World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v7.i29.2913

*World J Hepatol* 2015 December 18; 7(29): 2913-2919
ISSN 1948-5182 (online)


MINIREVIEWS

# Clinical applications of squamous cell carcinoma antigen-immunoglobulins M to monitor chronic hepatitis C

Andrea Martini, Andrea Gallotta, Patrizia Pontisso, Giorgio Fassina

Andrea Martini, Patrizia Pontisso, Department of Medicine, University of Padua, 35122 Padua, Italy

Andrea Gallotta, Giorgio Fassina, Xeptagen S.p.A., VEGA Science Park, 30175 Marghera, Venice, Italy

Author contributions: Martini A designed the study, acquired the data and drafted the article; Gallotta A made critical comments and reviewed the manuscript; Pontisso P and Fassina G conceived the study and made the final critical revision of the manuscript; all authors have seen and approved the final version of the manuscript.

Conflict-of-interest statement: Fassina G (Chief Scientific Officer) and Gallotta A (Senior Scientist) are employees of Xeptagen S.p.A.



Correspondence to: Dr. Giorgio Fassina, Xeptagen S.p.A., VEGA Science Park, Building Auriga, Via delle Industrie 9, 30175 Marghera, Venice, Italy. fassina@xeptagen.com
Telephone: +39-041-5093910

Received: July 10, 2015
Peer-review started: July 12, 2015
First decision: September 8, 2015
Revised: September 30, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: December 18, 2015

## Abstract

Hepatitis C virus (HCV) is the main cause of chronic liver disease and cirrhosis in Western countries. Over time, the majority of cirrhotic patients develop hepatocellular carcinoma (HCC), one of the most common fatal cancers worldwide - fourth for incidence rate. A high public health priority need is the development of biomarkers to screen for liver disease progression and for early diagnosis of HCC development, particularly in the high risk population represented by HCV-positive patients with cirrhosis. Several studies have shown that serological determination of a novel biomarker, squamous cell carcinoma antigen-immunoglobulins M (SCCA-IgM), might be useful to identify patients with progressive liver disease. In the initial part of this review we summarize the main clinical studies that have investigated this new circulating biomarker on HCV-infected patients, providing evidence that in chronic hepatitis C SCCA-IgM may be used to monitor progression of liver disease, and also to assess the virological response to antiviral treatment. In the last part of this review we address other, not less important, clinical applications of this biomarker in hepatology.

**Key words:** Hepatitis C virus; Treatment; Prognosis; Squamous cell carcinoma antigen-immunoglobulins M; Cirrhosis



**Core tip:** A high public health priority need is the development of biomarkers to screen for liver disease progression in hepatitis C virus (HCV)-positive patients. Serological squamous cell carcinoma antigen-immunoglobulins M has shown the ability to identify patients with progressive liver disease and patients at higher risk of hepatocellular carcinoma development. In this review we summarize the main clinical studies performed using this new circulating biomarker for monitoring cirrhosis progression in HCV-positive patients and to evaluate virological response to antiviral treatment.

Martini A, Gallotta A, Pontisso P, Fassina G. Clinical applications of squamous cell carcinoma antigen-immunoglobulins M to monitor chronic hepatitis C. *World J Hepatol* 2015; 7(29): 2913-2919 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i29/2913.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i29.2913

## INTRODUCTION

Liver cirrhosis is an increasing cause of morbidity and mortality in Europe and the United States. It is the fourth most common cause of death in adults worldwide and the major reason for more than 5500 liver transplants in Europe each year[1]. The main causes of cirrhosis in Western countries are infection with hepatitis C virus (HCV), alcohol abuse, and, increasingly, non-alcoholic fatty liver disease (NAFLD)[2]. In sub-Saharian Africa and in most parts of Asia, infection with hepatitis B virus (HBV) represents the most common cause of cirrhosis[2]. The prevalence of this advanced liver disease is difficult to assess and probably higher than reported, because the initial stages are asymptomatic until cirrhosis with clinical decompensation occurs, therefore the disorder is often undiagnosed[2]. In line with these findings, about 90% of individuals with viral hepatitis in Europe are not aware of their status[1]. Moreover, the prevalence of NAFLD is 2%-44% in the European population and even higher (42.6%-69.5%) in people with type 2 diabetes[1].

Hepatocellular carcinoma (HCC), one of the main complications of cirrhosis, and the leading cause of death among these patients, is the sixth most common neoplasm and the third most frequent cause of cancer death[3]. Whereas the survival of patients with most malignancies has enhanced over the last decade, 5-year survival rate of patients with HCC has not improved sufficiently and remains less than 10%[4]. The poor outcome of patients with HCC is related to the late detection of the cancer, with the majority of patients diagnosed at advanced stages of disease[4]. It has been demonstrated that HCC surveillance of population at risk increases survival, because of detection of tumours amenable to curative therapies[5-7]; in fact, surveillance is recommended by international guidelines[8].

A major problem with HCC detection and surveillance is the lack of reliable biomarkers. Table 1 summarizes the sensitivity and specificity of the serological markers currently available for HCC diagnosis.

Alpha-fetoprotein (AFP) is the most widely used serum marker for HCC diagnosis and surveillance; however, not all HCCs secrete AFP (about 32%-59% of patients with the tumour have normal AFP levels)[4]. Furthermore, AFP may be elevated in patients with chronic liver disease in the absence of HCC, making this biomarker inadequate for surveillance tests[4]. Indeed, European and American guidelines consider AFP too inaccurate to survey patients at risk of HCC and recommend the use of ultrasound (US) alone[9,10]. US sensitivity depends on many factors, including the quality of the US machine, the experience of the examiner, and also the patient[11]. In patients with liver cirrhosis, regenerative nodules may be hard to distinguish from HCC using US, and the sensitivity of this imaging technique to detect early HCC lies between 32% and 65%[11]. For this reason, some authors, as well as Asian guidelines, suggest the use of AFP in HCC surveillance[12,13].

From 1990's, especially in Japan, new biomarkers for HCC diagnosis and surveillance have been explored. Among these, des-$\gamma$ carboxy-prothrombin, an abnormal prothrombin protein, has been considered[14], but the results indicate that its sensitivity is highly dependent on tumour size[15]. The clinical utility of lens culinaris agglutinin-reactive fraction of AFP-L3 in early prediction of HCC development in patients with chronic HBV or HCV infection was also recently evaluated[16]. It was shown that several factors (gender, age, race, and presence of more advanced liver disease) are independent predictors of increased levels of this biomarker, which also lacks in sensitivity, specificity, and predictive values required for routine HCC surveillance[17].

Another biomarker that has been developed in recent years is Osteopontin, a molecule expressed by transformed malignant cells, also evaluated for colon and pancreatic cancer[18]. The majority of the studies analyzing osteopontin for the diagnosis of HCC was retrospective and included a range of 30 to 179 patients with HCC. The reported sensitivity of osteopontin for HCC was 86%, with a specificity of 86%, resulting in a diagnostic accuracy comparable to that of AFP. Further validation studies are needed to use this marker in daily clinical routine[18]. On the basis of the above considerations, a reliable biomarker to complement US in detecting early HCC still represents a crucial unmet need.

In recent years relevant emphasis has been ascribed to innate or natural immunity, which acts as the first line of defense, and also as the link between acquired immunity and immunological memory[19]. Poly-reactive natural auto-antibodies [immunoglobulins M (IgM)] can bind, with low affinity and high avidity, different markers that are expressed during cancer growth[20]. The presence of IgM-linked immune-complexes with diagnostic value has been found recently in different human tumours, including colon[21] and prostate cancer[22], and also in other pathologic conditions[23]. For liver disease, the diagnostic value of squamous cell carcinoma antigen-IgM (SCCA-IgM) immune-complex in serum has been demonstrated in several studies[24,25].

In this review we summarize the role of SCCA-IgM as a novel promising tool to monitor liver disease evolution and response to antiviral treatment in patients with chronic hepatitis C; we also analyze the value of this biomarker for the diagnosis and prognosis of HCC. An overview of the main observations on SCCA-IgM behaviour in clinical settings is presented in Table 2.

**Table 1 Sensitivity and specificity (%) of various biomarkers for hepatocellular carcinoma diagnosis**

| Biomarker | Sensitivity | Specificity |
|---|---|---|
| SCCA-IgM[57] | 52-89 | 50-82 |
| AFP[58] | 41-65 | 80-94 |
| Osteopontin[58] | 87 | 82 |
| DCP[58] | 23-89 | 95 |
| AFP-L3[58] | 37-60 | 90-92 |

AFP: Alpha-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen-immunoglobulins M.

## SCCA

SCCA belongs to the clade B subset of the serpins family[26]. SCCA1 (SERPINB3) and its isoform SCCA2 (SERPINB4) are over-expressed in squamous cell carcinoma (SCC) of the uterine cervix, lung, head and neck, rectal colon, pancreatic and liver tumors[27-30]. The isoform that has been better evaluated in literature is SERPINB3, which has showed functional connection with tumorigenesis. This isoform was found to prevent cell death through its binding to complex 1 of the mitochondrial respiratory chain or *via* suppression of c-JUN, as a response to different types of stress, such as UV, radiation, chemotherapy, tumour necrosis factor-alpha and natural killer cells[31-34]. Moreover, its inflammatory and pro-tumorigenic role has been revealed demonstrating its ability to enhance interleukin-6 effects through nuclear factor κB pathway in response to Rat Sarcoma Viral Oncoprotein (coding RAS gene) stimuli[29].

SCCA1 and 2 are undetectable in normal hepatocytes, but their expression progressively increases from chronic liver disease to dysplastic nodules and HCC[35]. In particular, SERPINB3 is more expressed in high-grade dysplastic nodules and in HCC than in large regenerative nodules, suggesting a role in hepatocarcinogenesis[36]. Furthermore, this serpin was identified in the majority of hepatoblastomas, with the highest levels in tumours of more advanced stage[37]. In HCC, high expression of SERPINB3 is significantly associated with early tumour recurrence, and shows a better prognostic significance than other clinical and histological variables[38]. These important clinical findings were confirmed at the molecular level: SCCA expression in liver tumor has been correlated with liver regeneration activity (expressed by MIB-I-labeling index)[39], and increased proliferation was also documented in hepatoma cell lines over-expressing SERPINB3 and in a mouse model transgenic for this serpin[39,40]. Recent data indicate that SERPINB3 is highly expressed in the hepatic stem/progenitor cell compartment of both fetal and adult livers[41]; moreover, after induction by HIF2-alpha in an hypoxic environment[42], SERPINB3 was shown to be crucial for tumour invasiveness and metastasis, since it promotes epithelial-mesenchymal transition[39] and transforming growth factor-beta production[43] (Figure 1).

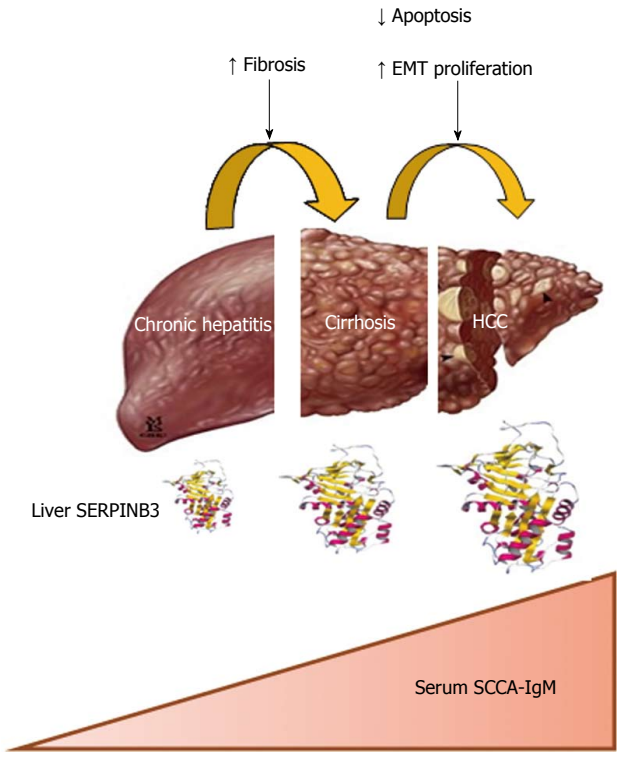


**Figure 1 Schematic representation of SERPINB3 behavior in the liver and of serological squamous cell carcinoma antigen-immunoglobulins M levels in chronic liver disease.** SCCA-IgM: Squamous cell carcinoma antigen-immunoglobulins M; HCC: Hepatocellular carcinoma; EMT: Epithelial-mesenchymal transition.

## SCCA-IGM IN HCV-POSITIVE PATIENTS

Recently, an ELISA assay has been developed to detect serological SCCA isoforms (SERPINB3 and SERPINB4) complexed with natural IgM[24]. The clinical usefulness of monitoring SCCA-IgM immune-complexes in chronic liver disease has been evaluated in several studies. In 2008 Biasiolo *et al*[44] observed that SCCA-IgM was detectable, at presentation, in 33% of untreated patients with histologically proven chronic hepatitis, but not in healthy control subjects. After a median period of six years, the same patients underwent a second liver biopsy and an increased level of the immune-complex was observed in 75% of cases with progressive disease (defined as an increase in fibrosis score $\geqslant$ 2 during follow-up in untreated patients). On the other hand, SCCA-IgM levels were substantially stable in patients with no disease progression during the same interval, and no difference in the level of the biomarker was detected in regard to the etiology of chronic liver disease[44].

In chronic HCV infection the presence of non-alcoholic steatohepatitis (NASH) at the histological level reflects a more severe clinical and pathological state than steatosis alone, being associated with a more rapid progression of fibrosis[45]. Recently, the relationship between SCCA-IgM and NASH was investigated in 91 patients with chronic hepatitis C: In patients with histological diagnosis of NASH the immune-complex levels were elevated and

**Table 2 Overview of the main observations on squamous cell carcinoma antigen-immunoglobulins M behaviour in clinical settings**

| Ref. | Main observation |
|---|---|
| SCCA-IgM in monitoring chronic liver disease | |
| Biasiolo *et al*[44] | Significant increase of SCCA-IgM levels over time in 75% of untreated patients with chronic hepatitis and with histologically proven liver disease progression (fibrosis score increase ⩾ 2) after six years of follow-up |
| Martini *et al*[46] | In patients with chronic hepatitis C, SCCA-IgM was found an independent predictor of histologically proven non alcoholic steatohepatitis |
| SCCA-IgM and antiviral treatment | |
| Giannini *et al*[48] | In chronic hepatitis C treatment with standard therapy, only patients who achieved sustained viral response showed a significant decrease in median values of SCCA-IgM up to one year of follow-up |
| Fransvea *et al*[49] | Reduction of SCCA-IgM levels during the first month of standard antiviral therapy was an independent predictor of sustained viral response |
| Martini *et al*[46] | Significant reduction of SCCA-IgM, lasting up to 6 mo of follow-up, was observed only in HCV-positive patients with sustained response to standard therapy |
| SCCA-IgM in diagnosis and prognosis of HCC | |
| Pontisso *et al*[51] | Significant increase over time of SCCA-IgM only in patients with early cirrhosis (histologically proven) who developed HCC within four years of follow up |
| Buccione *et al*[52] | In HCV-positive patients with overt cirrhosis, SCCA-IgM negativity (cut off ⩽ 200 AU/mL) accurately identified patients at low risk of liver cancer development in the subsequent year |
| Beneduce *et al*[24] | SCCA-IgM showed higher sensitivity for the diagnosis of HCC, compared to AFP |
| Pozzan *et al*[53] | In patients with HCC, SCCA-IgM levels were found an independent predictor of survival. A reduction in SCCA-IgM levels was correlated with response to HCC treatment |

HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; AFP: Alpha-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen-immunoglobulins M.

associated with more severe steatosis (> 33%), while in HCV-negative patients with steatosis and NASH SCCA-IgM was barely detectable. These results were probably due to a higher production of IgM in HCV positive patients, followed by an amplification of the ELISA signal as a result of a lower threshold required for B-cell activation after the engagement of CD81 by the HCV-E2 protein[46].

The association between SCCA-IgM and NASH in HCV positive patients was confirmed at univariate and multivariate logistic regression analysis. Among the various clinical aspects that were considered, only HCV genotype 3 was identified as an additional independent variable significantly associated with NASH[46]. Furthermore, a close correlation between the intensity of SCCA-1 expression in the liver and SCCA-IgM levels in the serum was documented in serum and liver samples from the same patients. Indeed, in cases of negative serological SCCA-IgM, SCCA-1 detection in the corresponding liver biopsy was weak, even in the presence of steatosis. On the other hand, the serpin was highly expressed in patients with elevated values of SCCA-IgM in serum[46].

## SCCA-IGM AND ANTIVIRAL TREATMENT

Combination therapy of pegylated interferon-alpha (PEG-IFNα) and ribavirin results in complete viral eradication in about 50% of patients with chronic HCV infection. However, a substantial number of patients show no significant response to therapy or develop viral relapse after the cessation of IFN-based therapy[47].

The first evidence of the behavior of SCCA-IgM during antiviral treatment with PEG-IFN and ribavirin was obtained from a longitudinal study in 2010[48]. Giannini *et al*[48] demonstrated that in patients with HCV-related cirrhosis who achieved sustained virological response (SVR) after standard treatment with PEG-IFN and ribavirin, there was a significant decrease in serum levels of SCCA-IgM at the end of treatment, and up to one year of follow-up, when compared to baseline. In null responders (NR) baseline values of serological SCCA-IgM were not statistically different from SVR patients, but during follow-up SCCA-IgM levels did not show significant changes compared to baseline[48]. In 2012, in a multicentre prospective study, 103 patients with HCV chronic infection undergoing antiviral treatment with PEG-IFN and ribavirin were enrolled to test the efficacy of SCCA-IgM as a marker of response[49]. This study confirmed that the reduction of SCCA immune-complexes was significantly different between patients that showed SVR and those who did not. Moreover, the decreased serological concentration of the biomarker was an independent predictor of SVR in regard to HCV genotype and age[49]. In addition, the behavior of SCCA-IgM in relation to antiviral therapy was recently confirmed in 91 patients with chronic hepatitis C. In the subgroup of patients who reached SVR and had the baseline positivity to SCCA-IgM, the serological values significantly decreased after six months of treatment and remained persistently low even at six months of follow-up after treatment. In NR patients, no significant variation in SCCA-IgM serum values was observed after six months of treatment or at six months after the end of therapy[46].

These studies clearly demonstrate that the termination of HCV-associated liver damage determines a progressive decline of SCCA-IgM serological levels, therefore this biomarker could be used as a surrogate marker to monitor active disease resolution.

## SCCA-IGM AND HCC

One of the most important and yet unmet clinical needs

in hepatology is the availability of serological markers to identify patients with chronic hepatitis and cirrhosis at higher risk of HCC development. In liver cirrhosis the rate of HCC progression is 3%-4% every year[50]; the identification of the subgroup of patients with possible HCC development within the next few years would allow the development of a personalized clinical management characterized by more effective early therapeutic interventions. In order to explore this possibility, SCCA-IgM was analyzed in a retrospective, longitudinal study that was preliminary conducted in a cohort of HCV-infected patients with early stage of cirrhosis, defined on the basis of histological findings[51]. This population was divided in two groups with similar clinical characteristics and no significant difference in the absolute value of the immune-complex at baseline. The first group included 16 cirrhotic patients who developed HCC during a median follow up of 4 years, while the second group included 17 control patients with cirrhosis who did not develop HCC during the same period. The progressive increase of SCCA-IgM over time was remarkable in cirrhotic patients who eventually developed HCC, while figures remained unchanged in the majority of the cirrhotic patients without evidence of liver cancer during the same time interval. The increase of AFP, measured in parallel in the same serum samples, was not significantly different in patients who developed HCC and in those without liver tumor progression. Accordingly, the predictive value of SCCA-IgM variation was found to be significantly better than that of AFP for predicting the progression to HCC (AUC: 0.821 *vs* 0.654)[51].

These data were in line with another retrospective study performed by Buccione *et al*[52]. The aim of this study was to evaluate whether the levels of SCCA-IgM in serum could identify HCV patients with clinical signs of cirrhosis at risk of HCC development. The study involved 57 cirrhotic patients, during a median period of 48 mo. The baseline value of serological SCCA-IgM was nearly 4-fold higher in patients who developed HCC than in those who did not, and the SCCA-IgM value $\leqslant$ 200 AU/mL accurately identified patients at low risk of liver cancer in the subsequent year, with a negative predictive value of 97%[52]. These results suggest that in patients with evident cirrhosis the assessment of this biomarker can improve the diagnostic process: The subgroup of patients at higher risk of liver tumor development, that need a constant monitoring, can be identified based on SCCA-IgM positivity.

In regard to the diagnostic value of SCCA-IgM for HCC, a cross sectional study performed by Beneduce *et al*[24] have demonstrated the positivity of this biomarker in the vast majority of HCC serum samples (70% sensitivity *vs* 42% sensitivity of AFP), whereas all healthy control samples were negative. Although no correlation with HCC etiology was found, the authors observed that the amount of circulating SCCA-IgM at different stages of liver disease reflected the extent of SCCA over-expression detected by immunohistochemistry in liver specimens. Moreover, SCCA-IgM positivity did not overlap with that of AFP, suggesting that the combination of these two biomarkers could improve the sensitivity for detecting HCC without compromising the diagnostic specificity[24].

In summary, the prognostic role of SCCA-IgM has been explored in patients with chronic hepatitis and cirrhosis, documenting a higher risk of fibrosis progression and liver tumor development.

Until recently, no data were available on the prognostic role of this biomarker in HCC prognosis. This aspect was addressed in a recent study by Pozzan *et al*[53], who retrospectively analyzed the serum of 327 patients, including patients with cirrhosis and HCC. In this study the ability of SCCA-IgM to predict liver cancer prognosis was proved for the first time. Indeed, the negativity of this biomarker was able to identify HCC patients with longer overall and progression-free survival. Median survival was 48 mo for patients with low SCCA-IgM (< 130 AU/mL) and 26 mo for those with high SCCA-IgM (> 130 AU/mL). The levels of the biomarker at four weeks were stable or increased in treated patients with stable disease or tumor, and reduced in patients with complete response; patients with partial response showed an intermediate behavior. In the same study, AFP was not able to predict complete response. The significant impact of SCCA-IgM determination in defining patient prognosis was confirmed also by data showing that SCCA-IgM levels and tumor size were the only identified independent predictors of overall survival[53]. Although these findings must be confirmed in further studies, they are supported by recent data demonstrating that liver tumors with high SCCA-1 tissue expression exhibit higher early recurrence after surgical resection[38].

## FUTURE PERSPECTIVES

Recent innovations in antiviral therapy for HCV have resulted in a remarkable improvement in SVR rates, better acceptability, and decreased duration of treatment compared to IFN and ribavirin-based therapy[54]. The improvement in the antiviral efficacy of the new drug regimens promises higher cure rates with fewer side effects and shorter times of treatment compared to the old standard of care, but it is more expensive and requires major investments[55]. Although recent data have demonstrated that treatment of chronic HCV infection with one of the new oral drug regimens can reduce HCV-related complications and is cost-effective in most patients[56], treating all eligible patients could have an enormous economic impact for both private and public resources[56]. As a consequence, one of the emerging needs is to identify patients that will benefit the most from the new antiviral regimens[56]. Up to now, no biomarkers have been proposed to define the subset of HCV-infected patients with more aggressive disease. In a context of inadequate resources, SCCA-IgM could be used as a support tool to prioritize patients who will benefit the most from these new drugs.

In conclusion, although further studies are needed to confirm the data, serological SCCA-IgM is emerging as a very useful tool in different clinical settings of liver disease.

## REFERENCES


1 **Blachier M**, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; **58**: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]

2 **Tsochatzis EA**, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014; **383**: 1749-1761 [PMID: 24480518 DOI: 10.1016/S0140-6736(14)60121-5]

3 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]

4 **Lok AS**, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010; **138**: 493-502 [PMID: 19852963 DOI: 10.1053/j.gastro.2009.10.031]

5 **Trevisani F**, De Notariis S, Rapaccini G, Farinati F, Benvegnù L, Zoli M, Grazi GL, Del PP, Di N, Bernardi M. Semiannual and annual surveillance of cirrhotic patients for hepatocellular carcinoma: effects on cancer stage and patient survival (Italian experience). *Am J Gastroenterol* 2002; **97**: 734-744 [PMID: 11922571]

6 **Zhang BH**, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004; **130**: 417-422 [PMID: 15042359]

7 **McMahon BJ**, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, Dunaway E, Williams J. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. *Hepatology* 2000; **32**: 842-846 [PMID: 11003632]

8 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051]

9 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

10 **European Association For The Study Of The Liver**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

11 **Singal A**, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, Marrero JA. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009; **30**: 37-47 [PMID: 19392863 DOI: 10.1111/j.1365-2036.2009.04014.x]

12 **Poon D**, Anderson BO, Chen LT, Tanaka K, Lau WY, Van Cutsem E, Singh H, Chow WC, Ooi LL, Chow P, Khin MW, Koo WH. Management of hepatocellular carcinoma in Asia: consensus statement from the Asian Oncology Summit 2009. *Lancet Oncol* 2009; **10**: 1111-1118 [PMID: 19880065 DOI: 10.1016/S1470-2045(09)70241-4]

13 **Lee E**, Edward S, Singal AG, Lavieri MS, Volk M. Improving screening for hepatocellular carcinoma by incorporating data on levels of α-fetoprotein, over time. *Clin Gastroenterol Hepatol* 2013; **11**: 437-440 [PMID: 23247324 DOI: 10.1016/j.cgh.2012.11.029]

14 **Li C**, Zhang Z, Zhang P, Liu J. Diagnostic accuracy of des-gamma-carboxy prothrombin versus α-fetoprotein for hepatocellular carcinoma: A systematic review. *Hepatol Res* 2014; **44**: E11-E25 [PMID: 23834468 DOI: 10.1111/hepr.12201]

15 **Nakamura S**, Nouso K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, Toshikuni N, Tanaka H, Miyake Y, Matsumoto E, Shiratori Y. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *Am J Gastroenterol* 2006; **101**: 2038-2043 [PMID: 16848811]

16 **Schütte K**, Schulz C, Link A, Malfertheiner P. Current biomarkers for hepatocellular carcinoma: Surveillance, diagnosis and prediction of prognosis. *World J Hepatol* 2015; **7**: 139-149 [PMID: 25729470 DOI: 10.4254/wjh.v7.i2.139]

17 **Sterling RK**, Wright EC, Morgan TR, Seeff LB, Hoefs JC, Di Bisceglie AM, Dienstag JL, Lok AS. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. *Am J Gastroenterol* 2012; **107**: 64-74 [PMID: 21931376 DOI: 10.1038/ajg.2011.312]

18 **Wan HG**, Xu H, Gu YM, Wang H, Xu W, Zu MH. Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2014; **38**: 706-714 [PMID: 25034355 DOI: 10.1016/j.clinre.2014.06.008]

19 **Rubin B**. Natural immunity has significant impact on immune responses against cancer. *Scand J Immunol* 2009; **69**: 275-290 [PMID: 19281540 DOI: 10.1111/j.1365-3083.2008.02220.x]

20 **Vollmers HP**, Brändlein S. Natural antibodies and cancer. *J Autoimmun* 2007; **29**: 295-302 [PMID: 17826951]

21 **Castaldi F**, Marino M, Beneduce L, Belluco C, De Marchi F, Mammano E, Nitti D, Lise M, Fassina G. Detection of circulating CEA-IgM complexes in early stage colorectal cancer. *Int J Biol Markers* 2005; **20**: 204-208 [PMID: 16398401]

22 **Beneduce L**, Prayer-Galetti T, Giustinian AM, Gallotta A, Betto G, Pagano F, Fassina G. Detection of prostate-specific antigen coupled to immunoglobulin M in prostate cancer patients. *Cancer Detect Prev* 2007; **31**: 402-407 [PMID: 18035503]

23 **Marcello A**, Wirths O, Schneider-Axmann T, Degerman-Gunnarsson M, Lannfelt L, Bayer TA. Circulating immune complexes of Abeta and IgM in plasma of patients with Alzheimer's disease. *J Neural Transm* (Vienna) 2009; **116**: 913-920 [PMID: 19415450 DOI: 10.1007/s00702-009-0224-y]

24 **Beneduce L**, Castaldi F, Marino M, Tono N, Gatta A, Pontisso P, Fassina G. Improvement of liver cancer detection with simultaneous assessment of circulating levels of free alpha-fetoprotein (AFP) and AFP-IgM complexes. *Int J Biol Markers* 2005; **19**: 155-159 [PMID: 15255549]

25 **Zuin J**, Veggiani G, Pengo P, Gallotta A, Biasiolo A, Tono N, Gatta A, Pontisso P, Toth R, Cerin D, Frecer V, Meo S, Gion M, Fassina G, Beneduce L. Experimental validation of specificity of the squamous cell carcinoma antigen-immunoglobulin M (SCCA-IgM) assay in patients with cirrhosis. *Clin Chem Lab Med* 2010; **48**: 217-223 [PMID: 20001440 DOI: 10.1515/CCLM.2010.044]

26 **Suminami Y**, Kishi F, Sekiguchi K, Kato H. Squamous cell carcinoma antigen is a new member of the serine protease inhibitors. *Biochem Biophys Res Commun* 1991; **181**: 51-58 [PMID: 1958219]

27 **Kato H**, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. *Cancer* 1977; **40**: 1621-1628 [PMID: 332328]

28 **Kato H**. Expression and function of squamous cell carcinoma antigen. *Anticancer Res* 1996; **16**: 2149-2153 [PMID: 8694535]

29 **Catanzaro JM**, Sheshadri N, Pan JA, Sun Y, Shi C, Li J, Powers RS, Crawford HC, Zong WX. Oncogenic Ras induces inflammatory cytokine production by upregulating the squamous cell carcinoma antigens SerpinB3/B4. *Nat Commun* 2014; **5**: 3729 [PMID: 24759783 DOI: 10.1038/ncomms4729]

30 **Vidalino L**, Doria A, Quarta S, Zen M, Gatta A, Pontisso P. SERPINB3, apoptosis and autoimmunity. *Autoimmun Rev* 2009; **9**: 108-112 [PMID: 19332150 DOI: 10.1016/j.autrev.2009.03.011]

31 **Katagiri C**, Nakanishi J, Kadoya K, Hibino T. Serpin squamous cell carcinoma antigen inhibits UV-induced apoptosis via suppression of c-JUN NH2-terminal kinase. *J Cell Biol* 2006; **172**: 983-990 [PMID: 16549498]

32 **Murakami A**, Suminami Y, Hirakawa H, Nawata S, Numa F, Kato H. Squamous cell carcinoma antigen suppresses radiation-induced cell death. *Br J Cancer* 2001; **84**: 851-858 [PMID: 11259103]

33 **Suminami Y**, Nagashima S, Murakami A, Nawata S, Gondo T, Hirakawa H, Numa F, Silverman GA, Kato H. Suppression of

a squamous cell carcinoma (SCC)-related serpin, SCC antigen, inhibits tumor growth with increased intratumor infiltration of natural killer cells. *Cancer Res* 2001; **61**: 1776-1780 [PMID: 11280721]

34 **Suminami Y**, Nagashima S, Vujanovic NL, Hirabayashi K, Kato H, Whiteside TL. Inhibition of apoptosis in human tumour cells by the tumour-associated serpin, SCC antigen-1. *Br J Cancer* 2000; **82**: 981-989 [PMID: 10732775]

35 **Pontisso P**, Calabrese F, Benvegnù L, Lise M, Belluco C, Ruvoletto MG, Marino M, Valente M, Nitti D, Gatta A, Fassina G. Overexpression of squamous cell carcinoma antigen variants in hepatocellular carcinoma. *Br J Cancer* 2004; **90**: 833-837 [PMID: 14970861]

36 **Guido M**, Roskams T, Pontisso P, Fassan M, Thung SN, Giacomelli L, Sergio A, Farinati F, Cillo U, Rugge M. Squamous cell carcinoma antigen in human liver carcinogenesis. *J Clin Pathol* 2008; **61**: 445-447 [PMID: 17893121]

37 **Turato C**, Buendia MA, Fabre M, Redon MJ, Branchereau S, Quarta S, Ruvoletto M, Perilongo G, Grotzer MA, Gatta A, Pontisso P. Over-expression of SERPINB3 in hepatoblastoma: a possible insight into the genesis of this tumour? *Eur J Cancer* 2012; **48**: 1219-1226 [PMID: 21737255 DOI: 10.1016/j.ejca.2011.06.004]

38 **Turato C**, Vitale A, Fasolato S, Ruvoletto M, Terrin L, Quarta S, Ramirez Morales R, Biasiolo A, Zanus G, Zali N, Tan PS, Hoshida Y, Gatta A, Cillo U, Pontisso P. SERPINB3 is associated with TGF-β1 and cytoplasmic β-catenin expression in hepatocellular carcinomas with poor prognosis. *Br J Cancer* 2014; **110**: 2708-2715 [PMID: 24809782 DOI: 10.1038/bjc.2014.246]

39 **Quarta S**, Vidalino L, Turato C, Ruvoletto M, Calabrese F, Valente M, Cannito S, Fassina G, Parola M, Gatta A, Pontisso P. SERPINB3 induces epithelial-mesenchymal transition. *J Pathol* 2010; **221**: 343-356 [PMID: 20527027 DOI: 10.1002/path.2708]

40 **Villano G**, Quarta S, Ruvoletto MG, Turato C, Vidalino L, Biasiolo A, Tono N, Lunardi F, Calabrese F, Dall'olmo L, Dedja A, Fassina G, Gatta A, Pontisso P. Role of squamous cell carcinoma antigen-1 on liver cells after partial hepatectomy in transgenic mice. *Int J Mol Med* 2010; **25**: 137-143 [PMID: 19956912]

41 **Villano G**, Turato C, Quarta S, Ruvoletto M, Ciscato F, Terrin L, Semeraro R, Paternostro C, Parola M, Alvaro D, Bernardi P, Gatta A, Pontisso P. Hepatic progenitor cells express SerpinB3. *BMC Cell Biol* 2014; **15**: 5 [PMID: 24517394 DOI: 10.1186/1471-2121-15-5]

42 **Cannito S**, Turato C, Paternostro C, Biasiolo A, Colombatto S, Cambieri I, Quarta S, Novo E, Morello E, Villano G, Fasolato S, Musso T, David E, Tusa I, Rovida E, Autelli R, Smedile A, Cillo U, Pontisso P, Parola M. Hypoxia up-regulates SERPINB3 through HIF-2α in human liver cancer cells. *Oncotarget* 2015; **6**: 2206-2221 [PMID: 25544768]

43 **Turato C**, Calabrese F, Biasiolo A, Quarta S, Ruvoletto M, Tono N, Paccagnella D, Fassina G, Merkel C, Harrison TJ, Gatta A, Pontisso P. SERPINB3 modulates TGF-beta expression in chronic liver disease. *Lab Invest* 2010; **90**: 1016-1023 [PMID: 20212457 DOI: 10.1038/labinvest.2010.55]

44 **Biasiolo A**, Chemello L, Quarta S, Cavalletto L, Bortolotti F, Caberlotto C, Beneduce L, Bernardinello E, Tono N, Fassina G, Gatta A, Pontisso P. Monitoring SCCA-IgM complexes in serum predicts liver disease progression in patients with chronic hepatitis. *J Viral Hepat* 2008; **15**: 246-249 [PMID: 18248333 DOI: 10.1111/j.1365-2893.2007.00935.x]

45 **Bedossa P**, Moucari R, Chelbi E, Asselah T, Paradis V, Vidaud M, Cazals-Hatem D, Boyer N, Valla D, Marcellin P. Evidence for a role of nonalcoholic steatohepatitis in hepatitis C: a prospective study. *Hepatology* 2007; **46**: 380-387 [PMID: 17659580]

46 **Martini A**, Fattovich G, Guido M, Bugianesi E, Biasiolo A, Ieluzzi D, Gallotta A, Fassina G, Merkel C, Gatta A, Negro F, Pontisso P. HCV genotype 3 and squamous cell carcinoma antigen (SCCA)-IgM are independently associated with histological features of NASH in HCV-infected patients. *J Viral Hepat* 2015; **22**: 800-808 [PMID: 25611978 DOI: 10.1111/jvh.12394]

47 **McHutchison JG**, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER, Galati JS, Bacon BR, Davis MN, Mukhopadhyay P, Koury K, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; **361**: 580-593 [PMID: 19625712 DOI: 10.1056/NEJMoa0808010]

48 **Giannini EG**, Basso M, Bazzica M, Contini P, Marenco S, Savarino V, Picciotto A. Successful antiviral therapy determines a significant decrease in squamous cell carcinoma antigen-associated (SCCA) variants' serum levels in anti-HCV positive cirrhotic patients. *J Viral Hepat* 2010; **17**: 563-568 [PMID: 19840364 DOI: 10.1111/j.1365-2893.2009.01217.x]

49 **Fransvea E**, Trerotoli P, Sacco R, Bernabucci V, Milella M, Napoli N, Mazzocca A, Renna E, Quaranta M, Angarano G, Villa E, Antonaci S, Giannelli G. SCCA-IC serum levels are predictive of clinical response in HCV chronic hepatitis to antiviral therapy: a multicentric prospective study. *J Viral Hepat* 2012; **19**: 704-710 [PMID: 22967101 DOI: 10.1111/j.1365-2893.2012.01604.x]

50 **Prorok PC**. Epidemiologic approach for cancer screening. Problems in design and analysis of trials. *Am J Pediatr Hematol Oncol* 1992; **14**: 117-128 [PMID: 1530116]

51 **Pontisso P**, Quarta S, Caberlotto C, Beneduce L, Marino M, Bernardinello E, Tono N, Fassina G, Cavalletto L, Gatta A, Chemello L. Progressive increase of SCCA-IgM immune complexes in cirrhotic patients is associated with development of hepatocellular carcinoma. *Int J Cancer* 2006; **119**: 735-740 [PMID: 16550605]

52 **Buccione D**, Fatti G, Gallotta A, Loggi E, Di Donato R, Testa L, Saitta C, Santi V, Di Micoli A, Erroi V, Frigerio M, Fazio V, Picciotto A, Biasiolo A, Degos F, Pontisso P, Raimondo G, Trevisani F. Serum Scca-IgM as a Predictor of Hepatocellular Carcinoma in Patients with Liver Cirrhosis. *OJGas* 2012; **2**: 56-61 [DOI: 10.4236/ojgas.2012.22012]

53 **Pozzan C**, Cardin R, Piciocchi M, Cazzagon N, Maddalo G, Vanin V, Giacomin A, Pontisso P, Cillo U, Farinati F. Diagnostic and prognostic role of SCCA-IgM serum levels in hepatocellular carcinoma (HCC). *J Gastroenterol Hepatol* 2014; **29**: 1637-1644 [PMID: 24635038 DOI: 10.1111/jgh.12576]

54 **Alberti A**, Piovesan S. The evolution of the therapeutic strategy in hepatitis C: features of sofosbuvir and indications. *Dig Liver Dis* 2014; **46** Suppl 5: S174-S178 [PMID: 25458777 DOI: 10.1016/j.dld.2014.09.028]

55 **Younossi Z**, Henry L. The impact of the new antiviral regimens on patient reported outcomes and health economics of patients with chronic hepatitis C. *Dig Liver Dis* 2014; **46** Suppl 5: S186-S196 [PMID: 25458773 DOI: 10.1016/j.dld.2014.09.025]

56 **Chhatwal J**, Kanwal F, Roberts MS, Dunn MA. Cost-effectiveness and budget impact of hepatitis C virus treatment with sofosbuvir and ledipasvir in the United States. *Ann Intern Med* 2015; **162**: 397-406 [PMID: 25775312 DOI: 10.7326/M14-1336]

57 **Montagnana M**, Danese E, Lippi G. Squamous cell carcinoma antigen in hepatocellular carcinoma: Ready for the prime time? *Clin Chim Acta* 2015; **445**: 161-166 [PMID: 25840050 DOI: 10.1016/j.cca.2015.03.031]

58 **Rich N**, Singal AG. Hepatocellular carcinoma tumour markers: current role and expectations. *Best Pract Res Clin Gastroenterol* 2014; **28**: 843-853 [PMID: 25260312 DOI: 10.1016/j.bpg.2014.07.018]

**P- Reviewer**: Hokama A, Larrubia JR **S- Editor**: Song XX
**L- Editor**: A **E- Editor**: Liu SQ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com