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Serum models accurately predict liver related clinical outcomes in chronic hepatitis C

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ABSTRACT:

Background and Aim: This study developed liver outcome scores in chronic hepatitis C (CHC) that directly predict liver related death, hepatocellular carcinoma (HCC) and liver decompensation.

Methods: 617 CHC patients were followed for a mean of six years and randomized into a training set (n=411) and a validation set (n=206). Clinical outcomes were determined using a population based data-linkage system.

Results: In the training set, albumin, gamma-glutamyl transpeptidase (GGT), hyaluronic acid (HA), age and sex were in the final model to predict five year liver related death (AUROC 0.95). Two cut points (4.0, 5.5), defined three risk groups with an incidence rate for liver related death of 0.1%, 2% and 13.2% respectively ($p<0.001$). Albumin, GGT, HA, age and sex were used to predict five year liver decompensation (AUROC 0.90). A cut point of 4.5 gave a sensitivity of 94% and a specificity of 84% to predict five year decompensation and defined two groups with an incidence rate for decompensation of 0.2% and 5.8% respectively ($p<0.001$). Alkaline phosphatase, α2-macroglobulin, age and sex were used to predict five year HCC occurrence (AUROC 0.95). A cut point of 8 had a sensitivity of 90% and specificity of 88% to predict five year HCC occurrence and defined two groups with an incidence rate for HCC of 0.2% and 5.6% respectively ($p<0.001$). Similar results were obtained using the validation set.

Conclusions: All three liver outcome scores had excellent predictive accuracy and were able to stratify risk into clinical meaningful categories for CHC patients.

Key words: serum model; liver related death; liver decompensation; hepatocellular carcinoma.

INTRODUCTION:

Hepatitis C virus (HCV) infection affects about 180 million people worldwide and predisposes these patients to complications of cirrhosis, hepatocellular carcinoma and early death.[1] Patients with chronic hepatitis C (CHC) had a three times higher risk of overall death and a 17 times higher risk of liver related death than the general population.[2,3] However, identifying those CHC patients who are at higher risk of developing liver related morbidity and mortality is problematic. This is due to the variable natural history of HCV with its prolonged and predominantly asymptomatic early phase and variable later progression. Histopathological stage of liver fibrosis has been used to stratify risk in CHC patients, but liver biopsy is an invasive procedure and has problems with sampling error and risk of serious complications.[4-6]

Non-invasive clinical tests are used to predict the severity of liver fibrosis and these are serum tests and radiological tests.[7] Only a few of the serum fibrosis panels have been shown to be associated with liver related clinical outcomes.[8-13] Transient elastography was significantly correlated with the development of liver decompensation, hepatocellular carcinoma (HCC) and death for patients with chronic liver disease.[14,15] This data suggests that non-invasive methods have the potential to predict clinical outcomes.

The potential advantages of serum liver panels that have been developed to directly predict clinical outcomes are that they will incorporate additional analytes that are not useful in predicting fibrosis but will be useful in predicting clinical end points. These additional

analytes may be associated with other factors such as portal hypertension, coagulopathy, protein synthetic dysfunction and renal failure that are known to predict liver related outcomes. Disease specific models have been developed for primary biliary cirrhosis and primary sclerosing cholangitis.[16,17]

The aim of this study was to develop simple serum liver outcome scores that directly predict the risk of liver related death, HCC and decompensation in a large group of CHC patients who had long term clinical follow up data available. The new liver outcome scores were compared with existing serum fibrosis models.

METHODS:

Patient recruitment:

CHC patients who attended the outpatient HCV liver clinics at Sir Charles Gairdner Hospital from 1997 to 2012 and had a Hepascore and other routine blood tests performed were included. Sir Charles Gairdner Hospital is a tertiary referral centre for liver disease and the state liver transplantation service. CHC was defined as positive HCV RNA on two occasions greater than six months apart. Exclusion criteria included co-infection with hepatitis B or HIV; hemochromatosis, α1-antitrypsin deficiency, Wilson disease or autoimmune liver disease; previous liver transplantation; and episodes of liver decompensation and HCC before enrolment. The study was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee and the Western Australia Department of Health Human Research Ethics Committee.

Candidate serum markers:

Twelve serum markers were analysed and these included: hyaluronic acid (HA), bilirubin, gamma-glutamyl transpeptidase (GGT), α2-macroglobulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelet count, prothrombin time, international normalized ratio (INR), alkaline phosphatase (ALP), creatinine and albumin. Bilirubin, AST, ALT, GGT, ALP and albumin were measured on an automated biochemistry analyser (Hitachi 917, Roche Diagnostics). HA (Wako, Germany) and α2-macroglobulin (Dako, USA) are performed on a fully automated chemistry analyser (Olympus AU2700, Beckman). All analyses were performed at a central laboratory, PathWest Laboratory Medicine, Perth.

Hepascore was calculated as y/ (1-y); where y= exp [-4.185818 - (0.0249 * Age) + (0.7464 * Sex) + (1.0039 * α2-macroglobulin (g/l)) + (0.0302 * HA (ug/l)) + (0.0691 * bilirubin (umol/l)) - (0.0012 * GGT(IU/l))].[21] The APRI was calculated as AST(U/L) /upper normal * 100/platelet count (109/L).[22] The Lok index was calculated as y/ (1-y); where y= exp [-5.56 - 0.0089 * platelet (10^3/mm^3) +1.26 * AST/ALT +5.27 * INR].[23] The FIB-4 was calculated as age (years) *AST (U/L) /((platelets (10^9/L) * (ALT (U/L))$^{1/2}$).[24]

Clinical outcomes:

Long term follow up of patients was obtained from the Western Australian Data Linkage Unit. This population-based data linkage system links health related datasets including the state cancer register, the state hospital morbidity database and the state mortality records.[25] The Hospital Morbidity Data System has 100% coverage for hospital admissions with a record linkage success rate >99%.[25] The hospital admission diagnosis and the cause of death were recorded using ICD 9 (before 1997) and ICD 10 (after 1997) classification codes.

The endpoints included: Liver related death (death from liver failure, variceal bleeding, HCC, liver disease was the major contributing factor) or liver transplantation; First episode of liver decompensation (ascites, hepatic encephalopathy, variceal bleeding, hepatorenal syndrome, spontaneous bacterial peritonitis); and development of HCC. Patients were followed from the day of entry to each end point or the end of study.

Statistical analysis:

Patients were randomised into a training set and a validation set (2:1 ratio). Cox regression analysis was used to model survival and predict liver related death, liver decompensation and HCC respectively. The candidate variables for each model were those factors that had a significant association with each end point with p value less than 0.05 using a univariate cox model. Age and sex were also included in all models as they are known factors that could affect both survival and serum marker results. The final models were chosen using the backwards selection method. Harrell's C statistic was used to evaluate the predictive ability of models [26]. ROC curve analysis was also used to test the ability of the final model to predict the risk of each clinical meaningful time point (three, five and ten years) to develop each end point and cut points were defined using Youden index. The survival probability for each risk group within the new models was calculated using Kaplan-Meier curves and a significance difference was defined with the log rank test. Area under ROC curve (AUROC) was calculated for each of the new models and this was compared with the serum fibrosis panels Hepascore, APRI, FIB-4 and Lok index. The incident rate of end points were calculated and compared using the Z test.

RESULTS:

The CHC patient population included 617 patients with all stages of liver disease as determined by serum fibrosis models and 60% had mild liver disease (Hepascore ≤ 0.5). 411 patients were randomised to the training set and 206 to the validation set. Patients' characteristics of the two groups are shown in Table 1 and there was no significant difference between the two groups. Patient follow up was for a mean of six years (range 0.1–14.1). There were 22 liver related deaths or liver transplantations, 23 HCC's and 27 episodes of decompensation by the end of follow up. Forty seven patients had a sustained viral response (SVR) during follow up. These patients with SVR were included in the model development and validation because these patients are still at risk of developing adverse clinical outcomes for an unknown period of time. A sensitivity analysis that censored patients with SVR was performed and found no significant effect on the results.

Training set

Liver related death was significantly associated with HA, GGT, bilirubin, α2-macroglobulin, platelet count, INR, prothrombin time, AST, ALP, albumin, and creatinine. These were included with age and sex in the initial predictive model (Table 2). The final model was [Liver Outcome Score (LOS)]: LOS_death = -0.1792 * albumin (g/L) +0.0042 * GGT (U/L) + 0.0041 * HA (ug/L) + 0.0377 * age + 0.4492 (if sex= male) + 8 and achieved a Harrell's C statistic of 0.95 (Table 2). The AUROC for LOS_death to predict three year, five year and ten year liver related death was 0.96 (95% CI, 0.91–1.00), 0.95 (95%CI, 0.90–1.00) and 0.95 (95% CI, 0.91–0.99) respectively. The mean LOS_death value in the training set was 2.99 (range: 0.23–9.85). A cut point of 5.5 had a sensitivity of 80.0% and specificity of 96.5% to predict three year liver related death. A cut point of 4.0 had a sensitivity of 92.9% and specificity of 85.1% to predict ten year liver related death. Using these cut points, patients

were categorised into low, moderate and high risk group (< 4.0, 4.0–5.5, ≥5.5) with the annual incident rate of 0.1%, 2.02% and 13.2% respectively. A significant difference of liver related survival was found between groups (P<0.0001) (Figure 1).

Liver decompensation was significantly associated with ten variables (HA, GGT, bilirubin, α2-macroglobulin, platelet count, INR, prothrombin time, AST, ALP, and albumin) and these were included with age and sex as candidate variables in the initial model (Table 2). The final model: was LOS_decompensation = 0.0031 * HA (ug/L) + 0.0030 * GGT (U/L) + 0.0562 * age - 0.5342 (if sex= male) - 0.1870 * albumin (g/L) + 9 and had a Harrell's C statistic of 0.91 (Table 2). The AUROC for LOS_decompensation to predict three year, five year and ten year decompensation was 0.96 (95%CI, 0.93–0.99), 0.90 (95%CI, 0.80–1.00) and 0.89 (95%CI, 0.80–0.98) respectively. The mean LOS_decompensation value in the training set was 3.59 (range: 0.39–9.23). A cut point of 4.5 achieved a sensitivity of 100% and a specificity of 83.4% to predict three year decompensation. The same cut point achieved a sensitivity of 94.4% and a specificity of 83.5% to predict five year decompensation and a sensitivity of 85.7% and specificity of 84.2% to predict ten year decompensation. Patients were therefore categorised into low and high risk group of developing decompensation with annual incidence rate of 0.15% and 5.58% respectively (Table 3). A significant difference in decompensation free survival was found between these two groups (p<0.0001) (Figure 1).

The development of HCC was significantly associated with eleven variables (HA, GGT, bilirubin, α2-macroglobulin, platelet count, INR, prothrombin time, AST, ALT, ALP, and albumin) and these were included with age and sex as candidate variables to predict HCC (Table 2). The final model was: LOS_HCC = 1.731 (if sex= male) + 0.0093 * ALP (U/L) + 0.6408 * α2-macroglobulin (g/L) + 0.1350 * age - 4 had a Harrell's C statistic of 0.95. The AUROC for LOS_HCC to predict three year, five year and ten year HCC development was

0.94 (95%CI, 0.90–0.99), 0.95 (95%CI, 0.91–0.99) and 0.93 (95%CI, 0.89–0.98) respectively. The mean LOS_HCC value in the training set was 5.75 (range: 0.45–12.01). A cut point of 8 had a sensitivity of 88.9% and a specificity of 87.7% to predict three year HCC development. The same cut point had a sensitivity of 90.0% and specificity of 87.9% to predict five year HCC and a sensitivity of 80% and specificity of 88.6% to predict ten year HCC development and. Patients were therefore categorised into low and high risk group of developing HCC with annual incidence rate of 0.15% and 5.78% respectively (Table 3). A significant difference of HCC free survival was found between these two groups ($p<0.0001$) (Figure 1).

Validation set

Patients in the validation set were followed for a mean of six years (range 0.2–14.1). Seven patients had a liver related death, six had liver decompensation and seven developed HCC. In this group the AUROC of LOS_death to predict three year, five year and ten year liver related death was 0.94 (95%CI, 0.89–1.00), 0.96 (95%CI, 0.92–1.00) and 0.95 (95%CI, 0.91–0.99) respectively. The AUROC of LOS_decompensation to predict three year, five year and ten year decompensation was 0.94 (95%CI, 0.85–1.00), 0.95 (95%CI, 0.85–1.00) and 0.87 (95%CI, 0.76–0.99) respectively. The AUROC of LOS_HCC to predict three year, five year and ten year HCC was 0.92 (95%CI, 0.84–1.00), 0.93 (95%CI, 0.87–1.00) and 0.94 (95%CI, 0.90–0.99) respectively.

Comparison with other serum models:

The predictive ability of the LOS panel was compared with Hepascore, APRI, FIB-4 and the Lok index (Table 3). The LOS panel had the best ability to predict liver related death, HCC and liver decompensation among serum models. Individual comparison showed that LOS_death was significantly better than Hepascore ($p=0.0009$) to predict liver related death.

LOS_HCC was significantly better than all other serum models to predict HCC development (Figure 2).

DISCUSSION:

The development of simple serum liver panel models that are able to stratify CHC patients into a hierarchy of risk levels of adverse clinical outcomes is of considerable clinical significance. The strengths of this study were the inclusion of a large number of well characterized CHC patients with active infection, a broad spectrum of disease severity (Hepascore range: 0.02–1.0) and a long follow up time of up to 14 years. Furthermore, the final LOS panel was validated in a separate cohort. The LOS panel had a high accuracy to predict five year liver related death, liver decompensation and HCC with an AUROC of 0.95, 0.90 and 0.95 respectively. Cut points were determined to identify patients at higher risk for each clinical outcome and these resulted in a high sensitivity and specificity. Using the defined cut points, those patients categorised in the high risk group had a significantly increased risk of adverse clinical outcomes, especially within the first five years of follow up. The low risk group had excellent survival for more than ten years. The annual incidence rate for high risk group for liver related death, HCC development and liver decompensation was 13.2%, 5.58% and 5.78% respectively. These rates were significantly higher than that of the low or moderate risk group.

Two previous studies re-analysed the HALT-C cohort to develop models to directly predict clinical outcomes. The HALT-C cohort only included CHC patients with advanced liver fibrosis and follow up of 3.5 years.[27,28] The first study used an increased Child-Turcotte-Pugh score, decompensation, HCC and all-cause mortality as a composite endpoint and analysed the value of direct serum fibrosis markers. The second study excluded HCC from the composite endpoint and analysed simple serum markers. Neither study performed AUROC or

sensitivity and specificity analysis of the models. A third study developed a serum model (HCC-4) that included age, alpha-fetoprotein, platelet count and GGT to predict the risk of HCC development in CHC patients with a mean of 6.8 years follow up and the AUROC was 0.802.[29] However, none of these studies validated the developed serum models in a separate cohort.

Few studies have evaluated the ability of serum fibrosis models to predict clinical outcomes. In alcoholic liver disease serum fibrosis models had a moderate ability to predict liver related death and in CHC they had a higher accuracy than Metavir stage to predict liver related death and events.[8,9] In general serum fibrosis models have at best a moderate accuracy to predict liver related survival and liver complications.[10-13] Similar results were found in this present study for FIB-4, APRI and the Lok index. The LOS panels had a clear advantage with their superior predictive ability and broad applicability for CHC patients with all grades of fibrosis severity. Moreover, specific models were built to predict liver related death, HCC and decompensation and this has allowed different serum markers to be included in each model. HA and GGT are well recognised serum markers associated with clinical decompensation and were included in the liver related death and liver decompensation LOS models. ALP and alpha-macroglobulin were independent predictors of HCC development and were included in the LOS_HCC model.

This study's limitations include the lack of clinical data and lifestyle information (BMI, smoking, alcohol consumption) therefore adverse outcomes may have been influenced by these confounding factors. However the outcomes were still accurately predicted by the LOS panels. Secondly, patient outcomes were recorded by the Western Australian Data Linkage Unit. Clinical follow up data such as blood test, endoscopy reports and ultrasound reports

were not available for analysis. However the quality of clinical outcome data collected by the unit has been validated and is highly accurate.[25]

In summary, this study developed three LOS models to predict liver related death, liver decompensation and HCC respectively for chronic hepatitis C patients. The predictive ability of the LOS panel was better than the currently used fibrosis models. The use of the LOS panels will potentially improve clinical care by allowing the optimum use of expensive directly-acting antiviral agents before the onset of significant clinical complications.[30] In addition these models would also be potentially valuable in determining the start of ultrasound screening for HCC and for assessing the presence of complications of portal hypertension. Future studies are required to validate these models in addition to the presently accepted clinical criteria used in CHC patients.

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Table 1: Patients characteristics

Characteristics	Training set (n=411)	Validation set (n=206)	P value
Age (year)	44 (10)	43 (11)	0.366
Gender M/F	284/127	133/73	0.256
HA (ug/L)	77 (137)	74 (141)	0.745
GGT (U/L)	95 (122)	87(106)	0.374
Bilirubin (umol/L)	12 (7)	11 (16)	0.746
α2-macroglobulin (g/L)	2.74 (1.08)	2.69 (1.03)	0.547
Platelet count (10^9/L)	220 (81)	224 (92)	0.557
INR	1.0 (0.1)	1.0 (0.1)	0.676
Creatinine (umol/L)	76.5 (1.7)	78.5 (3.01)	0.551
prothrombin time (secs)	8.9 (1.4)	8.7 (1.3)	0.066
ALT (U/L)	117 (101)	119 (140)	0.844
AST (U/L)	76 (60)	78 (111)	0.723
ALP (U/L)	85 (51)	88 (45)	0.709
Albumin (g/L)	44 (9.5)	43 (3.9)	0.280
Hepascore	0.48 (0.33)	0.45 (0.33)	0.343
APRI	1.03 (1.35)	1.04 (1.60)	0.930
Lok index	0.27 (0.21)	0.27 (0.22)	0.814
FIB-4	2.01 (2.59)	2.02 (2.97)	0.976
Follow up year	6.0 (3.0)	6.2 (3.1)	0.429
Liver related death	15 (3.65%)	7 (3.40%)	0.874
HCC	16 (3.89%)	7 (3.40%)	0.760
decompensation	21 (5.11%)	6 (2.91%)	0.255
Sustained viral response	29 (7.06%)	18 (8.74%)	0.552

Note: Continuous variables were presented as mean (standard deviation) and categorical variables were presented as count (percentage).

HA, hyaluronic acid; M, male; F, female; GGT, gamma-glutamyl transpeptidase; INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; HCC, hepatocellular carcinoma.

Table 2: Univariate and multivariate analysis to predict each end point.

	Coefficient , p value					
	Liver related death		Decompensation		Hepatocellular carcinoma	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
HA	0.006 p<0.001	0.0041 P=0.004	0.006 P<0.001	0.0031 P=0.017	0.005 P<0.001	
GGT	0.004 P<0.001	0.0042 P<0.001	0.003 P<0.001	0.030 P=0.005	0.003 P<0.001	
Bilirubin	0.071 P<0.001		0.052 P=0.004		0.068 P<0.001	
α2-macroglobulin	0.669 P=0.001		0.510 P=0.004		0.904 P<0.001	0.6408 P=0.007
Platelet count	-0.021 P<0.001		-0.013 P<0.001		-0.020 P<0.001	
INR	8.093 P<0.001		6.255 P<0.001		7.125 P<0.001	
Prothrombin time	0.275 P=0.002		0.215 P=0.010		0.287 P<0.001	
ALT	0.035 P=0.073		0.0002 P=0.915		0.004 P=0.011	
AST	0.012 P<0.001		0.009 P<0.001		0.011 P<0.001	
creatinine	0.005 P=0.008		-0.007 P=0.681		0.001 P=0.857	
ALP	0.009 P<0.001		0.009 P<0.001		0.008 P<0.001	0.0093 P<0.001
albumin	-0.313 P<0.001	-0.1792 P=0.067	-0.333 P<0.001	-0.1870 P=0.034	-0.181 P<0.001	
age	0.104 P<0.001	0.0377 P=0.148	0.118 P<0.001	0.0562 P=0.019	0.152 P<0.001	0.1350 P<0.001
sex	0.600 P=0.353	0.4492 P=0.516	-0.315 P=0.484	-0.5342 P=0.266	1.095 P=0.148	1.731 P=0.102

HA, hyaluronic acid; GGT, gamma-glutamyl transpeptidase; INR, international normalized ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; HCC, hepatocellular carcinoma.

Table 3: Comparison of the accuracy of LOS panel with other models to predict liver related outcomes.

Models	AUROC (95% CI)		
	Liver related death	Decompensation	HCC
LOS_death	0.95 (0.92-0.97)	-	-
LOS_decompensation	-	0.88 (0.81-0.96)	-
LOS_HCC	-	-	0.94 (0.90-0.97)
Hepascore	0.87 (0.82-0.93)	0.80 (0.71-0.90)	0.84 (0.78-0.90)
APRI	0.89 (0.84-0.94)	0.75 (0.64-0.87)	0.84 (0.75-0.92)
FIB-4	0.92 (0.87-0.96)	0.84 (0.77-0.92)	0.87 (0.82-0.93)
Lok index	0.86 (0.75-0.96)	0.81 (0.71-0.91)	0.80 (0.70-0.90)

Note: Serum models were compared using the whole cohort.

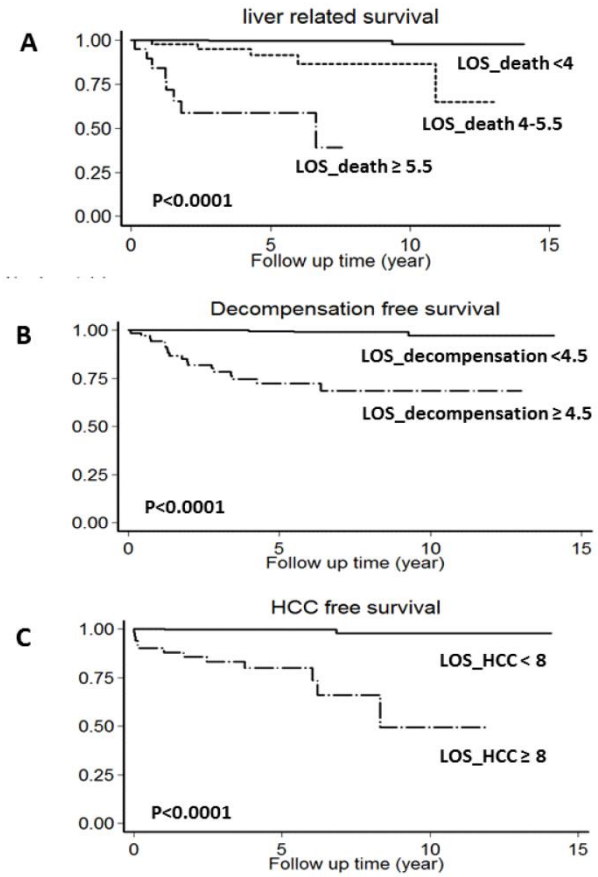

Figure1. Survival curves according to LOS panel. (A): Liver related survival (B): Decompensation free survival (C): HCC free survival.

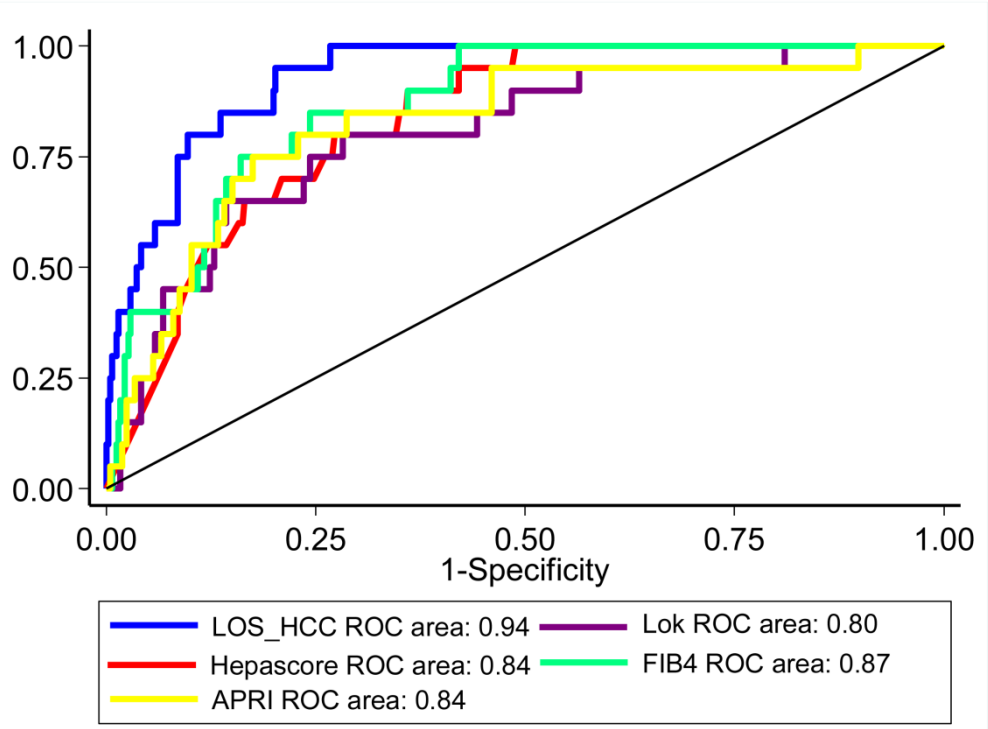

Figure2. ROC curves of serum models to predict HCC development.