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REVIEW

Emergent Biomarkers in Hepatocellular Carcinoma: Advancements in Early Detection and Surveillance

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Abstract

Hepatocellular carcinoma (HCC) ranks prominently among the predominant causes of cancer-related mortalities globally. Its latent clinical presentation often culminates in diagnoses at advanced stages. Given this challenge, there is an imperative to refine diagnostic methodologies to ameliorate its prognostic outcomes. Presently, the diagnostic cornerstone for early-stage HCC hinges on ultrasonography coupled with alpha-fetoprotein serological evaluations. Nonetheless, the instrumental role of biomarkers in this realm warrants emphasis. Conventional biomarkers, including alpha-fetoprotein, manifest suboptimal sensitivity and specificity metrics, rendering them insufficient for the nuanced demands of early HCC detection. Contemporary technological innovations have catalyzed the identification of prospective biomarkers, engendering renewed optimism for precocious HCC detection. This article elucidates established and nascent biomarkers pertinent to HCC detection, underscoring their prospective utility in the surveillance and diagnosis of incipient HCC stages. We postulate that these biomarkers may crystallize into quintessential clinical tools in the forthcoming era.

Introduction

As of 2020, epidemiological data designated primary liver cancer as the sixth most prevalent malignancy worldwide, with hepatocellular carcinoma (HCC) accounting for a substantial 75-86% of these cases (Singal et al., 2023). HCC's clinical trajectory is often cryptic, resulting in a lamentable trend where the majority of diagnoses occur at advanced disease stages (Xu et al., 2022). This diagnostic delay not only exacerbates the global oncological burden but also solidifies HCC as the fourth primary etiology of cancer-associated mortalities (Global Burden of Disease Cancer Collaboration, 2017). Forecasts from the World Health Organization project that HCC's mortality toll will likely eclipse one million by the year 2030 (Yang et al., 2022).

Predominantly, HCC emerges against the backdrop of

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chronic hepatic pathologies. Notably, cirrhosis is concurrently diagnosed in approximately 80% of HCC-afflicted individuals (Singal et al., 2023). Several etiological determinants—including chronic hepatitis B and C viral infections, aflatoxin exposure, metabolic dysregulations like obesity and diabetes, and alcohol overconsumption—have been implicated in HCC's pathogenesis (Zhou et al., 2020). Cumulatively, these factors precipitate hepatic insult, instigating a cascade of hepatic damage, regenerative cycles, inflammation, fibrosis, and, ultimately, tumorigenesis (Shimada et al., 2019). Timely diagnostic interventions can pave the way for surgical resections, which, when executed during early disease stages, demonstrate appreciable therapeutic efficacy (Anwanwan et al., 2020). Nevertheless, the extant diagnostic modalities, characterized by their suboptimal accuracy, cast a shadow on HCC prognosis.

In the contemporary clinical milieu, alpha-fetoprotein remains the predominant HCC biomarker. However, its inherent limitations in sensitivity and specificity (Chalasani et al., 2018) necessitate its concomitant application with ultrasonography for efficacious HCC monitoring in high-risk demographics (Singal et al., 2023). Despite being economically viable, this combinatorial approach yields a sensitivity of merely 63% (Chen et al., 2020), underscoring the exigency for more precise diagnostic tools. AFP-L3 and des-γ-carboxyprothrombin, though demonstrating enhanced diagnostic performance in conjunction with alpha-fetoprotein (Seo et al., 2015), are yet to secure an endorsement for HCC monitoring from regulatory bodies such as the FDA (Singal et al., 2023). This evidences an undeniable void in the domain of efficacious HCC biomarkers.

From a taxonomic perspective, HCC biomarkers can be stratified into four distinct categories: embryonic and glycoprotein antigens, enzymes and isoenzymes, genes, and cytokines (Zong et al., 2020). Recently, the scientific community has pivoted its focus toward serum-derived biomarkers, with proteins like Golgi protein 73, osteopontin, and glypican-3 under rigorous investigation (Wang et al., 2020; Zekri et al., 2020; Desert et al., 2022). The innovative realm of "liquid biopsy" technology has emerged as a potential game-changer, offering a pantheon of tools such as circulating tumor cells, circulating tumor DNA, microRNA, and extracellular vesicles. These tools elucidate the phenotypic and genotypic intricacies of primary tumors (Ye et al., 2019). Given their potential, these nascent biomarkers may revolutionize HCC management strategies. This review endeavors to synthesize the clinical implications of both traditional and emergent biomarkers in HCC's early diagnosis and ongoing surveillance.

Protein biomarkers and hepatocellular carcinoma

1. Alpha-fetoprotein

1.1. Production of alpha-fetoprotein

The gene encoding alpha-fetoprotein (AFP) resides in the 4q13.3-qter locus of the human chromosome. AFP, a glycoprotein unique to the fetal stage, is predominantly synthesized by the fetal liver and certain epithelial cells of the digestive tract. Throughout fetal maturation, AFP permeates the maternal bloodstream via the placental interface. Corresponding with fetal development, AFP concentrations surge, subsequently experiencing a precipitous decline postpartum to reach basal levels observed in healthy adults (Gitlin et al., 1978). Notwithstanding its negligible presence in adult physiology, pathological conditions such as hepatic malignancies and related liver diseases can instigate aberrant cellular proliferation and differentiation in the liver, consequently augmenting AFP synthesis and secretion (Forner et al., 1956) (Figure 1).

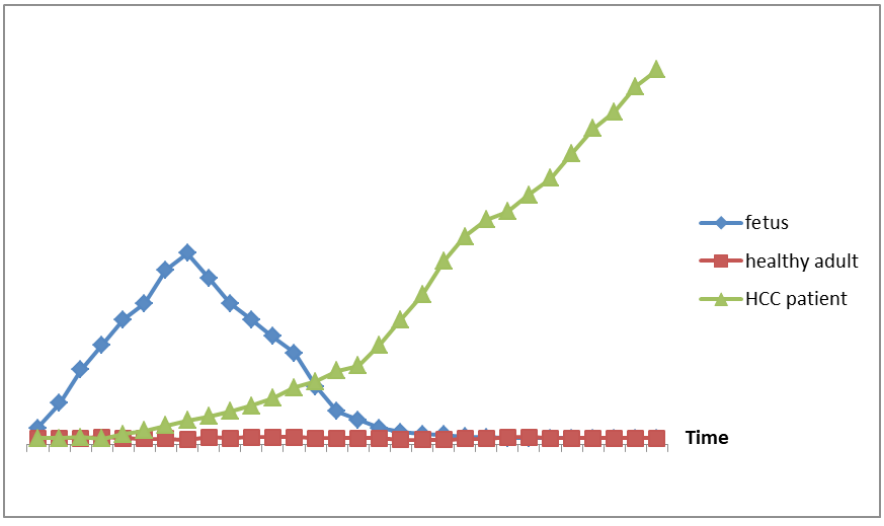

Figure 1. The relative levels of AFP in fetuses,postnatal to healthy adults and HCC patients

1.2. Alpha-fetoprotein and hepatocellular carcinoma

Alpha-fetoprotein (AFP) was first identified in human fetal serum by Bergstrand and Czar in 1956 (Bergstrand et al., 1956). For over half a century, AFP has been a cornerstone biomarker for HCC surveillance, representing the most ubiquitously employed indicator for the disease (Kulik et al., 2019). A pivotal study conducted in 1987 longitudinally observed 909 HCC patients, examining AFP concentrations and their correlation with tumoral progression. The findings indicated a higher prevalence of elevated AFP levels in tumors exceeding 5 cm in diameter. Moreover, HCC cases with heightened AFP levels were more predisposed to vascular invasion compared to their low AFP counterparts (Peng et al., 2004).

However, given the relatively low incidence of HCC in the general populace and the modest positive predictive value of AFP standing at 25.1% (Galle et al., 2019), it is noteworthy that approximately 30% of HCC patients exhibit AFP levels below the threshold of 20 ng/mL (Baig et al., 2009). Consequently, the exclusive reliance on AFP for HCC risk stratification in the general population is not advocated (Singal et al., 2023).

Beyond its diagnostic implications, AFP also holds therapeutic relevance in HCC management. The Milan criteria, currently the predominant guidelines in numerous countries for ascertaining liver transplantation eligibility (Morgul et al., 2020), have been juxtaposed with AFP concentrations. Research affirms that AFP-negative individuals often present with smaller neoplastic sizes and preserved hepatic function. Additionally, AFP-negativity emerges as an auspicious prognostic indicator for liver transplantation, suggesting that such patients derive augmented benefits from the transplant procedure (An et al., 2015).

While there remains room for refinement in AFP's utility for HCC diagnosis and therapeutic stratification, its role is anticipated to evolve, with enhancements in precision and broader application in future endeavors (Hu et al., 2022).

1.3. AFP-L3

Alpha-fetoprotein (AFP) demonstrates varying affinities to Lens Culinaris Agglutinin (LCA), leading to the categorization of serum AFP into three distinct subtypes: AFP-L1, AFP-L2, and AFP-L3. Each subtype undergoes unique glycosylation modifications at the molecular level (Qiu et al., 2020). While AFP-L1 and AFP-L2 are typical subtypes found in healthy adults, AFP-L3, often referred to as lens-responsive AFP, predominates in patients with hepatic malignancies, most notably hepatocellular carcinoma (HCC) (Park et al., 2017).

For early HCC diagnosis and therapeutic monitoring, AFP-L3 levels are commonly expressed as a fraction of the total AFP concentration. An elevated proportion of AFP-L3 generally correlates with a heightened likelihood of liver cancer manifestation (Zhou et al., 2021). Currently, a threshold of 10% serves as the conventional cut-off for AFP-L3 test positivity in HCC cases. A specificity surge is observed when AFP-L3 levels exceed 15% (Hanif et al., 2022). Some research indicates that AFP-L3 concentrations rise between 3-28 months preceding positive imaging indications, boasting a predictive accuracy for HCC as impressive as 94% (Liu et al., 2022).

By 2014, the Japanese Society of Liver Diseases had endorsed AFP-L3 as an HCC monitoring biomarker. Furthermore, in scenarios where traditional AFP readings are negative, AFP-L3 presents a viable alternative (Wang et al., 2020). Subsequent FDA approval in 2015 further solidified its utility (Qiu et al., 2020; Wang et al., 2020). However, while AFP-L3 exhibits commendable specificity (92.0%) for HCC surveillance, its sensitivity ranges between a modest 18.8% and 37.0% (Lee et al., 2021), suggesting that sole reliance on AFP-L3 percentages within total AFP may not be optimal for initial HCC screening.

To bolster AFP-L3's detection sensitivity, innovative methodologies have been developed, encompassing the micro-total laboratory system technique (Zhou et al., 2021), the aptamer sensor approach (Zhao et al., 2023), and a refined procedure for evaluating AFP-L3 subtype ratios (Li et al., 2020). These cutting-edge, high-sensitivity techniques show promise in refining the surveillance and diagnostic accuracy for HCC.

2. Golgi protein 73 (GP73)

Golgi protein 73 (GP73) is a transmembrane glycoprotein primarily localized on the Golgi apparatus membrane. It is also referred to by several designations including the 73 kDa Golgi protein, Golgi membrane protein 1 (GOLM1), or carcinoembryonic antigen-associated cell adhesion molecule 1 (CEACAM1) (Wang et al., 2020). Upon cleavage of its N-terminal region by proprotein convertase, GP73 is released extracellularly and subsequently becomes detectable in serum. Notably, its expression is markedly upregulated in hepatocytes (Gatselis et al., 2020).

A comparative study encompassing 30 liver cancer patients, 30 liver cirrhosis patients, and 30 healthy controls demonstrated that GP73 exhibits both high sensitivity and specificity, suggesting its potential utility as an early HCC biomarker (ElZefzafy et al., 2021). A pivotal study by Shaker et al. delineated that, at a cutoff value of 96.9 ng/mL, GP73 manifested a remarkable sensitivity of 96.9%. This contrasts with the comparatively diminished sensitivity of AFP at 9%, using a cutoff value of 75.92 ng/mL. Consequently, GP73 surpasses AFP in diagnostic efficacy for HCC surveillance (Shaker et al., 2020).

Intriguingly, GP73 possesses several potential glycosylation sites. Advanced proteomic methodologies have facilitated

the identification of heterogeneously fucosylated GP73, enabling precise quantification of serum GP73 levels. Recent innovations in nanotechnology have further refined these assays, enhancing their diagnostic sensitivity and specificity for HCC (Li et al., 2022; Liang et al., 2023). Nonetheless, elevated GP73 expression has been observed in other malignancies, including lung cancer, prostate cancer, and melanoma. Thus, the solitary use of GP73 as a definitive biomarker for HCC diagnosis is currently not recommended (Wang et al., 2020).

3. Des gamma carboxy prothrombin (DCP)

Des-γ-carboxy prothrombin (DCP), commonly known as Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II), is an aberrant prothrombin variant and a precursor secreted by hepatocellular carcinoma (HCC). Notably, DCP is devoid of the γ-carboxyl moiety found in the prothrombin molecule. As a result, it lacks the capability to bind calcium ions, rendering it inactive in coagulation processes (Pang et al., 2023).

The relevance of DCP in liver cancer was first reported by Liebman et al in 1984, observing that over 90% of liver cancer patients manifested a pronounced expression of DCP (Liebman et al., 1989). Subsequently, its application in liver cancer monitoring has gained traction. An investigation led by Feng et al., which analyzed serum DCP levels in 168 HCC patients, 150 patients with benign liver disease, and 153 healthy controls, revealed a conspicuous elevation of serum DCP levels in HCC patients compared to the latter groups (Feng et al., 2021).

Interestingly, DCP levels were found to be elevated in a significant proportion of AFP-negative liver cancer patients. Moreover, liver cancer could be ruled out in individuals exhibiting normal DCP levels, even when liver nodules were detected through ultrasound (Liu et al., 2020). The optimal cutoff value for serum DCP, as a monitoring metric for HCC risk, has been determined to be > 50 mAU/mL (Caviglia et al., 2023).

Comparative studies have yielded varied results regarding the superiority of DCP over AFP in HCC surveillance. While certain studies advocate for the dominance of DCP, others suggest a contrary stance. However, integrating both AFP and DCP has been shown to enhance diagnostic efficacy, delivering a heightened positive predictive value for HCC (Hemken et al., 2019). The GALAD model, a diagnostic paradigm in HCC, has incorporated DCP as an integral serum biomarker to gauge HCC risk in patients (Huang et al., 2022).

4. Glypican-3 (GPC3)

Glypican-3 (GPC3) is a cell-surface heparan sulfate glycoprotein. Notably, its highly negatively charged side chain facilitates the recruitment of essential ligands from the tumor microenvironment. Scientific investigations have ascertained that GPC3 functions as a coreceptor in hepatocellular carcinoma (HCC), endorsing cell proliferation through modulation of the Wnt/β-catenin signaling pathway (Shih et al., 2020).

Given the phenomenon of cell surface shedding characteristic of GPC3, it is detectable in the circulatory system. A study conducted by Liu et al demonstrated that serum levels of GPC3 in HCC patients were markedly elevated compared to those in healthy individuals, with an expression rate ranging between 70% and 100%. Furthermore, it was observed that GPC3 levels were elevated in approximately 50% of HCC patients who exhibited normal AFP values. Consequently, GPC3 exhibits promising potential as a biomarker for the early diagnosis of liver cancer (Liu et al., 2020).

5. Osteopontin (OPN)

Osteopontin (OPN) is a phosphorylated glycoprotein ubiquitously expressed across various cells and tissues. Notably, it's also identified as transformation-associated protein phosphatase. Interestingly, hepatocytes do not typically express OPN under normal conditions (da Costa et al., 2015). Elevated expression of OPN has been identified in multiple malignancies, including but not limited to lung, breast, gastric, and hepatocellular carcinoma (HCC) (Sun et al., 2018).

A prospective pilot study from 2012, which monitored HCC patients, revealed that OPN levels increased a year prior to the HCC diagnosis. This elevation was observed even in AFP-negative HCC patients, suggesting notable sensitivity (Shang et al., 2012). An evaluation involving 322 patients—comprising 105 with chronic hepatitis, 116 with cirrhosis, and 101 diagnosed with HCC—underscored OPN's potential as a diagnostic biomarker for HCC (Zhu et al., 2020).

Furthermore, research indicates that OPN might facilitate the generation of reactive oxygen species, thereby bolstering the proliferation and migration of HCC cells. This implicates OPN's significant role in both the onset and metastasis of HCC (Wu et al., 2022). Comparative studies have posited that OPN might outperform AFP in predicting HCC risk; the area under the curve (AUC) for AFP stands at 0.67, while that for OPN is slightly higher at 0.71. Impressively, combining both OPN and AFP raises the AUC to 0.73 (Khan et al., 2021).

Nevertheless, as of the current literature, OPN has yet to be incorporated into standard HCC surveillance guidelines (Singal et al., 2023). As such, there's a clear mandate for additional research to elucidate OPN's definitive role in HCC monitoring.

6. Aldo-keto reductase protein 1B10

Aldo-Keto Reductase 1B10 (AKR1B10) is an enzyme integral to the reduction of carbonyl compounds, specifically reliant on nicotinamide adenine dinucleotide phosphate (NADP) for its activity within the human system. This enzyme is paramount

in catalyzing reductions involving aldehydes, ketones, and quinones. Predominantly, its expression is observed in the gastrointestinal epithelium (Endo et al., 2021).

In 1998, investigations unveiled a heightened expression of AKR1B10 in HCC patients (Scuric et al., 1998). This finding was corroborated by Stefan et al., who determined an overexpression of AKR1B10 in early-stage, well-differentiated primary liver tumors (Heringlake et al., 2010). These discoveries position AKR1B10 as a potential early-stage biomarker for HCC monitoring.

The role of oxidative stress, particularly induced by reactive oxygen species (ROS), is pivotal in liver tumor pathogenesis. Chronic liver ailments typically manifest with an abundant production of ROS, culminating in hepatic oxidative stress. As liver disease progresses, AKR1B10 levels exhibit a concomitant rise (Ma-On et al., 2017). Contemporary research elucidates AKR1B10's capability to mitigate oxidative stress, thereby safeguarding hepatocytes (Liu et al., 2019). This understanding offers novel perspectives in HCC prophylaxis.

7. Dickkopf-1 (DKK1)

DKK1, a Wnt signaling modulator, is part of the secretory protein family. It inhibits Wnt signaling by participating in the formation of the Fz-Wnt-LRP5/6 complex (Suda et al., 2022). Recent studies by Yang, using a subcutaneous xenograft tumor model, identified that DKK1 potentiates the Akt/β-catenin signaling cascade, subsequently augmenting PD-L1 expression, which in turn encourages tumor growth (Yang et al., 2023). Shen et al elucidated that DKK1 has merit as an HCC monitor, especially during early stages when AFP levels remain subdued. This presents a potential in distinguishing AFP-negative HCC patients and aids in differential diagnosis between HCC and other chronic liver maladies (Shen et al., 2012). Using serum DKK1 singularly or in conjunction with other markers enhances early HCC detection, suggesting its superiority over AFP for early HCC diagnosis (Younis et al., 2019).

8. Midkine (MDK)

MDK, a fundamental heparin-binding growth factor, also recognized as neurite growth-promoting factor 2, is predominantly expressed during embryogenesis but is barely detectable in adult tissues. Altered apoptosis patterns and heightened hepatocyte proliferative activity are pivotal mechanisms in HCC development. MDK has been identified as an overexpressed gene in HCC (Zhang et al., 2020). Multiple studies confirm its sensitivity (0.85-0.86) and specificity (0.75-0.83) for HCC diagnosis (Zhang et al., 2019; Zhang et al., 2020). Further, Mi et al ascertained that elevated MDK expression is indicative of unfavorable prognosis and recurrence in combined hepatocellular cholangiocarcinoma, a rare primary liver cancer variant (Gowhari Shabgah et al., 2021).

9. Squamous cell carcinoma antigen (SCCA)

SCCA, a protein from the serine protease inhibitor family, is predominantly observed in squamous cell carcinoma cells and is a standard marker for diagnosis and monitoring of squamous cell carcinoma (Gomaa et al., 2009). Research indicates the efficacy of combining SCCA with AFP as a biomarker for HCC's early detection (Giannelli et al., 2005). Additionally, international studies reported an overexpression of SCCA in HCC tissues, potentially benefitting the identification of high-risk patients (Giannelli et al., 2005). Both SCCA and its immune complex, SCCA-IgM, have demonstrated diagnostic precision for HCC and have been proposed for screening (Liu et al., 2018).

10. Annexins

Annexins are a diverse family of Ca and charged phospholipid binding proteins, implicated in intracellular calcium regulation (Monastyrskaya et al., 2007). Specifically, Annexin A2 (ANXA2), a member of the Annexin A family, demonstrates heightened sensitivity to Ca and is ubiquitously expressed. Research reveals elevated ANXA2 expression in early HCC stages, and its levels rise concomitant with HCC progression. Clinical evidence supports the notion that circulating ANXA2 surpasses AFP in prognostic accuracy for HCC (Herrera-López et al., 2023). Moreover, ANXA1 and ANXA5 expressions escalate during liver cancer onset and are preferentially elevated in early nodules and HCC cells.

11. Soluble urokinase-type plasminogen activator receptor (suPAR)

SuPAR, a widespread membrane protein, participates in various biological processes, with aberrant expression linked to multiple diseases, including tumors and inflammations (Loosen et al., 2021). An international study postulated that suPAR levels in non-cirrhotic HCC patients were elevated compared to those with mild hepatic inflammation caused by fat accumulation. For HCC monitoring, suPAR levels exceeding 9.56 ng/ml demonstrated a sensitivity of 76.0% in high-risk EASL subgroups, proposing its application as an early alert for potential liver cancer patients (Chounta et al., 2015).

12. Thioredoxin reductase 1 (TXNRD1)

TXNRD1, an enzyme pivotal for sustaining cellular redox equilibrium, belongs to the thioredoxin reductase family. Its primary role involves the catalyzation of the reduction of oxidized thioredoxin, with repercussions on cell growth, differentiation, apoptosis, and immune response (Nordberg et al., 2001). Huang et al identified an upregulation of TXNRD1 in HCC, which fosters HCC growth and metastasis via the Akt/

mTOR signaling cascade (Huang et al., 2022).

13. Heat shock protein (HSP)

HSPs, proteins elicited in response to diverse cellular stressors, function primarily to shield cells from stress-induced damages, thus ensuring intracellular stability (Yamada et al., 2021). Research has identified that HSPA4 expression distinguishes tumor tissues from normal ones. Its role might be protective for HCC cells, fostering their proliferation and progression, suggesting its potential as a monitoring marker for HCC (Shang et al., 2021).

14. Cartilage oligomeric matrix protein(COMP)

COMP is a protein encoded by the human gene COMP, also known as tendon hook band protein (TSP-5), which belongs to one of the extracellular matrix glycoproteins.It can interact with other components of the extracellular matrix, playing an important role in maintaining the stability of extracellular matrix, and is mainly found in the oligomeric matrix of cartilage and tendon.It is usually not expressed in normal liver tissue (Xiao et al., 2004). A recent study found that serum COMP levels were elevated in patients with HCC, and with the increase of serum COMP concentration, HCC tumors grew and multiplied. Its combination with GP73 showed good efficacy in predicting the risk of developing HCC in patients with cirrhosis (Cui et al., 2022).

15. Glutamine synthetase (GS)

The main function of GS is to combine ammonia and glutamic acid to from glutamine, thus maintaining the balance of intracellular nitrogen metabolism. Its expression level is closely related to the functional status of liver cells. Liu et al confirmed that the level of GS increases gradually with the development of HCC, which has diagnostic value for AFP-negative patients (Liu et al., 2020).

In addition, there are cytokeratin 19 (Zhang et al., 2022), fibroblast growth factor 3 (FGF3) (Hu et al., 2007), mannose-binding lectin 2 (MBL2) (Su et al., 2016), AXL (Tsuchiya et al., 2015), hepatocyte growth factor (HGF), transforming growth factor-β1, vascular endothelial growth factor, α-l-fucosidase (AFU) (Gomaa et al., 2009), high mobility group box 3, phenylalanyltryptophan and glycocholate (Singh et al., 2020) can be used for the monitoring of HCC. However, there is no single biomarker that can be used for accurate early diagnosis of HCC (Singal et al., 2023)(Table 1).

16. Cartilage oligomeric matrix protein (COMP)

COMP is a glycoprotein encoded by the human COMP gene, alternatively termed the tendon hook band protein (TSP-5). This protein is classified among the extracellular matrix glycoproteins. COMP's crucial role lies in its interactions with other extracellular matrix components, thus ensuring matrix stability. Predominantly located within the oligomeric matrices of cartilage and tendons, its expression is typically absent in healthy liver tissue (Xiao et al., 2004). Recent research identified elevated serum COMP levels in hepatocellular carcinoma (HCC) patients, correlating with HCC tumor proliferation. Notably, when combined with GP73, COMP can effectively predict HCC risk in cirrhotic patients (Cui et al., 2022).

17. Glutamine synthetase (GS)

GS enzymatically catalyzes the amalgamation of ammonia with glutamic acid, producing glutamine. This process is fundamental in regulating intracellular nitrogen homeostasis. Liu et al observed an ascending GS level trend associated with HCC progression, suggesting its diagnostic potential, especially in AFP-negative cases (Liu et al., 2020).

Furthermore, several other potential markers, including cytokeratin 19 (Zhang et al., 2022), fibroblast growth factor 3 (FGF3) (Hu et al., 2007), mannose-binding lectin 2 (MBL2)

Table 1. Some biomarkers used for HCC diagnosis

Biomarkers	AUC	Sensitivity (%)	Specificity (%)	References
AFP	0.780	61	87	[43]
AFP-L3	0.755	34	92	[88]
GP73	0.909	59	95	[38]
DCP	0.890	71	84	[87]
OPN	0.700	83	56	[52]
AKR1B10	0.900	72.2	95.7	[87]
DKK1	0.877	80	60	[88]
MDK	-	85	83	[68]
CK19	0.867	87.5	86.2	[83]
HMBG3	0.791	75.6	81.6	[87]
AFP+AFP-L3+DCP	0.85	62	93	[43]
AFP+GPC3	0.91	86	89	[43]

Notes: AUC: area under curve; AFP: alphafetoprotein; AFP-L3: alpha-fetoprotein lens culinaris agglutinin 3; GP73: golgiprotein 73; DCP: des γ carboxy prothrombin; OPN: osteopontin; AKR1B10: Aldo-Keto reductase 1B10; DKK1: dickkopf-1; MDK: Midkin; CK19: cytokeratin-19; HMBG3: GPC3: glypican-3

(Su et al., 2016), AXL (Tsuchiya et al., 2015), hepatocyte growth factor (HGF), transforming growth factor-β1, vascular endothelial growth factor, α-l-fucosidase (AFU) (Gomaa et al., 2009), high mobility group box 3, phenylalanyltryptophan, and glycocholate (Singh et al., 2020), have been proposed for HCC surveillance. However, no singular biomarker offers precise early HCC detection (Singal et al., 2023) (Table 1).

Lipid biomarkers and hepatocellular carcinoma

α-Linolenic acid, a dietary free fatty acid, undergoes conversion in hepatocytes to produce long-chain n-3 PUFA. Observations have revealed a decrement in its levels in cirrhotic patients who later developed HCC, highlighting its diagnostic temporal relevance (Griffitts et al., 2010). Lysophosphatidylserine (LysoPS), a phospholipid resultant from phosphatidylserine hydrolysis, and its association with elevated expression in HCC, suggests its potential as a liver injury marker (Uranbileg et al., 2020). Enhancing HCC detection in cirrhotic patients might be achieved by integrating arachidic acid and n-3 docosapentaenoic acid with OPN and AFP (Khan et al., 2021). Notably, while elevated vaccenic acid and erucic acid plasma concentrations have been identified in HCC patients compared to cirrhotic controls (Muir et al., 2013), comprehensive studies in this domain are still warranted.

Other metabolites and hepatocellular carcinoma

Urine metabolites, derivatives of human metabolic processes, serve as indicators of metabolic and health statuses and offer valuable insights for disease early detection and screening, besides blood-based markers (Guan et al., 2021). Urinary metabolomic studies discerned over 200 distinct metabolites between HCC patients and healthy individuals, with Acetyl-DL-Leucine being notably upregulated (Xie et al., 2022). Bile acid dynamics are intricately tied to the liver, gallbladder, and intestine, making them pivotal for hepatobiliary disease diagnostics (Sun et al., 2021). Accumulation of bile acids can accelerate HCC progression in both mouse and human models, further accentuating their potential as biomarkers (Colosimo et al., 2022).

Liquid biopsy and hepatocellular carcinoma

1. Circulating tumor DNA (ctDNA)

1.1. Introduction to ctDNA

Liquid biopsy, a non-invasive diagnostic approach, assesses various biomarkers within body fluids. ctDNA embodies one such method, comprising DNA fragments released into circulation upon tumor cell death or division. This technique enables insights into the tumor's genetic landscape. When juxtaposed with conventional tumor biopsies, ctDNA's non-invasiveness and capability for dynamic tumor monitoring stand out (Liao et al., 2016).

1.2. Biomarkers for HCC-associated ctDNA Detection

Identifying ctDNA among the larger cfDNA pool remains a challenge. Tumor-specific mutations within circulating cfDNA signify ctDNA presence (Ye et al., 2019). Recent investigations unveiled ctDNA attributes such as levels, copy number variations, gene integrity, mutations, and DNA methylation as potential HCC biomarkers (Wu et al., 2020). A notable 20-fold increase in cfDNA concentrations was documented in HCC patients relative to healthy subjects (Yang et al., 2011). Liao et al revealed diagnostic potential in cfDNA mutation analyses by monitoring TERT, CTNNB9, and TP1 gene mutations (Liao et al., 2016). Methylation patterns in ctDNA, specifically in the early cancer stages, highlight its potential for early diagnosis. For instance, serum SEPT9 methylation discrepancies were observed between HCC patients and controls. This methylation, alongside its correlation with HCC progression, emphasizes its diagnostic merit (Kotoh et al., 2020). 5-Hydroxymethylcytosine (5 hmC) characterization within serum cfDNA could augment HCC early diagnosis sensitivity (Cai et al., 2021), albeit requiring methodological advancements.

2. Circulating tumor cells

CTCs, or tumor cells detached from the primary site, circulate in the bloodstream, providing insights into the tumor's genetic profile (Kleiner et al., 1999). Unlike ctDNA or cfDNA, cells deliver comprehensive data, encompassing DNA, RNA, and proteins (Deng et al., 2022). Initial CTC detection dates back 150 years by Ashworth in metastatic cancer patients (Lin et al., 2021), and their prevalence has been confirmed in early and advanced HCC patients (Wan et al., 2019). In HCC diagnostics, epithelial marker EpCAM and mesenchymal-related markers are used to identify CTCs (Hua et al., 2022). Despite the myriad of cells shed from primary tumors, a limited subset evades the immune response, necessitating the concurrent utilization of other biomarkers with CTCs for effective HCC diagnosis (Wan et al., 2021).

3. Extracellular vesicles (EVs)

Extracellular vesicles (EVs) are membrane-bound entities secreted by cells into the extracellular matrix. They can be subdivided into three primary categories based on their size and biogenesis, namely: exosomes, microvesicles, and apoptotic bodies (György et al., 2011). EVs secreted by tumors serve as signaling mediators between the tumor cells and stromal cells

in the tumor microenvironment. Notably, these vesicles are detectable in various body fluids and demonstrate resistance to biodegradation (O'Driscoll et al., 2015), positioning them as promising biomarkers for tumor identification. Specifically, EVs produced by hepatocellular carcinoma (HCC) are implicated in cell growth modulation, tumor microenvironment regulation, and influencing HCC progression and metastasis. Furthermore, analyzing HCC-associated RNA in EVs presents a novel biomarker approach for HCC detection and diagnosis (Costanzi et al., 2021). Long non-coding RNAs (lncRNAs), which are RNA molecules exceeding 200 nucleotides in length without protein-coding capacity, have emerged as significant contributors to tumor development. Their tumor-specific expression profiles accentuate their potential as tumor biomarkers (Wang et al., 2020). Notably, the EV-derived lncRNA EV-LINC00853 showcases promising sensitivity in early HCC diagnosis, especially in patients with unaltered AFP levels, indicating its potential as an early HCC diagnostic biomarker (Kim et al., 2020).

4. Exosomes

Exosomes are a subset of EVs originating from the endosomal system and are secreted through the fusion of multivesicular bodies with the plasma membrane. Characteristically, these vesicles measure between 50-100 nm in diameter and encapsulate a diverse range of biomolecules, including proteins, nucleic acids, and lipids, crucial for intercellular communication (Liu et al., 2021). Tumor-derived exosomes have been linked to the promotion of tumorigenesis and metastasis through mechanisms like dendritic cell maturation inhibition and the suppression of T cell-driven immune responses. Their distinctive structural and functional attributes underscore their potential as diagnostic biomarkers for malignancies (Chen et al., 2018). Sun et al found the exosomal non-coding long RNA-LINC00161 to be significantly upregulated in HCC patient serum using reverse transcription PCR, suggesting its prospective utility as an HCC biomarker (Sun et al., 2018).

5. Circulating microRNA

MicroRNAs (miRNAs) are short non-coding RNA entities approximately 21-25 nucleotides long. Their biogenesis originates from DNA transcription, and they undergo subsequent processing to achieve maturity. They can bind to messenger RNA (mRNA) molecules, inhibiting translation or expediting degradation, thus playing an instrumental role in post-transcriptional gene regulation. Intriguingly, a single miRNA can influence the expression of numerous genes (Matsuyama et al., 2019; Annese et al., 2020). The diagnostic significance of miRNA has been affirmed in multiple tumors. Moreover, changes in miRNA expression patterns have been observed throughout HBV-related HCC development, predominantly in its initial stages (Gao et al., 2011). Circulating miRNAs, present in blood and other bodily fluids, are safeguarded from enzymatic degradation either by being housed within microvesicles or through association with RNA-binding proteins. This resilience enables them to maintain stability through varied conditions, such as temperature fluctuations and pH alterations (Ji et al., 2019). Hence, gauging target miRNA concentrations can provide invaluable disease insights. MiR-16, a microRNA pivotal in cell cycle regulation, exhibits tumor-suppressive activities across diverse cancer types (Su et al., 2019). Fang et al contrasted circulating miR-16 levels in HCC patient serum against controls, revealing its potential as an early HCC diagnostic marker (Fang et al., 2022). MiR-122, abundant in the liver, is instrumental in liver function and metabolism. While its role in HCC development is established, its diagnostic precision for HCC remains moderate (Bandiera et al., 2015). A recent meta-analysis highlighted the differential impact of circulating miR-122 on HCC in healthy controls, but its differentiation efficacy for cirrhosis or dysplastic nodule patients was found wanting (Zhao et al., 2020). Comprehensive studies are imperative to elucidate its mechanism and diagnostic potential.

Composite markers for HCC surveillance

The GALAD model is a prognostic framework developed for the early detection of liver cancer. It integrates parameters such as gender, age, AFP-L3 percentage, total AFP value, and DCP to provide a holistic risk assessment, thereby facilitating earlier liver cancer identification (Johnson et al., 2015). However, while the GALAD model is predictive, its sensitivity and accuracy have varied across different cohort studies (as represented in Fig. 2) (Berhane et al., 2016). Thus, when employing the GALAD model for early liver cancer detection, a comprehensive assessment integrating other clinical data and test results is paramount. The BALAD scoring system, introduced by Toyoda et al in 2006, incorporates HCC-associated biomarker levels such as AFP, AFP-L3, and DCP, along with bilirubin and albumin scores to gauge patients' basal liver functionality (Toyoda et al., 2006). This model has gained validation across various geographies and has been recommended for predicting diagnosis and prognostication in HCC patients (Wongjarupong et al., 2021). Nonetheless, its clinical implementation warrants rigorous validation.

Other biomarkers in progress

1. High-throughput omics and HCC

High-throughput omics refers to techniques that harness high-

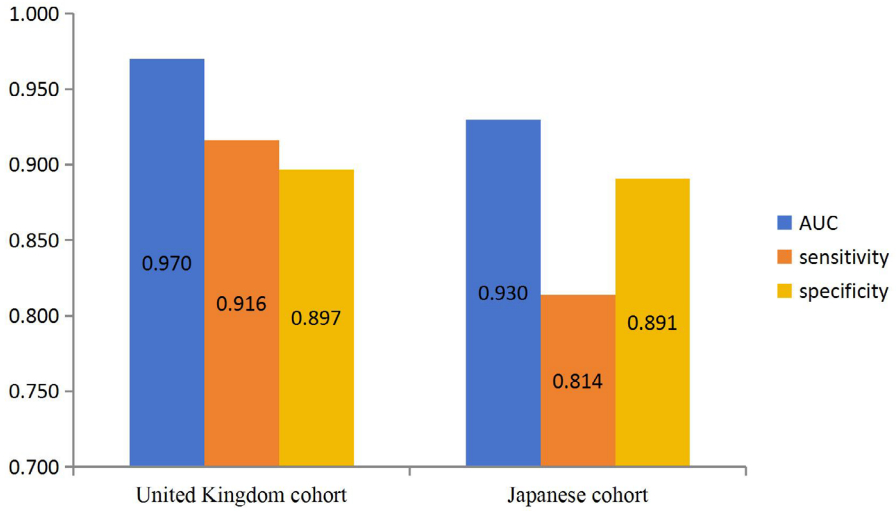

Figure 2. Diagnostic value of BALAD model under different cohort

throughput technologies combined with extensive data analysis to elucidate the molecular constituents, interactions, and functionalities within organisms. This spectrum encompasses genomics, transcriptomics, proteomics, and metabolomics. A salient characteristic of this approach is its proficiency in concurrently analyzing numerous molecules within a condensed timeframe, thus furnishing holistic biological insights. Utilizing high-throughput omics augments our comprehension of biological intricacies, illuminates gene-phenotype associations, aids in novel biomarker discovery, deepens disease mechanism insights, and lays the foundation for personalized medical interventions (Zhang et al., 2009). Within the realm of HCC biomarkers and proteomics, new potential biomarkers have been discerned. Specifically, the Hcp70/Hsp90 tissue protein and heterogeneous ribonucleoprotein C1/C2 have been postulated as potential HCC biomarkers, while complement C3a has emerged as a candidate biomarker for HCV-associated HCC in humans (Pei et al., 2009). Various studies, employing bioinformatics, have sifted through pivotal genes in HCC to discern potential early diagnostic biomarkers. Analyses have pinpointed several central genes intertwined with HCC's onset and progression, notably FOXM1, CCNA2, AURKA, CDKN3, among others (Luk et al., 2011; Wang et al., 2020).

2. Intestinal Microbiome and HCC

The intestinal microbiome embodies the collective of microorganisms residing within the human gastrointestinal tract. Research indicates that perturbations in the intestinal microbial composition are linked to specific liver ailments, including cirrhosis (Shen et al., 2017). Recent investigations have showcased how the intestinal microbiome can catalyze HCC development in animal models (Schnabl et al., 2014). A subsequent study probing the gut microbiome's potential as a non-invasive HCC biomarker divulged heightened fecal microbial diversity in HCC-afflicted patients. Notably, an uptick was observed in actinomycetes and 13 other genera, inclusive of Parabacteroides, in early-stage HCC and cirrhosis. Concurrently, there was a decline in butyrate-producing genera coupled with an escalation in lipopolysaccharide-producing genera (Ren et al., 2019), alluding to its plausible utility in detecting incipient HCC.

Conclusions

While the aforementioned biomarkers hold promise in facilitating early HCC diagnosis, their sensitivity and specificity constraints remain a challenge. Presently, no singular biomarker suffices the comprehensive requirements for early HCC detection. The quintessential HCC biomarker would empower clinicians to diagnose the disease during its latent phase, subsequently guiding the optimal therapeutic strategy. Current biomarkers fall short of this ideal, emphasizing the exigency for continued research to refine existing markers and unearth novel, robust biomarkers. Such endeavors are imperative to elevate the precision and dependability of HCC's early diagnosis.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

ML and YX contributed to study concept and design. XL, ZZ, TJ, WD, SW,WC,LY, XB, YL, YL and LZ collected and sorted out the literature. XL, ZZ and TJ drew pictures. XL, ZZ, TJ ,DW and SW wrote the first draft. ML edited the English version. ML and YX approved the submitted version after modification. All authors contributed to the article and approved the submitted version. All authors read and approved the final manuscript.

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