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Original Article

Surveillance Imaging and GAAD/GALAD Scores for Detection of Hepatocellular Carcinoma in Patients with Chronic Hepatitis

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Abstract

Background and Aims: Early detection of hepatocellular carcinoma (HCC) is crucial for improving survival in patients with chronic hepatitis. The GALAD algorithm combines gender (biological sex), age, α-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3), and protein induced by vitamin K absence or antagonist-II (PIVKA-II) for HCC detection. Similarly, the GAAD algorithm incorporates gender (biological sex), age, AFP, and PIVKA-II. This study aimed to assess the clinical utility of AFP-L3 in the GALAD algorithm and its potential synergies with ultrasound. We compared the clinical performance of GALAD with GAAD; AFP; AFP-L3; and PIVKA-II, with or without ultrasound, in Taiwanese adults. ***Methods:*** A total of 439 serum samples were analyzed using a Cobas® e 601 analyzer (healthy controls, n = 200; chronic liver disease controls, n = 177; HCC cases, n = 62). Performance was assessed through receiver operating characteristic curve analyses to calculate the area under the curve. ***Results:*** The area under the curve for differentiating early-stage HCC from patients with chronic liver disease was optimal for PIVKA-II (84.9%), GAAD (79.8%), and GALAD (79.4%), with slightly improved performance for detecting all-stage HCC. Clinical performance was unaffected by disease stage or etiology. Sensitivity for early-stage HCC was highest for GAAD (57.6%) and GALAD (57.6%). Sensitivity for each strategy was further enhanced when combined with ultrasound, regardless of disease stage or etiology ($P < 0.01$). ***Conclusions:*** These findings indicate that the role of AFP-L3 in the GALAD algorithm is minimal, supporting the use of GAAD for HCC detection. A combination of GAAD, GALAD, or PIVKA-II with ultrasound may improve diagnostic efficiency compared with recommended strategies.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second most common cause of cancer-related death in Asia.[1] In 2020, there were approximately 609,596 new cases of liver cancer in Asia, with an age-standardized rate of 11.6 per 100,000 persons.[1] Taiwan alone reported 10,988 new cases in 2020,[2] equating to nearly 30 people receiving a diagnosis of liver cancer daily.[2] HCC is often associated with cirrhosis caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, particularly in Asia.[3,4] For example, in Taiwan, between 2011 and 2019, viral hepatitis-induced HCC accounted for 78.7% of all HCC cases in males and 79.5% in females.[5]

Many countries in Asia have initiated a series of population-wide interventions aimed at preventing HCC associated with HBV and HCV infections,[6–8] with nine out of 11 countries in the South-East Asian region reporting a vaccine coverage rate of ≥90% by 2019.[7,9] The implementation of these vaccination programs has since led to a decrease in the

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HBV carriage rate and a reduction in the risk of developing HCC.[6,8] Antiviral therapy has also been shown to reduce the risk of HCC.[10] However, the risk of HCC persists even after HCV eradication.[11] Recently, metabolic dysfunction-associated steatohepatitis and metabolic dysfunction-associated steatotic liver disease have emerged as contributing factors to the development of HCC across the Asia–Pacific region, driven by unhealthy dietary choices and increasingly sedentary lifestyles.[12]

HCC is usually asymptomatic until its advanced stages, when the tumor becomes unresectable, resulting in a poor prognosis at diagnosis.[13] Surveillance is essential to improve timely detection of HCC.[13,14] The Asian Pacific Association for the Study of the Liver recommends six-monthly surveillance using ultrasound along with serum α-fetoprotein (AFP) biomarker measurements in high-risk patients, including those with HBV, HCV, and metabolic dysfunction-associated steatohepatitis.[13] Similar strategies are included in the Taiwan Liver Cancer Association and the Gastroenterological Society of Taiwan guidelines, which both recommend six-monthly ultrasound in high-risk patients, with or without tumor biomarker testing.[14]

Research has shown that the sensitivity of ultrasound for detecting HCC when used in isolation is poor.[15] However, performance improves when ultrasound is combined with serum biomarkers, such as AFP.[15] While ultrasound and AFP are guideline-recommended in Asia, their combination can often yield false-positive or indeterminate results, with some studies suggesting they may only identify around 63% of patients with early-stage HCC.[15] Moreover, obstacles related to both patients and providers, such as limited access to ultrasound and trained operators in certain areas, have resulted in suboptimal adherence to surveillance guidelines in clinical practice.[16]

The clinically validated *in vitro* GALAD algorithm, which combines gender (biological sex) and age with measurements of serum biomarkers AFP, *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3), and protein induced by vitamin K absence or antagonist II (PIVKA-II), has shown excellent sensitivity and specificity in distinguishing HCC from chronic liver disease (CLD).[17,18]

However, the role and contribution of AFP-L3 in detecting HCC as part of the GALAD algorithm remain controversial, owing to evolving disease etiologies and antiviral treatment paradigms. Advances in antiviral HBV/HCV therapies have led to improved liver function and normalized post-treatment AFP levels. This has increased the specificity of AFP for HCC surveillance in patients with chronic inflammatory backgrounds,[19] potentially rendering the use of AFP-L3 obsolete.[20]

Moreover, comparisons between GALAD and the novel GAAD (gender [biological sex], age, AFP, PIVKA-II [previously DCP]) algorithm have demonstrated similar clinical performance in differentiating patients with HCC from those with CLD across different disease stages and etiologies,[21,22] further suggesting that AFP-L3 makes a negligible contribution to the GALAD algorithm. This was also reflected in the results of the first GALAD algorithm development study, which reported odds ratios of 1.05 and 1.04 for AFP-L3 in the discovery and validation datasets, respectively.[23]

In the current study, we compared the clinical performance of the GAAD and GALAD algorithms, as well as individual tumor biomarkers (AFP, AFP-L3, and PIVKA-II) with or without ultrasound, in patients with chronic hepatitis at a single Taiwanese center. This study addresses an important unmet need to understand the clinical utility of AFP-L3 in detecting early-stage HCC, both as part of an algorithm and in combination with imaging.

Methods

Study design

Adult participants were enrolled at a single center in Kaohsiung, Taiwan, between 2018 and 2020. Cohorts included healthy controls, CLD controls, and HCC cases. Patients with HCC were retrieved from those with CLD and received usual care in the outpatient department of the liver clinic. These patients had a first-time HCC confirmed by clinical or histological diagnosis, according to regional guidelines.[13] HCC cases were grouped using the Barcelona Clinic Liver Cancer staging system, with stages 0/A defined as early-stage HCC and stages B/C/D defined as late-stage HCC. The CLD and HCC etiologies included cirrhotic and non-cirrhotic chronic HBV or HCV. The CLD group comprised patients with HBV and HCV infections who received antiviral medication and showed no evidence of HCC occurrence one year after blood sampling. Healthy controls were seronegative for hepatitis B surface antigen and hepatitis C antibody, had normal serum enzyme levels, and exhibited no visible liver nodules on sonography. Patients were excluded if they had any of the following conditions: HCC with recurrence status, combined cholangiocarcinoma, or other extrahepatic malignancies. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee of Kaohsiung Medical University Hospital (IRB number: KMUHIRB-E(I)-20200127).

Serum sample collection and assessment

In accordance with national health insurance reimbursement coverage in Taiwan, patients with CLD were assessed every two to three months as part of their routine care. Upon diagnosis of HCC, blood samples were analyzed retrospectively, using samples collected within the preceding three months. This time window was applied to ensure that blood samples reflected the disease stage and tumor microenvironment at the time of positive HCC diagnosis. Blood samples were stored at −20°C and analyzed at the study site. Serum levels of PIVKA-II, AFP, and AFP-L3 were measured using an Elecsys® assay on a Cobas® e 601 analyzer for five experimental runs conducted between 2021 and 2022. Established cut-offs for the detection of HCC for each surveillance strategy were as follows: 20 ng/mL for AFP, 2.3 ng/mL for AFP-L3, 28.4 ng/mL for PIVKA-II, a score of 2.57 for GAAD, and 2.47 for GALAD (range 0–10 for both). Ultrasound was performed every six months for patients with CLD and every four to six months for cirrhotic patients.

GAAD and GALAD (Cobas) algorithm development

The algorithm development process for GAAD and GALAD (Cobas) has been previously described.[22,24] To summarize, multivariate analyses were conducted to identify the best-performing panel of biomarkers for the detection of HCC using two methods: lasso regression (no fixed panel size) and exhaustive search with logistic regression (fixed panel size of two to four biomarkers). The dataset from the algorithm development study (STOP-HCC-ARP) was used to train logistic regression models for the two best-performing clinical algorithms (GAAD and GALAD), using a diagnosis of HCC (Barcelona Clinic Liver Cancer-stage-independent) as the predictor variable. The GAAD and GALAD cut-offs correspond to the 90% specificity cutoff for aiding in the diagnosis of early-stage HCC.

Statistical analysis

Analyses were performed both with and without healthy

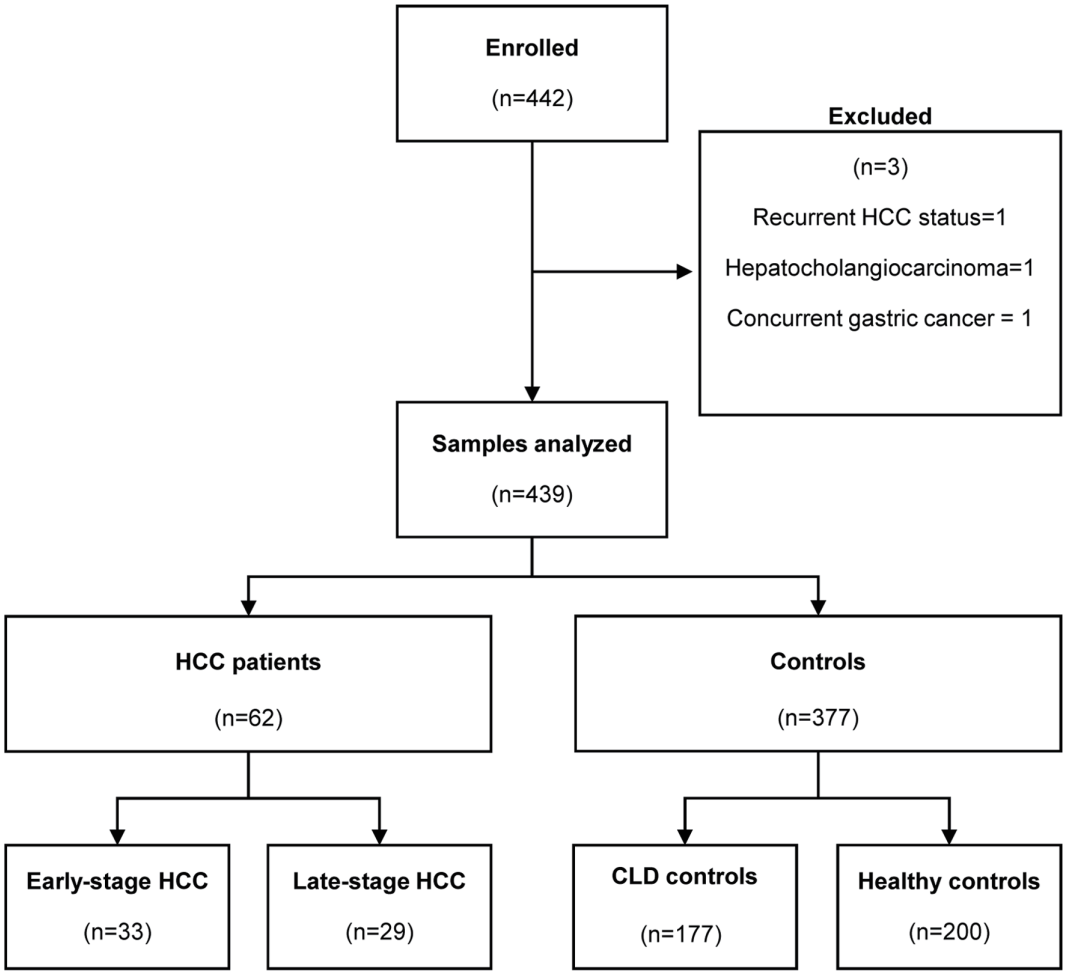

Fig. 1. Study design and sample disposition. CLD, chronic liver disease; HCC, hepatocellular carcinoma.

controls in the comparator group. To compare baseline demographics, *P*-values were calculated based on chi-squared tests for categorical variables and t-tests for numeric variables. Clinical performance was assessed using receiver operating characteristic (ROC) curve analyses to calculate the area under the curve (AUC). *P*-values for significantly larger AUCs were calculated using t-tests of 500 bootstrap replicates. For sensitivity analysis, the derived 95% confidence intervals were calculated from the binomial distribution using the Clopper–Pearson method.[25] Sensitivity for HCC across disease stages and etiologies was evaluated for each surveillance strategy, both alone and in combination with ultrasound. For evaluations with ultrasound, if either GAAD, GALAD, or ultrasound was positive, the result was considered positive, warranting further investigation.

Results

Study population

The study design and sample disposition are described in Figure 1. A total of 439 subjects had samples available for analysis, including 200 healthy controls, 117 patients with non-cirrhotic CLD (HBV = 67 and HCV = 50), 60 with cirrhotic CLD (HBV = 11 and HCV = 49), and 62 with HCC (HBV = 40, HCV = 20, and non-HBV/HCV = 2). Of the 62 patients with HCC, 33 had early-stage HCC and 29 had late-stage HCC. Further patient demographics are reported in Table 1.

Clinical performance

Box plots visualizing the distribution of GAAD and GALAD scores across HCC cases and controls by control group, disease stage, and etiology are shown in Figure 2. The distribution of GAAD and GALAD scores in the control groups was similar, regardless of whether healthy controls were included with CLD controls. The distribution of each score was higher in HCC patients compared with controls. For all-stage HCC, the median (IQR) scores were 4.55 (1.57–9.82) versus 0.34 (0.16–0.94) for GAAD, and 5.01 (1.55–9.85) versus 0.36 (0.17–0.95) for GALAD. Differences in GAAD and GALAD scores between controls and HCC groups were similar across etiologies. The distribution of AFP, AFP-L3, and PIVKA-II concentrations by control group, disease stage, and etiology are shown in Supplementary Figure 1.

ROC plots showed that for the differentiation of early-stage HCC from patients with CLD, AUCs were 84.9%, 79.8%, and 79.4% for PIVKA-II, GAAD, and GALAD, respectively. These were numerically higher than AFP and AFP-L3 alone (73.7% and 61.9%, respectively; Fig. 3A). Similar results were observed when healthy controls were included with CLD pa-

Table 1. Participant demographics

Patient characteristics	HCC (N = 62)	CLD controls (N = 177)	All controls* (N = 377)	*P*-value (HCC vs. CLD controls)
Age, years, mean (SD)	63.7 (10.0)	59.7 (13.0)	56.9 (14.8)	$P < 0.0001$
Sex, n (%)				$P = 0.038$
Female	17 (27.4)	77 (43.5)	224 (59.4)	
Male	45 (72.6)	100 (56.5)	153 (40.6)	
Weight, kg, mean (SD)	65.6 (11.3)	65.5 (14.1)	63.0 (13.0)	$P < 0.0001$
BMI, mean (SD)	25.4 (3.5)	25.0 (4.5)	24.4 (4.3)	$P < 0.0001$
Healthy control, n (%)	0 (0.0)	0 (0.0)	200 (53.1)	-
Disease etiology, n (%)				$P < 0.001$
Cirrhosis	38 (61.3)	60 (33.9)	60 (15.9)	
No cirrhosis	24 (38.7)	117 (66.1)	317 (84.1)	
HBV	40 (64.5)	78 (44.1)	78 (20.7)	
HCV	20 (32.3)	99 (55.9)	99 (26.3)	
Non-HBV/HCV	2 (3.2)	0 (0.0)	200 (53.1)	
BCLC stage, n (%)				
0	9 (14.5)	-	-	-
A	24 (38.7)	-	-	-
B	13 (21.0)	-	-	-
C	16 (25.8)	-	-	-
Biomarker/algorithm values, mean (range)				
AFP, ng/mL	2,401.8 (1.48–60,138)	5.98 (0.60–79.41)	4.32 (0.60–79.4)	$P = 0.064$
AFP-L3, ng/mL	62.53 (1.20–1,000)	1.36 (1.20–5.32)	1.28 (1.20–5.32)	$P = 0.012$
PIVKA-II, ng/mL	770.75 (13.17–10,108)	23.95 (3.78–319.60)	20.04 (3.78–319.60)	$P = 0.0027$
GAAD, score	5.37 (0.14–10)	1.12 (0.028–8.34)	0.76 (0.01–8.34)	$P < 0.0001$
GALAD, score	5.49 (0.16–10)	1.17 (0.031–8.33)	0.78 (0.02–8.33)	$P < 0.0001$

*All controls included healthy controls (n = 200) and CLD controls (n = 177). *P*-values were calculated based on chi-squared tests for categorical variables and t-tests for numeric variables. AFP, α-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; BMI, body mass index; CLD, chronic liver disease; GAAD, gender (biological sex), age, AFP, PIVKA-II; GALAD, gender (biological sex), age, AFP-L3, AFP, PIVKA-II; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PIVKA-II, protein induced by vitamin K absence or antagonist II; SD, standard deviation.

tients in the control group (Supplementary Fig. 2A). GALAD, GAAD, and PIVKA-II also showed AUCs between 85% and 90% for the discrimination of all-stage HCC from patients with CLD or from the combination of healthy controls and patients with CLD (Fig. 3B, Supplementary Fig. 2B). Lower AUCs were observed with AFP and AFP-L3.

Combined contingency tables for GAAD, compared with GALAD, ultrasound, and individual biomarkers for early-stage and all-stage HCC are shown in Supplementary Tables 1 and 2. Four early-stage HCC patients had normal PIVKA-II but presented with GAAD scores above the cut-off, whereas only two patients had elevated PIVKA-II and normal GAAD. Similarly, among all-stage patients, seven were GAAD+ with normal PIVKA-II, compared with four who were GAAD- with elevated PIVKA-II. GAAD and GALAD results were identical among all early-stage HCC patients. Two all-stage HCC patients were HCC-positive per the GALAD algorithm but negative with GAAD.

Clinical performance by disease etiology

ROC analysis showed that in patients with cirrhosis, the AUC for detection of early-stage HCC was highest with PIVKA-II (82.3%), followed by GAAD and GALAD, which exhibited comparable performance (78.3% and 78.2%, respectively; Fig. 4A). For the detection of all-stage HCC, the clinical performance of GALAD, GAAD, and PIVKA-II was similar (AUC: 84.6%, 83.5%, and 83.2%; Fig. 4B). In patients without cirrhosis, the AUC for differentiating CLD from early-stage HCC was highest for PIVKA-II (81.4%), and similar for AFP, GAAD, and GALAD (74.1%, 73.7%, and 73.2%, respectively; Fig. 4C). For detection of all-stage HCC, the AUC was highest for PIVKA-II, followed by GALAD and GAAD (89.9%, 83.3%, and 83.2%, respectively; Fig. 4D).

In patients with HBV etiology, PIVKA-II showed the best performance with an AUC of 87.7% (Fig. 4E) for differentiating early-stage HCC and 88.9% for all-stage HCC (Fig. 4F). The clinical performance of GAAD and GALAD in this cohort was similar for both early-stage HCC (AUC: 77.4% for both) and all-stage HCC (84.5% and 84.6%, respectively). GAAD showed the highest clinical performance for the differentiation of early-stage HCC with HCV etiology from CLD (88.3%), followed by GALAD (87.7%; Fig. 4G). Notably, AFP alone performed markedly better in the HCV subset than in the HBV subset for the diagnosis of early-stage HCC

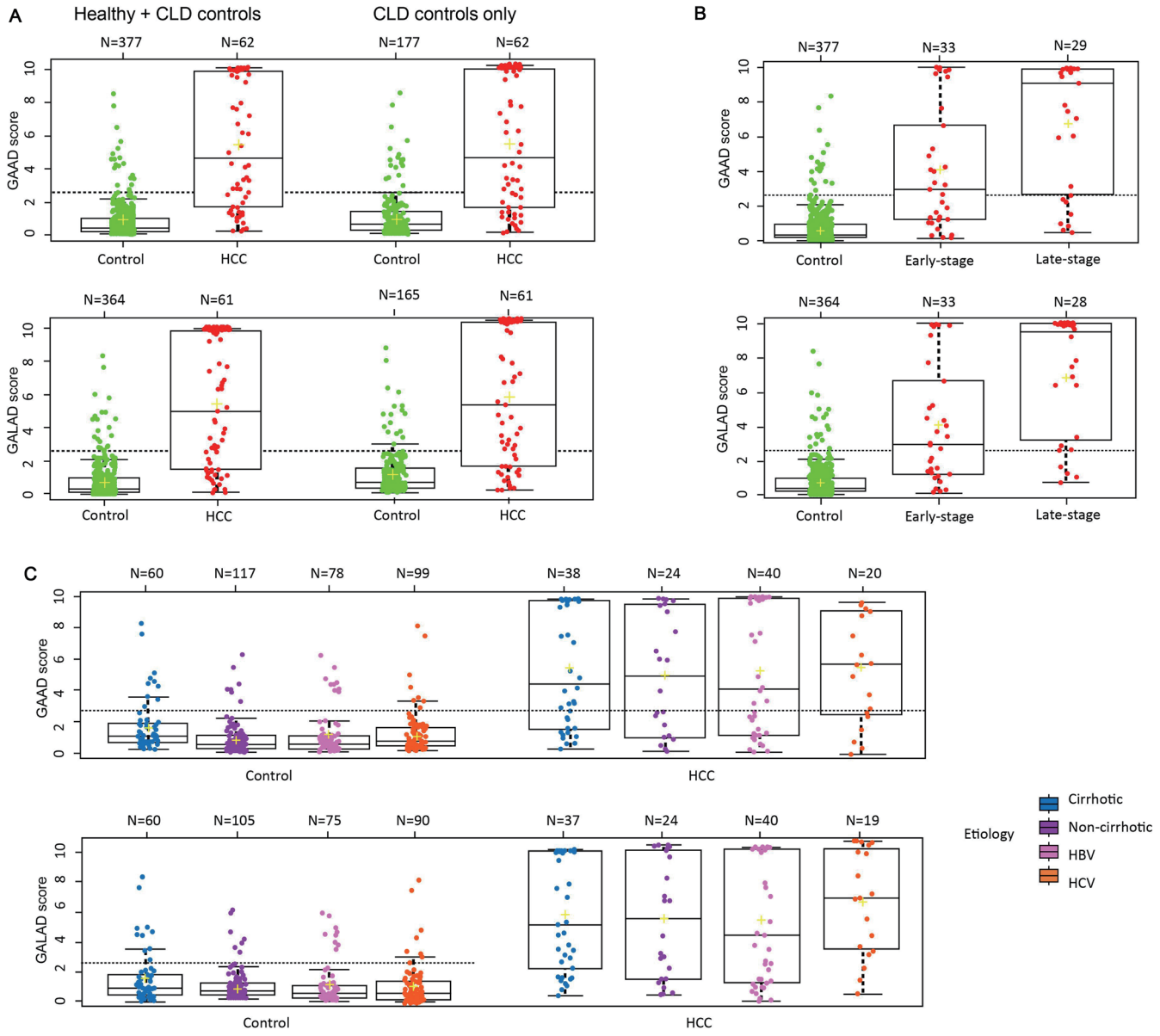

Fig. 2. Distribution of GAAD and GALAD scores by (A) healthy and CLD controls, CLD controls, and HCC; (B) BCLC stage; and (C) etiology. AFP, α-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist II; BCLC, Barcelona Clinic Liver Cancer; CLD, chronic liver disease; GAAD, gender (biological sex), age, AFP, PIVKA-II (previously DCP); GALAD, gender (biological sex), age, AFP-L3, AFP, PIVKA-II (previously DCP); HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

(85.3% vs 68.4%). For the detection of all-stage HCC with HCV etiology in CLD controls, GALAD showed the best performance (91.2%), followed by GAAD and PIVKA-II (89.4% and 88.3%, respectively; Fig. 4H).

Clinical performance with the addition of ultrasound

The sensitivity of ultrasound alone varied across etiologies and demonstrated lower performance than the GAAD and GALAD algorithms alone across all stages and etiologies (with the exception of non-cirrhosis). When combined with ultrasound, sensitivity increased for all surveillance strategies (algorithms and individual biomarkers), irrespective of disease stage or etiology (Fig. 5). The greatest improvements were observed when combining ultrasound with individual biomarkers, AFP, and AFP-L3, in the non-cirrhotic and HCV subgroup analyses. Similarly, the positive and negative predictive values of each surveillance strategy increased or remained the same when used in combination with ultrasound, irrespective of disease stage or etiology (Supplementary Tables 3 and 4). Combined contingency tables for ultrasound versus other surveillance strategies are reported in Supplementary Tables 5 and 6.

Discussion

Detection of HCC in the early stages of the disease is crucial for improving treatment opportunities and prognosis. This study reports on the diagnostic performance of poten-

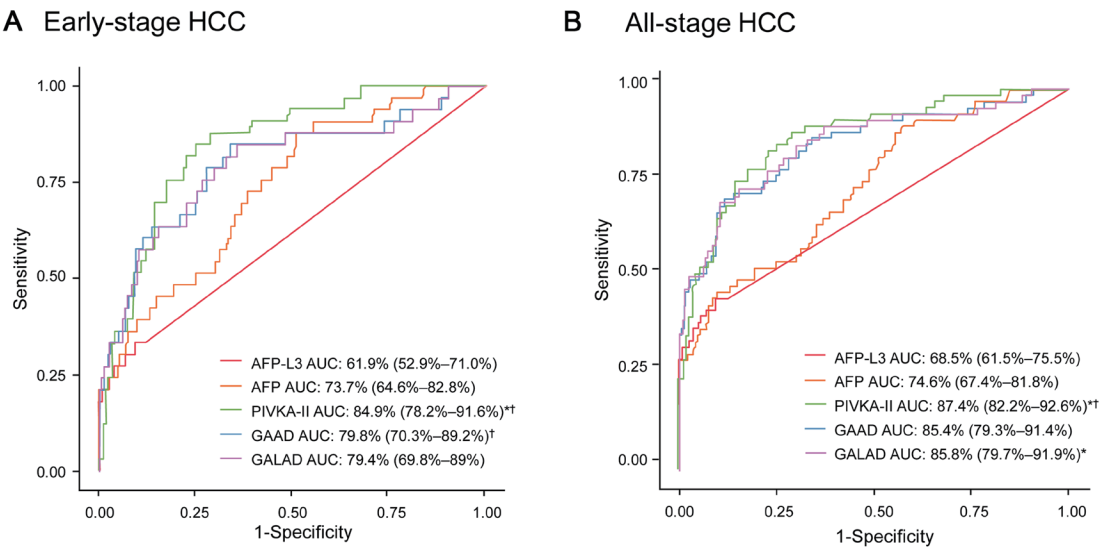

Fig. 3. Receiver operating characteristic plots of GAAD and GALAD algorithms (Cobas) and Elecsys AFP, AFP-L3, and PIVKA-II assays for discriminating between early- (A) and all-stage (B) HCC patients and CLD controls. *P < 0.0001 vs. GAAD; †P < 0.0001 vs. GALAD. P-values for comparisons between other surveillance strategies were non-significant. AFP, α-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein; AUC, area under the curve; CLD, chronic liver disease; GAAD, gender (biological sex), age, AFP, PIVKA-II; GALAD, gender (biological sex), age, AFP-L3, AFP, PIVKA-II; HCC, hepatocellular carcinoma; PIVKA-II, protein induced by vitamin K absence or antagonist II.

tial HCC surveillance strategies, GAAD, GALAD, AFP, AFP-L3, and PIVKA-II, with or without ultrasound, in Taiwanese adults with cirrhotic or non-cirrhotic HBV/HCV infections. In our predominantly male study cohort, individuals with HCC exhibited a 2:1 ratio for HBV and HCV etiologies, aligning with findings from previous studies that reported the prevalence of HBV and HCV in Taiwan and Asia as a whole.[5,26]

GAAD and GALAD showed similar clinical performance for the detection of HCC across disease stages and etiologies, including comparable sensitivity for differentiating early-stage HCC from CLD. This is consistent with the findings of a previous prospective multicenter evaluation of the clinical performance of GAAD, which found that the AUC values for GAAD and GALAD were 91.3% and 91.3%, respectively, for the detection of early-stage HCC, and 94.8% and 94.7%, respectively, for the detection of all-stage HCC in patients with CLD.[21] This suggests that the AFP-L3 variable in the GALAD score may have made a negligible contribution. These results correlate with a prior study assessing the utility of biomarker combinations in diagnosing HCC, which demonstrated significantly higher clinical performance for AFP and PIVKA-II compared with the combination of PIVKA-II, AFP, and AFP-L3 (AUCs 0.753 and 0.690, respectively; P = 0.001).[27] Furthermore, the prospective ESCALON study also concluded that AFP-L3 contributed minimally to the detection of early-stage HCC in two large multicenter cohorts from Europe and Latin America.[28] The value of AFP-L3 in algorithms combining demographic characteristics and serum biomarkers remains unclear. Indeed, the AFP-L3 biomarker showed reduced clinical performance compared with other surveillance strategies across all disease stages and etiologies.

Beginning in 2000, the National Health Insurance Administration of Taiwan initiated a healthcare enhancement program that incorporated the use of PIVKA-II for the surveillance of patients with cirrhosis and those undergoing curative therapy for HCC.[7] Notably, since 2020, this program has expanded to include reimbursement for the semi-annual assessment of PIVKA-II in individuals with cirrhosis and those receiving curative therapy for HCC.[7] Although the AUC of PIVKA-II was significantly larger than that of GAAD for the detection of early- and all-stage HCC, both performed comparably across disease stages and etiologies. This suggests that they may play complementary roles in detecting early-stage HCC, with GAAD identifying some early-stage cases undetected by PIVKA-II. Interestingly, Piratvisuth *et al*. demonstrated similar results for the performance of PIVKA-II in diagnosing HCC, but higher AUCs for AFP and GAAD.[21] These discrepancies may be attributed to differences in study designs and populations. The Piratvisuth *et al*. study included global regions and patients with non-viral etiologies, whereas our study focused exclusively on a Taiwanese population with viral etiologies, with the majority of patients in the HCC cohort having an HBV etiology. As reported in other studies of Asian populations, higher levels of AFP are found in HBV-infected patients compared with other viral etiologies.[29,30] The presence of the HBV protein, which may induce AFP receptor regulation, has been proposed as a contributing factor for increased AFP levels.[31] This may also explain the variable performance in detecting early-stage HCC for the single biomarkers AFP and AFP-L3 between viral etiologies observed in the present study, with higher AUCs in the HCV versus HBV subset.

To a lesser extent, both algorithms that included AFP as a component showed improved performance in patients with HCV etiology compared with those with HBV. The clinical performance of GAAD, GALAD, AFP, AFP-L3, and PIVKA-II improved when combined with ultrasound. This aligns with the findings of previous reports on AFP and GALAD.[15,18] For example, the GALADUS score, which combines GALAD with ultrasound, demonstrated superior clinical performance compared with GALAD alone for detecting HCC in a prospective cohort of patients with cirrhosis or chronic HBV infection.[18] The use of ultrasound in combination with other surveillance strategies was also supported by contingency data, which showed that several HCC cases undetected by ultrasound

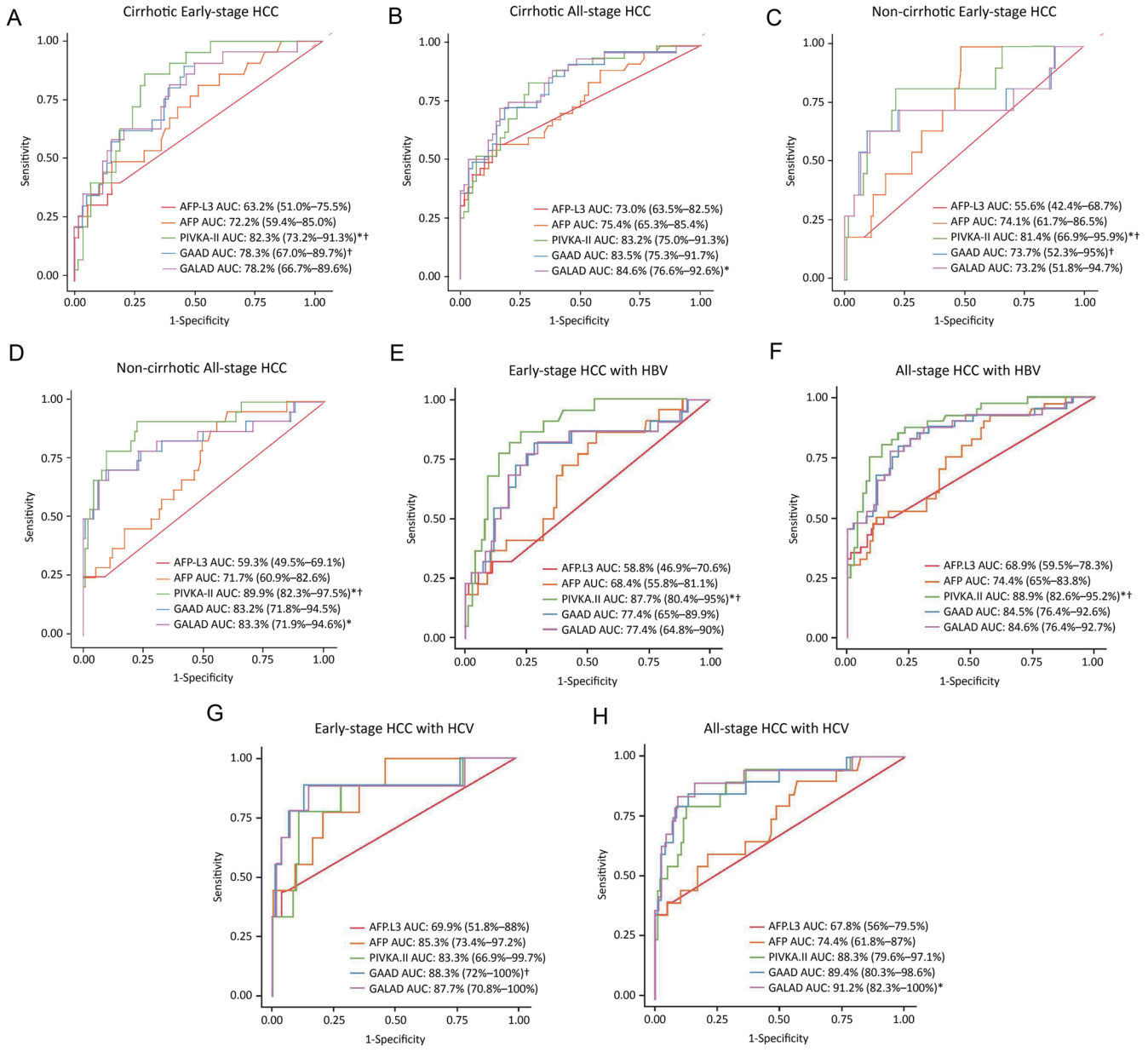

Fig. 4. Receiver operating characteristic plots of GAAD and GALAD (Cobas) algorithms and Elecsys AFP, AFP-L3, and PIVKA-II assays for differentiation of cirrhotic CLD controls and early-stage (A) or all-stage (B) HCC patients, differentiation of non-cirrhotic CLD controls and early-stage (C) or all-stage (D) HCC patients, differentiation of CLD controls and early-stage (E) or all-stage (F) HCC patients with HBV, and differentiation of CLD controls and early-stage (G) or all-stage (H) HCV etiologies. **P* < 0.0001 vs. GAAD; †*P* < 0.0001 vs. GALAD. *P*-values for comparisons between other surveillance strategies were non-significant. AFP, α-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein; AUC, area under the curve; CLD, chronic liver disease; GAAD, gender (biological sex), age, AFP, PIVKA-II; GALAD, gender (biological sex), age, AFP-L3, AFP, PIVKA-II; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PIVKA-II, protein induced by vitamin K absence or antagonist II.

were identified by GAAD, GALAD, and PIVKA-II.

Interestingly, GAAD, GALAD, and PIVKA-II outperformed AFP alone in detecting both early- and all-stage HCC, despite the inclusion of AFP in HCC surveillance guidelines.[13] Therefore, a combination of GAAD, GALAD, or PIVKA-II with ultrasound may improve diagnostic efficiency compared with ultrasound alone or in combination with AFP. Nonetheless, it is crucial to consider not only the effectiveness of these diagnostic measures but also their practical implementation in clinical settings. When considering PIVKA-II, its application in the Asia-Pacific region presents certain challenges. For instance, factors such as the determination of cut-off values remain unclear. There is an urgent need for further evidence and a broader international consensus to establish standardized cut-off values and improve reference ranges. Additionally, conducting cost-effectiveness studies is essential to validate the broader integration of these strategies into clinical practice. Notably, previous studies have established GAAD cut-off values and compared the cost-effectiveness of GAAD, with or without ultrasound, against the use of ultrasound and

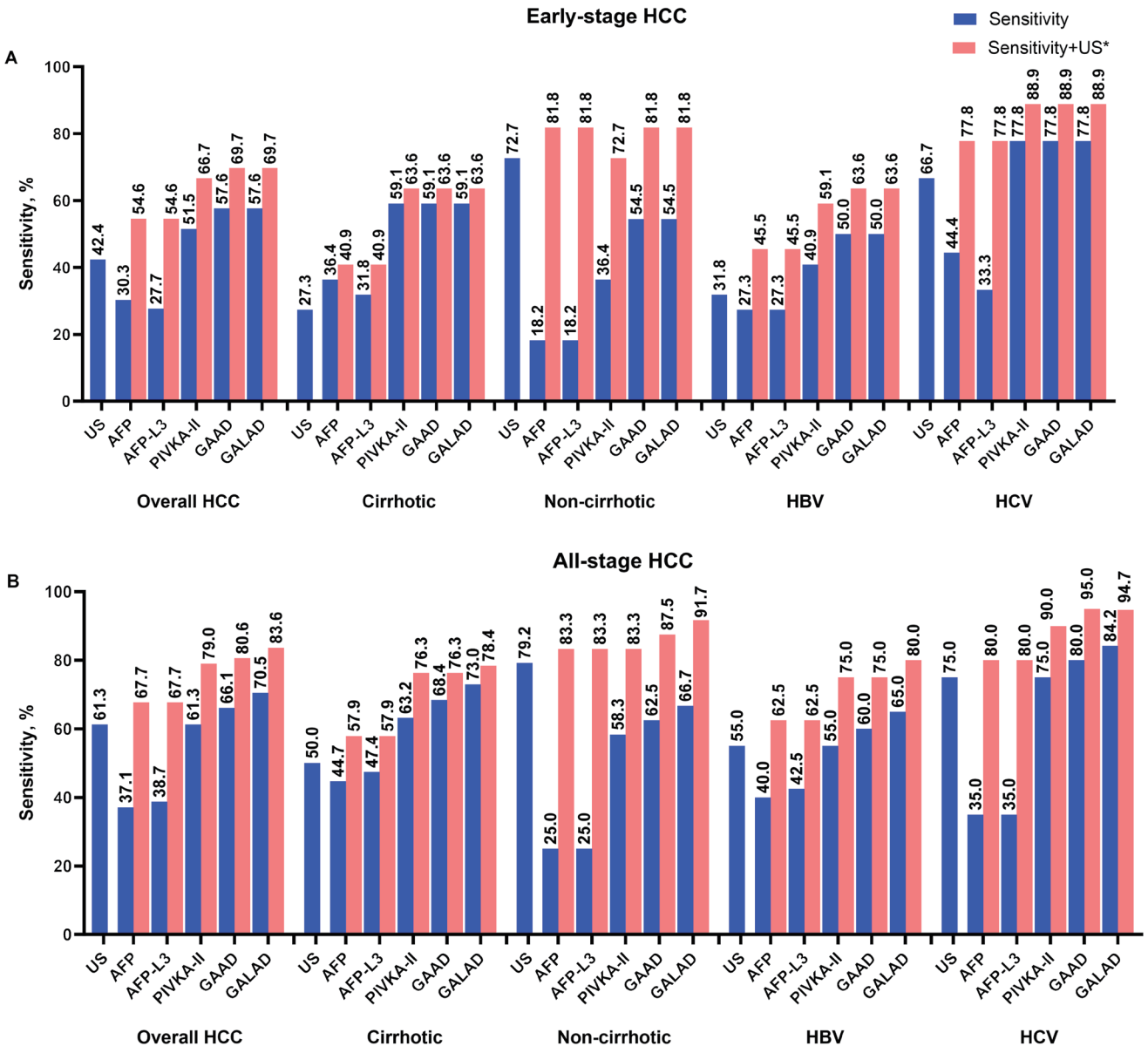

Fig. 5. Sensitivity of US, GAAD, and GALAD (Cobas) algorithms and Elecsys AFP, AFP-L3, and PIVKA-II assays for discriminating between early-stage (A) and all-stage (B) HCC patients and controls by etiology. *Sensitivity of the assay/algorithm when combined with ultrasound. AFP, α-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein; GAAD, gender (biological sex), age, AFP, PIVKA-II; GALAD, gender (biological sex), age, AFP-L3, AFP, PIVKA-II; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PIVKA-II, protein induced by vitamin K absence or antagonist II; US, ultrasound.

AFP for HCC screening in cohorts from the UK, Switzerland, and China.[32–34]

A limitation of our study is that the enrolled patients were not matched for age or gender, resulting in an older, predominantly male cohort. However, this population was intended for HCC surveillance, and our study aimed to reflect the real-world population that would undergo HCC surveillance in Taiwan. The issue of age and gender bias in the GAAD algorithm has been investigated previously.[21] After performing several simulations to address this bias between HCC cases and CLD controls for the GAAD score, the study found that results remained stable when cases were matched across all investigated sample sizes. Further validation through larger phase 3/4 case-matched studies is required to assess the benefit-to-harm ratio of GAAD-based surveillance.

Conclusions

These findings support the utility of the novel GAAD algorithm as a complementary tool in early-stage HCC detection for benign CLD patients undergoing HCC surveillance, with the potential to improve treatment opportunities and reduce mortality.

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COBAS and ELECSYS are trademarks of Roche. GAAD is a CE-marked digital tool for aid in diagnosis of early-stage HCC.

All other product names and trademarks are the property of their respective owners. Editorial support for this manuscript, under the direction of the authors, was provided by Katie Williams, MSc, and Sean Littlewood, MRes, of inScience Communications, Springer Healthcare Ltd, UK, and was funded by Roche Diagnostics GmbH.

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Conflict of interest

WLC: Member of advisory boards for Gilead, AbbVie, Vaccitech, PharmaEssentia; speaker for Gilead, AbbVie, BMS, Roche, and has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2022. CFH: Speaker for AbbVie, BMS, Gilead, Merck, and Roche, JFH: Consultant for Roche, Gilead, Sysmex, and Aligos; speaker for AbbVie, Gilead, and Sysmex; and has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2022. MLYu: Research grants from AbbVie, Gilead, Merck, and Roche Diagnostics; consultant for AbbVie, BMS, Gilead, Roche, and Roche Diagnostics; and speaker for AbbVie, BMS, Eisai, Gilead, Roche, and Roche Diagnostics; and has been an Associate Editor of *Journal of Clinical and Translational Hepatology* since 2023. AS is an employee of Roche Diagnostics International AG. KK is an employee of Roche Diagnostics GmbH. MLYeh has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2022. The other authors have no conflict of interests related to this publication.

Author contributions

Conceptualization (AS, KK, MLYu), formal analysis (CFH, KK, AS, MLYu), investigation (WLC, CYD, CFH, CIH, JFH, MYH, PYH, TYJ, PCL, YHL, CWW, YJW, MLYeh, MLYu), and data curation (CFH, KK, AS, MLYu). All authors contributed to the review and editing. All authors have approved the final version and publication of the manuscript.

Ethical statement

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee of Kaohsiung Medical University Hospital (IRB number: KMUHIRB-E(I)-20200127). All participants provided written informed consent.

Data sharing statement

Requests concerning the data supporting the findings of this study can be directed to rotkreuz.datasharingrequests@roche.com for consideration.

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