Supplementary Material

Direct comparison of five serum biomarkers in early detection of hepatocellular carcinoma

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Supporting information:

(b) HCC vs. CHB+LC; (c) HCC vs CHB; (d) Early-stage HCC vs CHB+LC+HC; (e) Early-stage HCC vs CHB+LC; (f) Early-stage HCC vs LC

Figure S6. Comparison of apparent and .632+ adjusted receiver operating characteristics (ROC) curves for CENPF in discriminating: (a) HCC vs. CHB+LC+HC; (b) HCC vs. CHB+LC; (c) HCC vs CHB; (d) Early-stage HCC vs CHB+LC+HC; (e) Early-stage HCC vs CHB+LC; (f) Early-stage HCC vs LC

Figure S7. Comparison of .632+ adjusted receiver operating characteristics curves of two prediction algorithms (AFP+DCP and AFP+DPC+age+sex) for discriminating: (a) HCC vs. CHB+LC+HC; (b) HCC vs. CHB+LC; (c) HCC vs CHB; (d) Early-stage HCC vs CHB+LC+HC; (e) Early-stage HCC vs CHB+LC; (f) Early-stage HCC vs LC

Abbreviations: AUC=area under the curve. HCC=hepatocellular carcinoma; HC=healthy control; CHB=chronic hepatitis B virus infection; LC=liver cirrhosis

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Table S1. Clinicopathologic characteristics of patients with HCC, CHB or LC

Variable	HCC (N=202)	CHB (N=215)	LC (N=226)
AFP (n, %)			
≤20 ng/ml	103 (51.0)	172 (80.0)	199 (88.1)
>20 ng/ml	99 (19.0)	43 (20.0)	27 (11.9)
HBV-DNA (n, %)			
Negative	47 (23.3)	129 (60.0)	160 (70.8)
Positive	36 (17.8)	72 (33.5)	44 (19.5)
Missing	119 (58.9)	14 (6.5)	22 (9.7)
HBsAg (n, %)			
Negative	27 (13.3)	0 (0.0)	6 (2.7)
Positive	132 (65.3)	184 (85.6)	184 (81.4)
Missing	43 (21.3)	31 (14.4)	36 (15.9)
HBeAg (n, %)			
Negative	36 (17.8)	98 (45.6)	136 (60.9)
Positive	123 (60.9)	86 (40.0)	54 (17.8)
Missing	43 (21.3)	31 (14.4)	36 (21.3)
HBsAb (n, %)			
Negative	120 (59.4)	153 (71.2)	164 (72.6)
Positive	39 (19.3)	31 (14.4)	24 (10.6)
Missing	43 (21.3)	31 (14.4)	36 (15.9)
HBeAb (n, %)			
Negative	45 (22.3)	97 (45.1)	55 (24.3)
Positive	114 (56.4)	87 (40.5)	135 (59.7)
Missing	43 (21.3)	31 (14.4)	36 (15.9)
HBcAb (n, %)			
Negative	9 (4.5)	0 (0.0)	3 (1.3)
Positive	149 (73.8)	184 (85.6)	187 (82.7)
Missing	44 (21.8)	31 (14.4)	36 (15.9)
HCV-DNA (n, %)			
Negative	133 (65.8)	31 (14.4)	100 (44.2)
Positive	14 (6.9)	0 (0)	2 (8.9)
Missing	55 (27.2)	184 (85.6)	124 (54.9)

Abbreviations: AFP,alpha-fetoprotein; CHB, chronic hepatitis B; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBsAb, hepatitis B surface antibody; HBeAg, hepatitis B e antigen; HBeAb, hepatitis B e antibody; HBcAb, hepatitis B core antibody; HCC, hepatocellular carcinoma; LC, liver cirrhosis

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Table S2. Association between the serum levels of six markers and clinicopathological factors in HCC patients

Variable	AFP		AFP-L3		DCP		SCCA		CENPF	
	Median (1^{st} Qu. -3^{rd} Qu.)	P-value¶	Median (1^{st} Qu. -3^{rd} Qu.)	P-value¶	Median (1^{st} Qu. -3^{rd} Qu.)	P-value¶	Median (1^{st} Qu. -3^{rd} Qu.)	P-value¶	Median (1^{st} Qu. -3^{rd} Qu.)	P-value¶
Age (years)										
≤55	35.6(6.7-330.7)	0.245	0.50(0.50-30.49)	0.904	222.0(52.3-2611.0)	0.329	113.9 (47.9-200.6)	0.133	169.9 (105.9-256.1)	0.662
>55	15.4(3.6-282.9)		0.50(0.50-29.46)		266.0(33.0-1432.0)		133.5 (67.7-390.4)		155.8 (82.3-267.1)	
Sex										
Male	15.1(4.4-238.7)	0.084	22.5(0.5-30.1)	0.439	248.5(39.0-1970.0)	0.851	119.7 (51.3-281.4)	0.484	167.2 (97.4-269.1)	0.594
Female	104.4(103-478.2)		1.7(0.5-36.0)		314.0(40.3-1322.0)		138.1 (71.6-216.8)		150.8 (109.6-205.8)	
HBsAg										
Positive	4.1 (2.2-23.2)	0.063	0.5 (0.5-0.5)	0.420	28.0 (21.0-50.0)	0.4700	150.6 (77.6-312.6)	0.056	142.3 (69.8-209.4)	0.412
Negative	7.8 (2.72-273.5)		0.5 (0.5-0.5)		35.0 (28.5-61.0)		75.9 (58.3-175.5)		143.8 (64.1-523.5)	
HBeAg										
Positive	15.0(4.3-248.8)	0.218	0.50(0.50-21.8)	0.239	212.0(40.0-2122.0)	0.608	135.2(67.7-303.7)	0.186	150.8(78.0-226.6)	0.324
Negative	67.6(8.4-545.0)		3.2(0.50-59.9)		391.0(55.0-2020.0)		111.7(63.9-179.2)		185.7(126.2-259.3)	
HCV infection										
Positive	17.7(4.9-337.9)	0.912	0.50(0.50-22.0)	0.437	251.0(42.0-2083.0)	0.852	135.9(68.9-296.0)	0.069	162.8(97.7-238.7)	0.784
Negative	20.4(5.7-167.7)		1.6(0.50-42.2)		337.0(36.5-1164.0)		84.8(41.4-141.6)		187.9(74.3-416.0)	
TNM tumor stage										
Stage I	13.2(3.3-184.5)	**0.034**	0.5(0.5-0.8)	**0.002**	71.5(30.0-518.0)	**<0.001**	135.9(67.6-289.0)	**0.036**	138.9(70.9-192.1)	**0.039**
>Stage I	48.6(6.4-346.6)		9.6(0.5-41.3)		542.0(67.5-4126.0)		71.8(47.7-86.9)		192.3(100.1-320.0)	

¶ The differences between the two groups were examined by the Wilcoxon Test.

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Table S3a. Diagnostic performance of the combination of AFP with CENPF or SCCA for detecting HCC

Marker combination	**HCC vs LC+HC**		**HCC vs LC**		**HCC vs HC**	
	AUC¶	SEN at 90% SPE§	AUC¶	SEN at 95% SPE§	AUC¶	SEN at 90% SPE§
AFP+CENPF	0.77 (0.72-0.87)	46.2 (28.6-64.6)	0.68 (0.63-0.80)	35.3 (20.9-55.0)	0.90 (0.45-0.70)	79.3 (67.3-90.9)
AFP+SCCA	0.63 (0.56-0.79)	34.0 (20.0-56.8)	0.60 (0.54-0.75)	29.2 (17.9-52.6)	0.82 (0.43-0.72)	70.1 (57.9-85.7)

Table S3b. Diagnostic performance of the combination of AFP with CENPF or SCCA for detecting Early-stage HCC

Marker combination	**Early-HCC vs LC+HC**		**Early-HCC vs LC**		**Early-HCC vs HC**	
	AUC¶	SEN at 90% SPE§	AUC¶	SEN at 95% SPE§	AUC¶	SEN at 90% SPE§
AFP+CENPF	0.61 (0.52-0.79)	27.5 (9.1-53.0)	0.53 (0.41-0.71)	20.6 (0-41.7)	0.84 (0.44-0.79)	68.3 (44.4-91.7)
AFP+SCCA	0.62 (0.54-0.78)	31.7 (19.4-55.6)	0.59 (0.53-0.74)	30.0 (17.5-52.8)	0.82 (0.44-0.72)	71.1 (56.4-87.1)

Abbreviations: AUC, area under the curve; CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; LC, liver cirrohosis; SEN, sensitivity; SPE, specificity

¶ AUC was adjusted for potential overfitting by .632+ bootstrap method

§.632+ bootstrap adjusted sensitivity at cutoffs yielding 90% specificity

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Table S4. Diagnostic performance of marker combinations for discriminating early-stage hepatocellular carcinoma and liver cirrhosis

Marker combination	Early-stage HCC vs decompensated LC		Early-stage HCC vs compensated LC		P-value*
	Apparent AUC [95% CI]	.632+ AUC [95% CI]	Apparent AUC [95% CI]	.632+ AUC [95% CI]	
AFP+AFP-L3	0.63 [0.52-0.74]	0.61 [0.39-0.75]	0.62 [0.50-0.74]	0.61 [0.37-0.77]	0.904
AFP+DCP	0.81 [0.75-0.86]	0.73 [0.71-0.87]	0.84 [0.78-0.90]	0.80 [0.53-0.88]	0.422
AFP-L3+DCP	0.63 [0.52-0.74]	0.61 [0.41-0.78]	0.81 [0.70-0.90]	0.76 [0.36-0.80]	0.018
AFP+AFP-L3+DCP	0.72 [0.61-0.82]	0.68 [0.48-0.82]	0.82 [0.73-0.91]	0.77 [0.33-0.80]	0.126

Abbreviations: AUC, area under the curve; HCC, hepatocellular carcinoma; LC, liver cirrhosis

* p-value for examining the differences between the AUC of the marker combination for discriminating early-stage HCC vs. decompensated LC and the AUC for discriminating early-stage HCC vs. compensated LC, using bootstrapping method (1000 bootstrap samples)

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Table S5. Diagnostic performance of AFP+DCP and their combination with age and sex for detecting hepatocellular carcinoma

Combination	AFP+DCP		AFP+DCP+age+sex	
	AUC¶	SEN at 90% SPE§	AUC¶	SEN at 90% SPE§
HCC vs LC+CHB+HC	0.87[0.68-0.84]	73.8[63.6-84.2]	0.92[0.73-0.88]	77.3[66.6-86.3]
HCC vs LC+CHB	0.84[0.67-0.83]	68.2[59.4-78.5]	0.91[0.74-0.88]	75.3[64.1-85.1]
HCC vs LC	0.83[0.68-0.84]	64.2[53.9-76.6]	0.87[0.70-0.86]]	64.2[53.9-76.6]
Early HCC vs LC+CHB+HC	0.79[0.73-0.88]	59.8[46.4-77.4]	0.88[0.80-0.93]	65.4[51.7-81.8]
Early HCC vs LC+CHB	0.77[0.71-0.86]	56.0[43.2-70.6]	0.87[0.79-0.92]	63.2[48.4-78.4]
Early HCC vs LC	0.75[0.71-0.87]	52.6[37.0-68.6]	0.81[0.71-0.89]	56.1[40.5-72.7]

Abbreviations: AUC, area under the curve; CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; LC, liver cirrohosis; SEN, sensitivity; SPE, specificity

¶ AUC was adjusted for potential overfitting by .632+ bootstrap method

§.632+ bootstrap adjusted sensitivity at cutoffs yielding 90% specificity

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Table S6. The regression equations and optimal probabilities of the combination of AFP and DCP

Group	No.	Regression model[¶] [ln(p/(1-p)]	Optimal probability[$]	Sensitivity (%)[§]	Specificity (%)[§]
HCC vs CHB+LC+HC	202 vs 644	0.004xAFP+0.004DCP-2.106	0.129	83.7	85.1
HCC vs CHB+LC	202 vs 441	0.003xAFP+0.003DCP-1.698	0.178	83.2	78.9
HCC vs LC	202 vs 226	0.003xAFP+0.002DCP-0.959	0.301	83.7	77.4
Early-HCC vs CHB+LC+HC	94 vs 644	0.004xAFP+0.002DCP-2.496	0.084	79.8	81.2
Early-HCC vs CHB+LC	94 vs 441	0.003xAFP+0.002DCP-2.102	0.119	76.6	76.6
Early-HCC vs LC	94 vs 226	0.003xAFP+0.001DCP-1.395	0.210	79.8	75.2

Abbreviations: CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; LC, liver cirrohosis;

[¶] The algorithm was constructed using logistic regression model

[$] The optimal probability was defined by threshold showing the highest Youden's index (i.e., sensitivity + speficity-1)

[§] Apparent sensitivity/specificity without correction for potential overfitting at respective optimal probability (defined by the Youden's index)

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Table S7. The regression equations and optimal probabilities of the combination of AFP, DCP, age and sex.

Group	No.	Regression model[¶] [ln(p/(1-p)]	Optimal probability[$]	Sensitivity (%)[§]	Specificity (%)[§]
HCC vs CHB+LC+HC	201 vs 636	0.005xAFP+0.003xDCP+0.09xAGE-1.648xSEX-4.661	0.200	86.6	83.2
HCC vs CHB+LC	201 vs 433	0.004xAFP+0.002xDCP+0.102xAGE-1.646xSEX-4.909	0.319	79.6	88.5
HCC vs LC	201 vs 225	0.004xAFP+0.002xDCP+0.079xAGE-1.512xSEX-3.378	0.398	79.6	83.1
Early-HCC vs CHB+LC+HC	94 vs 636	0.004xAFP+0.002xDCP+0.079xAGE-1.767xSEX-4.370	0.119	85.1	78.1
Early-HCC vs CHB+LC	94 vs 433	0.004xAFP+0.001xDCP+0.092xAGE-1.732xSEX-4.678	0.155	86.2	75.1
Early-HCC vs LC	94 vs 225	0.003xAFP+0.001xDCP+0.0068xAGE-1.587xSEX-3.164	0.296	69.1	84.9

Abbreviations: CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; LC, liver cirrohosis

[¶] The algorithm was constructed using logistic regression model, the age was continuous variable in years and the sex was categorical variable (male=1, female=2).

[$] The optimal probability was defined by threshold showing the highest Youden's index (i.e., sensitivity + speficity-1)

[§] Apparent sensitivity/specificity without correction for potential overfitting at respective optimal probability (defined by the Youden's index)

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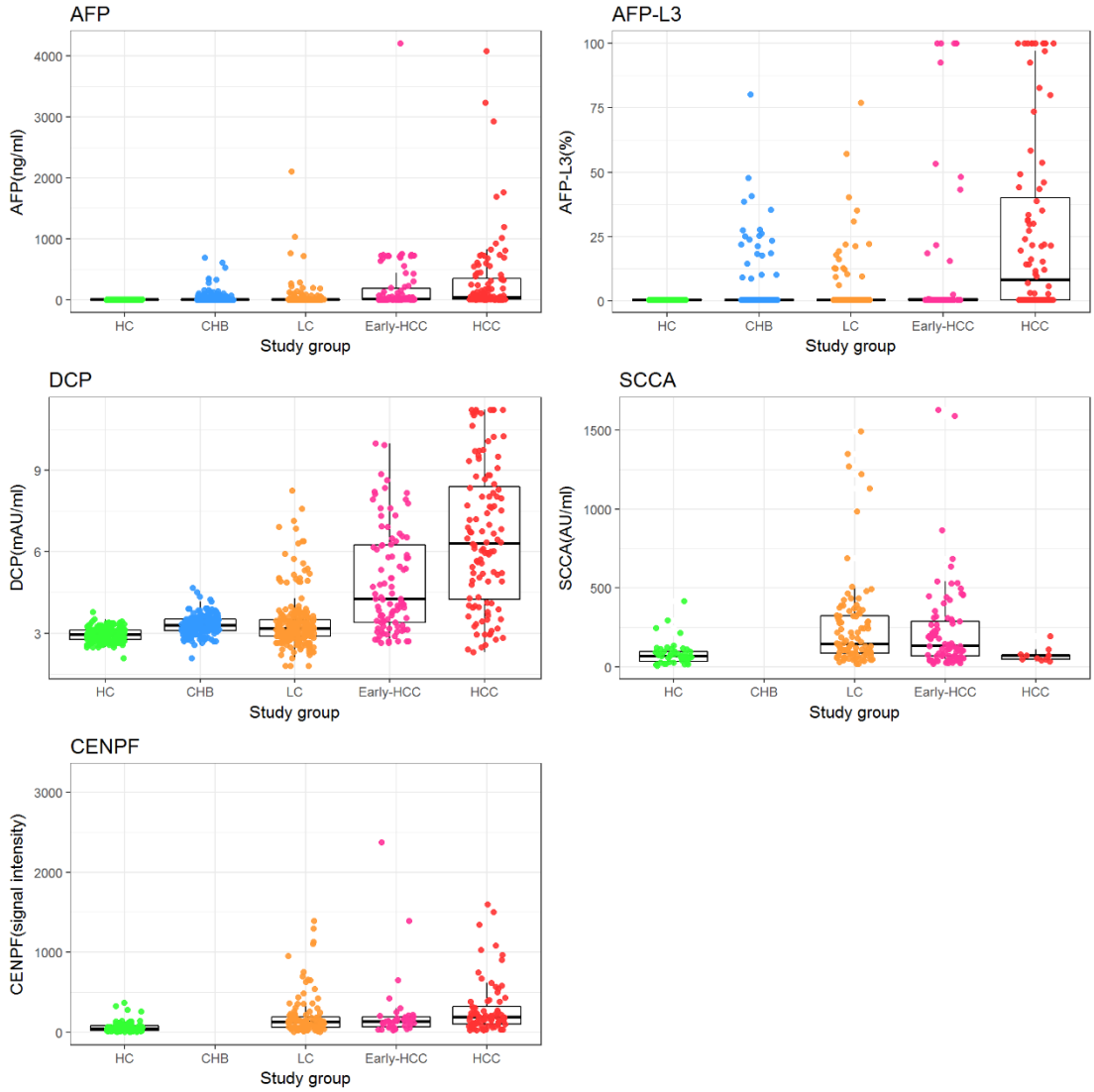

Figure S1. Scatter plot showing the expression intensity of AFP, AFP-L3, DCP, SCCA and CENPF-Ab between different study groups

Abbreviations: ROC=receiver operating characteristics. HCC=hepatocellular carcinoma; HC=healthy control; CHB=chronic hepatitis B virus infection; LC=liver cirrhosis

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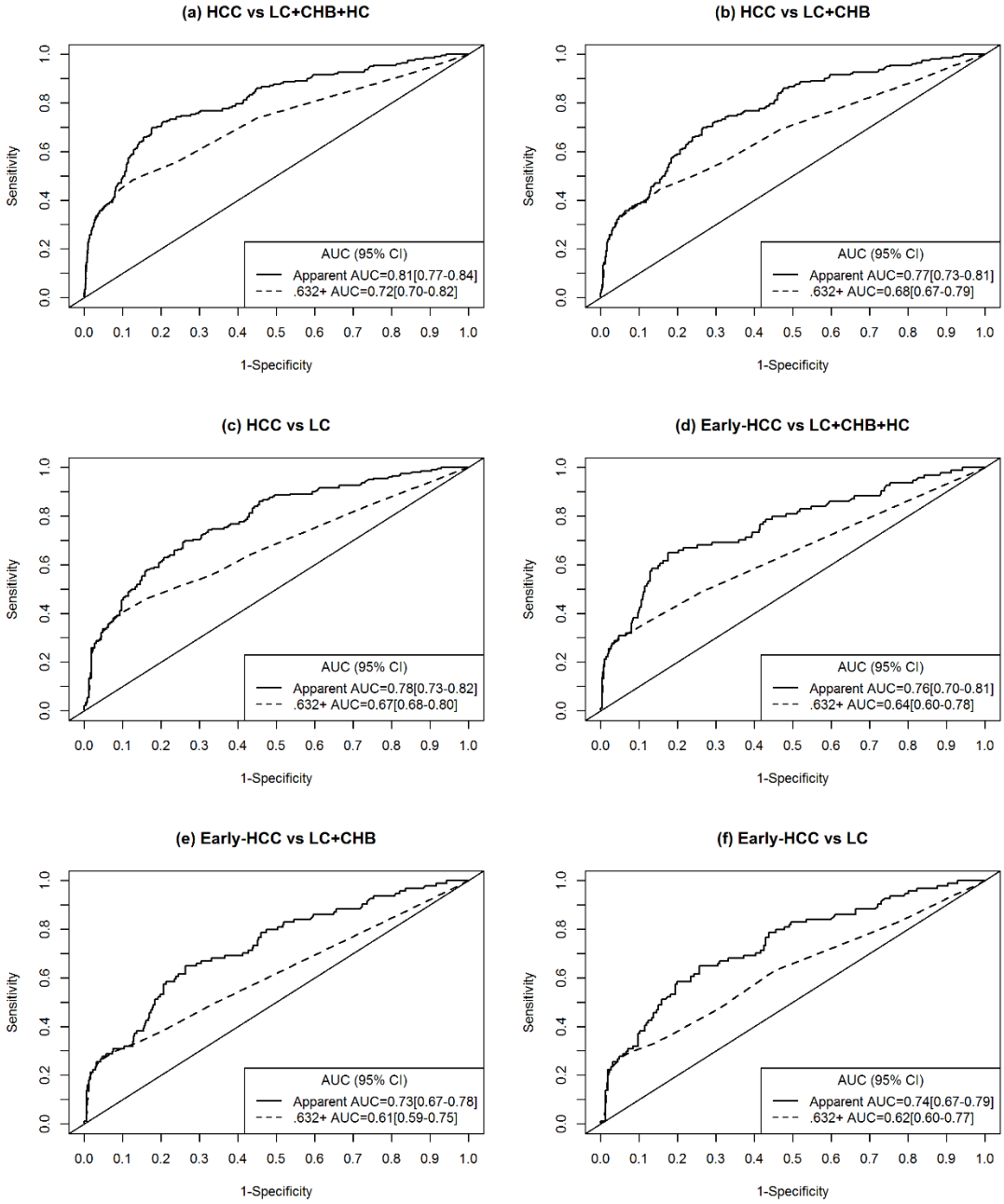

Figure S2. Comparison of apparent and .632+ adjusted receiver operating characteristics (ROC) curves for AFP in discriminating: (a) HCC vs. CHB+LC+HC; (b) HCC vs. CHB+LC; (c) HCC vs CHB; (d) Early-stage HCC vs CHB+LC+HC; (e) Early-stage HCC vs CHB+LC; (f) Early-stage HCC vs LC.

Abbreviations: AUC=area under the curve. HCC=hepatocellular carcinoma; HC=healthy control; CHB=chronic hepatitis B virus infection; LC=liver cirrhosis

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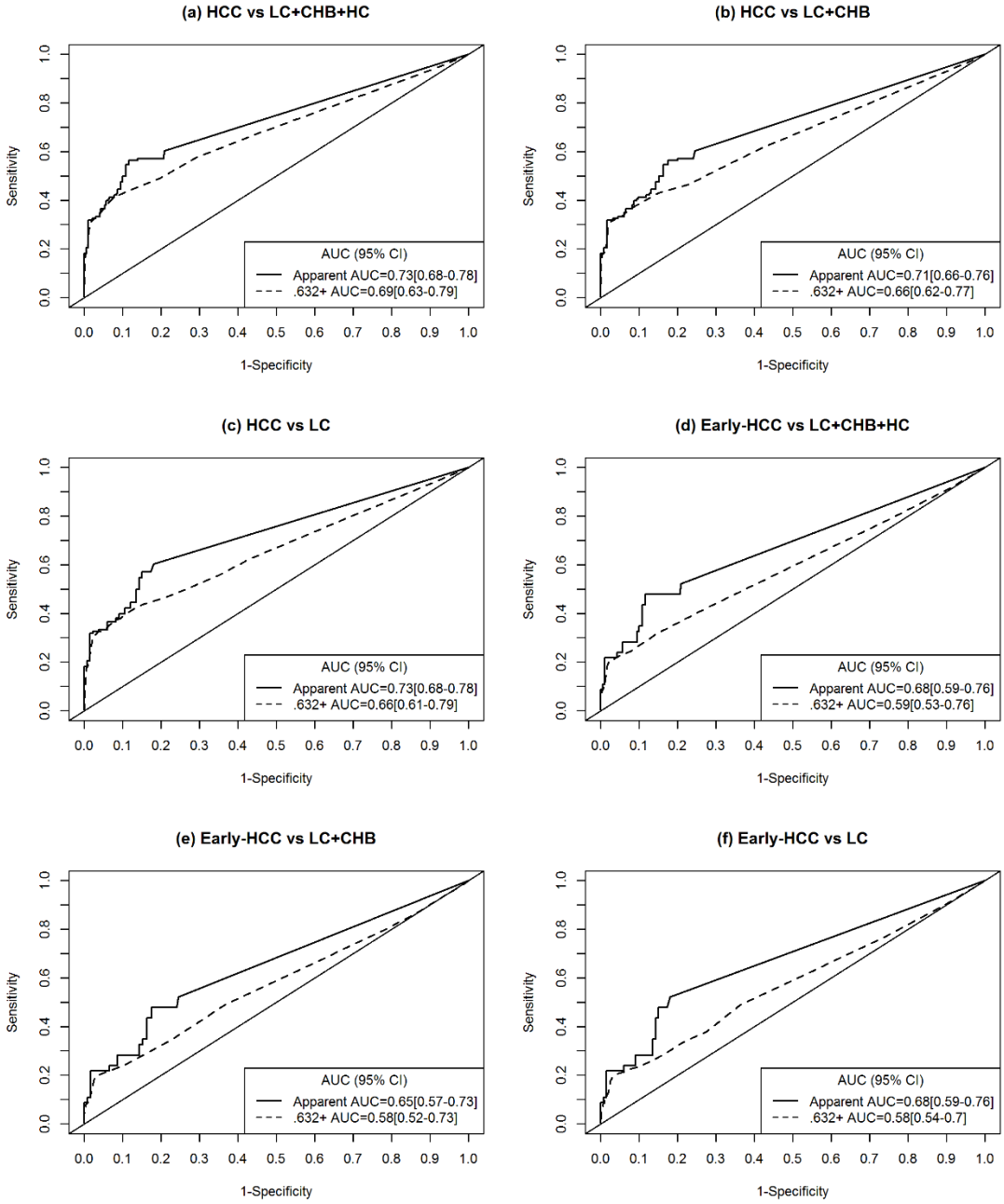

Figure S3. Comparison of apparent and .632+ adjusted receiver operating characteristics (ROC) curves for AFP-L3 in discriminating: (a) HCC vs. CHB+LC+HC; (b) HCC vs. CHB+LC; (c) HCC vs CHB; (d) Early-stage HCC vs CHB+LC+HC; (e) Early-stage HCC vs CHB+LC; (f) Early-stage HCC vs LC.

Abbreviations: AUC=area under the curve. HCC=hepatocellular carcinoma; HC=healthy control; CHB=chronic hepatitis B virus infection; LC=liver cirrhosis

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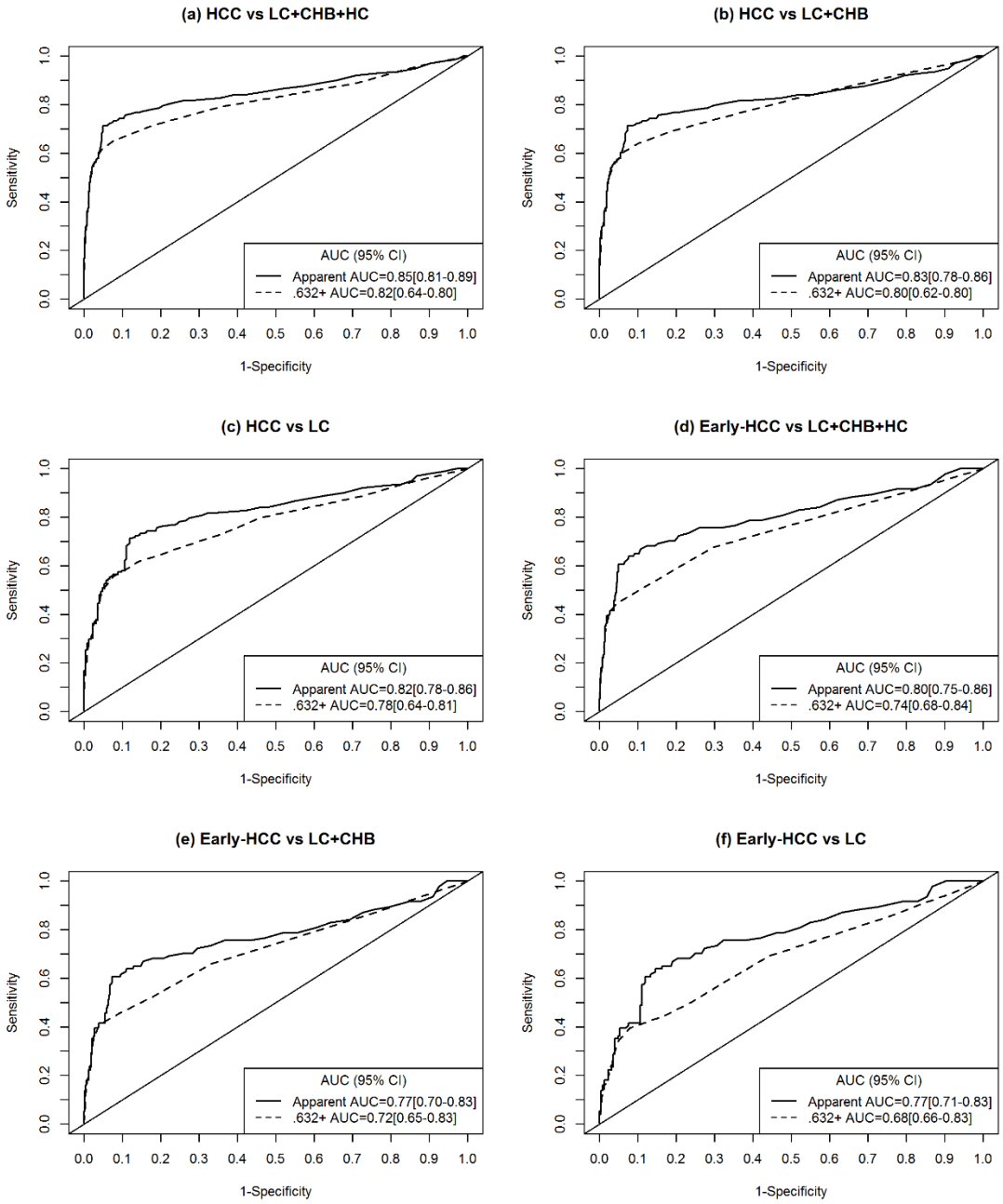

Figure S4. Comparison of apparent and .632+ adjusted receiver operating characteristics (ROC) curves for DCP in discriminating: (a) HCC vs. CHB+LC+HC; (b) HCC vs. CHB+LC; (c) HCC vs CHB; (d) Early-stage HCC vs CHB+LC+HC; (e) Early-stage HCC vs CHB+LC; (f) Early-stage HCC vs LC.

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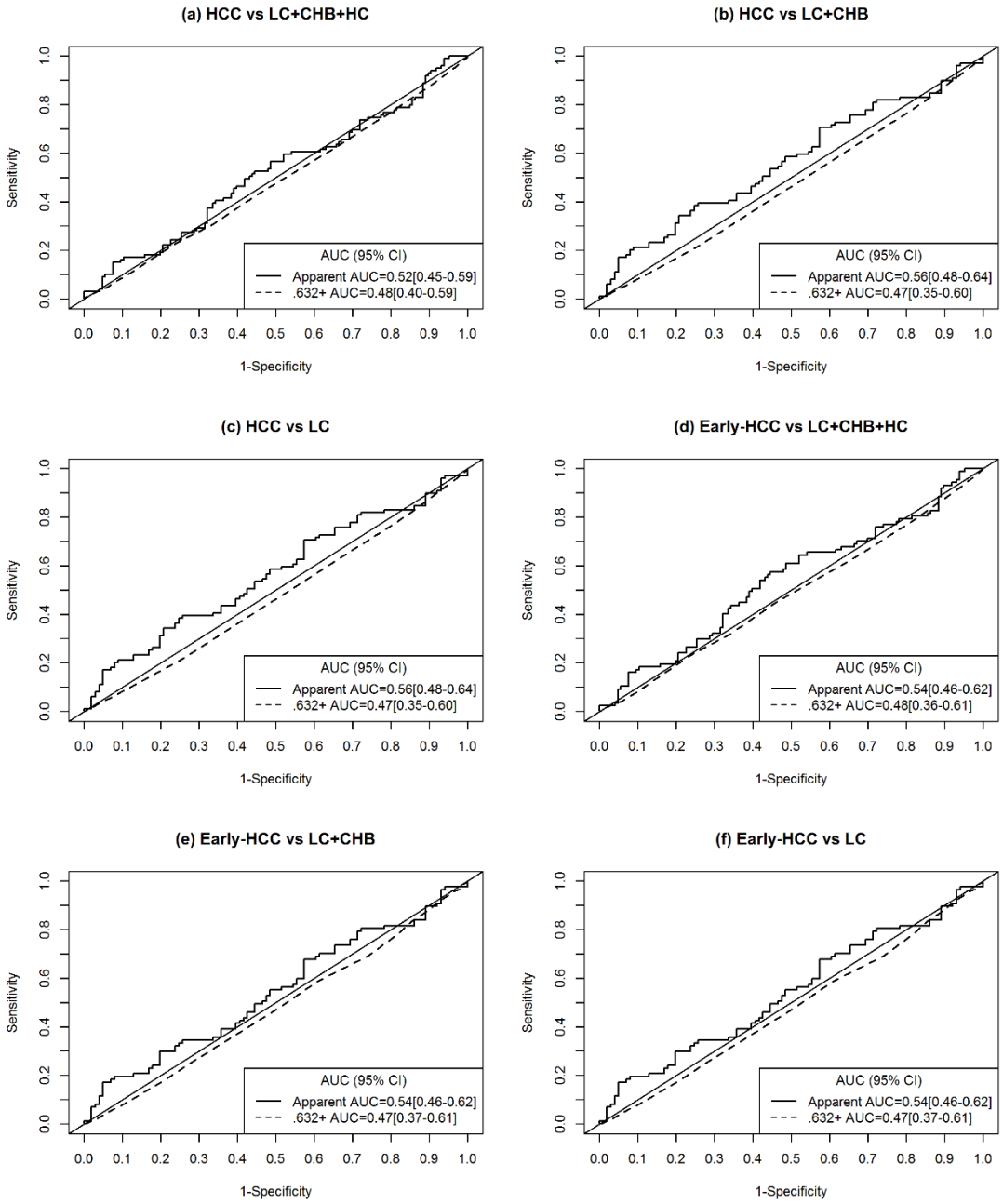

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Abbreviations: AUC=area under the curve. HCC=hepatocellular carcinoma; HC=healthy control; CHB=chronic hepatitis B virus infection; LC=liver cirrhosis

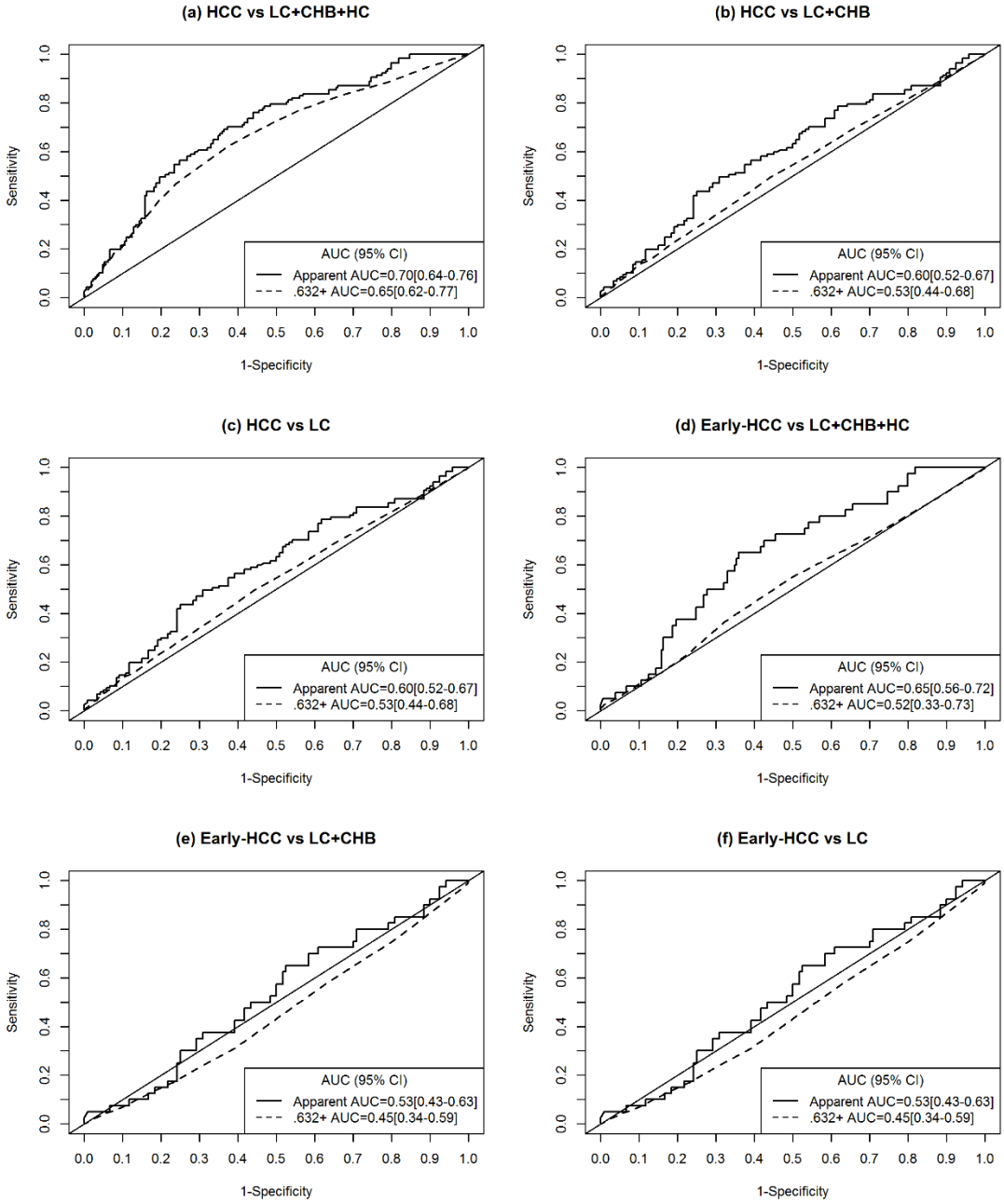

Figure S6. Comparison of apparent and .632+ adjusted receiver operating characteristics (ROC) curves for CENPF in discriminating: (a) HCC vs. CHB+LC+HC; (b) HCC vs. CHB+LC; (c) HCC vs CHB; (d) Early-stage HCC vs CHB+LC+HC; (e) Early-stage HCC vs CHB+LC; (f) Early-stage HCC vs LC.

Abbreviations: AUC=area under the curve. HCC=hepatocellular carcinoma; HC=healthy control; CHB=chronic hepatitis B virus infection; LC=liver cirrhosis

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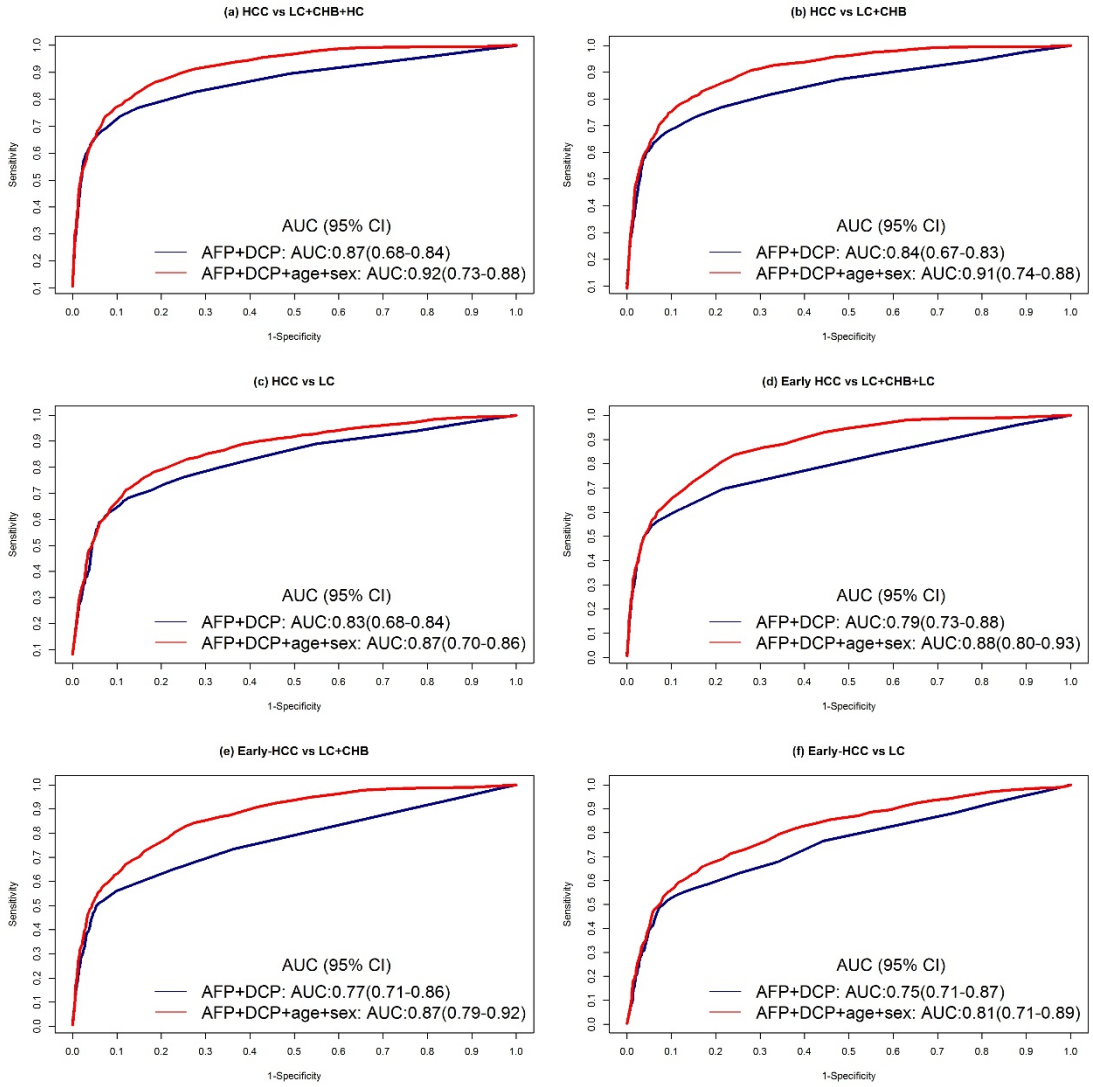

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Abbreviations: AUC=area under the curve. HCC=hepatocellular carcinoma; HC=healthy control; CHB=chronic hepatitis B virus infection; LC=liver cirrhosis