Diagnosis of Hepatocellular Carcinoma in Cirrhosis by Dynamic Contrast Imaging: The Importance of Tumor Cell Differentiation

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Dynamic contrast imaging techniques are considered the standard of care for the radiological diagnosis of hepatocellular carcinoma (HCC) in cirrhosis. However, the accuracy of radiological diagnosis depends largely on the degree of arterial hypervascularization, which increases with tumor size. Owing to the interplay and prognostic relevance of tumor vascularization and cell differentiation, we asked ourselves whether tumor grade also affects the outcome of radiological diagnosis. Sixty-two HCCs (47 of which measured 1-2 cm) were consecutively detected in 59 patients with compensated cirrhosis under surveillance with ultrasound and confirmed by way of echo-guided biopsy and concurrent investigations with contrast-enhanced ultrasound (CE-US), computed tomography (CT), and gadolinium magnetic resonance imaging (MRI). Tumor cell differentiation was evaluated using Edmondson-Steiner criteria in liver cores of 0.9-5.0 cm (median 1.6 cm). Eighteen (29%) HCCs were grade I (1.5 cm), 28 (45%) were grade II (1.5 cm), 16 (26%) were grade III (1.8 cm), and none were grade IV. Contrast wash-in and wash-out were concurrently demonstrated in 21 (34%) tumors by way of CE-US, including three (16%) grade I and 18 (41%) grade II-III (P = 0.08); in 32 (52%) tumors by way of CT, including three (16%) grade I and 29 (66%) grade II-III (P = 0.0006); and 28 (47%) tumors by way of MRI, including three grade I (16%) and 25 (57%) grade II-III (P = 0.01). Among 1- to 2-cm tumors, the radiological diagnosis was achieved in two of 16 grade I and 17 of 31 grade II-III tumors (P = 0.006).

Conclusion: Tumor grade, a relevant predictor of disease severity, influences the accuracy of dynamic contrast techniques in the diagnosis of HCC.

SURVEILLANCE WITH ABDOMINAL ULTRASOUND (US) OF PATIENTS WITH CIRRHOSIS, WHO ARE AT RISK OF HEPATOCELULAR CARCINOMA (HCC), IS THE STANDARD OF CARE TO DETECT SMALL, POTENTIALLY CURABLE TUMORS.1 A STANDARDIZED RECALL POLICY FOR LIVER NODULES DETECTED ON US EXAMINATION HAS BEEN ESTABLISHED THAT USES DYNAMIC CONTRAST IMAGING TECHNIQUES TO SHOW THE PATHOGNOMONIC PATTERN OF CONTRAST WASH-IN IN THE ARTERIAL PHASE FOLLOWED BY WASH-OUT IN THE VENOUS PHASE. NODULES THAT ESCAPE RADIOLOGICAL DIAGNOSIS CAN BE INVESTIGATED USING ECHO-GUIDED LIVER BIOPSY AND/OR AS ENHANCED FOLLOW-UP WITH IMAGING.2 Whereas two contrast imaging techniques with concordant wash-in/wash-out patterns are required for the diagnosis of ≤2 cm tumors, contrast-enhanced US (CE-US), spiral computed tomography (CT), or dynamic magnetic resonance imaging (MRI) alone suffices to diagnose >2 cm nodules.2,3 In a validation study performed by Forner and colleagues,4 the concurrent application of...
transplantation.6,7 with HCC, is a contraindication for orthotopic liver patients with HCV-related cirrhosis and, at variance carcinoma, a tumor that is increasingly seen in owing to a discrete number of false positive diagnoses Recently, the accuracy of CE-US has been questioned CT scan in the identification of small HCC nodules.5 showed better diagnostic performance of MRI than investigations by the same group in explanted livers was combined with gadolinium MRI, because previous techniques could be influenced by the degree of tumor cell diagnostic accuracy of dynamic contrast imaging tech- include the so-called very early HCC, are well standar- histology, physical examination, and complete blood count and biochemical tests, including serum alpha-fetoprotein (AFP; normal, ≤ 20 ng/mL) (IRMA; Abbott, North Chicago, IL) and markers for viral hepatitis and autoimmunity. In all patients, abdominal CT, MRI, and CE-US examinations and a US-guided FNB were performed within 2 months of detection of a liver nodule.

**Histology of Liver Nodules.** The diagnostic reference standard was histology. In each patient, an FNB was concurrently performed within the nodule and the surrounding liver parenchyma. The procedure was repeated in all cases with unsolved histological diagnosis (i.e., patients showing similar histological features of cirrhosis within and outside the nodule). A 21-gauge trenchant needle for microhistology (Biomol, HS Hospital Service, Italy) was used, and the diagnosis was made according to International Working Party criteria.10 Formalin-fixed,paraffin-embedded liver sections were examined by an experienced liver pathologist (G. R.) who was unaware of the results of the clinical and radiological examinations. All liver biopsy samples were re-evaluated by a second expert pathologist (M. R.) who was unaware of the clinical, radiologi- and pathological diagnoses. The criteria for diagnosing small and well-differentiated HCCs, which include the so-called very early HCC, are well standard- Table 1 shows the criteria used to distin- guish well-differentiated HCCs from high-grade dys- plastic nodules. Tumor cell differentiation was evaluated according to the Edmondson-Steiner grading system.13 Figure 1 shows the representative histological features of HCC grading of the series under study.

**Vascular Pattern of HCC.** Arterial hypervasculariza- tion (contrast wash-in) was a contrast hyperenhance- ment of the nodule (hyperechogenicity on US, hyperdensity on CT, hyperintensity on MRI) taking place during the arterial phase of the radiological examination, as compared with the surrounding liver parenchyma. Portal/venous contrast wash-out was a hypoen- hanced pattern of the nodule (hypoechochogenicity on US, hypodensity on CT, hypointensity on MRI) with respect to the surrounding liver parenchyma taking place during the portal/venous phase of the radiologi- cal examination. The typical radiological pattern of

| Features                  | High-Grade Dysplastic Nodules | Well-Differentiated HCC |
|---------------------------|-------------------------------|-------------------------|
| Thickness of plates       | Up to 2                       | >2*                     |
| Cell crowding             | 1.5-2                         | >2*                     |
| Uniformly increased N/C ratio** | No                          | Yes                    |
| Irregular thin trabecular pattern | No                          | Yes                    |
| Frequent acinar arrangement | No                           | Yes                    |
| Diffuse steatosis         | Rare                          | Frequent                |
| Stromal invasion          | Never detectable              | May be detectable       |
| Reticulin framework decrease/loss | Never detectable        | May be detectable       |
| Nodule in nodule growth pattern | Never detectable       | May be detectable       |

*Compared with surroundings.
**Nucleus to cytoplasmic ratio.
HCC was the presence of wash-in followed by wash-out of the contrast medium. According to the American Association for the Study of the Liver Disease guidelines, the radiological diagnosis of HCC in 1- to 2-cm HCC was the presence of the typical radiological pattern on two dynamic imaging techniques. For >2-cm nodules, a single dynamic study showing the typical vascular pattern for HCC is required. CT and MRI images were blindly and independently read by two experienced radiologists (L. V. F. and P. B.) who were unaware of the liver biopsy results.

**MRI.** MRI was performed with a 1.5-T system (Avanto; 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 multiphasic contrast-enhanced dynamic three-dimensional MRI of the whole liver with fat suppression. T1-weighted imaging included breath-hold in-phase gradient echo (175/5 TR/TE, 256 × 112 matrix, 70° flip angle) and out-of-phase gradient echo (175/2.38 TR/TE, 256 × 112 matrix, 70° flip angle). T2-weighted imaging included fat suppression sequences (1310/70 TR/TE, 320 × 192 matrix). Dynamic MRI was performed with a three-dimensional volumetric interpolated breath-hold examination sequence in an axial plane using the following parameters: 4.7/2.3 TR/TE, 320 × 157 matrix, 10° flip angle, 3-mm slice thickness. Gadolinium (Gadobenate Dimeglutamine [0.5 mmol/L]; Multihance, Bracco, Milan, Italy) was injected at a dose of 0.2 mL/kg at a rate of 2 mL/second. Arterial phase, portal venous, and delayed venous phase images were acquired approximately 30, 80, and 180 seconds from the start of contrast injection, respectively. A breathhold T1-weighted two-dimensional gradient echo with fat suppression MRI (4.7/2.3 TR/TE, 256 × 157 matrix) and three-dimensional volumetric interpolated breath-hold examination sequences were performed 2 hours after contrast injection (hepatocyte phase).

**CT.** CT was performed with a 64-detector CT scanner (Definition Siemens, Erlangen, Germany) at 2.5-mm slice thickness and a rotation time of 0.5 seconds. A total of 1.5 mg/kg iodinated contrast medium (Iomeron 400; Bracco, Milan, Italy) was injected with...
a 4.0 mL/second flow. In all patients, the acquisition time from the start of contrast injection and the start of acquisition sequences was 40 seconds for the arterial phase, 80 seconds for the portal venous phase, and 180 seconds for the delayed phase. Patients with an unsatisfactory acquisition of arterial phase were to repeat the examination using a bolus tracking technique.

**CE-US.** US studies were performed with a Philips iU22 system (Philips Ultrasound, Bothell, WA), using a multifrequency (5-2 MHz) convex transducer (C5-2). A preliminary gray-scale US examination of the upper abdomen was performed. On identifying the nodule, CE-US was performed with up to two bolus injections of 2.4 mL of a second-generation contrast agent (SonoVue; Bracco, Milan, Italy), having 8-µm microbubbles and stability for 6-8 minutes. The bolus was followed by a 10-mL saline flush. Low mechanical index (<0.1) was set for CE-US examination. Enhancement patterns were studied during the vascular phase for up to 3 minutes, including the arterial phase (0-35 seconds), portal phase (35-120 seconds), and late phase (120-180 seconds). All examinations were obtained and evaluated in real time by two expert echographers (M. F. and S. M.) and digitally stored and documented by a commercially available system or videotapes. Patients with a discrepant result were reevaluated in a dedicated reading session by the two echographers, who were unaware of the liver biopsy results.

**Statistical Analysis.** The baseline characteristics of the patients are expressed as the median and range or count and proportion. Comparisons between the vascular pattern and tumor cell differentiation of the nodules were performed using a Student t test or Mann-Whitney test for continuous variables and Fisher’s exact test for categorical variables. A conventional P value < 0.05 was considered statistically significant. Calculations were performed with the Stata version 10.0 statistical package (Stata 1944-2007, College Station, TX).

**Results**

Sixty-two HCC nodules were detected consecutively in 59 patients with cirrhosis who were under surveillance with US (Table 2). The diagnosis of HCC was histologically confirmed in liver biopsy cores ranging from 0.9 to 5.0 cm (median 1.6 cm). To assess intra-assay variation, 18 tumors (29%) were sampled twice during the same session, and the cores were blindly assessed for tumor cell differentiation by the same pathologist. Thirteen (72%) tumors yielded concordant readings (mean size 1.8 cm, weighted K 0.615), whereas in the five nodules with discordant results (mean size 1.8 cm), the worst grading was considered. Only one of the five discordant HCCs was a grade I versus grade II tumor, whereas the remaining four nodules were discordant for grade II versus grade III.

**Table 2. Demography of the 59 Patients with Compensated Cirrhosis and a De Novo HCC Nodule**

| Characteristic       | Male sex | Age, years | HCV-RNA | HBsAg | Alcohol | Other risk factors | HCC size |   |
|----------------------|----------|------------|---------|--------|---------|-------------------|----------|---|
|                      | 41 (69)  | 66 (44-85) | 42 (71) | 7 (12) | 4 (7)   | 6 (10)            | 0.5-1 cm | 3 (5) |
|                      |          |            |         |        |         |                   | 1-2 cm   | 47 (76) |
|                      |          |            |         |        |         |                   | 2-3 cm   | 12 (19) |

Data are presented as n (%) or median (range). Abbreviations: HBsAg hepatitis B surface antigen; HCV, hepatitis C virus.

**Table 3. Patient Characteristics Stratified According to Tumor Cell Grading (No Grade IV Tumors)**

| Characteristics            | Grade I | Grade II-III | P Value |
|----------------------------|---------|--------------|---------|
| HCC nodule                 | 18 (29%) | 44 (71%)     | –       |
| Male sex                   | 12 (66%) | 32 (73%)     | 0.75    |
| Age, years                 | 70 (52-83) | 64 (44-85) | 0.04    |
| HCV etiology               | 15 (83%) | 30 (68%)     | 0.35    |
| Child-Pugh class A         | 17 (94%) | 42 (95%)     | 1.0     |
| Serum AFP, ng/mL           | 8 (1-353) | 14 (2-2156) | 0.6     |
| Nodule size, cm            | 1.5 (1.1-2.5) | 1.6 (0.8-3.0) | 0.6     |
| 0.5-1 cm                   | 0        | 3            | 0.26    |
| 1-2 cm                     | 16       | 31           | –       |
| >2 cm                      | 2        | 10           | –       |
| Typical vascular pattern on CE-US | 3 (17%) | 18 (41%) | 0.08    |
| Typical vascular pattern on CT | 3 (17%) | 29 (66%) | 0.0006  |
| Typical vascular pattern on MRI | 4 (22%) | 25 (57%) | 0.01    |

Data are presented as n (%) or median (range). Abbreviations: HCV, hepatitis C virus.
and a bone metallic plaque, respectively.

Table 4. Correlation Between Tumor Size and Rates of Typical Vascular Pattern (Wash-in Followed by Wash-Out) for HCC in Contrast Imaging Techniques

| Tumor Size | No. of Nodules | Wash-In + Wash-Out Positives | Radiological Diagnosis of HCC |
|------------|----------------|-----------------------------|-----------------------------|
| 0.5-1 cm   | 3              | 0                           | CE-US                       |
| 1-2 cm     | 47             | 15                          | CT                          |
| >2 cm      | 12             | 6                           | MRI                         |

| *Two patients with HCC not investigated with MRI owing to claustrophobia and a bone metallic plaque, respectively.

Discussion

Tumor grade has clinical implications in HCC, because it correlates with well-established predictors of disease severity and recurrence after surgery, such as number and size of tumor nodules and portal invasion by tumor cells. The present study is the first to evaluate cell grading in small HCC nodules detected during surveillance of patients with cirrhosis, thus adding to the data regarding cell grading in both small and large HCC nodules in surgically resected livers. In our series of early detected tumors, the vast majority (71%) of the nodules were grade II and III, whereas none of the tumors was grade IV. The stratification of cell grading in early HCC nodules investigated before any treatment differs substantially from that reported in surgical specimens, where the HCC nodules were greater in size and more dedifferentiated (42%-60% grade II and III versus 28%-46% grade IV). Although a correlation has been demonstrated between cell grading and volume of the tumor in surgical studies,11 such a correlation was not apparent in our study, which only included HCCs <3 cm. Indeed, the median volume of tumors we investigated was the same across all the grading categories (no patient with grade IV tumors), each volumetric set of HCC (<1 cm, 1-2 cm, >2 cm) containing more grade II and III than grade I tumors. Although we acknowledge that medium to poorly differentiated HCC nodules can be more confidently diagnosed by FNB than well-differentiated tumors, our approach of comparing intranodular and extranodular tissue and the yield of liver cores

Table 5. Rates of Radiological Diagnosis of 1- to 2-cm Tumors with Single or Dual Imaging Techniques According to Tumor Cell Grading

| Imaging Techniques | Grade I | Grade II-III |
|--------------------|---------|-------------|
| CE-US              | 3 (19)  | 12 (39)     |
| CT                 | 3 (19)  | 18 (58)     |
| MRI                | 4 (25)  | 15 (52)*    |
| CE-US + MRI        | 1 (6)   | 6 (21)*     |
| CE-US + CT         | 1 (6)   | 8 (26)      |
| MRI + CT           | 2 (13)  | 11 (38)*    |
| Any dual combination | 2 (13) | 17 (55)     |

*Two patients with HCC not investigated with MRI owing to claustrophobia and a bone metallic plaque, respectively.

Table 6. Variables Associated with Radiological Diagnosis of HCC According to AASLD Criteria

| Features          | HCC Diagnosed (n = 29) | HCC Undiagnosed (n = 33) | P Value |
|-------------------|------------------------|--------------------------|---------|
| Male sex          | 23 (79)                | 20 (61)                  | 0.11    |
| Age >66 years     | 11 (41)                | 18 (55)                  | 0.19    |
| HCV-positive      | 21 (72)                | 24 (72)                  | 0.97    |
| Child-Pugh class A| 28 (97)                | 31 (94)                  | 0.63    |
| AFP >100 ng/mL    | 4 (14)                 | 3 (9)                    | 0.56    |
| Tumor size 1-2 cm | 18 (62)                | 29 (88)                  | 0.035   |
| Tumor grade 1     | 2 (7)                  | 16 (48)                  | 0.0003  |

Data are presented as n (%). Abbreviations: AASLD, American Association for the Study of Liver Diseases; HCV, hepatitis C virus.
of adequate length as those obtained with a trenc
needle, should have reasonably attenuated the risk of underestimation of tumor grade in our study. The lack of concordance we demonstrated in 28% of paired FNB examinations should not have subverted our correlation analysis in small tumors, because only one of the five discordant nodules was grade I versus grade II, whereas the remaining four nodules were discordant for grade II and III, to give a clinically meaningful discordance between paired FNB examinations of 5% only.

A previous study from our group comparing the accuracy of dynamic contrast imaging techniques and FNB to diagnose HCC in cirrhosis allowed us to assess whether tumor cell grading had any influence on the accuracy of dynamic contrast imaging techniques that are endorsed for the noninvasive diagnosis of HCC.9 To maximize the diagnostic accuracy of FNB, we used a 21-gauge trenchant needle for microhistology, resulting in tissue cores of 1.6 cm, on average. Moreover, by sampling all patients for both nodular and extranodular liver parenchyma, the differential diagnosis between low-grade tumors and dysplastic macrolregenerative nodules was eased.23 Finally, to evaluate the sensitivity of the study, a set of patients underwent two intranodule biopsies, and the biopsy specimens were blindly examined by two pathologists who were unaware of the clinical findings.

In our study, the diagnostic accuracy of dynamic contrast imaging techniques appeared to be attenuated in well-differentiated tumors compared with less differentiated tumors. This may have clinical implications, because the current standard of care for the radiological diagnosis of HCC, represented by the combination of CE-US and MRI, has been shown to have a sensitivity of 33.3% and a specificity of 100% in the setting of 0.5- to 2-cm tumors occurring in patients with cirrhosis.4 Similar figures were reported by other studies investigating the combinations of CE-US and CT, having a vaguely nodular appearance and an intact portal tract–based structure.34,35 In the original report, all those tumors were grade I and had a favorable outcome following hepatic resection compared with tumors of similar size with a distinctly nodular pattern that were made out by contrast imaging techniques. The latter tumors were more often dedifferentiated and tended to recur after hepatic resection.35 The paradigm of radiological diagnosis of HCC in cirrhosis rests on the excess unpaired arteries with respect to portal vein branches, which accounts for the typical vascular pattern of wash-in followed by wash-out, a feature that is expected to be increasingly detected in parallel with tumor growth. Our finding of low rates of contrast wash-in followed by wash-out in grade I tumors in general, and in particular in those <2 cm,
speaks in favor of a correlation between tumor cell grading and arterial vascularization of the tumor, even though it is unclear which of these variables drives the prognosis of HCC.\textsuperscript{11} Furthermore, the fact that small tumors not identified by contrast imaging have a benign prognosis ultimately calls for repeat liver biopsy examinations during the time the nodules remain unchanged at imaging, because this approach might help to improve early diagnosis of HCC.

The recent reclassification of small HCC, which resulted from a consensus meeting between eastern and western pathologists, emphasized the role of tumor grading and vascular remodeling in the classification and prognostication of HCC.\textsuperscript{11} Indeed, the most differentiated form of very early HCC, which is usually \(<2\) cm, displays grade I histology and grossly shows the vaguely nodular architecture mentioned before, is unlikely to infiltrate the portal vein system and to disseminate into the liver. Interestingly enough, this tumor is characterized by an incomplete neovascularization, whereby it often escapes detection by contrast imaging.\textsuperscript{2} Conversely, the small but more aggressive early HCC is characterized by a gross nodular architecture, a less differentiated histology, and a complete and extensive arterial neovascularization. The latter, unlike very early HCC, has a less favorable prognosis, because it is able to infiltrate the portal vein system and to disseminate into the liver in 27% and 10% of cases, respectively.\textsuperscript{8}

In conclusion, our study indicates that the accuracy of dynamic contrast imaging techniques to diagnose early HCC in cirrhosis is largely affected not only by the degree of arterial vascularization but also by cell grading of the nodule. Although this observation speaks in favor of a better prognosis for these nodules compared with those readily identified by radiological analysis, it further endorses the need for the histological examination of all small nodules arising in cirrhotic livers that are left undiagnosed by radiology.

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