HEPA-IC

code XG003

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

Hepatocellular Carcinoma (HCC) is one of the most frequent and lethal cancer forms worldwide, ranking four for incidence rate. Its prognosis is very poor, with less than 5% survival rate after five years from diagnosis. Early detection of HCC is still difficult due to the lack of adequate biomarkers to clearly differentiate HCC from benign liver lesion with high sensitivity and high specificity. The most widely used serologic marker to detect HCC is Alpha-Fetoprotein (AFP), which is elevated in a wide number of HCC patients (30-60%) but with low specificity (70-80%).

A new biomarker for HCC, Squamous Cell Carcinoma Antigen (SCCA) variants, has been recently identified in all surgically resected HCC but in none of the control normal livers, as detected by immunohistochemistry (figure 1) with anti-SCCA variants antibody (Hepa-Ab, Xeptagen) (1).

In HCC patient sera, SCCA variants are detected as circulating immune complexes (SCCA-IgM) with 70% sensitivity and 100% specificity versus healthy subjects. (2, 3, 4). Hepa-IC is a highly specific and sensitive ELISA assays for HCC detection designed to measure SCCA-IgM in patients sera (2, 3).

By using Hepa-IC the vast majority of HCC samples (70%) are strongly reactive (mean±SD = 2568.5±6797.3 AU/mL), while all healthy controls are negative (<120 AU/mL), expressing SCCA-IgM concentration in Arbitrary Units (AU) with a reference standard (figure 2). In cirrhotic patients SCCA-IgM are detected in 26% of cases but at lower levels (mean±SD = 147.5±348.3 AU/mL). Patients with chronic hepatitis C in only 18% of cases display presence of SCCA-IgM but at very low levels (mean±SD = 39.5±89.7 AU/mL). No correlation is found with AFP levels, which are significantly elevated (> 20 ng/mL) only in c.a. 42% of HCC patients. By using an AFP cut off value of 20 or 100 ng/mL, 96 or 80% respectively of HCC patients are positive for at least one marker. Table 1 shows a comparison of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) between SCCA-IgM and AFP levels in patients with liver pathologies and normal control.

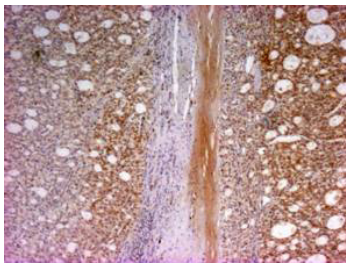

Figure 1: Histochemical appearance of Hepatocellular Carcinoma (HCC) nodules stained with anti-SCCA variants antibody (Hepa-Ab, Xeptagen)

SCCA-IgM may be used to monitor patients with cirrhosis evolving to HCC. Retrospective longitudinal studies have shown that SCCA-IgM increases over time in cirrhotic patients evolving to HCC while does not increase in cirrhotic patients not progressing to liver cancer (5, 6). In patients with chronic hepatitis of viral etiology SCCA-IgM complex behavior is associated with treatment response. In patients with sustained response, pretreatment abnormal levels of the immune complex become persistently negative after treatment, while remain unchanged or increased in those who are not responder to antiviral therapy.

HCC Early Detection
Surveillance of Cirrhotic Patients
Monitoring of Treatment

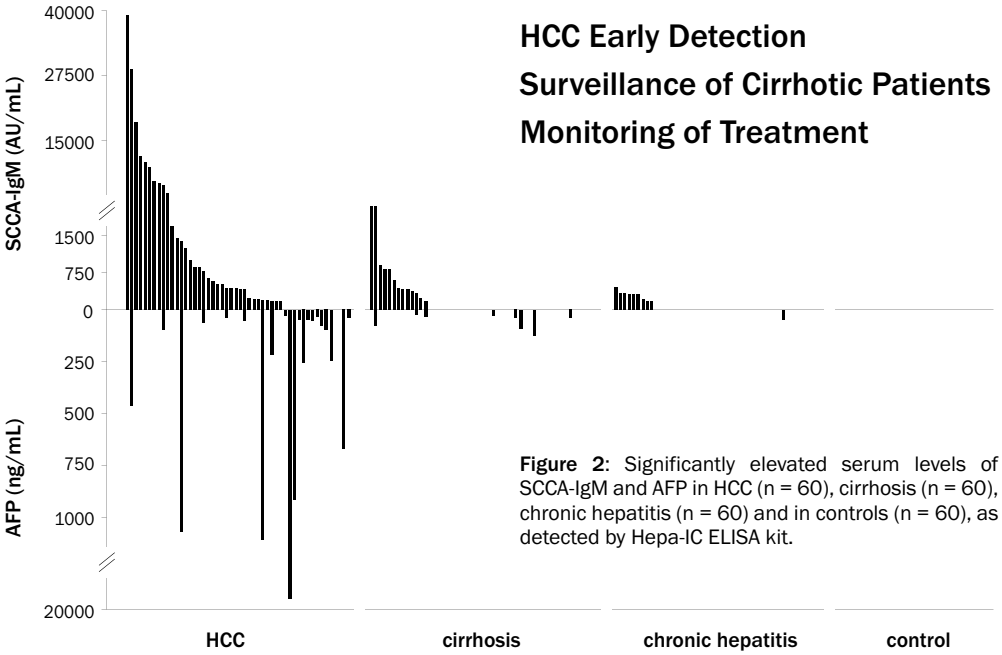

Figure 2: Significantly elevated serum levels of SCCA-IgM and AFP in HCC (n = 60), cirrhosis (n = 60), chronic hepatitis (n = 60) and in controls (n = 60), as detected by Hepa-IC ELISA kit.

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BIOMARKER	Sens	Spec	PPV	PNV
SCCA-IgM 120 AU/mL				
HCC vs Control		100%	100%	77%
HCC vs CR	70%	74%	73%	71%
HCC vs CH		82%	80%	73%
AFP 20 ng/mL				
HCC vs Control		100%	100%	63%
HCC vs CR	42%	84%	72%	59%
HCC vs CH		98%	95%	63%

Table 1: Comparison of sensitivity (Sens), specificity (Spec), positive predictive value (PPV), and negative predictive value (NPV) of SCCA-IgM and AFP, in differentiation of patients with HCC from those with cirrhosis (CR), chronic hepatitis (CH) and healthy subjects (Control).

REFERENCES

1. Pontisso P. *et al.* Br J Cancer, 2004, 90:833-7
2. Beneduce L. *et al.* J Hepatol, 2004, 40 (suppl.1):7
3. Beneduce L. *et al.* Dig Liver Dis, 2004, 36:A2-A3
4. Beneduce L. *et al.* Cancer, 2005, 103:2558-65
5. Pontisso P. *et al.* Int J Cancer, 2006, 119:735-40
6. Biasiolo A. *et al.* J Viral Hepat, 2008, 15:246-249

REAGENTS AND MATERIALS PROVIDED

XG003-PL: 96 wells multi-strip Assay-Plate, pre-coated with affinity purified rabbit anti-SCCA
XG003-Calibrator: Two vials of calibrator lyophilized from PBS. White powder. Exact concentration on label. Totally soluble.
XG-EA: 1.1 mL of Enzyme-conjugated goat anti-human IgM secondary antibody (Green cap) 10-fold concentrate solution in PBS containing 1% BSA.
XG-CH4: Chromogen: ABTS (2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic-acid)); Buffer: phosphate citrate. Light green tablet + white powder lyophilized from 0.05 M phosphate-citrate buffer, pH 5. Totally soluble.
XG-SB: 200 µL of Enzyme substrate: hydrogen peroxide solution 30% (w/w) in water. (Blue cap)
XG-DB5: Concentrated Dilution Buffer solution 5X, 10 mL. Once diluted, the working solution contains 1% BSA and 0.05% Tween 20 in PBS. The solution contains Proclin as preservative.
XG-WB2: Lyophilized Washing Buffer. Two white tablets. Once diluted, the working solution contains 0.05% Tween 20 in PBS. Totally soluble.

MATERIAL AND EQUIPMENT REQUIRED

Precision pipettes with disposable tips
Microplate washer
Microplate reader with a 405 ± 20 nm filter
Distilled or deionized water

BRIEF DESCRIPTION OF PROCEDURE

Calibration curve and samples : Reconstitute the lyophilized XG003-Calibrator with 440 µL of distilled water. Dispense 100 µL/well of standard calibrator (in duplicate), starting from the reconstituted solution and performing in-plate 2-fold serial dilutions in order to obtain a five-point calibration curve. Use XG-DB5 dilution buffer as diluent. For exact concentration of the reconstituted calibrator please refer to the concentration value (AU/mL) indicated on the XG003-Calibrator vial.
Dispense 100 µL/well of 8-fold diluted samples. Use XG-DB5 dilution buffer as diluent. Also dispense 100 µL/well of XG-DB5 dilution buffer as blank. Incubate the plate for 1h at room temperature. Wash 6x with XG-WB2 washing buffer.

Secondary antibody: Add 100 µL/well of XG-EA enzyme-conjugated antibody. Incubate 1h at room temperature. Wash 6x with XG-WB2 washing buffer.

ABTS substrate solution: Add 150 µL/well of freshly prepared ABTS substrate. Allow colour to develop in the dark at 37°C and then measure OD values of each well using an ELISA plate reader set to 405 nm.

PROCESSING OF THE RESULTS

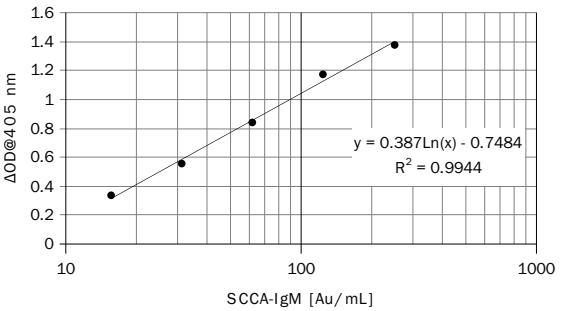

Figure 3: Range of linearity of a typical standard curve for SCCA-IgM after 20 minutes of substrate incubation.

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