Supplementary Materials


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│    1131 registered patients     │
│        (2016.4-2019.12)         │
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              │      ┌────────────────────────────────────────────────────────────┐
              │      │ 80 candidates were excluded:                               │
              │      │     22 patients with chronic hepatitis B(CHB) and          │
              │      │        alcohol-related liver disease (ALD) at the same time│
              ├─────→│     18 patients with liver failure                         │
              │      │     13 patients with esophageal and gastric variceal       │
              │      │        bleeding                                            │
              │      │      7 patients with drug-induced liver disease            │
              │      │     20 patients had no insufficient information of relevant│
              │      │        laboratory tests or clearly clinical diagnosis of   │
              │      │        hepatocellular carcinoma (HCC)                      │
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│ 1051 enrolled patients for following analysis:                                     │
│      784 patients without HCC: 561 CHB, 34 chronic hepatitis C, 56 nonalcoholic    │
│ fatty liver disease(NAFLD), 82 ALD, 51 autoimmune liver disease(AILD)              │
│      127 Early-stage HCC                                                           │
│      140 Late-stage HCC                                                            │
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│ Univariate and multivariate logistic regression analysis for factors associated   │
│    with abnormally elevated serum PIVKA-II levels in non-HCC and HCC patients,     │
│    respectively                                                                    │
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│ Analysis and comparison of PIVKA-II       │
│ levels in different subgroups             │
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│ Comparison of the diagnostic efficacy of PIVKA-II │
│ for early-stage and late-stage HCC in different    │
│ subgroups, respectively                           │
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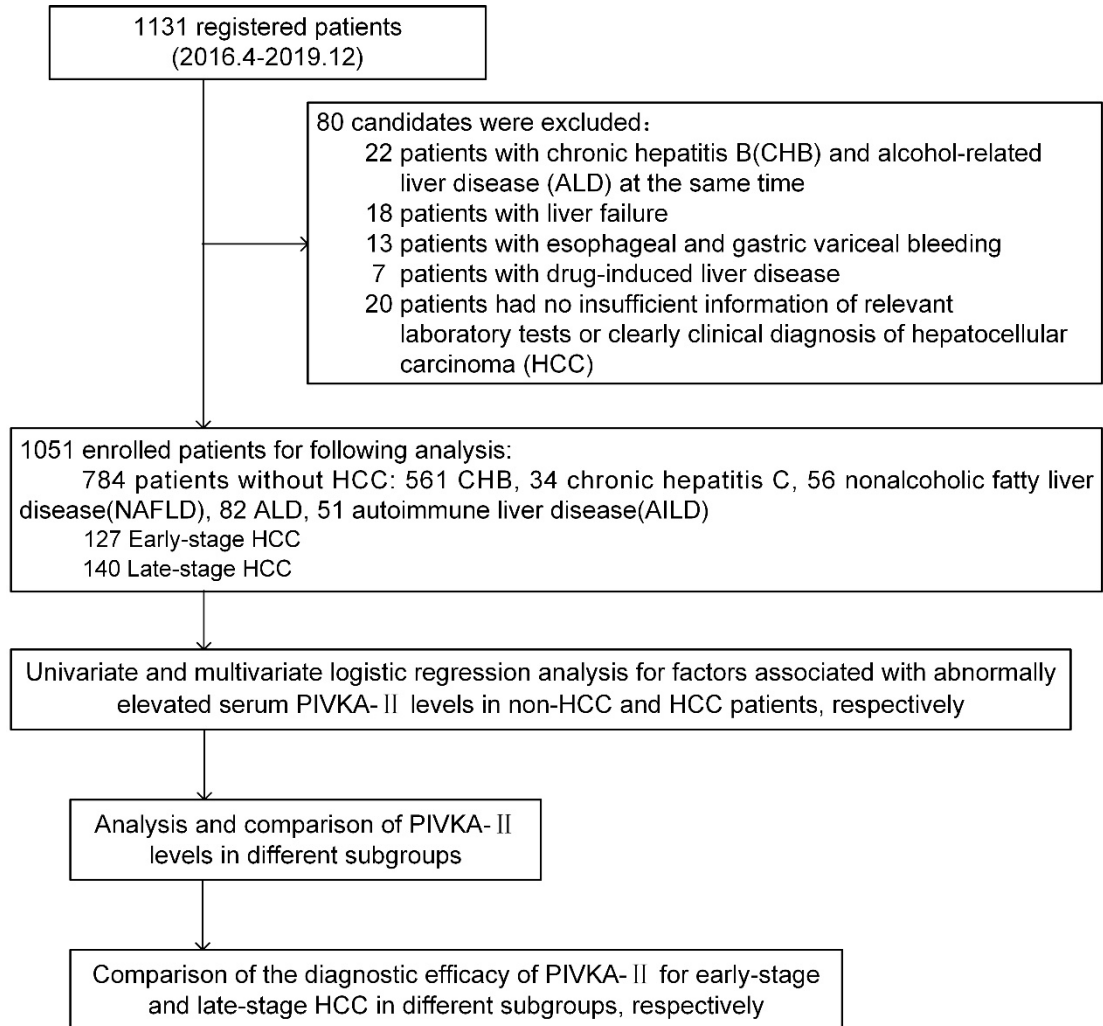
Supplementary Figure 1. The distribution and analysis process of patients enrolled in this study

Supplementary Table 1. Comparisons of the levels of serum protein induced by vitamin K absence or antagonist-II among non-, early-stage and late-stage hepatocellular carcinoma in different subgroups

Variables	Non-HCC	Early stage HCC	Late stage HCC	*P* value
Etiology				
Viral	23.0(18.0,31.0)	58.0(26.0,200.0)	5337(837.3,36245)	<0.001
Nonviral	27.0(20.0,38.0)	259.0(20.8,1613)	3033(223.8,44225)	<0.001
ALP				
≤1ULN	23.0(18.0,29.0)	50.0(25.0,190.5)	2129(238.0,28751)	<0.001
>1ULN	30.0(20.0,50.5)	62.5(27.8,406.8)	11992(1485,75000)	<0.001
TBIL				
≤1ULN	23.0(18.0,29.0)	59.0(28.0,216.0)	4018(462.0,32848)	<0.001
>1ULN	27.0(19.0,45.8)	55.5(22.5,234.0)	9448(991.0,58070)	<0.001
AST				
≤1ULN	23.0(19.0,29.0)	54.5(24.3,223.0)	593.0(100.5,6219)	<0.001
>1ULN	26.0(19.0,36.0)	58.0(32.0,234.0)	15164(1839,75000)	<0.001

HCC, hepatocellular carcinoma; ALP, alkaline phosphatase; TBIL, total bilirubin; AST, aspartate aminotransferase; ULN, upper limit of normal.

Supplementary Table 2. Performance characteristics of serum protein induced by vitamin K absence or antagonist-II for diagnosing late-stage hepatocellular carcinoma in different subgroups

Late HCC	AUC(95%CI)	Cutoff value (mAU/ml)	Se(%)	Sp(%)	LR+	LR-	*P* value
All Late	0.966(0.945,0.986)	94.0	87.86	95.92	21.52	0.13	
Etiology							0.556
Viral	0.970(0.950,0.990)	93.0	87.88	97.98	43.57	0.12	
Nonviral	0.944(0.860,1.000)	125.0	87.50	93.12	12.72	0.13	
ALP							0.894
≤1ULN	0.968(0.944,0.993)	46.0	91.36	92.67	12.47	0.093	
>1ULN	0.965(0.929,1.000)	364.0	89.83	98.48	58.99	0.10	
TBIL							0.457
≤1ULN	0.964(0.936,0.992)	49.0	90.53	95.47	19.99	0.099	
>1ULN	0.977(0.957,0.997)	114.0	93.33	93.48	14.31	0.071	
AST							0.201
≤1ULN	0.948(0.908,0.989)	46.0	87.76	91.60	10.45	0.13	
>1ULN	0.978(0.956,1.000)	483.0	92.31	99.52	191.54	0.077	

HCC, hepatocellular carcinoma; AUC, area under the ROC curve; CI, confidence interval; Se, sensitivity; Sp, specificity; LR+, positive likelihood ratio; LR-, negative likelihood ratio; ALP, alkaline phosphatase; TBIL, total bilirubin; AST, aspartate aminotransferase; ULN, upper limit of normal.
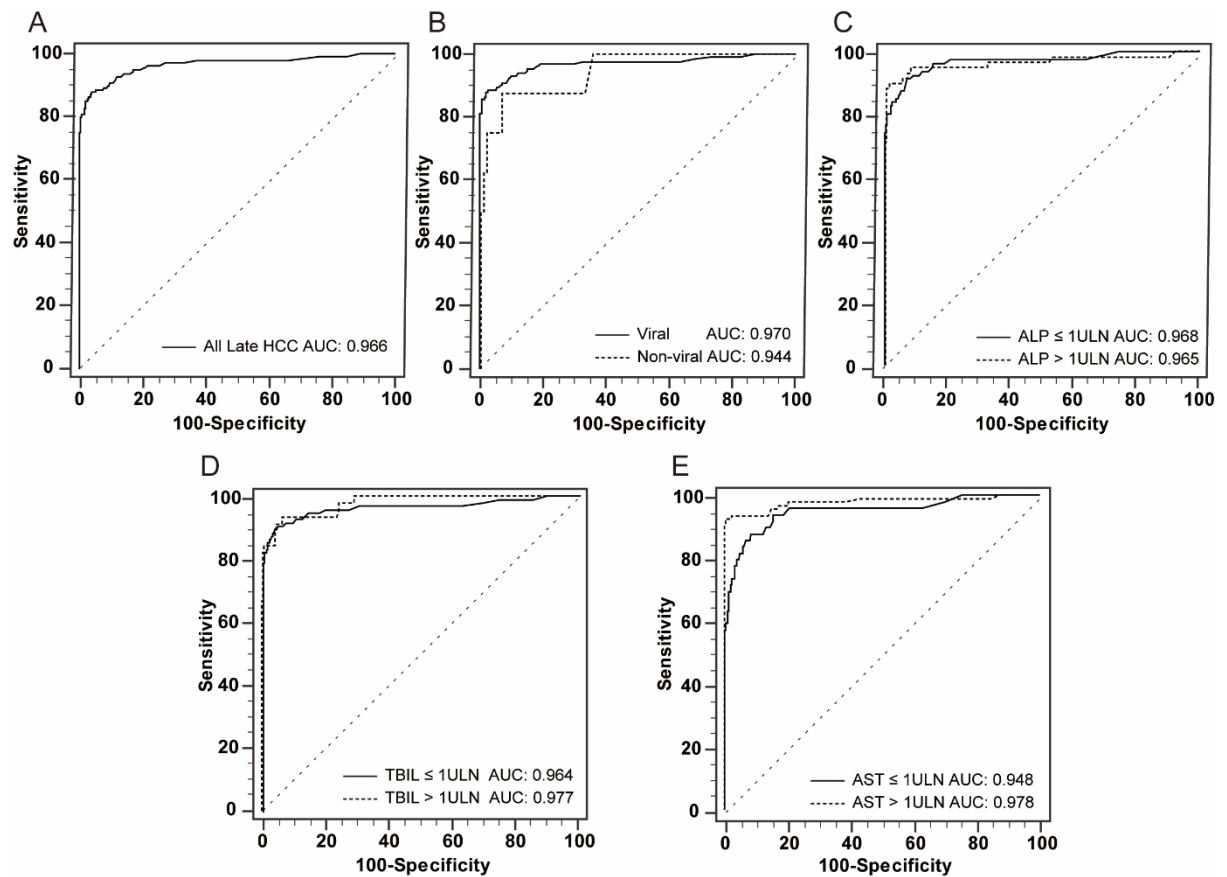
Supplementary Figure 2. Comparisons of ROC curves of serum PIVKA-II for diagnosing late-stage HCC in different subgroups. (A) For all late-stage patients. (B) Between viral and nonviral subgroups. (C) Between ALP ≤ 1×ULN and ALP > 1×ULN subgroups. (D) Between TBIL ≤ 1×ULN and TBIL > 1×ULN subgroups. (E) Between AST ≤ 1×ULN and AST > 1×ULN subgroups.
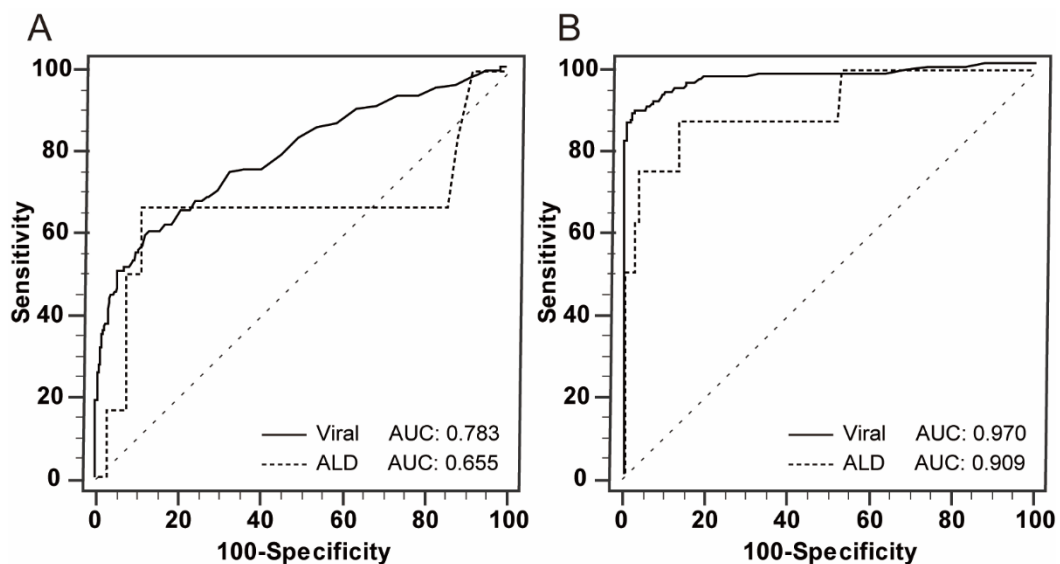
Supplementary Figure 3. Comparisons of ROC curves of serum PIVKA-II for diagnosing HCC between subgroups of viral and ALD etiology. (A) For early-stage HCC. (B) For late-stage HCC.

Supplementary Table 3. Performance characteristics of serum protein induced by vitamin K absence or antagonist-II in diagnosing early-stage hepatocellular carcinoma in subgroups of alkaline phosphatase and total bilirubin for patients with viral etiology

Viral Etiology	AUC(95%CI)	Cutoff value (mAU/ml)	Se(%)	Sp(%)	LR+	LR-	P value
ALP							0.3184
≤1ULN	0.800(0.742,0.857)	31.0	68.09	80.56	3.50	0.40	
>1ULN	0.731(0.608,0.854)	42.0	65.22	76.52	2.78	0.45	
TBIL							0.0121
≤1ULN	0.837(0.783,0.891)	27.0	79.27	73.40	2.98	0.28	
>1ULN	0.677(0.564,0.790)	42.0	57.14	80.37	2.91	0.53	

HCC, hepatocellular carcinoma; AUC, area under the ROC curve; CI, confidence interval; Se, sensitivity; Sp, specificity; LR+, positive likelihood ratio; LR-, negative likelihood ratio; ALP, alkaline phosphatase; TBIL, total bilirubin; ULN, upper limit of normal.

Supplementary Table 4. Performance characteristics of serum protein induced by vitamin K absence or antagonist-II in diagnosing early-stage hepatocellular carcinoma in subgroups of alkaline phosphatase and total bilirubin for patients with HBV alone

HBV alone	AUC(95%CI)	Cutoff value (mAU/ml)	Se(%)	Sp(%)	LR+	LR-	P value
ALP							0.3680
≤1ULN	0.794(0.735,0.853)	31.0	67.39	80.32	3.43	0.41	
>1ULN	0.732(0.610,0.854)	42.0	65.22	75.97	2.71	0.46	
TBIL							0.0151
≤1ULN	0.832(0.777,0.888)	27.0	78.75	73.07	2.92	0.29	
>1ULN	0.676(0.564,0.789)	42.0	57.14	80.19	2.88	0.53	

HCC, hepatocellular carcinoma; AUC, area under the ROC curve; CI, confidence interval; Se, sensitivity; Sp, specificity; LR+, positive likelihood ratio; LR-, negative likelihood ratio; ALP, alkaline phosphatase; TBIL, total bilirubin; ULN, upper limit of normal.