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CONSENSUS

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Chinese expert consensus statement on the clinical application of AFP/AFP-L3%/DCP using GALAD and GALAD-like algorithm in HCC

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Abstract

Background: Primary hepatocellular carcinoma (HCC) is one of the most prevalent world-wide malignancies. Half of the newly developed HCC occurs in China. Optimizing the strategies for high-risk surveillance and early diagnosis are pivotal for improving 5-year survival. Constructing the scientific non-invasive detection technologies feasible for medical and healthcare institutions is among the key routes for elevating the efficacies of HCC identification and follow-up.

Results: Based on the Chinese and international guidelines, expert consensus statements, literatures and evidence-based clinical practice experiences, this consensus statement puts forward the clinical implications, application subjects, detection techniques and results interpretations of the triple-biomarker (AFP, AFP-L3%, DCP) based GALAD, GALAD like models for liver cancer.

Conclusions: The compile of this consensus statement aims to address and push the reasonable application of the triple-biomarker (AFP, AFP-L3%, DCP) detections thus to maximize the clinical benefits and help improving the high risk surveillance, early diagnosis and prognosis of HCC.

KEYWORDS
alpha-fetoprotein (AFP), alpha-fetoprotein-L3% (AFP-L3%), des-gamma-carboxyprothrombin (DCP), gender-age-AFP-L3%-AFP-DCP (GALAD) model, hepatocellular carcinoma (HCC)

1 | INTRODUCTION

Primary liver cancer (PLC) is the sixth most common cancer worldwide in terms of the number of cases of 906,000 with an age-standardized incidence rate of 9.5/100,000, which mainly includes hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and mixed HCC-cholangiocarcinoma (HCC-ICC), with incidences of 93.0%, 4.3% and 1.6%, respectively.[1–3] PLC is the second cause of tumor-related deaths in China, with a mortality rate of 24.33 per 10,000 (males: 35.25 and females: 12.86 per 100,000), accounting for 13.94% of all sites of cancers, respectively.[4] In most cases, HCC develops with underlying chronic liver disease (CLD) and liver cirrhosis (LC). Several risk factors for HCC individuals have been identified, including aged over 40 years, male gender, presence of cirrhosis, a family history of HCC, high level of hepatitis virus B (HBV) replication, heavy alcohol consumption, smoking, diabetes, obesity and aflatoxin exposure.[5] Although the contribution of the different risk factors to PLC varies with region, chronic viral infection is still the leading cause of global PLC, accounting for 80% of HCC cases globally.[6] During recent decades, non-alcoholic fatty liver disease (NAFLD) is becoming the fastest-rising cause of HCC. Due to the wide coverage of HBV vaccination and the successful eradication of hepatitis C virus (HCV), NAFLD might become the most common underlying HCC etiology worldwide in the near future.[7,8] Although increasing from 10.1% in 2003–2005 to 12.1% in 2012–2015, the five-year survival for PLC was still 25% lower than all cancer survival estimates calculated with the same method (37.2%).[9] In China, the average medical expenditure per case for PLC diagnosis and treatment has increased from 21, 950 Chinese Yuan (CNY) in 2002 to 40, 386 CNY in 2011.[10] In 2019, the overall economic burden of PLC was estimated at 76.7 billion CNY (0.047% of the local GDP) in China.[10,11] Conclusively, the economic burden of PLC in China is substantial and will be consistently increased. Sustainable efforts in reducing the economic burden of PLC are urgently needed in China.

Because of the insufficient early warning and screening for HCC high-risk population, about 70%–80% of HCC patients are diagnosed at late stage, resulting in the five-year survival rate of HCC patients being extremely low.[6] Early detection of HCC is essential for curative treatment and the overall survival of HCC patients.[6] The alpha-fetoprotein (AFP), the Lens culinaris agglutinin reactive fraction of AFP (AFP-L3) and des-γ-carboxyprothrombin (DCP, another name: Protein induced by vitamin K absence or antagonist-II, PIVKAII) (hereafter referred to as "the triple-biomarker for HCC") are feasible biomarkers for HCC clinically. The growing evidences indicated that the use of the triple-biomarker combination together with demographic information, such as GALAD and GALAD-like (C-GALAD, C-GALAD II, GALAD-C, etc.), are more informative in HCC surveillance.[12–18] Recently, *Chinese guideline for liver cancer screening (2022)* by National Cancer Center recommended that AFP combined the ultrasound is still the most commonly used surveillance test.[2] The *Chinese guideline for diagnosis and treatment of hepatocellular carcinoma (2022)* has included the use of the GALAD and GALAD-like to enhance the diagnostic accuracy of HCC.[1] Although the *expert consensus on the multidisciplinary clinical application of AFP-L3* had implicated one of the triple-biomarker in 2017,[19] the importance of routine application of AFP-L3% and DCP in clinical practice was still not sufficient and well recognized. Based on the above situation, the background knowledge, clinical indications, application scenarios, detection technologies, result interpretations of the triple-biomarker and its derived algorithms are described in this statement aiming to improving the application rationality and feasibility and maximizing the clinical benefit of the triple-biomarker in form of algorithms.

2 | THE BACKGROUND AND CLINICAL SIGNIFICANCE OF THE TRIPLE-BIOMARKER FOR HCC

2.1 | Alpha-fetoprotein (AFP)

The initial AFP-related description called protein X of unknown nature was reported by Bergstrand and Czar in 1956.[20] AFP was firstly described in the HCC experimental mice by Abelev et al. in 1963. However, it was in 1964 that Tatarinov et al. described the increased AFP serum levels in HCC patients, which provided a brand-new clue at that time for the early detection and diagnosis of HCC.[21–23]

The glycoprotein AFP is derived from the yolk sac and liver of human fetuses whose molecular weight is 68–72 kDa. The concentrations of AFP peak at 12–16 weeks of pregnancy and then gradually decline. The silencers in the AFP gene transcriptional regulatory region are active after birth, which prevents the enhancers from activating AFP transcription.[24] After the age of 2 years, the AFP levels sharply drop to the normal adult range and remain low throughout the life cycle.[24] However, in the early stage of the malignant transformation of hepatocytes, the AFP gene in the hepatocytes is specifically activated, leading to the high expression level of AFP and participating various pathophysiological processes.[19] Ruoslahti et al. (1971)[25] pioneered the development of a quantitative detection technology of AFP using the radioimmunoassay (RIA). The lowest detection limit of the technology was 0.25 ng/mL.[25] The improvements of the AFP detecting technology initiated a burst of interest in AFP as a biomarker for HCC.[25] Since then, numerous techniques such as enzyme-linked immunosorbent assay (ELISA), rocket electrophoresis, fluorescent immunoassay, chemiluminescence and electrochemiluminescence (ECL), Raman spectroscopy and electrochemical immunosensor have been used for AFP determination.[26–30] Among these techniques, (E)CL could detect AFP at ng/mL level with short reaction time and high sensitivity without harmful radiation.

A randomized controlled trial conducted in individuals aged 35–59 years with HBV infection or a history of chronic hepatitis indicated that the combination of AFP testing and ultrasonography examination every six months led to a 37% reduction in HCC mortality.[31] Elevations of serum AFP in HCC were significantly

associated with the progression, recurrence and low overall survival rate of HCC.[32–34] Another study enrolled 78,743 HCC patients in Surveillance, Epidemiology, and End Results Program (SEER), indicating that AFP at diagnosis was an independent risk predictor associated with HCC pathological grade, progression and survival.[35]

The sensitivity and specificity of AFP largely depend on the cut-off values of AFP. The AFP at a cut-off value of 20 ng/mL for HCC surveillance achieved a sensitivity of 40%–60% and a specificity of 80%–90%.[36] Evidence from multi-center and retrospective studies in China shows that the sensitivity and specificity of AFP in HCC detection were 60%–70%, suggesting a 30%–40% false-negative and false-positive rate.[14,37,38] The higher the cut-off values of AFP, the lower the sensitivity and the higher specificity of AFP.[39] When using an AFP cut-off value greater than 100 ng/mL, the sensitivity was 31.2%, and the specificity 98.8%.[39] The sensitivity is reduced to 22.4% at cutoff levels greater than 200 ng/mL.[39] The *Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update* recommended that the cut-off value of AFP should be set at 200 ng/mL for surveillance programs when used in combination with ultrasonography.[40] The *Chinese guideline for diagnosis and treatment of hepatocellular carcinoma (2022)* declared that AFP ≥400 μg/L is highly suggestive of HCC after excluding pregnancy, chronic or active liver diseases, embryonal tumors of the gonads and gastrointestinal tumors.[1] A meta-analysis incorporated 32 studies (13,367 patients) characterized the sensitivity of ultrasonography for HCC, reported a pooled sensitivity of 45%, which increased to 63% after adding AFP.[41]

The sensitivity of AFP is lower in the setting of a solitary small HCC lesion compared with the large HCC. A multi-center study substantiated the low sensitivity (54%) of AFP in small HCC diagnosis.[42] Another multicenter study demonstrated that the sensitivity of AFP for HCC detection is 46% and was reduced to 23.4% in the small HCC (<2 cm) when 11 ng/mL was used as the cut-off value for HCC diagnosing.[43]

Due to the lack of sensitivity and specificity, AFP was withdrawn from the *European Association for the Study of the Liver (EASL) Policy Statement: risk-based surveillance for hepatocellular carcinoma among patients with cirrhosis (2023)*.[44] However, the *Chinese guideline for diagnosis and treatment of hepatocellular carcinoma (2022)*,[1] *Chinese guideline for liver cancer screening (2022)*,[2] *Chinese Guidelines for the prevention and treatment of chronic hepatitis B (2022)*,[5] *Clinical practice guidelines for hepatocellular carcinoma: The Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update*[45] continue to recommend the examination of abdominal ultrasonography and AFP repeated every 3–6 months for patients with cirrhosis, chronic hepatitis and a family history of HCC. Meanwhile, the *NCCN Clinical Practice Guidelines in Oncology for HCC (2023)* and *American Association for the Study of Liver Diseases (AASLD) practice guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma (2023)*[46] also continue to include AFP in HCC screening and surveillance decision trees.

[Consensus Statement 1] AFP is the most widely used HCC biomarker for the surveillance, early diagnosis and therapy monitoring. The clinical implication of AFP should be connected with the cut-off values. More attention should be paid to the additive examination for the AFP-negative high-risk populations.

2.2 | Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3)

Lectins affinity heterogeneities enable AFP classified into different glycoforms as measured by immunoblotting together with affinity electrophoresis. According to the different binding competence to Lens culinaris agglutinin (LCA), AFP could be divided into three glycoforms: AFP-L1 is a nonbinding fraction, AFP-L2 weak binding and AFP-L3 strong binding.[47] AFP-L1 is present in CLD and LC, constituting a majority fraction of total AFP in non-tumorigenic liver diseases.[48] AFP-L2 could be detected in maternal serum during pregnancy and yolk sac tumors.[49] AFP-L3 appears to be produced by carcinogenic hepatocytes.[49] The percentage of AFP-L3 in total AFP is called the fucosylation index of AFP or AFP-L3%. Several AFP-L3% detection methods are available, such as affinity adsorption assay, microfluidics-based immunofluorescence, affinity crossed immuno-electrophoresis, plant lectin affinity chromatography and affinity imprinting.[19]

The higher AFP-L3% were positively associated with the HCC progression,[50] the later clinical stages of HCC, extrahepatic metastasis and portal vein thrombosis.[51–53] The AFP-L3% was the independent predictor for the overall survival of HCC.[51–53] The preoperative positivity of AFP-L3% and continuously positive or negative-turn-positive of AFP-L3% after surgery indicated more aggressive tumor behavior, higher tumor recurrence and poorer clinical outcomes.[54,55] The sensitivity of AFP-L3% is associated with the clinical stage and its cut-off values.

For discriminating benign and malignant liver disease, the sensitivity of AFP can be improved by measuring the AFP-L3%.[19] The overall sensitivity of AFP-L3% for HCC detection was approximately 50%–60%.[49] The sensitivity of AFP-L3% increased with tumor enlargement and reached 80%–90% when the HCC tumor diameter ≥5 cm.[49] The increasing of the AFP-L3% is an important and somehow special event for small HCC.[49] In early-stage HCC detection, defined as a single tumor <3 cm, the sensitivity for AFP-L3% (cut-off: 5%) was 61.2% and the specificity 73.8%.[56] Another study indicated that HCC with tumor size <2 cm, AFP-L3% sensitivity was 46.7%.[57] A prospective follow-up cohort surveillance study showed that the AFP-L3% (>7%) sensitivity and specificity at −1 year were 34.3% and 74.7% respectively when the HCC patients at low AFP levels and in the absence of ultrasound findings.[58]

AFP-L3% immunological test systems is classified as class II (special controls) by the American Food and Drug Administration (FDA) for in vitro diagnostic use as an aid in the risk assessment of CLD for the development of HCC in conjunction with other laboratory findings, imaging studies and clinical assessment.[59] *Clinical practice guidelines for hepatocellular carcinoma: The Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update*

recommended that the abdominal ultrasound with concomitant measurements of the triple-biomarker (AFP, AFP-L3 and DCP) is described as a screening modality.[45] *Chinese guideline for liver cancer screening (2022)*[2] proposed AFP-L3% as an important supplementary measure for HCC detection. *Chinese guideline for diagnosis and treatment of hepatocellular carcinoma (2022)*[1] indicated the early diagnosis rate of HCC can be improved by measuring the AFP-L3%.

[Consensus statement 2] AFP-L3% is an important complement to AFP in discriminating malignancy from non-neoplastic hepatic regeneration. Using different cutoff value, AFP-L3% can contribute to the HCC early detection, supplemental surveillance for HCC high-risk populations and HCC prognostic monitoring.

2.3 | Des-gamma-carboxyprothrombin (DCP)/ protein induced by vitamin K absence or antagonist-II (PIVKA II)

DCP, also known as PIVKA II, is a defective prothrombin protein that absent vitamin K-dependent activity formed as the result of the absence of the 1–10 glutamic-acid residues in the N-terminus, which resulted in no biological coagulation activities of DCP.[60] Blanchard et al.[61] (1981) developed specific RIA for native and abnormal human prothrombin. Again Liebman et al.[62] (1985) applied the same approach identifying 91% of biopsy-confirmed HCC (69 of 76). Motohara et al.[63] (1985) developed a more sensitive DCP measurement (ELISA), which could detect as low as 0.13 U/mL DCP. With the progression of the measurement technology, the novel immunoassay using the automatic chemiluminescence immunoassay enable the more sensitive detection.[64,65] Since 2015, several chemiluminescence methods had been approval by the National Medical Products Administration (NMPA) for robust and high-throughput detection which are more feasible for clinical application.

The growing research suggested that the sensitivity of DCP (72.7%) generally stayed superior to AFP (67.7%) for HCC detection.[66] A retrospective data indicated that increased levels of DCP above 40 mAU/mL were observed in 62% HCC patients, while 47% HCC had AFP values above 20 ng/mL.[67] Our multi-center study comprising 1034 patients indicated that the accuracy of DCP for distinguishing HCC from non-HCC was 6.2–9.7% higher than that of AFP, and the accuracy of DCP was further elevated in HBV-HCC, which was 12.3%–20.67% higher than that of AFP.[68] Additionally, in data from the NASH research group of the Ministry of Health, Labor, and Welfare,[69] as well as in the data from the Japan Study Group of NAFLD,[70] DCP consistently exhibited a higher positive rate compared to AFP.

Meanwhile, DCP has specific predictive value for recurrence and microvascular invasion (MVI) of HCC, with a particular efficacy on monitoring postoperative recurrence in AFP-negative HCC patients.[37,68] In a French cohort, DCP level > 90 mAU/mL (HR: 3.5; 95% CI: 1.081–11.8; $p = 0.043$) was the independent risk factor of MVI in HCC patients with a sensitivity of 70% and a specificity of 63% for MVI prediction.[71] Twenty patients (74.1%) showed elevated levels of DCP (>40 mAU/mL) at recurrence, which suggested DCP as a valuable marker in HCC recurrence assessment.[60]

In conclusion, DCP plays an important role in the diagnosis and prognosis prediction in HCC and is particularly applicable to the clinical management of AFP-negative HCC. In China, the *Chinese guidelines for the prevention and treatment of chronic hepatitis B (2022)*,[5] *Chinese guideline for liver cancer screening (2022)*,[2] *Chinese guideline for diagnosis and treatment of hepatocellular carcinoma (2022)*,[1] *Chinese guideline for stratified screening and surveillance of primary liver cancer (2020)*[72] and *Chinese consensus statement on secondary prevention of primary liver cancer (2021)*[73] all recommend DCP as one of the supplemental screening and companion biomarkers for HCC. The Asian-Pacific Association for the Study of the Liver (APASAL) and Japan Society of Hepatology (JSH) have also confirmed the role of DCP in HCC high-risk populations screening and auxiliary diagnosis.[40,45]

[Consensus 3] DCP is effective in HCC high-risk surveillance, early diagnosis and stratified management for HCC. The parallel application of DCP and AFP could improve sensitivity and be especially useful in postoperative follow-up for AFP-negative HCC patients.

3 | THE JOINT UTILIZATION OF AFP, AFP-L3% AND DCP

Growing evidences demonstrated that the triple-biomarker (AFP, AFP-L3%, DCP) combination significantly improves the accuracy of early detection of HCC, which is of great significance for improving the five-year overall survival rate of HCC.

3.1 | The implications of AFP-L3% and DCP in population with low AFP level

TU et al.[74] reported that elevated AFP-L3% appeared 3–24 months earlier than positive imaging finding, and the accuracy of AFP-L3% predicting HCC was 95.0%. A retrospective study included 416 patients (260 with HCC and 156 without HCC) with AFP levels <20 ng/mL, indicating that the sensitivity for DCP and AFP-L3% was 50.0% and 41.0%, respectively.[56] Combined DCP and AFP-L3% could improve the sensitivity to 69.9% with a specificity of 71.9%.[56] The sensitivity of AFP-L3% and DCP identifying AFP-negative small HCC (<2 cm) was 46.7% and 40%, respectively[57]; furthermore, the joint detection of the triple-biomarker could improve the sensitivity to 86.7%.[57] A prospective follow-up study enrolled 106 CLD patients without HCC and followed for >12 months. The cumulative incidence of HCC was 10.5% at 5 years and 19.6% at 10 years. For patients with AFP < 20 ng/mL, multivariate logistic regression analysis revealed that AFP-L3% ≥ 4.9% (HR: 11.608; 95% CI: 2.422–55.629; $p = 0.002$) and DCP ≥25 mAU/mL (HR: 3.936; 95% CI: 1.088–14.231; $p = 0.037$) were risk factors of hepatocarcinogenesis.[75]

3.2 | Clinical indications of the triple-biomarker in HCC diagnosis and stratification

The published data suggested that the combination of AFP, AFP-L3% and DCP can improve the sensitivity to approximately 90% for detecting early-stage, small-sized, single HCC tumors in patients with low AFP levels.[57] HCC with positive DCP carried larger tumor size. At HCC tumor diameters of <3 cm, 3-5 cm and >5 cm, the positivity of DCP was 74%, 83%, and 96%, respectively. In contrast, the AFP-positive rate was 48%, 57%, and 65%, respectively.[76] The positivity of AFP, AFP-L3% and DCP increased in parallel with progression of the tumor stage, in which the prevalence of elevated DCP is more remarkable.[77] In addition, there was a striking decreasing of DCP after curative hepatectomy.[68] Combined AFP, AFP-L3% and DCP enable better assessment of the clinical outcomes and curative effect for HCC.[78]

Chronic HBV infection is the major high-risk factor for HCC, responsible for 80% of HCC cases in China.[79] While, HBV infection only accounted for 45% HCC etiology in some region.[80] A large-scale retrospective cohort from Shanghai Eastern Hepatobiliary Surgery Hospital displayed that the positive rate of AFP and AFP-L3% was 62% and 45% in HBV-related HCC, significantly higher than those of non-HBV-related HCC.[19] Since the HBV load can significantly influence the predictive value of AFP applied solely, we displayed that the DCP may be more suitable for identifying HCC with HBV infection background in China.[68] But whether or how the DCP clinical effectiveness influenced by the etiology still needs to be explored in future.

At present, the joint clinical application of the triple-biomarker had been included in several guidelines or consensus statements as was shown in Table 1.

[Consensus 4] Joint detection of AFP, AFP-L3% and DCP is recommended in HCC. The triple-biomarker combination is not only beneficial for the differentiation between the benign and malignant liver diseases in high-risk population thus improving the early diagnostic efficacy, but also provide indicators for clinical risk stratification and prognosis management (recurrence/survival).

4 | THE ALGORITHMS FOR THE COMBINED APPLICATION OF THE TRIPLE-BIOMARKER (AFP, AFP-L3% AND DCP) IN HCC

4.1 | GALAD algorithm

Johnson et al.[18] (2014) were the first to develop a novel HCC diagnostic algorithm based on gender, age, AFP-L3%, AFP and DCP in a British cohort, defined as the GALAD score. The score was calculated as following:

$$Z = -10.08 + 0.09 \times Age + 1.67 \times Gender\ (Male = 1; Female = 0) + 2.34 \times log_{10}(AFP) + 0.04 \times AFP\_L3 + 1.33 \times log_{10}(DCP)$$

The GALAD algorithm demonstrated exceptional overall performance with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.97, significantly higher than AFP (AUC: 0.88), AFP-L3% (AUC: 0.84) and DCP (AUC: 0.90) alone. The AUC was maintained in HCC early detection (AUC: 0.96).[18] BERHANE et al.[17] (2016) validated the GALAD algorithm in international multi-center cohorts that enrolled 6834 patients (2430 with HCC and 4404 with CLD) from British, Germany, Japan and Hong Kong, with an overall AUC ranging from 0.93 to 0.97, sensitivity 81.4%–91.6% and specificity 88.2% to 89.7%. In an Italy cohort, the diagnostic efficacy of GALAD (AUC: 0.98) was better than the triple-biomarker combination only (AUC: 0.95).[92] Studies from different groups indicated that the AUC of the GALAD for HCC detection were all above 0.95.[93–95]

In China, our group has pioneered the clinical validation of GALAD in a large-scale multi-center cohort ($N = 7664$). Compared with AFP (AUC: 0.826, 95% CI: 0.816–0.836), AFP-L3% (AUC: 0.763, 95% CI: 0.752–0.773), DCP (AUC: 0.919, 95% CI: 0.912–0.925) and triple-biomarker combination only (AUC: 0.943, 95% CI: 0.937–0.948), the GALAD (AUC: 0.960, 95% CI: 0.955–0.964) had the highest AUC for HCC identification.[37] Several other GALAD clinical validation studies in China also supported the diagnostic efficacy in HCC identifying, which the AUC value all above 0.90.[96–98]

Geographically, HCV infection, alcoholic liver disease and NASH are the leading cause of HCC in Europe and America. A multicenter case–control study was conducted in German and Japan to evaluate the diagnostic power of GALAD in NASH-related HCC. The results revealed that the sensitivity and specificity of GALAD in early HCC within Milan criteria were 84.0% and 90.9%, respectively.[13] Furthermore, the mean score of GALAD was elevated in NASH-related HCC 1.5 years early before the definite diagnosis of HCC.[13] A recent study by Yang et al.[99] (2019) found that the AUC of GALAD in HBV-related HCC (AUC: 0.94, 95% CI: 0.88–0.99) was superior to that in HCV (AUC: 0.89, 95% CI: 0.86–0.92), alcohol (AUC: 0.89, 95% CI: 0.81–0.97) and non-viral/alcohol related HCC (AUC: 0.88, 95% CI: 0.81–0.96). While the results from Schotten[98] and Berhane[17] exhibited that the GALAD showed similar performances in discriminating HBV and HCV related HCC, independent of viral load. According to our large-scale clinical research from five centers in China, the GALAD identified HCC at an AUC of 0.935 with a sensitivity of 83.78% and a specificity of 88.69%, which outperformed AFP, AFP-L3 and DCP.[14] Meanwhile, GALAD could stratify HCC patients into two distinct subgroups with low or high overall survival and recurrence risk,[14] which suggests the role of GALAD in prognosis assessment, risk stratification and disease management. The AUC of GALAD for HBV-related HCC from another retrospective cohort was 0.911, which is consistent with our investigation.[100]

In summary, the diagnostic efficacy of the GALAD algorithm significantly outperformed AFP, AFP-L3%, DCP, and their combination without the algorithm. The diagnostic accuracy of GALAD, whether influenced by the disease background, still needs more evidence-based studies to determine.

TABLE 1 The compilation of liver disease-related guidelines or consensus statements including joint clinical application of the triple-biomarker (AFP, AFP-L3%, DCP).

No.	Guidelines/Consensus	Related descriptions	Publisher	Publish time
1	Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update[81]	USG, AFP, DCP, AFP-L3 or their combinations have long been used as surveillance tests for HCC in Asian countries.	APASL	Jan. 2016
2	Expert consensus statement on the clinical application of multidisciplinary alpha fetoprotein heterogeneity[19]	• The detection of AFP-L3% is suitable for the dynamic monitoring of disease progression and therapeutic response in the high-risk populations of CLD, liver fibrosis and liver cirrhosis and HCC patients. • The detection of AFP-L3% can assist in predicting and diagnosing PLC in low AFP-level populations. • The detection of AFP-L3% can contribute to differentiating benign and malignant liver diseases. • The detection of AFP-L3% can be used as an independent predictor factor for monitoring the prognosis and recurrence of PLC.	Shanghai Society of Molecular Diagnostics, Shanghai Medical Association	May 2017
3	Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update[40]	• AFP is not recommended as a confirmatory test in small HCC. • DCP has been recognized as not only a highly specific marker for HCC but also a predictor of prognosis of HCC patients. • DCP showed better diagnostic performance than AFP in diagnosis of early HCC. • The presence of elevated AFP-L3% is correlated with HCC tumor shorter doubling time, and raised serum DCP levels might be indicative of microinvasion.	APASL	July 2017
4	EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma[82]	Serological tests that have been investigated or are under investigation for early diagnosis of HCC include AFP, DCP, AFP-L3%, alpha-fucosidase and glypican.	EASL	July 2018
5	NCCN Clinical Practice Guidelines in Hepatobiliary Cancers (Version 1 0.2020)[83]	A case–control study involving patients with hepatitis C enrolled in the large, randomized HALT-C trial who developed HCC showed that a combination of AFP and DCP is superior to either biomarker alone as a complementary assay to screening.	NCCN	March 2020
6	Expert consensus statement on multidisciplinary diagnosis and treatment of precancerous lesions of hepatocellular carcinoma (2020)[84]	• AFP-L3 only appears to be produced by carcinogenic hepatocytes, and its proportion (AFP-L3%) increases with the degree of cancerous lesions. Therefore, AFP-L3% can also be the biomarker for PLC detection. • DCP is an abnormal prothrombin protein produced in HCC. DCP is feasible for clinical application as a tumor marker for PLC.	Liver Cancer Study Group, Chinese Society of Hepatology, Chinese Medical Association	March 2020
7	Guidelines of Chinese Society of Clinical Oncology (CSCO) hepatocellular carcinoma (2020)[85]	AFP-negative HCC (ANHCC) patients account for 30% of the whole HCC patients. In AFP-negative population, AFP-L3%, DCP, AFU and plasma-free microRNA can be helpful in early diagnosis of HCC.	Chinese Society of Clinical Oncology	July 2020
8	Expert consensus statement on biomarker detection and application in hepatocellular carcinoma (2020)[86]	• Serum AFP, AFP-L3%, and DCP are commonly used and important biomarkers for diagnosing HCC. • US examination in combination with serum AFP, AFP-L3% and DCP can further improve the early screening and detection rate of HCC in AFP-negative population. Dynamic monitoring of AFP in combination with AFP-L3%, DCP and liver inflammation indicators could improve the differential diagnosis accuracy of HCC with AFP levels mildly elevated.	Molecular Diagnostics Group, Chinese Society of Laboratory Medicine, Chinese Medical Association	Dec. 2020
9	Guideline for stratified screening and surveillance of primary liver cancer (2020)[72]	Serum AFP combined with AFP-L3% and DCP measurement can improve the early detection rate of HCC (B2).	Chinese Preventive Medicine Association	Jan. 2021

TABLE 1 (Continued)

No.	Guidelines/Consensus	Related descriptions	Publisher	Publish time
10	Consensus statement on the secondary prevention for primary liver cancer (2021)[87]	• Serum AFP is a preferred biomarker for screening early-stage HCC (A1). • AFP combined with DCP and AFP-L3% can improve the detection rate of HCC (B2). • Dynamic monitoring of AFP in combination with AFP-L3%, DCP could improve the early detection rate of HCC with AFP levels mildly elevated (B2).	Chinese Society of Hepatology, Chinese Medical Association	March 2021
11	Management of Hepatocellular Carcinoma in Japan: JSH Consensus Statements and Recommendations 2021 Update[88]	• AFP, AFP-L3% and DCP are frequently elevated in HCC, and the positive rate of these markers increases with disease progression • Extremely high-risk group: 1. Ultrasound every 3–4 months 2. AFP, AFP-L3% and DCP every 3–4 months 3. Dynamic CT/MRI every 6–12 months (optional) • High-risk group: 1. Ultrasound every 6 months 2. AFP, AFP-L3% and DCP every 6 months	JSH	June 2021
12	Expert consensus statement on viral hepatitis health management (2021)[89]	DCP, AFP and AFP-L3% are the essential and complementary biomarkers for HCC.	Chinese Society of Health Management	Aug. 2021
13	Expert consensus statement on the role of hematological markers in the early clinical screening of hepatocellular carcinoma[90]	Combined use of AFP-L3% and DCP can improve the early detection of HCC with low AFP levels.	Chinese Preventive Medicine Association,	Sept. 2021
14	Guidelines for the Diagnosis and Treatment of Hepatocellular Carcinoma (2022)[1]	Serum AFP is commonly used and important for the diagnosis and treatment response monitoring of HCC. In AFP-negative population, AFP-L3, PIVKA II, GALAD, GALAD-like and plasma-free microRNA can be helpful to HCC early diagnosis.	China National Health Commission	Feb. 2022
15	China guideline for liver cancer screening (2022)[2]	• AFP-L3 is a strong LCA binding fraction and produced by carcinogenic hepatocytes. The AFP-L3% can be used to exclude the factors contributing to the upregulation of AFP. • DCP can be used as a complementary indicator to AFP in identifying AFP-negative early-stage HCC.	National Cancer Center	Aug. 2022
16	The consensus statement on tertiary prevention of primary liver cancer (2022)[91]	• The preoperative high levels of AFP and/or AFP-L3% and DCP are independent risk factors for HCC recurrence. • AFP with or without AFP-L3% and DCP combined with abdominal ultrasound or MRI and dynamic enhanced CT are routinely used for monitoring HCC recurrence. • Those with suspicious nodules or those with AFP >20 ng/mL and/or AFP-L3% >10%, DCP >40 mAU/mL during routine surveillance should initiate the enhanced HCC recurrence surveillance process.	Chinese Society of Hepatology, Chinese Medical Association	Oct. 2022
17	Guidelines for the prevention and treatment of chronic hepatitis B (2022)[5]	• AFP and AFP-L3 are important biomarkers for HCC detection. • DCP is another important indicator for diagnosing HCC and complementary to AFP.	Chinese Society of Hepatology, Chinese Medical Association	May 2023

4.2 | GALAD-like algorithm

As mentioned above, GALAD has been validated in multiple cohorts from China. Meanwhile, the optimized algorithms containing the same factors nominating as C-GALAD,[101] GALAD-C[16] and C-GALAD II[102] have been investigated in China, where HBV is the leading cause of HCC. In our recent study, 10,359 eligible participants, including HCC and CLD, were recruited from five medical centers to optimize the GALAD performance defined as C-GALAD.[101] The results indicated that the AUC of C-GALAD for HCC was 0.952 (95% CI: 0.947–0.956), superior to GALAD (AUC: 0.925, 95% CI: 0.919–0.931). Furthermore, our results suggested that C-GALAD was significantly associated with the postoperative prognosis of HCC.[101] Another study from China displayed again that the C-GALAD for HCC identification was better than the single parameter of the triple-biomarker.[102] Liu et al.[16] evaluated the diagnostic performance of GALAD and developed new algorithms, GALAD-C and GAAP, using logistic regression analysis. The GALAD-C and GAAP algorithms performed similarly (AUC of GALAD-C: 0.922; GAAP: 0.914), and both outperformed GALAD (AUC:0.891), DCP (AUC: 0.869), AFP (AUC: 0.750) and AFP-L3% (AUC: 0.711) for discriminating HCC from CLD. A clinical cohort including 229 HCC, 2317 CLD and 982 healthy control from eight centers were retrospectively collected to construct the C-GALAD II algorithm including age, gender, AFP, AFP-L3%, DCP, platelet (PLT) and total bilirubin (TBIL).[103] The C-GALAD II could accurately predict the risk of HCC with the AUC of 0.954 and 0.943 in the training and validation cohort, respectively, which was better than the GALAD algorithm.[103]

[Consensus 5] The performances of the GALAD, C-GALAD and C-GALAD-II algorithms, which integrated age, gender and the triple-biomarker (AFP, AFP-L3%, and DCP) are superior to AFP, AFP-L3 and DCP used alone in term of diagnosis, therapeutic assessment and prognosis monitoring. The dynamic application of GALAD and GALAD-like algorithms is beneficial for clinical decisions in HCC management.

5 | THE QUALITY ASSURANCE AND REFERENCE INTERVAL FOR THE MEASUREMENT OF THE HCC TRIPLE-BIOMARKER

5.1 | The quality assurance of the HCC triple-biomarker

The quality assurance of the HCC triple-biomarker in the clinical laboratory is a multifaceted issue including personnel, equipment, reagent supplies and environmental conditions.[104] The quality assurance requirements for clinical immunology tests published by the national as well as regional clinical laboratory supervision departments are appropriate for the triple-biomarker detection technically.

Limited by the detection methodology of AFP-L3%, the detection of AFP-L3% is one of the major sticking points of the HCC triple-biomarker. The detection methods of AFP-L3 include affinity adsorption assay, microfluidics-based immunofluorescence, affinity crossed immuno-electrophoresis, plant lectin affinity chromatography and affinity imprinting. The lectin affinity adsorption centrifugation method is convenient as it does not need special equipment. However, the manual procedure is both manual and time-consuming. Both the microfluidics-based immunofluorescence and MPCLIA methods for AFP-L3% assay demonstrate robustness and yield consistently stable AFP-L3 results. Furthermore, as the antibodies used by different detection systems might target different antigen epitopes, and different sources of antibody/LCA might have different affinity and calibrator traceability, the result and reference interval of AFP-L3% vary among different detection systems.[104] In addition, attention also needs to be paid to the comparability of AFP value, which is the denominator for the AFP-L3% among different devices. The possible impact of AFP-L2 on AFP-L3% results, such as the case in reproductive embryonal tumors that cause an increase in AFP-L2, may lead to a false elevation of AFP-L3%.

The sample factors, comprising the correct collection of blood specimen, the separation of serum / plasma and the conditions of storage, are among the principal factors affecting the quality of clinical detection.[104] Samples should be tested in time. If the tests cannot be performed within 24 hr, the serum/plasma should be separated and stored at 2–8°C for no more than 7 days. The samples should be frozen at −20°C if they need to be stored for more than 7 days and −80°C for more than 6 months, avoiding repeated freezing and thawing. In addition, the presence of sample hemolysis, severe jaundice and lipidemia may interfere with the test results.

[Consensus 6] There are reliable stable detection methods approved by NMPA for AFP, AFP-L3% and DCP. The clinical laboratories can adopt the appropriate detection methods (systems) according to their own feasibilities, and should comply with the guideline for clinical immunology detection published by the national and local quality control supervision departments. One should pay attention to the quality assurance for the whole process including the pre-analytical, analytical and post-analytical processes, and be aware of the possible differences from different detection systems while explain the results.

5.2 | The clinical cut-off value of GALAD and GALAD-like algorithm

The GALAD algorithm has been validated by several domestic and international multi-center clinical studies. However, the cut-off values of GALAD-like algorithms need to be validated by the third-party multi-center studies independently. The establishment and validation of the cut-off values for the HCC triple-biomarker-derived algorithms based on Chinese populations are crucial for the future clinical application of the algorithms. The composition and proportion of the enrolled populations, the disease grading and staging of enrolled patients, and the differences and

standardization in detection methodology are among the critical factors which might affect the cut-off values and diagnostic efficiency and are also among the key reasons for the different cut-off values and clinical diagnostic efficiency obtained from different studies. The HCC cohort should include a pathologically confirmed early HCC and should enroll both the disease (such as CLD or LC) and healthy controls.

Referring to the Chinese standard "Define and determine the reference intervals in clinical laboratory WS/T402-2012," the factors that may affect the results should be excluded and ensure the homogeneity of research subjects when the cut-off values were set. A non-parametric approach to estimating the reference interval requires a minimum of 120 subjects, and if subgroups are required, then a minimum of 120 individuals per group are needed. If there are outliers, they should be supplemented after removing the outliers.[105] Generally, the appropriate optimal cut-off value is determined by the maximum Youden index (sensitivity + specificity-1) of the ROC curves. The diagnostic sensitivity (number of true positives/number of gold standard positives), specificity (number of true negatives/number of gold standard negatives), positive predictive value (number of true positives/number of diagnostic positives) and negative predictive value (number of true negatives/number of diagnostic negatives) of the algorithms at the cut-off value are available. The AUC could reflect the general diagnostic efficiency of the algorithm. Attention should be paid to the 95% confidence interval (CI) of the diagnostic efficiency indicators of the algorithm, and the differences in diagnostic efficiency may result from different detection systems and populations. The current published cut-off values and clinical diagnostic efficiency of GALAD algorithm are summarized in Table 2. At present, the cut-off values for the GALAD algorithm are recommended to refer to those obtained and validated in the large-scale international cohort studies or obtained through the multi-center studies in Chinese populations. The cut-off values can also be established according to the consensus standard as described above and should be continuously optimized in clinical practice. The cut-off values of the GALAD-like algorithm are recommended to refer to the existing clinical multi-center study in China, preferably self-established based on the consensus standard as described above. The dynamic changes of the score of GALAD and GALAD-like algorithms are important for clinical interpretation as well.

[Consensus 7] At present, the cut-off values for the GALAD algorithm are recommended to refer to those obtained and validated in the large-scale international cohort study or obtained through the multi-center studies in Chinese populations. The cut-off values can also be established according to the consensus international standard and should be continuously optimized in clinical practice. The cut-off values of the GALAD-like algorithm are recommended to refer to the existing clinical multi-center study in China, preferably self-established based on the consensus international standard. The dynamic changes of the score of GALAD and GALAD-like algorithms are important for clinical interpretation as well.

6 | THE ECONOMIC EVALUATION OF THE TRIPLE-BIOMARKER JOINT APPLICATION FOR HCC SURVEILLANCE

Due to the insufficient regular surveillance of the high-risk populations, the early detection rate of HCC is low in China. Screening for the HCC high-risk populations is crucial for the early detection, diagnosis and treatment of HCC and is also the key to improving the prognosis.

The main HCC high-risk populations in China include HBV/HCV infection, heavy alcohol consumption, NASH, aflatoxin exposure, the presence of cirrhosis, a family history of HCC and males aged 40 years and over.[1] the HCC triple-biomarker joint application as the routine screening tool for HCC high-risk populations recommended by The Japan Society of Hepatology has achieved good effect in clinical practice, which is worthy of our reference.[45]

A meta-review of 43 studies on HCC screening cost-effectiveness highlighted biannual ultrasound and AFP as the most cost-effective strategy.[107] HANNAH et al.[108] reported that cirrhosis population-wide screening for HCC is likely to be cost-effective and risk-stratified screening using a serum biomarker test may also be cost-effective. Another cost-effectiveness analysis aiming to help decision-making by the health authority in Taiwan, China, revealed that screening the HCC high-risk individuals (CHB/CHC) with the two-stage screening intervention is considered potentially cost-effective compared with opportunistic screening in the target population of an HCC endemic area.[109] Rimal et al. reported that the inclusion of the assay for AFP-L3% and erythroagglutinating phytohemagglutinin (E-PHA)-reactive AFP (AFP-P4), as well as aspartate aminotransferase (AST), zinc sulfate turbidity test (ZTT) and AFP in mass screening for HCC, was found highly cost-effective.[110] Currently more evidences are required from the health economics evaluation studies of HCC screening in Chinese general populations. Compared with other malignancies, the availability of HCC screening technology in China is relatively low. The awareness as well as the application of the novel HCC biomarkers, including AFP-L3% and DCP, is insufficient. More evidence-based scientific health economic evaluation of the HCC triple-biomarker and its algorithms in HCC screening is anticipated in future.

[Consensus 8] Semi-annual detection of AFP, AFP-L3% and DCP combined with abdominal ultrasound might be cost-effective for HCC high-risk population screening. However, more evidences of the cost-effectiveness evaluations are still required.

7 | PROBLEM AND PROSPECT

Optimizing the strategy for HCC high-risk population, early warning and diagnosis is essential for improving the five-year survival rate. Establishing the scientific HCC screening strategy, which is especially suitable for grassroots screening, is an effective way to optimize the existing diagnosis strategy. The combined detection and

TABLE 2 Summary of the published cut-off values and diagnostic performances of the GALAD algorithm.

No.	The inclusion of populations			AUC (95% CI)	Cut-off value	Sensitivity	Specificity	Ref.
	HCC	Non-HCC	Regional distribution					
1	**Total: 394** Alcohol-HCC: 108 HCV-HCC: 43 HBV-HCC: 30 HBV/HCV-HCC: 2 NASH-HCC: 29 Other etiologies HCC: 182	**Total: 439** Alcohol-CLD: 70 HCV-CLD: 74 HBV-CLD: 58 HBV/HCV-CLD: 6 NASH-CLD: 93 Other etiologies CLD: 138	**British cohort: 833** Birmingham cohort: 670 Newcastle cohort: 163	All stage HCC: 0.9662 Early-stage HCC: 0.9553	−0.63 −0.63	93.00 86.00	89.00	18
2	**Total: 2430** HCV-HCC: 1179 HBV-HCC: 493 Alcohol-HCC: 652 Other etiologies HCC: 106	**Total: 4404** HCV-CLD: 1682 HBV-CLD: 1066 Alcohol-CLD: 1283 Other etiologies CLD: 52 Cholangiocarcinoma / Pancreatic cancer: 229 Healthy control: 92	**International multi-center cohort: 6834** Japan cohort: 4476 German cohort: 1278 British cohort: 833 China (Hong Kong) cohort: 247	British cohort all-stage HCC: 0.97 (0.96–0.98) British cohort early-stage HCC: 0.93 (0.90–0.96) Japan cohort all stage HCC: 0.93 (0.92–0.94) Japan cohort early-stage HCC: 0.91 (0.90–0.92) Japan cohort HCV-HCC: 0.92 (0.91–0.93) Japan cohort HBV-HCC: 0.93 (0.92–0.95) German cohort all-stage HCC: 0.94 (0.93–0.96) German cohort HCV-HCC: 0.93 (0.90–0.97) German cohort HBV-HCC: 0.94 (0.91–0.98)	−0.63 −0.63 −1.95 −1.95 −1.95 −1.95 −0.68 −0.68 −0.68	91.60 80.20 81.40 82.10 88.8 79.6 88.40 83.60 78.10	89.70 89.70 89.10 81.60 75.3 92.1 88.20 86.50 94.00	17
3	**Total: 5919**	**Total: 1745**	**China (Shanghai) cohort: 7664**	0.960 (0.955–0.964)	−0.33	91.9	86.8	37
4	**Total: 602** HBV-HCC: 513 Other etiologies HCC: 89	**Total: 923** CLD: 468 Healthy control: 367 ICC: 88	**China multi-center cohort: 1525** Fujian cohort: 295 Yunnan cohort: 127 Shanghai cohort: 1103	All-stage HCC: 0.903 (0.884–0.920) BCLC 0/A early-stage HCC: 0.869 (0.845–0.891)	−1.82 −3.20	75.99 84.62	87.98 73.34	14
5	**Total: 292** HCV-HCC: 124 NAFLD-HCC: 51 Alcohol-HCC: 65 HBV-HCC: 38 Other etiologies HCC: 14	**Total: 649** HCV-CLD: 233 NAFLD: 154 Alcohol-CLD: 128 HBV-CLD: 88 Other etiologies CLD: 46	**International multi-center cohort: 941** American cohort: 598 Europe/Asia cohort: 343	0.92 (0.90–0.95)	−1.18	85.00	87.00	93

TABLE 2 (Continued)

No.	The inclusion of populations			AUC (95% CI)	Cut-off value	Sensitivity	Specificity	Ref.
	HCC	Non-HCC	Regional distribution					
6	**Total: 344** Alcohol-HCC: 38 HBV-HCC: 57 HCV-HCC: 170 NASH-HCC: 30 Viral/Alcohol-HCC: 41 Other etiologies HCC: 8	**Total: 592** Alcohol-CLD: 85 HBV-CLD: 50 HCV-CLD: 273 NASH: 49 Viral/Alcohol-CLD: 101 Other etiologies CLD: 34	**American multi-center cohort: 936** Mayo Clinic cohort: 291 NCI EDRN cohort: 645	Mayo Clinic cohort: 0.95 (0.93–0.97)	−0.76	91.00	85.00	99
				Mayo Clinic HCV-HCC: 0.95 (0.90–1.00)	−0.53	94.00	85.00	
				Mayo Clinic NASH-HCC: 0.89 (0.81–0.97)	−0.85	87.00	84.00	
				Mayo Clinic BCLC 0-A HCC: 0.92 (0.88–0.96)	−1.18	92.00	79.00	
				EDRN cohort: 0.88 (0.85–0.91)	−0.17	76.00	86.00	
				EDRN HBV-HCC: 0.94 (0.88–0.99)	−1.25	83.00	100.00	
				EDRN HCV-HCC: 0.89 (0.86–0.92)	−0.17	79.00	85.00	
7	**Total: 152** NASH-HCC: 152	**Total: 594** NASH/NAFLD: 594	**International multi-center cohort: 746** German cohort: 357 Japan cohort: 389	All-stage HCC: 0.96 (0.94–0.98)	−1.334	91.2	90.91	13
				Early-stage HCC (Milan criteria): 0.91 (0.84–0.97)	−1.334	84	90.91	
				BCLC A early-stage HCC: 0.92 (0.86–0.98)	−1.334	86.21	90.91	
				BCLC A early-stage HCC without LC: 0.94 (0.86–1.00)	−1.334	85.71	93.41	
8	**Total: 242** HBV-HCC: 135 HCV-HCC: 106 Alcohol-HCC: 1	**Total: 383** HBV-CLD: 149 HCV-CLD: 133 Alcohol-CLD: 1 ICC/HCC-ICC: 50 Healthy control: 50	**China (Jilin) cohort: 625**	0.89 (0.86–0.92)	0.946	81.8	79.9	16
9	**Total: 196** HBV-HCC: 52 HCV-HCC: 84 Other etiologies HCC: 60	**Total: 377** HBV-CLD: 130 HCV-CLD: 139 Other etiologies CLD: 108	**Germany (Duisburg) cohort: 573**	All etiology HCC: 0.97 (0.96–0.98)	−0.63	88.30	95.00	106
				HBV-HCC: 0.96 (0.93–0.98)	−0.63	76.90	95.40	
				HCV-HCC: 0.98 (0.96–1.00)	−0.63	89.30	95.70	
				Other etiologies HCC: 0.99 (0.97–1.00)	−0.63	96.70	93.50	

(Continues)

TABLE 2 (Continued)

No.	The inclusion of populations		Regional distribution	AUC (95% CI)	Cut-off value	Sensitivity	Specificity	Ref.
	HCC	Non-HCC						
10	**Total: 122** Alcohol-HCC: 41 HBV-HCC: 11 HCV-HCC: 27 NAFLD-HCC: 26 Other etiologies HCC: 17	**Total: 145 LC** Alcohol-LC: 51 HBV-LC: 11 HCV-LC: 18 NAFLD-LC: 34 Other etiologies LC: 31	**Germany (Berlin) cohort: 267**	Training cohort: 0.9023 (0.8584–0.9463) Validation cohort: 0.8970 (0.8231–0.9710) All enrollment populations: 0.9000 (0.8620–0.9380)	−0.814 −0.550 −0.637	82.50 81.08 81.20	83.33 88.10 85.51	95
11	**Total: 80** HBV-HCC: 68 Alcohol-HCC: 5 Other etiologies HCC: 7	**Total: 227** HBV-CLD: 40 HBV-LC: 67 Fatty-LC: 6 Alcohol-LC: 18 Other etiologies LC: 9 Healthy control: 87	**China (Hangzhou) cohort: 307**	0.925 (0.890–0.960)	−0.63	95	57.1	96
12	**Total: 122** HCC with LC: 45 HBV-HCC: 88 HCV-HCC: 3 Fatty-HCC: 1 Other etiologies HCC: 34	**Total: 125** LC: 46 HBV-CLD: 72 HCV-CLD: 4 Fatty liver disease: 20 Other etiologies CLD: 26	**China (Guangzhou) cohort: 247**	0.899 (0.833–0.941)	−1.2	77.9	91.2	97

algorithms application of the HCC triple-biomarker (AFP, AFP-L3% and DCP) can effectively improve the diagnostic efficacy and clinically precise management. As mentioned above, GALAD, C-GALAD and C-GALAD II algorithms based on Chinese populations have been validated to be beneficial for improving the diagnostic efficacy recently. However, the following problems still exist: 1. The weights of the algorithm parameters have different assignments in different studies, manifested in different algorithmic models by different research groups. 2. The cut-off values of the algorithms varied in different studies and thus need to be optimized and validated in further multi-center studies. 3. Different values of the HCC triple-biomarker obtained from different methodologies may affect the stability of the algorithms. The standardization of different methodologies (systems) should be strengthened. The methodology background of the algorithms constructed could not be ignored when implemented clinically.

The HCC triple-biomarker (AFP, AFP-L3% and DCP) based on the serum immunology tests and the algorithms of GALAD and GALAD-like are feasible technologically with reasonable costs and personalized character, which is easy to implement in the areas with immunological testing services. The algorithms of GALAD and GALAD-like are beneficial for HCC surveillance and precise clinical management. Further large-scale prospective studies are still needed to optimize and validate the cut-off values appropriate for Chinese populations and to obtain evidence-based health economics evaluation.

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CONFLICT OF INTEREST STATEMENT

All authors claim that there is no conflict of interest including a desire for financial gain, prominence, professional advancement or a successful outcome.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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