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HEPATOCELLULAR CARCINOMA DIAGNOSTIC BIOMARKERS: INTEGRATIVE MOLECULAR AND CLINICAL ADVANCES (2025 REVIEW)

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Abstract: Hepatocellular carcinoma (HCC) remains the most common primary malignancy of the liver and a leading cause of cancer mortality worldwide. Traditional diagnostic strategies centered on alpha-fetoprotein (AFP) and imaging have limited sensitivity, especially for early-stage disease. Recent molecular advances have transformed the diagnostic paradigm, incorporating glycoproteins such as Golgi protein 73 (GP73) and glypican-3 (GPC3), coagulation markers such as des-gamma-carboxy prothrombin (PIVKA-II), and liquid biopsy components including circulating tumor DNA (ctDNA) and microRNAs (miRNAs). These biomarkers, alongside radiomic and metabolomic innovations, offer accuracy levels previously unattainable in hepatology. This review integrates findings from sixteen recent peer-reviewed studies (2024–2025) to delineate diagnostic efficacy, mechanistic relevance, and clinical implications, highlighting the emergence of multi-marker and AI-assisted detection systems.

Keywords:-Hepatocellular carcinoma (HCC); liver cancer; diagnostic biomarkers; alpha-fetoprotein (AFP); Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II); des-gamma-carboxy prothrombin (DCP); Golgi Protein 73 (GP73); Glypican-3 (GPC3); microRNAs (miRNAs); long noncoding RNAs (lncRNAs); circulating tumor DNA (ctDNA); cell-free DNA (cfDNA); liquid biopsy; radiomics; metabolomics; artificial intelligence (AI); machine learning; molecular diagnostics; multi-omics integration; early detection.

1. Introduction

Hepatocellular carcinoma represents nearly 85% of primary liver cancers, with over 850,000 new cases annually (Feng & Xu, Frontiers in Medicine, 2025). Its global distribution mirrors the prevalence of chronic hepatitis B and C infections, alcohol-related liver disease, and the rapidly growing burden of metabolic-associated steatotic liver disease (MASLD). The asymptomatic course of early HCC contributes to late diagnosis, resulting in poor prognosis and limited eligibility for curative treatment.

For decades, alpha-fetoprotein (AFP) served as the principal biochemical screening tool. However, its limited sensitivity (60–70%) and frequent elevation in benign hepatic conditions have driven the search for superior diagnostic alternatives (Marrero et al., Hepatology, 2024). The recent proliferation of multi-omics and liquid biopsy technologies has revealed new diagnostic candidates that capture molecular hallmarks of hepatocarcinogenesis.

The studies published between 2024 and 2025 represent a convergence of biochemistry, molecular genetics, and artificial intelligence (AI), emphasizing precision hepatology. This review synthesizes that body of evidence to describe how integrated biomarker approaches—

combining traditional serological markers with genetic, proteomic, and imaging-based signatures—are reshaping early HCC detection.

2. Alpha-Fetoprotein: A Historical Cornerstone

Alpha-fetoprotein remains the most widely available biomarker for HCC, reflecting re-expression of fetal glycoproteins by malignant hepatocytes. Yet, its stand-alone diagnostic performance remains modest. Marrero et al. (2024) reported a sensitivity of 65% and specificity of 88%, noting improved performance when AFP levels exceed 400 ng/mL.

The discovery of **AFP-L3**, a glycoform that selectively binds Lens culinaris agglutinin, significantly improved diagnostic precision. Ji et al. (Clinical Cancer Research, 2025) demonstrated that AFP-L3 achieved 62% sensitivity and 92% specificity, outperforming total AFP in differentiating HCC from cirrhotic nodules. The AFP-L3 to total AFP ratio, particularly above 10%, has been endorsed by both EASL and Japanese guidelines as an indicator of malignant transformation (Kudo et al., Cancers, 2025).

3. PIVKA-II: Refining Serum-Based Diagnostics

Des-gamma-carboxy prothrombin (DCP), also termed Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II), has gained substantial recognition as an independent and complementary biomarker to AFP. Zhang et al. (Journal of Clinical and Translational Hepatology, 2025) documented its superior diagnostic precision, with sensitivity between 70% and 76%, specificity near 94%, and an AUC of 0.86.

Unlike AFP, PIVKA-II reflects tumor-specific prothrombin synthesis defects rather than liver inflammation, making it highly specific for malignancy. Elevated PIVKA-II levels correlate with vascular invasion, tumor aggressiveness, and recurrence after curative therapy (Li et al., Molecular Cancer, 2025). Its integration into combined algorithms (AFP + PIVKA-II) now achieves over 90% diagnostic accuracy, endorsed by the **2025 AASLD guidelines**.

4. Glycoprotein Markers: GP73 and GPC3

Among glycoproteins, Golgi Protein-73 (GP73) and Glypican-3 (GPC3) have emerged as vital diagnostic and prognostic biomarkers. GP73, normally expressed at low levels in healthy hepatocytes, is markedly upregulated during malignant transformation. Ji et al. (2025) demonstrated that GP73 achieved sensitivity between 73% and 80% and specificity near 90%. Its expression persists even in AFP-negative HCC, making it indispensable for comprehensive diagnosis.

Similarly, GPC3, a heparan sulfate proteoglycan found on the hepatocyte membrane, is highly expressed in HCC but absent in benign conditions. Lucaciu et al. (International Journal of Molecular Sciences, 2025) reported GPC3's specificity exceeding 91%, with diagnostic accuracy surpassing 84%. These findings establish GP73 and GPC3 as superior adjuncts to AFP in detecting early or atypical presentations.

5. MicroRNAs as Epigenetic Indicators

MicroRNAs (miRNAs) regulate post-transcriptional gene expression and are stable in plasma, making them attractive non-invasive biomarkers. Antony et al. (Egyptian Liver Journal, 2025) identified **miR-21**, **miR-122**, and **miR-221** as particularly informative in differentiating early

HCC from cirrhotic states, demonstrating pooled sensitivity and specificity values of 86% and 92%, respectively.

Similarly, Tobaruela-Resola et al. (Journal of Physiology and Biochemistry, 2025) reported that dysregulated miRNA panels yielded diagnostic AUCs above 0.90 in MASLD-related HCC. These findings underscore miRNAs' clinical value as **liquid biopsy-based tools**, especially in patients with negative AFP results.

6. Long Noncoding RNAs and Emerging Molecular Biomarkers

Beyond miRNAs, long noncoding RNAs (lncRNAs) represent another regulatory layer in HCC development. Mir et al. (Asian Pacific Journal of Cancer Biology, 2025) explored lncRNA MSC-AS1, revealing its overexpression in 87% of tumor samples and diagnostic accuracy around 88%. This lncRNA influences Wnt/β-catenin and PI3K/AKT pathways, providing mechanistic insights into its biomarker potential.

Parallel investigations into exosomal RNA and methylation markers continue to expand the biomarker repertoire, facilitating multi-analyte precision diagnostics.

7. Liquid Biopsy and ctDNA Innovations

The advent of circulating tumor DNA (ctDNA) has revolutionized non-invasive cancer detection. Yang et al. (Biochimica et Biophysica Acta – Reviews on Cancer, 2025) demonstrated that ctDNA methylation patterns of RASSF1A, SEPT9, and GSTP1 achieved sensitivity up to 92% and specificity of 96%, outperforming conventional serum markers. Chan et al. (Eurasian Journal of Medicine and Oncology, 2025) further showed that cfDNA levels correlate with tumor burden and recurrence risk, supporting their role in surveillance after resection. These findings indicate that liquid biopsy may soon replace tissue-based histology for initial diagnosis and monitoring.

8. Radiomics: The Imaging Biomarker Frontier

Recent advances in imaging analytics have established radiomics as an emerging biomarker platform. Blake et al. (ResearchGate Preprint, 2025) utilized MRI-based radiomic algorithms to identify textural and perfusion features that discriminate early HCC from benign nodules with diagnostic accuracy exceeding 93%. Integration of these imaging-derived data into AI models alongside AFP, GP73, and ctDNA enhanced classification accuracy to over 95%.

Such multimodal models are expected to become the foundation of AI-assisted hepatocellular carcinoma diagnostics, capable of predicting tumor development before radiological visibility.

9. Metabolic and Proteomic Perspectives

The intersection of metabolism and tumor biology has attracted growing interest. Surma and Bułdak (Frontiers in Endocrinology, 2025) described how specific lipidomic and bile acid signatures in MASLD predict carcinogenic progression, achieving diagnostic AUC values near 0.91 when combined with AFP. Similarly, Adugna et al. (Immunity, Inflammation & Disease, 2025) identified osteopontin, YKL-40, and glycation-related serum proteins as early biomarkers in HBV-induced hepatocarcinogenesis. These metabolic markers hold promise for etiology-specific diagnostic panels.

10. Integrated and AI-Assisted Biomarker Models

Modern research emphasizes the integration of multiple biomarker classes. Li et al. (Molecular Cancer, 2025) developed a composite model incorporating AFP, GP73, PIVKA-II, and miR-21, achieving 94% sensitivity and 95% specificity (AUC = 0.96). The GAAD score (Gender, Age, AFP, DCP), introduced by Abenavoli et al. (Journal of Clinical and Experimental Hepatology, 2025), further improved early-stage detection across diverse etiologies. AI-driven models now employ gradient boosting and deep learning to fuse serological, genetic, and radiomic features, offering predictive accuracies surpassing 95%.

11. Comparative Diagnostic Accuracy Summary

Biomarker	**Sensitivity (%)**	**Specificity (%)**	**AUC (Accuracy)**	**Source**
AFP	61–68	82–90	0.78	Marrero et al., 2024
AFP-L3	62	92	0.83	Ji et al., 2025
PIVKA-II	70–76	89–94	0.86	Zhang et al., 2025
GP73	73–80	90	0.88	Ji et al., 2025
GPC3	69	91	0.84	Lucaciu et al., 2025
miRNAs (miR-21/122)	82–88	91–94	0.91	Antony et al., 2025
ctDNA methylation	85–92	93–96	0.94	Yang et al., 2025
Radiomics (MRI)	83–90	92	0.93	Blake et al., 2025
Composite Model (AFP+DCP+GP73)	91–94	95–97	0.96	Li et al., 2025

12. Global Integration and Policy Implications

The 2025 Cancers (MDPI) roadmap (Kudo et al., 2025) recommends regional biomarker tailoring: AFP and PIVKA-II for HBV-endemic Asia, GP73 and AFP-L3 for metabolic HCC in Europe, and miRNA/cfDNA panels for resource-limited regions. China's national Guidelines for Primary Liver Cancer (2025) now officially recognize ctDNA-based models as tier-one diagnostic tools (Cheng et al., Zhonghua Wai Ke Za Zhi, 2025).

These policy shifts illustrate the rapid transition from single-marker to multi-modal, molecularly guided screening frameworks, supported by artificial intelligence and bioinformatics infrastructure.

13. Limitations and Future Outlook

Despite impressive advances, several barriers persist. HCC's biological heterogeneity complicates universal biomarker standardization. Most studies remain geographically concentrated, limiting external validation. The cost and infrastructural requirements of molecular assays, especially ctDNA and multi-omics panels, restrict accessibility in low-income regions. Moreover, ethical issues surrounding AI-assisted diagnostic decision-making require careful regulation. The next decade will need global harmonization of biomarker thresholds, integration with electronic medical systems, and cost-effective miniaturized diagnostic platforms.

Conclusion

The diagnostic paradigm of hepatocellular carcinoma has shifted decisively toward a multi-analyte, precision-based model. Alpha-fetoprotein alone no longer defines the standard; rather, it serves as a component within broader integrated frameworks including PIVKA-II, GP73, GPC3, miRNAs, and ctDNA. These combined biomarkers achieve sensitivities and specificities exceeding 90%, with AI-assisted multimodal systems offering unprecedented predictive reliability. The convergence of molecular biology, imaging analytics, and computational intelligence signals the emergence of a biopsy-independent era in HCC diagnostics—one that promises earlier detection, improved prognosis, and global equity in liver cancer care.

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