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SELEZIONE ARTICOLI SU APPLICAZIONI CLINICHE

ELISA Kit for the detection of Squamous Cell Carcinoma Antigen (SCCA) variants Immune Complexes (SCCA-IgM) in Hepatocellular Carcinoma (HCC)
MONITORAGGIO PAZIENTI CIRROTICI


VALUTAZIONE DELLA RISPOSTA TERAPEUTICA


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. 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 (T1), 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 (T2) 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 pre-coated 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.

The progressive increase of SCCA-IgM immune complexes in cirrhosis is associated with development of hepatocellular carcinoma. 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. 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.

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 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 ultrasonography results, confirmed by CT scan, by magnetic resonance when indicated and by ultrasonography-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 (T1), 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 (T2) 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 pre-coated 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. The
The 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 \( (\Phi) \) was calculated using the following formula:

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

Stratified 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\textsuperscript{®} 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 \( (\Phi) \) was remarkably higher in cirrhotic patients who eventually developed HCC compared to those who did not progress to liver cancer (Fig. 2a). The distribution of \( \Phi \) values in group A \( (\Phi \text{ mean } ± \text{ SD } = 280.05 ± 606.71 \text{ (U/ml/year) } \text{ reflected both an increase of the initial SCCA-IgM value at } T_1 \) \text{ in } 6/8 \text{ 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 \( (T_1) \) \( (\Phi \text{ mean } ± \text{ SD } = -37.92 ± 95.94 \text{ (U/ml/year), } p = 0.0408) \). The median \( \Phi \) values in the 2 groups showed a significant difference (group A \( \Phi \text{ mean } ± \text{ SD } = -37.92 ± 95.94 \text{ (U/ml/year), } p = 0.0408) \text{ Median test} \) 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 \( (\phi) \text{ was } >20 \text{ (U/ml/year), while in } 94\% \text{ of the cases (16/17) } \Phi \text{ values remained almost unchanged or decreased.} \)

AFP increase in individual cases was not significantly different in both groups \((11.89 ± 23.27 \text{ (IU/ml/year vs. } 3.67 ± 11.46 \text{ (IU/ml)/year))}\).
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 (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).
biomarkers indicates that the prognostic accuracy measured as the area under the ROC curves (AUROC) was higher for SCCA-IgM (0.821) than for 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 dysplasia13,14 and hepatocyte proliferation rate15,16 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-

**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).
SCCA-IGM immune complexes in cirrhotic patients and development of hepatocellular carcinoma

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

<table>
<thead>
<tr>
<th>Age</th>
<th>Alive (n = 12)</th>
<th>Dead (n = 4)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median follow-up</td>
<td>3 years</td>
<td>1.5 years</td>
<td>0.036</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>70.08 ± 9.20</td>
<td>68.75 ± 3.40</td>
<td>0.692</td>
</tr>
<tr>
<td>Median</td>
<td>73.5</td>
<td>69.5</td>
<td></td>
</tr>
<tr>
<td>M/F</td>
<td>9/3</td>
<td>3/1</td>
<td>1.00</td>
</tr>
<tr>
<td>Φ [SCCA (U/mL)/year]</td>
<td>278,090 ± 628,494</td>
<td>351 ± 703,545</td>
<td>0.849</td>
</tr>
<tr>
<td>Median (range)</td>
<td>52 (0–2137)</td>
<td>32.5 (–65 to 1404)</td>
<td></td>
</tr>
<tr>
<td>Φ [AFP (IU/mL)/year]</td>
<td>0.86 ± 13.22</td>
<td>29.26 ± 36.76</td>
<td>0.055</td>
</tr>
<tr>
<td>Median (range)</td>
<td>2.2 (–34.8 to 19)</td>
<td>17 (0.2–70.6)</td>
<td></td>
</tr>
</tbody>
</table>

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 become a useful prognostic parameter in cirrhotic patients, to support clinical decisions.

References


Monitoring SCCA-IgM complexes in serum predicts liver disease progression in patients with chronic hepatitis

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SUMMARY. About 30% of the patients with chronic hepatitis develop a progressive liver disease and one of the most intriguing issues is the detection of noninvasive markers for fibrosis stage and disease progression. High levels of squamous cell carcinoma antigen (SCCA)-immunoglobulin M (IgM) are detectable in hepatocellular carcinoma and their increase in cirrhotic patients can predict tumour development. As SCCA-IgM can also be detectable at low percentages in patients with chronic hepatitis, the aim of this study was to assess SCCA-IgM complexes in relation to disease outcome in this group of patients. An ELISA assay was used to determine the presence of SCCA-IgM in 188 patients with chronic hepatitis and in 100 controls. An additional serum sample was available after a median period of 6 years in 57 untreated patients: these patients were subdivided in group A, including eight patients with a fibrosis score increase ≥2 in a second liver biopsy and group B, including 49 patients without fibrosis progression during a similar follow up. SCCA-IgM complexes were detectable in 63 of 188 (33%) patients but in none of the controls. A significant increase of SCCA-IgM levels over time was observed in patients with fibrosis progression (mean ± SD: 117 ± 200 U/mL/year), but not in those without histologic deterioration (mean ± SD: –8.8 ± 31 U/mL/year, P < 0.0001). In conclusion, monitoring SCCA-IgM levels over time appears a useful approach to identify patients with chronic hepatitis at higher risk for cirrhosis development.

Keywords: chronic hepatitis outcome, fibrosis progression, serpin, squamous cell carcinoma antigen.

INTRODUCTION

Chronic infection by hepatitis B (HBV) and hepatitis C (HCV) viruses is one of the main causes of chronic hepatitis, representing a relevant health problem worldwide. Natural history studies indicate that advanced fibrosis and cirrhosis arise in about 20–40% of the patients with chronic HBV or HCV [1], usually decades after virus infection [2]. In chronically infected patients, the precise definition of the hepatic fibrosis stage is considered one of the most important parameters to assess the risk of disease progression. At present, liver biopsy represents the gold standard to assess presence, type and stage of liver fibrosis [3,4]. Despite its primary role, this procedure has a number of widely recognized limitations, such as invasiveness, difficult standardization and high cost [5,6]. For these reasons, the last decade has been focused on direct and indirect noninvasive markers able to provide accurate information about liver fibrogenetic activity and fibrosis stage in patients with potentially progressive hepatic disease [7]. When chronic infection has reached the stage of cirrhosis, in most of the cases a well-compensated phase occurs for a long time, before development of complications. Epidemiological data have demonstrated that liver cirrhosis, regardless of its aetiology, is the most important risk factor for the development of primary liver cancer [8]. Recent findings indicate that monitoring the behaviour of the immune complex squamous cell carcinoma antigen (SCCA)-immunoglobulin M (IgM) in serum can predict HCC development in patients with cirrhosis [9]. Since it has been shown that SCCA-IgM complexes are also detectable in a low percentage of patients with chronic hepatitis [10], the aim of the present study was to assess the behaviour of this marker in serum, in relation to disease outcome in patients with chronic hepatitis.

PATIENTS AND METHODS

Patients

The study was conducted in 188 patients with histologically proven chronic hepatitis (M/F: 105/83; mean age ± SD:...
Table 1 Clinical and epidemiological characteristics of the patients with chronic liver disease and of the control group

<table>
<thead>
<tr>
<th></th>
<th>Chronic hepatitis</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>188</td>
<td>100</td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>44 ± 13</td>
<td>36 ± 9</td>
</tr>
<tr>
<td>Sex. M/F</td>
<td>105/83</td>
<td>66/34</td>
</tr>
<tr>
<td>Aetiology (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>148/188 (79)</td>
<td>–</td>
</tr>
<tr>
<td>HBV</td>
<td>40/188 (21)</td>
<td>–</td>
</tr>
</tbody>
</table>

44 ± 13 years) followed up in our Institution. Serum samples from 100 blood donors (M/F: 66/34; mean age ± SD: 36 ± 9 years) were collected as controls and stored at −20 °C until use. Table 1 summarizes clinical and epidemiological characteristics of the studied population, where most of the patients were infected with HCV. Serum samples were obtained at presentation, when no antiviral treatment was carried out by any of the patients. An additional serum sample was available after a median period of 6 years (range: 2–21 years) in only 57 untreated patients (because of nonclinical indication to treatment or patient refusal). These patients underwent a second liver biopsy during follow-up and were divided, on the basis of their histologic findings, in the following two groups: group A, which included eight patients with disease progression defined by a fibrosis score increase ≥2 during follow-up, and group B, which included 49 patients without histologic evidence of disease progression during the same time interval (fibrosis score increase <2). Both groups had similar clinical profile at presentation, as shown in Table 2. Informed consent was obtained from all patients before liver biopsy and blood sample collection.

Virologic markers

HBsAg, HBeAg and the presence of serum anti-HBc, anti-HBe and anti-HBs antibodies were evaluated by ELISA using commercially available kits (Abbott Diagnostics, North Chicago, IL, USA). Anti-HCV antibody reactivity was determined by a third generation ELISA (Ortho Diagnostics, Raritan, NJ, USA) and confirmed by recombinant immunoblot assay (Ortho Diagnostics). Serum HCV RNA was determined by the Amplicor HCV monitor assay (Roche Diagnostics, Branchburg, NJ, USA).

SCCA-IgM detection in serum

SCCA-IgM immune complexes levels were determined in serum using an ELISA assay (Hepa-IC, Xeptagen, Italy), according to the manufacturer’s instructions.

In patients’ follow-ups the increase of SCCA-IgM over time (φ) was evaluated using the formula:

\[
φ = \frac{SCCA-IgM(T2) - SCCA-IgM(T1)}{(T2 - T1) \text{ years}}
\]

where T1 refers to the time at presentation and T2 refers to the second time point.

Statistics

Statistical analysis was carried out using the Student’s t-test, the Fisher exact test, the nonparametric Mann–Whitney test and the chi-square test, when appropriate. The level of significance was set as a P < 0.05. All analyses were performed using GraphPad InStat software (San Diego, CA, USA).

RESULTS

At presentation, circulating SCCA-IgM immune complexes were detectable in 63 of 188 (33%) patients with chronic hepatitis, but in none of the control group. The number of SCCA-IgM positive patients was not significantly different between patients infected with HBV (27.5%, 11/40) and HCV (34%, 50/148). Mean age (47 ± 13 years vs 42 ± 14 years, P = 0.05) and sex distribution (M/F: 1.18 vs 1.63, P = 0.750) were similar in patients with and without the presence of SCCA-IgM complex.

Figure 1 shows the distribution of SCCA-IgM levels at the first (T1) and second (T2) serum test time in the two groups of patients followed over time. Levels of the complex were substantially stable over time in patients without disease progression (median value T1 = 108 U/mL, T2 = 94 U/mL), while an increase was detected in 75% of the

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patients with progressive disease (median value $T_1 = 313 \text{ U/mL}$, $T_2 = 707 \text{ U/mL}$, $P = 0.014$).

To better analyse these results, the increase of SCCA-IgM over time ($\phi$) was calculated for each patient. A significant increase of SCCA-IgM during follow up was detected in patients with chronic hepatitis and progressive disease, but not in those without histologic progression ($\phi$ mean $\pm$ SD: $117 \pm 200 \text{ U/year}$ vs $-8.8 \pm 31 \text{ U/year}$, $P < 0.0001$).

As displayed in Figure 2, the distribution of $\phi$ values in this latter group of patients, mainly characterized by negative values, reflects a decrease of this parameter over time in the majority of the patients without disease progression.

**DISCUSSION**

Despite a marked decrease in the incidence rate of all types of viral hepatitis in the past 15 years [11,12], several thousand deaths from cirrhosis and HCC attributable to chronic HBV and HCV infection occur each year [13,14]. In clinical practice, one of the most relevant goals is the early identification of the subgroup of patients with increased risk of histological progression of liver disease [15]. The risk and the speed rate of fibrosis progression can be influenced by virus-related, host-related and environmental factors, and the role of co-factors, such as alcohol, metabolic disorders and viral co-infections, has been widely demonstrated [16–18]. However, in individual patients the art of predicting fibrosis [19] is only partially based on the presence of these factors. The prognostic assessment is indeed more reliably estimated on the basis of serological parameters, such as levels of transaminases and of other markers able to evaluate liver function (i.e. prothrombin activity, platelet count) [20]. Also liver ultrasound, although less specific, is useful to assess disease progression during long-term follow-up [21] and liver stiffness, assessed by the fibroscan, appears a promising tool [22]. However, liver biopsy is still the gold standard for liver fibrosis staging, despite its limitations. With the aim to greatly reduce the need to apply liver biopsy, many direct and indirect biomarkers (Fibrotest, APRI, AAR, Forns’index) of liver fibrosis have been recently proposed [7]. To date, no serological marker has been identified as an adequate marker to predict fibrosis progression in individual patients.

Recent findings indicate that immunoglobulins of the IgM class can form complexes with the SCCA, a serin protease inhibitor (serpin) [10]. This circulating immune complexes (SCCA-IgM), has been considered a new biomarker for HCC, because it has been detected at high levels in the majority of the patients with primary liver cancer. Further studies have proposed that the SCCA-IgM complex could be used to monitor patients with cirrhosis to identify those at higher risk of HCC development [9]. As it has been shown that SCCA-IgM complexes are also detectable in a low percentage of patients with chronic hepatitis [10], we have evaluated its behaviour in patients with chronic hepatitis followed up in our Institution. The results of the study indicate that SCCA-IgM complexes were detectable in about one-third of the patients, independently of viral aetiology, age, and sex. Although the number of untreated patients with liver disease evolution was rather small, monitoring the SCCA-IgM complexes over time revealed a marked difference between the two groups of patients, recalling the behaviour recently found in cirrhotic patients with and without HCC progression [9]. SCCA-IgM levels increased in the majority of the patients with histologic deterioration, while values of the immune complexes remained substantially stable or slightly decreased in most of the patients without disease progression during the same interval.
In conclusion, monitoring SCCA-IgM complex behaviour over time could become a useful approach to predict disease outcome in individual patients with chronic hepatitis. Further studies are needed to define adequate frequency of testing and/or clinically relevant values to better address optimal treatment in individual patients.

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IgM-Linked SerpinB3 and SerpinB4 in Sera of Patients with Chronic Liver Disease

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Abstract

**Background:** Epidemiological studies indicate that a growing number of cirrhotic patients will develop hepatocellular carcinoma (HCC) in the next decade. Recent findings have demonstrated that Squamous cell carcinoma antigen 1 (SCCA1) and 2 (SCCA2) isoforms, now classified as serpinB3 and serpinB4, are over-expressed in HCC, but not in normal liver. As reported, high levels of circulating SCCA-IgM immunocomplexes in patients with cirrhosis are significantly associated with HCC development.

**Aim:** To ascertain whether IgM-linked SCCA isoforms circulate in patients with chronic liver disease, compared to total SCCA-IgM levels.

**Methodology and Findings:** 79 patients with chronic liver disease were studied, including 17 patients with chronic hepatitis, 36 patients with cirrhosis and 26 with HCC. 28 blood donors were used as control. Monoclonal antibodies against serpinB3 and serpinB4 were used as catcher antibodies to set up specific ELISA assays, while total SCCA-IgM immunocomplexes were detected by commercially available ELISA assay. Overall, the results revealed a better diagnostic sensitivity of total SCCA-IgM assay, compared to both serpinB3 and serpinB4 IgM-linked assays. SerpinB4-IgM median values obtained with SCC103 antibody were moderately higher in patients with cirrhosis than in those with HCC, median values: 0.168 (IQR 0.140–0.427) vs. 0.140 (IQR 0.140–0.278), (p = 0.177). A trend toward decreasing serpinB4-IgM/serpinB3-IgM median ratio was observed in patients with advanced liver disease, being 1.08 in patients with HCC, 1.10 in patients with cirrhosis and 1.40 in patients with chronic hepatitis (p = 0.079).

**Conclusions:** IgM-linked SCCA isoforms in serum of patients with chronic liver diseases were quantified for the first time. Although the number of patients was limited, this preliminary study reveals that the relative balance of the two serpin isoforms is altered in HCC and it is characterized by a lower serpinB4-IgM/serpinB3-IgM ratio, determined by lower serpinB4 levels.

Introduction

SerpinB3 and serpinB4 isoforms, also known as squamous cell carcinoma antigen 1 and 2 (SCCA1 and SCCA2) belong to over-serpin/claide B serpin family [1]. Over 1500 serpin members have been identified in humans, plants, bacteria, archea and poxviruses to date [2,3].

Genomic cloning of these two isoforms revealed that they are highly homologous, 91% identical at the amino acid level [4,5], share conserved tertiary structure, and use a unique conformational rearrangement for their inhibitory activity [6,7]. However, serpinB3 and serpinB4 show distinct properties and substrates: serpinB3 is a papaain-like cysteine proteinase inhibitor, while serpinB4 is a chymotrypsin-like serine proteinase inhibitor [8,9]. Little is known concerning the regulation of their gene expression. Both isoforms are broadly co-expressed in the spinous and granular layers of normal squamous epithelium, in several organs including tongue, tonsil, oesophagus, uterine cervix, vagina, the conducting airways, Hassall’s corpuscles of the thymus and some areas of the skin [10]. Regarding their role in normal epithelia, it has been suggested that SCCA isoforms may protect from bacterial and viral cystein proteases [11], mast cell chymase [12] and may also prevent cellular apoptosis of the cornified layer.

It has been demonstrated that SCCA isoforms are often overexpressed in neoplastic cells of epithelial origin [13], although their biological role in cancer cell is still unclear. It has been reported that both serpinB3 and serpinB4 protect neoplastic cells from apoptosis [14] and that serpinB3 promotes tumour growth [5,15–16], epithelial to mesenchymal transition and cell proliferation [17].
Overexpression of SCCA isoforms has been also described in HCC and in highly displastic liver nodules, but not in normal liver [18–20]. In addition, high levels of SCCA-IgM linked complexes, but not of the free SCCA protein, have been described in serum of patient with HCC [21].

To date, little information is available about the profile of expression of SCCA isoforms in patients with cancer. Some authors have demonstrated a selective expression of serpinB4 mRNA in squamous cell carcinoma (SCC) tissues from uterine cervix when compared to normal tissue or SCC tissues from oesophagus or skin [22–24]. Serological studies have reported elevated serum levels of serpinB4 isoform, ascribed to direct release from tumour cells [25,26]. However, there is still conflicting information about the prevalent circulating SCCA isoform and additional studies have not confirmed these data [27].

According to the new theory about cancer immunosurveillance, now updated as immunooditing [28–30], natural IgMs seem to play an important role in the innate immune response, not only against infectious agents, but also in the immunosurveillance against tumour cell growth. Multivalent IgMs bear a characteristic capacity to bind a wide range of post-transcriptionally modified tumour antigens and they all induce cancer-specific apoptosis, by triggering the intrinsic apoptotic pathway [31]. Several studies have demonstrated that tumour released antigens can react with the natural IgM class of immunoglobulins and form circulating immune complexes in different human tumours. The described immunocomplexed antigens include CEA in colorectal cancer [32], PSA in prostate cancer [33], AFP, SCCA and DPC in liver cancer [21,34,35]. It has been also shown that these circulating immunocomplexes provide a better diagnostic performance than the corresponding free biomarker.

The purpose of this study was to evaluate the occurrence of serum immunoreactivity of IgM linked serpinB3 and serpinB4 isoforms in patients with different extent of chronic liver disease, compared to total SCCA-IgM levels.

Results

The results obtained in the study, expressed as descriptive parameters, are summarized in Table 1. OD median value of serpinB3-IgM was 0.130 in each group of patients. The values of serpinB4-IgM obtained with SCC103 antibody, which recognises the serpin-protexase complex, were slightly lower in patients with HCC, compared to patients with cirrhosis (median values: 0.140 [IQR 0.140–0.278] vs 0.168 [IQR 0.140–0.427 p = 0.177]). Similar and not significantly different values of serpinB4-IgM obtained with SCC104 antibody were found comparing all groups of studied patients.

SerpinB3-IgM complex was positive in 2 out of 17 (12%) patients with chronic hepatitis, in 10 out of 36 (28%) patients with cirrhosis and in 6 out of 26 (23%) patients with HCC. Similar values of reactivity were obtained for serpinB4-IgM detected immobilizing SCC103 antibody on the plate of the dedicated ELISA. A little gain in positivity rate for the detection of serpinB4-IgM in the three analyzed groups of patients was obtained using the other serpinB4 specific monoclonal antibody SCC104Ab. Using this latter antibody 5 out of 17 (29%) patients with chronic hepatitis, 15 out of 36 (42%) patients with cirrhosis and 9 out of 26 (35%) patients with HCC were positive. When total SCCA-IgM levels, regarded as the reference biomarker, were measured, 53% of the patients with chronic hepatitis, 47% of the cirrhotic patients and 58% of the patients with HCC were positive, showing a better sensitivity of this reference test, when compared with the sensitivity of the three above-listed assays. All healthy subjects were negative for total SCCA-IgM ELISA, while a 7% of positivity rate was found using the other ELISA assays.

Circulating levels ofAFP were also measured, and were positive in 4 out of 26 (15%) patients with cirrhosis (median value 8.2 IU/ml) and in 13 out of 26 (50%) patients with HCC (median value 19.8 IU/ml, p = 0.02). In the group of patients with liver cirrhosis the distribution pattern of IgM-linked immunocomplexes was further analyzed in relation to liver tumour progression. Seventeen patients developed liver cancer during follow-up (group A) and these patients (Figure 1, panel A) showed higher, but not statistically significant, OD median values for all circulating biomarkers, when compared to patients without histological evolution (n = 19, group B) (Figure 1, panel B).

Several reports indicate that serpinB4, corresponding to the acidic isoform, is the main circulating isoform in patients with epithelial cancers [40,41,26] and an elevated serpinB4/serpinB3 mRNA ratio, detectable in different cancer cells [22,23,42], has been described as a poor prognostic factor for early-stage cervical cancer. In the present study, we have therefore evaluated the ratio between the different IgM-linked serpin isoforms in the three groups of patients.

In patients with chronic hepatitis the serpinB4-IgM/serpinB3-IgM median ratio was 1.40 (range 1.0–4.5), in patients with cirrhosis it was 1.10 (range 0.9–6.2), and in patients with HCC it was 1.08 (range 0.4–4.2). A progressive decrease of median ratio was observed in patients with more advanced liver disease, although no statistical difference was reached (Figure 2). These data are in keeping with the finding that 16 out of 26 patients with HCC (62%) and 15 out of 36 patients with cirrhosis (42%) were found below the LoD value for SerpinB4-IgM.

Discussion

In the present study a simple assay to quantify the two isoforms serpinB3 and serpinB4 circulating as IgM-linked immunocomplexes in patients with different extent of chronic liver disease was set-up. The choice of analyzing the IgM-linked instead of the free isoforms was derived by the results obtained previously, when the assay to detect SCCA-IgM reactivity was initially described and performed better than that for the detection of free SCCA protein in serum [21]. Isoform-specific immunoenzymatic assays have been set up, using commercially available monoclonal antibodies. The aim of this investigation was to assess the pattern of expression of IgM-linked SCCA isoforms in chronic liver disease and to define whether they might provide any clinical advantage compared to total circulating SCCA-IgM levels.

To the best of our knowledge, this is the first study on the behaviour of circulating SCCA-IgM isoforms in patients with chronic liver diseases. We have recently shown a progressive increase over time of total SCCA-IgM immunocomplex in sera of untreated patients with progressive forms of chronic hepatitis [43] and in cirrhotic patients who developed liver cancer during follow-up [44]. Although the number of patients was limited, we have attempted to evaluate whether the reactivity for IgM-linked serpin isoforms was distributed differently in patients with chronic hepatitis and in cirrhotic patients with or without liver cancer progression. The small number of patients did not allow to identify significant differences between serpinB3-IgM and serpinB4-IgM behaviour, despite cirrhotic patients who developed HCC showed a trend to higher reactivity for all circulating biomarkers.
The majority of previous reports concerning SCCA levels in serum of patients with epithelial cancers describe serpinB4 as the dominant serological isoform [37,40,26]. These data were confirmed by histological studies, showing an elevated expression of serpinB4 in cancer tissues [22,41], as well as an elevated serpinB4/serpinB3 mRNA ratio in cervical carcinoma [41,23] and in head–neck cancer [45]. This effect was explained as a result of a possible protective role of serpinB4 from inflammation and apoptosis of tumour cells, probably due to direct inhibition of cathepsin G [23].

As for SCCA-IgM isoforms and liver cancer, our findings clearly document a gradient decrease of serpinB4-IgM/serpinB3-IgM ratio when comparing patients with different extent of liver disease, despite not reaching statistically significant differences due to the limited number of the patients included in the study. This trend was mainly a consequence of lower serum levels of serpinB4-IgM in patients with liver tumour, where the majority of them showed values below the detection limit for this assay. Despite our findings are not in agreement with published studies [37,40,26], it should be noted that current literature on this subject refers to the analysis of SCCA free isoforms in cancers of epithelial origin, where the corresponding normal tissues express physiologically this serpin [46,10], while normal liver does not [18]. Since SerpinB3 has been found to induce epithelial to mesenchymal transition and increased proliferative and invasive potential [17], while no parallel information is available for SerpinB4, further studies to define the precise biological activities of the two isoforms, beside their antiprotease activity, are warranted.

In conclusion, the relative balance of the two serpin isoforms seems to be altered in HCC and characterized by a lower serpinB4-IgM/serpinB3-IgM ratio, determined by lower serpinB4 levels.

### Materials and Methods

#### Patients

Serum samples from 28 blood donors and from 79 patients with different extent of liver disease, including 17 patients with histologically proven chronic hepatitis, 36 patients with histologically proven liver cirrhosis and 26 patients with HCC, were analyzed. None of the patients underwent antiviral treatment at least 12 months before serum sample collection. Patients with cirrhosis underwent regular liver ultrasound screening and were classified as Child A at the time of serum collection. The diagnosis of HCC was based on the presence of hepatic focal lesion >2 cm detected by liver ultrasound and confirmed by computed tomography and/or magnetic resonance as imaging techniques [36]. The final diagnosis was confirmed by histopathological analysis on ultrasound-assisted fine needle biopsy, when indicated.

In patients with cirrhosis, two groups were identified on the basis of HCC progression during follow-up: group A included 17 patients who developed HCC during a follow-up median period of 4 years, while the remaining 19 patients (group B) showed no histologic evidence of disease progression during the same time interval. Table 2 summarizes the main epidemiological and clinical characteristics of the study population: both groups of cirrhotic patients showed similar clinical profiles in terms of mean age, sex distribution and aetiology. Overall, patients were prevalently male, with mean age ranging from 53 to 65 years and most of them were HCV infected (85%).

The study was performed according to the principles expressed in the Declaration of Helsinki. Serum samples were collected after obtaining a signed informed consent from the patients, as approved by our institutional Ethics Committee. Serum samples were obtained from whole blood collected into Vacutainer tubes.

---

**Table 1. IgM linked SerpinB3 and SerpinB4 isoforms, compared to total SCCA-IgM in patients with different extent of chronic liver disease.**

<table>
<thead>
<tr>
<th></th>
<th>Chronic hepatitis</th>
<th>Cirrhosis</th>
<th>HCC</th>
<th>Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SerpinB3-IgM (SCC111)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (OD405 nm)</td>
<td>0.130</td>
<td>0.130</td>
<td>0.130</td>
<td>0.130</td>
</tr>
<tr>
<td>IQR</td>
<td>0.130–0.172</td>
<td>0.130–0.221</td>
<td>0.130–0.175</td>
<td>0.130–0.130</td>
</tr>
<tr>
<td>95th percentile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% positivity</td>
<td>12</td>
<td>28</td>
<td>23</td>
<td>7</td>
</tr>
</tbody>
</table>

| **SerpinB4-IgM (SCC103)** |                   |           |     |                  |
| Median (OD405 nm)       | 0.207             | 0.168     | 0.140 | 0.154            |
| IQR                     | 0.140–0.275       | 0.140–0.427 | 0.140–0.278 | 0.140–0.249 |
| 95th percentile         |                   |           |     |                  |
| % positivity            | 12                | 28        | 23  | 7                |

| **SerpinB4-IgM (SCC104)** |                   |           |     |                  |
| Median (OD405 nm)       | 0.126             | 0.135     | 0.125 | 0.125            |
| IQR                     | 0.125–0.227       | 0.125–0.285 | 0.125–0.319 | 0.125–0.125 |
| 95th percentile         |                   |           |     |                  |
| % positivity            | 29                | 42        | 35  | 7                |

| **Total SCCA-IgM** |                   |           |     |                  |
| Median (AU/mL)        | 166               | 134       | 209  | 115              |
| IQR                   | 80–511            | 80–552    | 118–287 | 109–129         |
| 95th percentile       |                   |           |     |                  |
| % positivity          | 53                | 47        | 58  | 0                |

IQR = interquartile range (25th–75th percentile).

The percentage of positivity for each immunocomplex was determined as the fraction number of patients with immunocomplex levels above the defined cut-off value (95th percentile).

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BD Diagnostics, USA) after centrifugation for 15 min at 2000 ×g. Serum was aliquoted into cryovials and stored at −80°C until use.

ELISA Assay for IgM-linked serpinB3 and serpinB4 Isoforms

Three available monoclonal antibodies (CanAg Diagnostics, Gothenburg Sweden) specific for the two different isoforms of this serpin were utilized: SCC111 antibody, recognizing serpinB3 by Western blot, although a slight cross-reactivity for SerpinB4 in “in solution” assays has been also described recently [37]. SCC103 and SCC104 antibodies which reacted only with serpinB4. In particular, the SCC103 monoclonal antibody reacted with serpinB4 complexed with specific proteinase: cathepsinG, while SCC104 did not recognize the serpinB4 complex, but only the free form of the serpin. Polystyrene high binding immunoplates (Sigma Aldrich, Milano, Italy) were coated with 100 µl/well of each monoclonal anti-human serpin antibody diluted in phosphate-buffered saline (PBS) at a concentration of 10 µg/ml and incubated overnight at 4°C. The wells were blocked with 1% bovine serum albumin (BSA)/PBS and incubated at room temperature for 2 h. After washing with PBS-0.05% Tween, 100 µl of serum samples were added at 1:8 dilution in PBS-0.05% Tween containing 1% BSA and incubated at room temperature for 1 h. The presence of serpinB3-IgM or serpinB4-IgM complexes were revealed by the addition of 100 µl/well of peroxidase-conjugated anti-human IgM (Sigma Aldrich, Milano, Italy) at a 1:1000 dilution for 1 h. After washing, the enzyme reaction was developed with 100 µl/well of an 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid-diammonium salt)/(ABTS) and hydrogen peroxidase as chromogenic substrates. Plates were analyzed by measuring the optical density at 405 nm on a microtiter plate reader (Tecan, USA). Each sample was tested in duplicate and each run was performed including positive and negative controls. Intra-assay coefficient of variation, calculated by repeated analysis (n = 5) of 3 samples, was 4.6% for serpinB3-IgM assay, 6.2% for serpinB4-IgM (SCC104) assay and 3.8% for serpinB4-IgM (SCC103) assay. The inter-assay coefficients of variation, estimated from five independent runs with 3 samples tested in duplicate, were less than 15% for each assay. Cut-off OD values, calculated as 95th percentile on the distribution curve of the specific assay in 28 healthy subjects, were: OD 0.200 for serpinB3-IgM; OD 0.199 for serpinB4-IgM (SCC104Ab) and OD 0.384 for

Figure 1. Serpin-IgM isoforms in cirrhotic patients with (panel A) and without (panel B) HCC evolution. Data in the box-plot graphs represent median, upper and lower OD values for serpinB4-IgM (SCC 103), serpinB4-IgM (SCC 104) and serpinB3-IgM.

doi:10.1371/journal.pone.0040658.g001
serpinB4-IgM (SCC103Ab). The percentage of positivity for each immunocomplex was determined as the fraction number of patients with immunocomplex levels above the defined cut-off value. The detection limit values (LoD), for each assay, were calculated following the indications of the EP17-A guidelines [38]. LoD was determined by the formula:

\[
\text{LoD} = \text{LoB} + 1.645 \times (\text{SD of low concentration sample})
\]

LoB (limit of blank) values were assayed with 10 replicates of the blank reagent (PBS) that contains no analyte. LoD values, calculated with five replicates of a sample known to contain a low concentration of analyte, were OD 0.130 for serpinB3-IgM assay, OD 0.125 for serpinB4-IgM (SCC104Ab) assay, and OD 0.140 for serpinB4-IgM (SCC103Ab) assay. These figures were used for all the calculations when the patients value was below the detection limit.

**ELISA Assay for total SCCA-IgM**

Total SCCA-IgM immunocomplexes were also detected in the corresponding serum samples by commercial ELISA Kit (Hepa-IC, generous gift of Xeptagen S.p.A., Venice, Italy) according to manufacturer’s instructions. In this assay a polyclonal anti-human SCCA antibody was used as capture antibody assuring the detection of all SCCA isoforms [21,39]. The amount of total SCCA-IgM complex was expressed in arbitrary Units/ml (AU/ml). Cut-off value, calculated as 95th percentile, was 156 AU/ml. The assay displayed intra-assay and inter-assay coefficients of variation lower than 10% [21].

**Statistical Analysis**

Considering that most variables were not normally distributed, quantitative data were summarized as median and interquartile range (IQR). Comparisons between groups were performed using the non-parametric Mann Whitney U-test and, when more than two groups had to be compared at the same time, the Kruskal-Wallis analysis of variance was performed. Qualitative data were summarized as percentages and the Fisher’s exact test was used for differences in frequencies. A 2 tailed p-value of <0.05 was considered statistically significant. All analyses were performed using GraphPad InStat 3.0 software (San Diego, CA, USA).

### Table 2. Epidemiological and clinical characteristics of the patients included in the study.

<table>
<thead>
<tr>
<th></th>
<th>Chronic hepatitis</th>
<th>Cirrhosis</th>
<th>HCC</th>
<th>Healthy subjects</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>17</td>
<td>19</td>
<td>26</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Age (years, mean±SD)</td>
<td>53±13</td>
<td>63±14</td>
<td>65±12</td>
<td>64±12</td>
<td>39±9</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>11/6</td>
<td>12/5</td>
<td>11/8</td>
<td>19/7</td>
<td>17/11</td>
</tr>
<tr>
<td>Aetiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>viral</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>83%</td>
<td>ns</td>
</tr>
<tr>
<td>non viral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17%</td>
</tr>
</tbody>
</table>

*p values according to Kruskall-Wallis ANOVA.

*p = 0.98 (according to Mann-Whitney U test) when patients with cirrhosis and with HCC were compared.

doi:10.1371/journal.pone.0040658.g002
Acknowledgments

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Author Contributions

Conceived and designed the experiments: AB PP. Performed the experiments: AB NT MGR SQ CT GV. Analyzed the data: LB GF CM. Contributed reagents/materials/analysis tools: LB GF. Wrote the paper: AB PP.

References

27. SerpinB3-IgM and SerpinB4-IgM in Liver Diseases
Serum Scca-IgM as a predictor of hepatocellular carcinoma in patients with liver cirrhosis

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³Dipartimento di Medicina Interna, Policlinico Universitario di Messina, Messina, Italia
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ABSTRACT

Aberrant Squamous Cell Carcinoma Antigen (SCCA) expression is an early hepatocarcinogenetic event and circulating SCCA-IgM complexes are elevated in most HCC patients. We evaluated whether serum SCCA-IgM levels can identify HCV +ve cirrhotic patients at low HCC risk. In this retrospective study we enrolled 29 cirrhotic patients in whom serum SCCA-IgM was measured 8 - 69 months (median 31) before HCC diagnosis, and 28 cirrhotic patients who remained HCC-free, with SCCA-IgM measured 15 - 68 months (median 48) before the study end. The best discriminating value of SCCA-IgM was calculated and tested in predicting HCC diagnosis within 12, 24 and 36 months. Sensitivity analysis, considering different HCC incidence, was conducted to identify the patient subgroup with an annual cancer risk below the threshold of a cost-effective semiannual surveillance with ultrasound. Cumulative HCC incidence at 12, 24 and 36 months was 7.0%, 15.7% and 26.3%, respectively. SCCA-IgM levels were higher in HCC than in cirrhotic patients [median: 381 (95% C.I.: 50 - 5289) vs. 100 (70 - 493) AU/mL, P = 0.005]. The SCCA-IgM value ≤ 200 AU/mL accurately identified patients at low risk of HCC development in the subsequent year (sensitivity 75%, specificity 62%, positive predictive value 13% and negative predictive value 97%). Considering an annual HCC incidence ≤ 3%, patients with SCCA-IgM ≤ 200 AU/mL (60% of the whole patients) had an HCC risk below the accepted threshold of a cost-effective surveillance (1.5%). In conclusion, provided that our provocative results are confirmed in larger studies, SCCA-IgM serum measurement could permit implementation of a two step (with different costs) surveillance: an initial serological surveillance, based on the annual monitoring of this biomarker, and the conventional surveillance by semiannual US when SCCA-IgM becomes >200 AU/mL. This could improve the cost/effectiveness of surveillance of HCV infected patients at risk of HCC.

Keywords: SCCA-IgM; HCC Risk Assessment; Surveillance Cost/Effectiveness

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer, the third cause of cancer-related death worldwide and the first cause of mortality in cirrhotic patients [1]. Chronic hepatitis C virus (HCV) infection represents the most important risk factor for HCC in Western countries and in Japan [2]. In HCV infected cirrhotic patients the annual incidence of HCC ranges from 1% to 8%, being higher in Japan (4% - 8%), intermediate in Italy (2% - 4%) and lower in the USA (1.4%) [3]. The prognosis of HCC patients still remains unsatisfactory, the 5-year survival rate being less than 10% both in Europe [4] and the USA [5]. Surveillance of patients at risk of developing HCC, based on the periodic repetition of liver ultrasound (US), makes it possible to detect most cancers at an early stage, still amenable to curative treatments which can greatly improve the prognosis of these individuals [6-10]. Therefore, regular surveillance of patients at risk of HCC is currently recommended by both Western [11] and Eastern [12,13] practical guide-
lines for HCC management.

The cost/effectiveness of surveillance is highly dependent on the incidence of HCC and, in the case of cirrhotic patients, decision analysis studies suggest that surveillance becomes cost-effective when the HCC risk is 1.5% per year or greater [14,15]. Nonetheless, a prospective study in Western patients with cirrhosis did not report a satisfactory cost/effectiveness of a semiannual program based on US and alpha-fetoprotein (AFP) determination [7]. This is due to the fact that the risk of HCC development has not yet been clearly defined in an individual basis, and patients with an insufficient risk are included in surveillance. It would therefore be important to exclude these patients in order to save costs. On this prospect, composite systems based on demographic and clinical factors have been proposed to stratify patients according to the HCC risk [16-19] but they have not received external validation and have not entered into clinical practice. To date, no serological marker able to accurately predict tumour development in cirrhotic patients has been found.

Squamous Cell Carcinoma Antigen (SCCA) is a serine protease inhibitor detectable in the spinous and granular layers of normal squamous epithelium but also expressed by neoplastic epithelial cells [20]. An overexpression of SCCA variants (SCCA-1, SCCA-2 and SCCA-PD) has been described in HCC tissue but not in normal liver [21], and a recent study has shown that SCCA is more expressed in cirrhotic tissue adjacent to high grade dysplastic nodules or HCC than in cirrhotic tissue proximal to large regenerative nodules or low grade dysplastic nodules. Thus, SCCA overexpression has been claimed to be an early event in hepatocarcinogenesis [22]. Noteworthy, the serum concentration of circulating immune complexes composed of SCCA and Immunoglobulin M (SCCA-IgM IC) paralleled the extent of SCCA overexpression in liver specimens [23,24], and in a small group of HCV infected patients with cirrhosis progressively increasing levels of serum SCCA-IgM complex were able to identify subjects at higher risk of HCC development [25]. Exploiting such a predictive ability in an opposite way, this biomarker could be utilized to identify inappropriate candidates for surveillance.

Our study aimed at assessing the ability of serum SCCA-IgM levels to segregate, according to the risk of HCC development, HCV infected patients with liver cirrhosis to identify those with a risk below the threshold of a cost-effective US surveillance.

2. PATIENTS AND METHODS

2.1. Patients

Among cirrhotic patients followed up from April 1994 to January 2010 in 5 Centres, we retrospectively selected those meeting the following inclusion criteria: 1) infected by HCV (± other aetiologic factors), 2) aged ≥ 50 years, 3) maintained under semiannual surveillance, based on US (± alpha-fetoprotein determination), 4) availability of one blood sample stored at −20°C, collected at least 6 months before HCC occurrence (cases) or the date (January 2009) of study end (controls), 5) control patients were also required to remain tumour-free during the subsequent year, 6) no interferon treatment in the 6 months prior to the collection of the index blood sample. The 57 patients fulfilling these criteria were divided into 2 groups: HCC group encompassing 29 patients developing HCC during the study period, and control group including 28 patients who remained tumour-free.

2.2. HCC Diagnosis and Staging

HCC was diagnosed by histology or according to the non-invasive criteria proposed by the EASL and thereafter the AASLD guidelines [11]. Prior to the availability of these criteria, the non-invasive diagnosis was based on an AFP value > 200 ng/mL coupled with an imaging work-up suggestive of HCC and definitely confirmed by the patient follow-up. The tumour was staged as “non-advanced” if its burden met the Milano criteria or “advanced” if beyond them [26].

2.3. Serologic Testing

Circulating SCCA-IgM levels were determined in frozen serum samples, stored at −20°C, using an ELISA assay kit (Hepa-IC, Xeptagen SpA, Marghera, Venezia, Italy).

2.4. Statistical Analysis

Continuous variables were expressed as mean ± SD or median and 95% C.I. and discrete variables as absolute and relative frequencies.

Continuous non-parametric variables were compared with the Mann-Whitney U test, and discrete variables with the χ² test or Fisher’s exact test, as appropriate.

The Receiver Operating Characteristic (ROC) curve and the corresponding area under the curve (AUC) were calculated to assess the accuracy of the seromarker in distinguishing HCCs from cirrhosis. The best cut-off value was chosen as the value with the highest Youden index [(sensitivity + specificity) − 1] and, in the event of equality, the value with higher sensitivity was chosen.

To assess the ability of SCCA-IgM to predict HCC occurrence in clinical practice, we simulated a prospective study protocol with patient assessment every 12 months. The simulation is depicted in Figure 1. In practice, patients who developed HCC during the 12, 24 and 36 months following the SCCA-IgM measurement were shifted to the HCC group, leaving the HCC-free indi-
Figure 1. Annual occurrence of hepatocellular carcinoma (HCC) in the studied population at 12, 24 and 36 months from SCCA-IgM determination.

individuals in the cirrhotic group. Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values of the SCCA-IgM were calculated. PPV and NPV were also calculated simulating HCC incidences expected in clinical practice.

A sensitivity analysis was then conducted to test the ability of SCCA-IgM to identify, according to HCC incidence in the whole patient population, the subgroup with an HCC risk below 1.5%/year, which is considered the threshold of a cost-effective surveillance [11].

A 2-tailed P value < 0.05 was considered statistically significant. Statistical analysis was performed using the SPSS 17.0 statistical package (Chicago, IL), Microsoft Excel program and MedCalc 11 software.

2.5. Ethics

The database management conforms to current Italian legislation on privacy and the study conforms to the ethical guidelines of the Declaration of Helsinki. All patients had provided informed consent to register and manage their data in an anonymous way in our database. The study was approved by the ethics committee of the participating Institutions.

3. RESULTS

3.1. Patients’ Characteristics (Table 1)

The HCC group and the control group did not differ for age and gender. In both groups, a few individuals were co-infected with HBV or declared a heavy alcohol intake as cofactors of liver disease. No significant differences were found in the distribution of Child-Pugh classes, with a predominance of patients with well preserved liver function. Most patients did not receive any antiviral therapy, without differences between groups. Nearly 70% of cases had a non-advanced HCC (within the Milano criteria) at the time of diagnosis.

Table 1. Demographic and clinical characteristics of patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCC patients (n = 29)</th>
<th>Cirrhotic patients (n = 28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.5 ± 8.2</td>
<td>65.8 ± 10.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Gender (males)</td>
<td>18 (62.1%)</td>
<td>16 (57.1%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Aetiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>25 (86.2%)</td>
<td>23 (82.1%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>HCV + HBV</td>
<td>2 (6.9%)</td>
<td>3 (10.7%)</td>
<td></td>
</tr>
<tr>
<td>HCV + alcohol</td>
<td>2 (6.9%)</td>
<td>2 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>Child-Pugh class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>27 (93.1%)</td>
<td>22 (78.6%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>B</td>
<td>2 (6.9%)</td>
<td>6 (21.4%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Antiviral therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no therapy</td>
<td>28 (96.6%)</td>
<td>26 (92.9%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>lamivudine + adefovir</td>
<td>1 (3.4%)</td>
<td>2 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>Median follow-up (months)</td>
<td>31 (68 - 4)</td>
<td>48 (68 - 15)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Tumour size (cm)</td>
<td>2.5 ± 1.3</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Milano criteria</td>
<td>within 20 (69.0%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>beyond 9 (31.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus; HBV: hepatitis B virus; SCCA-IgM: Serpin Squamous Cell Carcinoma Antigen-IgM complexes; n.s.: not statistically significant.

3.2. SCCA-IgM Performance

Blood samples used for SCCA-IgM determinations were collected from 8 to 69 months (median 31 months) before HCC detection in the HCC group, and from 15 to 68 months (median 48 months) prior to the study end in the control group. Median SCCA-IgM values were higher in the HCC group than in the control group [381 AU/ml (95% C.I. 117 - 615) vs. 100 AU/ml (95% C.I. 80 - 146); P = 0.004].

The ROC curve of SCCA-IgM levels found from 8 to 69 month prior to the cancer diagnosis (HCC patients) or the end of the study (HCC-free patients) is reported in Figure 2. The best discriminating value of 200 AU/ml (Youden Index 46.4%) had an overall sensitivity of 57.1%, an overall specificity of 89.3% and, considering the HCC prevalence in our population (51%), a PPV of 84.2% and an NPV of 67.6%. The PPV and NPV were also calculated for the cumulative incidence of HCC observed at 12 (7.0%), 24 (15.7%) and 36 (26.3%) months after SCCA-IgM measurement (Table 2).

Notably, in this model the NPV for HCCs occurring within the year following the SCCA-IgM determination rose to 97%, and it was still 91% for HCCs occurring within 24 months. When the HCC prevalence was set at 3% to simulate the annual incidence expected in clinical practice, the NPV for HCC occurrence within 12 and 24
Figure 2. Receiver Operating Characteristic (ROC) curve and corresponding area under the curve (AUC) of SCCA-IgM values measured 8 - 69 months prior to cancer diagnosis (HCC patients) or end of the study (HCC-free patients). The dot indicates the best cut-off value (200 AU/mL).

Table 2. Sensitivity (Se), Specificity (Sp), Positive (PPV) and Negative (NPV) Predictive Values of the best discriminating value of SCCA-IgM measured 12, 24 and 36 months prior to the HCC occurrence (cases) or the study end (controls).

<table>
<thead>
<tr>
<th>Timing (months)</th>
<th>HCC cumulative incidence (%)</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>–12</td>
<td>7.0</td>
<td>75</td>
<td>62</td>
<td>13</td>
<td>97</td>
</tr>
<tr>
<td>–24</td>
<td>15.7</td>
<td>67</td>
<td>65</td>
<td>26</td>
<td>91</td>
</tr>
<tr>
<td>–36</td>
<td>26.3</td>
<td>60</td>
<td>67</td>
<td>39</td>
<td>82</td>
</tr>
</tbody>
</table>

SCCA-IgM: Serpin Squamous Cell Carcinoma Antigen-IgM complexes.

The sensitivity analysis showed that, when annual incidence of HCC in the whole population was 3%, cirrhotic patients with SCCA-IgM ≤ 200 AU/ml had a tumour incidence of 1.2%, not enough for implementing a cost-effective semiannual surveillance with US [11] (Table 3). The accepted threshold of 1.5% was indeed crossed by patients with SCCA-IgM ≤ 200 AU/ml only when the tumour incidence was at least 4% in the whole population.

Table 3. Sensitivity analysis according to different annual incidences of HCC. With an overall incidence ≤ 3%, cirrhotic patients with SCCA-IgM ≤ 200 AU/ml have a risk of developing hepatocellular carcinoma (HCC) below the threshold (1.5%) of a cost-effective semiannual surveillance with liver ultrasound.

<table>
<thead>
<tr>
<th>Annual incidence of HCC (%)</th>
<th>Whole population</th>
<th>Patients with SCCA-IgM ≤ 200 AU/ml (N = 34)</th>
<th>Patients with SCCA-IgM &gt; 200 AU/ml (N = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.8</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>7.4</td>
<td></td>
</tr>
</tbody>
</table>

4. DISCUSSION

Several studies indicate that surveillance of individuals at risk of HCC increases the chance of detecting tumours at a stage amenable to curative or effective treatments, thus improving the prognosis of these patients [6-9,27]. Since the cost/effectiveness of this procedure is highly dependent on the incidence of HCC [14,28], the decision to enter a patient into a surveillance program should be determined by his/her level of HCC risk. Nowadays, the individual risk of HCC development in patients infected...
by HCV (the main cause of this tumour in the Western world and in Japan) has not yet been clearly defined. Therefore, although a Markov model would indicate that semiannual surveillance with US and AFP is cost-effective [28], this assumption has not been confirmed by a prospective observational study, principally including anti-HCV positive patients, where the estimated cost of each year of life gained exceeded 100,000 $ [7]. To optimize the cost-effectiveness of surveillance, we should be able to identify patients in whom surveillance is not worthwhile due to a very low HCC risk. The NPV becomes a crucial indicator for this purpose.

Some predictive indices have been proposed in HCV infected patients, but these studies do not report the NPV and only considered patients after [29] or during antiviral therapy [30]. The availability of inexpensive, easy to measure serological markers, able to accurately define this risk over a given—and relatively long-time span would be instrumental for this purpose. Since the aberrant expression of SCCA is an early event in hepatocarcinogenesis [22] and the circulating levels of the IgM immune-complexes parallel the extent of its tissue expression [23], monitoring SCCA-IgM is a promising tool for this perspective.

The prospective approach we simulated made it possible to test the performance of SCCA-IgM measured 12 months prior to HCC detection. The value of 200 AU/ml showed a valuable NPV of 97% one year before cancer diagnosis, despite the high HCC prevalence (7%) observed in our model. Moreover, reducing the HCC prevalence to a value simulating the tumour incidence expected in clinical practice (3%), the NPV reached 98.8%, and the sensitivity analysis revealed that, at this cancer prevalence, the SCCA-IgM can confidently identify HCV-infected cirrhotic patients with a too low risk of developing HCC in the subsequent year to enter a semiannual surveillance based on US. This ability, however, disappears when the HCC incidence in the whole population is 4% or higher. Therefore, provided that the expected annual risk of HCC is ≤3% in a given population of HCV infected patients (as can be anticipated in patients with advanced fibrosis/early cirrhosis), SCCA-IgM serum measurement could permit implementation of two step (with different costs) surveillance: an initial serological surveillance, based on the annual monitoring of this biomarker, and the conventional surveillance by semiannual US when SCCA-IgM becomes >200 AU/mL (Figure 3). Using this model, in our series, 34/57 (60%) cases would have been initially excluded from a non cost-effective semiannual surveillance, and annually postponed until this practice becomes cost-effective, without incurring an unacceptable risk of HCC underdiagnosis (as suggested by an NPV 99%).

This pilot study, however, has several limitations: 1) it is a retrospective investigation, suffering therefore of the typical methodological biases of these studies; 2) it is based on a small sample size; 3) our population included nine patients (16%) with HBV infection or heavy alcohol intake as cofactors of liver disease. The small sample size prevented the possibility to obtain reliable results on SCCA-IgM performances in the subgroups. On the other hand, the aetiological panel of our HCV-infected patients reproduces that into which daily clinical practice runs; 4) our results cannot be generalized to patients with non-HCV related cirrhosis or younger than 50 years (excluded from the study).

Therefore, the results of our seminal study, overturning the ordinary use of a new and promising onco-marker from an indicator of a high risk condition to a practice investigation of patients at risk of developing HCC: possible practical application of SCCA-IgM annual monitoring to improve the cost-effectiveness of surveillance of HCV-infected patients with an expected annual HCC incidence ≤3%.

This pilot study, however, has several limitations: 1) it

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Surveillance for hepatocellular carcinoma in patients with cirrhosis improves outcome. 


Gastroenterology, 130, 417-422. doi:10.1053/j.gastro.2004.04.0552-0


SCCA-IGM ARE PREDICTIVE OF HEPATOCELLULAR CARCINOMA DEVELOPMENT IN PATIENTS WITH HCV CIRRHOSIS. A PROSPECTIVE STUDY.

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¹Department of Medicine, University of Padua, Italy,
²Xeptagen, VEGA Park, Venice, Italy

Introduction and aims. In chronic hepatitis C increasing SCCA-IgM levels were found predictive of fibrosis progression and in HCV cirrhosis high levels of this biomarker were associated with an increased risk of HCC development in a recent multicenter cross-sectional study. Aim of the study was to assess the clinical significance of SCCA-IgM in cirrhotic patients in a prospective study.

Materials and Methods. 71 patients with cirrhosis (M/F: 53/18) were consecutively enrolled and followed up for a median period of 53 months at our Institution. Etiology was HCV in 37%, HBV in 17%, alcohol in 44% and metabolic or unknown in 2% of the cases. The majority of the patients (69%) were Child A. SCCA-IgM was measured in serum at presentation by ELISA (Hepa-IC, Xeptagen).

Results. SCCA-IgM was more frequently detected in HCV cirrhosis than in the remaining patients (38% vs 13%, p= 0.02). During follow up 11/26 HCV patients developed HCC (median time: 11 months). In this group the positivity of the biomarker at presentation was significantly associated with HCC development (70% vs 25%, p=0.04), but not with other cirrhosis complications. Kaplan–Meier curves confirmed a lower HCC-free survival in HCV cirrhotic patients positive for SCCA-IgM, compared to negative cases (p=0.03). The relative risk of HCC development was 2.7 in HCV cirrhosis (95% CI=1.6-4.6) and increased up to 3.2 (95% CI= 1.1-9.6) in the subset of SCCA-IgM positive patients.

Conclusions. In patients with HCV cirrhosis SCCA-IgM was highly predictive of HCC development and may be considered as a prognostic tool for the subclassification of cirrhotic patients.
Successful antiviral therapy determines a significant decrease in squamous cell carcinoma antigen-associated (SCCA) variants’ serum levels in anti-HCV positive cirrhotic patients*

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SUMMARY. Aberrant squamous cell carcinoma antigen (SCCA) expression is an early event in hepatocarcinogenesis, and increasing serum levels of SCCA variants IgM immune complexes (SCCA-IgM IC) have been found in cirrhotic patients developing hepatocellular carcinoma (HCC). We longitudinally evaluated a cohort of cirrhotic patients with hepatitis C virus infection (HCV) who underwent pegylated interferon (PEG-IFN) and ribavirin treatment. SCCA-IgM IC levels were assessed in the sera of 33 cirrhotic patients with HCV (21 males, median age 57 years) before, at the end and at 6-month and 1-year follow-up after treatment with PEG-IFN and ribavirin. SCCA-IgM IC serum levels (arbitrary units/mL, AU/mL) were evaluated according to treatment outcome: sustained virological response (SVR) vs nonresponse (NR). Overall, 15 patients obtained a SVR to antiviral therapy (45%). There was no significant difference in baseline SCCA-IgM IC serum levels between SVR and NR patients. When compared to baseline (451.2 AU/mL), SVR patients showed a significant decrease in median SCCA-IgM IC serum levels at the end of treatment (186.8 AU/mL, \( P = 0.013 \)) and at both 6-month (96.8 AU/mL, \( P < 0.001 \)) and 1-year follow-up (52.4 AU/mL, \( P < 0.001 \)), while no significant modification was observed in NR patients. In patients with HCV-related liver cirrhosis, successful antiviral therapy is associated with a dramatic and significant decrease in SCCA-IC serum levels. Because of the pathophysiological correlation between SCCA and liver carcinogenesis, it is hypothesized that in patients with liver cirrhosis, SVR may be accompanied by a decreased proliferative stimulation.

Keywords: cirrhosis, hepatitis C virus, peg-interferon, ribavirin, squamous cell carcinoma antigen.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a leading cause of end-stage liver disease and hepatocellular carcinoma (HCC) [1] worldwide. In patients chronically infected with HCV, liver cirrhosis is the most important factor for the development of HCC [2]. Longitudinal evaluation of patients with compensated cirrhosis as a result of HCV infection has shown that the yearly development rate of HCC ranges between 2% and 4% and that in these patients, HCC is the most frequent liver-related complication [3,4]. In patients with cirrhosis and HCV, antiviral therapy with pegylated interferon (PEG-IFN) and ribavirin is aimed at halting disease progression and decreasing the incidence of HCC [5]. Some studies have thus shown that successful antiviral therapy decreases the rate of HCC development in these patients.

Squamous cell carcinoma antigen (SCCA) is a serine protease inhibitor that is physiologically found in the spinous and granular layers of normal squamous epithelium and is also typically expressed by neoplastic cells of epithelial origin [6]. Recently, overexpression of SCCA variants (SCCA-1, SCCA-2 and SCCA-PD), as detected by immunohistochemistry, has been described in resected specimen of HCC tissue but not in normal liver [7]. Furthermore, SCCA
overexpression seems to be an early event in hepatocarcinogenesis, as in cirrhotic patients, both the prevalence and the intensity of immunostaining for SCCA were significantly greater in dysplastic nodules than in large regenerative nodules [8]. Noteworthy, the serum concentration of circulating immune complexes composed by SCCA and immunoglobulin M (SCCA-IgM IC) paralleled the extent of SCCA overexpression in liver specimens, thus making of serum SCCA-IgM IC an interesting marker for diagnosis of HCC [9,10]. More recently, it has been shown that progressive increase over time in serum SCCA-IgM IC can be observed in patients with chronic hepatitis C with worsening disease stage and in cirrhotic patients who develop HCC [11,12]. There are no data in the literature on the possible modification of serum SCCA-IgM IC in patients with HCV infection undergoing antiviral therapy. In this setting, monitoring of serum SCCA-IgM IC may improve our understanding of the antiproliferative action of successful antiviral treatment and patients’ outcome.

In this study, our aim was to evaluate the behaviour of serum SCCA-IgM IC in a cohort of cirrhotic patients with HCV who were treated with PEG-IFN and ribavirin so as to evaluate whether successful antiviral therapy may be associated with a modification in SCCA-IgM IC serum levels.

PATIENTS AND METHODS

Study cohort

In this study, we included 33 patients chronically infected with HCV who underwent PEG-IFN and ribavirin combination antiviral therapy at our Institution between March 2002 and April 2007 and who fulfilled the following clinical and biochemical criteria: age between 18 and 70 years, clinical or histological diagnosis of compensated cirrhosis, no history of previous decompensation of liver disease, ultrasound examination of the abdomen negative for focal liver lesions, serum bilirubin <2 mg/dL, serum albumin >3.5 g/dL, prothrombin activity >80%, serum creatinine <1.3 mg/dL, haemoglobin >12 g/dL in men and >11 g/dL in women, leucocyte count >3000/mm$^3$ or absolute neutrophil count >1000/mm$^3$ and platelet count >100 000/mm$^3$.

Patients were excluded if they had other causes of liver disease, previous organ transplantation, pre-existing uncontrolled psychiatric disease, seizure disorders, severe cardiovascular disease, haemoglobinopathies, haemophilia, poorly controlled diabetes, autoimmune diseases, intravenous drug use and/or alcohol abuse, positivity for human immunodeficiency virus infection and if they were unable to use contraception.

This study was investigator-driven, was carried out in the clinical practice and was not supported by any pharmaceutical company. Informed consent was obtained from all patients.

**Treatment protocol**

Patients were treated with either PEG-IFN α2a (180 μg/week subcutaneously) or PEG-IFN α2b (1.5 μg/kg/week subcutaneously) plus oral weight-based ribavirin in two separated doses (total dose was 800 mg/day for patients weighing <65 kg, 1000 mg/day for patients with a body weight ranging from 65 to 85 kg and 1200 mg/day for patients weighing 85 kg or more). Selection of PEG-IFN was at the discretion of the study physicians in charge of the patients, as this was not a randomized study. Treatment duration was 24 weeks for genotype 2 and genotype 3 patients, and 48 weeks for genotype 1 and 4 patients. At week 12, treatment was discontinued in patients who did not clear HCV-RNA or who had a reduction in viral load lower than 2.0 log when compared to baseline, and at week 24 treatment was discontinued in genotype 1 and 4 patients who were still HCV-RNA positive [13]. Sustained virological response (SVR) was assessed on the basis of serum HCV-RNA negativity at 24 weeks of follow-up even if treatment was discontinued before the assigned schedule as a result of side effects or noncompliance to therapy. Nonresponse (NR) to treatment was defined as lack of HCV-RNA clearance during treatment and at follow-up [13]. Serum HCV-RNA was measured by Amplicor HCV Monitor (Roche, Milan, Italy. Cut-off limits, quantitative test: 600 IU/mL; qualitative test: 50 IU/mL). HCV genotype was determined before treatment in all patients with the INNO-LiPA HCV II kit (Bayer Diagnostics, Emeryville, CA, USA).

**Clinical evaluation**

Clinical and biochemical evaluations were performed at baseline, week 2, week 4 and then monthly thereafter while on treatment, at 24 weeks follow-up and then every 6 months thereafter. Ultrasonographic examination of the liver- and serum α-fetoprotein determination was carried out at baseline and every 6 months while on treatment.

**SCCA-IgM IC assay**

Serum samples for SCCA-IgM IC were obtained at baseline, at the end of treatment and at 6-month and 12-month follow-up and stored at ~80 °C. Circulating SCCA-IgM IC 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 IC 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 [9–11]. The amount of SCCA-IgM IC were expressed in arbitrary units/
mL (AU/mL), as previously reported by other authors [9–11].

**Statistical analysis**

Continuous variables are shown as median and 95% confidence interval for the median, and discrete variables are shown as absolute value and percentage. Comparison of continuous variables has been carried out with nonparametric tests for paired (Wilcoxon test) or nonpaired (Mann–Whitney U-test) samples. Discrete variables were compared using the Fisher’s exact test. Correlation between variables was carried out with Spearman’s rank correlation test. A two-tailed P < 0.05 was considered statistically significant. Statistical analysis was carried out with MedCalc software version 5.00.019 (MedCalc Software, Mariakerke, Belgium).

**RESULTS**

Table 1 shows the main characteristics of the study population. Patients were prevalently males (64%) and infected with HCV genotype 1 (58%). Serum SCCA-IgM IC levels were similar in males (n = 21, 253.2 AU/mL, 98.5–457.4 AU/mL) and females (n = 12, 203.1 AU/mL, 67.5–687.7 AU/mL) and showed no statistically significant correlation with age and biochemical variables (aspartate aminotransferase, alanine aminotransferase, serum albumin, serum bilirubin, serum prothrombin activity and serum z-fetoprotein, Table 2).

In the whole population, we observed a significant decrease when compared to baseline (253.2 AU/mL, 98.5–457.4 AU/mL) in serum SCCA-IgM IC levels at the end of PEG-IFN and ribavirin therapy (143.5 AU/mL, 85.1–244.6 AU/mL; P = 0.011) and at both 6-month (137.2 AU/mL, 86.8–255.4 AU/mL; P = 0.043) and 1-year follow-up (61.2 AU/mL, 29.0–110.5 AU/mL; P = 0.001, Fig. 1). A SVR to antiviral treatment was obtained in 15 patients (45%). Baseline median serum SCCA-IgM IC levels tended to be higher in SVR patients when compared to NR, although there was no statistically significant difference between the two groups. Furthermore, there were no significant differences in SCCA-IgM IC serum levels between SVR and NR patients at all the time points of the study (end of treatment, 6-month and 1-year follow-up, Table 3).

Longitudinal evaluation of serum SCCA-IgM IC levels according to treatment outcome showed that SVR to antiviral therapy was associated with a statistically significant decrease in serum SCCA-IgM IC levels when compared to baseline. Among SVR patients, the decrease in serum SCCA-IgM IC levels when compared to baseline was already evident at the end of treatment (baseline: 451.2 AU/mL vs end of treatment: 186.8 AU/mL; P = 0.013) and was more marked at both 6-month (96.8 AU/mL; P < 0.001) and 1-year follow-up (52.4 AU/mL; P < 0.001). In NR patients, SCCA-IgM IC serum levels showed no significant modifica-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Years</td>
<td>57 (49–64)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male/female</td>
<td>21 (64)/12 (36)</td>
</tr>
<tr>
<td>ALT</td>
<td>IU/L</td>
<td>111 (70–129)</td>
</tr>
<tr>
<td>AST</td>
<td>IU/L</td>
<td>77 (58–99)</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/L</td>
<td>44 (42–47)</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>µM</td>
<td>13.6 (11.9–15.3)</td>
</tr>
<tr>
<td>Prothrombin activity</td>
<td>%</td>
<td>98 (92–101)</td>
</tr>
<tr>
<td>z-Fetoprotein</td>
<td>µg/L</td>
<td>8.4 (4.8–22.6)</td>
</tr>
<tr>
<td>HCV genotype</td>
<td>1/non-1</td>
<td>19 (58)/14 (42)</td>
</tr>
<tr>
<td>SCCA-IgM IC</td>
<td>AU/mL</td>
<td>253.2 (98.5–457.4)</td>
</tr>
</tbody>
</table>

Data are shown as median and 95% confidence interval for the median for continuous variables and as absolute count and percentage for categorical variables. ALT, alanine aminotransferase; AST, aspartate aminotransferase; SCCA-IgM IC, squamous cell carcinoma antigen IgM immune complexes; AU, arbitrary units; HCV, hepatitis C virus.

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 2 Correlations between serum squamous cell carcinoma antigen immunoglobulin M immune complexes levels and study variables at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>rs</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.075</td>
<td>0.702</td>
</tr>
<tr>
<td>ALT</td>
<td>−0.137</td>
<td>0.471</td>
</tr>
<tr>
<td>AST</td>
<td>−0.087</td>
<td>0.648</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.149</td>
<td>0.476</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td></td>
<td>0.392</td>
</tr>
<tr>
<td>Prothrombin activity</td>
<td>0.194</td>
<td>0.341</td>
</tr>
<tr>
<td>z-Fetoprotein</td>
<td>0.013</td>
<td>0.963</td>
</tr>
</tbody>
</table>

P = 0.011 and at both 6-month (137.2 AU/mL, 86.8–255.4 AU/mL; P = 0.043) and 1-year follow-up (61.2 AU/mL, 29.0–110.5 AU/mL; P = 0.001, Fig. 1). A SVR to antiviral treatment was obtained in 15 patients (45%). Baseline median serum SCCA-IgM IC levels tended to be higher in SVR patients when compared to NR, although there was no statistically significant difference between the two groups. Furthermore, there were no significant differences in SCCA-IgM IC serum levels between SVR and NR patients at all the time points of the study (end of treatment, 6-month and 1-year follow-up, Table 3).

Longitudinal evaluation of serum SCCA-IgM IC levels according to treatment outcome showed that SVR to antiviral therapy was associated with a statistically significant decrease in serum SCCA-IgM IC levels when compared to baseline. Among SVR patients, the decrease in serum SCCA-IgM IC levels when compared to baseline was already evident at the end of treatment (baseline: 451.2 AU/mL vs end of treatment: 186.8 AU/mL; P = 0.013) and was more marked at both 6-month (96.8 AU/mL; P < 0.001) and 1-year follow-up (52.4 AU/mL; P < 0.001). In NR patients, SCCA-IgM IC serum levels showed no significant modifica-

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**Fig. 1** Serum squamous cell carcinoma antigen immunoglobulin M immune complexes levels at baseline, at the end of pegylated interferon and ribavirin therapy and at 6-month and 1-year follow-up in the whole population.
tion when compared to baseline at both the end of treatment and during follow-up (Figs 2a,b). In SVR, decrease in serum SCCA-IgM IC levels was not correlated with modification in serum aminotransferase and \( \alpha \)-fetoprotein levels (data not shown).

Three patients developed HCC during follow-up (two NR and one SVR). When compared to baseline, serum SCCA-IgM IC levels showed an increase in both NR patients (baseline → end of treatment → end of follow-up: patient 1: 12.6 → 38.6 → 44.4 AU/mL; patient 2: 391.7 → 853.6 → 2019.1 AU/mL), while the SVR patient showed a blunted decrease in SCCA-IgM IC levels (451.2 → 374.8 → 230.4 AU/mL). Among the NR patients who did develop HCC, serum \( \alpha \)-fetoprotein was consistently within the normal range (i.e., ≤20 µg/L) in one and showed a nonsignificant decrease in the other one (baseline → end of treatment → end of follow-up: patient 1: 9.2 → 13.3 → 9.0 µg/L; patient 2: 64.8 → 53.3 → 20.2 µg/L), while in the SVR patient who developed HCC it was consistently normal (10.3 → 7.8 → 9.4 µg/L).

DISCUSSION

Aberrant SCCA expression seems to be an early event in human hepatocarcinogenesis, and dysplastic nodules of cirrhotic patients have an increased prevalence and intensity of immunostaining for SCCA than large regenerative nodules [7,8]. Furthermore, serum levels of SCCA-IgM IC are correlated with expression of SCCA in liver resected specimens of patients with HCC [9], and increasing SCCA-IgM IC serum levels have recently been demonstrated in the sera of untreated chronic hepatitis C patients with progressive disease as well as in cirrhotic patients who develop HCC [11,12]. It has been reported that SCCA isoforms protect neoplastic cells from apoptosis, and SCCA1 seems to promote tumour growth [14–16].

We assessed serum SCCA-IgM levels in a series of cirrhotic patients with compensated disease who underwent PEG-IFN and ribavirin antiviral therapy. Because therapy with PEG-IFN – besides its immunomodulatory and antiviral effects – should have antiproliferative properties [17–19], and successful antiviral treatment is associated with a decreased risk of HCC [20,21], we sought to investigate the behaviour of SCCA-IgM IC during PEG-IFN and ribavirin treatment and in the course of follow-up in a series of patients with HCV-related cirrhosis. Our aim was to evaluate whether successful antiviral therapy was associated with a decrease
in SCCA-IgM IC serum levels. Patients with liver cirrhosis were selected as it was anticipated that their SCCA-IgM IC serum levels should have been elevated [11,12], and liver cirrhosis rather than chronic hepatitis is a risk factor for HCC in patients with HCV infection.

In this study, we observed that serum SCCA-IgM IC levels were elevated in the majority of patients, with no statistically significant difference between baseline values of SVRs and nonresponders to treatment. We observed that serum SCCA-IgM IC levels decreased during antiviral therapy and continued to decrease during both 6-month and 1-year follow-up. However, the main result of our study was that the drop in serum SCCA-IgM IC levels was statistically significant in patients who obtained a SVR to antiviral therapy alone. Indeed, at the end of treatment, serum SCCA-IgM IC levels were already significantly reduced when compared to baseline in SVR patients but not in nonresponders. Furthermore, at both 6- and 12-months follow-up, SCCA-IgM IC serum levels significantly declined further when compared to pretreatment values in SVR patients alone, while in nonresponders there was no significant modification respect to baseline. This reduction in SCCA-IgM IC levels was not associated with modification of amino-transferase and α-fetoprotein, and therefore cannot be interpreted as a by-product of decreased necroinflammatory activity. Although SCCA-IgM IC serum levels tended to be lower both during treatment and follow-up in SVR when compared to nonresponders, the results of our study show that its levels cannot be used to predict response to PEG-IFN and ribavirin therapy, at least in patients with cirrhosis. HCC was diagnosed in three cirrhotic patients during follow-up, and SCCA-IgM IC levels showed a constant increase during both treatment and follow-up in the two NR patients who developed HCC, while in the SVR patient who did develop HCC SCCA-IgM IC levels were already high at baseline and showed a blunted decline during successful antiviral treatment. Noteworthy, in these three patients, serum α-fetoprotein levels did not follow the same behaviour but rather showed a decreasing pattern or were consistently within the normal range. These findings further support the possible use of serum SCCA-IgM IC levels for the identification of cirrhotic patients at risk of developing HCC [9–11]. A limitation to this study is the fact that SCCA-IgM IC serum levels at the diagnosis of HCC were not available.

In summary, we have observed that antiviral therapy with PEG-IFN and ribavirin determines a significant decrease in SCCA-IgM IC serum levels of cirrhotic patients with HCV, and most importantly we have shown that this decrease is significant in patients who obtain a SVR to antiviral treatment alone. Noteworthy, we have found that SCCA-IgM IC serum levels do not predict response to antiviral therapy, at least in cirrhotic patients. Due to the relatively small number of events observed, it was not possible to draw firm conclusions on the association between development of HCC and behaviour of SCCA-IgM IC serum levels during treatment. Larger studies are needed so as to better evaluate the relationship between longitudinal modification of serum SCCA-IgM IC levels and development of HCC in cirrhotic patients with HCV infection.

CONFLICT OF INTEREST

No conflicts of interest exist.

FINANCIAL SUPPORT

None.

REFERENCES

14. Schneider SS, Schick KE, Fish E et al. A serine proteinase inhibitor locus at 18q21.3 contains a tandem duplication of


SCCA-IC serum levels are predictive of clinical response in HCV chronic hepatitis to antiviral therapy: a multicentric prospective study


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SUMMARY. The combination of pegylated interferon (Peg-IFN) and ribavirin is currently the gold standard therapy in patients with HCV chronic infection. The duration of therapy, as well as the therapeutic dosage, depend on the genotype. Identification of the genotype and rapid virological response (RVR) are widely accepted as the most important predictors of clinical outcome during antiviral therapy but to optimize cost-benefits and to reduce possible side effects, further prognostic factors are needed. Squamous cell carcinoma antigens immunocomplex (SCCA-IC) has been reported to be increased in the serum of patients with liver cancer. In this multicentric prospective study, we investigated the serum levels of SCCA-IC in 103 patients with HCV chronic infection. Serum HCV-RNA was detected before the beginning of treatment, after 4, 12, 24 or 48 weeks, and at week 24 during follow-up. RVR, early virological response and sustained virological response (SVR) were assessed following the international guidelines. SCCA-IC levels were higher in responders (238 AU, interquartile difference 130–556 AU) and decreased significantly to 125 AU (70–290 AU). The mean baseline value in nonresponders was 149 AU (86.5–306.5 AU), but after 4 weeks of treatment the serum levels decreased to 115 AU (80–280 AU): the profile of reduction was different between patients with or without a positive SVR. Logistic regression with SVR as dependent variable identified as significant independent variables: the reduction in SCCA-IC after 1 month (OR = 4.82; 95% CI 1.39–16.67; \( P = 0.131 \)) and a genotype other than 1 (OR = 0.094; 95% CI 0.21–0.42; \( P = 0.002 \)); sex and age were also significant factors influencing SVR. SCCA-IC seems to be a reliable independent prognostic marker of therapeutic effectiveness in anti-HCV positive patients undergoing antiviral therapy.

Keywords: antiviral therapy, chronic C hepatitis, prognostic factor, SCCA-IC.

INTRODUCTION
The gold standard treatment for chronic hepatitis C, to prevent or delay progression to liver cirrhosis and hepatocellular carcinoma (HCC), is currently the combination of pegylated interferon-\( \alpha \) (Peg-IFN-\( \alpha \)) with ribavirin [1,2]. The HCV genotype and a rapid virological response (RVR) have been widely recognized as the two most important prognostic factors for the response to antiviral therapy [3,4]. The international guidelines for the treatment of HCV patients have suggested that these prognostic factors may be usefully applied to tailor the therapeutic regimen and optimize the cost-benefit ratio [5]. Nevertheless, additional prognostic factors are urgently needed in clinical practice.

Recently, in a preliminary study, it was reported that serum levels of squamous cell carcinoma antigen immuno-complex (SCCA-IC) were decreased in patients with HCV-related cirrhosis who responded to antiviral therapy [6]. However, the limited number of patients so far investigated, and the lack of studies in patients with chronic HCV-related hepatitis do not
allow definite conclusions to be drawn as regards the validity of this test in patients undergoing antiviral therapy. In particular, there are no data available correlating the use of SCCA-IC with RVR, widely recognized as the gold standard marker of clinical response to antiviral therapy.

SCCA-IC is an immuno-complex whereby IgM immunoglobulins link the serin inhibitor protease SCCA [7,8]. SCCA was first reported to be expressed in the liver of HCC patients and for this reason, was proposed as a potential biomarker for the detection of HCC [9]. SCCA-IC serum levels have been demonstrated to show a better diagnostic capacity than SCCA and have therefore been proposed for use in combination with alpha-fetoprotein as an additional biomarker for HCC detection [10].

Aim of this study was to test the utility of SCCA-IC as a marker of response in patients with HCV chronic infection undergoing antiviral therapy. A multicentric prospective study was designed, in which serum samples were collected at baseline before starting the therapy, at RVR, early virological response (EVR) and 6 months after the end of therapy, and a cohort of patients with HCV chronic infection undergoing antiviral therapy were enrolled.

MATERIALS AND METHODS

Patients

During the period 2007–2010, 103 patients referred to the following centres: Unit of Internal Medicine ‘C. Frugoni’ and of Infectious Diseases, University of Bari; Unit of Gastroenterology, University of Pisa; and Unit of Gastroenterology, University of Modena And Reggio Emilia were enrolled in this study.

Patients with liver cirrhosis, haematological abnormalities (haemoglobin level <12 g/dl in women and <13 g/dl in men; neutrophil count <1.5 × 103 cells/mL; platelet count <90 × 103 cells/mL), pre-existing severe psychiatric conditions (especially depression), severe cardiac disease, haemoglobinopathies, haemophilia, autoimmune diseases, human immunodeficiency virus (HIV) co-infection, previous liver transplantation and other causes of liver disease (hepatitis B virus infection, alcohol dependence) were excluded. Women unable or unwilling to practise contraception were also excluded.

Study design

In total, 103 patients were recruited, 61 men and 42 women. Mean age was 53.4 year (±12.7 year) in men and 58 year (±9.5 year) in women, with a significant difference between the two groups (t-test = 2.05; P = 0.04).

Blood samples were collected at baseline before the beginning of therapy, after 4, 12, 24 and 48 weeks during treatment and at 24 weeks of follow-up. The study was not supported by any pharmaceutical company. Informed consent was obtained from all patients.

Patients were treated with weekly subcutaneous pegylated interferon (Peg-IFN)-x2α (180 μg/week) or Peg-IFN-x2b (1.5 μg/kg/week) plus oral ribavirin at a dosage of 800–1200 mg/day depending on pretreatment body weight (800 mg/day for weight <60 kg, 1000 mg/day for weight ≥ kg and <75 kg; and 1200 mg/day for weight ≥75 kg). This study was not randomized, and the physicians in charge of the patients chose the Peg-IFN at their own discretion. Patients with genotype 2 and 3 were treated for 24 weeks, and those with genotype 1 and 4 for 48 weeks. Virological response was evaluated at weeks 4, 12, 24 and 48 during treatment and at 24 weeks of follow-up by qualitative PCR (Amplicor: Roche Diagnostic System, France), with a sensitivity of 50 UI/mL. HCV genotype was determined before treatment in all patients with the INNO-LIPA HCV II kit (Bayer Diagnostics, Emeryville, CA, USA). Sustained virological response (SVR) was defined as the absence of detectable HCV-RNA in serum by qualitative PCR at the end of therapy and at week 24 of follow-up. Early virological response was defined as undetectable serum HCV-RNA or a reduction in HCV-RNA levels by at least 2 logs from baseline values at week 12 of treatment. Rapid virological response, was defined as undetectable serum HCV-RNA at week 4 of treatment. Patients with measurable HCV-RNA by qualitative PCR at the end of the follow-up period were considered nonresponders.

SCCA-IC measurement

SCCA-IC serum concentrations were determined in sera previously collected and stored at −20 °C until use. Serum concentrations of SCCA-IC were measured as in our previous works, using ELISA commercial kits purchased from Xepha-gen (Padua, Italy).

Statistical analysis

Categorical variables are summarized as count and percentage. Chi-squared test was used to evaluate differences between independent groups. Comparisons were made by parametric tests if normally (Gaussian) distributed, non-parametric tests otherwise. Quantitative variables are summarized as mean and standard deviation. Differences of SCCA-IC, lacking a Gaussian distribution, are summarized as median and interquartile range and comparison was performed by Kruskal–Wallis for comparing multiple independent groups or Friedman test for nonparametric repeated measure analysis of variance. Multiple comparisons between paired or independent groups were performed using Wilcoxon test and P-values were adjusted taking into account the number of comparisons according Bonferroni.

The percentage variation, determined as the ratio between the difference of SCCA-IC at time t less SCCA-IC at time t-1, divided by SCCA-IC at time t-1, was calculated to evaluate the reduction in SCCA-IC concentrations between two
consecutive time points. The percentage variation was then classified in two classes according to the median value. A logistic regression model was built to evaluate the effect on SVR of the class of SCCA-IC reduction (more than 12% after the first month and after the third month, more than 7% at the last follow-up visit), genotype (1 vs other), adjusted for age (classified as <58 vs ≥58 year) and sex (M vs F).

To evaluate the concordance of percentage of SVR patient predicted by SCCAIC classes and prediction of SVR by RVR and EVR was performed the McNemar test to compare paired percentage. A P-value < 0.05 was considered statistically significant. All analyses were performed with software SAS 9.2 for PC (SAS Institute, Cary, NC, USA).

RESULTS

There were 63 responders (Table 1); mean age was 53.5 year (±11.9 year) for responders and 58.3 year (±10.7 year) for nonresponders; this difference resulted statistically significant (t-test = 2.1; P = 0.038) (Fig. 1). There were no statistically significant differences in the percentage of responders between men and women: 62.3% (38/61) vs 59.5% (25/42) (χ² = 0.08; P = 0.7767).

Genotype 1 was observed in 41.3% (26/63) of responders, vs 90% (36/40) of non responders: the percentage of genotype 1 was significantly different between responders and nonresponders (χ² = 24.24; P < 0.0001). The median value of SCCA-IC by genotype and response is shown in Table 2 and profile by time of genotype 1 patients is shown in Fig. 2. Responders with all genotypes had higher levels of SCCA-IC than nonresponders. Furthermore, in responders, the level of SCCA-IC showed a tendency to decrease, whereas in nonresponders the SCCA-IC level appeared to remain the same at each time point. The median level of SCCA-IC at baseline resulted lower respect to overall median value, but profile for responders patients resulted similar (like a parallel line) until the last determination, where values for genotype 1 and overall sample resulted the same. Nonresponders patients with genotype 1 had identical value respect to overall sample, because the most part of nonresponders patients was genotype 1.

The initial value of SCCA-IC (Fig. 2) in responders was 238 AU (130–556), this decreased to 188 AU (116–400) after 1 month, to 134 AU (90–385) after 3 months and reached the value 125 AU (70–290) at the end of follow-up. In nonresponders, the initial value was 149 AU (86.5–306.5) and remained substantially unchanged after 1 month, 142.5 AU (82.5–286.5), showing a slight decrease after 3 months 115 AU (80–280) and remaining at this level until the end of follow-up: 119 AU (82–260). The difference in concentration between responders and nonresponders at the first month resulted statistically significant (P = 0.0031), while at the other time points it was not significant (P > 0.05). The decrease between consecutive time points resulted statistically significant (P < 0.001) in the responders group: the baseline-first month difference was 43 AU (15–101); first – third month, 38 AU (10–68); third month – end of the study, 14 AU (3–45). In the nonresponders group, the decrease at consecutive time points resulted significant between baseline-first month (5 AU: 0–14.5; P < 0.001), but not at subsequent time points, when the reduction was 0.5 AU (0–14.5) and 0 AU (9 to –8). Median reduction resulted significantly different between responders and nonresponders (P < 0.0001 at each comparison) at every time point. These results allowed us to conclude that the profile of reduction was different between patients that would achieve a positive SVR as compared to nonresponders.

To evaluate the effect of SCCA-IC reduction in SVR, a logistic regression model was built with the presence of SVR as dependent variable, while the independent variables were the class of SCCA-IC reduction after 1, 3 months and at the end of follow-up, genotype 1 vs others, sex (M vs F) and age (<58 vs ≥58). The model resulted statistically significant (Table 3; P < 0.0001). Sex was not shown to be a statistically significant variable, while age younger than 58 increased the probability of achieving SVR (OR = 9.66; CI95% 2.21–42.15; P = 0.0025). An OR = 0.094 (CI95% 0.21–0.42; P = 0.002) was shown for

**Table 1** Main characteristics of study sample for sustained virological response (SVR) and nonresponders patients

<table>
<thead>
<tr>
<th>SVR</th>
<th>NO SVR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 63</td>
<td>N = 40</td>
<td></td>
</tr>
<tr>
<td>Age (SD)</td>
<td>53.5 year (11.9)</td>
<td>58.3 year (10.7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38 (60.3%)</td>
<td>23 (57.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>25 (39.7%)</td>
<td>17 (42.5%)</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>26 (41.3%)</td>
<td>36 (90.0%)</td>
</tr>
<tr>
<td>2</td>
<td>24 (38.1%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>3</td>
<td>10 (15.9%)</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>3 (4.7%)</td>
<td>1 (2.5%)</td>
</tr>
</tbody>
</table>

Table 2 Median (interquartile range) of SCCA-IC according to genotype and response at the end of the study period

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SVR YES</th>
<th>Other Genotype</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>205.5 (126–370)</td>
<td>307 (140–570)</td>
<td></td>
</tr>
<tr>
<td>First month</td>
<td>179.5 (118–303)</td>
<td>200 (110–448)</td>
<td></td>
</tr>
<tr>
<td>Third month</td>
<td>132 (90–204)</td>
<td>153 (81–406)</td>
<td></td>
</tr>
<tr>
<td>End of follow-up</td>
<td>124.5 (72–200)</td>
<td>156 (67–340)</td>
<td></td>
</tr>
<tr>
<td>SVR NO</td>
<td>149 (85.5–300)</td>
<td>240 (102.5–409)</td>
<td></td>
</tr>
<tr>
<td>First month</td>
<td>142.5 (82.5–271.5)</td>
<td>232.5 (91.5–409)</td>
<td></td>
</tr>
<tr>
<td>Third month</td>
<td>115 (80–255)</td>
<td>231 (90–399.5)</td>
<td></td>
</tr>
<tr>
<td>End of follow-up</td>
<td>114 (82–250)</td>
<td>224 (99–344)</td>
<td></td>
</tr>
</tbody>
</table>
genotype 1, suggesting that with this genotype patients are more likely to be nonresponders. Predictive factors of SVR were the reduction after first month, after the third month and at follow-up. No effect of interaction between time points and genotype resulted statistically significant, and this parameter was therefore removed from the model. Looking at the odds ratio, it seems that a reduction of more than 12% between the baseline value and the first time point increases by 4.82-fold the probability of SVR (95% CI 1.39–16.67; \( P = 0.131 \)). A reduction between the first month and third month of more than 12% increases the probability of SVR 15-fold (3.44–66.74; \( P = 0.0003 \)), and a further reduction of more than 7% until the end of the study period increases the probability of SVR 9-fold (95% CI 2.22–42.15; \( P = 0.043 \)).

Taking into account only genotype 1 patients reduction in SCCA-IC between first month and baseline resulted with a borderline significativity (OR = 3.65, CI95% 0.97–13.76, \( P = 0.055 \)), while reduction between third month and first month remain statistically significant (OR = 9.09, CI95% 2.085–39.69, \( P = 0.003 \)).

Figure 3 shows the probability of response for each value of percentage reduction in SCCA-IC between baseline and the first month for the whole sample and for genotype 1
The probability of SVR obtained with the univariate logistic model (independent variable SVR Y/N and dependent percentage reduction in SCCA-IC as continuous variable) is increased by more than 50% for small levels of reduction starting from 7% to 8% as compared to the baseline value. For genotype 1, the probability of being a responder patient at the end of follow-up resulted more than 50% for higher value of reduction (at least 15%). Positive predictive value of SVR of SCCA-IC reduction was 69.8% (44 with reduction / 63 SVR patients), while RVR 79.4% (50 RVR/63 SVR) and EVR 96.8% (61 EVR/63 SVR). There was a statistically significant difference between predictive value of class of SCCA-IC and EVR (McNemar test 17.0, \( P < 0.0001 \)), but not respect to RVR (McNemar test 1.8, \( P = 0.179 \)). This results are confirmed in the subgroup of genotype 1 patients: predictive value of class of SCCA-IC reduction was 65.4% (17 with reduction / 26 SVR), while for RVR was 69.3% (18/26), that resulted not significantly different (\( P = 0.76 \)) and for EVR 96.1% (25/26) significantly different respect to class of SCCA-IC (\( P = 0.0047 \) (Table 4).

**DISCUSSION**

In this study, we have demonstrated that a decreased serum concentration of SCCA-IC after 4 weeks of antiviral therapy is an independent prognostic factor of therapeutic response. We base this conclusion on the following data: (i) the reduction in SCCA-IC was significantly different between patients achieving SVR or not; (ii) the profile of SCCA-IC is different between responders and nonresponders even with different genotypes; (iii) If the reduction is evaluated as percentage, the effect of the decrease between baseline and the first month value becomes even more noticeable.

The model evaluating the effect of predictors such as genotype and SCCA-IC reduction at each time point was shown to be well able to predict the response. The reduction at the first time point seems to be a good predictor with low variability, and the probability allows us to conclude that if a reduction is observed at the first follow-up visit the patient will achieve a positive clinical response.

The genotype shows a strong influence on obtaining a SVR, but does not affect the conclusions about SCCA-IC, that can therefore be considered a useful marker for monitoring patients when measured at each visit: before starting therapy and after the first and second follow-up visit. Thus, if

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**Table 3** Odds ratio, 95% confidence intervals and \( P \)-value obtained with the multivariate logistic regression model

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>CI 95%</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1 vs other</td>
<td>0.09</td>
<td>0.02–0.42</td>
<td>0.002</td>
</tr>
<tr>
<td>Reduction at first month &lt;12%</td>
<td>4.82</td>
<td>1.39–16.67</td>
<td>0.0131</td>
</tr>
<tr>
<td>Reduction at third month &lt;12%</td>
<td>15.16</td>
<td>3.44–66.74</td>
<td>0.0003</td>
</tr>
<tr>
<td>Reduction at the end of study period &lt;7%</td>
<td>4.08</td>
<td>1.04–15.97</td>
<td>0.0432</td>
</tr>
<tr>
<td>Age &lt; 58 year vs older</td>
<td>9.66</td>
<td>2.21–42.15</td>
<td>0.0025</td>
</tr>
</tbody>
</table>

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![Fig. 3](image-url) Probability of being a responder as a function of the SCCA-IC reduction. The x-axis shows the percentage variation obtained as SCCA-IC value at baseline less the value at the first month divided by the baseline value.
SCCA-IC serum levels reduce during therapy, the patient will achieve SVR. Should be noticed that genotype 1 subgroup has less probability to be a responder, but even in this group if a reduction in SCCA-IC level is observed a SVR could be obtained.

In a previous work by Giannini et al., [6] the authors reported that a higher SCCA-IC, but not SCCA, serum concentrations reduction in patients with liver cirrhosis was observed in responders to antiviral therapy. Probably because of the small sample size, they could not make an appropriate test of the significance of the reduction with respect to the future response. Moreover, in that work the genotype was not evaluated together with SCCA-IC in a multivariate model. In this article, instead, the authors focused on the definition of a parameter to predict SVR, so both genotype and repeated reductions of the marker have been evaluated in a multivariate model. This confirmed the importance of the reduction of the marker, more than the value of the marker itself, as an independent variable, specifically with respect to the genotype. Our data demonstrate that patients with SVR have a decreasing profile according to genotype. This suggests that SVR could be predicted by genotype, as already known, but that important information on a positive response can be gained if there is a reduction more than 12% at the first month as compared to the baseline value.

Therefore, an antigen immuno-complex such as SCCA-IC can predict the clinical outcome. It remains to be seen what molecular mechanism is related to the decrease in SCCA-IC, to explain its significance as a prognostic factor.

In other studies, SCCA-IC was reported as a new biomarker for early detection of HCC. However, while the sensitivity performance was very encouraging, the specificity was disappointing. In particular, patients without HCC but with underlying chronic liver disease had unexplainably high serum levels of SCCA-IC [8,11]. We hypothesize that SCCA-IC could be related to the liver fibrosis/inflammation status [12]. In fact, we detected increased levels of SCCA-IC in patients with systemic sclerosis (SS). In particular, among these patients, we found that those with fibrotic involvement of the lung displayed the highest serum levels of SCCA-IC, as compared with the other subsets of SS patients with a different clinical outcome [13]. Therefore, it is likely that SCCA-IC serum levels reflect the fibrosis-immunity-related status, which could explain why this parameter is a prognostic factor independently of the genotype or the RVR.

In conclusion, we report a new prognostic factor that can predict the therapeutic response in patients with chronic hepatitis C undergoing antiviral therapy. SCCA-IC can be easily measured using a commercially available ELISA kit. Finally, we suggest that being an independent prognostic factor, SCCA-IC may be useful to refine patients selection, discriminating those patients that will benefit from antiviral therapy and thus optimizing the cost-benefit ratio.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

Table 4 Comparison of positive predictive value (SVR) and negative predictive values (Not SVR) between reduction in SCCA-IC respect to rapid virological response (RVR) and early virological response (EVR) patients

<table>
<thead>
<tr>
<th></th>
<th>Reduction of SCCA at first month &lt;12%</th>
<th>RVR</th>
<th>P-value</th>
<th>EVR</th>
<th>P-value</th>
<th>Overall sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVR (n = 63)</td>
<td>44</td>
<td>50</td>
<td>79.4%</td>
<td>61</td>
<td>96.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Not SVR (n = 40)</td>
<td>9</td>
<td>6</td>
<td>15.0%</td>
<td>6</td>
<td>15.0%</td>
<td>0.1797</td>
</tr>
<tr>
<td>Genotype 1 subgroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVR (n = 26)</td>
<td>17</td>
<td>18</td>
<td>69.2%</td>
<td>25</td>
<td>96.2%</td>
<td>0.0047</td>
</tr>
<tr>
<td>Not SVR (n = 36)</td>
<td>8</td>
<td>4</td>
<td>11.1%</td>
<td>4</td>
<td>11.1%</td>
<td>0.045</td>
</tr>
</tbody>
</table>


Trerotoli P, Fransvea E, Angelotti U et al. Tissue expression of Squamous Cellular Carcinoma Antigen (SCCA) is inversely correlated to tumor size in HCC. *Mol Cancer* 2009; 8: 29.

median overall stiffness was 5.2 kPa (4.35–6.15) [IcSSc=5.8 (4.4–6.5); dcSSc=4.7 (4.3–5.8); p=0.310]. A good correlation between stiffness and duration of disease was found (r=0.49, p=0.01), without differences between the forms of disease. In particular, the stiffness raises of 0.2 kPa every year of disease in both forms. No correlation was found between TRAIL levels and stiffness or HGF values (pens). A good correlation was found between HGF values with duration of disease and stiffness in both forms of SSc (p<0.01), especially in dcSSc patients.

**Conclusions:** Increasing of stiffness value in pair with the duration of disease suggests a possible evolution of hepatic fibrosis in SSc patients. HGF values correlate with duration of disease and stiffness in both forms of SSc, and in particular in dcSSc patients.

### P.18.21

**ENHANCED LIVER FIBROSIS (ELF) TEST: A PROSPECTIVE STUDY OF NON-INVASIVE DIAGNOSTIC METHOD OF LIVER FIBROSIS**

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**Background and aim:** Fibrosis prediction is an essential part of the assessment and management of pts with chronic liver disease. The ELF Test consists of an algorithm of three fibrosis markers: hyaluronic acid, amino-terminal propeptide of type-III-collagen and inhibitor of matrix-metalloproteinase-1. The aim of our study was to evaluate a serological marker method-ELF Test in the assessment of liver fibrosis, comparing with liver biopsy.

**Material and methods:** We included 31 pts (14 M – 45%, 17 F – 55%; mean ± SD ages 52±11 yrs) with chronic C hepatitis (n=12/31 – 39%), chronic B hepatitis (n=10/31 – 32%) and non-alcoholic steatohepatitis (n=9/31-29%). A percutaneous liver biopsy specimen was obtained from all pts. Liver fibrosis stages were evaluated according to the Metavir scoring-system (F0–F4). Serum samples were analysed using the proprietary assays developed for ELF Test by Siemens Healthcare Diagnostics Inc. Results were entered into the established algorithm and expressed as discriminant scores for a comparison to Metavir histological staging. Liver fibrosis was classified in mild (ELF score ≤7.7), moderate (ELF score 7.8–9.8) and severe fibrosis (ELF score ≥9.9).

**Results:** We found the following distribution by Metavir: F1=7, F2=11, F3=4, F4=9. The ELF Test diagnosed mild fibrosis in 7 pts, moderate fibrosis in 17 pts and severe fibrosis in 7 pts. The accuracy according to AUROC for the diagnosis of significant fibrosis (F ≥2; ELF score ≥7.7) was 0.869 (95% CI 0.81–0.99; cut-off=8.49; p=0.0001), Se=0.87 and Sp=0.56, PPV=0.87 and NPV=0.87. The accuracy for the diagnosis of cirrhosis (F=4; ELF score ≥9.8) was 0.995 (95% CI 0.98–1.00; cut-off=9.03; p=0.0001), Se=0.96 and Sp=0.95, PPV=0.85 and NPV=0.97.

**Conclusions:** The ELF Test showed better efficacy in highlighting the presence of cirrhosis than in discriminating intermediate stages of liver fibrosis. Liver biopsy is still the gold standard in staging fibrosis. The ELF Test could be employed for a preliminary selection of patients eligible for biopsy and could be used during their follow-up.

### P.18.22

**SERUM FGF19 LEVELS ARE INDEPENDENTLY RELATED TO BMI IN HEALTHY BLOOD DONORS: AN INTERIM ANALYSIS OF AN ONGOING STUDY**


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**Background and aim:** Fibroblast growth factor 19 (FGF19) is an enteroenterone playing key roles in enterohematopoietic signaling, bile acid (BA) synthesis, gall-bladder motility and metabolic homeostasis. Aim of our study was to evaluate the correlation between serum fasting FGF19 and glucose, lipid metabolism and BMI in healthy subjects.

**Material and methods:** 285 blood donors were prospectively enrolled from our Transfusion Center, from January 2011 to March 2012. Exclusion criteria were: increased ALT, γGT or ALP, history of liver, GI or gallstone disease, previous abdominal surgery and treatment with metabolic or GI medications. All patients underwent lab-tests: fasting glucose and insulin, total cholesterol, HDL, LDL, triglycerides, ALT, γGT, ALP, fasting FGF19 serum level (ELISA assay), serum BA levels (HPLC-ESI-MS/MS in 150 subjects). Student t-test was used for the comparison of groups; multivariate analysis was used to identify variables independently related.

**Results:** 279 subjects (153M/126F; age 41.6±11.6 yrs) met the inclusion criteria. Mean BMI was 25.1±3.7 (male 25.6±3.1; female 23.9±3.9; P<0.001); mean fasting FGF19 was 124.8±84.2 pg/ml. Fasting serum FGF19 levels were significantly higher in subjects with BMI<25 (144.4±106.6 vs overweight subjects (102.9±76; P<0.001). FGF19 was inversely correlated with BMI (r = –0.245; P<0.001). 52 subjects presented insulin-resistance (HOMA-IR >2.5). Insulin resistance was not correlated with FGF19 level (131.7±102.9 no IR vs 112.6±89.1 IR subjects). Serum cholesterol and triglycerides did not correlate with FGF19 serum levels. On multivariate analysis, FGF19 was independently related to BMI (r=–.78; P<0.001).

**Conclusions:** Serum FGF19 was significantly lower in otherwise healthy overweight subjects and a linear inverse correlation between BMI and serum FGF19 was observed. The mechanisms responsible for these findings are probably related with a different bile acid homeostasis, and deserve further investigation.

### P.18.23

**SCCA-IgM: A BIOMARKER TO MONITOR THE OUTCOME OF THERAPY WITH SORAFENIB IN ADVANCED HCC**

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**Background and aim:** Circulating Squamous Cell Carcinoma Antigene (SCCA)-IgM complex has been found higher in patients with hepatocellular carcinoma (HCC) than in patients with chronic liver disease and cirrhosis. This study aimed to evaluate the ability of SCCA-IgM serum levels to monitor the efficacy of therapy with Sorafenib in patients with HCC.

**Material and methods:** Forty-two patients with a new diagnosis of HCC (stage B and C, according to BCLC score) were enrolled in a prospective study from April 2011 to July 2012 in two referral centres. The diagnosis of HCC was made according to guidelines AASLD 2010. All patients were treated with Sorafenib (800 mg/day). Response to therapy was evaluated with imaging techniques according to the mRECIST criteria. Serum SCCA-IgM levels were determined by the Hepa-IC kit (Xeptagen SpA, Marghera, Venezia, Italy) at basal time and after 3 months from the beginning of treatment. The quantification of the complex SCCA-IgM is expressed in Arbitrary Units (AU/ml).

**Results:** At basal time SCCA-IgM were detectable (>100 AU/ml) in serum of...
25/42 (59.5%) patients. Successively, the patients were divided in two groups: Group A (16 patients) who responded to therapy and Group B (9 patients) who did not respond to therapy. In Group A the mean value decreased from 276.75 AU/ml at T0 to 190.3 AU/ml at T3 (p < 0.001). In Group B mean value remained stable during the follow-up (252.4 AU/ml at T0 and 285.2 AU/ml at T3, p not significant).

**Conclusions:** These results suggest that the assessment of SCCA-IgM may be helpful in monitoring the outcome of HCC treatment with Sorafenib.

**P.18.24**

**CIRCULATING SCCA-IgM COMPLEX IS ABLE TO MONITOR THE SUCCESS OF LOCO-REGIONAL THERAPY IN HCC PATIENTS**

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**Background and aim:** About 3–4% of cirrhotic patients develops primary liver cancer every year. The serpin squamous cell carcinoma antigen (SCCA) is elevated in liver cancer specimens and circulating SCCA-IgM complexes have been described in patients with hepatocellular carcinoma (HCC). This study aimed to evaluate the ability of SCCA-IgM serum levels to monitor the efficacy of loco-regional treatments.

**Material and methods:** In two referral centres of South Italy, sixty-four patients with a new diagnosis of HCC at stage A and B, according to BCLC score, were enrolled in a prospective study from April 2011 to July 2012. The diagnosis of HCC was made according to the AASLD 2010 guidelines. All patients underwent loco-regional treatments such as Laser Thermal Ablation (LTA), Radio-Frequency (RF), Percutaneous Ethanol Injection (PEI), Transarterial Chemoembolization (TACE). Response to therapy was evaluated with imaging techniques according to the mRECIST criteria.

Serum SCCA-IgM levels were determined by the Hepa-IC kit (Xeptagen SpA, Marghera Venezia, Italy) at basal time and after 1 month from the beginning of treatment. The quantization of the complex SCCA-IgM is expressed in Arbitrary Units (AU)/ml.

**Results:** At basal time SCCA-IgM were detectable (> 100 AU/ml) in serum of 34/64 (53%) patients. These patients were divided in two groups: Group A (29 patients) who responded to therapy and Group B (5 patients) who didn’t respond to therapy. In Group A the mean value decreased from 263.83 AU/ml at T0 to 229.7 AU/ml at T1 (p < 0.05). In Group B mean value increased from 226.2 AU/ml at T0 to 273 AU/ml at T1 (p = not significant).

**Conclusions:** These results suggest that the assessment of SCCA-IgM may be helpful in monitoring the outcome of loco-regional treatments in patients with HCC.
HEPATOLOGY

Diagnostic and prognostic role of SCCA-IgM serum levels in hepatocellular carcinoma (HCC)

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Key words
diagnosis, hepatocellular carcinoma, prognosis, SCCA-IgM, serological biomarkers.

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Abstract

Background and Aim: The serpin squamous cell carcinoma antigen complexed with IgM (SCCA-IgM) has been reported as a promising serological marker for hepatocellular carcinoma (HCC). We aimed to further evaluate SCCA-IgM diagnostic accuracy and to determine its prognostic role.

Methods: SCCA-IgM levels were determined in 327 sera obtained from 81 HCC patients, 206 cirrhotics and 40 healthy blood donors (controls). Sensitivity, specificity, correlation with clinical and tumor parameters and with survival were evaluated.

Results: HCC patients had SCCA-IgM levels significantly higher than controls and cirrhotics (P < 0.0001). Sensitivity, specificity, positive and negative predictive values for HCC were 89%, 50%, 41% and 92%, respectively. In comparison, sensitivity and specificity for alphafetoprotein were 48% and 85%. SCCA-IgM levels were not significantly correlated with clinical or biological variables. With a cut-off of 130 AU/mL (receiver operating characteristic curves), SCCA-IgM proved efficient in the prediction of prognosis, identifying the patients with long overall survival (efficiency validated in the homogeneous subgroup of patients with intermediate-stage HCC undergoing transarterial chemoembolization) and predicting progression-free survival. A Cox multivariate analysis confirmed SCCA-IgM predictive value, identifying tumor size and SCCA-IgM levels as independent predictors of survival. A reduction in SCCA-IgM levels correlated with response to treatment.

Conclusions: SCCA-IgM is a sensitive marker of HCC in patients with cirrhosis even though lacking in specificity. The determination of the levels of the marker in HCC patients is highly efficient in predicting the patients’ prognosis, identifying those with long overall and progression-free survival and the responders and should be introduced in the clinical practice.

Introduction

Conventional biomarkers, including alphafetoprotein (AFP) and des-gamma-carboxyprothrombin (DCP), are of limited help in the diagnosis of hepatocellular carcinoma (HCC) and only partially useful in the definition of the patients’ prognosis.1 AFP, however, is still considered by some authors2,3 as a clinically meaningful instrument for HCC surveillance and diagnosis. This is based on the results of two randomized surveillance studies carried out in China, in which AFP determination led to a higher percentage of patients diagnosed at an early stage and, associated with ultrasound (US), reduced mortality.5 From the prognostic point of view, AFP has recently shown a relevant prognostic impact in specific settings, such as in patients on the waiting list for liver transplantation6 or in predicting response in patients undergoing loco-regional treatment.7 However, in a study based on the results obtained in a series of 1158 patients with HCC, we confirmed not only the low sensitivity of AFP in the diagnosis of HCC but also its limited prognostic value, since a multivariate analysis did not identify AFP as an independent predictor of survival.1

The discovery of a novel class of tumor markers constituted by circulating IgM antibodies forming immune complexes with specific cancer biomarkers has provided interesting opportunities for HCC patient management.8–19 IgM immune complexes can be detected in the serum of HCC patients as reported for squamous cell carcinoma antigen (SCCA),9–13,15 for AFP15–18 and for DCP,19 and the assessment of AFP-IgM, DCP-IgM and SCCA-IgM immune complexes has allowed a higher diagnostic performance than the determination of the free, not complexed, biomarker. According to the revised hypothesis of cancer immune surveillance,20 these immune-complexed biomarkers might be involved in
cancer immune-editing, reflecting the host immune-protective mechanisms, aimed at suppressing tumor growth.

SCCA-IgM was recently shown to be a predictive biomarker of HCC risk in patients with cirrhosis, because it was found that an increase in time of serum SCCA-IgM levels is associated with a higher incidence of HCC. SCCA-IgM also showed a prognostic role in patients with chronic HCV-related hepatitis in whom the marker predicted histologic progression and response to interferon treatment. In this study, we evaluated the efficiency of the determination of serum SCCA-IgM levels in identifying patients with HCC and defining their prognosis, in search of a biomarker to be used to modulate treatment aggressiveness, in parallel with the Barcelona Clinic Liver Cancer (BCLC)/American Association of the Study of Liver Diseases (AASLD) treatment and prognostic algorithm.

**Methods**

The study was retrospectively performed on sera prospectively collected from 327 patients providing informed consent to blood drawing, consecutively recruited between 2005 and 2009. The series included 81 HCC patients, 206 cirrhosis patients without HCC, and 40 blood donors as a healthy control group. Sera from cirrhotics were obtained at our outpatient clinic from patients with chronic liver disease fulfilling the following inclusion criteria:

- International normalized ratio (INR) higher than 1.20;
- White blood cell (WBC) lower than 4.40 × 10⁹/L; (at least two out of three criteria);
- Platelets (PLTs) below 150 × 10⁹/L; (at least two out of three criteria);
- US examination showing findings compatible with cirrhosis: coarse liver structure, irregular profiles, dilated portal tract, reduced flow velocity, enlarged spleen diameter (at least three out of five criteria).

The absence of HCC was confirmed by routine US examination (at the time of entry in the study and every six months), with a one-year negative follow-up. Sera from HCC patients were obtained from patients admitted in our unit for treatment purposes. The diagnosis of HCC was based on the 2005 AALSD guidelines, although, for a local policy, almost always (96%) finally biopsy-confirmed, with the main lesion or the most accessible and “safe for biopsy” lesion being selected for histological sampling. The clinical features of patients with HCC and with cirrhosis are shown in Tables 1 and 2, respectively.

As shown by a preliminary Kolmogorov–Smirnov test, the distribution of the data was not normal (P < 0.0001) and non-parametric tests were therefore used throughout the study. The diagnostic accuracy of SCCA-IgM was evaluated by the receiver operating characteristic (ROC) method and the area under the curve (AUC) calculated. With respect to the prognostic accuracy, the HCC study population was arbitrarily subgrouped into long-term and short-term survivors (> or < than 36 months). This choice was supported by the fact that in patients in BCLC/AASLD intermediate stage, that represented the majority of our sample, the expected survival is calculated at 36 months, and ranges between 20 to 40%. The clinical features of the two subgroups of patients are shown in Table 3.

The prognostic cut-off was again established with the ROC method at the value that maximized sensitivity and specificity. Overall survival (OS) curves were calculated by Kaplan–Meier method and compared by the log–rank test. The prognostic accuracy of SCCA-IgM levels was then evaluated, restricting the analysis only to patients with “intermediate stage” HCC who underwent transcatheter arterial chemoembolization (TACE), carried out using the standard selective/superselective method with lipiodol (5–10 mL) and epirubicin (10–40 mg). To further evaluate the prognostic value of SCCA-IgM, the correlation

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical, biological and tumoral features of patients with hepatocellular carcinoma (HCC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male 63 78</td>
</tr>
<tr>
<td></td>
<td>Female 18 22</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>66 ± 11</td>
</tr>
<tr>
<td>Status at the end of follow-up</td>
<td>Alive 25 31</td>
</tr>
<tr>
<td>Alive 25 31</td>
<td></td>
</tr>
<tr>
<td>Death 56 69</td>
<td></td>
</tr>
<tr>
<td>Etiology</td>
<td>HBV 9 11</td>
</tr>
<tr>
<td>HCV 41 51</td>
<td></td>
</tr>
<tr>
<td>Alcohol 18 22</td>
<td></td>
</tr>
<tr>
<td>Mixed 7 9</td>
<td></td>
</tr>
<tr>
<td>Others 6 7</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>No 3 4</td>
</tr>
<tr>
<td>Yes 78 96</td>
<td></td>
</tr>
<tr>
<td>Child–Pugh</td>
<td>A 54 67</td>
</tr>
<tr>
<td>B 26 32</td>
<td></td>
</tr>
<tr>
<td>C 1 1</td>
<td></td>
</tr>
<tr>
<td>AFP(ng/mL)</td>
<td>≤ 20 42 52</td>
</tr>
<tr>
<td>≥ 20 200 24 30</td>
<td></td>
</tr>
<tr>
<td>Number of nodules</td>
<td>≤ 3 58 72</td>
</tr>
<tr>
<td>≥ 3 and ≤ 5 16 20</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 7 8</td>
<td></td>
</tr>
<tr>
<td>Maximal size (cm)</td>
<td>≤ 3 53 65</td>
</tr>
<tr>
<td>&gt; 3 and ≤ 5 17 21</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 11 14</td>
<td></td>
</tr>
<tr>
<td>Portal thrombosis</td>
<td>No 76 94</td>
</tr>
<tr>
<td>Yes 5 6</td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td>No 80 99</td>
</tr>
<tr>
<td>Yes 1 1</td>
<td></td>
</tr>
<tr>
<td>Edmonson’s grading</td>
<td>1 42 54</td>
</tr>
<tr>
<td>2 34 44</td>
<td></td>
</tr>
<tr>
<td>3–4 2 2</td>
<td></td>
</tr>
<tr>
<td>First treatment</td>
<td>TACE 49 60</td>
</tr>
<tr>
<td>Surgery 2 3</td>
<td></td>
</tr>
<tr>
<td>Percutaneous ablation 19 23</td>
<td></td>
</tr>
<tr>
<td>Supportive cares 11 14</td>
<td></td>
</tr>
<tr>
<td>CLIP score</td>
<td>0 15 18</td>
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<tr>
<td>1 41 51</td>
<td></td>
</tr>
<tr>
<td>2 20 25</td>
<td></td>
</tr>
<tr>
<td>3–4 5 6</td>
<td></td>
</tr>
<tr>
<td>BCLC staging</td>
<td>very early/early 21 26</td>
</tr>
<tr>
<td>intermediate/advanced 60 74</td>
<td></td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HCV, hepatitis C virus; Mixed, HBV+HCV, HBV+alcohol, HCV+alcohol; Others, primary biliary cirrhosis, hemochromatosis, cryptogenic.
between SCCA-IgM levels and the progression-free survival (PFS) was calculated considering only patients in whom treatment (TACE) was effective (i.e., those with at least partial response, no stable disease, or disease progression at the one-month CT scanning, according to modified Response Evaluation Criteria In Solid Tumors [RECEIST] criteria). OS and PFS curves were calculated by Kaplan–Meier method and compared by the log–rank test. Finally, in a subgroup of 22homogeneous patients more recently treated by drug eluting beads-TACE (DEB-TACE), response to treatment was correlated with trends in time of SCCA-IgM levels, in samples obtained the day of treatment and the day of control CT scanning, performed as a routine four weeks later (chi-squared analysis).

The correlation between SCCA-IgM levels and the following variables was evaluated: number of nodules, tumor size (diameter of the largest lesion on CT scanning/nuclear magnetic resonance (NM)), grading according to Edmondson, Child–Pugh status (A, B, C), AFP values (<20, 20–200, >200 U/mL), BCLC staging (very early/early vs intermediate/advanced), presence or absence of thrombosis and metastasis. The impact of the same variables and of SCCA-IgM levels (> ≤ than the prognostic cut-off) on survival was first evaluated with univariate analysis; only the variables that were statistically significant or borderline in the univariate analysis (P < 0.1) were included in the Cox regression analysis to finally establish the independent predictors of survival. A P value < 0.05 was held as significant.

**SCCA-IgM assay.** The serum levels of SCCA-IgM immune complexes were assessed by commercial ELISA Kit (Hepa-IC, Xeptagen, Xeptagen SpA, Marghera, Venice, Italy) according to the manufacturer’s instructions. The amount of SCCA-IgM immune complexes was expressed in arbitrary units (AU/mL) by interpolation of samples absorbance on the calibration curves provided in the kit and results were processed with XEREPRO software (Xeptagen).

### Table 2 Clinical and biological features of patients with cirrhosis

<table>
<thead>
<tr>
<th>Gender</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>140</td>
<td>68</td>
</tr>
<tr>
<td>Female</td>
<td>66</td>
<td>32</td>
</tr>
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</table>

| Mean age (years) | 57 ± 11 |

<table>
<thead>
<tr>
<th>Etiology</th>
<th>n</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>HBV</td>
<td>39</td>
<td>19</td>
</tr>
<tr>
<td>HCV</td>
<td>124</td>
<td>60</td>
</tr>
<tr>
<td>Alcohol</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Mixed</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Child–Pugh</th>
<th>n</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>146</td>
<td>71</td>
</tr>
<tr>
<td>B</td>
<td>58</td>
<td>28</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>1</td>
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<table>
<thead>
<tr>
<th>AFP (ng/mL)</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>&lt; 20</td>
<td>175</td>
<td>85</td>
</tr>
<tr>
<td>≥ 20 e &lt; 200</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>≥ 200</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Sensitivity and specificity of SCCA-IgM determination for HCC were therefore 89% and 50% respectively, with a positive predictive value (PPV) and a negative predictive value (NPV) of 41% and 92%. On the other hand, sensitivity and specificity for SCCA-IgM of AFP, considering the established normality cut-off of 20 U/mL, were 48% and 85% respectively, with a PPV of 40% and NPV of 95%. Using a diagnostic AFP cut-off of 200 U/mL (Table 5), the gain in specificity was not substantial (from 45% to 50%).

#### Results

**Diagnostic accuracy.** Controls had median SCCA-IgM levels (40 AU/mL, C.I. 31–65) significantly lower than both cirrhotics and HCC patients (P < 0.0001). The difference between median SCCA-IgM levels in the two last groups of patients (cirrhosis 90.5 AU/mL [C.I. 80–111.2], HCC 130 [C.I. 121–170]), was highly statistically significant (P = 0.0001). Considering the cut-off for SCCA-IgM obtained by the ROC curves (89 AU/mL, AUC 66%), the levels of the immune complex were increased in 89% (72/81) of HCC patients and in 50% (104/206) of cirrhotics.

Sensitivity and specificity of SCCA-IgM determination for HCC were therefore 89% and 50% respectively, with a positive predictive value (PPV) and a negative predictive value (NPV) of 41% and 92%. On the other hand, sensitivity and specificity for HCC of AFP, considering the established normality cut-off of 20 U/mL, were 48% and 85% respectively, with a PPV of 40% and NPV of 95%. Using a diagnostic AFP cut-off of 200 U/mL (Table 5), the gain in specificity was not substantial (from 45% to 50%).

**Survival prediction.** The three-year survival of all the HCC patients, irrespective of the treatment they underwent, was 36% (median survival time 29 months, confidence interval [C.I.] 24–34). The AUC of SCCA-IgM in the prediction of survival was not particularly high (AUC = 0.63), the cut-off being fixed at 130 AU/mL. With the above cut-off, SCCA-IgM presented a 67% sensitivity and a 49% specificity in the prediction of long-term survival.

The survival curves of the two subgroups (SCCA-IgM levels lower or higher than the cut-off) again irrespectively of the treatment they underwent, was 36% (median survival time 29 months, confidence interval [C.I.] 24–34). The AUC of SCCA-IgM in the prediction of survival was not particularly high (AUC = 0.63), the cut-off being fixed at 130 AU/mL. With the above cut-off, SCCA-IgM presented a 67% sensitivity and a 49% specificity in the prediction of long-term survival.

The Kaplan–Meier curves obtained considering only the patients with intermediate stage HCC who underwent TACE showed an analogoustrend: median survival was 30 months (CI 29–66) for patients with SCCA-IgM < 130 AU/mL and 26 months (CI 22–30) for those with SCCA-IgM >= 130 AU/mL. Similarly, the hazard plot, describing the risk of death in the two groups, showed a forceps opening at 24 months (Fig. 1).

The Kaplan–Meier curves obtained considering only the patients with intermediate stage HCC who underwent TACE showed an analogous trend: median survival was 30 months (CI 29–66) for patients with SCCA-IgM < 130 AU/mL and 26 months (CI 22–30) for those with SCCA-IgM >= 130 AU/mL. Similarly, the hazard plot, describing the risk of death in the two groups, showed a forceps opening at 24 months (Fig. 1).

The Kaplan–Meier curves obtained considering only the patients with intermediate stage HCC who underwent TACE showed an analogous trend: median survival was 30 months (CI 29–66) for patients with SCCA-IgM < 130 AU/mL and 26 months (CI 22–30) for those with SCCA-IgM >= 130 AU/mL. Similarly, the hazard plot, describing the risk of death in the two groups, showed a forceps opening at 24 months (Fig. 1).
who showed a radiologic complete response to TACE treatment, confirmed the prognostic value of SCCA-IgM: median PFS was 14 months (C.I. 11–17) for patients with SCCA-IgM < 130 AU/mL and 6 (C.I. 5–7) for those with SCCA-IgM > 130 AU/mL, a difference that was highly statistically significant (log–rank \( P = 0.003 \)).

In the subgroup of 22 patients treated by DEB-TACE, a drop in SCCA-IgM levels significantly correlated with a complete or partial tumor response, as assessed by the CT scanning performed four weeks following treatment (\( P = 0.0039 \))(Table 6). Patients with stable disease or tumor progression had always stable or increased levels of the marker at four weeks, while patients with complete response always showed a reduction. Patients with partial response had an intermediate behavior. In the same series, AFP did not predict complete response (data not shown).

### Table 3. Clinical, Biological and Tumoral Features of Patients With Hepatocellular Carcinoma Subgrouped on the Basis of Scca-IgM Prognostic Cut-Off (> < 130 AU/ml)

<table>
<thead>
<tr>
<th></th>
<th>SCCA IgM</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 130 AU/ml</td>
<td>&gt; 130 AU/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
</tbody>
</table>
| Child–Pugh           | 36        | 90                   | 18    | 44      | \( \chi^2 = 24.224781 \)
| A                    | 39        | 90                   | 18    | 44      | \( \chi^2 = 24.224781 \)
| B                    | 4         | 10                   | 22    | 54      | \( \chi^2 = 24.224781 \)
| C                    | 0         | 0                    | 1     | 1       | \( \chi^2 = 24.224781 \)
| AFP (ng/ml)          |           |                      |       |         |
| < 20                 | 28        | 70                   | 14    | 34      | \( \chi^2 = 11.256037 \)
| 20 < e < 200         | 6         | 15                   | 18    | 44      | \( \chi^2 = 0.0036 \)
| ≥ 200                | 6         | 15                   | 9     | 22      | \( \chi^2 = 0.0036 \)
| Number of nodules    |           |                      |       |         |
| ≤ 3                  | 35        | 87                   | 23    | 56      | \( \chi^2 = 0.0036 \)
| > 3 and ≤ 5          | 5         | 13                   | 11    | 26      | \( \chi^2 = 0.0036 \)
| > 5                  | 0         | 0                    | 7     | 18      | \( \chi^2 = 0.0036 \)
| Maximal size (cm)    |           |                      |       |         |
| ≤ 3                  | 30        | 75                   | 23    | 56      | \( \chi^2 = 0.0036 \)
| > 3 and ≤ 5          | 7         | 18                   | 10    | 24      | \( \chi^2 = 0.0036 \)
| > 5                  | 3         | 7                    | 8     | 20      | \( \chi^2 = 0.0036 \)
| Portal thrombosis    |           |                      |       |         |
| No                   | 37        | 93                   | 39    | 95      | \( \chi^2 = 0.0036 \)
| Yes                  | 3         | 7                    | 2     | 5       | \( \chi^2 = 0.0036 \)
| Metastasis           |           |                      |       |         |
| No                   | 39        | 99                   | 41    | 100     | \( \chi^2 = 0.0036 \)
| Yes                  | 1         | 1                    | 0     | 0       | \( \chi^2 = 0.0036 \)
| Edmonson’s grading   | 1         | 22                   | 55    | 21      | \( \chi^2 = 0.0036 \)
| 2                    | 18        | 45                   | 18    | 44      | \( \chi^2 = 0.0036 \)
| 3–4                  | 0         | 0                    | 2     | 4       | \( \chi^2 = 0.0036 \)
| BCLC staging         | Very early/early | 12        | 30   | 9       | \( \chi^2 = 0.0036 \)
| Intermediate/advanced | 28        | 70                   | 32    | 78      | \( \chi^2 = 0.0036 \)

**n.s.** not significant.

### Table 4. Combination of SCCA-IgM and AFP testing. The diagnostic cut-off of SCCA-IgM and AFP were 89 AU/mL and 20 U/mL respectively

<table>
<thead>
<tr>
<th>Serological diagnosis</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCA-IgM</td>
<td>AFP</td>
</tr>
<tr>
<td>HCC (81)</td>
<td>Cirrhosis (206)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n %</th>
<th>n %</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>+</td>
<td>37</td>
<td>46</td>
</tr>
<tr>
<td>+</td>
<td>72</td>
<td>89</td>
</tr>
<tr>
<td>+</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Overall (+/-, +/-, -/+)</td>
<td>76</td>
<td>94</td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

### Table 5. Combination of SCCA-IgM and AFP testing. The diagnostic cut-off of SCCA-IgM and AFP were 89 AU/mL and 200 U/mL respectively

<table>
<thead>
<tr>
<th>Serological diagnosis</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCA-IgM</td>
<td>AFP</td>
</tr>
<tr>
<td>HCC (81)</td>
<td>Cirrhosis (206)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n %</th>
<th>n %</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>+</td>
<td>58</td>
<td>72</td>
</tr>
<tr>
<td>+</td>
<td>72</td>
<td>89</td>
</tr>
<tr>
<td>+</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Overall (+/-, +/-, -/+)</td>
<td>73</td>
<td>90</td>
</tr>
<tr>
<td>-</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Univariate and multivariate analysis. Only diagnosis (Cirrhosis/HCC) was significantly correlated with SCCA-IgM levels (\( Rho = 0.167, 0.010–0.0031, 95\% CI, \( P = 0.037 \), Spearman’s rank correlation analysis). In HCC, none of the clinical variables was significantly correlated with SCCA-IgM levels, including Child–Pugh status or AFP levels (\( P = 0.975 \)), in the univariate analysis.

At univariate analysis tumor size, number of nodules, Child–Pugh status, BCLC staging and SCCA-IgM were significantly correlated with a complete response to TACE treatment, confirmed by radiologic complete response to TACE treatment, as assessed by the CT scanning performed four weeks following treatment (\( P = 0.0039 \))(Table 6). Patients with stable disease or tumor progression had always stable or increased levels of the marker at four weeks, while patients with complete response always showed a reduction. Patients with partial response had an intermediate behavior. In the same series, AFP did not predict complete response (data not shown).
associated with survival (Table 7); in particular, AFP was not even close to being significantly associated with survival ($P = 0.45$). When the patients were subgrouped according to their SCCA-IgM levels ($<\text{ or } >$ the cut-off), patients with lower levels of the marker were significantly more frequently Child–Pugh A and with lower levels of AFP (see Table 3).

The Cox multivariate analysis showed that, amongst the five parameters, tumor size ($P < 0.001$, [HR] 2.47, C.I. 1.31–3.61) and SCCA-IgM levels ($P = 0.004$, Hazard ratio [HR] 2.34, C.I. 1.28–3.64), selected in this order in the “forward conditional” model, were identified as independent predictors of survival.

**Table 6** Correlation between trend in time of SCCA-IgM levels and response to DEB-TACE

<table>
<thead>
<tr>
<th>Disease</th>
<th>Stable/progression</th>
<th>Partial response</th>
<th>Complete response</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCA-IgM levels $\geq 6$</td>
<td>6 (100%)</td>
<td>6 (55%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>SCCA-IgM levels $&lt;6$</td>
<td>0 (0%)</td>
<td>5 (45%)</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

$P = 0.0039$, chi square 11.090.

$^1$/$\geq$ no variation or increase.

$^1<$ reduction.

**Table 7** Significant predictors of survival: the univariate analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard ratio</th>
<th>C.I.</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size</td>
<td>3.61</td>
<td>1.77–4.81</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of nodules</td>
<td>2.00</td>
<td>0.99–2.48</td>
<td>0.093</td>
</tr>
<tr>
<td>Child–Pugh status</td>
<td>2.02</td>
<td>1.06–3.75</td>
<td>0.031</td>
</tr>
<tr>
<td>BCLC Staging</td>
<td>1.41</td>
<td>0.94–2.10</td>
<td>0.088</td>
</tr>
<tr>
<td>SCCA-IgM</td>
<td>2.12</td>
<td>1.08–3.85</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Figure 1 Survival and hazard plot for the two subgroups of patients: in black, those with SCCA-IgM $>130$ AU/mL; in grey, those with SCCA-IgM $<130$ AU/mL. The curves of survival and hazard diverge starting at 24 months. $\geq$, $< 130$ AU/mL; $>$, $>130$ AU/mL.

Figure 2 Progression-free survival (PFS) curves for the two subgroups of intermediate-stage patients treated by TACE: in black, those with SCCA-IgM $>130$ AU/mL; in grey, those with SCCA-IgM $<130$ AU/mL. Patients with SCCA-IgM levels below the cut-off have a significantly longer PFS. $\geq$, $< 130$ AU/mL; $>$, $>130$ AU/mL.
**Discussion**

The search of reliable and efficient biomarkers for a diagnostic and prognostic evaluation of HCC is still an open issue. This search is clearly justified by the fact that the discovery of a biomarker efficient enough, both in terms of sensitivity and specificity, to avoid performing the often long list of examinations required for a diagnosis of HCC (US, CT scanning, NMR, liver biopsy) would dramatically cut costs and risks involved in the diagnostic algorithm. AFP is still universally used, although several studies have repeatedly demonstrated that both the sensitivity and the specificity of the marker are not high enough to justify its routine use, despite some recent attempt to rehabilitate the marker. Better performances are provided by AFP in defining the prognosis of HCC patients, and for instance the marker is used as a prognostic tool in one of the more widely adopted staging systems for HCC, the Cancer of the Liver Italian Program (CLIP) score, as well as in the post-treatment evaluation, because it correlates with radiologic response, disease-free and overall survival. However, on a large scale, even the prognostic definition in the individual patient lacks adequate efficiency, as we also recently confirmed.

As reported, immunohistochemical studies recently demonstrated an overexpression of SCCA variants (SCCA-1, SCCA-2 e SCCA-PD) in HCC tissues. SCCA is a member of the serine protease (serpin) inhibitors, and “in vivo” and “in vitro” studies demonstrate that it has a role in cancer development and progression because it interferes with apoptosis, prompts tumor growth and promotes epithelial-to-mesenchymal transition, thus facilitating tumor invasion. SCCA expression is an early event in hepatocarcinogenesis with a progressive increase of expression in cirrhosis, dysplastic nodes and HCC. This increase heralding, in cirrhotics, HCC development.

Our study confirms, at least in part, the results obtained with the use of SCCA-IgM in the diagnosis of HCC. With the indicated cut-off, the marker showed a high (89%) sensitivity, with however, a relatively low specificity (50%), as previously reported also in other studies, and the increased SCCA-IgM levels in cirrhotics is justified by the data obtained at immunohistochemistry.

Even though we do not support a diagnostic approach based on the combination of multiple diagnostic markers, which inevitably leads to associate an increased sensitivity to a lower specificity, it must be reported that the association of SCCA-IgM and AFP determination (> 20 U/mL) raised the sensitivity to 94% (5% higher than with SCCA-IgM alone) with a specificity of 45% (5% lower than with SCCA-IgM alone) and a high NPV (95%). With a diagnostic AFP cut-off (> 200 U/mL), the sensitivity was basically the same with SCCA-IgM alone.

In contrast with what was observed with respect to AFP, SCCA-IgM serum levels do not correlate with the patients’ tumor burden, in terms of number and size of the nodules, nor they appear to depend on tumor grading, as already reported or with the Child–Pugh status or the BCLC/AASLD stage. It seems therefore that the determinants for the expression of the marker are not linked to the extent of the neoplastic involvement or with the liver function. They appear to rather reflect the intrinsic biological activity of the tumor and/or the extent of the patients’ immune response.

If several studies already reported the diagnostic role of SCCA-IgM, much less information is available on its potential prognostic role. We subgrouped the series of HCC patients on the basis of the cut-off identified by using the ROC curves (SCCA-IgM >20 U/mL) with as the end-point, a short (<36 months) or long (>36 months) survival. As said, the 36 months survival limits was arbitrarily chosen, but this time point is routinely utilized in the BCLC/AASLD guidelines to define the survival of HCC patients in the intermediate stage, that were, in this study, the majority of the sample.

The difference in survival between patients with SCCA-IgM levels higher or lower than the cut-off was statistically significant (P = 0.018), with a median survival in patients with SCCA-IgM < 20 U/mL almost twice as high as in those with SCCA-IgM above the cut-off (48 vs 26 months). It is interesting to note that the survival curves of the two groups of patients remain substantially similar up to 24 months, to then diverge very clearly after that time point, to suggest that SCCA-IgM levels reflect a biologic feature potentially related to more aggressive liver tumors. Analogous results were obtained if considering only patients in intermediate stage undergoing TACE, a homogenous subgroup of HCC patients, thus confirming, with an internal validation carried out in patients in a specific stage and undergoing a specific treatment, the results obtained in the overall group. Additionally, SCCA-IgM levels determination showed a very clear correlation with PFS in patients with complete response, with a median time to progression of 14 months in patients with low versus six months in those with high SCCA-IgM levels. Again, this points to either a different biologic behavior of the tumor, with a higher aggressiveness mediated by the effect of the serpin on tumor growth and invasiveness, or to an adaptive immune response in patients with different levels of expression of the biomarker. The antigenic changes involved in malignant transformation in cancer cells are indeed recognized by the immune system and trigger an immune response that is aimed at controlling tumor growth. The significant impact of SCCA-IgM determination in defining the patients’ prognosis was confirmed, in this study, also by the data showing that, in the Cox analysis, SCCA-IgM levels were identified as independent predictors of OS, with a relative weight just slightly lower than that of tumor size. Finally, even though these data are to be interpreted with caution, given the small sample size, SCCA-IgM determination also demonstrated to efficiently and significantly predict response to treatment since a drop in the marker at four weeks from DEB-TACE was only observed in patient with complete or partial response while, conversely, no patient with stable disease or progression showed a reduction in SCCA-IgM levels, a predictive capacity not shown by AFP. A recent paper has described a prognostic impact of SCCA tissue expression in 61 patients with HCC in whom the expression was evaluated in either biopitic or surgical samples. The authors’ findings indicated that the absence of SCCA expression was correlated with a higher proliferation rate and was a significant independent negative predictor of survival.

These findings are in contrast with our own results, but it must be kept in mind that no correlation between serum SCCA-IgM levels and SCCA tissue expression has been documented in a study that specifically addressed the point and also that serum SCCA-IgM determination reflects not only the tumor biology but, as already discussed, also the patients’ immune response.

In summary, SCCA-IgM sensitivity is, in this study, quite encouraging, but the specificity is not high enough to justify the
use of the marker in HCC diagnosis, at least as a single marker. We, however, do not support the use of a “multiple-markers” approach that, in our mind, may ameliorate sensitivity, but with a cost of an even lower specificity and with an increase in direct (tests) and indirect (imaging procedure-related) costs. Overall, the role of the marker is to be confirmed in larger, prospectively collected, series of patients. On the other hand, the performance of SCCA-IgM in defining the prognosis of HCC patients is quite clear in terms of OS, PFS and treatment response. Obviously, additional studies, which should be aimed at confirming our data and analyzing the levels of the marker in relation also to different treatment methodologies on a larger patient sample, are again required.

References


16. Biasiolo A, Tono N, Quarta S, Beneduce L, Gatta A, Fassina G, Pontisso P. Squamous cell carcinoma antigen (SCCA)-IgM complex: SCCA1-IgM and SCCA-


