Clinica Chimica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Squamous cell carcinoma antigen in hepatocellular carcinoma: Ready for the prime time?

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ARTICLE INFO

Article history:
Received 18 February 2015
Received in revised form 23 March 2015
Accepted 25 March 2015
Available online xxxx

Keywords:
Hepatocellular carcinoma
Liver cancer
Squamous cell carcinoma antigen
SCCA
Alpha-fetoprotein

ABSTRACT

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and the third cause of cancer deaths. The leading predisposing condition is represented by an underlying viral hepatitis, mainly sustained by hepatitis B and C viruses. Since the cumulative risk of developing HCC can be as high as 30-fold in patients with infectious cirrhosis, a timely diagnosis is necessary for establishing an appropriate treatment in these patients. The armamentarium of diagnostic and prognostic biomarkers in patients with HCC currently entails alpha-fetoprotein (AFP) and a limited number of innovative biomarkers, among which squamous cell carcinoma antigen (SCCA) and its immune complexes are among the most widely investigated. The clinical data published so far and reviewed in this article seemingly suggest that neither total serum SSCA or its isoform 1 (i.e., SCCA1) may be ready for the prime time for management of patients with HCC. More interesting evidence has emerged from studies investigating the serum values of SCCA–IgM, since the diagnostic performance of this biomarker was found to be frequently superior to that of AFP and, even more importantly, the combination of SCCA–IgM and AFP was characterized by a much better sensitivity than either biomarker alone, with only a modest decrease of specificity. Larger studies are needed before these preliminary findings can be generalized, but the combined use of AFP and SCCA–IgM represents an appealing perspective in diagnosis and prognostication of HCC.

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1. Introduction on hepatocellular carcinoma

Hepatocellular carcinoma (HCC), also known as malignant hepatoma, is the most common form of primary liver cancer, affecting as many as 600,000 persons worldwide and ranked third among the leading cause of cancer deaths [1]. The prevalence is reportedly higher (2- to 3-fold) in men than in women. A broad geographic variability has been observed for HCC, which is mainly attributable to a different distribution and natural history of the leading predisposing conditions, that are mainly represented by hepatitis B (HBV) and hepatitis C (HPC) virus infections [2]. More specifically, the age-standardized prevalence is the highest in eastern and south-eastern Asia (approximately 10 to 20:100,000) as well as in middle and western Africa (approximately 8 to 20:100,000), intermediate in Southern Europe (approximately 3 to 10:100,000), and the lowest in developed countries such as those of the Americas, Australia and northern Europe (approximately 2.5 to 7.5:100,000). Importantly, the incidence of HCC has

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http://dx.doi.org/10.1016/j.cca.2015.03.031
0009-8981/

sharply increased in the past two decades in several developed countries as a consequence of a typical "cohort effect" attributable to the previous spread of HBV and HBC epidemics between the 1950s and the 1980s [2].

The leading predisposing factor for HCC is indeed represented by an underlying viral hepatitis, mainly sustained by HBV and HCV. This hypothesis is supported by the fact that chronic liver infection may trigger carcinogenesis by a recurrent turnover of hepatocytes, which is cause of inflammation and liver injury, and ultimately predisposes to development of liver cirrhosis. Accordingly, the prevalence of HBV-related HCC varies from 10% in high income countries up to 80% in low income countries, whereas that of HCV-related HCC varies from 20% in high income countries up to 30% in low income countries [2]. Italy is a notable exception of developed country with high incidence of HCV-related HCC, exhibiting a rate as high as 60% (compared to 13% of HBV-related HCC) [3]. Beside chronic viral hepatitis, which is per se responsible of over 70% of all forms of HCC around the globe, additional causes of cirrhotic liver disease have been identified, including alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), hereditary hemochromatosis, primary biliary cirrhosis, alpha-1-antitrypsin deficiency, oral contraceptives and aflatoxin poisoning, whose contributing rates to HCC are not clearly determined or exhibit broad worldwide heterogeneity (Table 1) [2,4]. All these conditions may trigger hepatocarcinogenesis through disruption of common genetic pathways including transforming growth factor-beta (TGF-β), epithelial cell adhesion molecule (EpCAM), Wnt/β-catenin, Notch and Hedgehog signaling systems [5].

Overall, the cumulative risk of developing HCC has been reported to be the highest (i.e., 20–30 fold) in patients with HCV-related cirrhosis and in those with hereditary hemochromatosis (i.e., 20-fold), intermediate in patients with primary biliary cirrhosis (i.e., 10–20) and HBV-related cirrhosis (i.e., 10–20 fold) or aflatoxin poisoning (i.e., 2–20 fold), and lower in all the other predisposing conditions [6]. In patients with viral-related cirrhosis, concomitant infection with HBV and HCV, or with HBV and HDV, increases by 2- to 6-fold the risk related to each infection alone, whereas alcohol abuse increases by 2- to 4-fold the risk compared with alcohol abstinence [6].

The prognosis of HCC is generally poor but varies widely around the globe, and this is mostly attributable to the underlying conditions and available therapeutic options (i.e., liver transplantation, radiofrequency ablation, chemoembolization). Accordingly, the 5-year survival of HCC patients is approximately 12% to 15% in US and Europe, but substantially decreases to 5% in low-income countries [2].

A timely diagnosis is necessary for establishing an early diagnosis and an appropriate treatment in these patients. The armamentarium of diagnostic and prognostic biomarkers in patients with HCC currently entails AFP and few other and less conventional tests. With the use of the traditional diagnostic thresholds (i.e., between 50 and 50 ng/mL), the leading drawbacks of AFP in the screening of HCC are represented by the low diagnostic sensitivity (as many as 40% HCCs do not produce suggestive amounts of AFP), combined with an equally unsatisfactory diagnostic specificity (values exceeding 200–400 ng/mL are not unusual in patients with chronic active HCV infection, liver toxic injury and other forms of hepatitis) [7]. This evidence led the way to a number of studies aimed to identify additional or complementary biomarkers, which may be useful in the clinical management of HCC patients [7], and mainly include s-carboxyprothrombin (DCP), EGF homology domains 2, interleukin-6 (IL-6), osteopontin, Golgi protein-73 (GP73), and, last but not least, circulating miRNAs [8–10]. Most of these biomarkers are in phase III studies, and they cannot be routinely offered to the clinical laboratories since their measurement still requires challenging, expensive and time consuming techniques.

Table 1
Leading predisposing factors for hepatocellular carcinoma (HCC) worldwide.

Cause	HCC—attributable rate	HCC—increased risk
Hepatitis B	10–80%	10–20 fold
Hepatitis C	10–30%	20–30 fold
Alcoholic liver disease	10–20%	2–4 fold
Hereditary hemochromatosis	<5%	20-fold
Primary biliary cirrhosis	<5%	10–20 fold
Oral contraceptives	<5%	2–3 fold
Non-alcoholic fatty liver disease (NAFLD)	30–40%	2–4 fold
Aflatoxin poisoning	<5%	2–20 fold

2. Genetics and biology of squamous cell carcinoma antigen

Recent molecular studies revealed that the squamous cell carcinoma antigen (SCCA) actually consists of two highly homologous proteins (SCCA1 and SCCA2) belonging to the serine protease inhibitors family. These proteins are encoded by two 1711 bp-long, tandemly arranged genes (*SCCA1* and *SCCA2*) located on chromosome 18q21.3, and alternatively known as serpin peptidase inhibitor, clade B (ovalbumin), member 3 gene (*SERPINB3*) or serpin peptidase inhibitor, clade B (ovalbumin), member 4 gene (*SERPINB4*) [11]. These two genes, which share a high degree of homology (up to 98%), encode for two 45 kDa, 390 amino acid-long proteins of the same name, which are 92% identical at the amino acid level. The first isoform, SCCA1, is neutral while the second isoform, SCCA2, is acidic [12,13]. Importantly, a third genetic variant of SCCA has also been identified in a minority of HCC cases, which is characterized by a G351A polymorphism in the reactive core of the protein [14].

A low level of genetic and biochemical differences between SCCA1 and SCCA2 predictably results in a modest variation in their biological activity: SCCA1 exerts inhibitory effects on serine proteinase and cysteine proteinase, while SCCA2 mainly inhibits serine proteinase, chymase and cathepsin G [15]. The SCCA is normally expressed by basal and parabasal layers of normal squamous epithelium, but is found to be overexpressed in epithelia of cancerous tissue [16]. By means of using a reverse transcription-polymerase chain reaction-based technique, Stenman et al. showed that although both proteins are co-expressed in normal and cancerous tissues, the acidic isoform SCCA2 is present in larger amount in the circulation of cancer patients [17].

Reliable evidence attests that *SERPINB3/4* expression progressively increases across a continuum of precancerous diseases, from chronic liver disease [18], dysplastic nodules [19] up to HCC [14], thus suggesting that these genes may be strongly involved in the complex process of hepatocarcinogenesis. In a recent study entailing direct assessment of both SCCA1 and SCCA2 mRNA expression in 93 HCCs, 93 paired adjacent non-cancerous tissues, 16 cirrhosis livers and 9 normal livers, Li et al. reported that total SCCA mRNA could be detected in 33% HCC tissues, in 9% adjacent non-cancerous tissues and 22% normal liver tissues, whereas no expression was detected in cirrhosis liver tissues, a fact that could be attributed to the low prevalence of viral-driven cirrhosis in this subset of patients [20]. Interestingly, the rate of SCCA2 expression was also confirmed to be higher (by nearly 3-fold) than that of SCCA1. The *SERPINB3/4* genes are also seemingly overexpressed in a subset of more aggressive forms of primary liver cancer characterized by early tumor recurrence [21], thus representing appealing genetic markers in patients with HCC.

A potential explanation for the overexpression of SCCA in HCC has recently been provided by Cannito et al. [22], who showed that hypoxia inducible factor (HIF)-2α, a protein frequently overexpressed in malignancy and strongly involved in the pathogenesis of cancer growth and spread, is effective to up-regulate transcription, synthesis and release of SCCA. Although the biological interplay between SERPINB3/4 and cancer has not been fully elucidated, hypothesis exists that these proteins may exert anti-apoptotic and proliferative effects by targeting the cascade upstream of caspase 3 or inhibiting tumor suppressive

Please cite this article as: Montagnana M, et al, Squamous cell carcinoma antigen in hepatocellular carcinoma: Ready for the prime time?, Clin Chim Acta (2015), http://dx.doi.org/10.1016/j.cca.2015.03.031