of the study. Serum samples were collected, under informed consent, at the time of clinical visits and stored at −20°C for further analysis. All the patients were histologically proven, Child A cirrhosis at the time of the first serum test (T_1), being the grade of inflammatory activity 6.33 ± 1.86 for the patients of group A and 5.55 ± 1.13 for the patients of group B ($p = 0.38$). A second serum test (T_2) was performed after a median interval of 3 years, corresponding to a median period of 2 years (range 1–4 years) before HCC diagnosis for the patients of group A. None of the patients was coinfected with HBV, and alcohol or drug abuse were excluded as potential liver disease cofactors.

Circulating SCCA-IgM immune complexes levels were determined using an ELISA assay kit (Hepa-IC, Xeptagen SpA, Italy) according to the manufacturer's instructions. Briefly, plates precoated with anti-human SCCA antibody were incubated with either serially diluted standards or serum samples, and the presence of SCCA-IgM complexes were revealed by the addition of enzyme-conjugated anti-human IgM. The plate was then washed and the substrate solution was incubated for 20 min. Subsequently, the plate was read on a microtiter plate reader at 405 nm.[8] The

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TABLE I – CLINICAL FEATURES AT PRESENTATION OF THE CIRRHOTIC PATIENTS WHO DEVELOPED HCC DURING FOLLOW-UP (GROUP A) AND OF THE CIRRHOTIC PATIENTS WHO DID NOT DEVELOP HCC DURING THE SAME TIME OF OBSERVATION (GROUP B)

	Group A (16 patients)	Group B (17 patients)	*p*
Age (years)[1]	69 ± 9	63 ± 11	0.060
M/F	11/5	10/7	0.818
Platelets (10^9/L)[1]	116.73 ± 56.84	117.75 ± 60.51	0.964
INR[1]	1.07 ± 0.3	1.13 ± 0.11	0.550
Bilirubin (μmol/L)[1]	21.24 ± 8.96	18.71 ± 6.3	0.367
Albumin (g/L)[1]	35.27 ± 6.42	38.98 ± 4.89	0.109
AFP(IU/mL)[1]	24.11 ± 59.04	10.91 ± 23.34	0.399
>20 IU/mL (%)	31%	18%	0.438

[1]Mean ± SD.

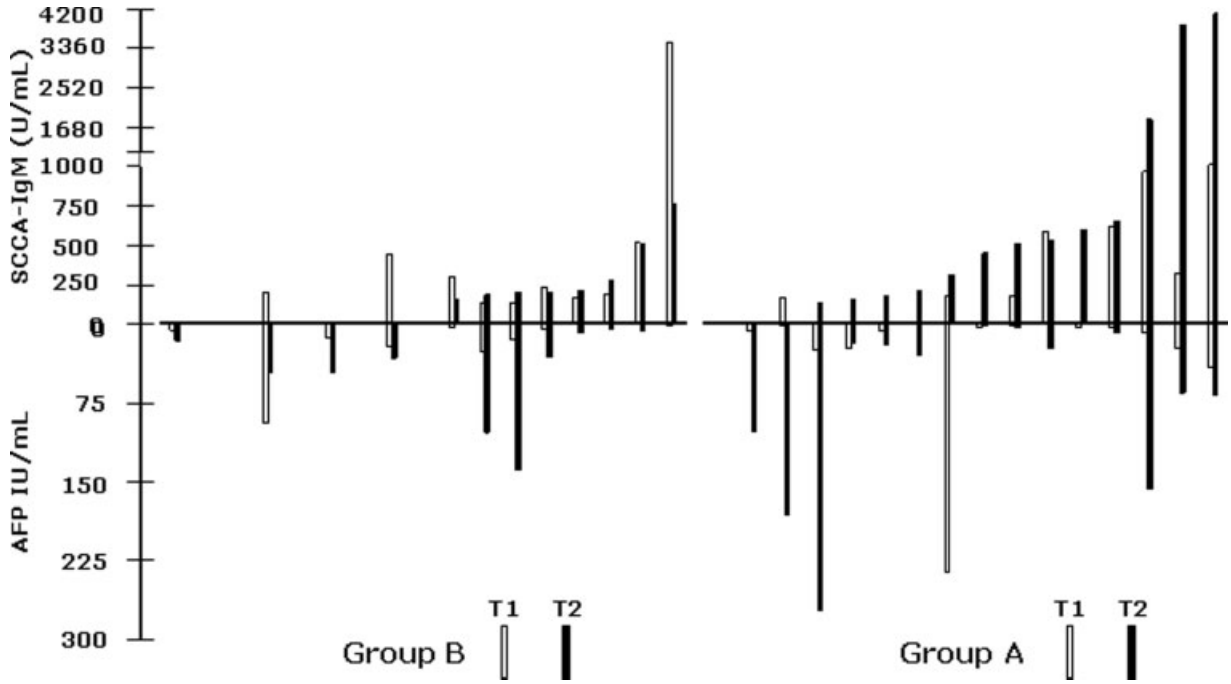

FIGURE 1 – Individual T_1 and T_2 data for patients who did not develop HCC during follow up (group B) and in the group of cirrhotic patients who developed HCC during the same interval of observation (group A) showing SCCA-IgM levels (upper bars) and AFP levels (lower bars).

amount of SCCA-IgM complexes were expressed in arbitrary Units/ml (U/ml). In the same serum sample alpha-fetoprotein (AFP) was also assessed using a solid phase ELISA assay (DRG International, USA).

The increase of SCCA-IgM and of AFP over time (Φ) was calculated using the following formula:

$$\Phi = \frac{[X - IgM]_{(T2)} - [X - IgM]_{(T1)}}{[(T_2) - (T_1)]}$$

Statistical analysis was carried out using the Student's *t*-test, the Fisher exact test, the Spearman correlation coefficient and the median test. The level of significance was set as $p < 0.05$. All analyses were performed using Analyse-it® software (England). The area under the receiver operating characteristic (ROC) curves were calculated and compared using the MedCalc software (Belgium).

At presentation, similar SCCA-IgM complexes reactivity was detectable in cirrhotic patients who developed HCC (group A) and in the group without HCC development during the same length of follow-up (group B) [mean ± SD: 267.40 ± 382.25 U/ml *vs.* 249.10 ± 446.90 U/ml, $p = 0.9006$]. Alpha-fetoprotein did not correlate with the presence of SCCA-IgM in the same serum sample ($r = -0.0010$), AFP values being similar in both groups at presentation in terms of mean level and of incidence of AFP levels >20 IU/ml, as described in Table I. Figure 1 shows T_1 and T_2 figures for both SCCA-IgM and AFP in individual patients.

The increase of SCCA-IgM over time (Φ) was remarkably higher in cirrhotic patients who eventually developed HCC compared to those who did not progress to liver cancer (Fig. 2*a*). The distribution of Φ values in group A (Φ mean ± SD = 280.05 ± 606.71 (U/ml)/year) reflected both an increase of the initial SCCA-IgM value at T_1 (in 6/8 patients) and the occurrence of SCCA-IgM neo-reactivity at T_2 in 6/8 patients who were undetectable at T_1, an event that did not occur in any of the 7 patients of group B who were undetectable at presentation (T_1) (group B Φ mean ± SD = −37.92 ± 95.94 (U/ml)/year, $p = 0.0408$). The median Φ values in the 2 groups showed a significant difference (group A = 42.11 (U/ml)/year, group B = 0 (U/ml)/year, $p = 0.0081$ Median test) as displayed in Figure 3. Figure 4 depicts the behavior of SCCA-IgM over time in the 2 groups of cirrhotic patients: in group A patients, the increase of SCCA-IgM over time (ϕ) was >20 (U/ml)/year in 75% of cases (12/16), while no change or slight decrement was observed in 25% of the patients (4/16). An opposite behavior was observed in patients of group B, where only 6% of the patients (1/17) presented Φ >20 (U/ml)/year, while in 94% of the cases (16/17) ϕ values remained almost unchanged or decreased.

AFP increase in individual cases was not significantly different in both groups (11.89 ± 23.27 (IU/ml)/year *vs.* 3.67 ± 11.46 (IU/ml)/

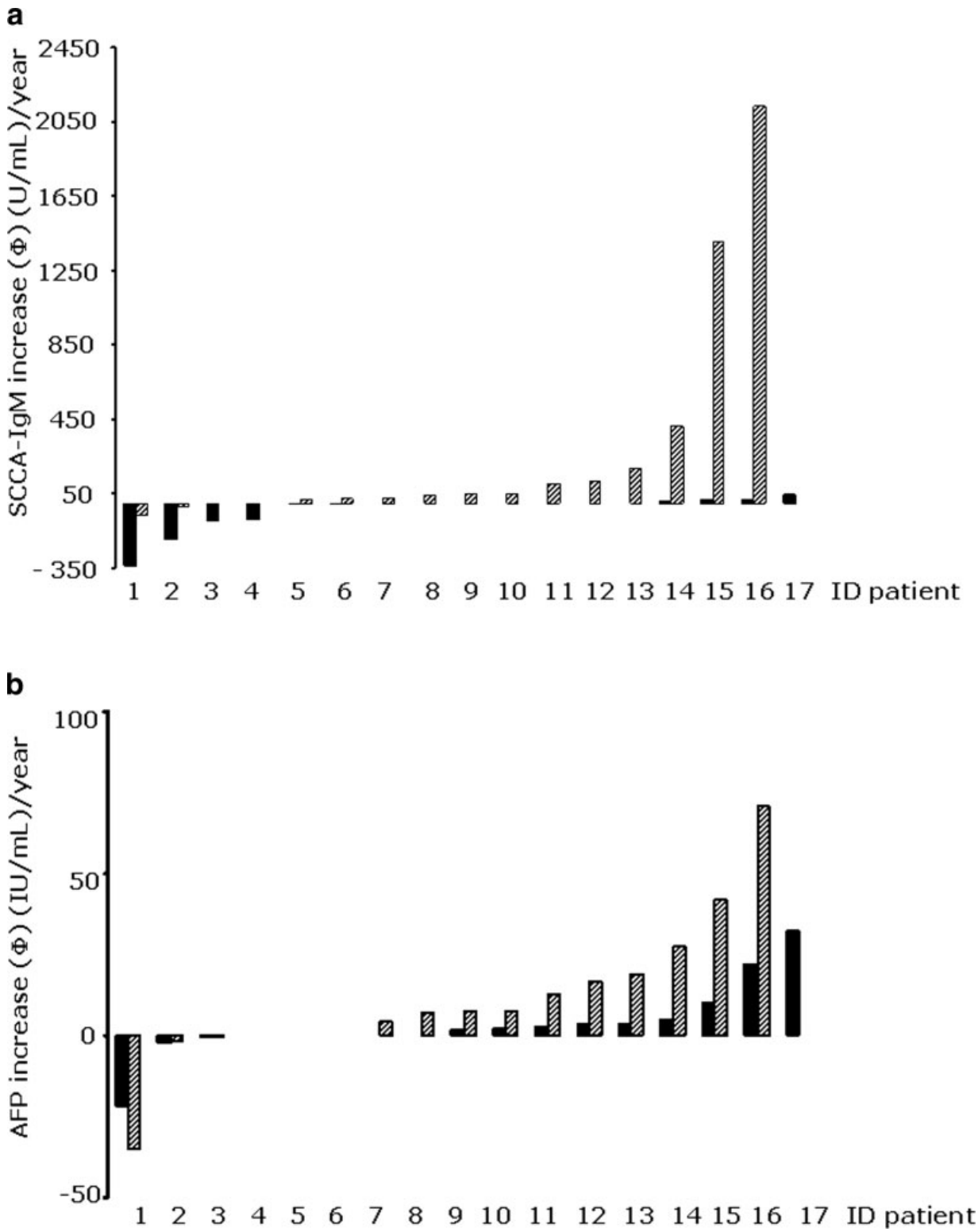

Figure 2 – Determination of the increase over time ((Φ) of SCCA-IgM (*a*) and of AFP (*b*) for each component of the group of cirrhotic patients who developed HCC during follow up (group A, ruled bars) and in the group of cirrhotic patients who did not develop HCC during the same interval of observation (group B, black bars).

year, $p = 0.2179$), although it was correlated with poor clinical outcome (Table II). Indeed, patients with shorter survival who died during follow-up, showed a trend towards higher levels of AFP increase over time, compared to patients still alive in group A, confirming the aggressive biological behavior previously associated with AFP elevation.[9]

Figure 5 depicts ROC curves of the increase over time (Φ) of SCCA-IgM and AFP and the rate of change in the levels of both

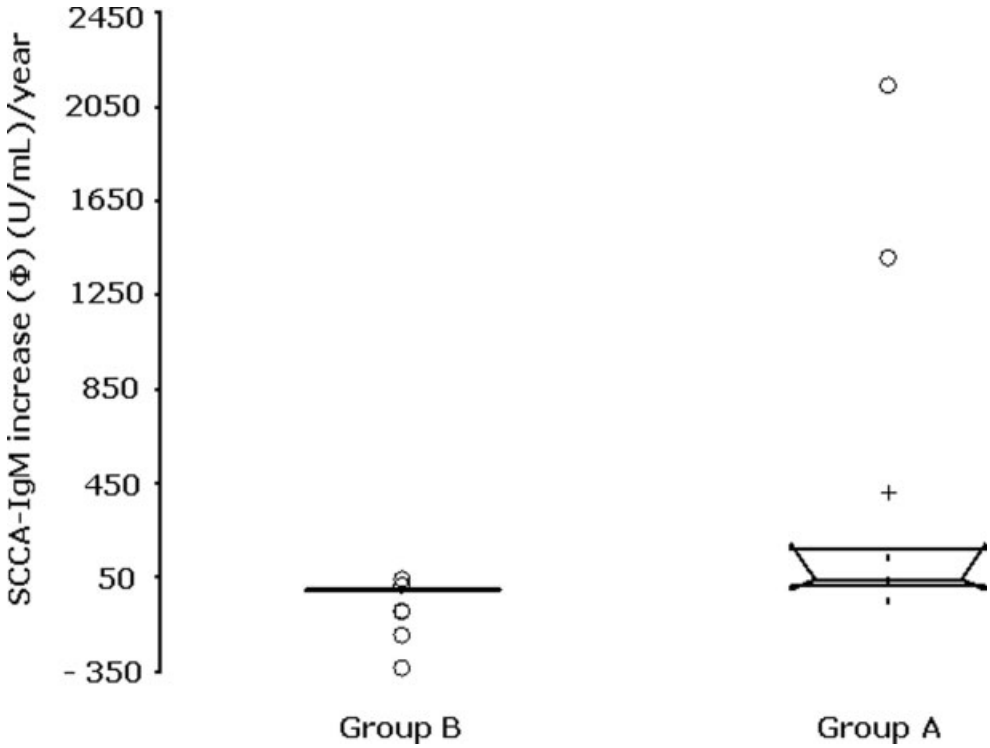

FIGURE 3 – Box plot for SCCA-IgM increase over time (Φ) in the group of cirrhotic patients who did not develop HCC during follow up (group B) and in the group of cirrhotic patients who developed HCC during the same interval of observation (group A). The box indicates the lower and upper quartile and the middle line indicates the median. Boxes are notched at the median with the lengths of the notches representing the 95% confidence interval. A dotted-line connects the observations within 1.5 inter-quartile ranges (IQRs) of the lower and upper quartile. Crosses represent the observations between 1.5 and 3.0 IQRs from the quartiles and circles represent points beyond this.

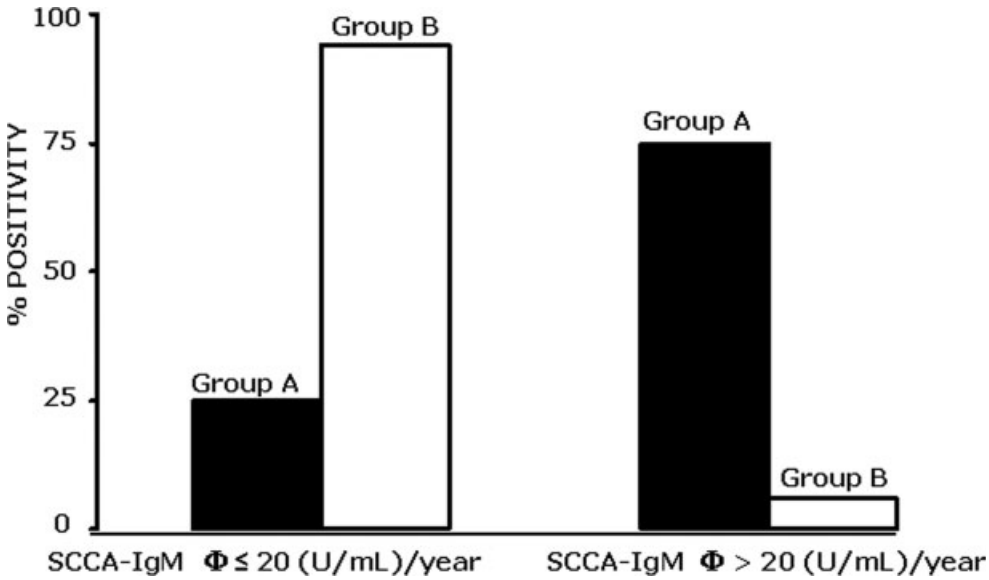

FIGURE 4 – Distribution of the cirrhotic patients in relation to different interval of SCCA increase (Φ). White bars refer to cirrhotic patients without occurrence of HCC (group B) and black bars indicate cirrhotic patients, having similar clinical characteristics and follow-up who developed HCC after at least 1 year from the end of the study (group A).

biomarkers indicates that the prognostic accuracy measured as the area under the ROC curves (AUROC) was higher for Φ SCCA-IgM (0.821) than for Φ AFP (0.654).

In clinical practice, one of the main unresolved issues for the management of patients with cirrhosis is that the individual risk of HCC development has not yet been clearly defined. Predictive factors have been considered in different studies, and scores with clinical and biological variables, including age, sex, HCV infection and genotype, prothrombin activity, platelet count and symptoms of portal hypertension have been proposed, allowing the identification of groups of patients with low or high risk of liver cancer development.[10–12] At the histological level, liver cell dysplasia[13,14] and hepatocyte proliferation rate[15,16] have been proposed as predictive factors of increased risk of liver cancer. These methods, however, are limited by the fact that they require liver biopsy and in daily practice this invasive procedure is not frequently performed in cirrhotic patients, where the diagnosis, excluding the early stage, is usually based on clinical findings. To date, no serological biomarkers are available to be used in surveillance programs. In the present study, we have assessed the behavior of the serpin SCCA, initially described in tumors of epithelial origin.[17] This biomarker has been recently detected also in the majority of cases of primary liver cancer where high amounts were observed at transcription and protein levels in neoplastic cells but not in normal liver.[6,7] Further studies have revealed that SCCA reactivity is also present in the liver in chronic hepatitis and in cirrhosis, although the extent of expression is usually lower than that observed in neoplastic cells, likely reflecting the regenerative ac-

TABLE II – INCREASE OVER TIME (Φ) OF SCCA AND AFP IN PATIENTS OF GROUP A IN RELATION TO CLINICAL OUTCOME

	Alive (n = 12)	Dead (n = 4)	p
Median follow-up	3 years	1.5 years	0.036
Age			
Mean ± SD	70.08 ± 9.20	68.75 ± 3.40	0.692
Median	73.5	69.5	
M/F	9/3	3/1	1.00
Φ [SCCA (U/mL)/year]			
Mean ± SD	278.090 ± 628.494	351 ± 703.545	0.849
Median (range)	52 (0–2137)	32.5 (−65 to 1404)	
Φ [AFP (IU/mL)/year]			
Mean ± SD	0.86 ± 13.22	29.26 ± 36.76	0.055
Median (range)	2.2 (−34.8 to 19)	17 (0.2–70.6)	

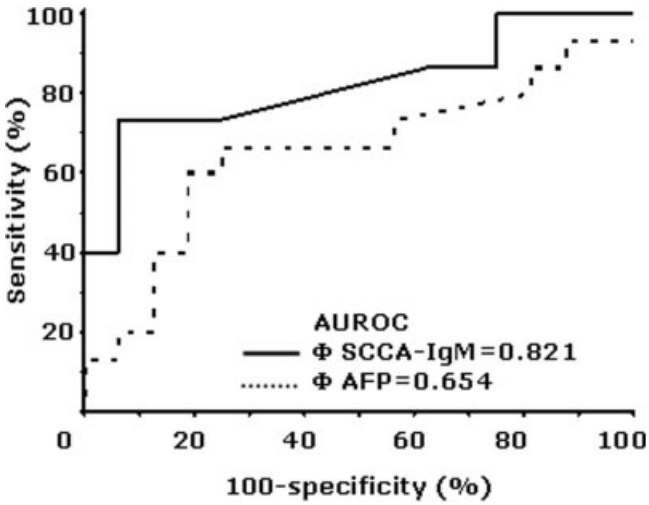

FIGURE 5 – ROC curves comparing the distribution of the increase of SCCA-IgM over time (Φ SCCA-IgM) and the increase of AFP over time (ΦAFP) in the group of cirrhotic patients who developed HCC during follow up (group A) *versus* the group of cirrhotic patients who did not develop HCC during the same interval of observation (group B).

tivity of the damaged liver.[8] Due to the availability of a standardized assay to measure circulating SCCA associated with IgM immunoglobulins, the main form of SCCA found in serum,[8] we have evaluated its behavior over time in 2 groups of cirrhotic patients with HCV infection, regularly attending the surveillance program for HCC development in our institution and presenting with similar clinical features and AFP levels. The results of the study indicate that the absolute value of the immune complexes at presentation was similar in cirrhotic patients with HCC progression and in nonprogressive patients. However, a remarkable difference between the 2 groups was found in the behavior of the SCCA-IgM complexes over time, since a progressive increase occurred in the majority of the patients who developed HCC after at least one year from the end of the study, while figures remained unchanged or slightly decreased in the majority of the cirrhotic patients without evidence of HCC during the same time interval. This behavior was observed at least one year before clinical diagnosis of HCC, suggesting that this *preclinical phase* might become a suitable window to specifically address new potentially effective therapies. The increase of AFP, measured in parallel in the same serum samples, was not significantly different in patients who eventually developed HCC and in those without liver tumor progression, although individual patients with shorter survival showed a trend towards higher AFP increase. The findings observed in this small series of cirrhotic patients recall similar results obtained when another serologic biomarker was used in a recently published study in men with localized prostate cancer,[18] where watchful waiting is one of the managing options.[19] The rate of rise in prostate specific antigen (PSA) levels was preoperatively assessed and the increase of PSA by more than 2.0 ng/ml during the year before diagnosis was found significantly associated with the risk of dying for prostate cancer.[18]

In conclusion, if the findings reported in the present study will be further confirmed in larger studies, monitoring SCCA-IgM complexes' behavior over time could became a useful prognostic parameter in cirrhotic patients, to support clinical decisions.

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