REVIEW

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Functional imaging of hepatocellular carcinoma

Hepatic Oncology

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Practice points

- Contrast-enhanced imaging lacks sensitivity and specificity for effective hepatocellular carcinoma management.
- Imaging of tumor physiology can provide entirely new clinically relevant information.
- Targeted contrast agents, if developed further, may provide a specific method of lesion characterization.
- These agents can be of particular importance in the early stages of drug development.
- Volumetric lesion assessment and imaging of lesion viability will likely improve RECIST1.1 and mRECIST methods.
- Further prospective translational trials in these techniques are warranted.

Imaging plays a key role in the clinical management of hepatocellular carcinoma (HCC), but conventional imaging techniques have limited sensitivity in visualizing small tumors and assessing response to locoregional treatments and sorafenib. Functional imaging techniques allow visualization of organ and tumor physiology. Assessment of functional characteristics of tissue, such as metabolism, proliferation and stiffness, may overcome some of the limitations of structural imaging. In particular, novel molecular imaging agents offer a potential tool for early diagnosis of HCC, and radiomics may aid in response assessment and generate prognostic models. Further prospective research is warranted to evaluate emerging techniques and their cost-effectiveness in the context of HCC in order to improve detection and response assessment.

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Hepatocellular carcinoma (HCC) is the most common primary liver tumor worldwide and the third most common cause of cancer-related death [1,2]. Most HCC arises in the context of liver cirrhosis secondary to hepatotropic viruses, alcohol excess, metabolic derangements and nonalcoholic steatohepatitis [3].

The diagnosis of HCC is based on a characteristic vascular enhancement profile using dynamic contrast-enhanced CT (DCE-CT), MRI (DCE-MRI), and in some cases, DCE-ultrasound (DCE-US) (American and European Associations for the Study of Liver Disease [AASLD and EASL] guidelines) (Figure 1) [4–6]. However, the cirrhotic liver is not homogenous but contains regenerative or dysplastic nodules, as well as HCC, presenting a challenge to conventional imaging

KEYWORDS

- CT • functional imaging
- hepatocellular carcinoma
- molecular imaging
- MRI • PET • radiomics
- ultrasound

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Figure 1. Flow chart for hepatocellular carcinoma management. Flow chart displaying the typical route of an HCC patient in our institution once a >1-cm lesion is detected on US screening. BCLC: Barcelona Clinic Liver Cancer; DCE-CT: Dynamic contrast-enhanced CT; HCC: Hepatocellular carcinoma; RFA: Radiofrequency ablation; TACE: Transarterial chemoembolization; US: Ultrasound.

techniques that have limited sensitivity in differentiating various pathological processes [7]. This is of particular importance in the assessment of early stage disease, where prompt intervention translates into long-term survival. Response assessment following locoregional therapies is similarly problematic. The primary objective of these therapies is to induce necrosis, and no

immediate change in tumor size is usually seen. Furthermore, the use of radiation or embolization material alters the vascular enhancement pattern of HCC, making detection of residual disease challenging. Response assessment to systemic therapies, such as the targeted agent sorafenib, is a further challenge [8]. Targeted agents produce a cytostatic rather than cytotoxic

response, where reduction in tumor size may take months [9]. Conventional imaging response criteria WHO and RECIST1.1 [10] describe changes in tumor size based on the sum of the greatest dimensional measurement of target lesions. Therefore, WHO and RECIST1.1 are limited in assessing therapeutic response to locoregional and systemic therapies in HCC, with recent studies showing a poor correlation between clinical outcome measures and conventional radiologic response measures [11]. This is a particular concern in early-phase clinical trials setting, delaying the clinical transition of potentially efficacious agents. Introduction of modified RECIST (mRECIST) [12] and EASL criteria, that assess viable tumor, as defined by arterial enhancement, is a step change in the assessment of tumor response. However, similar to RECIST1.1 and WHO, these modified response assessment criteria are surrogates of tumor viability on representative tumor slices only, and by no means represent whole tumour analysis. Moreover, novel drugs that target angiogenesis and perfusion may impact on contrast delivery, and mRECIST and EASL assessments.

More accurate imaging modalities are therefore urgently needed to facilitate not only clinical management but also early-phase research.

Functional imaging techniques enable the noninvasive assessment of tumor physiology, where changes in response to treatment occur earlier than changes in tumor size. These techniques may also aid in differentiating benign nodules and HCC. There are various functional imaging techniques available for both the delineation of malignant disease and therapeutic response. This review describes these techniques, discussing their application in HCC and providing an overview of the functional imaging characteristics of this disease through each imaging modality.

Ultrasound

Because of its high spatial resolution balanced against low cost and lack of radiation, biannual ultrasound (US) examination of the liver is typically used for surveillance of patients at high risk of developing HCC [4]. However, because HCC has a varied reflection pattern on US, sensitivity is low (60%) as reported by a systematic

Table 1. Detection of primary hepatocellular carcinoma with US, CT scan and MRI techniques.

Study (year)	Modality	HCC appearance	Sensitivity (%)	Specificity (%)	Ref.
US					
Colli <i>et al.</i> (2006)	B-mode US	Hyper-, hypo-, iso-intense	60	97	[5]
Ignee <i>et al.</i> (2005), Tanaka (1990), Golli <i>et al.</i> (1993)	Doppler	Hypervascular, with a centripetal bloodflow and/or 'basket-pattern'	–	–	[13–16]
Alam <i>et al.</i> (2013)	Doppler	Detection of portal vein thrombosis	81	100	
Ding <i>et al.</i> (2005)	DCE-US	Arterial enhancement followed by washout	92	87	[17]
CT					
Colli <i>et al.</i> (2006)	DCE-CT	Arterial enhancement followed by washout	68	93	[5]
Kim <i>et al.</i> (2009)	Perfusion maps	Higher AEF on quantitative color maps	88	–	[18]
MRI					
	T1 and T2	Hyper-, hypo-, iso-intense or mixed	–	–	
Colli <i>et al.</i> (2006)	DCE-MRI	Arterial enhancement followed by washout	81	85	[5]
Vandecaveye <i>et al.</i> (2009)	DW-MRI	Restricted diffusion	95	81	[19]
Ichikawa <i>et al.</i> (2010)	Gd-EOB-DTPA	Hypointense compared with surrounding liver parenchyma	68–80	–	[20]

AEF: Arterial enhancement fraction; DCE: Dynamic contrast enhanced; DW: Diffusion weighted; Gd-EOB-DTPA: Gadoteric-acid-disodium; HCC: Hepatocellular carcinoma; US: Ultrasound.

review of 14 studies evaluating surveillance US (Table 1) [5].

• Doppler & dynamic contrast-enhanced US

Doppler US techniques visualize vascular morphology by measuring changes in US frequency that occur when a moving object reflects a US-wave, and are applied clinically with relative ease. HCC lesions are typically hypervascular, with a centripetal bloodflow [13], and some studies report a ‘basket-pattern’ where the lesion is cupped by blood vessels that branch inside the tumor [14,15]. The presence and nature of portal vein thrombosis can also be determined; with a sensitivity (81%) and specificity (100%) compared with DCE-CT for identifying tumor invasion [16]. DCE-US may increase the accuracy of US-assessed vascularity and hemodynamics. In DCE-US, microbubble contrast agents are used to increase the echogenicity of blood. This enables noninvasive, real-time evaluation of vascular morphology, enhancement patterns and other hemodynamics derived from time-intensity curves. Following contrast injection, HCC displays arterial enhancement followed by a ‘wash-out’, shown as an enhancement defect (Figure 2). Characterizing HCC with DCE-US based on this pattern has reasonable sensitivity (92%) and specificity (87%) [5,17,21]. However, small HCCs (<2 cm) may not have developed sufficient vascularization and often have a different enhancement pattern, similar or identical to surrounding liver tissue [7]. Moreover, intrahepatic cholangiocarcinoma and various benign lesions

may also display this enhancement pattern [22]. SonoVue/Lumason has received regulatory approval in a number of countries. A systematic review on DCE-US for the characterization of focal liver lesions in a cirrhotic background concluded that there was no significant difference in sensitivity and specificity between DCE-CT, -MRI and -US. The authors argue that DCE-US could potentially be applied during surveillance to further characterize screen detected lesions as it is more cost beneficial compared to CT and MRI [23]. In our institution DCE-US is often used to further characterize indeterminate lesions on CT/MRI.

DCE-US has also been investigated as a marker of therapeutic response (Table 2). DCE-US time-intensity curves, specifically area under the curve, have shown potential in the assessment of response to radiofrequency ablation (RFA) [24], transarterial chemoembolization (TACE) [25] and sorafenib [26–28]. User-dependency and low reproducibility may limit the use of DCE-US for response assessment. Introduction of time-intensity curves, and 3D techniques that allow volumetric assessment of lesions [29], may overcome these limitations as they offer a more quantitative approach. It is important to note that DCE-US response assessment is based on contrast enhancement; therefore, tumors that do not initially display arterial enhancement may not be accurately evaluated with this technique. DCE-US can also be applied to guide RFA [30,31] and 3D DCE-US in particular shows promise in this area.

Figure 2. Dynamic contrast-enhanced ultrasound images of hepatocellular carcinoma at different time points. Dynamic contrast-enhanced ultrasound images of hepatocellular carcinoma, displaying arterial blood vessels cupping the tumor in a ‘basket-pattern’ (A), arterial enhancement (B) and washout (C) of a lesion in a cirrhotic liver. B-mode images with low mechanical index settings (D) allow some anatomical correlation of dynamic contrast-enhanced ultrasound images, although image quality with these settings is reduced it is necessary to avoid microbubble disruption. Images courtesy of Professor Adrian Lim, Imperial College London NHS trust.

Table 2. Hepatocellular carcinoma response assessment by various imaging modalities.

Study (year)	Modality	Treatment	Patients (n)	Result	Ref.
Farina <i>et al.</i> (2009)	DCE-US with bloodpool agent	Locoregional and sorafenib	37	Decrease of AUC of liver parenchyma between 0 and 7 days poststart of sorafenib treatment predicted occurrence of adverse events. Reduction of tumor AUC at day 14 was correlated with overall survival and progression-free survival	[24–27]
Moschouris <i>et al.</i> (2014)			20	1 day post-RFA, TAC with high peak followed by plateau indicate remaining intra- and peri-lesional tumor activity	
Frampas <i>et al.</i> (2013)			47	A combination of mRECIST and DCE-US was found to be feasible and have prognostic value post-TACE	
Sugimoto <i>et al.</i> (2013)			19	A decrease of lesion AUC by >40% 1 month post-targeted therapy indicated nonprogression at month 2	
Xia <i>et al.</i> (2008)	DCE-US with sonazoid	TACE	43	Sonazoid DCE-US 1 week post-treatment predicted response to TACE	[32]
Park <i>et al.</i> (2014), Hayashida <i>et al.</i> (2008)	DCE-CT	Locoregional, sorafenib and radiotherapy	61 40	mRECIST, washout phase is important postradiotherapy. Was more sensitive in detecting washout in this group	[33,34]
Lee <i>et al.</i> (2013)	Perfusion maps	TACE	76	Post-TACE, perfusion maps improved sensitivity of DCE-CT for detection of recurrent or residual HCC from 63 to 82%	[35]
Frampas <i>et al.</i> (2013)	Perfusion CT	Sorafenib	19	Was not able to accurately measure response in this group	[26]
Hayashida <i>et al.</i> (2008)	DCE-MRI	Locoregional and sorafenib	40	Was more accurate at detecting arterial enhancement than CT in this group mRECIST	[34]
Sahin <i>et al.</i> (2012), Kokabi <i>et al.</i> (2014)	ADC values	TACE and TARE	2018	TACE, increasing ADC values indicate a response. TACE, >30% increase in ADC 3 month post-treatment	[36,37]
Lin <i>et al.</i> (2012)	¹⁸ F-FDG	Surgical and locoregional	101	(¹⁸ F)-FDG PET was valuable in ruling out recurrence disease post-treatment, meta-analysis of five studies	[38]
Bieze <i>et al.</i> (2014)	¹⁸ F-choline	TACE, RFA, sorafenib	6	Decrease in standard uptake value (SUV) _{ratio} . Potential to assess recurrence post-TACE/RFA and identify new sites	[39]

ADC: Apparent diffusion coefficient; AUC: Area under the curve; DCE: Dynamic contrast enhanced; HCC: Hepatocellular carcinoma; RFA: Radiofrequency ablation; TAC: Time-activity curve; TACE: Transarterial chemoembolization; US: Ultrasound.

• Molecular imaging with US

Targeted molecular imaging, using specific ligands attached to microbubbles, is gaining increasing interest. While most research using targeted contrast agents is preclinical [40], Sonazoid (GE healthcare), an agent phagocytized by Kupffer cells, has shown to improve detection and characterization of liver lesions [41] and has regulatory approval in Japan. Sonazoid has been investigated as a response marker to TACE with some success [32].

There is considerable interest in developing biomarkers of angiogenesis, given its pivotal role in hepatocarcinogenesis. Integrins, a family of cell adhesion molecules, facilitate the interaction between tumor vasculature and extracellular matrix. $\alpha v\beta_{3/5}$ -integrins are overexpressed on tumor-related endothelial cells compared with mature vessels, where they interact with components of the extracellular matrix via the

tripeptide sequence arginine-glycine-aspartic acid (RGD) [42]. Utilizing the RGD sequence, Anderson *et al.* developed a contrast agent with high specificity for $\alpha v\beta_{3/5}$ in a breast cancer model [43]. This has been translated to an HCC *in vivo* model that could allow for the evaluation of multiple hepatic lesions in a single contrast bolus as the contrast agent an exciting concept given the lack of validated biomarkers of response to antiangiogenic therapies.

Another HCC-specific contrast agent undergoing *in vitro* evaluation is a monoclonal antibody to glypican-3 (GCP3) attached to polylactic-co-glycolic acid microbubbles. GPC3 is overexpressed in HCC [44], but not in healthy or cirrhotic tissue [45], thus making it suitable for lesion characterization. Anti-GCP3 strategies are also being developed in MRI and PET (discussed below) [46]. Furthermore, HAB18, that is overexpressed on tumor membrane, and

is associated with invasion and metastasis, is also being studied as an HCC-specific contrast agent using microbubble technology [47,48]. How clinically feasible these specific contrast agents will be is questionable due to cost–benefit, however, the ability to specifically target HCC may allow for therapeutic application of these agents. The use of microbubble agents for drug delivery is an active area of research, but goes beyond the scope of this review [40,49].

• US elastography

US elastography (USE) quantifies lesion stiffness, a mechanical property of tissue, measured in Pascal (kPa). Studies have shown that USE is able to distinguish between HCC and benign tissue [50,51], pathological tissue having lower elasticity than normal tissue. However, contradictory results have been reported [52,53], which may reflect reduced liver elasticity from cirrhosis, and heterogeneous elasticity profile of HCC itself. Due to the simplicity of USE, novel shear-wave techniques might prove a more robust and useful tool to apply during screening (Figure 3), though likely more in the context as a prognostic biomarker rather than a means of lesion characterization.

Important drawbacks of US-based techniques are their subjectivity, limited reproducibility and inability to assess the entire liver in a standardized manner, thus requiring additional contrast boluses, particularly in multifocal

disease. Obesity, nonalcoholic steatohepatitis, abdominal air and poor patient cooperation may complicate visualization of the liver entirely (Table 1), hampering accurate response assessment. Furthermore, mRECIST/EASL evaluation is not possible with DCE-US.

Multidetector CT

• Dynamic contrast-enhanced CT

Multidetector CT imaging is widely used for the assessment of liver lesions whereby three enhancement phases are obtained; arterial, portal and delayed phase [54], with HCC displaying characteristic arterial enhancement followed by washout (Table 1) [5]. However, a significant number of lesions do not display this enhancement pattern, and biopsy is often required particularly for small 1–2 cm nodules [7,55]. DCE-CT offers good reproducibility, and is widely used to evaluate treatment response in conjunction with mRECIST. As discussed, response assessment based on mRECIST may be limited. In a retrospective study of 61 patients with HCC receiving radiotherapy, washout rather than arterial enhancement was found to be important for the evaluation of treatment response, as external beam radiotherapy can induce hyper-enhancement of healthy liver parenchyma, making response assessment based on arterial enhancement difficult [33]. Response assessment following transarterial radioembolization (TARE) is also complicated. TARE can result

Figure 3. Shear-wave elastography of an hepatic lesion. The images display a novel shear-wave technique currently under development by Toshiba Medical Systems, applied to a hepatocellular carcinoma. Once the lesion is detected on B-mode ultrasound, shear waves are applied. As a quality control measure, a propagation map visualizes the propagation of these waves through the liver tissue. An elasticity map is then generated, displaying the regional stiffness of the selected region in kPa. Hepatocellular carcinoma shows higher stiffness than the surrounding tissue. Images courtesy of Professor Fuminori Moriyasu, Tokyo Medical University.

in peritumoral edema, necrosis and hemorrhage that may cause an apparent increase in tumor size that can last up to 3–6 months post-treatment. HCCs treated with TARE exhibit a maximum RECIST response 3 months post-therapy, while absence of arterial enhancement is visible after 1 month. Correlation with other imaging methods, such as diffusion-MRI and PET, may aid in response assessment in this situation, a particular need [56,57] given the increasing role of TARE in the management of HCC. Volumetric assessment of lesions has been attempted in the context of colorectal and pancreas cancer metastasis, by applying 3D algorithms to multidetector CT images retrospectively [58]. This study reported less inter observer variability compared with standard RECIST, however it is unclear how these algorithms would translate to lesions a cirrhotic liver.

• Perfusion CT & quantitative color maps

By continuously scanning a volume of liver, perfusion can be assessed with excellent temporal resolution. However, clinically this technique has not borne out, with changes in perfusion parameters, such as blood flow, blood volume and mean transit time not correlating with response following sorafenib or TARE [26,59]. The high-radiation dose associated with CTP further limits its use in clinical practice. Tumor enhancement can be assessed using quantitative color maps (QCM), which can be generated with commercially available software by subtracting unenhanced images from the arterial, portal and delayed-phase scans. This enables quantitative analysis of lesion enhancement by calculating the arterial enhancement fraction [18]. HCC lesions that are iso- or hypo-enhanced on arterial phase images can display higher arterial enhancement fraction than healthy liver tissue on QCM, if they show sufficient washout during the portal or delayed phase [18]. Kim *et al.* proposed an improvement in diagnostic accuracy using QCM, without an increase in radiation dose ($n = 93$) [18]. QCM was reported to improve response assessment following TACE compared with DCE-CT alone (Table 2) [35]. Because QCM does not increase radiation exposure, further research in the context of treatment response is warranted.

• Low voltage & dual energy CT

Low tube voltage scanning increases tissue and contrast agent attenuation, and may be more

sensitive to arterial enhancement than higher energy CT [60]. Dual energy CT can characterize tissue based on its spectral behavior, achieved by measuring tissue attenuation at different tube voltages and has been applied in the assessment of treatment response with some success [61]. The role of both low voltage scanning and dual energy imaging techniques in the clinical management of HCC requires further investigation, particularly with regards to their sensitivity and specificity when compared with standard imaging techniques.

The main disadvantage of CT compared with other imaging techniques remains radiation exposure: 25–35 mSv per DCE-CT study. Furthermore, DCE-CT has a relatively low sensitivity in diagnosing HCC (68%) (Table 1). The use of perfusion CT, and other novel approaches such as low voltage CT [60] and dual energy CT [61] may overcome these limitations, but need further validation in prospective studies.

MRI

• Dynamic contrast-enhanced MRI

DCE-MRI allows the evaluation of hepatic nodules based on their enhancement profile, similar to DCE-US and DCE-CT [5,62–63]. Gadolinium chelates are used as contrast agents, significantly reducing T1 decay time, increasing signal intensity. T1 images allow visualization of the contrast agent in the extravascular–extracellular space at different time intervals enabling the assessment of the enhancement profile of any given lesion. The overall sensitivity and specificity of DCE-MRI in the context of detecting HCC is 81 and 85%, respectively (Figure 4) [5]. However, sensitivity reduces with decreasing lesion size (<2 cm 50–80%; <1 cm 3–33%) [64].

• Diffusion-weighted MRI

DW-MRI assesses water motion within tissue and is routinely used in the clinical setting. DW-MRI images are obtained by applying balanced gradients to T2-weighted sequences. The amount of diffusion weighting can be increased or decreased by changing the ‘b-value’, an acquisition parameter. Signal intensity from stationary water molecules is maintained, while those that are in motion lose signal intensity. Pathological tissues typically have high signal intensity, because diffusion is often restricted, whereas healthy tissue has low signal intensity on DW-MRI. Low b-value images ($b < 150$ s/mm²) improve lesion detection compared with T2

Figure 4. Typical enhancement of hepatocellular carcinoma. Arterial (A), portal (B) and delayed (C) phase images of a dynamic contrast enhanced MRI scan displaying the characteristic hepatocellular carcinoma enhancement pattern; arterial enhancement followed by washout. A T2 (D) sequence shows this lesion as slightly hyperintense compared with the surrounding liver parenchyma.

images alone, whereas high b-value images ($b < 500 \text{ s/mm}^2$) can be used for lesion detection and characterization [65]. A study conducted in 55 patients concluded that high b-value DW-MRI ($b600 \text{ s/mm}^2$) improved detection and characterization of HCC lesions compared with DCE-MRI alone (Table 1) [19].

The total displacement of water molecules is described by the apparent diffusion coefficient (ADC), generated by calculating the ADC-value for individual voxels, using multiple b-value diffusion images. ADC-maps have the added benefit of reducing T2 interference, which may be present in lesions with a long T2 decay. ADC-values can be useful for the characterization of liver lesions, with ADC cut-off values of $1.4\text{--}1.6 \times 10^{-3} \text{ mm}^2/\text{s}$ having a reported sensitivity of 74–100%, and specificity of 77–100% for differentiating between benign and malignant lesions [19,65–66]. Increasing ADC-values indicate treatment response following TACE ($n = 74$) and TARE ($n = 18$) [36,37] (Table 2). Cirrhosis and benign cirrhotic nodules also alter

diffusion in the liver, which may interfere with the interpretation of diffusion-weighted imaging and account for the relatively large range in reported sensitivity and specificity.

Intravoxel incoherent motion (IVIM) is a DW-MRI-based technique, where multiple b-values are used to separate pseudodiffusion (D^*), perfusion fraction (f) and true molecular diffusion (D) [67]. This technique has been successfully applied to characterize liver lesions [68,69]. In a retrospective study ($n = 42$) comparing IVIM to ADC, low D-values indicated high-grade HCC with higher accuracy than ADC-values. Furthermore, f -values were correlated to the percentage of arterial enhancement [70]. IVIM-based imaging shows potential, and further prospective research is necessary to determine its role in the context of HCC.

• MRI elastography

MRI elastography (MRE) also measures tissue stiffness but, unlike USE, MRE allows assessment of the entire liver. Mechanical shear waves

are applied to the liver, and the propagation of these waves is measured using phase-contrast sequences [71,72]. MRE has mainly been applied to noninvasively assess liver fibrosis [73,74], but has been used to evaluate hepatic lesions [71,75]. Malignant lesions show significantly higher stiffness using MRE compared with normal liver, benign lesions and fibrotic liver (10.1 vs 2.3 kPa, 2.7 and 5.9 kPa, respectively). A cutoff of 5 kPa allowed for accurate distinction between benign and malignant tumors (100%), but some overlap between malignancy and fibrosis was still observed [71]. MRE has some benefits over USE, such as its applicability in obese patients, but the high cost and technical complexity effect cost-effectiveness of the technique. MRE is not able to differentiate between HCC, ICC or other malignant hepatic lesions. Further investigation should focus on the detection and characterization of small hepatic nodules and we feel there may be a particular role in the assessment of changes in elasticity of the liver and HCC following treatment.

• Volumetric functional MRI

mRECIST and EASL response guidelines describe changes in tumor size based on the sum of the longest axis measurement: however, this single dimension may not accurately portray the entire lesion. Feasibility of a 3D approach through semiautomated segmentation is described by Lin *et al.*, where quantitative and volumetric EASL and RECIST (qEASL and vRECIST) were applied post -ACE [76]. Volumetric assessment of DCE- and DW-MRI was superior compared with RECIST1.1, EASL, mRECIST and α -fetoprotein levels in retrospective studies ($n = 144$) [77,78]. Corona-Villalobos *et al.* found similar findings when applying this technique post-TACE-sorafenib therapy [79]. Although more experience with these techniques is required before clinical implementation, we believe whole lesion evaluation represents a step change in response assessment of HCC.

• Liver-specific contrast agents

Contrast agents specific for liver imaging include hepatobiliary agents and superparamagnetic iron oxide particles (SPIO). Hepatobiliary agents target healthy hepatocytes and are excreted through the biliary tract, while SPIO particles target the reticuloendothelial system [62]. Uptake and excretion of gadoxetic-acid-disodium

(Gd-EOB-DTPA) can be visualized dynamically, where HCC display no uptake of Gd-EOB-DTPA while normal hepatocytes do. Absorption of Gd-EOB-DTPA is mediated by organic anion – transporting polypeptide 8 (OATP8) [80] present on hepatocytes. OATP8 expression decreases with poor tumor differentiation [81], and evidence suggests uptake of Gd-EOB-DTPA correlates with a less aggressive tumor phenotype. [82]. In a multicenter trial, Gd-EOB-DTPA-MRI uptake combined with serum AFP levels, was reported to be prognostic following resection [83]. However, this was a retrospective study including multiple cohorts. Other studies report an improved detection rate using Gd-EOB-DTPA [20,84–85], and added value, especially in the detection of lesions <1 cm, compared with DCE-CT.

SPIO agents are phagocytized by Kupffer cells and induce local changes in the magnetic field that can be measured by T2/T2* sequences, they are not US FDA approved. Contrast is achieved as HCC contain less Kupffer cells than surrounding liver tissue. Detection rate of HCC can be increased using SPIO particles [86]. In a study of 114 patients with hepatic nodules, SPIO particles were reported to be useful in differentiating low-grade HCC and dysplastic nodules [87].

Contrast agents in preclinical stages of development include nanoparticles labeled with anti-GCP3 monoclonal antibody and gadolinium [88], which as previously discussed, has high specificity for HCC. This agent is currently undergoing *in vitro* development and may develop into a novel MR contrast agent.

Motion artefacts are a common problem in all forms of hepatic MRI scanning, especially in the presence of ascites. It is expected that, as scanning techniques improve, this will become a less limiting factor. High costs and technical complexity are further disadvantages of MRI techniques.

PET

PET imaging is a well-integrated technique in oncology, and is routinely used for diagnosis, staging and therapeutic response assessment in a number of malignancies. During PET, radiolabeled compounds are administered intravenously, at subphysiological doses, in order to assess differing characteristics of tumor biology such as proliferation, angiogenesis and apoptosis. PET has been extensively investigated in HCC with disappointing results, and in most

centers PET imaging remains a research tool. However, this may change with the advent of novel techniques, such as immuno-PET (Table 3).

• ¹⁸F-FDG

Gluconeogenesis, a marker of metabolism, is increased in malignant tissue and can be visualized using ¹⁸F-FDG, the most commonly used radiotracer in oncology. High hexokinase activity, and low glucose-6-phosphatase activity, mediates the phosphorylation of ¹⁸F-FDG intracellularly to ¹⁸F-FDG-6-phosphate, which becomes trapped. Hence, ¹⁸F-FDG uptake reflects rate of gluconeogenesis. A number of studies have shown a correlation between high uptake of ¹⁸F-FDG and high-grade tumors [92–94]. Others have indicated the prognostic value of ¹⁸F-FDG in selecting patients for liver transplantation [95], locoregional treatment [96] or sorafenib [97]. However, the rate of gluconeogenesis of normal hepatocytes and HCC is similar, resulting in high background uptake, making differentiation of HCC difficult. The reduction in glucose-6-phosphatase expression is dependent on degree of differentiation in liver lesions and is not absolute [98]. Furthermore, ¹⁸F-FDG uptake is increased in the presence of inflammation, a potential confounding factor as HCC develops in the presence of inflammation [92,99]. These factors negatively impact on the sensitivity of ¹⁸F-FDG-PET in detecting intrahepatic HCC (64%) (Table 2). ¹⁸F-FDG-PET has been reported to be inferior compared with other imaging modalities [89], and is not routinely used in clinical practice, except for the detection of extrahepatic disease in some centers [100].

• ¹⁸F-fluoro-thymidine

¹⁸F-FLT is a validated biomarker of proliferation, specific for malignancy [101]. Like thymidine,

¹⁸F-FLT enters the cell by both sodium-dependent active nucleoside transporters and by passive diffusion, and undergoes phosphorylation by thymidine kinase-1. Phosphorylated ¹⁸F-FLT is not incorporated into DNA and is trapped within the cytosol, such that the uptake of ¹⁸F-FLT correlates with rate of proliferation. An initial pilot study in HCC (n = 16) showed promising correlation between ¹⁸F-FLT uptake and cell proliferation and noninferiority compared with ¹⁸F-FDG. However, there is high physiological uptake of ¹⁸F-FLT within the liver, therefore making it difficult to characterize pathological hepatic lesions [102]. Kinetic spatial filtering (KSF), a temporal intensity information-based voxel clustering approach, utilizes tissue-specific time-activity curves to subtract those voxels that display a time-activity curves associated with normal tissue [103]. This leads to a reduction of background liver signal while maintaining signal from tumors. The application of KSF has been investigated for the evaluation of treatment response in liver metastases from a number of tumor types [103,104]. The same principle of KSF can be applied in the context of HCC, and may improve response assessment in the management of HCC using ¹⁸F-FLT (Figure 5). Investigation of this technique in HCC is ongoing within our research group. We believe that ¹⁸F-FLT-PET with application of the KSF may offer a unique biomarker for response to targeted therapies, particularly in the setting of early drug development.

• ¹¹C-acetate & ¹⁸F-acetate

Fatty acid synthesis is associated with tumor growth and invasiveness, and can be evaluated using ¹¹C-acetate and ¹⁸F-acetate. In a study of 32 patients with HCC, ¹¹C-acetate had a

Table 3. Detection of primary hepatocellular carcinoma by various PET tracers.					
Study (year)	PET	HCC appearance	Sensitivity (%)	Specificity (%)	Ref.
Wudel <i>et al.</i> (2003)	¹⁸ F-FDG	High uptake in poorly differentiated HCC, no significant uptake in well-differentiated HCC, background uptake in liver parenchyma	64	–	[89]
Park <i>et al.</i> (2008)	¹¹ C-acetate	High uptake in well-differentiated HCC, background uptake in liver parenchyma	75		[90]
Talbot <i>et al.</i> (2010)	¹⁸ F-choline	Increased uptake in well-differentiated HCC, low uptake in poorly differentiated HCC, high background uptake in liver parenchyma	88	62	[91]
Talbot <i>et al.</i> (2010)	¹⁸ F-FDG + (11)F-choline		94	–	[91]
Park <i>et al.</i> (2008)	¹⁸ F-FDG + (11)C-acetate		86	–	[90]

HCC: Hepatocellular carcinoma.

Figure 5. Kinetic spatial filtering of ^{18}F -FLT scan. ^{18}F -FLT scan of a patient with diffuse hepatocellular carcinoma (arrow) (A), note the high background uptake of liver parenchyma, which complicates image interpretation. When kinetic spatial filtering is applied (B), it is possible to assess the diffuse hepatocellular carcinoma in this patient.

sensitivity of 87% for detecting HCC [105], and in a larger study including 110 HCC lesions, an overall sensitivity of 75% was found [90]. While encouraging, subset analysis revealed a significantly lower sensitivity (32%) for the detection of small HCC lesions (1–2 cm). ^{18}F -acetate was studied as an alternative to ^{11}C -acetate due to its longer half-life (110 vs 20 min) and more favorable biodistribution [106]. However, no difference was observed in the uptake of ^{18}F -acetate by the tumor compared with ^{18}F -FDG [107]. The clinical value of a dual tracer approach combining ^{11}C -acetate and ^{18}F -FDG has also been investigated, the rationale being that ^{11}C -acetate has greater uptake in well-differentiated HCC while poorly differentiated HCC have increased uptake by ^{18}F -FDG. Combined they have an overall sensitivity of 86% for detecting intrahepatic HCC, but no increase in the detection of extrahepatic disease was observed [90]. Long scan times and increased radiation dose limits the clinical applicability of a dual tracer approach.

• ^{11}C -choline & ^{18}F -choline

Choline is a substrate for phosphatidylcholine, the key phospholipid in the cell membrane. During malignant transformation, overexpression of key enzymes involved in choline metabolism are seen (e.g., choline kinase- α), leading to increased phosphocholine and total choline containing compounds. Similar to ^{18}F -FDG and ^{18}F -FLT, radiolabeled choline is metabolized in the normal hepatocytes, resulting in high background uptake limiting its utility. Various studies have shown that ^{11}C -choline and

^{18}F -choline have increased uptake in moderately differentiated HCC, but low uptake in poorly differentiated HCC [91,108–109]. Talbot *et al.* prospectively compared ^{18}F -choline with ^{18}F -FDG, and concluded a significantly higher sensitivity of ^{18}F -choline for the detection of primary HCC (88 vs 67%) [91]. A later study emphasized the ability of ^{18}F -choline to detect extrahepatic disease, and potential to assess response in terms of local recurrence post-TACE and RFA, and progression of extrahepatic disease post-sorafenib [39]. Choline tracers have mainly been studied as a dual tracer to complement ^{18}F -FDG. A study that included 78 patients with HCC found that adding ^{11}C -choline increases the sensitivity of ^{18}F -FDG PET from 63 to 90% for the detection of HCC [109].

• Glypican-3-targeted PET ^{89}Zr - αGPC3 & ^{89}Zr -DFO-1G12

A novel approach to visualize HCC using PET is using immuno-PET; the application of radio-labeled antibodies. ^{89}Zr - αGPC3 is a radio-labeled antibody that targets GPC3, as previously described [44,110]. While GPC3-targeted US and MR contrast agents have shown feasibility *in vitro*, ^{89}Zr - αGPC3 has been tested *in vivo* in HepG2 inoculated mice with promising results [111]. Not only was assessment of HCC feasible using ^{89}Zr - αGPC3 , liver to lesion contrast was high. Background uptake of ^{89}Zr - αGPC3 may be higher in the presence of cirrhosis, however previous immunohistochemistry studies would suggest minimal uptake [45]. Similarly, the anti-GPC3 monoclonal antibody (clone

1G12) with ^{89}Zr (^{89}Zr -DFO-1G12) also displayed specific uptake in GPC3 positive HCC cells [112]. Monoclonal antibodies typically have a long circulation times which impacts image quality in PET imaging, and Sham *et al.* aimed to overcome this limitation by using F(ab')₂ fragments. Using immobilized ficin to digest αGPC3 they were able to create ^{89}Zr - αGPC3 -F(ab')₂, and apply this *in vivo*. Their results indicate an increased tumor-to-liver contrast ratio using this method [113]. Specific uptake of ^{89}Zr - αGPC3 and its fragmented version should be compared with each other, in cirrhotic models if clinical translation is to be achieved.

• PET/MRI

Studies in PET/MRI have shown feasibility with encouraging results in whole-body scanning and assessment of liver metastasis [114–116]. PET/MRI allows for multiparametric evaluation of liver lesions where, for example, tracer uptake and diffusion can be assessed simultaneously, which may lead to improved response assessment [117]. This could provide an extremely useful tool in early phase clinical trials. Without CT attenuation correction, SUV measurements are underestimated in PET/MRI, but segmentation-based MR attenuation correction reduces this bias [118]. The considerable cost of PET/MRI limits routine clinical use, and we feel that the role of PET/MRI is predominantly in the research setting.

Costs of PET imaging, short half-life of some radioactive tracers and high background uptake in normal hepatocytes limit the routine use of PET. Choline, acetate, thymidine and FDG-based tracers each have been investigated in HCC with varying results, and currently their role is limited to a research setting (Tables 2 & 3). Although the clinical application of PET in HCC thus far remains limited, it has potential in the early assessment of response to targeted therapy, of particular importance, in the drug development setting [119].

Radiomics & texture analysis

Radiomics involves the collation of quantitative imaging characteristics with the purpose of creating diagnostic and prognostic models [120]. This could be of particular importance in the context of HCC where biomarkers for patient stratification and prognosis are lacking. Aerts *et al.* describe tumor shape, intensity, texture and wavelet features, as characteristics

used to assess tumor phenotype in lung or head-and-neck malignancies based on CT imaging ($n = 1019$) [121]. These features can be assessed through TA, where statistical models are applied to analyze image gray levels. TA can be applied to all modalities and has been proposed as a biomarker for tumor heterogeneity, operating on the premise that heterogeneous tumors have more or different, variations in image gray-level patterns than homogenous tumors. US-TA has shown promise in lesion characterization [122] and response assessment [123]. In a breast-cancer model responders and nonresponders were correctly identified using US-TA, after 1 week of chemotherapy, with 100% sensitivity and 93% specificity [123]. MRI-TA can characterize liver hemangiomas and cysts [124], and ^{18}F -FLT PET TA is able to assess intratumor heterogeneity of proliferation in breast cancer, and may be used in response assessment [125]. TA is noninvasive, allowing for multiple measurements over time and allows whole lesion assessment (intratumor heterogeneity). One of the current research interests of our group is to assess the potential of MRI-TA to act as a predictive biomarker for survival and response to RFA in the context of HCC.

Quantitative imaging characteristics need to be correlated with histopathological findings and treatment outcomes [126]. A limitation of radiomics is the heterogeneity of clinical imaging protocols [120], which greatly impact the quantitative measurements. Standardized protocols should be used in prospective trials. Application of TA puts no additional burden on the patient and software development and standardized imaging protocols may lead to implementation to clinical practice. However, further prospective studies are required to correlate textural features derived from statistical models to tumor biology and verify the benefit of TA in the context of HCC.

Conclusion & future perspective

Although a reasonable sensitivity and specificity is reported for characterizing HCC using DCE-US, DCE-CT and especially DCE-MRI [5,17] based on its vascular enhancement profile, it is important to note that particularly small tumors, <2 cm, do not display the characteristic HCC enhancement profile [7]. Functional imaging may increase detection rate of early HCC, and aid in the characterization of hepatic nodules based on the assessment

of physiological properties. Furthermore, these novel technologies show potential in the assessment of treatment response. This is increasingly important for clinical management of HCC, as locoregional and novel-targeted therapies cannot be accurately assessed with structural imaging alone. The incorporation of functional characteristics in RECIST (mRECIST) has shown a more accurate reflection of tumor biology, but measuring other functional characteristics and 3D lesion analysis will further improve response assessment and clinical management of HCC in the future [25,127]. Emerging HCC-specific contrast agents, such as GPC3 targeting agents, will play a large role going forward. PET ligands and multiparametric PET/MRI can prove invaluable in the drug development process and should be developed further for these purposes,

while contrast agents for US and MRI may find uses in clinical practice. Staging systems of HCC will become more complex as new means of assessing and treating these lesions become available, and the clinical relevance and cost-effectiveness of each imaging method should be considered carefully before clinical implementation is considered.

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