



Combining serum AFP and CEUS LI-RADS for better diagnostic performance in Chinese high-risk patients

Wushuang Gong¹ · Jiaqi Wu¹ · Hong Wei¹ · Zhaopeng Jiang¹ · Ming Wan¹ · Chengwei Wu¹ · Weili Xue¹ · Rao Ma¹ · Xianli Zhou¹ · Hang Zhou¹

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Abstract

Purpose To evaluate and compare the diagnostic performance of revised contrast-enhanced ultrasound (CEUS) Liver Imaging Reporting and Data System version by combining LR-M category and serum alpha-fetoprotein (AFP) under different cut-off values.

Material and methods This retrospective study enrolled 152 high-risk patients with 152 histology-proven nodules. For revised LI-RADS, nodules in LR-M with different elevated AFP thresholds have been reclassified as the LR-5 category. The diagnostic performances of original and revised CEUS LI-RADS were evaluated and compared.

Results To compare with the original version, the sensitivity of revised LR-5 (adjusted with AFP value > 200 ng/ml or 400 ng/ml) for the diagnosis of hepatocellular carcinoma (HCC) improved from 52.5 to 69.2% or 65.0%, respectively (both $p < 0.001$) without compromising specificity (87.5% vs. 71.9% or 78.1%, respectively, both $p > 0.05$). For the diagnosis of non-HCC malignancy, the specificity of the LR-M after reclassification was improved (69.6% vs. 84.4% or 80.7%, respectively, both $p < 0.001$) with a non-significant sensitivity reduction (100.0 vs. 70.6% or 82.4%, respectively, both $p > 0.05$). After modification, the sensitivity of LR-5 also increased to 69.1% or 64.9% (both $p < 0.001$), while the specificity and PPV did not change (both $p > 0.05$) for larger nodules (> 20 mm).

Conclusion The diagnostic performance of CEUS LI-RADS can be further improved by reclassifying LR-M nodules with elevated AFP thresholds to LR-5.

Keywords Ultrasonography · Alpha-fetoprotein · Contrast agent · Hepatocellular carcinoma · Diagnosis

Abbreviations

ACR	American college of radiology	HCV	Hepatitis C virus
AFP	Alpha-fetoprotein	ICC	Intrahepatic cholangiocarcinoma
AUC	Area under the ROC curve	LI-RADS	Liver imaging reporting and data system
CEUS	Contrast-enhanced ultrasound	MLC	Metastasis liver carcinoma
CHC	Combined hepatocellular cholangiocarcinoma	NPV	Negative predictive value
HBV	Hepatitis B virus	PPV	Positive predictive value
HCC	Hepatocellular carcinoma	ROC	Receiver operating characteristic
		US	Ultrasound

Wushuang Gong and Jiaqi Wu were contributed equally to this manuscript.

✉ Xianli Zhou
hrbzhouxl@163.com

✉ Hang Zhou
zhouhang686686@163.com

¹ In-Patient Ultrasound Department, Second Affiliated Hospital of Harbin Medical University, Surgeons' Hall, No. 246, Xuefu Road, Nangang District, Harbin City, Heilongjiang, China

Introduction

Hepatocellular carcinoma (HCC) constitutes the most common primary liver malignancy, accounting for about approximately 80% [1]. Accurate differentiation of hepatic lesions has significant importance in clinical management [2–4]. With the development of contrast-enhanced media, imaging modalities can replace invasive biopsy as the confirmative diagnostic approach in high-risk populations of

HCC. Taking advantage of microbubbles within the intravascular space, contrast-enhanced ultrasound (CEUS) is recommended as the first-line diagnostic technology in the assessment of focal liver lesions (FLL) according to several international guidelines and recommendations [5–8]. Moreover, some western guidelines restricted the use of biopsy in cases of imaging uncertainty after examination with contrast-enhanced media (computed tomography, magnetic resonance imaging, CEUS) [9, 10]. Therefore, CEUS must be integrated with other methods.

The Liver Imaging Reporting and Data System (LI-RADS) version 2017 was developed by the American College of Radiology (ACR) to standardize terminology, reduce variance and errors in imaging interpretation, enhance communication between radiologists and clinicians, and create a structured report for the diagnosis of HCC [11, 12]. CEUS LI-RADS could assign the increasing probability of HCC from LR-1 to LR-5 and provide an LR-M class that encompasses probably or definitely malignancies but is not HCC-specific [11]. Previous studies demonstrated that CEUS LI-RADS category 5 had high specificity in predicting HCC, ensuring nearly 100% certainty [13, 14]. However, a significant drawback in CEUS LI-RADS is based on the fact that a high proportion of HCCs were still misdiagnosed as LR-M category, leading to a relatively lower sensitivity (44.8–64.2%) and accuracy (60.4–70.8%) of LR-5 [15]. Therefore, LR-M criteria must be revised to improve CEUS LI-RADS diagnostic performance.

Convenient and cost-effective serum alpha-fetoprotein (AFP) detection is frequently utilized for HCC diagnosis and surveillance [16]. However, different thresholds for AFP were adopted in the diagnosis of HCC. A study in the United States compared different cut-off values (20 ng/mL, 50 ng/mL, 100 ng/mL, 400 ng/mL) and found that when the threshold of AFP was 20 ng/ml, the sensitivity was approximately 60% with the specificity of 90%, while when the threshold was 400 ng/ml, the sensitivity was only 17%, but the specificity increased to 99.4% [16]. Moreover, an elevation of AFP also occurred in some patients involving chronic liver disease with non-cancerous causes [17]. Most guidelines discouraged the use of AFP alone due to its limited performance in diagnosing HCC, especially in patients with viral hepatitis [18, 19]. On the other hand, some experts have successfully incorporated the use of serum biomarkers in conjunction with CEUS characteristics to get an accurate diagnosis of FLLs [20, 21]. It should be noted that few studies focused on the role of serum AFP level in improving the diagnostic ability of CEUS LR-5 and LR-M.

Therefore, our objective was to combine different serum AFP cut-off values with the LR-M category to improve the current CEUS LI-RADS diagnostic performance.

Materials and methods

The retrospective study was approved by the Institutional Review Board, which waived the requirement of written informed consent.

Patient selection

The inclusion criteria were (a) high-risk factors (cirrhosis or chronic hepatitis B viral infection or current or prior HCC) for HCC according to ACR CEUS LI-RADS (v2017) guidelines [11], (b) visible FLL in the B-mode ultrasound (US), (c) pathological proven FLL, and (d) availability of serum AFP levels concentration within two weeks before any treatments for FLL. The exclusion criteria were (a) previously treated nodules or recurrent HCCs and (b) image degradation or missing CEUS data. Finally, 152 patients with 152 FLLs were eventually included in this study. A detailed flow-chart of patient selection is shown in Fig. 1.

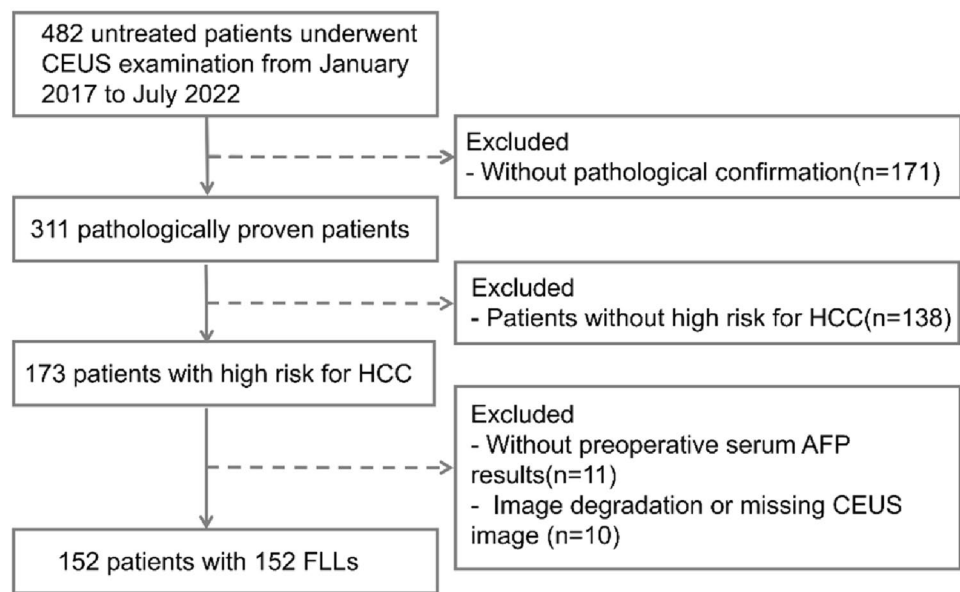
Standard reference

In this study, pathological results were used as the gold standard. Histological results were confirmed by ultrasound (US)-guided biopsy or surgical pathology within half a month after CEUS examination. If patients underwent both US-guided biopsy and surgery, surgical pathology results were used.

B-mode US and CEUS examination

This study was performed by ultrasound machines including Aplio 900 (Toshiba Medical Systems, Tokyo, Japan) and Aixplorer Ultrasound system (SuperSonic Imagine, Aix-en-Provence, France). Sonographers with more than six years of relevant clinical experience conducted the CEUS examination. Under the conventional B-mode US prior to CEUS, we collected the size, location, nodule echogenicity, and surrounding liver parenchyma. Then, all FLLs were examined using low mechanical index (MI) imaging and a dual-screen format after an injection of 2.4 ml SonoVue bolus followed by a 5 mL saline flush through the median cubital vein. The sonographer started the timer and saved the dynamic images after injecting the bolus of contrast agent. During the initial 60 s, we continually scanned the target nodule and the liver parenchyma surrounding it. To avoid continuous destruction of microbubbles, we swept the nodule at 15 s intervals and recorded for 4–6 min until the bolus cleared from circulation. The SonoVue could be reinjected 15 min after the first injection if the CEUS result was unclear. All imaging data were exported to a removable hard drive for later evaluation.

Fig. 1 Study population flow-chart. *Note: CEUS* contrast-enhanced ultrasound, *HCC* hepatocellular carcinoma, *AFP* alpha-fetoprotein, *FLLs* focal liver lesions



The CEUS process was classified into arterial (10–20 s to 30–45 s), portal venous (30–45 s to 120 s) and late phase (120–300 s).

Image interpretation

Two highly skilled ultrasound physicians with at least five years of CEUS experience independently reviewed the CEUS images. The nodules were classified according to CEUS LI-RADS (v2017). If no consensus was reached, the final categorization was achieved by another expert ultrasound physician with 10 years of experience in CEUS. Medical information was not disclosed to all readers.

Serum AFP examination

In the fasting state in the early morning, 5 ml of venous blood from the patients was detected by electrochemiluminescence immunoassay. In this study, we selected conventional AFP thresholds based on previous studies (20 ng/ml [22], 50 ng/ml [23], 100 ng/ml [16], 200 ng/ml [24], and 400 ng/ml [25]) and optimal thresholds derived from receiver operating characteristic (ROC) analysis according to our data.

Diagnostic criteria for modified CEUS LI-RADS

Criteria 1: The original CEUS LI-RADS v2017.

Criteria 2: LR-5 and LR-M with AFP > optimal threshold according to our data as a predictor of HCC, LR-M with AFP ≤ optimal threshold according to our data as a predictor of non-HCC malignancy.

Criteria 3: LR-5 and LR-M with AFP > 20 ng/ml as a predictor of HCC, LR-M with AFP ≤ 20 ng/ml as a predictor of non-HCC malignancy.

Criteria 4: LR-5 and LR-M with AFP > 50 ng/ml as a predictor of HCC, LR-M with AFP ≤ 50 ng/ml as a predictor of non-HCC malignancy.

Criteria 5: LR-5 and LR-M with AFP > 100 ng/ml as a predictor of HCC, LR-M with AFP ≤ 100 ng/ml as a predictor of non-HCC malignancy.

Criteria 6: LR-5 and LR-M with AFP > 200 ng/ml as a predictor of HCC, LR-M with AFP ≤ 200 ng/ml as a predictor of non-HCC malignancy.

Criteria 7: LR-5 and LR-M with AFP > 400 ng/ml as a predictor of HCC, LR-M with AFP ≤ 400 ng/ml as a predictor of non-HCC malignancy.

Statistical analysis

The SPSS software package version 23.0 and GraphPad Prism version 9.0 were used to perform the statistical analysis. Quantitative data were presented as medians ± standard deviation, while categorical data were identified as numbers and percentages. ROC was used to determine the optimal threshold for serum AFP level for HCC diagnosis. Pairwise comparisons of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by McNemar test or chi-square test if applicable. Diagnostic performance in diagnosing HCC were assessed by ROC curve analysis, sensitivity, specificity, PPV and NPV. A two-sided *p*-value less than 0.05 was established to be a significant difference.

Results

Patients and nodule characteristics

Based on inclusion criteria, the study population consisted of 152 patients with 152 FLLs (mean age, 56 years; range 27–78 years). Among all the patients, the mean maximum diameter of liver nodules was 42.5 mm (range 9.0–146.2 mm). The detailed clinical characteristics, including age, gender, nodule size, etiology of liver disease, serum AFP value, and pathological analysis, are presented in Table 1.

Imaging characteristics and CEUS LI-RADS categories

The CEUS imaging features and CEUS LI-RADS categories of nodules are shown in Table 2. Of the 152 nodules, there were 11 LR-3, 16 LR-4, 67 LR-5, and 58 LR-M nodules. Similar to expectations, the incidence of HCC from LR-3 to

LR-5 category was increased gradually (45.5%, 81.3% and 94.0%, respectively). Although all non-HCC malignancies were classified as LR-M category, 39 HCCs were also classified as LR-M due to early washout (<60 s).

Diagnostic performance of AFP alone to predict HCC

ROC analysis was used to determine the optimal cut-off value as 2.5 ng/ml. When using AFP alone to predict HCC, the diagnostic performances of different thresholds are shown in Online Resource 1. Under all thresholds, the AUC was from 0.53 to 0.63, and the accuracy was from 34.2 to 78.9%. Furthermore, different AFP values varied in sensitivity from 20.8 to 53.3% and specificity from 34.4 to 84.4%.

Diagnostic performance of the revised CEUS LI-RADS by combining with serum AFP

The AFP values of LR-M category with different pathologic types are shown in Fig. 2. FLLs in LR-M category with elevated AFP level were reclassified as LR-5 (Fig. 3). In contrast, LR-M FLLs with normal AFP did not change the classification (Fig. 4). The sensitivity and specificity of Criteria 1 to diagnose HCC were 52.5% and 87.5%, respectively. Criteria 2, Criteria 3, Criteria 4, and Criteria 5 improved the sensitivity of HCC diagnosis obviously (all $p < 0.001$). But the specificity of Criteria 2, Criteria 3, Criteria 4, and Criteria 5 dropped significantly (all $p < 0.05$). Moreover, both Criteria 6 and Criteria 7 elevated the sensitivity (69.2% vs. 52.5% and 65.0% vs. 52.5%, respectively) (both $p < 0.001$) without significantly reducing the specificity (71.9% vs. 87.5% and 78.1% vs. 87.5%, respectively) ($p > 0.05$). When it came to the diagnostic performance to identify non-HCC malignancy, Criteria 2, Criteria 3, Criteria 4, and Criteria 5 could elevate the specificity of LR-M (all $p < 0.001$), accompanied by a significant decrease in sensitivity (all $p < 0.05$). Criteria 6 and Criteria 7 could meanwhile increase the specificity of LR-M (84.4% vs. 69.6% and 80.7% vs. 69.6%, respectively) (both $p < 0.001$) without significant loss of sensitivity (70.6% vs. 100.0% and 82.4% vs. 100.0%, respectively) (both $p > 0.05$) (Table 3).

Diagnostic performance of the revised CEUS LI-RADS for FLLs size > 20 mm

In subgroup of liver nodules larger than 20 mm, the sensitivity and specificity of LR-5 in the diagnosis of HCC were 48.9% and 92.6%, respectively. Besides Criteria 6 and Criteria 7, Criteria 5 could also increase the sensitivity (69.1%

Table 1 Clinical and Pathologic Information (n = 152)

Characteristic	Value
Mean age (y)*	56 (27–78)
<i>Gender</i>	
Males	118 (77.6)
Females	34 (22.3)
Maximum Diameter (cm)*	42.5 (9.0–146.2)
HBV (+)	129 (84.9)
HCV (+)	14 (9.2)
Liver cirrhosis	108 (71.1)
<i>AFP (ng/ml) *</i>	
≤ 20 ng/ml	75 (49.3)
20–50 ng/ml	17 (11.2)
50–100 ng/ml	12 (7.9)
100–200 ng/ml	8 (5.3)
200–400 ng/ml	10 (6.6)
> 400 ng/ml	30 (19.7)
<i>Pathologic analysis</i>	
Hepatocellular carcinoma	120 (78.9)
Intrahepatic cholangiocarcinoma	5 (3.3)
Combined hepatocellular cholangiocarcinoma	4 (2.6)
Metastasis	8 (5.3)
Hemangioma	2 (1.3)
Dysplastic nodules	8 (5.3)
Other benign nodules	5 (3.3)

HBV hepatitis B virus, HCV hepatitis C virus, AFP alpha-fetoprotein. Unless otherwise indicated, data are number of nodules and numbers in parentheses are percentages

*Data in parentheses are range

Table 2 Imaging characteristics and CEUS LI-RADS categories of patients with different pathological types

	Nodules (n = 152)				
	Hepatocellular carcinoma (n = 120)	Intrahepatic cholangiocarcinoma (n = 5)	Combined hepatocellular cholangiocarcinoma (n = 4)	Metastasis (n = 8)	Benign nodules (n = 15)
<i>CEUS features</i>					
Wash-in patterns					
APHE	102 (85.0)	3 (60.0)	3 (75.0)	4 (50.0)	8 (53.3)
Rim-APHE	5 (4.2)	1 (20.0)	1 (25.0)	2 (25.0)	–
Iso-/Hypo-	13 (10.8)	1 (20.0)	–	2 (25.0)	7 (46.7)
Washout patterns					
Early WO	39 (32.5)	4 (80.0)	4 (100.0)	7 (87.5)	2 (13.3)
Marked WO	–	1 (20.0)	–	1 (12.5)	–
Late and mild WO	67 (55.8)	–	–	–	5 (33.3)
No WO	14 (11.7)	–	–	–	8 (53.3)
<i>CEUS LI-RADS categories</i>					
LR-3	5 (4.2)	–	–	–	6 (40.0)
LR-4	13 (10.8)	–	–	–	3 (20.0)
LR-5	63 (52.5)	–	–	–	4 (26.7)
LR-M	39 (32.5)	5 (100.0)	4 (100.0)	8 (100.0)	2 (13.3)

APHE arterial phase hyperenhancement, WO washout, CEUS contrast-enhanced ultrasound. LI-RADS Liver Imaging Reporting and Data System. Data are number of nodules and numbers in parentheses are percentages

LR-M category with elevated AFP

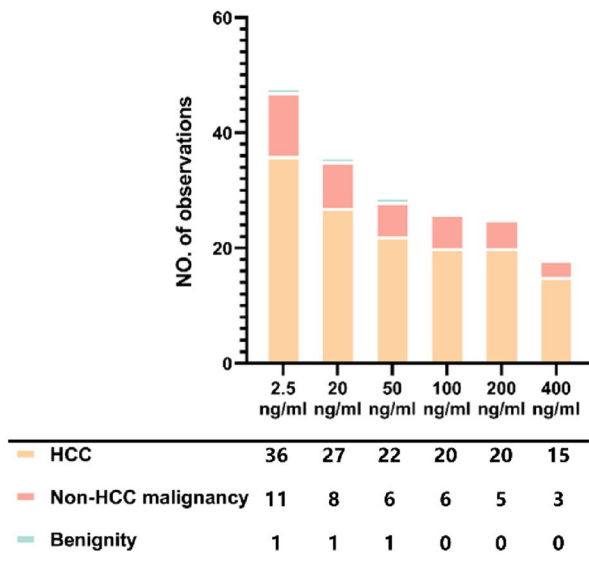


Fig. 2 Elevation of AFP in category LR-M among different pathological types. Note: HCC hepatocellular carcinoma, AFP alpha-fetoprotein. Data are number of nodules

vs. 48.9%, $p < 0.001$) without significant loss of specificity (74.1% vs. 92.6%, $p = 0.063$) (Table 4).

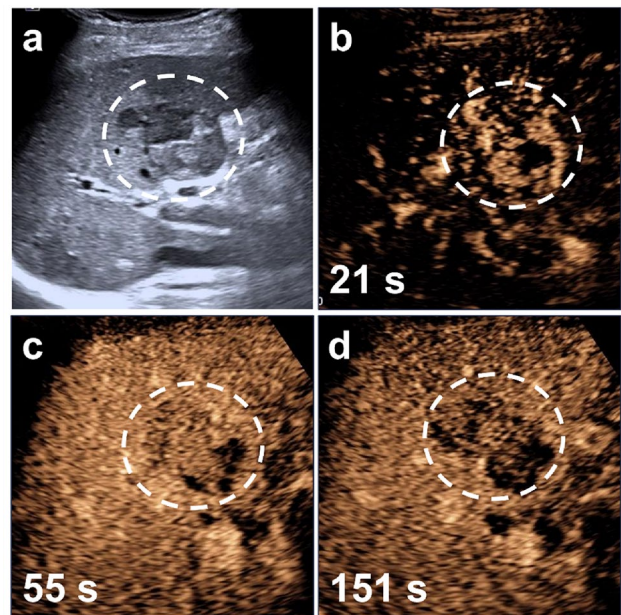


Fig. 3 HCC nodule with elevated serum AFP value (301.32 ng/mL). a A 46-mm hypoechoic lesion in a 66-year-old male patient with HBV; b The nodule showed APHE 21 s after SonoVue injection, and c early washout was observed at 55 s. d A mild washout was presented at 151 s. The nodule would be classified as CEUS LR-M (criteria 1 and 7) or reidentified as CEUS LR-5 (criteria 2, 3, 4, 5, 6)

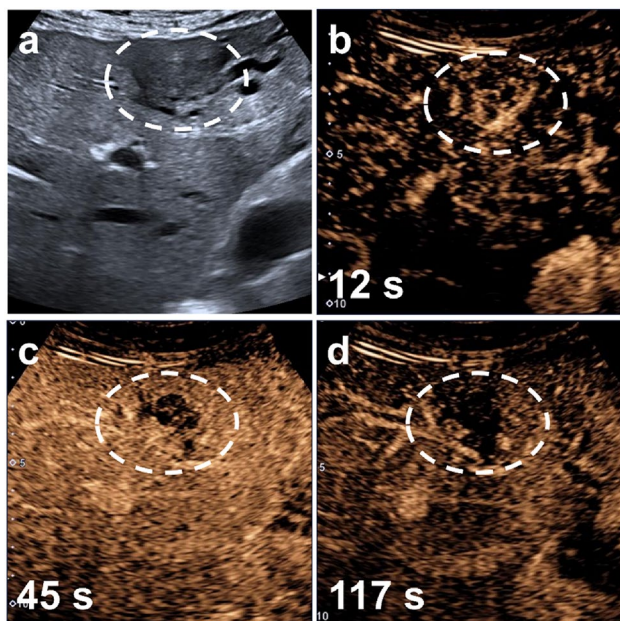


Fig. 4 ICC nodule with normal serum AFP value (1.83 ng/mL). **a** A 40-mm hypoechoic nodule in a 72-year-old female patient with HBV; **b** The nodule showed APHE 12 s after SonoVue injection, and **c** early washout was observed at 45 s. **d** A mild washout was presented at 117 s. The nodule would be classified as CEUS LR-M (criteria 1, 2, 3, 4, 5, 6, 7)

Discussion

In this retrospective study, we performed multiple comparative studies of 152 nodules based on combining CEUS LI-RADS and different serum AFP cut-off values in the diagnosis of HCC. Eventually, at thresholds of 200 ng/ml and 400 ng/ml, the sensitivity improved effectively ($p < 0.001$) without significant loss of specificity ($p = 0.063$ and 0.250).

In this study, the incidences of HCC in LR-3, LR-4, LR-5 category nodules were 45.5%, 81.3% and 94.0% respectively, which corresponded to a higher probability of HCC in CEUS LI-RADS [26]. Meanwhile, our research also demonstrated an excellent specificity of 87.5% and PPV of 94.0%, consistent with previous research ranging 78.5–96.0% and 92.7–98.0% [27–29]. Thus, CEUS LR-5 could accurately identify HCCs and avoid the use of percutaneous biopsy which may cause bleeding or implantation metastasis [30]. However, another study showed that 68.5% of HCCs were in the LR-M category, which caused a relatively poor sensitivity of 65.5% [31]. It was similar to our study of 67.2% and 52.5%, respectively. In order to improve the sensitivity of HCC detection and to reduce missed diagnoses, it is necessary for us to reclassify HCCs in the LR-M category. Several studies focused only on the modification of imaging characteristics, such as adjusting the time to define early washout [31] or combining the time and degree of early washout [27, 28], but few attention was paid to the role of serum biomarkers.

Table 3 Diagnostic performance of categories LR-5 and LR-M before and after recategorization

Category	Sensitivity	<i>p</i> Value	Specificity	<i>p</i> Value	PPV	<i>p</i> Value	NPV	<i>p</i> Value
<i>LR-5 as a predictor of HCC</i>								
Criteria 1#	52.5		87.5		94.0		32.9	
Criteria 2	82.5	<.001*	50.0	<.001*	86.1	.098	43.2	.276
Criteria 3	75.0	<.001*	59.4	.004*	87.4	.158	38.8	.495
Criteria 4	70.8	<.001*	65.6	.016*	88.5	.233	37.5	.578
Criteria 5	69.2	<.001*	68.8	.031*	89.3	.291	37.3	.590
Criteria 6	69.2	<.001*	71.9	.063	90.2	.386	38.3	.503
Criteria 7	65.0	<.001*	78.1	.250	91.8	.593	37.3	.574
<i>LR-M as a predictor of non-HCC malignancy</i>								
Criteria 1#	100		69.6		29.3		100	
Criteria 2	35.3	.001*	97.0	<.001*	60.0	.058	92.3	.006*
Criteria 3	52.9	.008*	90.4	<.001*	40.9	.323	93.9	.014*
Criteria 4	64.7	.031*	86.7	<.001*	37.9	.417	95.1	.030*
Criteria 5	64.7	.031*	84.4	<.001*	34.4	.619	95.0	.028*
Criteria 6	70.6	.063	84.4	<.001*	36.4	.488	95.8	.044*
Criteria 7	82.4	.250	80.7	<.001*	35.0	.552	97.3	.110

PPV positive predictive value, NPV negative predictive value, HCC hepatocellular carcinoma. Data in sensitivity, specificity, PPV, NPV and accuracy were shown as percentages

#The current LR-5 and LR-M criteria of CEUS LI-RADS v2017

*Statistically significant

Table 4 Diagnostic performance of categories LR-5 and LR-M before and after recategorization for FLLs size > 20 mm

Category	Sensitivity	p Value	Specificity	p Value	PPV	p Value	NPV	p Value
<i>LR-5 as a predictor of HCC</i>								
Criteria 1#	48.9		92.6		95.8		34.2	
Criteria 2	85.1	< .001*	51.9	.001*	86.0	.073	50.0	.145
Criteria 3	75.5	< .001*	63.0	.008*	87.7	.122	42.5	.385
Criteria 4	70.2	< .001*	70.4	.031*	89.2	.191	40.4	.493
Criteria 5	69.1	< .001*	74.1	.063	90.3	.258	40.8	.461
Criteria 6	69.1	< .001*	77.8	.125	91.5	.360	42.0	.383
Criteria 7	64.9	< .001*	81.5	.250	92.4	.454	40.0	.504
<i>LR-M as a predictor of non-HCC malignancy</i>								
Criteria 1#	100.0		63.8		29.6		100.0	
Criteria 2	37.5	.002*	97.1	< .001*	66.7	.031*	91.1	.012*
Criteria 3	56.3	.016*	88.6	< .001*	42.9	.275	93.0	.027*
Criteria 4	68.8	.063	83.8	< .001*	39.3	.378	94.6	.054
Criteria 5	68.8	.063	81.9	< .001*	36.7	.508	94.5	.051
Criteria 6	75.0	.125	81.9	< .001*	38.7	.391	95.6	.080
Criteria 7	81.3	.250	78.1	< .001*	35.1	.519	96.5	.120

PPV positive predictive value, NPV negative predictive value, HCC hepatocellular carcinoma. Data in sensitivity, specificity, PPV, NPV and accuracy were shown as percentages

#The current LR-5 and LR-M criteria of CEUS LI-RADS v2017

*Statistically significant

Serum AFP evaluation is a simple, rapid, and inexpensive means of HCC surveillance and it is also of great significance for evaluating treatment response [32]. However, the role of AFP alone is limited in the diagnosis of HCC [33]. One probable reason is that increased AFP level could reflect the severity of hepatic destruction and subsequent regeneration, which could be presented in patients with active hepatitis or cirrhosis even without HCC [16]. Elevated AFP (> 20 ng/mL) was observed in about 40.6% of high-risk patients without HCC, according to our data. Another explanation might be that AFP levels are normal (≤ 20 ng/ml) in about 30% to 40% of patients with HCC, which is consistent with our findings [32]. Consequently, combining imaging features and AFP seems promising in distinguishing FLLs. To improve the efficacy of differentiating the diagnosis of FLLs, Yang et al. tried to associate CEUS LI-RADS with serum biomarkers in diagnosing non-HCC malignancy [20]. Serum biomarkers were also associated with CT/MRI LI-RADS for the differentiation of non-HCC malignancies [34]. By combining serum AFP and LR-M criteria, we revised CEUS LI-RADS. A recent CEUS LI-RADS study illustrated that CEUS LR-3, 4, 5, M category combined with elevated level of AFP (AFP > 200 ng/ml) could significantly increase the sensitivity (79.6%) for the diagnosis of HCC while specificity remained high (96.6%) [35]. However, only 67.2% of FLLs in that study were confirmed by histological examination, which may hinder the validity of the gold standard. Furthermore, Li et al. did not evaluate the role of different AFP cut-off values in the CEUS

LI-RADS workup. In contrast, our study found that besides 200 ng/ml, incorporating AFP level higher than 400 ng/ml and LR-M category could also satisfy increasing sensitivity without compromising specificity for the diagnosis of HCC.

According to our data, at the AFP cut-off value of 200 ng/ml, there were still 2 CHCs, 2 ICCs and 1 metastasis erroneously reclassified to the LR-5 category. Some non-HCC malignancies with elevated AFP could be observed, which was consistent with previous findings [16]. Furthermore, another research showed that the usefulness of AFP for the diagnosis of HCC in small HCCs was limited [18]. In our subgroup analysis (nodules size > 20 mm), adjustment with AFP level > 100 ng/ml maintained a relatively higher specificity with improved sensitivity, just as other revised CEUS LR-5 did (combining AFP above 200 ng/ml or 400 ng/ml).

There were still several limitations in our study. Firstly, enrolling patients who originated from this single-center, retrospective study with histopathological confirmation may inevitably lead to a small sample size (152 nodules) and selection bias. Prospective validation of multi-center cohorts with large samples is required. Secondly, based on indications of CEUS LI-RADS v2017 [11], patients at high risk for HCC were enrolled in this study, which may be the main reason why there were relatively low incidences of other malignancies than HCC (11.2% vs. 78.9%) in this paper, which was similar to previous research (8.7% vs. 84.5%) [31]. Thirdly, since we enrolled high-risk patients strictly following ACR recommendations, the overwhelmingly high rate of hepatitis B virus infection (85%) might limit the application of our findings in

the western population. Moreover, the AFP presents several issues in patients with viral hepatitis (physiological increase without HCC), which may influence cut-offs and the applicability of our results. Fourth, the US-guided biopsy can cause false-negative results, which cannot eliminate the possibility of HCC [7]. Fifth, the different US machines adopted in this study may limit the interpretation of images by physicians. However, both machines used in this study were high-end instruments operated by experienced sonographers. In addition, all CEUS images were scanned and stored under the same protocol.

In conclusion, CEUS LR-5 demonstrated a high specificity but a low sensitivity in diagnosing HCC. A combination of LR-M category and serum AFP level at a cut-off value of 200 ng/ml or 400 ng/ml significantly improved CEUS LR-5's ability to diagnose HCCs and LR-M's ability to diagnose non-HCC malignancies.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11547-023-01614-9>.

Author contributions Conceptualization: JW; Data curation: WG, RM; Formal analysis: HW, RM; Funding acquisition: HZ; Investigation: JW, CW; Methodology: MW, WX; Project administration: HZ, XZ; Resources: JW, HW; Software: ZJ, WX; Supervision: HZ, XZ; Validation: MW, CW; Visualization: ZJ; Writing-original draft: WG, HZ; Writing-review and editing: WG, JW, HZ; all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee the Second Affiliated Hospital of Harbin Medical University.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Written informed consent was waived by the Institutional Review Board of the Second Affiliated Hospital of Harbin Medical University.

Consent to publish The authors affirm that human research participants provided informed consent for publication of the images in Figs. 3 and 4.

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