Analysis of Liver Tumors Using Preoperative and Intraoperative Contrast-Enhanced Ultrasound (CEUS/IOCEUS) by Radiologists in Comparison to Magnetic Resonance Imaging and Histopathology

Analyse von Lebertumorentitäten mittels präoperativer und intraoperativer Kontrastmittelsonografie (CEUS/IOCEUS) durch Radiologen im Vergleich zur Magnetresonanztomografie und zur Histopathologie

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Key words
CEUS, liver tumor, MRI, histopathology, IOCEUS

received 24.3.2016
accepted 29.11.2016

Bibliography
DOI http://dx.doi.org/10.1055/s-0042-124347
Published online: 2017 | Fortschr Röntgenstr 2017; 189: 431–440
© Georg Thieme Verlag KG Stuttgart · New York
ISSN 1438-9029

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ABSTRACT

Purpose To evaluate the diagnostic significance of preoperatively and intraoperatively performed contrast-enhanced ultrasound (CEUS/IOCEUS) in the diagnosis of liver tumors in comparison to magnetic resonance imaging (MRI) and histopathology.

Materials and Methods Retrospective analysis of 70/317 patients who underwent surgery for liver tumors between January 2012 and October 2015. Findings of CEUS and IOCEUS were compared to MRI. CEUS and IOCEUS were performed using multifrequency linear probes (1–5, 6–15 MHz) after bolus injection of 1–5 ml sulfur hexafluoride microbubbles. The histopathology after surgical resection, MRI morphology (T1, T2, VIBE, diffusion sequences) and wash-in/wash-out kinetics of CEUS were evaluated.

Results In 70 analyzed patient cases, 64 malignant liver lesions could be detected. 6 patients had benign liver lesions. Among the 64 malignant lesions, there were 28 metastases, 24 hepatocellular carcinomas (HCC), 9 cholangiocellular carcinomas (CCC) and 3 gallbladder carcinomas. 2 of the 6 benign liver lesions were hemangiomas, 2 were adenomas, 1 was an FNH and 1 was a complicated cyst. There was no significant difference when determining the lesion’s malignancy/benignity (p = 1.000). Furthermore, there was no statistical significance between preoperative CEUS and MRI regarding the general differential diagnosis of a tumor (p = 0.210) and the differential diagnosis classification between HCCs (p = 0.453) and metastases (p = 0.250). There was no statistical significance in tumor size (10 mm – 151 mm; mean 49 mm SD +/− 31 mm) and location (tumor size p = 0.579; allocation to liver lobes p = 0.132; segment diagnosis p = 0.121) between preoperatively performed CEUS and MRI. The combination of preoperative MRI and CEUS for lesion detection showed significant differences compared to CEUS or MRI only (p < 0.001 for CEUS; p = 0.004 for MRI). IOCEUS offered the substantial advantage of locating additional liver lesions (p = 0.004 compared to preoperative MRI, p = 0.002 compared to preoperative CEUS). In 10/37 cases (27%) IOCEUS could locate further liver lesions which had not been identified during CEUS and/or MRI preoperatively, so that operative therapy was adapted accordingly and resection was extended if necessary.

Conclusion CEUS proves to be a dynamic imaging method for preoperative diagnosis of liver tumors showing high diagnostic significance in the characterization of a tumor’s micro-
Introduction

To perform liver surgery successfully, even the smallest tumors must be reliably detected and characterized. Modern cross-sectional imaging methods, particularly MRI with liver-specific contrast agent, have greatly improved the detection and characterization of liver tumors even in our own workgroups [1, 2]. Numerous small lesions are visualized with MRI prior to surgery [2]. However, these lesions often require contrast-enhanced ultrasound (CEUS) for detection and characterization [3–11], but CEUS only achieves high diagnostic accuracy when performed by an experienced examiner [12–14].

Palpable tumors are additionally evaluated intraoperatively with ultrasound to decide whether resection of malignant lesions is necessary or whether a lesion appears benign [15].

In the case of deep lesions, it is often not possible to obtain findings via palpation. In the case of smaller non-cystic lesions, B-mode ultrasound alone is often not diagnostic [7, 16].

A DEGUM study showed the value of transabdominal liver tumor imaging with CEUS with a diagnostic accuracy for CEUS of >90% [3–5].

Although intraoperative CEUS has been possible for over 10 years [17], it is only performed in individual cases by experienced examiners [15].

Inclusion of an experienced radiologist in a liver surgery team is valuable for helping to make decisions in critical cases regarding expanded resection due to tumor location and characterization based on intraoperative CEUS (IOCEUS) in combination with the preoperative diagnosis based on MRI using liver-specific contrast agent [18, 19].

The goal of the following studies is to determine the value of IOCEUS performed by an experienced radiologist compared to histology and preoperative imaging.

Materials and Methods

This retrospective study is based on 70 cases of patients who underwent partial liver resection between January 2012 and October 2015 at the University Hospital Regensburg. These 70 cases were selected from a total of 317 cases of patients who underwent partial liver resection in the indicated time period (Fig. 1).

247 cases were not included for the following reasons: Surgery was not indicated by a tumor in 24 cases. The histological finding was not definitive in 21 cases. 111 cases with no determination of clear malignancy were excluded based on the CEUS diagnosis. Instead of complementary imaging with CEUS and MRI, CT was used as the reference imaging method in 91 cases.

The inclusion criterion for the 70 selected cases was a tumor finding suspicious for malignancy as the indication for surgery with availability of preoperative CEUS and MRI findings for determining tumor status as complementary imaging. Moreover, a

Citation Format

histopathological finding had to be available as the gold standard for determining the status of liver tumors. Imaging was supplemented by IOCEUS in 42 cases.

Written informed consent was available for each examination. Every case was discussed regarding a surgical therapy decision in an interdisciplinary tumor conference. Approval of the local ethics commission (University Regensburg) was obtained for the study.

Imaging techniques and examination procedure

**CEUS** In preoperative CEUS, 1.0 ml to 2.4 ml contrast agent consisting of sulfur hexafluoride microbubbles (SonoVue®, BRACCO) as a bolus with 10 ml NaCl were injected intravenously. In IOCEUS larger contrast agent quantities of 5 – 15 ml SonoVue® were used. The higher intraoperative dose can be attributed to an increased destruction rate of the microbubbles in ventilated patients in connection with the oxygen content of the inhalation gas [20]. The correlation to other protocols also yielded a higher contrast dose under surgical conditions [19].

High-end ultrasound systems (LOGIQ E9/GE and Ascendus/Hitachi) were used. Preoperative CEUS examinations were performed with convex multifrequency transducers (C1 – 5 MHz, C1 – 6 MHz), while multifrequency linear transducers (9 L transducer, 6 – 9 MHz; ML15 transducer, 6 – 15 MHz) with virtual adjustable convexity were used for IOCEUS. B-mode examination was followed by color-coded Doppler examination and power Doppler examination. CEUS was performed using a low MI technique (mechanical index <0.16) with amplitude modulation and phase inversion harmonic imaging (PIHI). Loops were documented in the arterial phase (15 – 45 seconds), the portal venous phase (46 – 90 seconds), the venous phase (91 – 120 seconds), and the late phase (3 – 5 minutes). In particular, the constellation of irregular early arterial hypervasculization and wash-out beginning in the portal venous phase and increasing to the late phase was considered a main criterion for malignancy [15]. To differentiate HCCs from CCCs, the contrast agent dynamics were evaluated. In concordance with the current literature [21], HCCs show irregular hypervasculization in the arterial phase, contrast enhancement to the portal venous phase, and wash-out in the late phase. CCCs show contrast enhancement in the arterial phase at the margins and central hypoenhancement. Increasing wash-out with portal venous hypoenhancement to non-enhancement in the late phase is seen in CCCs.

**MRI** All MRI examinations were performed with a gadoxetate disodium solution (Primovist®, BAYER) as the contrast agent with 5 ml to 20 ml being injected based on weight. A 3 T MRI system (Magneto Skyra, Siemens) was used. Imaging was performed using the VIBE 3D technique (Volume Interpolated Breathhold Examination) from the dynamic arterial phase (15 – 45 seconds) to the late phase (10 – 15 minutes). T1 and T2-weighted images with axial and coronal slices and a slice thickness of 5 mm were acquired. Adverse reactions to the contrast agent did not occur. However, one MRI examination had to be ended early due to claustrophobia so that only limited assessment was possible.

**Examiner and interpretation of images**

Preoperative contrast-enhanced ultrasound was performed by one experienced examiner (3000 ultrasound examinations per year for more than 10 years) at the interdisciplinary ultrasound center of the University Hospital Regensburg.

An experienced radiologist was responsible for the interpretation of all CEUS examinations. 1 of 5 surgeons specialized in liver surgery at the University Hospital Regensburg participated in each of the CEUS examinations.

An experienced MRI radiologist was responsible for interpreting the MRI examinations in consensus with various assistants.

**Statistical analysis**

The software SPSS (version 22.0, SPSS Inc., Chicago, USA) was used to create a database and for statistical evaluation. The significance analysis was performed with the McNemar and Wilcoxon tests and paired t-test. Values of $p < 0.05$ were considered significant.

**Results**

**Included cases and histopathology**

In the 70 included patients (43 men, 27 women, age 29 – 83 years, mean 62.9 SD +/- 11.9 years), the tissue for histopathological examination was obtained surgically.

64 malignant and 6 benign tumors were confirmed histopathologically (**Fig. 2**). The malignant tumors included 28 metas-
tases (including 25 with a colorectal primary tumor; 1 neuroendocrine tumor, 1 case of nasopharyngeal carcinoma, 1 case of breast cancer as the primary tumor), 24 HCCs, 9 CCCs, and 3 cases of gallbladder cancer. The group of benign lesions included 2 adenomas, 2 hemangiomas, 1 FNH, and 1 cyst. The size of the 70 tumors ranged from 10 mm to 151 mm with a mean of 49 mm SD +/- 31 mm.

For malignant tumors, the indication for surgery was always a high-grade suspicion of a malignant tumor in the liver with a curative intention (n = 64). 3 benign tumors were resected due to the size of the lesion (2 hemangiomas, 1 FNH > 100 mm). 3 benign tumors were incorrectly described as suspicious for malignancy based on the preoperative imaging resulting in a decision to perform surgical resection (2 adenomas, 1 complicated cyst). MRI classified both adenomas and the complicated cyst as malignant. CEUS characterized one of the two adenomas as suspicious for malignancy.

Determination of tumor status

63/70 lesions were correctly classified as malignant or benign based on CEUS. Therefore, a sensitivity of 90 % was calculated for CEUS. 7/70 diagnoses were assessed as "incorrect", including 6 cases in which the tumor status could not be clearly identified based on CEUS (Table 1).

The information provided by MRI as a complementary imaging method correlated with histology in 64/70 cases resulting in a sensitivity of 91.4 % for MRI. In 6/70 cases the MRI diagnosis was not identical with the histopathological diagnosis with the tumor entity not being defined in 3/70 cases (Table 2). No malignant lesion was incorrectly classified as benign on CEUS or MRI.

Malignant lesions

The test quality criteria for malignant lesions were as follows: For CEUS a sensitivity of 90.6 %, a positive predictive value (PPV) of 98.3 %, a specificity of 83.3 %, and a negative predictive value (NPV) of 83.3 %. MRI reached a sensitivity of 96.8 %, a PPV of 98.4 %, a specificity of 33.3 %, and an NPV of 33.3 %.

A significant difference between CEUS and MRI could not be determined (p = 1.000). Thus, the imaging methods were comparable with respect to the determination of tumor status (Fig. 3, Table 3).

Differentiation of tumors

With respect to the differentiation of tumors, the CEUS diagnosis corresponded with the histopathological diagnosis in 49/70 cases. This resulted in a sensitivity of 70 % for differential diagnosis. CEUS differential diagnosis was evaluated as "false" in 21/70 cases, while there was no differential diagnosis in 16/70 cases. MRI was correct in 55/70 cases, without a diagnosis in 9/70 cases and "false" in 15/70 cases. The sensitivity of MRI for the differentiation of tumors was thus 78.6 %. No significant difference in the diagnostic value for differential diagnosis could be determined (p = 0.210) (Fig. 3, Table 3).

In the evaluation of wrong diagnoses, 3 CCCs were classified incorrectly with the differentiation from metastases on CEUS and MRI being incorrect (Table 4). Clear differentiation between HCC and CCC was not possible on MRI in 2 cases (Table 4).

For the case groups HCC and metastasis, a separate comparison between CEUS and MRI was performed (Fig. 3, Table 3): HCC Among 24 cases of HCC, CEUS diagnosed 15/24 cases correctly (sensitivity 62.5 %). No diagnosis was made in 9/24 cases. There were no wrong diagnoses. MRI detected an HCC...
Correctly in 18/24 cases (sensitivity 75.0 %), did not yield a diagnosis in 5/24 cases, and diagnosed one case incorrectly (HCC incorrectly classified as CCC). There was no significant difference between CEUS and MRI regarding HCC diagnosis (p = 0.453).

Metastases In the case of 28 histologically confirmed metastases, CEUS was able to correctly detect 25/28 tumors, while MRI was able to identify all 28 tumors. The sensitivity of CEUS was thus 89.2 % and the sensitivity of MRI was 100 %. In 3/28 cases, no differential diagnosis was made based on the CEUS findings. There was no significant difference regarding the correct differential diagnosis of liver metastases (p = 0.250).

Tumor size

There was an average deviation regarding tumor size of +/- 3 mm for CEUS and +/- 4 mm for MRI. The macro-pathological measurement as the gold standard was used as the basis. The difference regarding the measurement of lesions with CEUS and MRI was

<table>
<thead>
<tr>
<th>analysis aspect</th>
<th>no. of cases in which an analysis was possible</th>
<th>number of correct CEUS diagnoses</th>
<th>number of correct MRI diagnoses</th>
<th>p-value</th>
<th>significant difference between CEUS and MRI?</th>
</tr>
</thead>
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<tr>
<td>tumor status</td>
<td>70</td>
<td>63 (90.0 %)</td>
<td>64 (91.4 %)</td>
<td>1.000</td>
<td>no</td>
</tr>
<tr>
<td>differential diagnosis</td>
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<td>49 (70.0 %)</td>
<td>55 (78.6 %)</td>
<td>0.210</td>
<td>no</td>
</tr>
<tr>
<td>HCC lesions</td>
<td>24</td>
<td>15 (62.5 %)</td>
<td>18 (89.2 %)</td>
<td>0.453</td>
<td>no</td>
</tr>
<tr>
<td>Metastases</td>
<td>28</td>
<td>25 (75.0 %)</td>
<td>28 (100 %)</td>
<td>0.250</td>
<td>no</td>
</tr>
</tbody>
</table>

**Fig. 3** Graphic display of the sensitivity of CEUS in comparison to MRI. In terms of the determination of malignancy/benignity, CEUS had a sensitivity of 90 % and MRI had a sensitivity of 91.4 %. For differential diagnosis, the sensitivity of CEUS was 70.0 %, while the sensitivity of MRI was 78.6 %. For the differential diagnoses “HCC” and “metastasis”, a sensitivity of 62.5 % and 89.2 %, respectively, was achieved for CEUS, while a sensitivity of 75.0 % and 100 %, respectively, was achieved for MRI.

**Table 3** Comparison of MRI with CEUS in terms of determination of benignity/malignancy, differential diagnosis as well as determination of HCCs and metastases (McNemar test).


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not significant according to the t-test for two paired samples (p = 0.579).

Location in relation to the liver lobes
In 68 cases the findings were able to be evaluated with respect to the location of the lesions ("only one or both liver lobes affected?"). The histopathological finding was used as the standard for determining correct assessment by CEUS and MRI. The results of preoperative CEUS and MRI corresponded to histopathology in 57/68 cases (83.8%). In 8/68 cases in which preoperative CEUS assumed lesions in only one liver lobe, MRI showed tumor in both liver lobes. In 3/68 cases in which MRI classified one liver lobe as tumor-free, preoperative CEUS disproved that finding (Fig. 5). A significant difference between CEUS and MRI was not determined (p = 0.132).

Intraoperative ultrasound confirmed whether one or both lobes were affected. In 42/70 cases in which IOCEUS examination was performed in addition to CEUS and MRI, there were only 2 cases in which the CEUS findings differed from the location of the tumor determined by IOCEUS and only 1 case in which the MRI findings differed from the IOCEUS results (4.7% and 2.3%, respectively).

Segmental location
In 53 cases segment data was available both in the preoperative CEUS findings and in the MRI findings. The correct location was determined from the resection decision and the subsequent histopathological evaluation. CEUS and MRI were evaluated based on whether they were able to detect a segment containing a lesion subsequently confirmed by histology. In 14/53 cases ultrasound was able to diagnose ≥ 1 additional segments than MRI as having "malignant infiltration" (26.4%). MRI was able to detect more segments affected by a tumor than ultrasound in 19/53 cases (35.8%) (Table 5). There was no significant difference between CEUS and MRI regarding segment diagnosis (p = 0.121).

Lesion detection
For the evaluation of lesion detection, the correct number of lesions was the maximum number visualized by an imaging modality and confirmed by histology. The number of lesions was simply specified as "multiple" in 7/70 cases on preoperative CEUS and in 8/70 cases on MRI. A concrete number of lesions were specified in the remaining cases. In 33/70 cases, both imaging methods detected the same number of lesions. In 16/70 cases, MRI identified more lesions, while ultrasound identified more lesions in 9/70 cases. However, there was no significant superiority of MRI over CEUS (p = 0.083) (Table 5).

Since the examination modalities are comparable with respect to lesion detection, the value of the preoperative use of a combination of MRI and CEUS was analyzed in the following. Using the combination of imaging methods, more lesions could be detected in 9/70 cases than with MRI and in 16/70 cases than with CEUS. The addition of complementary preoperative imaging yielded a significant advantage both for CEUS and MRI (p < 0.001 for CEUS and p = 0.004 for MRI).

Preoperative CEUS imaging was supplemented by IOCEUS in 42/70 cases. Lesions < 10 mm could not be visualized intraoperatively on B-mode imaging, thus necessitating the use of CEUS.

One lesion previously identified on preoperative CEUS could not be visualized in two cases. In all other cases, all preoperatively known lesions could also be detected during intraoperative ultrasound examination.

In 37/70 cases a comparison between intraoperative ultrasound and preoperative MRI regarding lesion detection was possible. All lesions visualized on MRI were identified. Lesions that could not be diagnosed on MRI were additionally seen in 10/37 cases (27%) (Table 5). In the evaluation, the number and segment allocation of the tumor lesions based on IOCEUS were correlated to the histopathological finding and confirmed based on size and location.

IOCEUS proved to be advantageous on a statistically significant level (p = 0.004) for the detection of tumor lesions not preoperatively visualized by MRI. A significant superiority of IOCEUS regarding lesion detection was also able to be determined compared to preoperative CEUS examination (p = 0.002). The surgical therapy decision was modified based on the IOCEUS diagnosis in all 10/37 cases in which lesions were detected in addition to those found on preoperative MRI and the resection was expanded.

Discussion
Our current study highlights the importance of the presence of a radiologist with CEUS experience for performing intraoperative ultrasound to ensure optimized liver surgery. It was also able to be shown that MRI and preoperative contrast-enhanced ultrasound are comparable in the diagnosis of liver tumors regarding tumor entity, tumor size, and tumor location. There were no significant differences regarding detection, localization, determination of tumor status, and differential diagnosis between CEUS and MRI. Only IOCEUS provided a significant advantage with respect to the detection of lesions that could not be diagnosed by MRI and preoperative CEUS, resulting in modification of the surgical therapy decision and expansion of the resection in 10/37 cases (27%).

The largest and most comprehensive analysis of CEUS and the diagnosis of liver tumors is the multicenter, prospective DEGUM study [3 – 5, 13]. It was able to be shown that CEUS has high diagnostic value for all benign and malignant liver tumor entities.
Histology was obtained whenever possible. The main criterion for the detection of malignant lesions was the combination of irregular arterial vascularization and increasing wash-out to the portal venous phase. Depending on the type of tumor, diagnostic accuracy of up to 100% was able to be achieved, while accuracy of up to >80% was achieved for most cases of HCC and CCC [3–5, 13].

The main criterion for benign lesions was nodular, centripetal/centrifugal contrast enhancement usually with a lack of contrast enhancement in the late phase. Diagnostic accuracy of 100% should be achieved in the case of benign lesions. However, irregularly vascularized adenomas, atypical partially thrombosed hemangiomas, and small regenerative nodules in the case of cirrhosis were difficult to classify [10]. Differential diagnosis can even be difficult on MRI in such cases regardless of their benign genesis. Thus, in cases of doubt they require histological confirmation via biopsy or follow-up.
Considering all tumor entities, the DEGUM study showed: CEUS is equivalent to contrast-enhanced CT of the liver and can provide advantages in the detection of small lesions (< 10 mm) [22]. However, the comparison to MRI was not performed with liver-specific contrast agent in all cases [3–5]. This highlights the importance of this study in which the comparison to MRI involves the use of liver-specific contrast agent (Primovist®, BAYER) in every case.

The results of this study have been confirmed by other current studies [1, 6–8, 10, 12, 15, 18]. CEUS was able to achieve a sensitivity of > 80 % for the differentiation between malignant and benign lesions in all specified studies. Studies that take differential diagnosis into consideration were able to identify advantages of CEUS particularly with respect to HCC diagnosis [22] as well as in cirrhotic liver tissue [8, 9]. A significant advantage of CEUS was shown for the diagnosis of colorectal liver metastases [11, 15] and for diagnostic imaging during/after chemotherapy [19, 22] and after ablation therapy [22, 23]. In studies using CT and MRI for comparison, no significant differences regarding the determination of tumor status were able to be shown [12].

Analyses examining intraoperative contrast-enhanced ultrasound showed the possibilities of IOCEUS for detecting additional tumors and small lesions (< 10 mm) [8, 15, 18, 19]. This was not possible in B-mode.

Intraoperative determination of the status of tumors was also important. This could only be assessed intraoperatively by CEUS, which highlights the need for intraoperative contrast administration. IOCEUS proved to have high diagnostic significance particularly in patients who underwent preoperative chemotherapy [24]. This study was able to confirm the results of other studies also in this regard in that it showed a significant advantage of IOCEUS for resection decisions compared to all preoperative imaging modalities [15, 19].

**Limitations of the study design**

There are limitations of a retrospective study design, for example with respect to data collection and the analysis of causal relationships.
Limitations of CEUS  Ultrasound is limited with respect to topographic regions of the liver that are also difficult to visualize on intraoperative CEUS in some cases (subdiaphragmatic segment VIII). Moreover, special ultrasound devices and a highly experienced examiner are needed to acquire high-quality contrast-enhanced ultrasound scans. In addition, ultrasound is a depth-dependent imaging modality that can reach its limits particularly in overweight patients. IOCEUS can ensure good visualization of the liver even in overweight patients but is subject to time constraints due to the surgical situation.

Limitations of MRI  MRI is dependent on the imaging technology and the field strength. Breathing artifacts can affect image quality particularly in liver diagnosis as a result of the proximity to the diaphragm. Because it is a time-intensive examination, good patient compliance is necessary. Moreover, absolute contraindications must be taken into consideration (i.e., older generation pacemakers, magnetizable objects) and contraindications in relation to gadolinium-containing intravenous contrast agent (from nephropathies to the rarely occurring nephrogenic systemic fibrosis) [24].

Finally a particularly valuable aspect of this study is that a comparative analysis of the imaging modalities CEUS and MRI in relation to liver tumors was only performed in cases in which the gold standard of a histopathological tumor diagnosis was available. Since this constellation only applied in 70 of 317 cases, this explains the low number of included cases but ensures the high quality and significance of this analysis. Moreover, not only preoperative imaging but also intraoperative imaging was included in this study, thus representing a further advantage. Based on the results of this study, a combination of CEUS and MRI with liver-specific contrast agent is recommended in the case of suspicion of a malignant tumor in the liver. Intraoperative CEUS can be an important part of resection planning.

Further prospective studies are needed to prove the value of intraoperative CEUS imaging in liver surgery.

Conclusion

In the preoperative diagnosis of liver tumors, CEUS is a dynamic imaging method with high diagnostic significance regarding dynamic tumor vascularization for evaluating the entity, size, and location of liver tumors. A combination of MRI and CEUS is highly advantageous for the detection of lesions. Intraoperative ultrasound with CEUS provides a significant advantage for the detection of lesions not diagnosed preoperatively and thus makes an important contribution to surgical therapy decisions.

CLINICAL RELEVANCE OF THE STUDY

- CEUS and MRI with liver-specific contrast agent (Primovist®) are equivalent preoperative imaging modalities with respect to the detection and status evaluation of liver tumors.
- Preoperative complementary imaging with MRI supplemented by CEUS is highly advantageous for detecting liver lesions compared to the use of only one of the two methods.
- IOCEUS can detect lesions and their dimensions not visualized by preoperative imaging when performed by an experienced examiner so that resection can be expanded as needed.

Conflict of Interest

No conflict of interest has been declared by the author(s).

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