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REVIEW ARTICLE

# Evaluating treatment response following locoregional therapy for hepatocellular carcinoma: A review of the available serological and radiological tools for assessment

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## Abstract

Hepatocellular carcinoma (HCC) is an aggressive primary malignancy of the liver and is the third most common cause of cancer-related global mortality. There has been a steady increase in treatment options for HCC in recent years, including innovations in both curative and non-curative therapies. These advances have brought new challenges and necessary improvements in strategies of disease monitoring, to allow early detection of HCC recurrence. Current serological and radiological strategies for post-treatment monitoring and prognostication and their limitations will be discussed and evaluated in this review.

## Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related global mortality.[1] HCC typically complicates a cirrhotic liver. Historically, patients frequently presented with late-stage malignancy, with an associated poor prognosis. Over the last 20 years, the institution of surveillance programs has led to detection at earlier stages of malignancy and cirrhosis, enabling an increasing number of patients to be offered potentially curative surgery.[2] Despite this, more than three-quarters of patients remain ineligible for resection.[3] To supplement this gap in therapy, there has been an expansion in locoregional therapeutic (LRT) options (Table 1).

The development of LRT options has brought new challenges. First, the definition of treatment failure is evolving, as longer survival may permit patients to receive repeated interventions,[17,18] or undergo "stage migration" and step up or down to other treatment modalities.[19,20] Second, LRT introduces irreversible intra-lesional imaging artifacts, which may confound the accurate radiologic evaluation of disease.

The complex nature of the surgical, medical, radiological, and oncological challenges posed by HCC treatment renders a multi-disciplinary approach to post-LRT disease evaluation essential. Collaborative assessment by a multidisciplinary team has been shown to improve outcomes[21] and is recommended by current societal guidelines.[5,6] Assessment of liver function prior to and following therapy is a critical part of appropriate patient selection and management, however, is beyond the scope of this review. In current practice, a multi-modal synthesis of clinical progress, assessment of measurement of serological makers, and imaging are used to best evaluate treatment effects and to individualize recurrence risk to inform surveillance strategies. This article will review the current strategies employed in the evaluation of treatment response and surveillance for HCC following locoregional therapy.

## Current methods for disease evaluation post-LRT

### *Serological markers*

*α-Fetoprotein.* α-Fetoprotein (AFP) is the most extensively studied biomarker in the setting of screening and diagnosis of early HCC, surveillance for early disease recurrence following therapy, and prognostication. AFP is a glycoprotein, with structural similarities to albumin. Produced by the fetal liver and yolk sac, levels decline rapidly after birth to adult serum levels of 5–20 μg/L.[22,23] AFP's physiological significance is unclear, but its role as a carrier protein and in immunoregulation has been hypothesized.[22] Despite being the most widely used biomarker for HCC, AFP levels are normal in up to 40% of HCC patients.[24]

AFP levels may be raised in patients with chronic liver disease or hepatitis in the absence of HCC. An observational study of 855 patients with advanced hepatitis C infection (without HCC) over a 42-month period reported that 24.5% of patients had at least one abnormally raised AFP serum level (20–199 μg/L) during 3-monthly screening, 2.3% had at least one AFP value ≥200 μg/L.[25] In the current era of nucleoside analogs and direct-acting antivirals against hepatitis B and C, these treatments have mitigated some confounding causes of elevated AFP.[26]

Post-treatment assessment. Both relative changes in AFP levels following therapy as well as the absolute level have been shown to predict outcomes for patients treated with LRT for HCC. In a retrospective study of 463 HCC patients treated with TACE and selective internal radiation therapy (SIRT), an AFP reduction of 50% or more was seen in 65% of patients and was associated with improved progression-free survival and overall survival (hazard ratio [HR] 4.2, 95% confidence interval [CI] 2.4–7.2 in non-responders and HR 5.5, 95% CI 3.1–9.9 in responders).[27] AFP responders had a long time to progress compared with non-responders (7.5 *vs* 2.7 months).

A 50% reduction of AFP at 7 days following radiofrequency ablation (RFA) therapy has been shown to predict disease-free survival[28] and overall survival in patients treated with TACE (34.9 *vs* 13.2 months).[29,30] Park *et al*. demonstrated that a 50% reduction in AFP after TACE therapy was associated with a longer time to progression and overall survival on multivariate analysis.[31]

A retrospective study from Japan of 416 patients showed that a pre- or post-ablation AFP > 100 ng/mL was associated with increased rates of recurrent tumor adjusting by tumor size, number, and platelet count.[32] A meta-analysis of HCC patients receiving RFA showed that an AFP > 20 ng/mL predicted worse overall survival (HR 1.46, 95% CI 1.25–1.70) but not when cut-offs of >200 or >400 ng/mL were used.[33,34] In patients treated with RFA a pre-treatment AFP > 400 ng/mL predicted distant tumor recurrence.[35]

*Lens culinaris agglutinin-A-reactive AFP.* In the context of HCC, AFP molecules show a greater affinity in binding to lens culinaris agglutinin A (LCA). With respect to LCA, AFP can be characterized by three different glycoforms L1: (non-bound to LCA), L2 (intermediate binding to LCA), and L3 (bound to LCA). Lens culinaris agglutinin-A-reactive AFP (AFP-L3) seems to be predominantly produced by HCC and so increased levels are more specific to HCC than raised total AFP levels, which could be associated with cirrhosis or chronic hepatitis.[36]

The utility of ALP-L3 in the surveillance and diagnosis of HCC has been investigated predominantly by Japanese researchers, leading to its inclusion in Japanese clinical guidelines.[37] Elsewhere, a multi-center North American study in surveillance of hepatitis C virus (HCV) patients comparing AFP and AFP-L3 fractions found that the incidence of HCC was higher in patients with elevated AFP-L3 fraction than in those with globally elevated AFP.[38]

Post-treatment assessment. Like AFP, both the relative change as well as the absolute level of AFP-L3 predicts prognosis in patients with HCC following locoregional therapy. Huang *et al*. showed an AFP-L3% reduction of ≥20% in patients undergoing TACE was associated with improved overall survival (42.9 *vs* 15.4 months, $P < 0.0001$) and radiologic response by RECIST criteria ($P < 0.0001$).[39]

A 2014 meta-analysis of 15 studies and 4465 patients concluded that high pre-treatment serum AFP-L3 in HCC predicted

**Table 1** The characteristics of different locoregional therapies for hepatocellular carcinoma (HCC)

| Treatment | Mechanism of action | Indications | Contra-indications | Median survival benefit | Comments |
|---|---|---|---|---|---|
| PEI | Ablative, 95% absolute ethanol injected into the tumor causing protein denaturation and cellular dehydration, leading to necrosis | Single HCC <2 cm<br>Poor hepatic reserve precluding other ablative modalities<br>When other ablative therapies are unavailable or contraindicated, including concerns of a heat sink effect<br>Can be considered when HCC located in the proximity of major vessels or bile ducts | >3 tumors<br>CP C cirrhosis/uncontrollable coagulopathy/severe ascites<br>Extrahepatic disease<br>Main portal vein thrombosis | Early-stage disease: 60% 5-year overall survival[4] | Capsular or intra-tumoral septa may prevent the adequate distribution of the injected ethanol |
| RFA | Ablative electrode producing complete tumor necrosis performed under ultrasound guidance or laparoscopically | Potentially curative therapy for small, early-stage HCC <2 cm<br>May be considered as an alternative to surgery in tumors up to 4 cm, or 2–3 tumors ≤3 cm[5–7]<br>Bridging therapy to liver transplantation | Close proximity of surrounding vasculature, and bile ducts. Laparoscopically guided ablation could be considered<br>Subcapsular tumors have the risk of needle track seeding | Early-stage disease: Median overall survival of 60 months, a 5-year recurrence rate of 50–70% with RFA[8,9] | Can cause thermal injury to surrounding tissue |
| MWA | Complete tumor necrosis through electromagnetic microwaves agitating water molecules<br>Several needles can be used to perform ablation simultaneously, which may be able to achieve a larger ablation zone than RFA | Similar indications to RFA | Risk of thermal injury to surrounding organs | Early stage disease: 5-year survival 65%[9] | Can cause thermal injury to surrounding tissue |
| TACE<br>TAE | Transarterial embolization—bland particle embolization, chemo-embolization, or drug-eluting beads | Intermediate-stage HCC disease<br>May be used in combination with ablation, especially in tumors >2 cm<br>Bridging therapy to liver transplantation | Unfavorable arterial supply precluding successful hepatic artery catheterization<br>Absent portal vein flow<br>Decompensated Cirrhosis<br>Tumor burden >50% of liver<br>Untreated esophageal varices at high risk of bleeding | Median overall survival 19.4–37 months[10,11] | Considered to be a non-curative therapy |
| TARE | Intra-arterial delivery of Yttrium-90 ($^{90}$Y) microspheres, causing tumor necrosis | Intermediate or Advanced stage disease<br>Safe in portal vein thrombosis | Pulmonary shunting (estimated lung dose >30 Gy)<br>Extra-hepatic disease<br>Life expectancy <3 months<br>Decompensated cirrhosis | Advanced stage disease—Overall survival 8–9 months[12–14] | Three RCTs comparing TARE with sorafenib in advanced stage HCC failed to meet the primary endpoint of superior overall survival[12–14] |

(*Continues*)

**Table 1** (Continued)

| Treatment | Mechanism of action | Indications | Contra-indications | Median survival benefit | Comments |
|---|---|---|---|---|---|
| SBRT | Stereotactic external beam radiation with photons | Advanced stage disease (1–3 lesions, up to 5–6 cm)[15]<br>Child-Pugh A<br>Macrovascular invasion<br>Combination therapy with other treatments<br>Bridge to liver transplantation | Decompensated cirrhosis | Median overall survival 17 months[16] | Can cause radiation-induced liver injury |

BCLC, Barcelona clinic liver cancer disease stage; IRE, irreversible electroporation; MWA, microwave ablation; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; SBRT, stereotactic beam radiotherapy; TACE, transarterial chemo-embolization; TAE, transarterial embolization; TARE, transarterial radio-embolization.

poorer overall and disease-free survival.[40] Nouso *et al*. demonstrated that even in patients with AFP < 20 ng/mL, an AFP-L3% of >10% was present in 13.3% of these patients and conferred worse 5-year survival (69.4% *vs* 41.1%, $P = 0.001$).[41]

Tateishi et al. found that those with an AFP-L3% of >15% pre- and post-ablation were associated with increased rates of tumor recurrence (HR 1.52, 95% CI 1.06–2.18 and HR 4.25, 95% CI 1.42–12.74% respectively).[32] Another study showed a baseline AFP-L3% >15% in patients treated with RFA was associated with worse overall survival (HR 1.45, 95% CI 1.11–1.91, $P = 0.008$).[35] Patients with a baseline AFP-L3% of >24.4% were associated with worse 2-year survival (37.5% *vs* 78.6%, $P = 0.01$).[42]

While data exists to show that AFP-L3 can help predict prognosis following locoregional therapy, there is less evidence demonstrating the utility of AFP-L3 to aid in post-treatment surveillance to help monitor and detect early HCC recurrence.

*Des-γ-carboxyprothrombin (protein induced by vitamin K absence or antagonist-II).* Des-γ-carboxyprothrombin (DCP) is an abnormal prothrombin molecule produced by post-translational carboxylation of the prothrombin precursor in malignant cells and is also elevated in the setting of vitamin K deficiency and thus is also referred to as protein induced by vitamin K absence or antagonist-II (PIVKA-II).[43,44] Absent in the serum of healthy individuals, DCP can be detected in patients with HCC. DCP's role as an autologous growth factor in angiogenesis and paracrine stimulation of HCC has also been suggested.[45]

DCP has been identified as a predictor of microvascular invasion and is associated with poorer histological differentiation[46,47] portal vein invasion.[48] DCP may be elevated in the presence of normal AFP values and therefore multiple studies have investigated its efficacy in the diagnosis of HCC in combination with AFP and other diagnostic markers.[49,50]

Post-treatment assessment. In a Japanese retrospective cohort of 142 patients treated with TACE, an elevated DCP > 100 mAU/mL post-treatment was associated with poorer survival based on multivariate analysis (HR 8.14, 95% CI 2.46–26.94), and a poorer disease control rate defined as a composite endpoint of complete response and partial response.[51] In patients receiving percutaneous ethanol injection (PEI), pre-treatment DCP > 100 mAU/mL was associated with angioinvasion[48] and in another study DCP > 20 mAU/mL was associated with tumor recurrence.[52] In patients treated with RFA, a pre-treatment DCP > 400 mAU/mL was associated with poorer overall survival, local tumor progression and distant recurrence.[35] Tateishi *et al*., postulated that in some cases an elevated DCP > 100 mAU/mL following ablation may indicate liver injury rather than HCC recurrence and was associated with increased mortality.[32]

In a subgroup analysis of 115 treatment-naive patients receiving TACE therapy, with a baseline DCP > 200 mAU/mL, a reduction of 50% following treatment was associated with improved overall survival.[39] No difference in overall survival or progression-free survival was found in patients with a lower baseline DCP included. Park *et al*. demonstrated that a 50%

reduction in DCP after TACE was associated with a longer time to progression and overall survival (HR 0.26, 95% CI 0.14–0.49 and HR 0.12, 95% CI 0.05–0.29).[31]

While DCP shows promise in predicting prognosis following locoregional therapy, further evidence is required to demonstrate its clinical utility in post-treatment surveillance.

***Genomic markers following LRT.*** Recent advances in technology have enabled high throughput genome-wide analyses of biopsy/resection specimens to further characterize the HCC genome and epigenome.[53,54] The data these studies have generated has contributed to a greater understanding of the genetic environment in HCC. This has led to the interest in cell-free tumor DNA (ctDNA) biomarkers for HCC.

ctDNA refers to DNA derived from tumor cells (undergoing apoptosis or necrosis) that can be found in extracellular compartments (e.g., plasma, urine, CSF). In patients with cancer, ctDNA levels relative to levels of background cell-free DNA are not only dependent on tumor size and proliferation but also influenced by other factors such as ctDNA half-life and rate of clearance in the liver or kidneys. Methods of ctDNA analysis include genomic and epigenetic alterations including loss of heterozygosity, somatic mutations in oncogenes and tumor suppressor genes, cancer-specific methylation changes, and microsatellite alterations.[55–62]

One such example in HCC monitoring is the use of telomerase reverse transcriptase (*TERT*) promoter mutations in ctDNA. Mutant *TERT* is recognized as a contributor to carcinogenesis in more than 90% of cancer types.[63] TERT promoter mutations are one of the most frequently recognized in HCC, present in 30–60% of patients.[64] Hirai *et al*. demonstrated that in patients with advanced HCC undergoing treatment with TACE or chemotherapy, the presence of TERT promoter mutations correlated with large tumor size and high DCP. The overall survival of patients with TERT promoter mutations was shorter (HR 1.94, 95% CI 1.18–3.24). Those with a higher fractional abundance (≥1%) of mutant alleles had shorter survival than those with a low (<1%) fractional abundance.[64] Other studies have demonstrated that TERT promoter mutations are associated with shorter disease-free survival[65,66] and poor overall survival.[66–68] Some studies have not shown prognostic value for a TERT promoter mutation.[69] There is limited evidence for the use of circulating TERT mutations to monitor patients with HCC following locoregional therapy. Other molecular mutations that have been identified in HCC and their relative frequencies are detailed in Table 2.

**Table 2** Common genetic aberrations in hepatocellular carcinoma

| Gene | Frequency of aberration[†] | Pathway |
|---|---|---|
| TERT promoter | ~60% | Telomere maintenance |
| TP53 | 3–40% | P53 pathway |
| CTNNB1 | 11–41% | Wnt pathway |
| AXIN1 | 5–19% | Wnt pathway |
| ARID1A | 4–17% | Chromatin remodeling |
| CDKN2A | 7–8% | Cell cycle |
| ARID2 | 5–7% | Chromatin remodeling |
| FGF3, FGF4 or FGF19 | 4–5.6% | FGF pathway |

[†]Includes genetic mutations or deletions.
Adapted from Khemlina et al.[70]

In contrast to genetic mutations, epigenetic processes allow the regulation of gene expression without changing the underlying genetic sequence. DNA methylation involves the addition of a methyl group to cytosine bases within the genome, which typically leads to gene silencing.[71] Methylation of tumor-suppressing genes is well recognized as an early step in cancer pathogenesis[72] and is studied as a biomarker to assist with diagnosis and prognostication of HCC.[73] A large cohort study of 1098 HCC patients from China investigating the use of a panel of ctDNA methylation markers in plasma produced a diagnostic and prognostic prediction model for HCC with an area under the curve (AUROC) of 0.97 (95% CI 0.93–0.98) and 0.76 respectively.

Research of molecular biomarkers for the management of HCC is gaining momentum, but a comprehensive review is beyond the scope of this article. Further work is required to determine molecular biomarkers that may add to prognostication, post-LRT surveillance, and detection of early recurrence and minimal-residual disease in these patients.

## Radiological treatment evaluation

*Lesional imaging characteristics following therapy.* In recent years, radiological techniques have predominated as the primary method for disease evaluation post-LRT. The radiological diagnostic challenge is compounded when surveillance is required following HCC treatment. LRT induces macroscopic changes in the lesion that create new imaging characteristics. Recognition of the physical changes occurring in a treated HCC lesion as well as complications and artefactual findings related to LRT is essential to assess and evaluate treatment response.

RFA induces coagulative necrosis in the treated tumor as a result of the thermal energy delivered. The region of coagulative necrosis is seen as an area of hypo-enhancement on contrast computed tomography (CT) and MRI; the lack of enhancement is key to the assessment of treatment effect as it is interpreted as a surrogate for tumor necrosis. Successful curative treatment with RFA demands an ablative margin around the HCC lesion.[74] CT/MRI may demonstrate a "target" appearance following RFA due to differences in attenuation/signal between the tumor and ablated parenchyma. When present, this appearance allows for assessment of the ablative margin.[75] Without this discrepancy between zones, assessment of the ablative margin becomes difficult and relies on the radiologist's assessment of pre- and post-RFA images with reference to nearby landmarks such as vessels and the liver surface.[76]

In radiological evaluation following TACE, tumor shrinkage is a major indicator of the LRT effect.[76] Conventional TACE utilizes Lipiodol (Guerbet LLC, Indiana) emulsification for the delivery of cytotoxic drugs. The distribution of radio-opaque Lipiodol retention within the tumor site is used as a proxy of tumor necrosis on CT and can be established in the immediate post-procedural period through a non-contrast scan.[76,77]

Incomplete Lipiodol retention can represent untreated regions of HCC *or* complete necrosis. Local recurrence may manifest as increasing lesion size or vascularity (suggested by arterial enhancement) following TACE. Lipiodol interference artifact on follow-up CT imaging can mask the development of arterial enhancement (representing recurrent viable tumor) following TACE.[78] TACE using drug-eluting beads (DEB-TACE) does not require the use of Lipiodol and therefore results in decreased density and intensity of the treated HCC and loss of arterial enhancement.[79]

The therapeutic effect of the Yttrium-90 microspheres deployed during TARE is not instantaneous. Radiation-induced development of free radicals and subsequent DNA damage accumulates over time, leading to apoptosis and necrosis in the target lesion. In a cohort of 42 patients undergoing TARE, the reported time to radiographic response (decrease in tumor size or enhancement) on CT was 30–120 days following treatment.[80] In addition, when followed up early, treated tumors may either show a reduction in size, appear stable, or may appear larger, limiting the utility of dimension-based evaluation of the disease.[81] If the evaluation of tumor response is conducted by imaging size criteria alone partial response rates of 23.8–50% have been reported following treatment.[82,83]

Following stereotactic body radiotherapy (SBRT) treatment, lesions may exhibit peritumoral arterial and venous hyperenhancement consistent with radiation-induced liver disease (RILD).[84] There may be little change in tumor enhancement up to the first 12 months following treatment[85,86] and up to 40% of successfully treated HCCs based on explant histology, can have persistent enhancement. Therefore, the size increase is the best method for SBRT treatment failure.

Common to RFA, TACE, TARE, and SBRT; the hypoattenuated treated HCC lesion can be accompanied by a hyperenhancing rim of transient peri-ablation hyperemia on subsequent imaging.[87] This may persist for up to 12 months, when present it can obscure small residual tumor deposits or be mistaken for progressive disease on follow-up imaging.[81,88,89] Therefore, caution must be made in its initial interpretation, for this reason, follow-up imaging is not recommended until 1 month following treatment.

## Imaging modalities used for post-treatment surveillance

*Contrast-enhanced ultrasound.* Conventional ultrasound scanning has no role in disease monitoring following LRT. Contrast-enhanced ultrasound (CEUS) utilizes contrast media containing gas-cored microbubbles encapsulated by a lipid monolayer or cross-linked albumin.[76] Circulating US contrast can be utilized as a tool for assessing the vascularity of treated lesions following LRT.

Unlike CT imaging, CEUS is not confounded by artifacts caused by Lipiodol retention. Its use, therefore, has been investigated in the detection of residual tumors 1-week following TACE.[90] CEUS detected positive enhancement, suggestive of residual blood supply and disease, in 58.1% ($n = 43$) of lesions 1-week following TACE and was more sensitive than dynamic CT in that regard (39.5% detection rate). In the post-RFA setting the sensitivity of CEUS at day 1 for residual tumor detection has also been reported as superior to CT (27% *vs* 20%),[91] however difficulties with identifying the tumor boundary on CEUS may limit its utility following RFA in assessing the ablative margin.[92] Additional advantages of CEUS over other imaging techniques include its lack of patient exposure to ionizing radiation and the low risk of contrast reaction.

At this time, CEUS is not widely available as a modality for post-LRT surveillance, it requires specialist operator knowledge which greatly impacts its sensitivity for detecting residual or progressive disease. Patient factors including body habitus and disease factors such as tumor location may also limit its surveillance efficacy. CEUS is also limited to the assessment of a single lesion and, in comparison to cross-sectional imaging, lacks the ability to restage.

*Contrast-enhanced CT.* CT has been the predominant method of disease evaluation post-LRT. CT benefits from wide availability, assessor familiarity, and lower cost than MRI. However, its sensitivity in detecting residual disease, particularly following Lipiodol-based TACE, is limited. Aside from lesion size-based evaluation, CT relies on arterial contrast enhancement as a proxy for the viability of residual disease. Lipiodol artifacts may mask contrast enhancement in small pockets of residual disease. Significant Lipiodol artifact has been reported up to 2 months following therapy, blurring accurate evaluation of the treated lesion.[78] Lipiodol retention has been used as an immediate indicator of the area of the treatment on non-contrast CT following TACE, however concerns regarding loss of emulsification of doxorubicin in vivo raise doubt as to whether Lipiodol distribution accurately reflects the area subject to chemotherapy.[78] Following DEB-TACE, hypodensity at the site of treated HCC is expected with no residual arterial enhancement. Arterio-portal shunts are commonly detected following TACE as hyper-attenuated regions on CT, these may be misinterpreted as residual or recurrent disease reducing the specificity of CT evaluation in this context (Fig. 1).[76]

These issues are reflected in studies that compare the radiological assessment of response following LRT with histological examination. Kim *et al*. compared CT-evaluated "complete response" to the histological evaluation of explanted livers following liver transplantation in patients who had received RFA or TACE. Following analysis of the resected specimens, they reported a positive predictive value of 69.0% to determine the completeness of treatment.[93]

Single photon emission computed tomography (SPECT) is a nuclear medicine scan producing a 3D image by gamma detection of a radioisotope. It may be used following TARE to map the distribution of the radioactive microspheres correlating to the treated region.[94] The SPECT image can then be geometrically correlated with CT images to provide anatomical relations.

*Contrast-enhanced MRI.* MRI has become the modality of choice for post-LRT imaging in HCC. MRI offers higher resolution images of the treated liver than CT and is less susceptible to the artefactual characteristics induced by Lipiodol following TACE.[95,96] The use of imaging subtraction techniques in MRI can also improve post-treatment evaluation of disease by reducing the effect of T1 hyperintensity induced by coagulative hemorrhagic necrosis within the lesion.[97]

The addition of gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) as a contrast agent may increase

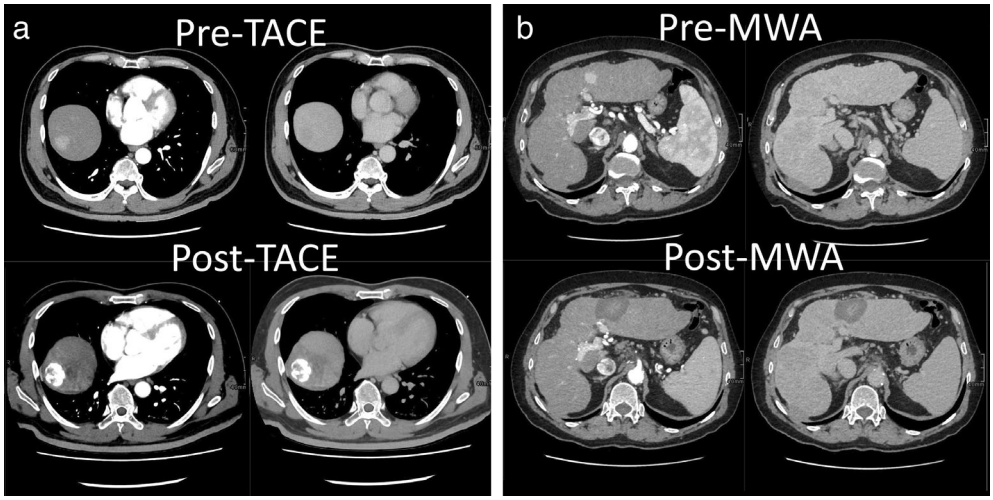


**Figure 1** Radiological appearances of HCC treated with TACE and MWA. (a) Arterial and delayed phases pre-TACE (top row) showing arterial enhancement and washout, respectively, in segments 7/8. Complete lipiodol deposition post-treatment (bottom row). (b) Arterial and delayed phases pre-ablation (top row) showing arterial enhancement and washout, respectively, in segment 2/3. Post-treatment changes with no residual enhancement and ablation zone covering the site of the previous HCC (bottom row). HCC, hepatocellular carcinoma; MWA, microwave ablation; TACE, transarterial chemo-embolization.

the accuracy of MRI for the detection of residual disease post-LRT. Gd-EOB-DTPA is a liver-specific contrast agent; when injected intravenously, approximately half is absorbed by functional hepatocytes and is then excreted via the biliary system, the remainder acts as a conventional intravascular contrast agent. A 2015 meta-analysis reported that Gd-EOB-DTPA outperformed CT in the diagnosis of HCC.[98]

Estimations of the sensitivity and specificity of MRI as a modality for post-LRT residual/recurrent disease detection vary, largely depending on the imaging protocol employed and the study population. For example, Bolog *et al*. reported that double contrast-enhanced MRI (SPIO and Gd-DTP contrast agents) had a sensitivity and specificity of 92% and 100% respectively in detecting a residual viable tumor in a cohort of 30 lesions following treatment with TACE, imaging diagnoses were correlated with histopathology and angiographic results.[99] In a more recent study, following RFA, Gd-EOB-DTPA-enhanced MRI had a sensitivity of 83–88% and specificity of 94% for the detection of recurrent disease.[100]

Akin to CT imaging, MRI evaluation post-LRT relies on a somewhat imprecise visual comparison of pre- and post-treatment images guided by lesional or adjacent liver landmarks. As mentioned, its sensitivity decreases with decreasing size of the interrogated region. Gd-EOB-DTPA administration is contra-indicated in patients with significant chronic kidney disease.

*Functional imaging.* Positron emission tomography with fluorine-18 deoxyglucose (FDG-PET) detects the increased metabolic activity of tumors through glucose metabolism. It has a role in the detection of a number of solid organ tumors. However, FDG avidity in HCC lesions appears low, and the sensitivity of PET/CT for the detection of HCC is poor (36% for HCC diagnosis in a 2016 study).[101]

Other novel metabolic tracers have been investigated in the post-treatment evaluation of HCC. 11C-acetate is metabolized into acetyl-CoA which is preferentially used by cancer cells to build membrane fatty acid.[102] Dual modality scans with FDG-PET and 11C-acetate PET have been shown to predict treatment response in patients receiving TACE and bevacizumab.[103] Patients receiving Y90 TARE for inoperable HCC, who were avid to 11 C-acetate, demonstrated better treatment response as measured by a reduction of post-treatment metabolic burden.[96]

Prostate Specific Membrane Antigen (PSMA) was first found to be overexpressed in prostate cancer cells and has since been validated as a diagnostic target. There is increased immunohistochemical expression in other cancer cell types, including in HCC[104] and a prospective pilot study demonstrated that 68Ga-PSMA PET-CT performed better than FDG PET-CT for identifying both intra and extrahepatic HCC.[104] Further research is required to determine whether PSMA-PET may assist with post-treatment monitoring of HCC.

While emerging data show that may be a role for functional imaging as a prognostic indicator of vascular invasion or extra-hepatic disease recurrence,[105,106] its use in post-LRT surveillance is not currently recommended by societal guidelines.[5]

## Response evaluation guidelines for post-LRT imaging

***Radiological response assessment criteria.*** Standardized radiological frameworks have been developed as guidelines for assessing treatment response and detecting recurrence in treated lesions. Initial guidelines focused on the anatomic evaluation of treated lesions by uni- or bi-dimensional size measurement; "Response Evaluation Criteria in Solid Tumors" (RECIST); and the "WHO criteria" response assessment for solid tumors. A reduction in lesion size correlates to a therapeutic

response.[107,108] However, it is increasingly evident that post-treatment tumor size may not always reflect tumor viability. Patients without significant radiological tumor shrinkage have been found to have partial or even complete treatment response on subsequent histological examination following resection.[109,110] These findings may be explained by a delay between the inducement of tumor necrosis and radiological shrinkage of the lesion.[111] Despite this, imaging criteria such as RECIST are frequently used to assess treatment response, particularly in research settings.

Newer and revised guidelines, such as the "modified Response Evaluation Criteria in Solid Tumor for HCC" (mRECIST) and the "European Association for the Study of the Liver" guidelines (EASL), have attempted to address these issues by including an evaluation of intravenous arterial contrast enhancement of the treated lesion as an indicator of tumor viability in addition to lesion size measurement.[5,112,113] Unlike healthy hepatocytes, HCCs receive their vascular supply predominantly from the hepatic artery, rather than a portal vein. This unique biology allows non-invasive diagnosis in the presence of hepatic arterial enhancement and portal venous and delayed phase washout and forms the basis of hepatic artery-directed LRT such as TAE, TACE,[114] and SIRT. The mRECIST framework has benefited post-LRT HCC assessment by standardizing CT/MRI imaging protocols, mandating independent review by multiple radiologists, and formalizing response categorization. The inclusion of arterial enhancement as a surrogate of tumor viability has more closely aligned the radiological assessment of disease following LRT with actual outcomes of progression in comparison to anatomic-size-based assessments alone.[111,115,116] As such the use of mRECIST for disease assessment following LRT is supported by current societal guidelines. Using a similar rationale, Choi et al. suggested incorporating the decrease in the enhancement of gastrointestinal stromal tumors (GIST) in the tumor response assessment following imatinib. The Choi criteria, and subsequently modified Choi criteria, have been used in the assessment of GIST treatment response rather than RECIST criteria.[117]

The Liver Imaging Reporting and Data Systems (LI-RADS) diagnostic algorithm developed by the American College of Radiology has been endorsed and supported by the American Association for the Study of Liver Disease (AASLD).[118] LI-RADS Treatment Response (TR) was developed to assess tumor response on a lesion-by-lesion level compared to mRECIST which assesses overall tumor burden.

These frameworks continue to suffer from some limitations. mRECIST criteria can only be applied in the evaluation of typical hypervascular HCC lesions, rather than tumors that are infiltrative, iso, or hypoenhancing on arterial phase imaging. In the latter cases, response assessment relies on local expertise and may rely on an assessment of size changes only. Assessment of enhancement changes on portal venous and delayed phases rather than the arterial phase may be of benefit. However, a consensus on such an approach is still lacking.

Post-LRT lesions may illustrate atypical enhancement rendering them unsuitable for mRECIST evaluation. In addition, mRECIST still utilizes bi-dimensional size criteria in the assessment of enhancing or non-enhancing areas of treated lesions and can therefore be limited in tumors with heterogeneously distributed patterns of necrosis as can be found in poorly differentiated tumors. mRECIST can only be used to evaluate lesions >1 cm. In the context of LRT, treatment is often cytostatic rather than cytotoxic and hence radiological response may take significantly longer to become evident limiting the application of mRECIST in initial disease response assessment. In addition, lesions post-LRT may exhibit a phenomenon of "pseudo-progression" where the hepatoma initially increases in size and may be accompanied by new lesions in the initial response to treatment.[119]

***Timing of assessment.*** No strong evidence exists to support a standardized radiological follow-up regime for patients treated with LRT. By convention, patients undergoing LRT undergo cross-sectional imaging surveillance at three monthly intervals for 2 years minimum.[120] Patients receiving TARE undergo imaging follow-up at 3 months to allow for the delay in radiographic response associated with this LRT.

## Discussion

Assessment of HCC recurrence following LRT relies on the evaluation of laboratory tests and imaging. While AFP is the best-established serum marker and may inform disease recurrence and prognosis in patients who receive locoregional therapy, it is notably undetectable in 40% of patients.[5,6] Novel serum markers such as AFP-L3 and DCP have now been incorporated into some national guidelines for screening of early HCC.[37] Furthermore, the use of a composite risk score comprising of select serum markers and imaging may have the potential to improve the sensitivity and specificity of these markers in isolation, such as the GALAD score[121,122] in the setting of early disease, with a notable paucity of evidence for the use of composite risk scores for disease monitoring.[123] While there is some data to support the use of these serum markers to assist in prognostication in patients following LRT, the use of these tests has not been widely adopted outside of the research setting.

More recently, advances in next-generation genome sequencing have allowed the detection of ctDNA to become more accessible and affordable for clinical use. Identification and monitoring of ctDNA may allow the personalization of medicine in these patients, potentially assisting in the identification of early recurrence and prognostication. A challenge in developing ctDNA as a useful biomarker in HCC stems from heterogeneity in tumor genotypes, mutations vary not only between individuals but also even between different foci of the same tumor.[54] Additionally, reliable detection of ctDNA requires highly sensitive assays as the relative abundance of ctDNA (signal) is low compared with high concentrations of circulating non-tumor DNA (noise).

Surveillance imaging following therapy remains the mainstay of HCC recurrence detection as well as prognostication. Standardized frameworks endorsed by international societies such as mRECIST can assist in the assessment of HCC following therapy.[5,6] However, treated lesions may show atypical enhancement and lesional artifacts characteristic of different treatments such as lipiodol deposition following TACE, perivascular odema following TARE, and hyperenhancement following SBRT. These changes vary depending on treatment modality and may complicate mRECIST evaluation. As novel therapies for HCC are developed, response assessment modalities must be updated to ensure accurate assessment of lesion response.

The use of novel serum and molecular markers in combination with radiological surveillance could assist in the early detection of HCC recurrence following locoregional therapy. It is worth considering a scenario where novel serum markers suggest possible HCC recurrence, but radiological modalities are unable to detect local or distant recurrence, it remains unclear whether current locoregional or systemic modalities may be offered in the absence of a clearly treatable target.

Further research is required to determine whether this surveillance strategy can improve the sensitivity and specificity of detection, whether this could help rationalize surveillance intervals and burden of investigations, and patient outcomes including morbidity and mortality, quality of life, and cost-effectiveness.

## Conclusion

The accurate detection of disease progression in patients with HCC following locoregional therapy remains challenging but is evolving rapidly. In combination with functional assessment of the underlying liver, this is important to inform ongoing investigation and treatment. The challenge for the future post-LRT assessment of disease will be combining the evaluation of radiological response, functional assessment, and novel biomarkers which may further inform biology and prognosis, which may in turn allow proactive changes in management.

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