

Systematic Review Article

EMERGING CIRCULATING BIOMARKERS FOR EARLY DETECTION OF HEPATOCELLULAR CARCINOMA: A SYSTEMATIC REVIEW

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and a major cause of cancer-related mortality worldwide. Since early-stage HCC is often asymptomatic, diagnosis is frequently delayed, limiting curative treatment options. Current surveillance using abdominal ultrasonography with or without alpha-fetoprotein (AFP) has suboptimal sensitivity for early detection. Consequently, several novel circulating biomarkers, including AFP-L3, des-gamma-carboxy prothrombin (DCP)/Vitamin K Absence or Antagonist-II (PIVKA-II), Golgi protein 73 (GP73), glypican-3 (GPC3), osteopontin (OPN), circulating tumor DNA (ctDNA), circulating microRNAs (miRNAs), exosomal biomarkers, and multi-biomarker panels, have emerged as promising diagnostic tools. This systematic review aims to critically evaluate the evidence on these emerging circulating biomarkers and identify those with the greatest potential for early detection and clinical application in HCC. **Objectives:** To identify emerging circulating biomarkers for early hepatocellular carcinoma, evaluate and compare their diagnostic performance, assess the utility of individual biomarkers and biomarker panels, and identify promising candidates for clinical application.

Materials and Methods: This systematic review was conducted in accordance with the PRISMA guidelines. A comprehensive literature search was performed in PubMed/MEDLINE, Scopus, Web of Science, Cochrane Library, and Google Scholar using predefined keywords related to hepatocellular carcinoma and circulating biomarkers. Articles published from 2009 were included in results. Original research studies, Randomized controlled trials, cohort studies, comparative observational studies, evaluating circulating biomarkers for early HCC detection in adults were included. Studies were selected through title and abstract screening followed by full-text review, and the selection process was documented using a PRISMA flow diagram. Data on study characteristics, biomarker type, sample size, diagnostic performance, sensitivity, specificity, and Area Under Curve (AUC) were extracted using a standardized form. The findings were synthesized narratively.

Results: Circulating microRNAs (miRNAs), methylated DNA markers (MDMs), and serum biomarker-based prediction models demonstrated promising diagnostic performance for hepatocellular carcinoma (HCC). Individual and multi-miRNA panels, particularly miR-21, miR-122, miR-16, and miR-375, achieved AUCs ranging from 0.759 to 0.996, showing utility in detecting early-stage and alpha-fetoprotein (AFP)-negative HCC. MDM panels and the CO-Methylation Amplification Real-Time PCR-Based Methylated DNA Marker Logistic Regression (COMET-LR) model achieved

AUCs up to 0.96, 95% sensitivity, and 97.4% specificity. The Gender, Age, AFP-L3%, AFP, and Des- γ -carboxyprothrombin (GALAD) and Gender, Age, AFP-L3%, AFP, DCP, and Ultrasound (GALADUS) scores outperformed ultrasound and AFP, with AUCs up to 0.98. The Gender, Age, Methylation, AFP, and DCP (GAMAD) model showed the highest diagnostic accuracy (AUC = 0.952), supporting the potential of multimarker approaches for non-invasive early HCC detection.

Conclusion: Overall, circulating microRNAs (miR-21, miR-122, miR-16, and miR-199a), methylated DNA markers (MDMs), and biomarker-based models such as GALAD and GALADUS show strong potential as non-invasive biomarkers for the early detection of HCC. Combining these biomarkers with AFP consistently improved diagnostic accuracy, particularly for early-stage and AFP-negative HCC. However, large multi-centre prospective studies are needed to validate these findings, standardize biomarker assays, and confirm their clinical utility before routine implementation.

Keywords: Hepatocellular carcinoma, Circulating biomarkers, Early detection, Alpha-fetoprotein, Circulating tumor DNA, MicroRNA, Exosomes, Diagnostic accuracy.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and a leading cause of cancer-related mortality worldwide. Because early-stage HCC is often asymptomatic, many patients are diagnosed at advanced stages when curative treatment options are limited. Early detection is therefore essential to improve survival and clinical outcomes.

Current surveillance relies on abdominal ultrasonography with or without serum alpha-fetoprotein (AFP). However, AFP has limited sensitivity and specificity for detecting early-stage HCC, highlighting the need for more accurate diagnostic biomarkers. Recent advances have identified several promising circulating biomarkers, including AFP-L3, des-gamma-carboxy prothrombin (DCP)/Vitamin K Absence or Antagonist-II (PIVKA-II), Golgi protein 73 (GP73), glypican-3 (GPC3), osteopontin (OPN), circulating tumor DNA (ctDNA), circulating microRNAs (miRNAs), exosomal biomarkers, and multi-biomarker panels, which may improve early diagnosis.

Zhou J et al. (2011) reported that a seven-miRNA plasma panel (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801) showed high diagnostic accuracy for HCC, with AUCs of 0.864 in the training cohort and 0.888 in the validation cohort. It also differentiated HCC from healthy controls (AUC 0.941), chronic hepatitis B (0.842), and cirrhosis (0.884), supporting its potential as a non-invasive biomarker for early HCC detection.^[1]

A serum microRNA classifier (Cmi) showed high diagnostic performance for hepatocellular carcinoma (HCC), accurately identifying small-size, early-stage, AFP-negative, and preclinical HCC. The classifier outperformed α -fetoprotein (AFP), highlighting its potential as a non-invasive biomarker for early HCC detection (Lin XJ et al., 2015).^[2]

An eight-miRNA plasma panel (miR-20a-5p, miR-25-3p, miR-30a-5p, miR-92a-3p, miR-132-3p, miR-185-5p, miR-320a, and miR-324-3p) differentiated HBV-related hepatocellular carcinoma (HCC) from HBV-positive cancer-free controls with 86.6% sensitivity and 64.6% specificity. Meta-analysis further identified a four-miRNA panel (miR-20a-5p, miR-320a, miR-324-3p, and miR-375) as a promising preclinical biomarker for early HCC detection, with improved performance when combined with α -fetoprotein (AFP) (Wen Y et al., 2015).^[3]

Although numerous studies have evaluated these biomarkers, their diagnostic performance remains variable, and the evidence has not been comprehensively synthesized. Therefore, this systematic review aims to evaluate the current evidence on emerging circulating biomarkers for the early detection of HCC and identify biomarkers with the greatest potential for clinical application.

Objectives

To identify emerging circulating biomarkers for the early detection of hepatocellular carcinoma, compare their diagnostic accuracy, evaluate the clinical utility of individual biomarkers and multi-biomarker panels, and determine the most promising candidates for routine clinical application.

MATERIALS AND METHODS

This systematic review was conducted in accordance with the PRISMA guidelines. A comprehensive literature search was performed in PubMed/MEDLINE, Scopus, Web of Science, Cochrane Library, and Google Scholar using predefined keywords, including hepatocellular carcinoma, circulating biomarkers, early detection, alpha-fetoprotein (AFP), circulating tumor DNA (ctDNA), microRNA (miRNA), exosomes, and diagnostic accuracy. Articles published from the year 2009 were included in results. Original research studies, randomized controlled trials,

cohort studies, and comparative observational studies evaluating circulating biomarkers for early HCC detection in adults were included. Studies without extractable data or clearly defined outcome measures, conference abstracts, letters, Editorials were excluded. Studies were selected through title and abstract screening followed by full-text review, and the selection process was documented using a PRISMA flow diagram. Data on study characteristics, biomarker type, sample size, diagnostic performance, sensitivity, specificity, and AUC were extracted using a standardized form. Titles, abstracts, and full texts were independently screened by two reviewers, with disagreements resolved by consensus. Data extraction and quality assessment were performed independently using standardized tools appropriate to study design. Due to heterogeneity among studies, the findings were synthesized narratively.

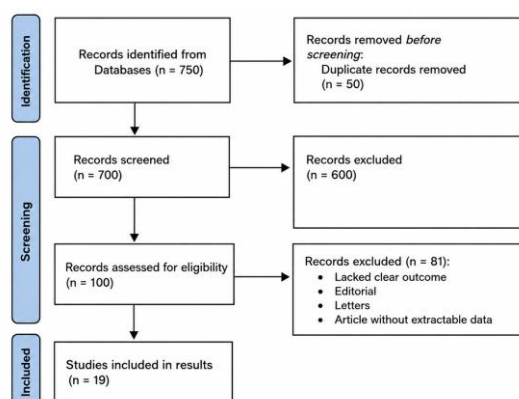


Figure 1: PRISMA flow diagram of study selection process

RESULTS

Plasma microRNA-21 (miR-21) levels, measured by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), decreased significantly after curative resection in patients with HCC ($p=0.0125$), indicating an association with tumor burden. Patients with HCC had significantly higher plasma microRNA-21 levels than those with chronic hepatitis and healthy controls (both $p<0.0001$). Diagnostic performance was strong, with an AUC of 0.773 for distinguishing HCC from chronic hepatitis and 0.953 for distinguishing HCC from healthy individuals. MicroRNA-21 outperformed α -fetoprotein, and combining both biomarkers further improved diagnostic accuracy (Tomimaru Y et al., 2012).^[4]

Serum miR-21 levels were significantly elevated in patients with HCC compared with healthy controls, chronic hepatitis B, and liver cirrhosis (all $P<0.0001$). Diagnostic analysis showed good accuracy for identifying HCC (AUC=0.849, sensitivity 82.1%, specificity 83.9%) and retained strong performance in Alpha-Fetoprotein (AFP)-negative HCC cases (AUC=0.831). Serum miR-21

also effectively differentiated HCC from chronic hepatitis B and liver cirrhosis, and was significantly associated with advanced clinical stage and distant metastasis. These findings indicate that miR-21, particularly when combined with AFP, may improve the diagnosis of HCC, including AFP-negative disease (Guo X et al., 2017).^[5]

Serum miRNA (miR) profiling identified miR-122 as a potential biomarker for HCC. Serum miR-122 levels were significantly higher in patients with HCC than in healthy controls ($p<0.001$) and decreased significantly one month after surgical resection, suggesting an association with tumor burden. Although miR-122 levels were slightly higher in HCC patients than in individuals with chronic hepatitis B, the difference was only marginally significant ($p=0.043$). These findings indicate that serum miR-122 may be a useful non-invasive marker for HCC detection in healthy populations but is more reflective of liver injury than HCC-specific changes in patients with chronic hepatitis B virus (HBV) infection (Qi P et al., 2011).^[6]

Serum miR-21, miR-122, and miR-223 levels were significantly higher in patients with HCC than in healthy controls ($P = 7.48 \times 10^{-13}$, 6.93×10^{-9} , and 3.90×10^{-12} , respectively). However, these miRNAs were also significantly elevated in chronic hepatitis, with miR-21 and miR-122 showing higher levels than in HCC ($P = 3.99 \times 10^{-4}$ and 4.97×10^{-8} , respectively). Receiver Operating Characteristic (ROC) analysis indicated good discrimination of liver disease from healthy controls but limited ability to distinguish HCC from chronic hepatitis, suggesting these miRNAs are biomarkers of liver injury rather than HCC-specific markers (Xu J et al., 2011).^[7]

Serum Dickkopf-1 (DKK1) levels were significantly elevated in patients with HCC. ROC analysis showed good diagnostic accuracy, with AUCs of 0.848 and 0.862, and maintained strong performance for early-stage HCC (AUCs 0.865 and 0.896) and AFP-negative HCC (AUCs 0.841 and 0.869). DKK1 also differentiated HCC from chronic HBV infection and cirrhosis (AUCs 0.834 and 0.873). Combining DKK1 with AFP further improved diagnostic accuracy (AUCs 0.889 and 0.888), supporting its role as a complementary biomarker for HCC diagnosis (Shen Q et al., 2012).^[8]

In a multi-centre case-control study, AFP demonstrated superior diagnostic performance for early-stage HCC (AUC = 0.80, 95% CI: 0.77–0.84) compared with Des- γ -carboxy prothrombin (DCP) (AUC = 0.72) and AFP-L3% (AUC = 0.66). An AFP cutoff of 10.9 ng/mL yielded 66% sensitivity for early-stage HCC and 65% sensitivity for very early-stage HCC (AUC = 0.78, 95% CI: 0.72–0.85) (Marrero JA et al., 2009).^[9]

A 13-miRNA serum signature accurately distinguished HBV infection, HBV-positive HCC, and control groups. Additionally, a 3-miRNA panel (miR-25, miR-375, and let-7f) effectively

differentiated HCC from healthy controls, with miR-375 alone demonstrating excellent diagnostic performance (AUC = 0.96, sensitivity = 100%, specificity = 96%), highlighting its potential as a non-invasive biomarker for HCC detection (Li LM et al., 2010).^[10]

The Gender, Age, AFP-L3%, AFP, and DCP (GALAD) score demonstrated excellent diagnostic accuracy for HCC in patients with nonalcoholic steatohepatitis (NASH), achieving an AUC of 0.96, outperforming AFP (0.88), AFP-L3% (0.86), and DCP (0.87). For early-stage HCC within the Milan criteria, the GALAD score showed an AUC of 0.91, with 68% sensitivity and 95% specificity at a cutoff of -0.63. Additionally, GALAD scores increased up to 1.5 years before HCC diagnosis, supporting its value for early HCC surveillance (Best J et al., 2020).^[11]

A six-marker methylated DNA marker (MDM) panel (HOXA1, EMX1, AK055957, ECE1, PFKP, and CLEC11A) demonstrated excellent diagnostic performance for HCC, with an AUC of 0.96 (95% CI: 0.93–0.99), 95% sensitivity, and 92% specificity. The panel detected 75% of stage 0, 93% of stages A and B, and 100% of stages C and D HCC cases, outperforming alpha-fetoprotein (AFP) (AUC: 0.94 vs. 0.80; $P < 0.0001$), highlighting its potential as a non-invasive biomarker for HCC detection (Kisiel JB et al., 2019).^[12]

A CO-Methylation Amplification Real-Time (COMET) PCR-based methylated DNA marker (MDM) logistic regression model (COMET-LR) showed excellent diagnostic performance for HCC, with 90.0% sensitivity and 97.4% specificity. The model maintained high sensitivity in early-stage, small (<3 cm), AFP-negative, and Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II)-negative HCC, and outperformed AFP, AFP-L3, PIVKA-II, and established prediction models (GALAD, Doylestown, and ASAP), highlighting its potential as a non-invasive tool for early HCC detection (Yang T et al., 2025).^[13]

The Gender, Age, AFP-L3%, AFP, and DCP (GALAD) score demonstrated excellent diagnostic accuracy for HCC, with an AUC of 0.95, 91% sensitivity, and 85% specificity, and maintained high performance for early-stage HCC (AUC = 0.92, 92% sensitivity, 79% specificity). In the Early Detection Research Network (EDRN) cohort, the GALAD score achieved an AUC of 0.88. Combining GALAD with ultrasound (GALADUS score) further improved diagnostic accuracy (AUC = 0.98, 95% sensitivity, 91% specificity), outperforming ultrasound alone (Yang JD et al., 2019).^[14]

In patients with cirrhosis, the longitudinal Gender, Age, AFP-L3%, AFP, and DCP (GALAD) score showed the highest accuracy for HCC detection (c-statistic = 0.85) compared with single-time point GALAD (0.79) and alpha-fetoprotein (AFP; 0.77). At 90% specificity, longitudinal GALAD achieved 66.7% sensitivity versus 54.8% for single-time point

GALAD and 40.5% for AFP. For early-stage HCC, longitudinal GALAD demonstrated 69.2% sensitivity, supporting its value for HCC surveillance (Singal AG et al., 2022).^[15]

In patients with non-diagnostic alpha-fetoprotein (AFP) levels (<200 ng/mL), AFP-L3% and des-γ-carboxyprothrombin (DCP) improved the detection of HCC. AFP-L3% was positive in 36.7% and DCP in 43.0% of HCC cases, while combined AFP-L3%/DCP testing identified 60% of patients. The combination of AFP, AFP-L3%, and DCP increased the overall diagnostic sensitivity to 84%, with DCP showing 100% specificity (Hadziyannis E et al., 2013).^[16]

A six-circulating microRNA (miRNA)-based diagnostic score (miR-184, miR-532-5p, miR-221-3p, miR-5589-5p, let-7b-3p, and miR-26b-3p) demonstrated excellent accuracy for detecting HCC, particularly early-stage HCC, with an AUC of 0.9535. The model outperformed alpha-fetoprotein (AFP) and was successfully validated in three independent cohorts (AUCs = 0.9780, 0.9961, and 0.9681), supporting its potential as a reliable non-invasive biomarker for HCC diagnosis and postoperative monitoring (Yu B et al., 2022).^[17]

Serum microRNA-16 (miR-16) and microRNA-122 (miR-122) were significantly elevated in early-stage HCC and demonstrated good diagnostic accuracy (AUC = 0.798 and 0.759, respectively). Combining miR-16 and miR-122 with alpha-fetoprotein (AFP) improved diagnostic performance (AUC = 0.862), while miR-16 effectively differentiated HCC from liver cirrhosis (AUC = 0.936), supporting their potential as non-invasive biomarkers for early HCC detection (Fang Y et al., 2022).^[18]

Serum microRNA-16 (miR-16) levels were significantly lower in HCC than in chronic hepatitis C virus (HCV) patients ($P = 0.033$), with 57.5% sensitivity and 70% specificity for HCC detection. Combining miR-16 with alpha-fetoprotein (AFP) improved sensitivity to 85% and diagnostic accuracy to 87.5%. Additionally, microRNA-199a (miR-199a) and miR-16 were significantly associated with tumor size and number, suggesting their potential as biomarkers for HCC progression (El-Abd NE et al., 2015).^[19]

Piratvisuth T et al. (2022) reported that a multimarker panel combining alpha-fetoprotein (AFP), Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II/DCP), and either AFP-L3, insulin-like growth factor-binding protein 3 (IGFBP3), cartilage oligomeric matrix protein (COMP), or matrix metalloproteinase-3 (MMP3), together with age and sex, improved the detection of both early- and all-stage HCC. The panel demonstrated diagnostic performance comparable to the Gender, Age, AFP-L3%, AFP, and DCP (GALAD) score.^[20]

Marsh TL et al. (2025) reported that the Gender, Age, AFP-L3%, AFP, and DCP (GALAD) score outperformed alpha-fetoprotein (AFP) for detecting HCC within 12 months before diagnosis, achieving

an AUC of 0.78 versus 0.66 for AFP ($P < 0.001$). At 82% specificity, GALAD demonstrated 62% sensitivity, compared with 41% sensitivity for AFP ($P = 0.001$), highlighting its superior performance for early HCC detection.^[21]

Wu H et al. (2026) developed the Gender, Age, Methylation, AFP, and DCP (GAMAD) model by integrating circulating tumor DNA (ctDNA) methylation, alpha-fetoprotein (AFP), Protein Induced by Vitamin K Absence or Antagonist-II

(PIVKA-II/DCP), age, and sex for early HCC detection. The model achieved 80.5% sensitivity, 90.4% specificity, and an AUC of 0.934 in the validation cohort. In the independent test cohort, GAMAD demonstrated an AUC of 0.952, with 86.5% sensitivity for stage 0/A and 91.7% sensitivity for stage B-C HCC, outperforming the Gender, Age, AFP-L3%, AFP, and DCP (GALAD) model.^[22]

Table: Summary of Key Findings of Included Articles

Author (Year)	Key Finding
Tomimaru Y et al. (2012)	Plasma miR-21 was elevated in HCC, decreased after surgery, and outperformed AFP; combining both improved diagnostic accuracy.
Guo X et al. (2017)	Serum miR-21 showed good diagnostic accuracy and improved detection of AFP-negative HCC when combined with AFP.
Qi P et al. (2011)	Serum miR-122 was elevated in HCC but primarily reflected liver injury in chronic HBV infection.
Xu J et al. (2011)	miR-21, miR-122, and miR-223 distinguished liver disease from healthy controls but limited ability to distinguish HCC from chronic hepatitis.
Shen Q et al. (2012)	DDK1 demonstrated good diagnostic accuracy and improved HCC detection when combined with AFP.
Marrero JA et al. (2009)	AFP was more sensitive than DCP and AFP-L3% for detecting early-stage HCC.
Li LM et al. (2010)	A 13-miRNA signature accurately detected HCC, and miR-375 showing excellent diagnostic performance.
Best J et al. (2020)	The GALAD score outperformed AFP, AFP-L3, and DCP and enabled early HCC detection.
Kisiel JB et al. (2019)	A six-marker methylated DNA marker (MDM) panel showed excellent accuracy and outperformed AFP.
Yang T et al. (2025)	The COMET-LR model demonstrated high sensitivity and specificity, outperforming AFP and other prediction models.
Yang JD et al. (2019)	The GALAD score outperformed ultrasound alone, while GALADUS further improved diagnostic accuracy.
Singal AG et al. (2022)	Longitudinal GALAD showed superior sensitivity for HCC surveillance in patients with cirrhosis.
Hadziyannis E et al. (2013)	Combining AFP, AFP-L3%, and DCP improved diagnostic sensitivity of HCC, with DCP showing 100% specificity.
Yu B et al. (2022)	A six-miRNA diagnostic score accurately detected early-stage HCC and outperformed AFP.
Fang Y et al. (2022)	miR-16 and miR-122, especially when combined with AFP, improved early HCC detection.
El-Abd NE et al. (2015)	miR-16 combined with AFP improved HCC detection, while miR-199a and miR-16 reflected disease progression.
Piratvisuth T et al. (2022)	A multimarker panel comprising AFP, PIVKA-II/DCP, AFP-L3, IGFBP3, COMP, or MMP3, together with age and sex, improved early- and all-stage HCC detection and performed comparably to the GALAD score.
Marsh TL et al. (2025)	The GALAD score outperformed AFP for early HCC detection (AUC 0.78 vs. 0.66), with 62% sensitivity versus 41% for AFP at 82% specificity.
Wu H et al. (2026)	The GAMAD model achieved 80.5% sensitivity, 90.4% specificity, and AUC 0.934 in validation, and AUC 0.952 in the test cohort, outperforming the GALAD model.

CONCLUSION

Guo X et al. (2017) concluded that microRNA-21 (miR-21), especially when combined with alpha-fetoprotein (AFP), could serve as a valuable biomarker for improving the diagnosis of HCC, including AFP-negative disease.^[5]

Qi P et al. (2011) suggested that serum microRNA-122 (miR-122) is a promising non-invasive biomarker for HCC detection in healthy individuals but primarily reflects liver injury rather than HCC-specific changes in patients with chronic HBV infection.^[6]

Yu B et al. (2022) concluded that the six-circulating microRNA (miRNA)-based diagnostic score is a promising non-invasive biomarker for early-stage HCC detection and postoperative recurrence monitoring.^[17]

Fang Y et al. (2022) concluded that circulating microRNA-16 (miR-16) and microRNA-122 (miR-122) are promising non-invasive biomarkers for the early detection of HCC.^[18]

El-Abd NE et al. (2015) concluded that combining serum microRNA-16 (miR-16) with AFP improved

the detection of HCC in hepatitis C virus-positive patients, while microRNA-199a (miR-199a) and miR-16 showed potential as biomarkers of disease progression.^[19]

Kisiel JB et al. (2019) reported that plasma-based methylated DNA markers (MDMs) showed high accuracy for HCC detection, with further validation required to establish their clinical utility.^[12]

Yang JD et al. (2019) reported that the GALAD score outperformed ultrasound for hepatocellular carcinoma detection, while GALADUS score further improved diagnostic accuracy.^[14]

Singal AG et al. (2022) suggested that the GALAD score has high sensitivity for HCC detection in patients with cirrhosis. However, larger Phase III studies are required to confirm its clinical utility.^[15]

Marrero JA et al. (2009) concluded that AFP was more sensitive than DCP and AFP-L3% for detecting early and very early-stage HCC using a cutoff value of 10.9 ng/mL.^[9]

Overall, the included studies indicate that circulating microRNAs (miR-21, miR-122, miR-16, and miR-199a), methylated DNA markers (MDMs), and biomarker-based prediction models such as GALAD

and GALADUS are promising non-invasive tools for the early detection and surveillance of HCC. Several studies demonstrated that combining these biomarkers with AFP improved diagnostic accuracy, particularly for early-stage and AFP-negative HCC. Although these biomarkers showed encouraging sensitivity and specificity, most authors emphasized the need for further validation before routine clinical implementation. Large-scale, multi-centre, prospective studies with standardized biomarker assays and diverse patient populations are required to validate these findings, establish optimal diagnostic cutoffs, and assess the long-term clinical utility and cost-effectiveness of these biomarkers in routine HCC screening and surveillance.

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