Differentiation of dysplastic nodules from hepatocellular carcinoma in patients with liver cirrhosis by gadobenate dimeglumine-enhanced MR imaging with dynamic and hepatobiliary phase

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Purpose

To assess whether gadobenate dimeglumine (Gd-BOPTA) - enhanced MR imaging with dynamic and hepatobiliary phase may differentiate dysplastic nodules (DNs) from hepatocellular carcinomas (HCCs) in patients with liver cirrhosis.
Methods and Materials

Study population

This was a single-centre retrospective study. The institutional review board of our hospital approved this retrospective study and waived the requirement for informed consent.

Through a review of the database and records of our radiology department, we retrospectively identified patients with liver cirrhosis who were scanned by Gd-BOPTA-enhanced MR imaging between April 2006 and April 2011. Inclusion criteria for the present study were: hepatocellular nodule diameter ≤ 1 cm and ≤ 3 cm; a diagnosis of HCC or DNs based on histology; availability of multiphasic liver MR studies including unenhanced, hepatic arterial phase (HAP), portal venous phase (PVP), equilibrium (EP) and hepatobiliary phase (HP) imaging. Of the 101 patients who were deemed initially eligible for the study, 26 patients were excluded for the following reasons: (a) history of previous adjuvant treatment such as transcatheter arterial chemoembolization, or radiofrequency ablation prior to histology (n=11 patients); (b) suboptimal preoperative multiphasic liver MR studies due to respiratory artifacts (n=5); (c) lack of histologic confirmation (n=10).

Therefore, we finally included 75 cirrhotic patients (mean age, 55 years ± 12 [standard deviation]; median age, 61 years; age range, 48-78 years) - 47 men (mean age, 65 years ± 11; median age, 68 years; age range, 48-78 years) and 28 women (mean age, 62 years ± 14; median age, 67 years; age range, 55-76 years) -. The difference in median age between the male and female patients was not found to be statistically significant (P < .05, Nonparametric Mann-Whitney U test performed after Shapiro-Wilk test results failed to show a normal distribution for age data). All patients had a definite diagnosis of liver cirrhosis (Child-Turcotte-Pugh class A or B) related to viral infection (hepatitis B [n = 15 patients], hepatitis C [n = 80] or both [n = 10]) or alcohol abuse (n = 10), obtained by means of biopsy or unequivocal imaging findings including irregular liver margins and nodulations.

MR imaging

All patients underwent MR imaging which was performed by a superconducting magnet operating at 1.5 T (Achieva, 1.5T release 2.1.3.4, Philips Medical Systems, Best, The Netherlands) with a peak gradient amplitude of 30 mT/m and a peak slew rate of 150 mT/m. Images were acquired in the transverse plane with a combined four-channel anteroposterior phased-array surface coil. Parallel imaging with a sensitivity-encoding (SENSE) technique with a factor of two was employed. A three-quarter field of view was used in the phase-encoding direction. Presaturation pulses were applied above and below the imaging volume to diminish flow artifacts.
MR imaging parameters are detailed in Table 1.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>T2-w HASTE*</th>
<th>T2-w SPAIR#</th>
<th>T1-w FFE§</th>
<th>T2-w FFE§</th>
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<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<td>Respiratory triggered</td>
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<td>No</td>
<td>No</td>
<td>Yes</td>
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<td>54</td>
<td>18</td>
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<td>Repetition time (msec)</td>
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<td>448</td>
<td>332</td>
<td>350</td>
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<td>1.54</td>
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<td>10</td>
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<td>70</td>
<td>/</td>
<td>/</td>
<td>/</td>
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<td>2</td>
<td>1</td>
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<td>(300-420) x (300-420)</td>
<td>(300-420) x (300-420)</td>
<td>(300-420) x (300-420)</td>
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<td>256 x 256</td>
<td>432 x 432</td>
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<td>-3</td>
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<td>Slice number</td>
<td>30</td>
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<td>30</td>
<td>30</td>
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</tr>
</tbody>
</table>

**Table 1**: Table 1

**References**: Department of Radiology, Cattinara Hospital - Trieste/IT

MR imaging was performed before and after Gd-BOPTA injection (0.1 mmol/kg; 2 mL/sec) through a forearm or antecubital vein at 2 mL/sec through an 18-gauge intravenous catheter by using an automated injector (Spectris MR Injector; Medrad, Indianola, Pa) followed by 20 mL of saline at 2 mL/sec. Between the precontrast and dynamic image acquisitions, an MR fluoroscopic sequence for contrast bolus chase (TR/TE, 4/0.87 msecs, flip angle 40°, slice thickness 80 mm, field-of-view was 530 x 530 mm with a matrix of 256 x 128, acquisition time 0.512 seconds) was performed and yielded a subtracted coronal two-dimensional projection of the abdominal aorta every second. The HAP was started with a 5-seconds delay from the contrast visualization in the abdominal aorta. The PVP, EP, and hepatobiliary phase (HP) initiated respectively at 70 seconds, 180 seconds, and 1 hour after the start of injection of contrast agent. Subtraction of unenhanced images from contrast-enhanced images was performed to improve the detection of lesion enhancement in lesions appearing hyperintense on T1-w sequences. All acquisitions were performed during suspended respiration at end expiration to optimize image coregistration for subtraction algorithms.

**Visual Image analysis**
All MR images were retrospectively assessed in consensus by two abdominal radiologists with 14 and 9 years of experience in hepatobiliary imaging, respectively, who were aware that the patients had been given a histopathologic diagnosis of DN or HCC. Information regarding tumor location was also provided to the reviewers to allow lesion-by-lesion analysis. All readings were performed on a Picture Archiving and Communications System (PACS) - integrated workstation (19-inch TFT display, resolution 2560 x 1600 pixels, Ebit Sanità AET, Genoa, Italy) at a central location. The two readers were free to use processing tools such as windowing, gradation adjustment or magnification and to scroll the MR images. Each pulse MR sequence was evaluated independently per patient (not in a random fashion).

Each lesion was located in a liver segment [16, 17]. Uniform criteria were adopted to define hepatocellular nodule signal intensity on unenhanced MR imaging sequences and vascularity on contrast-enhanced MR imaging sequences. Hepatocellular nodules that were hypointense on to the background hepatic parenchyma T2*-weighted FFE MR sequences were considered siderotic. For analysis of tumor enhancement features, the observers determined the following: (a) the signal intensity of the nodules compared with that of adjacent liver parenchyma on T1-weighted and T2-weighted unenhanced MR imaging sequences; (b) the relative intensity of the nodules compared with that of the adjacent liver parenchyma during all phases (HAP, PVP, EP, and HP); (c) temporal changes in degree of enhancement during the HAP, PVP, and/or EP; and (d) the enhancement pattern in the HAP.

Nodules displaying higher, similar (comparable) or lower intensity compared to the adjacent liver parenchyma (within 3 cm from the outer nodule border) at the visual analysis were defined as hyper-, iso- or hypovascular, respectively. The nodule intensity on arterial phase was assessed on subtracted images in nodule hyperintense on T1-w sequences. The readers determined the presence or absence of six imaging findings known to be suggestive of DN according to univariate analysis (Table 3). Hereafter, the six findings are denoted by the following terms: T2 hyperintensity, fat containing (intranodular fat on dual-echo T1-w FFE sequence), T1 hypointensity, arterial phase hyperintensity, washout, and hepatobiliary phase hypointensity. To reduce learning bias, a set of sample images from six different cases representing the MR findings to be analyzed were shown to the readers in advance. Discrepant interpretations were resolved by consensus through the involvement of an additional reader with similar experience in MR imaging.

Lesions that showed an inhomogeneous enhancement pattern on contrast-enhanced images were categorized according to the intensity of the predominant parts of the lesions (>50%).

Quantitative Image analysis

In addition, to ensure accurate classification of relative lesion intensity, MR intensity was quantified by region of interest (ROI) cursors placed on each and on the liver
parenchyma. One of the authors (with 3 years of experience in hepatobiliary imaging), who was not involved in the visual analysis, measured liver signal intensity, tumor signal intensity, and the SD of the background noise on the HAP, PVP, EP and HP images. For heterogeneous lesions, the ROIs were chosen in more homogeneous areas. Areas of intratumoral necrosis or hemorrhage were excluded from ROI. The mean area of the ROIs for lesions was 0.5 cm$^2$ (range, 0.2-2.4 cm$^2$). The mean area of the ROIs for the normal liver parenchyma was 2.0 cm$^2$ (range, 1.8-2.3 cm$^2$). A difference of more than 10 intensity units between the signal of the tumor and that of the liver was considered important. Background noise was measured just ventral to the right anterior abdominal wall (inside the phased-array coil) along the phase-encoding direction where respiratory or motion-related artifacts were absent. On the basis of values recorded on HAP, PVP, EP, and HP images, the lesion-to-liver contrast-to-noise ratio (CNR) was calculated as follows: lesion signal intensity $\#$ liver signal intensity) / SD of background noise.

**Reference standard**

Within 15 days after MR the percutaneous US-guided biopsy was performed with 18 - 20-gauge modified Menghini needles and stained with hematoxylin/eosin and the Masson trichrome method. A pathologist with 15 years of experience in pathologic examination of hepatobiliary diseases retrospectively reviewed the pathology slides and the macroscopic pictures of the resected specimen with no knowledge of the imaging findings. All specimens were cut, fixed in formalin, embedded in paraaffin, and stained with hematoxylin-eosin. Tumor diameter, tumor differentiation, presence of necrosis, amount of central fibrous stroma, proportion of cellular area, and cholangiolocellular component were determined. All nodules were histologically diagnosed according to the recommendation of the International Working Party [1]. HCCs were classified according to the definition of the World Health Organization (high differentiation, G1; moderate differentiation, G2; and poor differentiation, G3) [18]. In all cases, tumor grade was defined by the poorest degree of differentiation identified within the tumor upon pathologic analysis of the entire specimen.

**Statistical analysis**

A biostatistician participated in the statistical analysis performed by a computer software package (XLSTAT, version 2010.5.08, Addinsoft, NY, USA). To determine the differences in clinicopathologic and radiologic features according to MR imaging enhancement features (e.g. atypical arterially enhancing DNvs vs typically arterially enhancing HCC), the $\chi^2$ test with Yates correction was used.

To assess the effect of the potential risk factors separate logistic regressions [19] were first conducted to determine the relationship between each MR imaging finding, considered as potential predictor variables, and the probability of DN diagnosis. The MR imaging findings included in the analysis were nodule intensity on T1 and T2-w
sequences and nodule intensity after Gd-BOPTA injection on HAP, PVP-EP, and HP. The outcome variable was DN diagnosis. These regressions produced odds ratios (OR) [20, 21] with 95% confidence intervals.

Then, multivariable logistic regression analysis was performed with all MR imaging findings with the highest ORs entered simultaneously into the analysis to assess how well the MR imaging findings jointly predicted the probability of the malignant diagnosis. The goodness of fit of the model was determined by calculating the Nagelkerke $R^2$ and the area under the receiver operating characteristic curve. A high Nagelkerke $R^2$ and high area under the receiver operating characteristic curve values indicate a high goodness of fit. The results of quantitative analysis were analyzed by means of a Mann-Whitney-type test for independent samples. For all tests a $P$ value < .05 was considered to indicate a statistically significant difference.
Results

We retrospectively identified 82 hepatocellular nodules including 57 HCCs (diameter, 2-5 cm) and 25 DNs (diameter, 1-3 cm). The mean HCC diameter was 16.1 ± 6.47 mm (range 10-30 mm), while the mean DNs diameter was 13.6 ± 7.46 cm (range: 1-3 cm). HCCs revealed a well-differentiated pattern (n=46) or a moderately - poorly differentiated pattern (n=11) at histology. DNs revealed a low-grade pattern (n=17) and an high-grade pattern (n=8) on histology.

DNs showed T2 hyper- (5/25), or iso-hypointensity (20/25), and T1 hyper- (18/25), or iso-hypointensity (7/25). DNs showed iron content with evidence of T2* hypointensity (n=3 cases), or intranodular fat content (n=1 case) on dual echo sequence. DNs revealing T2 hyperintensity revealed HAP hyperintensity (n=3) or iso-hypointensity (n=22), PVP-EP hypointensity (n=1) or iso-hyperintensity (n=4), and HP iso-hyperintensity (n=3) or hypointensity (n=2). Those DNs with T2 hyperintensity revealed an high-grade pattern on histology. DNs revealing T1 iso-hyperintensity revealed HAP iso-hyperintensity (n=18) but with evidence of contrast enhancement after image subtraction only in 1 case, and PVP-EP and HP iso-hyperintensity.

HCCs showed T2 hyper- (n=55), or hypointensity (n=2), with evidence of intranodular on dual-echo MR sequence in 10 cases. HCCs revealed HAP hyper- (n=42), iso- (n=8), or hypointensity (n=7), PVP - EP hypo- (n=50) or isointensity (n=7), and HP hypo- (n=51), iso- (n=3), or hyperintensity (n=3).

Univariate Logistic Regression Analysis

Nodule T2 hypointensity with HAP iso-hypointensity (Odds Ratio -OR- 64.07; 95% CIs 12.50 - 328.39; P<.05) or HP iso-hyperintensity (OR 75.12, 95% CIs:12.86 - 438.66; P<.05), or with T1 iso-hypointensity and HAP iso-hypointensity (OR 50.4; 95% CIs 8.47 - 299.71) or T1 iso-hypointensity and HP iso-hyperintensity (OR 56.61; 95% CIs: 8.74 - 366.60) were the most significant imaging findings related to DN diagnosis.

Multivariable Logistic Regression Analysis

On multivariate regression analysis, the Nagelkerke $R^2$ was 0.75-0.85, and the area under the receiver operating characteristic curve was 0.6-0.93, indicating that the fit of the overall model was good. Table 5 shows the diagnostic performance for DN diagnosis of the different variable combinations. For the multivariate model, the average residual squared error was 0.0764.
Based on the results of the univariate and multivariable logistic regression analysis we were able to identify four main patterns of DNs on MR images: (a) T2 iso-hypointensity and HP iso-hyperintensity (Figure 1); (b) T2 iso-hypointensity and HAP iso-hypointensity (Figure 2); (c) T2 iso-hypointensity, T1 iso-hyperintensity and HP iso-hyperintensity (Figure 3). Atypical patterns for DNs include T2 hyperintensity (Figure 4) and HP hypointensity (Figure 5 and Figure 6).

**Quantitative Analysis**

On quantitative analysis (Table 2), the difference between DNs and HCCs in the CNR was found significant only on HP.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>HCC</th>
<th>DN</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAP</td>
<td>27.43±18.2</td>
<td>11.20±15.82</td>
<td>.06</td>
</tr>
<tr>
<td>PVP</td>
<td>4.59±9.80</td>
<td>2.13±6.93</td>
<td>.73</td>
</tr>
<tr>
<td>EP</td>
<td>4.05±11.76</td>
<td>-0.41±5.09</td>
<td>.59</td>
</tr>
<tr>
<td>HP</td>
<td>-6.27±9.11</td>
<td>4.22±7.01</td>
<td>.03</td>
</tr>
</tbody>
</table>

Table 2 - Note: - Results of quantitative analysis.

CNR = contrast-to-noise-ratio; HCC = hepatocellular carcinoma; DN = dysplastic nodule; HAP: arterial phase; PVP: portal venous phase; EP: equilibrium phase; HP: hepatobiliary phase.

* Mann-Whitney U test
Fig. 1: Figure 1a-l. 65-year-old woman with HCV-virus related liver cirrhosis with low-grade dysplastic nodule. (a-d) The nodule (arrow) appears hypointense on T2-weighted fast spin-echo (a) and hyperintense on T1-weighted in-phase (b) and out-of-phase FFE MR imaging sequences (c), and presents a clear siderotic nature on T2-weighted fast field echo [FFE] MR imaging sequences (d). (e-l) MR images obtained by three-dimensional fat-suppressed T1-weighted High Resolution Isotropic Volume Examination (THRIVE) sequence before and after Gd-BOPTA injection (0.1 mmol/kg; 2 mL/sec). (e-g) Before contrast injection (e) the nodule (arrow) appear slightly hyperintense, while after contrast injection during the hepatic arterial phase (f) the nodule appears isointense to the adjacent liver. The image subtraction does not show any nodule enhancement during the hepatic arterial phase (g). The nodule appears isointense during portal venous (h), equilibrium (i), and hepatobiliary phase (l).

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Fig. 2: Figure 2a-g. 63-year-old man with HCV-and HBV-virus related liver cirrhosis and low-grade dysplastic nodule. (a-c) The nodule (arrow) appears isointense on T2-weighted fast spin-echo (a) and T1-weighted in-phase (b) and out-of-phase (c) fast field echo [FFE] MR imaging sequences. (d-g) Contrast-enhanced MR imaging obtained by three-dimensional fat-suppressed T1-weighted High Resolution Isotropic Volume Examination (THRIVE) sequence before and after Gd-BOPTA injection (0.1 mmol/kg; 2 mL/sec). The nodule (arrow) appears hypointense during arterial phase (d), and isointense during portal venous (e) and equilibrium phase (f), and becomes almost isovascular to the adjacent liver during the hepatobiliary phase (g).

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**Fig. 3:** Figure 3a-i. 35-year-old man with HCV-virus related liver cirrhosis and high-grade dysplastic nodule. (a-c) The nodule (arrow) appears hypointense on T2-weighted fast spin-echo (a) and hyperintense on T1-weighted in-phase (b) and out-of-phase FFE MR imaging sequences (c). (d-l) MR images obtained by three-dimensional fat-suppressed T1-weighted High Resolution Isotropic Volume Examination (THRIVE) sequence before and after Gd-BOPTA injection (0.1 mmol/kg; 2 mL/sec). Before contrast injection (d) the nodule (arrow) appear slightly hyperintense, while after contrast injection during the hepatic arterial phase (e) the nodule appears isointense to the adjacent liver. The image subtraction does not show any nodule enhancement (f). The nodule appears isointense during portal venous (g), equilibrium (h), and hepatobiliary phase (i).
**Fig. 4:** Figure 4a-h. 55-year-old man with HCV-virus related liver cirrhosis and high-grade dysplastic nodule. (a-d) The nodule (arrow) appears hyperintense on T2-weighted half-Fourier fast spin-echo (a) and T2-weighted FFE MR imaging sequence (b), and hyperintense on T1-weighted in-phase (c) and out-of-phase (d) fast field echo [FFE] MR imaging sequences. (e-h) Contrast-enhanced MR imaging obtained by three-dimensional fat-suppressed T1-weighted High Resolution Isotropic Volume Examination (THRIVE) sequence before and after Gd-BOPTA injection (0.1 mmol/kg; 2 mL/sec). The nodule (arrow) appears hypointense during arterial (e), portal venous phase (f), and equilibrium phase (g), and becomes hyperintense to the adjacent liver during the hepatobiliary phase (h).

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Fig. 5: Figure 5a-g. 60-year-old woman with HCV-virus related liver cirrhosis and low-grade dysplastic nodule. (a-c) Unenhanced MR images. The nodule (arrow) appears typically hyperintense on T1-weighted in-phase (a) and out-of-phase FFE MR imaging sequences (b) and is not visible on T2-w image. (d-g) Contrast-enhanced MR imaging obtained by three-dimensional fat-suppressed T1-weighted High Resolution Isotropic Volume Examination (THRIVE) sequence acquisition before and after Gd-BOPTA injection (0.1 mmol/kg; 2 mL/sec). The nodule (arrow) appears slightly hyperintense during the hepatic arterial phase (d), and appears isointense during portal venous phase (e), and hypointense during the equilibrium (f) and hepatobiliary phase (g).

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**Fig. 6:** Figure 6a-f. 50-year-old man with HCV-virus related liver cirrhosis and low-grade dysplastic nodule. (a, b) Unenhanced MR images. The nodule (arrow) appears hyperintense on T1-weighted in-phase (a) and hypointense on the out-of-phase FFE MR imaging sequences (b) revealing a microscopic fat content, and appearing hypointense on T2-weighted MR images (c). (d-f) Contrast-enhanced MR imaging obtained by three-dimensional fat-suppressed T1-weighted High Resolution Isotropic Volume Examination (THRIVE) sequence before and after Gd-BOPTA injection (0.1 mmol/kg; 2 mL/sec). The nodule (arrow) appears isointense to the adjacent liver during the hepatic arterial phase (d), and appears hypointense during portal venous phase (e), and hepatobiliary phase (f).

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Conclusion

The combination of nodule appearance on T2-weighted MR imaging and nodule enhancement after contrast injection may identify some patterns related to DN diagnosis on Gd-BOPTA-enhanced MR imaging.
References


31) Sun HY, Lee JM, Shin CI, et al. Gadoxetic acid-enhanced magnetic resonance imaging for differentiating small hepatocellular carcinoma (< or = 2 cm in diameter) from arterial enhancing pseudolesions: special emphasis on hepatobiliary phase imaging. Invest Radiol 2010; 45 (2): 96 - 103


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