Improving the Early Detection of Hepatocellular Carcinoma in Patients with Chronic Liver Disease: An Integrated C-GALAD Model and Cell-Free DNA Approach

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ABSTRACT

Background/Aims: *Chronic liver disease (CLD) confers an increased risk of hepatocellular carcinoma (HCC), but individual biomarkers have limited diagnostic performance, necessitating a combined approach. The study aimed to evaluate the value of a combined strategy integrating the C-GALAD (gender, age, lens culinaris agglutinin-reactive alpha-fetoprotein [AFP-L3], and des-gamma-carboxy prothrombin [DCP]) model with circulating cell-free DNA (cfDNA) for the early detection of progression from CLD to HCC.*

Materials and Methods: *A total of 226 patients (HCC, n = 57; CLD without HCC, n = 169) were enrolled. Pretreatment blood samples were collected, and AFP, AFP-L3, and DCP levels were measured; C-GALAD scores were calculated. Plasma cfDNA was extracted and quantified. Baseline clinical characteristics (age, sex, etiology, and cirrhosis) and laboratory parameters (platelet count, total bilirubin, serum albumin [ALB], AFP, AFP-L3, and DCP) were compared between groups. Multivariate logistic regression and receiver operating characteristic curve analysis were performed to identify independent diagnostic factors and evaluated diagnostic performance.*

Results: *The HCC group had a higher prevalence of cirrhosis, lower ALB levels, and higher AFP, AFP-L3, and DCP levels than the CLD group. Median C-GALAD scores were 51 (interquartile range [IQR], 43-57) in the HCC group and 51 (IQR, 46-58) in the CLD group, whereas cfDNA concentrations were 40.25 (IQR, 33.41-48.19) and 31.62 (25.90-35.45) ng/mL, respectively (both P < .001). Multivariable logistic regression identified cirrhosis (adjusted odds ratio [OR], 3.14; 95% CI, 1.18-8.37; P = .022), C-GALAD score (adjusted OR, 2.32; 95% CI, 1.73-3.10; P < .001), and cfDNA concentration (adjusted OR, 1.20; 95% CI, 1.12-1.30; P < .001) as independent predictors of HCC. The area under the curve (AUC) was 0.850 for C-GALAD (sensitivity, 66.67%; specificity, 89.94%) and 0.799 for cfDNA. The combined model achieved an AUC of 0.917, with a sensitivity of 82.46%, specificity of 91.72%, accuracy of 89.38%, positive predictive value of 77.05%, and negative predictive value of 93.94%.*

Conclusion: *The combined C-GALAD and cfDNA strategy significantly improves the accuracy of early HCC detection in patients with CLD, demonstrating excellent diagnostic performance.*

Keywords: *C-GALAD model, circulating cell-free DNA, chronic liver disease, early diagnosis, hepatocellular carcinoma*

INTRODUCTION

Hepatocellular carcinoma (HCC) remains a malignancy with persistently high incidence and mortality worldwide. Its asymptomatic progression during the early stages often leads to late-stage diagnosis and poor prognosis.[1-3] Chronic liver disease (CLD)—including chronic hepatitis B (CHB), chronic hepatitis C (CHC), alcohol-related liver disease (ALD), and nonalcoholic fatty liver disease (NAFLD)—is a major risk factor, with affected individuals at significantly elevated risk of HCC progression.[4,5] Timely detection and intervention at the precancerous or early stage can substantially improve 5-year survival, highlighting the urgent need for precise and efficient early diagnostic strategies.[6]

Current early diagnostic approaches include serum biomarkers and imaging. Classic markers such as alpha-fetoprotein (AFP), lens culinaris agglutinin-reactive AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP) have notable performance limitations, particularly across different etiologic backgrounds.[7,8] AFP exhibits low sensitivity for early HCC and may show false-positive elevations in patients with active CLD without malignancy, leading to missed diagnoses or overdiagnosis.[9] Imaging

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modalities (e.g., ultrasonography, contrast-enhanced computed tomography [CT], and magnetic resonance imaging [MRI]) can detect space-occupying lesions but have limited sensitivity for small nodules (<1 cm) and face challenges in distinguishing benign from malignant lesions, particularly in patients with CLD and complex histological changes.[10-12] Thus, no single modality can meet the requirements for clinical precision, highlighting the need for multimarker strategies.

The C-GALAD (gender, age, AFP-L3, and DCP) model is a scoring system based on gender, age, and serum markers including AFP, AFP-L3, and DCP. This model was derived by refitting the original GALAD formula for the Chinese population and aims to improve HCC diagnostic accuracy by integrating clinical and laboratory parameters.[13] Previous studies have demonstrated that the original GALAD outperforms traditional markers.[14] Circulating cell-free DNA (cfDNA), a liquid biopsy biomarker released from apoptotic or necrotic tumor cells, can be detected early during tumorigenesis and offers high specificity and noninvasive approach across cancers, representing a novel avenue for early HCC diagnosis.[15] However, each approach has individual drawbacks. C-GALAD relies on traditional markers that may remain normal in some patients with early HCC, potentially delaying diagnosis; its performance in patients with nonhepatitis B virus (HBV) CLD remains insufficiently validated, raising concerns about its generalizability across etiologies.[16,17] cfDNA detection, although not directly limited by etiology, is influenced by tumor stage and burden and, when used alone, carries a risk of underdetection.[18] Combining the integrative clinical parameters of C-GALAD with the cross-etiologic molecular sensitivity of cfDNA may overcome etiology-specific limitations and improve diagnostic accuracy and generalizability across HBV, hepatitis C virus (HCV), alcohol-related, and NAFLD etiologies.

Given the clinical diversity of CLD etiologies and the limited generalizability of existing diagnostic tools, the present study systematically evaluated a combined C-GALAD plus cfDNA strategy for early HCC diagnosis in CLD populations, aiming to establish a precise diagnostic approach with cross-etiologic adaptability, thereby facilitating risk stratification, early intervention, and ultimately improved prognosis.

Main Points

- *The C-GALAD (gender, age, lens culinaris agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin) score and circulating cell-free DNA (cfDNA) levels were significantly elevated in patients with hepatocellular carcinoma (HCC) and identified as independent diagnostic factors.*
- *Individually, the C-GALAD model and cfDNA demonstrated moderate diagnostic performance, with area under the curve (AUC) values of 0.850 and 0.799, respectively.*
- *The combined C-GALAD and cfDNA model significantly improved diagnostic accuracy, achieving an AUC of 0.917.*
- *This integrated strategy demonstrated high sensitivity (82.46%) and specificity (91.72%) for detecting early-stage HCC in patients with chronic liver disease.*
- *The combined approach overcomes the etiological specificity limitations of existing tools and provides a promising "pan-etiology" strategy for early HCC screening.*

MATERIALS AND METHODS

Patients

A total of 226 patients with CLD treated at the First Hospital of Putian between March 2023 and March 2025 were enrolled. Participants were divided into an HCC group and a CLD without HCC group. The enrollment process is shown in Figure 1. Inclusion criteria were as follows: (1) age 18 years or older; (2) patients with HCC confirmed by pathologic biopsy were required to have a documented history of CLD, and the diagnosis had to meet the criteria of the "Standard for diagnosis and treatment of primary liver cancer (2022 edition)," with disease classified as Barcelona Clinic Liver Cancer (BCLC) stage 0 or A; (3) CLD included CHB, CHC, alcohol-related liver disease or NAFLD.[19] Chronic HBV infection was confirmed by serum hepatitis B surface antigen positivity for more than 6 months; chronic HCV infection was diagnosed by a positive HCV antibody test and confirmed by detection of HCV RNA; ALD was diagnosed based on chronic heavy alcohol intake (men ≥56 g/day or ≥196 g/week; women ≥42 g/day or ≥98 g/week of pure alcohol) after exclusion of other causes of liver disease; and NAFLD was diagnosed by imaging or histology after exclusion of other potential causes of secondary hepatic fat accumulation. For patients without HCC, the absence of intrahepatic space-occupying lesions was confirmed by abdominal ultrasonography, contrast-enhanced CT, or contrast-enhanced MRI; if a lesion was present, needle biopsy was performed to confirm its benignity. Additional inclusion criteria were no history of liver transplantation; no antiviral, hepatoprotective, radiotherapy, chemotherapy, immunotherapy, or other treatment that might affect test indicators within the past 3 months; complete clinical data; and the ability to cooperate with regular follow-up.[4-7,20-23]

Exclusion criteria were as follows: (1) concurrent other malignancies; (2) end-stage liver cancer; (3) coinfection

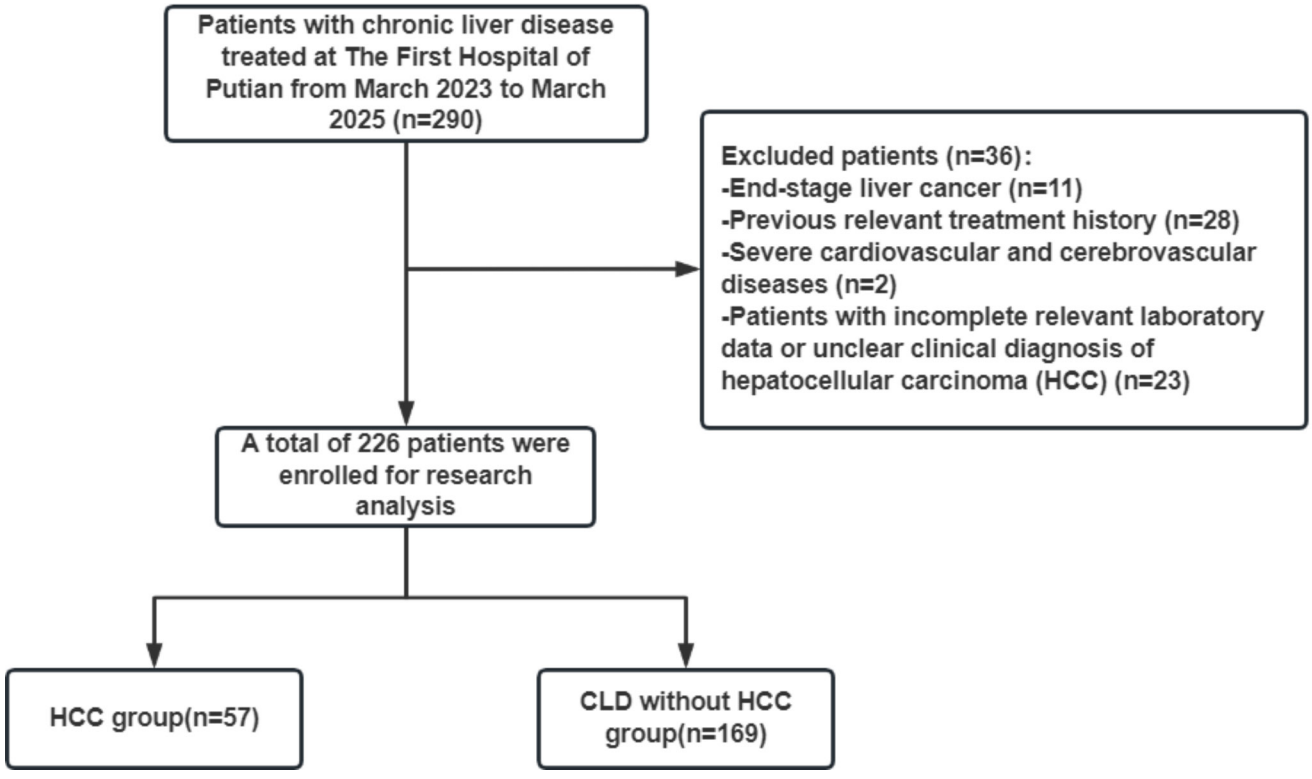

Figure 1. Flowchart of patient enrollment.

with HBV and HCV, to avoid potential interference with viral replication and host immune response; (4) severe concurrent cardiovascular or cerebrovascular diseases, renal disease, pulmonary disease, or autoimmune diseases, as these conditions may trigger systemic inflammation and cellular damage, potentially interfering with serum marker levels; (5) active concurrent infections, which may cause nonspecific elevations in markers such as cfDNA; (6) obstructive jaundice; (7) a history of prior treatment (liver resection, liver transplantation, transarterial chemoembolization, radiofrequency ablation, anti-angiogenic drugs, or warfarin therapy); (8) pregnancy or lactation; (9) cognitive impairment or mental illness that precluded cooperation with sample collection and follow-up.

This study was approved by the Ethics Committee of the First Hospital of Putian (Approval No.202112PT76; approval date: December 6, 2021), and written informed consent was obtained from each participant. No artificial intelligence was used during the preparation of the manuscript.

Cirrhosis Diagnosis Criteria

Cirrhosis was diagnosed according to the Chinese guidelines on the management of liver cirrhosis (2019 edition). The specific criteria were as follows: (1) histologic evidence of cirrhosis; (2) endoscopic findings of esophagogastric or ectopic varices; (3) imaging findings (CT, ultrasound, or liver stiffness measurement) suggestive of cirrhosis or portal hypertension; or (4) in patients without histology, endoscopy, or imaging, a diagnosis could be made if at least 2 of the following 4 criteria were met: (a) platelet count (PLT) <100 × 10^9/L without other identifiable cause; (b) serum albumin (ALB) <35 g/L after exclusion of other causes such as renal disease or malnutrition; (c) international normalized ratio greater than 1.3 or prolonged prothrombin time after discontinuation of anticoagulant or thrombolytic therapy for more than 7 days; or (d) aspartate aminotransferase-to-platelet ratio index greater than 2 in adults. Fulfillment of any 1 of the above criteria was sufficient for a diagnosis of cirrhosis.

Laboratory Methods

Before any treatment, 5 mL of blood was collected from all patients. Blood samples were obtained before pathological examination, when HCC status had not yet been confirmed. Blood samples were allowed to clot at room temperature for 30 minutes, followed by centrifugation at 5000 rpm (approximately 2795 × *g*) for 10 minutes at

4°C to separate the serum. The isolated serum was immediately aliquoted and stored at −80°C until subsequent analysis. Serum AFP levels were measured using a Cobas 6000 automatic biochemical analyzer (Roche Diagnostics, Basel, Switzerland) with the corresponding AFP detection kit. AFP-L3 was separated using the microcentrifugation column method, and its level was determined using the Cobas 6000 analyzer and AFP-L3 detection kit. Serum DCP levels were measured by enzyme-linked immunosorbent assay (ELISA) using a Thermo Scientific microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) and a DCP ELISA kit. Reference ranges were as follows: AFP < 20 μg/L, AFP-L3 < 10%, and DCP < 40 mAU/mL. The C-GALAD score was calculated according to the established formula (13): score = −11.501 + 0.733 × [gender (male = 1, female = 0)] + 0.099 × [age (years)] + 0.073 × [AFP-L3 (%)] + 0.840 × log10[AFP (ng/mL)] + 2.346 × log10[DCP (mAU/mL)]. Positive thresholds were defined as AFP greater than 20 μg/L, AFP-L3 greater than 10%, and DCP greater than 40 mAU/mL. Additionally, a 3-mL fasting venous blood sample was collected from patients with CLD into anticoagulant tubes. Plasma cfDNA was extracted using a Serum/Plasma Circulating DNA Extraction Kit (Tiangen Biochemical Technology Co., Ltd., Beijing, China) and stored at −20°C. Primers targeting the stably expressed housekeeping gene *GAPDH* (Sangon Biotech Co., Ltd., Shanghai, China) were used for quantification. A standard curve was constructed using serial dilutions (1 : 10, 1 : 100, 1 : 200, 1 : 400, 1 : 800) of human genomic DNA as the standard (purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). Following a single freeze–thaw cycle, the extracted DNA samples were subjected to amplification using a real-time quantitative polymerase chain reaction instrument. The reaction conditions were as follows: 95°C for 3 minutes, followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. After completion of the reaction, the obtained cycle threshold (Ct) values were applied to the standard curve to calculate the corresponding DNA concentration. The final Ct value was taken as the mean of 3 replicate wells, and standard curves with correlation coefficients (R^2) ≥ 0.99 were considered acceptable. Only quantitative cfDNA concentration was measured; no molecular sequencing or profiling analysis was performed.

Statistical Analysis

Data analysis was conducted using R software (version 4.2.2) (R Foundation for Statistical Computing; Vienna, Austria) and MSTATA software (www.mstata.com), and GraphPad Prism (version 10.0.0) (GraphPad Software; Boston, MA, USA) was used for graphical representation. The Kolmogorov–Smirnov test was used to assess the normality of data distribution. Normally distributed continuous data are expressed as mean ± SD, and group differences were assessed using the *t*-test. Non-normally distributed continuous variables are reported as median and interquartile range (IQR), and the Mann–Whitney *U*-test was used for intergroup comparisons. Categorical variables are summarized as counts and percentages, with differences assessed using the chi-square test or Fisher's exact test, as appropriate. Receiver operating characteristic (ROC) curve analysis was performed to establish optimal diagnostic thresholds for HCC. The cutoff point yielding the maximum Youden's index was selected, and the corresponding accuracy, sensitivity, and specificity were calculated. Initially, univariate logistic regression was used to screen for factors associated with HCC. Variables identified as significant in this initial analysis were subsequently entered into a multivariate logistic regression model to identify independent risk factors. A 2-sided $P < .05$ was considered statistically significant.

RESULTS

Baseline Characteristics of the Patients

A total of 57 patients with HCC and 169 patients with CLD without HCC were enrolled in the study. Baseline characteristics are summarized in Table 1. No significant differences were observed in age or sex between the groups ($P = .518$ and $P = .616$). Regarding etiology, the distributions of HBV, HCV, ALD, and NAFLD were comparable between the groups (all $P > .05$). The prevalence of liver cirrhosis was significantly higher in patients with HCC (66.7%) than in patients with CLD without HCC (40.2%) ($P = .001$). Among patients with HCC, 32 (56.14%) had solitary tumors and 25 (43.86%) had multiple tumors (≥2); tumor diameters were <3 cm in 19 (33.33%), 3-5 cm in 17 (29.82%), and ≥5 cm in 21 (36.85%) patients; and 24 cases (42.11%) met the Milan criteria for early-stage HCC. Laboratory analysis revealed no statistically significant differences in PLT or total bilirubin between the groups ($P = .573$ and $P = .067$, respectively). However, ALB concentrations were significantly lower in the HCC group ($P = .028$). Conversely, serum levels of AFP, AFP-L3, and DCP were all significantly higher in the HCC group than in the CLD without HCC group (all $P < .001$; Figure 2).

Expression Levels of C-GALAD Score and Cell-Free DNA in Hepatocellular Carcinoma and Chronic Liver Disease Without Hepatocellular Carcinoma Groups

Both C-GALAD score and plasma cfDNA concentration were measured and compared between the groups.

Table 1. Clinical Characteristics of Patients with HCC and CLD Without Patients with HCC

Clinical Characteristics	HCC (n = 57)	CLD Without HCC (n = 169)	*P*
Age, years	51 (43, 57)	51 (46, 58)	.518
Gender			.616
Male	40 (73.68%)	117 (69.23%)	
Female	17 (29.82%)	52 (30.77%)	
Etiology			.573
HBV	34 (59.65%)	77 (45.56%)	
HCV	12 (21.05%)	40 (23.67%)	
ALD	8 (14.04%)	29 (17.16%)	
NAFLD	6 (5.26%)	23 (13.61%)	
Liver cirrhosis	38 (66.67%)	68 (40.23%)	.001
Number of tumors		N/A	N/A
Solitary	32 (56.14%)	N/A	N/A
≥2	25 (43.86%)	N/A	N/A
Tumor size		N/A	N/A
<3 cm	19 (33.33%)	N/A	N/A
3-5 cm	17 (29.82%)	N/A	N/A
≥5 cm	21 (36.85%)	N/A	N/A
Early-stage HCC (Milan criteria)	24 (42.11%)	N/A	N/A
Laboratory parameters			
PLT (×10^9/L)	152.46 ± 36.34	157.83 ± 32.47	.296
TBIL (μmol/L)	16.76 ± 2.22	17.35 ± 2.31	.091
ALB (g/L)	33.68 ± 5.34	35.70 ± 6.13	.027
AFP (μg/L)	249.24 (70.47, 975.57)	21.66 (15.30, 26.83)	<.001
AFP-L3 (%)	9.42 ± 1.69	6.76 ± 1.04	<.001
DCP (mAU/mL)	407.14 (55.25, 1347.90)	30.47 (28.23, 40.67)	<.001

AFP, alpha-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive alpha-fetoprotein; ALB, serum albumin; ALD, alcohol-related liver disease; CLD, chronic liver disease; DCP, des-gamma-carboxy prothrombin; HBV, hepatitis B virus, HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; PLT, platelet count; TBIL, total bilirubin.

As shown in Figure 3, the median C-GALAD score was 51 (IQR, 43-57) in the HCC group (n = 57) and 51 (IQR, 46-58) in the CLD without HCC group (n = 169). A statistically significant difference in C-GALAD score was observed between the groups ($P < .001$). Similarly, cfDNA analysis showed a median level of 40.25 ng/mL (IQR, 33.41-48.19) in the HCC group, which was significantly higher than the median level of 31.62 ng/mL (IQR, 25.90-35.45) in the CLD without HCC group ($P < .001$). These results indicate that both markers have significant discriminatory ability, underscoring their potential as biomarkers for distinguishing HCC from CLD.

C-GALAD Score and Cell-Free DNA Are Independent Predictors of Hepatocellular Carcinoma

,Multivariable logistic regression analysis was performed with HCC occurrence as the dependent variable and the statistically significant factors from Table 1 (cirrhosis, ALB, C-GALAD score, and cfDNA level) as independent variables. The results indicated that liver cirrhosis, C-GALAD score, and cfDNA level were significantly associated with HCC risk. Patients with liver cirrhosis had a significantly higher risk of HCC than those without cirrhosis (adjusted OR, 3.14; 95% CI, 1.18-8.37; $P = .022$). A negative association was observed between serum ALB and HCC risk (adjusted OR, 0.95; 95% CI, 0.87-1.03), although this association was not statistically significant ($P = .178$). Each unit increase in C-GALAD score was strongly associated with higher HCC risk (adjusted OR, 2.32; 95% CI, 1.73-3.10; $P < .001$). Similarly, each unit increase in cfDNA level was significantly associated with increased HCC risk (adjusted OR, 1.20; 95% CI, 1.12-1.30; $P < .001$). Detailed results are presented in Table 2 and Figure 4.

Diagnostic Value of Combining C-GALAD and Cell-Free DNA for Hepatocellular Carcinoma Progression in Patients with Chronic Liver Disease

ROC curve analysis was used to assess the performance of C-GALAD score, cfDNA, and their combination in differentiating HCC from CLD without HCC. Results are detailed in Table 3 and Figure 5. The C-GALAD score exhibited favorable diagnostic efficacy, with an area under the curve (AUC) of 0.850 (95% CI: 0.790-0.910). At the optimal cutoff value of 1.236, the sensitivity was 66.67%, specificity was 89.94%, Youden's index was 0.5661, and overall accuracy was 80.47%. cfDNA also demonstrated diagnostic potential, with an AUC of 0.799 (95% CI, 0.726-0.872). Using a cutoff of 35.80, sensitivity was 70.18%, specificity was 77.51%, Youden's index was 0.4769, and accuracy was 75.66%. Notably, the integrated model combining C-GALAD and cfDNA demonstrated the highest diagnostic performance, with an AUC of 0.917. At the optimal cutoff of 0.3116, the combined model achieved a sensitivity of 82.46%, specificity of 91.72%, Youden's index of 0.7418, and accuracy of 89.38%. This integrated approach also demonstrated optimized positive and negative predictive values (positive predictive value [PPV]: 77.05%; negative predictive value [NPV]: 93.94%). In summary, the integration of the

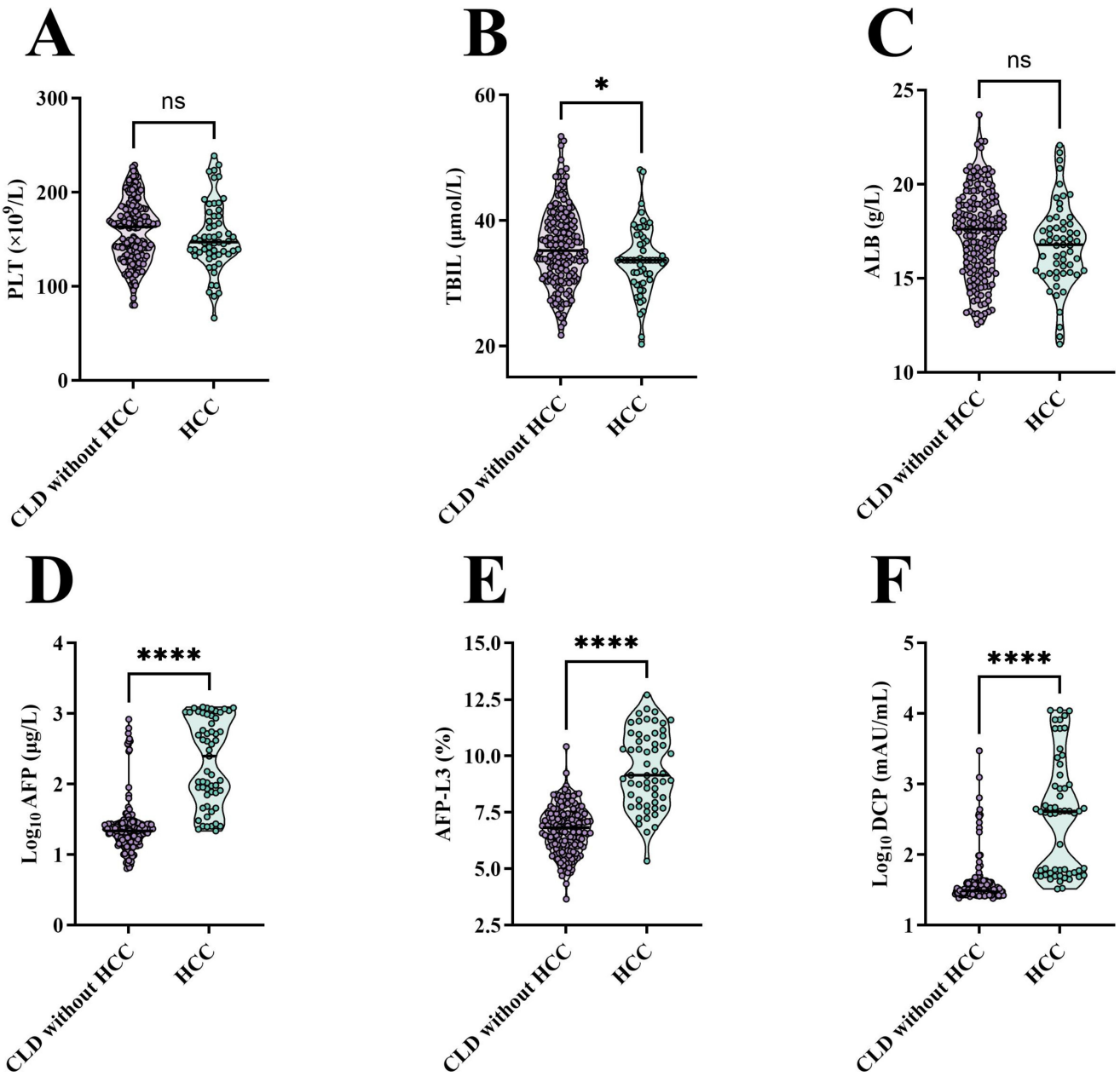

Figure 2. Comparison of serum markers between the CLD without HCC group and the HCC group. (A) Platelets; (B) TBIL; (C) ALB; (D) AFP; (E) AFP-L3; (F) DCP. *P < .05; ****P < .0001. ALB, serum albumin; AFP, alpha-fetoprotein; CLD, chronic liver disease; DCP, des-gamma-carboxy prothrombin; HCC, hepatocellular carcinoma; TBIL, total bilirubin.

C-GALAD score and cfDNA effectively combines molecular and clinical data, enhancing the precision of early HCC detection in patients with CLD.

DISCUSSION

HCC represents one of the most aggressive end-stage complications of CLD. Most patients are diagnosed at intermediate or advanced stages, precluding curative interventions and resulting in poor prognosis.[24,25] Therefore, early and accurate screening is critical for improving outcomes and reducing mortality.[26] Conventional screening tools have notable limitations: serum AFP is prone to false-positive and false-negative results due to benign liver conditions such as hepatitis activity and cirrhosis, and its performance as a single test remains suboptimal.[27,28] The C-GALAD model, a composite scoring

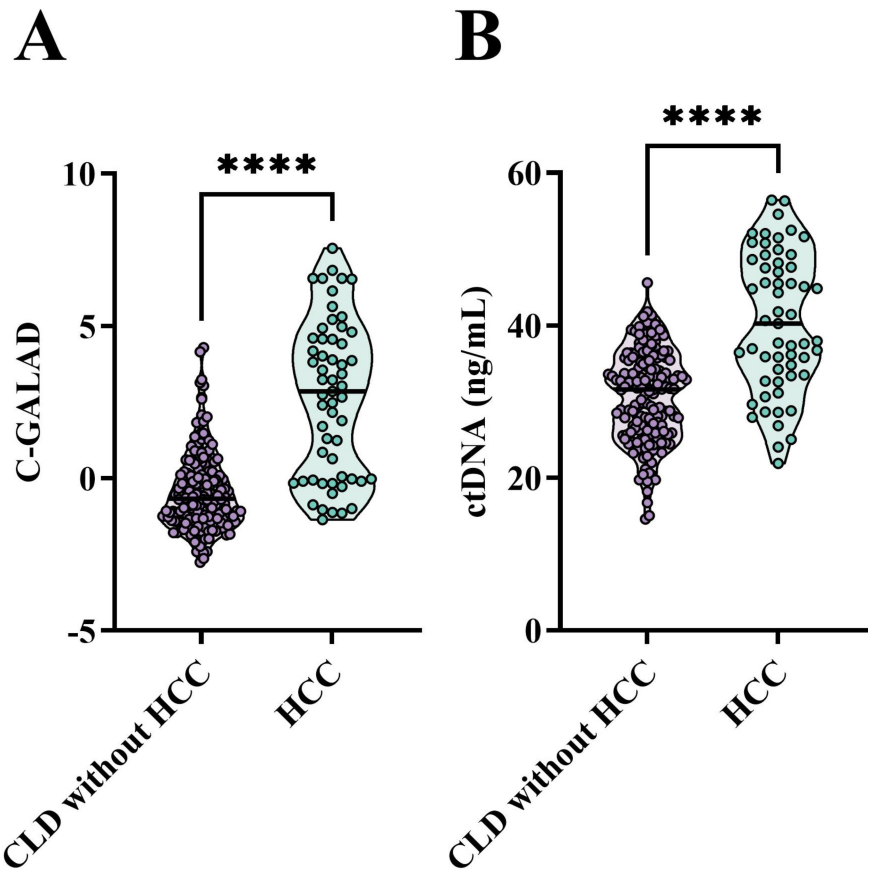

Figure 3. Expression levels of the C-GALAD score and cfDNA in patients with CLD without HCC and HCC. (A) C-GALAD scores in the 2 groups. (B) cfDNA levels in the 2 groups. ****$P < .0001$. cfDNA, cell-free DNA; C-GALAD, gender, age, lens culinaris agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin; CLD, chronic liver disease; HCC, hepatocellular carcinoma.

Table 2. Multivariable Logistic Regression Analysis for Independent Predictors of HCC Diagnosis

Characteristics	N	Event N	OR	95% CI	*P*
Liver cirrhosis					
No	120	19	Reference	—	—
Yes	106	38	3.14	1.18, 8.37	.022
ALB	226	57	0.95	0.87, 1.03	.178
C-GALAD	226	57	2.32	1.73, 3.10	<.001
cfDNA	226	57	1.20	1.12, 1.30	<.001

Model fit statistics: null deviance = 255; null *df* = 225; log-likelihood = −59.2; AIC = 128; BIC = 146; deviance = 118; residual *df* = 221; number of observations = 226.

ALB, serum albumin; cfDNA, cell-free DNA; C-GALAD, gender, age, lens culinaris agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin; HCC, hepatocellular carcinoma; OR, odds ratio.

system integrating multiple established serum markers, overcomes the limitations of single-biomarker testing.[8] Meanwhile, circulating cfDNA, an emerging liquid biopsy marker, sensitively reflects malignant transformation and genomic aberrations.[18] Based on a comparative analysis of 226 patients with CLD, the present study demonstrates that the combined C-GALAD and cfDNA strategy significantly enhances early diagnostic efficacy for the progression of CLD to HCC, thereby offering a novel and efficient screening approach.

Both the C-GALAD score and cfDNA levels were significantly higher in the HCC group than in the non-HCC group, and both were identified as independent diagnostic predictors. These findings are consistent with previous reports and further support the diagnostic potential of these markers across diverse CLD etiologies. The

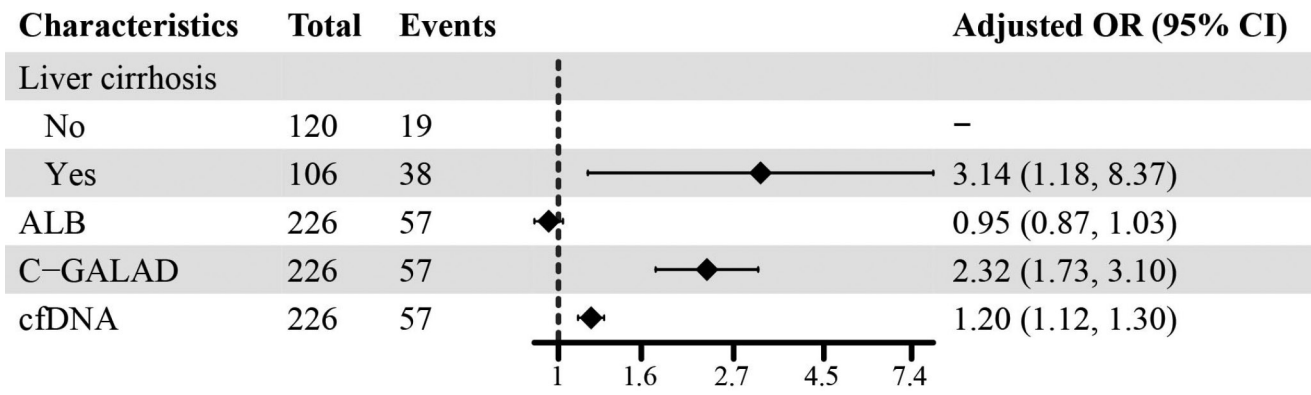

Figure 4. Forest plot of multivariable logistic regression analysis for independent factors associated with HCC in patients with CLD. CLD, chronic liver disease; HCC, hepatocellular carcinoma.

C-GALAD model integrates gender, age, AFP, AFP-L3, and DCP, thereby incorporating multidimensional information to enhance diagnostic performance compared with single biomarkers. Previous studies have reported an AUC greater than 0.8 for this model in HBV-related HCC, significantly outperforming AFP alone.[13] In the present mixed-etiology cohort (CHB, CHC, alcohol-related liver disease, and NAFLD), a significant difference in C-GALAD scores remained between HCC and non-HCC groups, suggesting cross-etiologic applicability. However, the standalone AUC was 0.850, indicating room for improvement, possibly because of baseline variations in biomarker expression across etiologies. For example, in NAFLD progressing to nonalcoholic steatohepatitis, hepatocellular lipid accumulation induces oxidative stress and chronic inflammation, leading to necrosis and regenerative AFP elevation, which may interfere with diagnostic accuracy.[29]

Table 3. ROC Curve Analysis of C-GALAD and cfDNA for the Diagnosis of HCC in CLD

Parameter	C-GALAD	cfDNA	Combined Prediction
AUC (95% CI)	0.850 (0.790-0.910)	0.799 (0.726-0.872)	0.917
Accuracy	0.8047	0.7566	0.8938
Youden's index	0.5661	0.4769	0.7418
Sensitivity	0.6667	0.7018	0.8246
Specificity	0.8994	0.7751	0.9172
Cutoff value	1.236	35.80	0.3116
PPV	0.6909	0.5128	0.7705
NPV	0.8889	0.8851	0.9394

AUC, area under the curve; cfDNA, cell-free DNA; C-GALAD, gender, age, lens culinaris agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin; HCC, hepatocellular carcinoma; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating curve.

cfDNA, comprising tumor-derived DNA fragments carrying molecular signatures, offers noninvasiveness and the potential for early detection.[30] A 2021 meta-analysis by Zhang et al[31] (38 studies) reported a pooled AUC of 0.86 for cfDNA-related indicators (concentration, integrity, mutations) in HCC diagnosis, whereas a 2023 meta-analysis by Wang et al[32] showed a sensitivity of 76%, a specificity of 72%, and an AUC of approximately 0.78 for cfDNA concentration alone. In the present study, cfDNA levels were higher in the HCC group, with a standalone AUC of 0.799, consistent with prior liquid biopsy studies (e.g., Lin et al., AUC 0.82).[33] Some studies have detected cfDNA mutations before imaging abnormalities appear.[34] However, cfDNA alone achieved a specificity of only 77.51%, likely because of nonspecific elevations resulting from hepatocyte damage and apoptosis in patients with CLD, explaining its suboptimal performance as a standalone test.[35]

The core innovation of the present study lies in demonstrating that the combined C-GALAD and cfDNA strategy significantly outperforms either marker alone. The combined model achieved an AUC of 0.917, with a sensitivity of 82.46%, a specificity of 91.72%, and an overall accuracy of 89.38%, along with optimized PPVs and NPVs. Previous research has shown that C-GALAD performs better in populations with viral hepatitis but has reduced specificity in patients with alcohol-related liver disease and NAFLD because of nonspecific elevations of AFP and DCP resulting from benign liver injury.[36,37] In contrast, cfDNA primarily reflects apoptosis and necrosis and exhibits more balanced diagnostic performance across

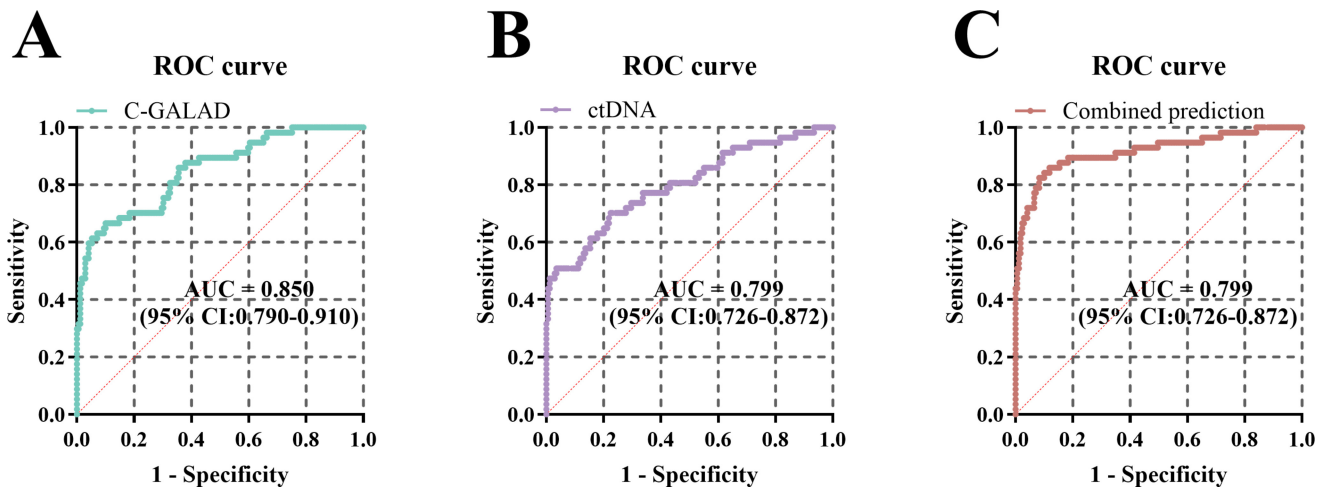

Figure 5. ROC curve analysis of C-GALAD, cfDNA, and their combination for diagnosing HCC. (A) C-GALAD, (B) cfDNA, (C) combined prediction. The predicted probability was obtained using C-GALAD and cfDNA as covariates in a binary logistic regression model. cfDNA, cell-free DNA; c-GALAD, gender, age, lens culinaris agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin; HCC, hepatocellular carcinoma; ROC, receiver operating curve.

etiologies.[38] The present findings support this pattern: in the mixed-etiology cohort, C-GALAD alone (AUC, 0.850) left room for further improvement, whereas cfDNA demonstrated favorable cross-etiologic applicability. The superior performance of the combined strategy derives from the complementary strengths of its components: the C-GALAD model integrates clinical and biochemical information but is susceptible to baseline variations across etiologies, whereas cfDNA detects tumor-specific molecular alterations with greater etiological universality, thereby offsetting the limitations of C-GALAD in non-HBV populations. This dual-tier approach—capturing clinical abnormalities via C-GALAD and molecular aberrations via cfDNA—minimizes diagnostic errors resulting from etiological heterogeneity and nonspecific biomarker elevations and offers promise as a pan-etiologic screening strategy for early HCC detection across diverse backgrounds, including CHB, CHC, ALD, and NAFLD.

Numerous multimarker diagnostic studies have been conducted internationally, with performance varying widely across combinations. Combinations of conventional biomarkers (AFP, AFP-L3, and DCP) have yielded AUCs of 0.80 to 0.87 across multiple cohorts.[39] Composite scoring models such as GALAD and C-GALAD have achieved AUCs of 0.85-0.90 in populations predominantly with viral hepatitis and are endorsed by several guidelines for screening high-risk populations.[40,41] Liquid biopsy-based combinations (cfDNA, miRNA, and exosomes) are emerging areas of research.[27] The combined C-GALAD and cfDNA strategy in the present study (AUC, 0.917) demonstrated performance comparable to that of leading combined diagnostic approaches and may be particularly suitable for early screening in CLD populations with complex etiologies.

From a clinical perspective, this combined strategy offers significant advantages. Peripheral blood sampling is simple, noninvasive, and more suitable for long-term follow-up than imaging with contrast-enhanced CT or MRI, particularly in primary care and resource-limited settings. The high sensitivity and NPV facilitate early identification of patients with HCC while ruling out individuals at low risk, thereby facilitating precision risk stratification. Individuals with positive results for both markers (high risk) can proceed to imaging and biopsy, whereas those with negative results may undergo follow-up at extended intervals, reducing the healthcare burden. Moreover, the strategy maintains excellent diagnostic efficacy for early-stage HCC (BCLC 0/A), enabling curative treatments such as surgery or ablation, which are crucial for improving prognosis.

Several limitations warrant consideration. First, the present study was conducted at a single-center study and had a relatively small sample size, potentially introducing selection bias; multicenter studies with larger sample sizes are needed. Second, diagnostic performance was not analyzed separately by etiologic subgroup (CHB, CHC, NAFLD, etc.); future studies should conduct detailed subgroup analyses, as in Sun et al[19], to optimize model parameters for each etiology. Third, cfDNA measurement in the

present study assessed total cfDNA concentration without identifying specific tumor-associated targets or performing molecular sequencing, which may have limited its specificity. Subsequent research could incorporate next-generation sequencing to identify HCC-specific mutations, further enhancing diagnostic precision.[42,43] Finally, the lack of long-term follow-up prevented assessment of the impact of the combined strategy on patient outcomes; prospective follow-up studies are needed to validate its value for recurrence monitoring and treatment response evaluation.

In conclusion, the combined C-GALAD and cfDNA strategy effectively integrates clinical, biochemical, and molecular information, overcoming etiology-specific limitations and improving early HCC detection in patients with CLD. This noninvasive, broadly applicable, and precise approach holds promise as a tool for HCC screening and risk stratification. Future multicenter, large-scale prospective studies are warranted to further validate its clinical value, optimize protocols, and facilitate broader application, ultimately contributing to reduced HCC-related mortality.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

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