

# Diagnosis of Hepatic Nodules 20 mm or Smaller in Cirrhosis: Prospective Validation of the Noninvasive Diagnostic Criteria for Hepatocellular Carcinoma

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This study prospectively evaluates the accuracy of contrast-enhanced ultrasound (CEUS) and dynamic magnetic resonance imaging (MRI) for the diagnosis of nodules 20 mm or smaller detected during ultrasound (US) surveillance. We included 89 patients with cirrhosis [median age, 65 years; male 53, hepatitis C virus 68, Child-Pugh A 80] without prior hepatocellular carcinoma (HCC) in whom US detected a small solitary nodule (mean diameter, 14 mm). Hepatic MRI, CEUS, and fine-needle biopsy (gold standard) (FNB) were performed at baseline. Non-HCC cases were followed (median 23 months) by CEUS/3 months and MRI/6 months. FNB was repeated up to 3 times and on detection of change in aspect/size. Intense arterial contrast uptake followed by washout in the delayed/venous phase was registered as conclusive for HCC. Final diagnoses were: HCC (n = 60), cholangiocarcinoma (n = 1), and benign lesions (regenerative/dysplastic nodule, hemangioma, focal nodular hyperplasia) (n = 28). Sex, cirrhosis cause, liver function, and alpha-fetoprotein (AFP) levels were similar between HCC and non-HCC groups. HCC patients were older and their nodules significantly larger ( $P < 0.0001$ ). First biopsy was positive in 42 of 60 HCC patients. Sensitivity, specificity, and positive and negative predictive values of conclusive profile were 61.7%, 96.6%, 97.4%, and 54.9%, for MRI, 51.7%, 93.1%, 93.9%, and 50.9%, for CEUS. Values for coincidental conclusive findings in both techniques were 33.3%, 100%, 100%, and 42%. Thus, diagnosis of HCC 20 mm or smaller can be established without a positive biopsy if both CEUS and MRI are conclusive. However, sensitivity of these noninvasive criteria is 33% and, as occurs with biopsy, absence of a conclusive pattern does not rule out malignancy. These results validate the American Association for the Study of Liver Disease (AASLD) guidelines. (HEPATOLOGY 2007;47:000-000.)

Hepatocellular carcinoma (HCC) is the 6th most common cancer worldwide and the third most frequent cause of death of cancer.<sup>1</sup> In more than 90% of the cases, HCC complicates liver cirrhosis, and in

these patients HCC constitutes the leading cause of death.<sup>2-6</sup> HCC incidence at 5 years may exceed 25%,<sup>5,7-9</sup> and the sole approach to achieve long-term survival is to detect the tumor at an early stage when effective therapy can be applied. Accordingly, all guidelines recommend performing screening for HCC in those patients with cirrhosis who would be treated if diagnosed with this condition.<sup>10,11</sup> Screening is based on ultrasound (US), and unequivocal diagnosis of a US-detected nodule within a cirrhotic liver represents a major clinical challenge. Biopsy confirmation has several limitations. Location of the tumor, clotting disorders, and ascites may prevent needle insertion, and it is not free of risks (bleeding or seeding). Finally, it is flawed by false-negative results caused by sampling error or to the unfeasibility of confidently distinguishing between dysplastic changes and well-differentiated HCC. This raises the need of well-defined noninvasive criteria that would allow an accurate diagnosis based on the imaging characterization. This need is of paramount relevance in settings such as transplantation. False-positive diagnosis results in unfair priority granting to patients without HCC or, what is even worse, to indicate transplant in the absence of HCC.<sup>12-14</sup> The European

Abbreviations: AASLD, American Association for the Study of Liver Diseases; AFP, alpha-fetoprotein; CEUS, contrast-enhanced ultrasound; FNB, fine-needle biopsy; FPR, false-positive rate; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging; US, ultrasound; VIBE, Volumetric Interpolated Breath-hold Examination.

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Association for the Study of Liver Diseases 2000 Conference proposed a set of criteria to establish HCC diagnosis in patients with cirrhosis.<sup>10</sup> These were based on the coincidental observation of a hypervascular arterial profile of the nodule by 2 dynamic imaging techniques. To reduce the risk of false-positive diagnosis in small nodules, the noninvasive criteria were restricted to tumors larger than 2 cm in a cirrhotic liver. Accordingly, below this cutoff a biopsy diagnosis was mandatory. These criteria have been widely applied and have been clinically useful in patients with large HCC. Thereby, their wrong implementation in noncirrhotic livers or by using single reports of isolated imaging techniques have brought disappointing results.<sup>15</sup> The same occurs if the diagnostic accuracy of noninvasive criteria is solely tested in patients with positive biopsy.<sup>15</sup> In practice, biopsy is just performed in patients with non-diagnostic imaging techniques, and hence, such a comparison is flawed. As said, the usefulness of the criteria based on detection of contrast uptake has been confirmed, and the sole concern relies on the potential anecdotal false-positive diagnosis that could be raised in small benign lesions such as atypical hemangioma.<sup>16</sup> Intense arterial uptake might be present in nodules other than HCC. Nodules larger than 2 cm are unlikely to be wrongly classified as HCC, but this risk has raised the need to refine the specific characteristics that a nodule within a cirrhotic liver should exhibit to establish HCC diagnosis. Several studies have shown that the characteristic HCC profile includes the intense arterial uptake but is followed by contrast washout in the delayed venous phase.<sup>17</sup> The recognition of the diagnostic value of contrast washout allowed the refinement of the criteria as reflected in the recent American Association for the Study of Liver Diseases (AASLD) guidelines<sup>11</sup> and in the unpublished consensus of the European Association for the Study of the Liver experts that met in 2005. According to these new criteria, it is possible to establish HCC diagnosis if a nodule within a cirrhotic liver exhibits intense arterial uptake followed by washout in the venous phase. Dynamic imaging techniques include contrast-enhanced ultrasound, computed tomography, and magnetic resonance imaging (MRI). In nodules larger than 2 cm, a single imaging technique would be enough. However, and trying again to prevent false-positive HCC diagnosis in tiny lesions, it was recommended that in nodules between 1 and 2 cm, the diagnosis should require the coincidental findings of 2 techniques. If these criteria are not met, biopsy diagnosis is recommended.

These criteria were derived from the results of several cohort studies with diagnostic confirmation by evolutionary findings or biopsy or explant correlations. However, they have never been prospectively validated, and this is

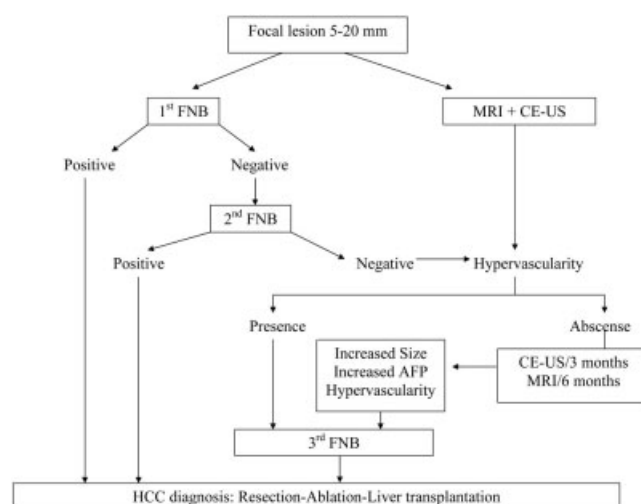


Fig. 1. Diagnostic algorithm followed in the study.

especially relevant in small hepatic nodules detected during surveillance in cirrhosis. Accordingly, this study prospectively evaluates the accuracy of contrast-enhanced ultrasound (CEUS) and MRI for the diagnosis of solitary nodules of 20 mm or smaller detected during surveillance in patients with cirrhosis and, thus, validate the AASLD diagnostic algorithm.

## Patients and Methods

Between November 2003 and August 2006, we prospectively included asymptomatic patients with Child-Pugh A-B cirrhosis with no history of HCC in whom a new solitary, well-defined, solid nodule between 5 and 20 mm was detected by screening ultrasound (US). Patients with poor liver function who would have undergone transplantation even without HCC diagnosis and those with significant comorbidities were excluded because no clinical decision/treatment would be indicated on HCC diagnosis. Patients with severe clotting alterations or contraindications to perform MRI, CEUS, or fine-needle biopsy were also excluded. The protocol was approved by the Ethics Committee for Clinical Research.

Figure 1 summarizes the diagnostic algorithm. On signing informed consent, we registered all demographic data and obtained a blood sample to determine liver, renal, and hematology parameters. Patients were examined by dynamic MRI and CEUS with second-generation contrast agent, and finally submitted to fine-needle biopsy (FNB). Biopsy result was considered the gold standard. If a conclusive diagnosis was not achieved, a second FNB was done. If the report was again negative for malignancy and did not raise a conclusive diagnosis, the policy was decided according to the imaging pattern. If any imaging technique evidenced arterial hypervascularization, a third

FNB was indicated. All the other cases and those with a third negative biopsy for malignancy were followed with CEUS every 3 months and MRI every 6 months. If growth or hypervascularization was detected, a new FNB was performed.

**MRI Imaging Technique.** MRI was performed with a 1.5-T system (Symphony, Siemens Medical Systems, Erlangen, Germany) using a phased-array torso coil for signal detection. All patients underwent transverse T1-weighted and T2-weighted MRI and multiphase contrast-enhanced dynamic 3-dimensional MRI of the whole liver with fat suppression. T1-weighted imaging included breath-hold in-phase gradient echo (100/5.24 TR/TE,  $256 \times 134$  matrix,  $70^\circ$  flip angle) and out-of-phase gradient echo (100/2.38 TR/TE,  $256 \times 134$  matrix,  $70^\circ$  flip angle). T2-weighted imaging included breath-hold haste (1100/116 TR/TE,  $256 \times 144$  matrix). Dynamic MRI was performed with a 3-dimensional Volumetric Interpolated Breath-hold Examination (VIBE) sequence in axial plane by using the following parameters: 4.3/2,  $25^\circ$  flip angle,  $256 \times 106$  matrix, slice thickness of 3 mm. Gadolinium (gadodiamide 0.5 mmol/L Omiscan-Amersham) was injected at a dose of 0.2 mL/kg at a rate of 2 mL/s. Bolus tracking technique was used to obtain arterial-phase images, approximately 20 seconds after contrast injection. Portal venous and delayed venous phase images were acquired 60 to 65 and 100 to 110 seconds thereafter. A breath-hold T1-weighted 2-dimensional gradient echo with fat suppression MRI (160/2.6 TR/TE,  $256 \times 115$  matrix) was performed 5 minutes after contrast injection.

**CEUS Technique.** US studies were performed using Sequoia 512 equipment (Acuson, Mountain View, CA). Baseline hepatic US was performed with a multifrequency 4C1 convex and 4V1 sectorial array probe to identify the target nodule. On identifying the lesion, CEUS was performed using the contrast coherent imaging (CCI, Siemens) with the 4C1 convex array probe. A low mechanical index ( $<0.2$ ) was selected to avoid the microbubbles disruption. CEUS explorations were performed after the administration of 2.4 mL SonoVue (Bracco, Italy). This bolus was repeated if the first exploration was not evaluable. Enhancement patterns were studied during the vascular phase up to 3.5 minutes, including the arterial (0-49 seconds), portal (50-179 seconds), and late phase ( $>180$  seconds).

**Fine-Needle Biopsy.** FNB was performed using a 20-gauge spinal needle (Yale Spinal BD medical, NJ). Several back and forth passages were done after insertion of the needle. When technically feasible because of location and accessibility, a core-biopsy was performed using an 18-gauge needle-biopsy (Monopty; Bard Inc, Covington,

UK). Specimens were routinely processed and stained with hematoxylin-eosin. Diagnosis of HCC was made according to the International Working Party criteria.<sup>18</sup>

**Image Interpretation.** MRI studies were read by 2 radiologists experienced in imaging of the liver (C.A. and J.R.A.) who were unaware of the results of the biopsy. Nodular lesions were categorized as follows: (1) *conclusive HCC diagnosis*: nodules displaying intense contrast enhancement in the arterial phase and washout in 1 of the venous phases (portal or delayed), (2) *suggestive of HCC, but nonconclusive*: nodules showing enhancement during the hepatic arterial phase without washout; even if pseudocapsule of fatty content were demonstrated, lesions were still included in this category; (3) *dysplastic foci*: nodules with hyperintensity on T1-weighted images with isointensity/hypointensity during all 3 phases of dynamic study; (4) *regenerative nodules*: lesions only seen at delayed phases as low intensity; (5) *nonspecific hypervascular nodules*: contrast-enhanced nodules only seen during the arterial phase of the dynamic study, and (6) *hemangioma*: nodules with hyperintensity in T2-weighted images with centripetal contrast uptake after arterial phase that persists in delayed phases.

CEUS were performed by 3 expert radiologists (R.V., L.B., and C.B.). Explorations were recorded and blindly reviewed by at least 2 radiologists. Categorization of doubtful explorations was achieved by consensus. Lesions were defined as follows: (1) *conclusive HCC*: nodules showing intense contrast uptake during the arterial phase followed by washout in portal and/or venous phase; (2) *suggestive of HCC, but nonconclusive*: nodules showing early enhancement during the hepatic arterial phase without washout in venous phase; (3) *dysplastic/regenerative nodules*: nodules with no contrast enhancement during the 3 phases; and (4) *hemangioma*: early centripetal contrast uptake after arterial phase that persists in delayed phases.

**Categorization of the Results of Imaging Pattern for HCC Diagnosis.** According to the previously depicted definitions, we stratified the findings of imaging techniques as hypovascular (no specific contrast enhancement of the nodule as compared with surrounding liver), suspicious (arterial hypervascularization regardless of washout), or conclusive (arterial hypervascularization followed by venous washout). Therefore, nodules classified as suspicious for HCC include those defined as conclusive and those categorized as suggestive but nonconclusive.

**Validation of the AASLD Criteria.** Those nodules in which both CEUS and MRI depicted a conclusive pattern were classified as "AASLD criteria positive." Nodules not displaying this coincidental profile were classified as "AASLD criteria negative."

**Table 1. Patients' Characteristics Considering the Final Diagnosis**

	All Patients	HCC Nodules	Non-HCC Nodules	Differences
Number of patients	89	60	29	
Age median [range] (years)	65 [37-83]	66.5 [37-83]	60 [38-78]	$P = 0.032$
Male/female	53/36	37/23	16/13	NS
Child-Pugh A/B	80/9	52/8	28/1	NS
HCV/HBV/Ethanol/PBC/others	68/6/10/1/4	45/4/8/1/2	23/2/2/0/2	NS
AST median [range] (U/L)	81 [25-322]	82 [25-204]	82 [26-322]	NS
ALT median [range] (U/L)	70 [16-537]	65 [16-210]	85.5 [21-537]	NS
Prothrombin ratio median [range] (%)	78.5 [35-100]	78 [35-100]	80.5 [49-98]	NS
Bilirubin median [range] (mg/dL)	1 [0.3-4.1]	1 [0.3-4.1]	0.7 [0.3-4.1]	NS
Platelets median [range] ( $10^9/L$ )	04 [31-286]	101 [32-286]	139 [31-270]	NS
Albumin median [range] (g/dL)	39 [26-48]	39 [28-48]	40 [29-46]	NS
Baseline AFP median [range] (ng/mL)	8 [1-1154]	8.5 [1-1154]	5 [1-170]	NS
Nodule size (mm):				
<10	13	2	11	
10-15	44	29	15	
16-20	32	29	3	$P < 0.0001$
Nodule pattern:				
Hypoechoic	47	34	13	
Isoechoic	7	7	0	
Hyperechoic	35	19	16	NS
Halo: yes / no	29/60	26/34	3/26	$P = 0.008$

Continuous variables expressed as a median/range and qualitative variables as total count.

**Statistical Analysis.** Baseline characteristics of the patients are expressed as median and range or count and proportion. Comparison of patients with HCC and patients with non-HCC nodules was done by using the Student  $t$  test or the Mann-Whitney test for continuous variables and the chi-squared test/Fisher's exact test for categorical variables. A conventional  $P$  value = 0.05 was considered statistically significant. Calculations were done with the SPSS package (SPSS, Inc. 1989-1995, Chicago, IL).

## Results

A total of 89 patients with liver cirrhosis were included. Their characteristics are summarized in Table 1. Median age was 65 years; most had cirrhosis caused by hepatitis C virus infection ( $n = 68$ ; 76.4%) with preserved liver function (Child-Pugh class A: 80). Median baseline alpha-

fetoprotein (AFP) was 8 ng/mL (range, 1-1154) and was greater than 20 ng/mL in 23.2% of patients. Median size of the nodules was 14 mm (range, 7-20); 13 (14.6 %) were smaller than 10 mm, 44 (49.4 %) were 10-15 mm, and 32 (36 %) were 16-20 mm. Most nodules were hypoechoic ( $n = 47$ ; 52.8%). Final diagnosis of the 89 nodules is summarized in Table 2: Sixty patients were diagnosed with HCC (67.4%), whereas 1 was diagnosed with cholangiocarcinoma (1.1%). Twenty-four lesions were classified as regenerative nodules/dysplastic nodules (27%). Three cases corresponded to hemangioma (3.4%) and 1 to focal nodular hyperplasia (1.1%). Patients with nonmalignant nodules were followed for a median of 23 months (range, 4-41) to ensure the benign nature of the nodule.

There were no significant differences between patients diagnosed with HCC and patients with non-HCC nod-

**Table 2. Final Diagnoses of the 89 Nodules**

		<10 mm	11-15 mm	16-20 mm	Total
HCC nodules	HCC	2	29	29	60
	Well-differentiated	0	15	9	24
	Moderately differentiated	2	8	15	25
	Poorly differentiated	0	6	4	10
	Unknown degree	0	0	1	1
Non-HCC nodules	Cholangiocarcinoma	0	1	0	1
	Regenerative/dysplastic nodule	8	13	3	24
	Hemangioma	3	0	0	3
	Focal nodular hyperplasia	0	1	0	1
<b>Total</b>		13 (14.6%)	44 (49.4%)	32 (36%)	89

Size categorized as <10 mm; 10-15 mm, and 16-20 mm.

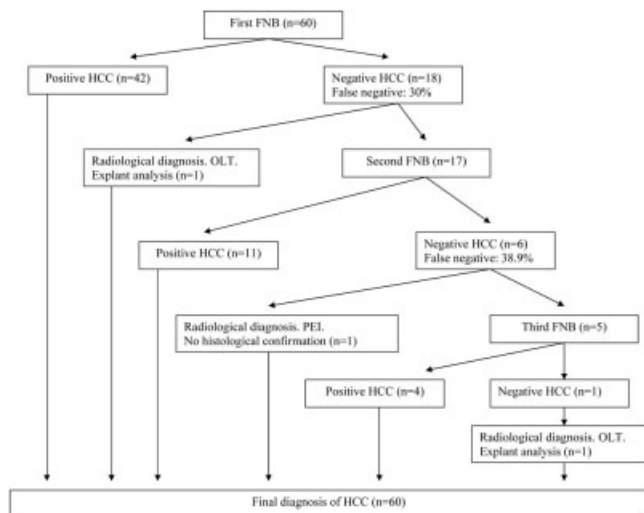


Fig. 2. Accuracy of FNB for HCC diagnosis.

ules in sex, etiology, Child-Pugh class, laboratory parameters, and presence of ascites. Patients with HCC were older (66.5 versus 60 years;  $P < 0.05$ ). The median AFP of HCC patients was 8.5 (range, 1-1154) and 5 (range, 1-170) in the non-HCC cohort. AFP exceeded 20 ng/dL in 24% of the HCC patients, whereas this was registered in 21% of non-HCC patients (nonsignificant difference). There were no differences in the baseline US echogenicity of the nodules. However, 26 of the HCC exhibited a US halo, whereas this was observed in only 3 of the 29 non-HCC nodules ( $P = 0.008$ ). In addition, HCC nodules were significantly larger than non-HCC: only 2 of 13 nodules smaller than 10 mm were diagnosed as HCC, whereas this was the case in 29 of the 32 nodules between 16 and 20 mm ( $P < 0.0001$ ). Twenty-four (40%) of the HCCs were well differentiated, and 25 (42%) were moderately differentiated. In the single HCC case in which repeated biopsies were not diagnostic, the differentiation degree could not be registered. There was no significant association between tumor size and differentiation degree.

**Diagnostic Accuracy of FNB for HCC.** The accuracy of FNB for HCC diagnosis is summarized in Fig. 2. FNB was not obtained in 5 patients because of confident diagnosis by imaging or technical issues related to proper definition of the nodules at the time of puncturing; all of

them were diagnosed as non-HCC nodules (2 hemangiomas and 3 regenerative nodules, 4 of 5 smaller than 10 mm). Accordingly, 84 of the nodules underwent biopsies at least once. In 43 of them, a core-biopsy was obtained together with fine-needle aspiration. The first tissue sampling established the HCC diagnosis in 42 of the 60 cases with HCC. This represents a false-negative rate of 30% at first assessment. A second sampling was done in 17 of these 18 false-negatives, and it established the HCC diagnosis in 11 (61.6%). In 1 patient, the second FNB was not performed because of ascites and liver failure; both CEUS and MRI evidenced nodule growth beyond 2 cm with characteristic dynamic pattern (hypervascularization with washout). The explant analysis demonstrated a poorly differentiated HCC.

One of the 6 patients with 2 consecutive negative biopsies showed a significant growth of the nodule in the dome of the right lobe. Computed tomography and MRI were conclusive for HCC, and ablation was indicated. After initial success, the tumor recurred 9 months later, and diagnosis was thus secured. In 4 cases, HCC was diagnosed at the third biopsy. Median time between the first and third biopsy was 9.6 months (range, 3-23). In all of these cases the third biopsy was indicated because of nodule growth. The patient with 3 negative FNB exhibited nodule growth exceeding 2 cm. Imaging profile was diagnostic for HCC and was listed for transplantation with explant confirmation. There was no significant relationship between diagnostic accuracy of biopsy and nodule size or differentiation degree.

**CEUS and MRI.** Table 3 summarizes the diagnostic accuracy of CEUS and MRI. CEUS was not evaluable in 3 nodules because of lung interposition; these 3 nodules were finally diagnosed as HCC.

Twenty-four of the 89 nodules were hypovascular by both techniques. Only 2 of the 24 (8.3%) hypovascular nodules were finally diagnosed of HCC, whereas 22 of 29 non-HCC nodules were hypovascular at CEUS and MRI.

Considering the suspicious criteria, CEUS detected 47 of 60 HCC (sensitivity = 78.3%). Four of the 29 non-HCC nodules were misdiagnosed as HCC [false-positive rate (FPR) = 13.8%; specificity = 86.2%]. At MRI, 51 of the 60 HCC were properly diagnosed (sensitivity =

Table 3. Diagnosis Accuracy of CEUS and MRI

		Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Likelihood Ratio
CEUS	Suspicious (n = 51)	78.3%	86.2%	92.2%	71.4%	5.682
	Conclusive (n = 33)	51.7%	93.1%	93.9%	50.9%	7.519
MRI	Suspicious (n = 54)	85%	89.7%	94.4%	74.3%	8.264
	Conclusive (n = 38)	61.7%	96.6%	97.4%	54.9%	18.182

Imaging diagnosis categorized as suspicious (arterial hypervascularization regardless of washout) or conclusive (arterial hypervascularization followed by venous washout). The nodules classified as suspicious include those defined as conclusive and those categorized as suggestive but nonconclusive of HCC.

**Table 4. Diagnosis Accuracy of Combined CEUS and MRI**

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
<b>CE-US suspicious MRI suspicious (n = 40)*</b>	66.7%	100%	100%	59.2%
<b>CE-US suspicious MRI conclusive (n = 29)†</b>	48.3%	100%	100%	48.3%
<b>CE-US conclusive MRI suspicious (n = 28)‡</b>	46.7%	100%	100%	47.5%
<b>CE-US conclusive MRI conclusive (n = 20)</b>				
<b>AASLD criteria positive</b>	33.3%	100%	100%	42%

Imaging diagnosis categorized as suspicious (arterial hypervascularization regardless of washout) or conclusive (arterial hypervascularization followed by venous washout). The nodules classified as suspicious include those defined as conclusive and those categorized as suggestive but nonconclusive of HCC.

\*In 3 cases (1 nodule 10–15 mm and 2 nodules 16–20 mm), both techniques suggestive but inconclusive for HCC.

†Nine cases (1 nodule < 10 mm, 3 nodules 10–15 mm, and 5 nodules 16–20 mm); the CEUS was suggestive but inconclusive for HCC.

‡Eight cases (3 nodules 10–15 mm and 5 nodules 16–20 mm); the MRI was suggestive but inconclusive for HCC.

85%), whereas 3 of 29 non-HCC nodules were erroneously classified as HCC (FPR = 10.3%; specificity = 89.7%).

The application of conclusive criteria reduced sensitivity and increased specificity. CEUS accurately diagnosed 31 of the 60 HCC (sensitivity = 51.7%) with specificity raising to 93.9%. A false-positive HCC diagnosis was registered in a 14-mm cholangiocarcinoma and in a 16-mm angioma (FPR = 6.1%). MRI properly characterized both nodules and was able to accurately diagnose 37 of the 60 HCC nodules (sensitivity = 61.7%). There was a single false-positive HCC diagnosis at MRI that was registered in a 12-mm regenerative nodule. This nodule vanished during follow-up that currently goes beyond 30 months (FPR = 2.6%; specificity = 97.4%).

The diagnostic accuracy for HCC diagnosis increased in parallel with nodule size. Thereby, conclusive findings at MRI in nodules larger than 15 mm had a sensitivity of 76% with 100% specificity.

**Evaluation of AASLD Criteria.** Combination of both techniques offers the best accuracy. Table 4 shows the sensitivity and specificity stratifying findings according to type of pattern by both techniques. The sensitivity of coincidental conclusive findings fitting into the AASLD definitions is markedly reduced (33.3%), but specificity reaches 100%. However, if both techniques would at least be suspicious (1 of them could be conclusive and the other not, or both suspicious), the sensitivity ranges between 46.7% and 48.3%. Because there are only 3 nodules with coincidental suggestive findings, the 100% specificity is not robust enough.

The results in nodules smaller than 10 mm are less informative for HCC diagnosis because only 2 of the 13 nodules were finally diagnosed as HCC. The AASLD guidelines recommend a wait-and-see policy in these very small nodules, and our data also support this aspect of the recommendations for HCC screening and diagnosis.

## Discussion

Confident diagnosis of cancer is a critical step before treatment indication. In most of the malignant diseases, diagnosis is based on biopsy sampling, but development of better imaging techniques and specific tumor markers have permitted establishment of the diagnosis of malignancy in the absence of pathology confirmation. These noninvasive diagnostic criteria are relevant for the management of patients with suspicion of HCC when the detected nodule is of small size. Early small HCC are usually composed of well-differentiated hepatocytes,<sup>19</sup> and this turns the confident diagnosis through examination of FNB samples into a pathology challenge. Their reading requires major expertise, and even so, it is usual to assume a high rate of false-negative reports. Accordingly, some unique studies where such false-negative results are not observed have not been reproduced elsewhere and should be looked at with caution.<sup>20</sup> Studies to evaluate the diagnostic capacity of imaging techniques offer limited information because they just include patients with already diagnosed HCC, either by imaging techniques or by biopsy. In that way, the population is biased by excluding those with nontypical imaging or negative biopsy. Such studies merely serve to validate the usefulness of any technique to establish vascularization<sup>21</sup> but provide no data about diagnostic sensitivity and specificity of any technique for the diagnosis of small nodules within a cirrhotic liver. This information is critical to establish reliable diagnostic criteria for HCC, and for this reason we designed this prospective investigation. It included small ( $\leq 2$  cm) solitary nodules detected during follow-up US. These represent the target of screening programs. Larger nodules are easily diagnosed, and their treatment is less effective. Tumor growth beyond 2 to 3 cm is usually associated with microscopic vascular invasion/satellites, which are major predictors of recurrence after initial effective therapy.<sup>22</sup>

In our study, diagnosis was based on pathology even if this would require repeated biopsy. In addition, in pa-

tients without malignant diagnosis despite repeated biopsy we established an active follow-up policy to detect evolutionary changes such as growth or change in imaging pattern. If this would be observed, new biopsy procedures and intensive evaluation would be repeated. As a whole, the study aimed to define the nature of small nodules detected during screening in a cirrhotic liver and to evaluate the diagnostic accuracy of imaging techniques for small HCC, hence aiming to prospectively validate the noninvasive diagnostic criteria as recently proposed by AASLD.

We have recruited a cohort of patients with cirrhosis in whom a solitary nodule 2 cm or smaller was detected during US screening. The prevalence of HCC in our study was 67.4% (60/89), there being no previous prospective studies with similar design against which to compare this figure. Previous investigations have included all nodule sizes or just those nodules that show vascular uptake or those with positive biopsy. In our study, the sole criteria for selection was detection of a solitary nodule 2 cm or smaller, and this makes our investigation highly novel and unique. Patients were prospectively recruited on detection of a nodule between 5 and 20 mm on US screening, and the sole requirement to classify a nodule as a target was its recognition in at least 2 US views from different angles. If we would have recruited patients with multiple nodules, the prevalence of HCC might have been higher and the same would have happened if the US detection of hepatic nodules had only registered those with a highly suspicious imaging appearance. The same would apply if cases had been selected because of an arterial blood flow pattern on baseline US-Doppler or immediately after CEUS. Clearly, detection of such small solitary nodules requires expert explorers, but this request for quality and expertise should be the case in any diagnostic intervention in patients. In addition to HCC nodules, we had 28 benign nodules (most of them classified as regenerative/dysplastic nodules, but also including 1 focal nodular hyperplasia and 3 angioma). Finally, we had a single case of cholangiocarcinoma, a neoplasm that while not as frequent as HCC may also appear in a cirrhotic liver.<sup>23</sup> In summary, our cohort offers a representative and large enough population to offer reliable data.

The study offers several relevant findings. The first of them is the low likelihood of nodules smaller than 10 mm being an HCC (only 2 of 13 nodules in our cohort) that together with the difficulty of obtaining an FNB in these tiny nodules reinforces the recommendation of close follow-up before initiating an active workup proposed by the AASLD. Secondly, as previously known, a first FNB is able to establish HCC in a major proportion of patients (70% in our series). However, in a relevant number, the

diagnosis will require a second or third sampling, either at baseline or during follow-up when some evolutionary change reinforces the suspicion of malignancy. Tumor location and risk considerations prevented both approaches in all cases, and this stresses that the study reflects the difficulties of real clinical practice. Nevertheless, our most important result is that imaging techniques are accurate enough to establish diagnosis without requesting biopsy. Sensitivity and specificity figures are encouraging, but for HCC diagnosis the tool to be used in practice has to exhibit a 100% specificity to avoid false positives. This is why the AASLD criteria were developed, and in our study we unequivocally validate their value.

Our data show that the use of a single technique may raise the wrong diagnosis, and this affects both CEUS and MRI. Not surprisingly, if using only suspicious pattern (arterial contrast uptake regardless washout), the number of false positives (4 for CEUS and 3 for MRI in separate patients) is higher than when using the conclusive definitions (arterial uptake followed by washout). In this last scenario, we had 2 false positives for CEUS (one 14-mm cholangiocarcinoma and one 16-mm angioma) and 1 false positive for MRI (a regenerative nodule that experienced spontaneous necrosis and disappeared during follow-up). When both diagnostic techniques were used together and coincidental findings were requested, the false positives were eliminated. This indicates that even in experienced hands the reading of dynamic imaging techniques in tiny hepatic nodules is not easy, and as a consequence, the prudent guideline requirement to have coincidental findings to establish HCC diagnosis is worth being maintained. If false-positive diagnoses occur in experienced centers, the likelihood for such a mistake will surely be higher in nonreferral settings.<sup>12</sup> It could be argued that MRI specificity in nodules larger than 15 mm was 100%, and hence MRI could be enough in these larger nodules. The same could be argued for the detection of a suggestive pattern by 1 technique while the other was conclusive. The number of cases in each of these instances is limited and until further data are obtained it is advisable to remain on the safe side. The experience accumulated in the United Network for Organ Sharing shows that there is a major need to homogenize the diagnostic and staging criteria for HCC to avoid the misuse of organs.<sup>12</sup> Thus, before proposing a diagnostic policy that would be properly applied in a very limited number of sites, it is preferable to retain a cautious approach and recommend the current guidelines.

Obviously the application of stringent diagnostic criteria results in a limited sensitivity that implies that in up to 67% of the patients with HCC 2 cm or smaller the diagnosis will rely on a positive biopsy. Improvement in

the information offered by imaging techniques may allow the incorporation of new definitions and further reduce the need for biopsy, but these additional parameters are not available. The lack of sensitivity raises an important observation. Some authors have suggested that if a small nodule in a cirrhotic liver does not exhibit contrast uptake in the arterial phase, its malignant nature is ruled out and no additional explorations have to be performed. This concept has been proved wrong.<sup>21</sup> Such nodules may already be HCC, and patients may benefit from treatment leading to long-term cure, an option that might be gone with larger tumor size. If the suspicion of HCC is delayed until detection of arterial vascularization or further growth, the tumor stage is often more advanced. Pathology studies in HCC smaller than 20 mm in size have shown that the risk of microscopic vascular invasion and satellite nodules significantly increases when tumor size exceeds 2 cm and acquires the characteristic arterial blood supply.<sup>19</sup> Hence, the diagnosis of the very early HCC or carcinoma *in situ* entity that has the highest likelihood for cure with resection or ablation requires diagnosis before that afforded by the current capability of imaging techniques.<sup>22</sup> Obviously, diagnosing by biopsy and pathology reading of minute nodules will remain a challenge, and this has prompted several groups facing this problem to develop research programs to investigate new diagnostic tools based on immunostaining,<sup>24</sup> gene expression assessment,<sup>25,26</sup> or protein profiling.<sup>27</sup> Several promising results have been raised through the study of surgical samples, but no validation in biopsy tissues from small tumors is available, and this is where future research will have to focus.

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