Abstract. Many hepatocellular carcinoma (HCC) patients do not qualify for curative surgical intervention and are instead treated with locoregional therapies (LRTs) including ablative and endovascular therapies. Assessment of imaging response is essential in the management of HCC for determining efficacy of therapy and as a surrogate marker for improved survival. The established morphological image biomarkers for tumor burden measurement continue to be applied, as size measurement can easily be used in clinical practice. However, in the setting of liver-directed LRTs for HCC, simple tumor morphological changes can be less informative and usually appear later than biologic changes. Functional imaging (such as perfusion and diffusion imaging, PET-CT/MR and MR spectroscopy) has the potential to be a promising technique for assessment of HCC response to LRTs. Although promising, none of these functional imaging biomarkers have gone through all the required steps of standardization and validation and established accepted criteria for clinical practice.

1. Introduction

Liver cancer is a highly prevalent disease worldwide and one of the leading causes of cancer death in the world (1). An estimated 782,500 new liver cancer cases and 745,500 deaths occurred worldwide during 2012, with China alone accounting for approximately 50% of the total number of cases and deaths. Despite the declining rate for liver cancer in China, population growth and ageing still led to a large and rising number of new cases in 2015 (2). Most (70-90%) primary liver cancers occurring worldwide are hepatocellular carcinoma (HCC). Because of the presence of advanced disease or poor liver function, many HCC patients are not candidates for curative surgical treatments (resection or transplantation). These patients may be eligible for treatment with locoregional therapies (LRTs) including ablative and endovascular therapies, and/or with cytostatic targeted molecular systemic therapies such as sorafenib, which achieve some survival benefits for unresectable HCC (3). Accurately determining tumor response after therapy has become essential in the management of HCC for determining efficacy of therapy (4), subsequent therapeutic planning and as a surrogate marker for improved survival (5). Lack of objective response after one or more LRTs is associated with poor survival, although it may be influenced by the type of LRTs used (6-8) and pattern of tumor progression (intrahepatic or extrahepatic) (9).

Here, we review various proposed clinical response criteria of HCC to LRTs from morphological to functional imaging.
biomarkers, assess their accuracy for determining tumor response after LRTs and discuss their challenges in clinical practice.

2. Morphological imaging response criteria (size-based) of HCC to LRT

In 1981, the World Health Organization (WHO) proposed the first tumor response criterion after therapy (WHO criterion) (10) (Fig. 1). In 2000, the Response Evaluation Criteria in Solid Tumors 1.0 (RECIST version 1.0) was proposed (11) and updated (RECIST version 1.1) in 2009 (12) (Table I and Fig. 2), which addressed the shortcoming of the WHO criterion. The objective the WHO and RECIST response criteria assess overall tumor burden using morphological tumor-size measurements on imaging and require assessment at baseline and on follow-up imaging. Objective response assessment is based on morphological tumor-size change after therapy and classified into complete response (CR), partial response (PR), no change (NC) and progressive disease (PD) (Table I).

LRTs for HCC induce tumor necrosis and reduced vascularity. Some LRTs initially lead to even an increase in apparent tumor size due to extensive necrosis. This is a major limitation of morphological (size-based) imaging response criteria for assessing response of HCC to LRTs. Therefore, morphological (size-based) imaging response criteria for determination of therapeutic success that rely solely on change in tumor size after therapy may be inappropriate to HCC treated with LRTs (13).

In addition, more objective imaging response criteria that are specific to the therapy type have been developed (14-20).

In summary, for the determination of therapeutic success of HCC to LRTs, morphological (size-based) imaging measurements using WHO, RECIST 1.0 and RECIST 1.1 criteria may not be applicable because these therapies result in tumor necrosis regardless of change in size.

3. Enhancement-based functional imaging response criteria of HCC to LRTs

In 2000, The European Association for the Study of the Liver (EASL) proposed determination of therapeutic success of HCC to LRTs should measure the size of residual viable tumor rather than the overall size of the tumor (14) (Table I and Fig. 3). In 2005, the American Association for the Study of Liver Diseases (AASLD) guideline cited this concept (15). In 2010, the EASL published the modified response evaluation criteria in solid tumors (mRECIST) criteria for HCC response to LRTs based on measuring only the viable enhancing areas of the HCC after LRTs, excluding portion of necrosis. It is as an amendment and update to the AASLD-JNCI Expert Panel Criteria and are based on the most recent 2009 RECIST 1.1 and EASL 2000 criteria (16) (Table I and Fig. 4). The mRECIST criteria assesses HCC at baseline, after LRTs and
Table I. Criteria for response assessment after locoregional therapy in patients with hepatocellular carcinoma.

<table>
<thead>
<tr>
<th>Type of tumor response</th>
<th>WHO criteria (after 4 weeks)</th>
<th>RECIST criteria (after 4 weeks)</th>
<th>EASL criteria (after 4 weeks)</th>
<th>mRECIST criteria (after 4 weeks)</th>
<th>RECICL criteria (after 3 months)</th>
<th>Criteria proposed by Choi (after 2 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response (CR)</td>
<td>Disappearance of all target lesions</td>
<td>Disappearance of all target lesions</td>
<td>Absence of enhanced tumor areas, reflecting complete tumor necrosis</td>
<td>Disappearance of any intratumoral arterial enhancement in all target lesions</td>
<td>100% tumor-necrotizing effect or 100% tumor size reduction rate</td>
<td>Disappearance of all lesions; No new lesions</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>≥50% decrease</td>
<td>≥30% decrease</td>
<td>≥50% decrease of enhanced areas</td>
<td>≥30% decrease in the sum of diameters of viable (enhancement in arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions</td>
<td>The tumor-necrotizing effect or tumor size reduction rate is between 50% and &lt;100%</td>
<td>A decrease in size of ≥10% or a decrease in tumor density (HU) ≥15% on CT; No new lesions; No obvious progression of non-measurable disease lesions</td>
</tr>
<tr>
<td>Stable disease (SD)</td>
<td>Any cases that do not qualify for either partial response or progressive disease</td>
<td>Any cases that do not qualify for either partial response or progressive disease</td>
<td>Any cases that do not qualify for either partial response or progressive disease</td>
<td>Any cases that do not qualify for either partial response or progressive disease</td>
<td>Any cases that do not qualify for either partial response or progressive disease</td>
<td>Does not meet the criteria for CR, PR, or PD; No symptomatic deterioration attributed to tumor progression</td>
</tr>
<tr>
<td>Progressive disease (PD)</td>
<td>≥25% increase or new lesions</td>
<td>≥20% increase or new lesions</td>
<td>≥25% increase enhance lesions or new enhance lesions</td>
<td>≥20% increase in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started</td>
<td>The tumor growth ≥25% regardless of the necrotizing effect, or emergence of a new lesion (categorized into 3 groups: intrahepatic solitary lesion, intrahepatic multiple lesions, and vascular invasion/extrahepatic spread)</td>
<td>An increase in tumor size of ≥10% and does not meet criteria of PR by tumor density (HU) on CT; New lesions; New intratumoral nodules or increase in the size of the existing intratumoral nodules</td>
</tr>
</tbody>
</table>

overall response. In the process of clinical application, high quality arterial-phase enhanced CT/MR imaging is required. There are also other response criteria for HCC response to LRTs based on enhancement CT/MRI exam. In 1994, the Liver Cancer Study Group of Japan used a similar system (RECIST) (17) to assess direct treatment effect and overall disease status after therapy, which was updated in 2004, and again in 2009 (18,19). In 2007, Choi et al (20) proposed the Choi Response Criteria for HCC response to antivascular therapy based on tumor density and volume.

Therefore, the EASL and the AASLD-JNCI and mRECIST criteria suggested assessing response criteria for HCC response to LRTs based on the size of viable enhancing tumor rather than overall tumor size including area of necrosis. These criteria have been shown to correlate well with histopathologic response than WHO and RECIST criteria. Previous studies of patients with HCC treated with ablation (21,22), TACE (6-8,21,23-26), and transarterial radioembolization (TARE) with Y90 (24,25) have shown that mRECIST and EASL criteria have excellent intercriterion concordance and are more accurate at predicting complete histopathologic response (23) and survival after therapy than WHO and RECIST criteria (25).

First of all, a major shortcoming of enhancement-based models of imaging response systems for HCC response to LRTs is that they assess all target and non-target lesions in the entire liver without taking into consideration that different tumors in the same patient are not treated at the same time (27). Thus, the overall prognosis in such patients is unclearly determined by the behavior of the treated or untreated tumors. Recent studies have shown one or two primary target lesion responses to LRTs correlate well with disease progression and survival in patients with solitary and multifocal HCC (25,28,29). Additionally, the EASL and mRECIST criteria do not take into consideration the change of the overall tumor size after LRTs assessing change in size of viable enhancing tumor rather than tumor size or the residual viable tumor size (31). Since the therapeutic effects on the tumor microvascular environment alter tissue perfusion, physiologic imaging techniques such as dynamic contrast-enhanced ultrasound (D-CEUS), CT or MR perfusion imaging begins to play a critical role in the evaluation of therapies that result in decreased enhancement without necrosis (32,33).

The fundamental principle of perfusion imaging is based on DCE imaging techniques that compute the temporal changes in tissue enhancement after intravenous administration of contrast media. A variety of imaging protocols have been proposed for perfusion imaging and the protocol selection should be made on the availability of the scanner technology and the pertinent physiologic parameter of interest. The computed perfusion parameters are dependent on the scan protocol and the mathematical model/software for image processing (34,35). The commonly described perfusion CT parameters include blood flow (BF), blood volume (BV), permeability surface area (PS), time to peak enhancement (TTP) and transfer constant (Ktrans). Similarly for perfusion MR, transfer constant (Ktrans) is the most accepted quantitative surrogate end point from compartment models (36,37).

CEUS and D-CEUS were acknowledged to be a feasible examination for evaluating dynamic changes in tumor vascularity in patients with HCC undergoing antiangiogenic target therapy (38) and chemoembolization (39). Previously, HCC shows hyper-vascularised tumor enhancement type. After treatment, it shows lack of contrast enhancement, whereas still viable tumor shows arterial-enhancing and subsequent washout (40). CEUS and D-CEUS could be used to identify the result of HCC response to antiangiogenic therapies, predict tumor responses and patient survival (38). The times to peak intensity, mean transit time (MTT) values and area under the curve (AUC) levels, correlated well with tumor responses and survival rates (32). In addition, AUC, time to peak intensity and slope of wash-in were positively associated with progression free survival (PFS) (32). In fact, CEUS and D-CEUS are low cost and good safety examinations can provide both morphological and functional data. In addition, its important role in clinical application has been recently highlighted by EFUMB guidelines (41). For example this panel of experts recognised the important role of CEUS in the very early evaluation of ablative treatment as a guidance for immediate retreatment of residual unablated tumor (41). More recently, three-dimensional CEUS technique (3D CEUS) has been reported to improve the study of tumor vascularity, thus, allowing the response evaluation of HCC treatments in the three orthogonal planes. Nevertheless, 3D CEUS may be limited by the spatial resolution of the current 3D probes in the assessment of therapeutic response of HCC treated with
ablative treatments compared to conventional CEUS (42). Additionally, the best timing and the best quantitative dynamic parameters for the assessment HCC response to LRTs are still unclear.

On perfusion CT, HCC has been reported to show substantially higher perfusion (high BF, BV and PS with low MTT) compared to normal liver tissue (43). After antiangiogenic drugs or HCC directed therapies, decrease in tumor perfusion parameters has been shown within days of initiation of treatment (43,44). Similarly, Zhu et al (43) have shown that HCC nodules showing more substantial reduction in tumor permeability (Ktrans) on perfusion MR soon after sunitinib, had better long-term outcome. Liang et al (45) reported Signal parameters of DCE-MRI over tumor and liver parenchyma correlated with tumor response and survival, respectively, in HCC patients receiving radiotherapy combination with an anti-angiogenic agent.

With CT, relatively high radiation dose and limited coverage of the anatomy are two major drawbacks of perfusion technique. Several efforts are being made with low dose scanning approaches (46). Likewise, there is no consensus on a scanning protocol or a mathematical model specific for HCC. Since the liver has a dual arterial and portal venous perfusion, the scan protocols should ideally include dual inputs to estimate quantitative perfusion parameters for hepatic tumors. However, due to larger tumor burden in advanced HCC and frequent occurrence of angioinvasion into the portal venous system, single arterial input is often applied as a simplifying assumption (47). More recently, volume perfusion CT (VPCT) enables quantification of perfusion in tumor tissue in absolute values by measuring flow and concentration of iodinated contrast medium during a time period within blood vessels and tissue generating time density curves (TDC) (48). This technique is also designed to calculate separately hepatic arterial and portal venous blood flow to the liver and liver tumors based on input functions obtained by regions of interest (ROIs) set in the spleen and the portal vein, the former representing a substitute for direct hepatic arterial measurements. VPCT have been used to characterize HCC (49) and monitor the HCC response to TACE and analysis of TACE-impact on tumor and uninvolved liver parenchymal perfusion at day one post-TACE (50).

MR imaging has several advantages over CT, including the lack of ionizing radiation. Therefore, it has the ability to image whole organs repeatedly and dynamically with high temporal resolution, and the possibility of repeating the study multiple times after treatment. A variety of imaging protocols, other than DCE imaging technique after intravenous gadolinium contrast, have been proposed for perfusion MR imaging. Transcatheter intraarterial perfusion (TRIP) MR imaging involves direct catheter-based intraarterial injection of contrast material (51), which offers a functional alternative to conventional digital subtraction angiography in the assessment of tumor perfusion changes during TACE (52). Recently, unenhanced MR perfusion imaging using the arterial spin labeling (ASL) technique was also introduced to quantify perfusion in the liver (53). This perfusion method, which does not require the use of contrast media, is a non-invasive MR perfusion technique that may offer great potential as an alternative imaging method for pure liver portal perfusion (53).

There are several drawbacks in liver perfusion MRI technique. General challenges confronting perfusion MR include lack of accepted standards of image acquisition and analysis, variable reproducibility and no established response evaluation criteria. Furthermore, unlike the linear relationship between iodine concentration and Hounsfield units on CT, the relationship between gadolinium concentration and signal intensity (SI) is non-linear with MR imaging, complicating quantitative perfusion measurements.

In summary, the possibility of evaluating tissue vascularization through perfusion imaging has led to the exploration of these imaging techniques as new assessment tools in order to measure the effectiveness of intraarterial therapy with or without antiangiogenic therapies for HCC. Different imaging biomarkers for assessing response to therapy in HCC derive from different imaging technique and the protocol selection should be made on the availability of the scanner technology and the pertinent physiologic parameter of interest. Further studies are warranted to determine the still unclear aspects such as the best timing and the best quantitative dynamic parameter for the assessment of response to HCC treatment.

5. Diffusion-weighted MR imaging for assessing response of HCC to LRTs

Diffusion-weighted MR imaging (DWI) has the unique ability of being able to provide information that reflects tissue cellularity and cellular membrane integrity (54). Moreover, apparent diffusion coefficient (ADC) measurement on an ADC map can be quantified by acquiring images with a different gradient duration and amplitude (i.e., b-value). DWI and ADC maps reflect the water molecule diffusion in tissue and can discriminate viable tumor from necrotic tissue. Viable tumor cells have intact membranes that restrict water molecules, whereas necrotic tissue shows increased water molecule diffusion as a result of cell membrane disruption (55). This makes it an attractive and useful technique for the assessment of tumor response after LRTs in patients with HCC.

The visual assessment of DWI, which includes images at higher b-values ≥500 sec/m², may aid to distinguish the different components of HCC (viable and necrotic components) following LRTs. As a general observation, necrotic HCC tissues (liquefaction or coagulation necrosis) secondary to LRTs typically show lower signal intensity on higher b-value images than viable tissues. ADC has also been used for early evaluation and prediction response to LRTs (56-64). An increase in ADC values has been reported following radioembolization (56,57) and chemoembolization (58-62) in the early post-treatment period (a few days up to 2 weeks) with measurable differences before and after treatment (58-62), but the treatment effect was noted 1-3 months after treatment.

The role of the pretreatment ADC value in predicting the response to LRTs have also been investigated with discordant results (58,65), which may be related to the nature of tumors with or without necrotic tissue before treatment (66). Recent studies have also shown that pre-treatment ADC values as well as changes in ADC values after treatment may provide useful information for predicting survival for patients with unresectable hepatocellular carcinoma (63,67-69). Vandecaveye et al (63) reported that 1-month response deter-
mined with apparent diffusion coefficient is an independent predictor of outcome for HCC treated with chemoembolization. Several studies have also shown that the pretreatment ADC values of liver malignancy can be a predictive factor of tumor response to RFA therapy (70); Mori et al (70) reported that the signal intensity of HCC on the ADC map was strongly associated with outcome after RFA. Hypointensity on the ADC map was the strongest independent factor related to recurrence and survival after RFA, even for small HCC (70).

In summary, previous studies of DWI in monitoring HCC response to LRTs, have uniformly reported increasing ADC during therapy onset proceeding anatomic size changes (71). DWI should be recommended as a routine method for evaluation of HCC response to LRTs, however, not in substitution but rather in combination with enhancement (EASL, mRECIST) criteria (72), because at this point, this technology is evolving with no accepted protocols and quantified standards, although the National Cancer Institute (NCI) has recognized the potential of this technique and has proposed consensus guidelines for DWI to meet minimum standards for its use as an effective image biomarker (73). In patients with contraindication to contrast agents or with slight-enhancement lesions, DWI can be considered a reasonable alternative to enhancement (EASL, mRECIST) criteria. However, further technological improvements (i.e., intravoxel incoherent motion diffusion-weighted MRI with bi-exponential diffusion model) and technique standardization are still required to use DWI at its full potential (74-76).

6. Positron emission tomography (PET)/PET-computed tomography (PET-CT) imaging for assessing response of HCC to LRTs

PET is a quantitative imaging modality and 18F-fluorodeoxyglucose (18F-FDG), a glucose analog, is the most commonly used PET tracer in clinical practice. 18F-FDG-PET has been considered to be a very useful non-invasive tool for diagnosis, tumor staging and monitoring of treatment responses in various malignancies (77). Recent studies have shown that PET-CT is useful in assessing HCC characterization. Low 18F-FDG uptake is seen in well-differentiated HCC, whereas high 18F-FDG uptake is observed in moderately to poorly differentiated HCC (78). Overall, the FDG-PET sensitivity in detecting HCC is lower (50-70%) than other liver tumors. Standardized uptake value (SUV) is the accepted semi-quantitative biomarker of tracer uptake in PET. There is growing evidence that in PET-positive HCC, early metabolic response may reflect molecular changes and predict long-term outcome after completion of therapy (79-84). A SUV on 18F-FDG PET-CT imaging can serve as an independent prognostic factor in HCC and may predict tumor recurrence after TACE (79,83). 18F-FDG uptake was an independent prognostic factor for PFS and OS in HCC patients treated with TACE or concurrent intra-arterial chemotherapy with external beam radiotherapy (CCRT). Especially, in HCCs with high 18F-FDG uptake, patients treated with CCRT showed better survival than those treated with TACE (Fig. 5). 18F-FDG PET-CT may help determine the treatment modality for intermediate-to-advanced stage HCCs (80). An early interim PET-CT after TACE may have prognostic value for HCC patients treated with TACE and radiotherapy (83,84). Therefore, the European Organization for Research and Treatment of Cancer (EORTC) has defined response assessment criteria for PET (85). EORTC has also suggested that the initial region of interest for SUV measurements should contain only viable tumors and be used consistently on the subsequent scans. To overcome some of the limitations of the EORTC criteria, Wahl et al (86) even proposed modified criteria with more stringent requirements for tumor response assessment with PET.
In summary, PET and PET-CT have proven value in the imaging-based diagnosis of recurrent disease following LRTs of liver malignancies, and repeat treatment is often initiated solely based on this imaging modality. $^{18}$F-FDG is not a tumor-specific tracer and the reproducibility of SUV is influenced by the time of image acquisition from tracer injection. Given the limitations of FDG in HCC, other tracers with different molecules including choline-based tracers are being investigated (87).

7. Other novel functional imaging biomarkers for assessing response of HCC to LRTs

MR spectroscopy (MRS) is a promising non-invasive technology. Hydrogen-1 ($^1$H) and phosphorus-31 ($^{31}$P) MRS are the most common in vivo used in clinic. $^1$H-MRS has been used in the assessment of the effectiveness of chemoembolization treatment for HCC (88-91). The mean cho/lipid ratios were significantly decreased after TACE in HCC. Choline levels decreased significantly after treatment (88). The lipid value after TACE was proportionally increased because the iodized oil used for TACE has a substantial lipoid component (88). The studies of $^{31}$P-MRS assessing the effectiveness of chemoembolization treatment for HCC have shown that a significant decrease in nucleoside triphosphates (NTP) and an increase in Pi had been observed in the early phase of chemotherapy or chemoembolization in liver tumors (representing necrosis of tumor cells), followed by changes in phosphodiesters (PDE) and phosphomonooester (PME) levels (92,93). Decreases in the PDE/NTP ratio and the PDE/TPC ratio that occurred after treatment were the most remarkable changes secondary to chemoembolization. In the responsive group, the PDE/TPC ratio was significantly decreased after chemoembolization, whereas the NTP/TPC ratio was significantly increased. In the non-responsive group, phosphorus metabolism had no significant changes after treatment (94). There are several technical limitations in the assessment of the effectiveness for HCC by MRS. This technology is evolving with no accepted protocols and quantified standards. Image distortions and susceptibility artifacts were unavoidable to some degree. New imaging sequences also need to develop to improve image quality (95).

Integrated PET-MRI is a new imaging modality, combining the advantages of FDG-PET with the ability of MRI to detect small liver tumors without CT radiation exposure. Recent results show that detection sensitivity for hepatic metastases, through post hoc fusion of FDG-PET images and 1.5 Tesla contrast-enhanced (Gadolinium) MRI obtained from two different scanners, is significantly higher than for PET-CT (96). PET-MRI appears to offer higher lesion conspicuity and diagnostic confidence compared to PET-CT (97), and this additional information can influence clinical management of cancer patients. The combined advantages of detection of smaller hepatic tumors with a higher sensitivity and detection of focal FDG uptake suggestive for local tumor progression indicates that PET-MRI could provide complementary information and facilitate improved clinical decision making (98). FDG PET-MRI could potentially improve the accuracy of (early) detection of progressive disease, and thus, allow swifter and more effective decision-making regarding appropriate treatment (99).

8. Conclusion

It is important that early and accurate assessment of the efficacy of HCC following intra-arterial (i.e., chemoembolization and radioembolization) and ablative (i.e., radiofrequency ablation and cryoablation) therapies in making therapeutic decisions, such as whether to repeat, interrupt or completely terminate therapy. Functional imaging has an important position in assessing tumor response in locoregional therapy for HCCs, which induce biologic changes that may be detected by functional imaging much earlier than morphological imaging. An ideal imaging biomarker should be able to detect an immediate response to any therapeutic regimen in one examination. Although promising, none of these functional imaging biomarkers have gone through all the required steps of standardization and validation and established accepted criteria for clinical practice. At present in clinical practice, different imaging biomarkers for assessing response to therapy in HCC derive from different imaging technique and the protocol selection should be made on the availability of the scanner technology and the pertinent physiologic parameter of interest. Therefore, it is unlikely to be the sole functional imaging biomarker of HCC response after LRTs. A combination of enhancement (EASL, mRECIST) criteria and functional imaging has been stated to be better in assessing therapeutic response of HCC after LRTs and in providing more information to guide future therapy.

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References


