ORIGINAL ARTICLE

Case Control Study

Combination of squamous cell carcinoma antigen immunocomplex and alpha-fetoprotein in mid- and long-term prediction of hepatocellular carcinoma among cirrhotic patients

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Abstract

BACKGROUND

The combination of alpha-fetoprotein (AFP) and squamous cell carcinoma antigen immunocomplex (SCCA-IgM) have been proposed for its use in the screening of hepatocellular carcinoma (HCC). Current screening programs for all cirrhotic patients are controversial and a personalized screening is an unmet need in the

precision medicine era.

AIM
To determine the role of the combination of SCCA-IgM and AFP in predicting mid- and long-term appearance of HCC.

METHODS
Two-hundred and three cirrhotic patients (Child A 74.9%, B 21.2%, C 3.9%) were followed-up prospectively every six months to screen HCC by ultrasound and AFP according to European Association for the Study of the Liver guidelines. The estimation cohort was recruited in Italy (30.5%; 62/203) and validation cohort from Spain (69.5%; 141/203). Patients underwent to evaluate SCCA-IgM by enzyme-linked immunosorbent assay (Hepa-IC, Xeptagen, Italy) and AFP levels at baseline. Patients were followed-up for 60 mo, being censored at the time of the appearance of HCC.

RESULTS
There were 10.8% and 23.1% of HCC development at two- and five-years follow-up. Patients with HCC showed higher levels of SCCA-IgM than those without it (425.72 ± 568.33 AU/mL *vs* 195.93 ± 188.40 AU/mL, $P = 0.009$) during the five-year follow-up. In multivariate analysis, after adjusting by age, sex, aspartate transaminase and Child-Pugh, the following factors were independently associated with HCC: SCCA-IgM [Hazard ratio (HR) = 1.001, 95%CI: 1.000-1.002; $P = 0.003$], AFP (HR = 1.028, 95%CI: 1.009-1.046; $P = 0.003$) and creatinine (HR = 1.564 95%CI: 1.151-2.124; $P = 0.004$). The log-rank test of the combination resulted in 7.488 ($P = 0.024$) in estimation cohort and 11.061 ($P = 0.004$) in the validation cohort, and a 100% of correctly classified rate identifying a low-risk group in both cohorts in the two-year follow-up.

CONCLUSION
We have constructed a predictive model based on the combination of SCCA-IgM and AFP that provides a new HCC screening method, which could be followed by tailored HCC surveillance for individual patients, especially for those cirrhotic patients belonging to the subgroup identified as low-risk of HCC development.

Key Words: Squamous cell carcinoma antigen; Hepatocellular carcinoma prediction; Precision medicine; Stratification of cirrhotic patient

Core Tip: Current screening programs of hepatocellular carcinoma (HCC) for all cirrhotic patients are controversial and a personalized strategy is an unmet need in the precision medicine era. By studying circulating biomarkers in two-hundred and three cirrhotic patients followed-up for 60 mo, we found that the combination of circulating alpha-fetoprotein and squamous cell carcinoma antigen immunocomplex resulted in a 100% of correctly classified rate identifying a low-risk group of HCC at two years of follow-up in two different cohorts. This predictive model provides a new screening method, which could be followed by tailored HCC surveillance for individual patients.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common malignant primary liver tumor and the second leading cause of cancer-related death in the world, according to the World Health Organization[1].

Up to 90% of HCCs in the Western world seem to occur in patients with cirrhosis, with an annual incidence ranging from 2% to 4% with differences in age, gender, etiology and duration of the cirrhosis[2,3]. According to the Barcelona Clinic Liver Cancer stratification, patients diagnosed on stage 0 and A of HCC have a tremendously better five-year HCC-free rate (93%) than those patients diagnosed on the advanced stage (5%) due to the availability of curative therapies such as surgical resection or liver transplantation[4]. However, the vast majority of HCC patients are diagnosed at advanced stages[5] and only a small proportion of new HCC patients are diagnosed through the surveillance[6]. Tumor stage at diagnosis can be impacted by several factors in clinical practice, including low surveillance rates and compliance and delays in follow-up of abnormal screening tests[4]. Therefore, in order to diagnose HCC at the early stage, besides having an accurate diagnostic tool, an appropriate strategy of HCC surveillance specifically focusing on well-defined high-risk population is essential and indispensable.

Current guidelines[7,8] recommend HCC screening by abdominal ultrasound at 6-month intervals in cirrhotic patients. However, the practice guideline-recommended "one-size-fits-all" HCC screening program for early tumor detection is performed in less than 20% of the target population and its implementation in clinical practice is far from satisfactory due to multiple patient- and provider-related factors[9]. More importantly, the risk of developing HCC is likely not uniform across all cirrhotic patients[10,11]. Therefore, an individual HCC risk prediction followed by tailoring the personalized surveillance strategy is expected to overcome the challenge in the era of precision medicine[9,12].

SERPINB3 and SERPINB4, formerly known as squamous cell carcinoma antigen 1-2 (SCCA1/2), are two isoforms of Clade B Serine Protease Inhibitors that are found physiologically in the spinous and granular layers of normal squamous epithelium such as tongue, esophagus, lung and uterus among others, while become highly expressed in squamous cell carcinomas of these organs[13,14]. Recent evidences found the plasma levels of both SCCA[15] and immunoglobulin M complex (SCCA-IgM)[16] associated with liver tumor development, suggesting that monitoring of SCCA and SCCA-IgM levels might be useful for identifying cirrhotic patients at higher risk of developing HCC[15]. A large number of studies further supported the usefulness of SCCA-IgM for the diagnosis[17] and monitoring of chronic liver disease[18-20] including the histological response after antiviral treatments. A recent meta-analysis concluded that both SCCA and SCCA-IgM had a similar moderate diagnostic accuracy (0.7-0.9) for HCC screening; however, a combination of SCCA and SCCA-IgM was the best diagnostic option[17]. Pozzan *et al*[21] proved that SCCA-IgM alone was able to predict HCC-free and progression-free survival for intermediate-stage patients treated by transcatheter arterial chemoembolization. Lately, Biasiolo *et al*[22] showed that SCCA-IgM alone but not AFP was significant to predict the HCC-free survival in a prospective cohort. However, the previous study did not assess the combination of SCCA-IgM and AFP, and there was no external validation study that further confirmed those results. More importantly, the majority of previous studies were performed only in Italian cohorts with a dominant hepatitis C etiology by a uni-center design. The present study aims to evaluate the potential role of the combination of SCCA-IgM and AFP as a biomarker in the mid-term and long-term prediction of HCC among patients with cirrhosis by using a multi-center and internal-external-validation study design.

MATERIALS AND METHODS

Patients

From January 2007 to March 2016, 62 cirrhotic patients (30.5%; 62/203) were enrolled from the outpatient clinics of the *Azienda Ospedaliera di Padova* (Padova, Italy) as estimation cohort and 155 cirrhotic patients (69.5%; 141/203) were included at Valme University Hospital (Seville, Spain) as validation cohort. The study was retrospectively performed on prospectively collected sera. Patients were followed-up every six months for HCC screening according to European Association for the Study of the Liver guidelines[7]. The study was performed by following the ethical guidelines

expressed in the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. Human samples were collected after obtaining a signed informed consent as approved by the Ethical Committee of both hospitals.

Cirrhosis was diagnosed by documenting at least one of the following: clinical (esophageal varices, liver dysfunction, or previous ascites or variceal bleeding), pathological (liver biopsy) or radiological (coarse/nodular/lobar redistribution on ultrasound) markers of cirrhosis. Demographic, clinical and laboratory parameters were recorded at the first visit including age, sex, etiology of cirrhosis, aspartate transaminase (AST), alanine aminotransferase, bilirubin, albumin, creatinine and platelet levels. Patients with both chronic viral hepatitis and a history of alcohol intake were categorized as having viral hepatitis. Similarly, patients with steatohepatitis were included as alcoholic cirrhosis if alcohol was determined as the cause of liver disease in the clinical record. Non-alcoholic steatohepatitis, as well as autoimmune liver diseases such as autoimmune hepatitis, primary biliary cirrhosis or primary sclerosing cholangitis, were categorized as "Others". Follow-up time was censored at the last clinic visit, death, liver transplantation or diagnosis of HCC within the term of 60 mo. HCC was diagnosed without biopsy in the majority of the cases because of current clinical diagnostic approaches, including ultrasonography, computed tomography, magnetic resonance imaging were sufficient to diagnose HCC[7,8].

Sample storage and assays

Peripheral blood sample was collected from each patient at the time of the first clinic visit. Plasma and serum aliquots were stored in cryovials at -80°C after centrifugation for 10 min at 1500 ×*g* at 4°C. Serum AFP and SCCA-IgM were measured for each patient by an experienced technician who was blind to the clinical information. AFP levels were determined by an electrochemiluminescence immunoassay using an automatized analyzer Elecsys (Roche, Switzerland) and SCCA-IgM was measured in duplicate using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (Hepa-IC, Xeptagen, Venice, Italy). The amount of SCCA-IgM immune complexes was expressed in arbitrary units (AU)/mL by interpolation of samples absorbance on the calibration curves plotted with SCCA-IgM calibrators.

Statistical analysis

Cox proportional hazards regression was used to estimate the hazard ratio (HR) and CI. Comparisons between categorical variables were made by the Chi-square or Fisher test. Results are presented as frequencies and percentages for categorical variables, means ± SDs for normal continuous variables and median, quartile 1 and 3 for not normal continuous variables. Missing data was listwise deleted (complete-case analysis). Those factors showing statistical ($P < 0.05$) association to HCC in univariate analyses were combined in a backwards stepwise multivariable model. Factors not significant but of potential clinical relevance such as age and sex were also included in order to avoid confounding. In the estimation cohort, we used two-year follow-up data to perform the univariate and multivariate analysis to assess the factors independently associated with HCC-free survival because cirrhotic patients need to be screened at least every two years. Akaike's information criterion (AIC) was additionally computed to select the most robust predictors. The predictive cut-off of SCCA-IgM was established by means of receiver operating characteristic (ROC) curve method at a value that maximized specificity and sensitivity according to Youden index. The same AFP cut-off value derived from estimation cohort (5 ng/mL) was used in validation cohort. Categorical variables were compared by means of the Kaplan-Meier method, with curves compared using the log-rank test. The Harrell's concordance index (C-index) was used to assess the score's discrimination ability. C-index values and the corresponding 95%CIs were estimated for each main study time point. The sensitivity, specificity, positive predictive value and negative predictive value were calculated to demonstrate the predictive ability. SPSS (version 25.0; SPSS Inc., IL, the United States) and Stata 11 (StataCorp, College Station, TX) statistical packages were used.

RESULTS

Identification of the study cohort and baseline characteristics

The baseline characteristics and biochemical parameters of the overall cohort, as well as estimation and validation cohorts, are shown in Table 1. Briefly, a total of 203 patients with liver cirrhosis were included in the study, with 74.9% Child-Pugh A, 21.2%B, and 3.9% Child-Pugh C. The most common etiology of cirrhosis was alcohol (54.2%), followed by HCV (27.1%) and HBV (8.4%). HCC development was observed in 22 patients (10.8%) during the two-year follow-up (22.1 ± 5.11) and 47 patients (23.2%) during the five-year follow-up (41.9 ± 16.0 mo). The baseline values of serum SCCA-IgM were significantly higher in patients who developed HCC than in those who did not (514.17 ± 714.43 AU/mL *vs* 216.92 ± 233.51 AU/mL, *P* < 0.001) during the two-year follow-up, as well as AFP (23.91 ± 41.37 ng/mL *vs* 6.16 ±10.49 ng/mL, *P* < 0.001).

Identification of risk factors for HCC development

Univariate analysis showed that the levels of SCCA-IgM (*P* = 0.004), AFP (*P* < 0.001), AST (*P* = 0.021) and creatinine (*P* = 0.018) were associated with two-year HCC-free survival in the estimation cohort (Table 2). Nevertheless, Child-Pugh classification, platelets count and other biochemical parameters were similar between both groups of patients. By using a multivariate Cox regression, after adjusting for age, gender, AST and Child-Pugh, SCCA-IgM (HR = 1.001, 95%CI: 1.000-1.002; *P* = 0.003), AFP (HR = 1.028, 95%CI: 1.009-1.046; *P* = 0.003) and creatinine (HR = 1.564, 95%CI: 1.151-2.124; *P* = 0.004) were independently associated with increased two-year risk of HCC.

Internal estimation of the combination of SCCA-IgM and AFP

After multivariate analysis, the model including SCCA-IgM, AFP and creatinine was the most robust for the prediction of HCC development (AIC: 44.83); however, no statistical significance was observed in ROC curve analysis (*P* = 0.234) so the second model consisting of the combination of SCCA-IgM and AFP was chosen (AIC: 55.54). Therefore, we performed ROC curve to explore the ability of SCCA-IgM and AFP in predicting the patients with cirrhosis to develop HCC during the two-year follow-up. By establishing a cut-off of 124 AU/mL for SCCA-IgM (sensitivity of 75% and specificity of 76%) and using a cut-off of 5 ng/mL for AFP (sensitivity of 75% and specificity of 48%), we obtained AUROCs of 0.74 (95%CI: 0.55-0.93; *P* = 0.029) and 0.73 (95%CI: 0.52-0.95; *P* = 0.034), respectively. However, although the predictive ability of the combination of SCCA-IgM and AFP was also significant [AUROC 0.77 (95%CI: 0.63-0.92; *P* = 0.013)], we observed no statistical significance when comparing the combinatory model to SCCA-IgM (*P* = 0.669) or AFP (*P* = 0.715) alone (Figure 1).

This combination allowed us to stratify the cohort into low-risk group (AFP < 5 ng/mL and SCCA-IgM < 124 AU/mL), intermediate-risk group (AFP > 5 ng/mL or SCCA-IgM > 124 AU/mL) and high-risk group (AF P> 5 ng/mL and SCCA-IgM > 124 AU/mL). The predicted mean survival curves were compared by Kaplan-Meier at two- and five-years follow-up in the estimation cohort (Figure 2). Notably, we found that the low-risk group that was stratified by the combination of SCCA-IgM and AFP correctly identified a 100% of HCC-free survival rate in two-year followed-up which was further confirmed in the five-year follow-up (100%) (Figure 2C).

External validation

The same cut-off values were used for the validation cohort to confirm the results of the predictive ability of HCC-free survival. Again, the low-risk group showed a 100% of two-year and 96.2% of five-year follow-up of HCC-free survival rate (Figure 3C). However, there were no differences between the combination and SCCA-IgM or AFP alone in the comparative C-index estimates for the validation data cohort (Table 3), as are the results of the confirmatory analysis of the predictive ability of both the two- and five-year HCC-free survival.

For practical applications, we calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value and likelihood ratio (LR) of the combination of SCCA-IgM and AFP to demonstrate the predictive ability (Table 4). An LR- of 0 were obtained in both estimation and validation cohort in two-year follow-up, so the low-risk group of patients who did not develop HCC could be accurately ruled-out. The correctly classified rate increased from 75.3% (estimation cohort) to 78.8% (validation cohort) in two-year follow-up and from 61.1% (estimation cohort) to 68.5% (validation cohort) in five-year follow-up.

Table 1 Characteristics of included patients

	Global (*n* = 203)	Italian (*n* = 62) (Estimation cohort)	Spanish (*n* = 141) (Validation cohort)	Univariable analysis
Gender (Male)	73.4% (149/203)	74.2% (46/62)	73.0% (103/141)	0.865
mean age (yr)	57.93 ± 9.76	55.77 ± 10.51	58.87 ± 9.22	
Etiology				0.001
Alcohol	54.2% (110/203)	41.9% (26/62)	59.6% (84/141)	
HCV	27.1% (55/203)	38.7% (24/62)	22.0% (31/141)	
HBV	8.4% (17/203)	16.1% (10/62)	5% (7/144)	
Others	10.3% (21/203)	3.2% (2/62)	13.5% (19/141)	
Child-Pugh				0.340
A	74.9% (152/203)	64.5% (40/62)	79.4% (112/141)	
B	21.2% (43/203)	27.4% (17/62)	18.4% (26/141)	
C	3.9% (8/203)	8.1% (5/62)	2.1% (3/141)	
AST (IU/mL)	51.69 ± 38.49	69.17 ± 47.74	44.50 ± 31.44	0.001
ALT (IU/mL)	42.54 ± 38.68	61.09 ± 56.11	34.91 ± 25.16	0.000
Tot. Bilirubin (mg/dL)	1.60 ±1.87	1.94 ± 2.87	1.45 ± 1.22	0.215
Creatinine (mg/dL)	0.86 ± 0.68	0.98 ± 1.21	0.81 ± 0.22	0.292
Platelets (× 10^9/mL)	116.00 ± 58.10	100.53 ± 43.11	122.45 ± 62.32	0.005
Albumin (mg/dL)	3885.19 ± 586.66	3810.34 ± 613.79	3916.34 ± 574.96	0.248
AFP (ng/mL)	8.09 ± 17.50	12.00 ± 25.43	6.69 ± 12.82	0.101
SCCA-IgM (AU/mL)	249.13 ± 332.01	197.73 ± 431.13	271.73 ± 276.35	0.144
Two-year HCC (Yes)	10.8% (22/203)	12.9% (8/62)	9.9% (14/141)	0.530
Five-year HCC (Yes)	23.2% (47/203)	21.0% (13/62)	24.1% (34/141)	0.625

Comparisons between groups were made using the Mann-Whitney *U* test or the Student *t*-test for continuous variables, and the χ^2 test or the Fisher's exact test for categorical data. *P* values represent the statistical significance of the differences between both subsets. Data are expressed as numbers of patients (%) or mean ± SD. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HCV: Hepatitis C virus; HBV: Hepatitis B virus; AFP: Alpha-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen and its immune complexes; Child-Pugh: The Child–Turcotte–Pugh score or Child Criteria; HCC: Hepatocellular carcinoma.

DISCUSSION

In the present study, we revealed an enhanced HCC risk assessment by using the combination of SCCA-IgM and AFP serum levels. A low-risk subgroup of cirrhotic patients with 100% of internal-external validated two-year follow-up (mid-term) of HCC-free survival rate was correctly identified. This strategy may enable to personalize intensity of HCC screening. Moreover, a high HCC-free survival rate (96.2%) at five-year follow-up (long-term) further confirmed our proposed surveillance strategy with patients at low-risk of HCC development. Although prior studies have proposed SCCA-IgM for HCC prediction[21,22], our study is the first to internal-externally validate the proposed biomarkers. Validation is an important aspect of predictive model development, because of the performance of regression models is generally substantially higher in the estimation cohort than in validation cohort[23]. An inconsistency of correctly classified rate from estimation to validation cohorts further explains and highlights the urgent need of a well-defined cut-off developed by multi-center larger-population based studies in the future[17].

Combination of clinical symptoms, laboratory variables and molecular biomarkers have been investigated to develop HCC risk predictive models; however, their performance is still debated and not yet adopted in clinical practice. A recent disease-specific Toronto HCC Risk Index revealed that the 10-year cumulative incidence of HCC differed from etiologic category ranging from 22% to 5%, and further allowed to stratify patients into three groups according to the HCC risk estimation with a 10-year

Table 2 Univariable and multivariable analysis regarding two-year hepatocellular carcinoma disease-free survival in the estimation cohort

Covariate	Non-HCC (*n* = 54)	HCC (*n* = 8)	Univariable analysis HR (95%CI; *P* value)	Multivariable analysis HR (95%CI; *P* value)
Gender (Male)	75.9% (41/54)	62.5% (5/8)	0.571 (0.137-2.392; 0.444)	
mean age (yr)	55.96 ± 10.82	54.5 ± 8.5	0.987 (0.924-1.055; 0.706)	
Etiology (alcohol/HCV/HBV/other)	25/17/10/2	1/7/0/0	1.075 (0.481-2.405; 0.859)	
Child-Pugh (A/B/C)	35/14/5	5/3/0	0.922 (0.290-2.935; 0.891)	
AST (IU/mL)	63.94 ± 42.21	107.29 ± 69.83	1.013 (1.002-1.024; 0.021)	
ALT (IU/mL)	58.86 ± 55.48	77.29 ± 62.52	1.004 (0.993-1.015; 0.452)	
Tot. Bilirubin (mg/dL)	1.97 ± 3.05	1.75 ± 1.02	0.983 (0.734-1.316; 0.906)	
Creatinine (mg/dL)	0.83 ± 0.20	2.04 ± 3.44	1.363 (1.055-1762; 0.018)	1.564 (1.151-2.124; 0.004)
Platelets (× 10^9/mL)	102.25 ± 43.28	88.00 ± 42.89	0.992 (0.974-1.010; 0.387)	
Albumin (mg/dL)	3833 ± 625	3642 ± 525	1.000 (0.998-1.001; 0.394)	
AFP (ng/mL)	7.80 ± 9.25	40.38 ± 62.71	1.024 (1.010-1.038; 0.001)	1.028 (1.009-1.046; 0.003)
SCCA-IgM (AU/mL)	136.83 ± 163.44	608.75 ± 1093.53	1.001 (1.000-1.002; 0.004)	1.001 (1.000-1.002; 0.003)

Cox proportional hazards model was used to estimate the hazard ratios and CIs in the multivariable analysis. Data are numbers of patients (%) or mean ± SD. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HCV: Hepatitis C virus; HBV: Hepatitis B virus; AFP: Alfa-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen and its immune complexes; Child-Pugh score: The Child–Turcotte–Pugh score or Child Criteria; HCC: Hepatocellular carcinoma; HR: Hazard ratio.

Table 3 Predictive discrimination ability of the combination of squamous cell carcinoma antigen immunocomplex and alfa-fetoprotein as compared with squamous cell carcinoma antigen immunocomplex or alfa-fetoprotein alone in both estimation and validation cohorts

Total patients (*n* = 203)	Combination of SCCA-IgM and AFP (95%CI)	SCCA-IgM (95%CI; *P* value)	AFP (95%CI; *P* value)
Estimation cohort (*n* = 62)			
Two-year HCC-free survival	0.787 (0.620-0.955)	0.727 (0.526-0.927; 0.451)	0.705 (0.464-0.946; 0.398)
Five-year HCC-free survival	0.744 (0.613-0.876)	0.686 (0.535-0.837; 0.299)	0.705 (0.539-0.871; 0.581)
Validation cohort (*n* = 141)			
Two-year HCC-free survival	0.773 (0.659-0.887)	0.706 (0.588-0.827; 0.122)	0.748 (0.617-0.880; 0.701)
Five-year HCC-free survival	0.730 (0.648-0.813)	0.706 (0.623-0.788; 0.297)	0.646 (0.548-0.734; 0.067)

C-index values and the corresponding 95% CIs were estimated for each main study time point to assess the model's discrimination ability. *P* values represent the statistical significance of the differences between the combination and the squamous cell carcinoma antigen immunocomplex or alfa-fetoprotein alone. AFP: Alfa-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen and its immune complexes; HCC: Hepatocellular carcinoma.

incidence of HCC of 3%, 10% and 32%, respectively[10]. The AFP has been currently removed from the clinical practice guidelines because of its low PPV, which potentially results in "overdoing" the follow-up testing (*e.g.*, computed tomography, magnetic resonance imaging), in the frequently encountered patients with mildly elevated AFP[24]. However, El-Serag *et al*[24] constructed an AFP-based algorithm to identify patients at risk for HCC, and further suggested that the wide availability of AFP tests, high level of laboratory standardization and low cost made AFP still a feasible strategy to predict HCC. Moreover, three recent meta-analyses have proved the usefulness of the combination of AFP with SCCA-IgM[17], Des-gamma-carboxyprothrombin and Golgi protein 73[25,26] for hepatocellular carcinoma diagnosis, suggesting the combinations of biomarkers a feasible strategy of HCC screening. Therefore, the consideration remaining to us is not whether to use AFP for HCC screening and predicting or not, but how to use it appropriately.

Table 4 Operating characteristics for the combination of squamous cell carcinoma antigen immunocomplex and alfa-fetoprotein regarding two- and five-year hepatocellular carcinoma disease-free survival

Variables	Two-year incidence in validation cohort				Five-year incidence in validation cohort			
	Estimation cohort		Validation cohort		Estimation cohort		Validation cohort	
	Low-risk	High risk	Low-risk	High risk	Low-risk	High risk	Low-risk	High risk
Cut-off	AFP < 5 ng/mL and SCCA-IgM < 124 AU/mL	AFP > 5 ng/mL and SCCA-IgM > 124 AU/mL	AFP < 5 ng/mL and SCCA-IgM < 124 AU/mL	AFP > 5 ng/mL and SCCA-IgM > 124 AU/mL	AFP < 5 ng/mL and SCCA-IgM < 124 AU/mL	AFP > 5 ng/mL and SCCA-IgM > 124 AU/mL	AFP < 5 ng/mL and SCCA-IgM < 124 AU/mL	AFP > 5 ng/mL and SCCA-IgM > 124 AU/mL
True positive	8	4	14	7	13	5	33	12
False positive	33	8	101	21	28	7	82	16
True negative	21	46	26	106	21	42	25	91
False negative	0	4	0	7	0	8	1	22
Sensitivity	100%	50%	100%	50%	100%	38%	96%	35%
Specificity	39%	85%	20%	83%	43%	86%	23%	85%
PPV	20%	33%	12%	25%	32%	42%	29%	43%
NPV	100%	92%	100%	94%	100%	84%	96%	81%
LR+	1.64	3.38	1.26	3.02	1.75	2.69	1.27	2.36
LR-	0.00	0.59	0.00	0.60	0.00	0.72	0.13	0.76
Correctly classified	75.8%		78.8%		61.1%		68.5%	

PPV: positive predictive values; NPV: negative predictive values; LR: likelihood ratio.

By using the present combination of SCCA-IgM and AFP, we will enable rational allocation of the limited medical resources to the high-risk patients who most need to be screened, and avoid wasteful and unnecessary distribution to low-risk individuals who had 100% of HCC-free survival rate in the two-year follow-up. Moreover, the disordered PPV that was influenced by the low prevalence of HCC development through using current "one-size-fits-all" surveillance program, further strengthen the necessity of altering surveillance to a subgroup of high-risk population inside the cirrhotic patients that will ensure a high pre-test probability[27]. Currently there have not been any randomized controlled trial of HCC surveillance in patients with cirrhosis[6]. Cirrhotic patients are older, have more comorbidities and abdominal ultrasound has low sensitivity for HCC detection in a nodular cirrhotic liver. Several cohort studies demonstrated that surveillance was associated with increased early tumor detection, curative treatment option and it improved the overall survival[28]. In contrast, other studies reported that HCC surveillance was not associated with decreased HCC-related mortality, adding to the existing controversy surrounding the benefits of HCC surveillance[29,30]. Nevertheless, modifying HCC screening frequency according to estimated individual HCC risk by using the present combination of biomarkers may enable more efficient early tumor detection because of high-risk subjects are more likely develop HCC.

In this sense, the combination of SCCA-IgM and AFP, classifying a low-risk group with 100% of HCC-free survival, will enable us to exclude those patients from surveillance programs or to extend the intensity of screening to two years. This strategy will enable rational allocation of medical resources, cost-effective and accurate preventive intervention, which will substantially improve the dismal prognosis of HCC and will uphold the spirit of advancing with time in the era of precision medicine. Furthermore, a recent cost-effectiveness study has further verified that tailored HCC surveillance strategies according to estimated patient's risk stratification indeed revealed superior cost-effectiveness[31]. The present strategy of SCCA-IgM and AFP should be further implemented and verified in the clinical setting through future well-designed prospective studies. Moreover, an easy-to-use and outpatient-based
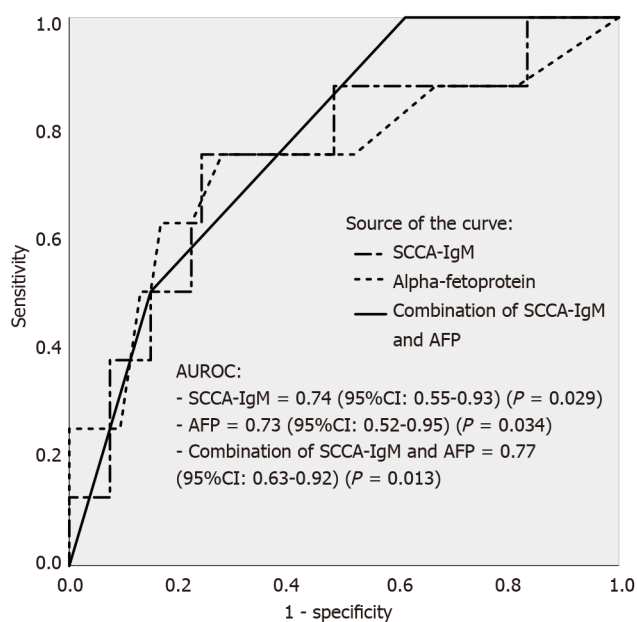
Figure 1 Receiver operating characteristic curves of the combination of squamous cell carcinoma antigen immunocomplex and alpha-fetoprotein as compared to squamous cell carcinoma antigen immunocomplex and alpha-fetoprotein in predicting two-year mortality in the estimation cohort. The clinical relevance of squamous cell carcinoma antigen immunocomplex and alpha-fetoprotein in patients with cirrhosis was determined by the calculation of the area under the receiver operating characteristic. Baseline serum levels distribution above the cut-off of the two biomarkers in patients who developed hepatocellular carcinoma *vs* patients who did not was compared. AUROC, area under the receiver operating characteristic. Comparison of the AUROCs estimated for each set. AFP: Alpha-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen immunocomplex.

instead of laboratory-based kit will optimize the performance of the combination of the present biomarkers.

There were several limitations in the present study. First, the present study did not used biopsy to ultimately confirm HCC. Second, the definition of cirrhosis was not reached from liver biopsies. This can lead to an underestimation of subclinical cirrhosis of the population studied. However, according to the current clinical practice guidelines there is no need to perform biopsy for the diagnosis of HCC and cirrhosis, and the ethic concern prohibited certain studies design to perform the biopsy[32]. In fact, the recent technological approach with typical radiological characteristics on contrast-enhanced cross-sectional imaging have a positive predictive value of almost 100%[33]. Third, lead time bias and length time bias were always a crucial consideration of diagnostic accuracy experimental design.

CONCLUSION

In summary, we have proved that the combination of SCCA-IgM and AFP enhanced the predictive value for detecting HCC, which could be followed by tailored HCC surveillance for individual patients, especially for those cirrhotic patients belonging to the subgroup identified as low-risk of HCC development.

A Estimation cohort: SCCA-IgM

B Estimation cohort: AFP

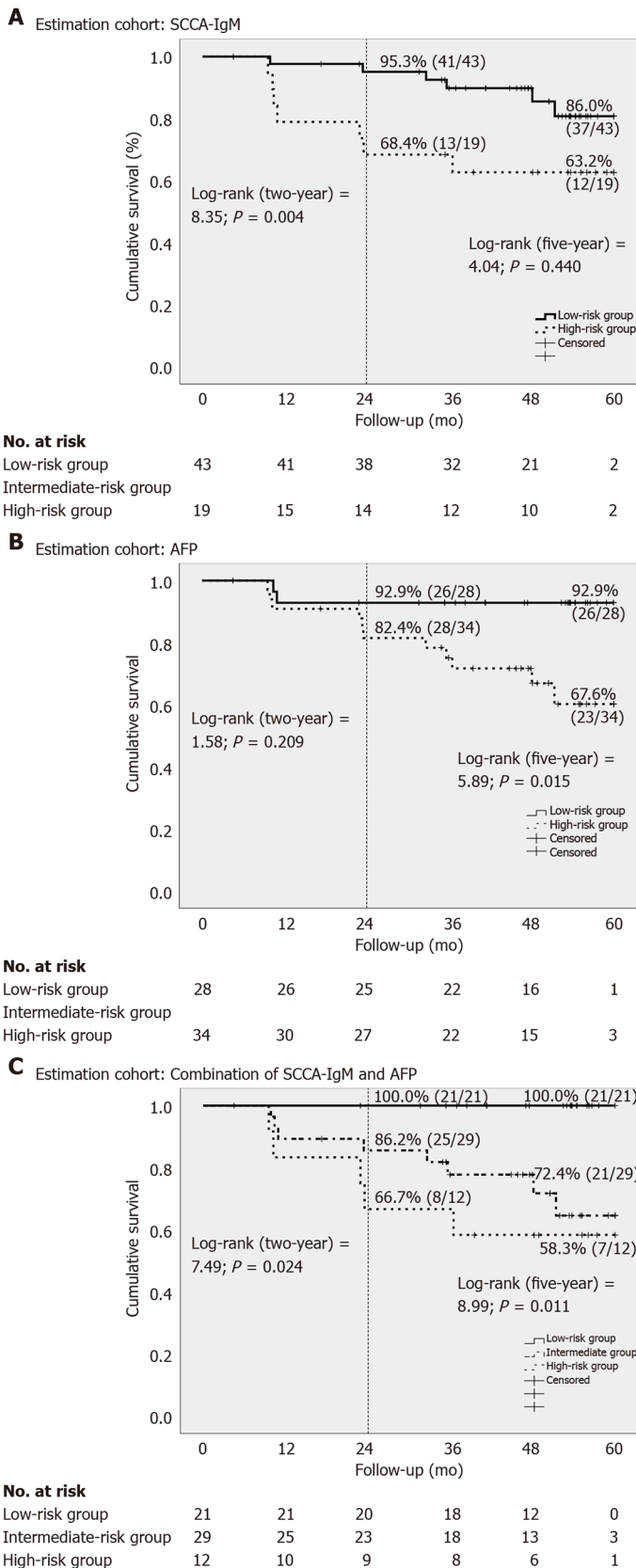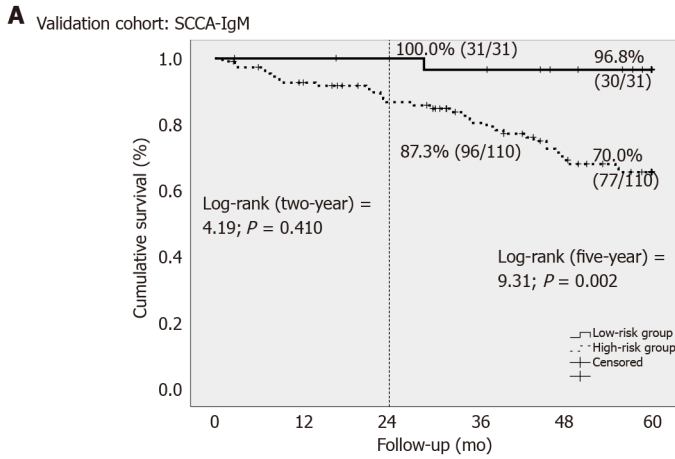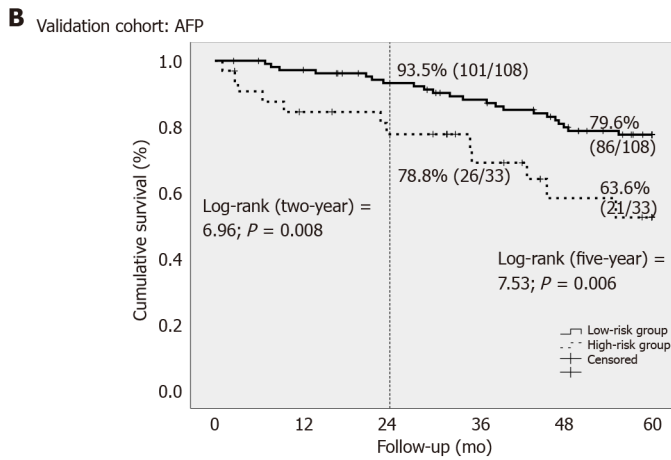**C** Estimation cohort: Combination of SCCA-IgM and AFP

Figure 2 Estimating two- and five-year hepatocellular carcinoma disease-free survival by using Kaplan-Meier method according to the squamous cell carcinoma antigen immunocomplex, alpha-fetoprotein and combination of those in estimation cohort. A: Squamous cell carcinoma antigen immunocomplex (SCCA-IgM); low-risk: < 124 AU/mL, high-risk: > 124 AU/mL; B: Alpha-fetoprotein (AFP); low-risk: < 5 ng/mL, high-risk: > 5 ng/mL; C: Combination of SCCA-IgM and AFP. AFP: Alpha-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen immunocomplex.

Figure 3 Estimating two- and five-year hepatocellular carcinoma disease-free survival by using Kaplan-Meier method according to the squamous cell carcinoma antigen immunocomplex, alpha-fetoprotein and combination of those both in validation cohort. A: Squamous cell carcinoma antigen immunocomplex (SCCA-IgM); low-risk: < 124 AU/mL, high-risk: > 124 AU/mL; B: Alpha-fetoprotein (AFP); low-risk: < 5 ng/mL, high-risk: > 5 ng/mL; C: Combination of SCCA-IgM and AFP. AFP: Alpha-fetoprotein; SCCA-IgM: Squamous cell carcinoma antigen immunocomplex.
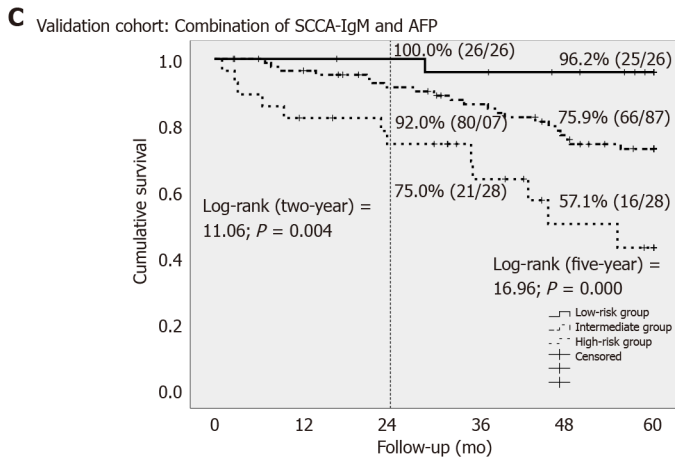
ARTICLE HIGHLIGHTS

Research background
Early diagnosis or prediction of hepatocellular carcinoma (HCC) development would have a major impact on the prognosis of patients under surveillance.

Research motivation
Current screening programs for HCC are far from being satisfactory due to patient- and provider-related factors. Individualizing the program according to the risk of HCC development could be a strategy to overcome these challenges in the era of precision medicine.

Research objectives
This study aimed to evaluate non-invasive biomarkers in the prediction of HCC among patients with cirrhosis.

Research methods
Retrospective cohort study analyzing the association of baseline serum biomarkers with the development of HCC in the mid- and long-term in cirrhotic patients of different etiologies.

Research results
Squamous cell carcinoma antigen immunocomplex (SCCA-IgM) serum levels are associated to the development of HCC at mid- long-term, independently of previously known predictors.

Research conclusions
A predictive model based on the combination of alpha-fetoprotein and SCCA-IgM levels could provide a new HCC screening method, optimizing surveillance for individual patients, especially for cirrhotic patients allocated in the low-risk group.

Research perspectives
Tailored HCC surveillance assessed by non-invasive biomarkers in individual patients would help to better allocate the resources to those patients at higher risk of developing HCC.

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