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Diagnosis of hepatocellular carcinoma

Asmaa I Gomaa, Shahid A Khan, Edward LS Leen, Imam Waked, Simon D Taylor-Robinson

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the commonest cancers worldwide. It is a major health problem and its incidence is increasing [1]. The presence of cirrhosis of the liver is the major risk factor and worldwide this is largely due to chronic hepatitis C virus (HCV) and hepatitis B virus (HBV) infection. The diagnostic modalities, especially with respect to hepatic imaging, have improved in recent years. This, along with HCC surveillance in patients with cirrhosis, has led to the detection of HCC at an earlier stage, when curative therapy is likely to be more successful.

The major diagnostic techniques for HCC include serum markers, various imaging modalities and histological analysis.

SERUM MARKERS FOR HCC

A number of serum markers have been proposed and several are currently used in commonplace clinical practice as a method for detecting HCC (Table 1).

Alpha-1 fetoprotein (AFP)

Under physiological conditions, AFP is a fetal-specific glycoprotein with a molecular weight of around 70 kDa. It is synthesized primarily by the embryonic liver, by cells of the vitelline sac and by the fetal intestinal tract in the first trimester of pregnancy. The serum concentration of AFP declines rapidly after birth and its expression is repressed in adults. Pathologically, patients with chronic liver disease, particularly those associated with a high degree of hepatocyte regeneration, can express...
AFP in the absence of cancer. Also, AFP is elevated in hepatocarcinogenesis, embryonic carcinomas and in gastric and lung cancer.

AFP has been used as a serum marker for HCC for many years. It was first described by Abelev et al. in the 1960s. The first quantitative serum assays for AFP were established by Ruohoslatt and Seppala. AFP is not elevated in all patients with HCC. Some patients with cirrhosis and/or hepatic inflammation can have an elevated AFP, even without the presence of a tumor. The test has a sensitivity of 39%-65%, a specificity of 76%-94%, and a positive predictive value of 9%-50% for the presence of HCC in previously published studies. The variation in sensitivity and specificity of AFP in the studies performed may be due to the diversity of patient populations examined, varying study designs and differing cut-off values for normality.

There is a debate in defining the AFP cut-off level for the diagnosis of HCC. An AFP value above 400-500 ng/mL has been considered to be diagnostic for HCC in patients with cirrhosis. However, such a cut-off value is problematic in absolute diagnostic terms, since high levels of this magnitude are not as common in the presence of smaller tumors (< 5 cm) and furthermore, only 30% of HCC patients have levels higher than 100 ng/mL in this context.

A case-control study of 170 HCC patients and 170 matched patients with chronic liver disease was conducted in Italy. The authors defined an AFP level of 16 ng/mL as the threshold that maximized sensitivity and specificity for the diagnosis of HCC. Using an AFP level of 20 ng/mL (the upper normal range) as the cut-off yielded equivalent sensitivity (60.0% vs 62.4%) and specificity (90.6% vs 89.4%). The positive and negative predictive values were 85% and 70%, respectively. The positive predictive value increased to 100% for patients with cirrhosis and/or hepatic inflammation can have an elevated AFP, even without the presence of a tumor. The test has a sensitivity of 39%-65%, a specificity of 76%-94%, and a positive predictive value of 9%-50% for the presence of HCC in previously published studies. The variation in sensitivity and specificity of AFP in the studies performed may be due to the diversity of patient populations examined, varying study designs and differing cut-off values for normality.

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In another study, conducted in 290 Chinese patients with chronic hepatitis B, 44 patients were found to have an elevated AFP (> 20 ng/mL). Of these, only six (14%) had HCC and the remaining 38 patients had an elevated AFP due to hepatitis B viral flares (n = 18), or due to unknown causes (n = 20). It is clear that using an AFP level of just above normal as a cut-off level gives a very low positive predictive value. Moreover, patients with chronic hepatitis B or C with reactivation were found to have AFP levels > 500 ng/mL.

AFP seems to be of prognostic value at the time of tumor diagnosis. A high AFP concentration (> 400 ng/mL) in HCC patients is associated with greater tumor size, bilobar involvement, portal vein invasion, and a lower median survival rate. According to a recent study, patients with serum AFP greater than 1000 ng/mL have a higher incidence of vascular invasion (61%) compared to patients with an AFP level less than or equal to 1000 ng/mL (32%). This may relate to the finding that well-differentiated tumours express lower levels of AFP.

In addition, AFP can be used as a marker for detecting tumour progression in patients with AFP-producing HCC. After treatment of the tumour, complete response is likely if the pre-treatment elevated AFP levels decline to and remain at normal levels during subsequent follow-up measurements. Reduction of AFP levels after palliative treatment, such as transarterial chemoembolization, usually indicates a favorable response to treatment. In addition, AFP is an excellent marker for detection of de novo HCC after treatment, if the new lesion is of the AFP-secreting variety.

A large multicenter study, based on both retrospective and prospective data collection, was carried out by Farinati et al. over a consecutive series of more than 1000 HCC patients. Only 18% of the studied patients had an AFP level of > 400 ng/mL. Moreover, patients with high AFP had poor survival. In this study, AFP was not a sensitive marker to detect the presence of HCC. Also, the prognostic value of AFP is limited, but it is correlated with the overall survival in untreated patients, or in those treated by liver transplantation or locoregional therapies.

**Lens culinaris agglutinin-reactive AFP**

There are three different AFP variants, differing in their sugar chains (AFP-L1, AFP-L2, AFP-L3). AFP-L1, the non-LCA-bound fraction, is the main glycoform of AFP in the serum of patients with non-malignant chronic liver disease. In contrast, Lens culinaris-reactive AFP, also known as AFP-L3, is the main glycoform of AFP in the serum of HCC patients and it can be detected in approximately one third of patients with small HCC (< 3 cm), when cut-off values of 10% to 15% are used. Its sensitivity and specificity ranges from 75% to 96.90% and 90% to 92%, respectively at the cut-off level of 15%.

In a study conducted in HCC patients with lesions less than 2 cm in size, using a cut-off level of 10% was diagnostic for the presence of HCC. AFP-L3 was found to be associated with poorly differentiated and advanced HCC. Higher AFP-L3 levels were found in hypervascular HCC, compared to iso- or hypovascular HCC. Elevated levels of AFP-L3 were associated with a shorter tumor doubling time in comparison with those with low levels of AFP-L3.

Moreover, AFP-L3 acts as a marker for clearance of HCC after treatment and as a predictor of recurrence.
failure to decline to the normal level indicates residual disease. Recurrence of HCC is expected when AFP-L3 levels increase to > 10% or rise after normalization with treatment[23]. Another study reported that AFP-L3 > 15% is a significant predictor for HCC recurrence[29].

AFP-L3 levels were found to be related to progression from moderately differentiated to poorly differentiated tumors. HCC patients with AFP-L3 > 10% had a higher frequency of poorly differentiated tumors[27]. Thus, this biomarker may be able to predict advanced tumor stage and a worse prognosis.

It is reported that an AFP-L3 level of 15% or more is correlated with HCC- associated portal vein invasion[29], both total serum AFP and AFP-L3 can be measured simultaneously[29], and estimating the AFP-L3/ AFP ratio is helpful in diagnosis and prognosis of HCC.

Des-gamma carboxyprothrombin (DCP)

DCP is an abnormal prothrombin protein that is found in the serum of patients with HCC and in patients with vitamin K-deficiency or on warfarin therapy. It is a “so-called” protein induced by vitamin K absence or antagonist- II (PIVKA-II) [29,30].

DCP is produced as a result of an acquired defect in the post-translational carboxylation of the prothrombin precursor (the 10 glutamic acid residues at the N terminus) in malignant cells[31]. The reduced activity of gamma-carboxylase was attributed to defective gene expression in HCC patients[3]. A DCP level of 40 mAU/mL is commonly used as a cut-off level, at which the rate of early detection of small HCC is improved[32]. Serum DCP was found to have a sensitivity of 48% to 62%, a specificity of 81% to 98%, and a diagnostic accuracy of 59% to 84% in diagnosing HCC in several large case-controlled studies.[3,7,32]

DCP is a well-recognized tumor marker used for the diagnosis of HCC. Its diagnostic accuracy has been investigated in multiple studies, with conflicting results. DCP has been reported to be more sensitive and specific than AFP in the diagnosis of HCC, especially in Eastern Asian countries and in North America[7,32,33]. Conversely, in Europe, studies have not shown these results. These discrepant results were related not only to racial factors but also to different etiological factors in liver disease. Even with these results, the measurement of both markers is suggested to increase diagnostic efficacy[3].

A recent study compared the performance characteristics of AFP, DCP and AFP-L3 in the diagnosis of HCC. DCP was significantly better than the other markers in differentiating HCC from cirrhosis, with a sensitivity of 86% and a specificity of 93%[34]. However, tumor size can affect the sensitivity and specificity of DCP in detecting HCC. According to a study by Nakamura and colleagues[35], the efficacy of DCP was lower than that of AFP in the diagnosis of small HCC tumors, although higher than that of AFP for large tumors.

Multiple studies have shown that DCP can be a useful indicator of vascular invasion in HCC patients[37,28,34]. Moreover, it was found to be a helpful marker for monitoring the effectiveness of treatment and the recurrence of HCC after treatment[17,27].

Alpha-L-fucosidase (AFU)

AFU is a glycosidase found in all mammalian cell lysosomes and is concerned with the degradation of a variety of fucose-containing fucoglyco-conjugates[38]. The activity of this lysosomal enzyme is detectable in the sera of healthy subjects. Increased activities are found in patients with HCC compared to healthy individuals and patients with chronic liver disease. Using a cut-off value of 870 nmol/mL, the sensitivity and specificity was 81.7% and 70.7%, respectively. Simultaneous determination of both AFP and AFU can increase the sensitivity to 82.6%. AFU activity was correlated with tumor size in patients with HCC[7,39,40]. According to another study conducted in 884 Chinese subjects[41], the AFU activity was significantly higher in HCC patients compared to patients with cirrhosis, chronic hepatitis, other malignant tumors, other diseases and healthy individuals. The sensitivity for AFU was 81.5% and the specificity was 85.4%. Furthermore, the persistently elevated AFU level in patients with cirrhosis adds to the detection of HCC at an earlier stage[32,38], owing to elevated activity of AFU at least 6 mo before the detection of HCC by ultrasonography in 85% of patients[39].

However, it should be noted that the prolonged storage of serum samples affects the enzyme activities over time[42]. Furthermore, AFU activity is not only elevated in primary HCC, but also in cases of colorectal cancer[43] and ovarian cancer[44], in addition to some non-malignant extrahepatic diseases, such as diabetes, pancreatitis, and hypothyroidism[29].

Glypican-3 (GPC3)

GPC3 is a cell-surface glycoprotein which is a member of the glypican family of glycosyl-phosphatidylinositol-anchored cell-surface heparin-sulfate proteoglycans[39]. GPC3 mRNA and protein are not detectable in normal tissues, except placenta and fetal liver, and they are expressed in the majority of HCCs[46,47]. Normally, GPC3 has a role in regulating cell proliferation and survival during embryonic development by modulating the activity of various growth factors. Also, it functions as a tumor suppressor[48,49].

GPC3 was expressed in 72% of HCC tissues, while it was lacking in hepatocytes from normal liver and non-malignant hepatic diseases. In addition, GPC3 was detected in sera from 53% of HCC patients, and it was not detected in the serum of patients with chronic hepatitis, or healthy individuals. No correlation was found between GPC3 and total AFP levels in patients with HCC. However, simultaneous measurement of GPC3 and total AFP increased the sensitivity without affecting the specificity[50].

Hippo and colleagues[51] found that serum levels of soluble GPC3 (sGPC3), the NH2-terminal portion of GPC3, were significantly higher in HCC patients, compared to patients with cirrhosis and healthy controls.
sGPC3 was better than AFP in detecting well- or moderately-differentiated HCC and the combination of both markers improved overall sensitivity from 50% to 72%.[52,53]

A recent study evaluated the level of GPC3 in 49 fine needle aspiration biopsies, using immunocytochemical staining. For the diagnosis of HCC in the cytoligical material, the sensitivity of GPC3 was 83.3%, the specificity 96%, and the positive predictive value and the negative predictive value were 95% and 85.7%, respectively. This high sensitivity and specificity enabled the delineation of HCC, distinct from other benign and malignant hepatic lesions, and from most metastatic lesions.[52] Recently, it has been suggested that GPC3 can induce oncogenesis through activation of the insulin-like growth factor II (IGF-II) signalling pathway.[53]

**Squamous cell carcinoma antigen (SCCA)**
SCCA, a member of the serpin (serine protease inhibitor) family, is physiologically expressed in the skin and other squamous epithelial cells.[54] High levels have been reported in tissues of head and neck cancer and other epithelial cancers.[55] It has also been reported to be over-expressed in HCC tissue and in serum from patients with HCC.[56]

Giannelli and colleagues measured serum SCCA in three patient groups: 120 patients with HCC, 90 with cirrhosis, and 41 healthy subjects. SCCA levels were significantly elevated in HCC patients, compared to patients with cirrhosis only or normal subjects. The sensitivity and specificity for SCCA were 84% and 49%, respectively, at the optimum cut-off value of 0.37 ng/mL.[56] The authors suggested that combination of the SCCA (high sensitivity/low specificity) and AFP (low sensitivity/high specificity) markers may be beneficial. According to a recent study, SCCA was found to be expressed in pre-malignant dysplastic nodules as well as malignant lesions.[57]

It has been reported recently that both AFP and SCCA can react with the IgM class of immunoglobulins to form the immunocomplexes AFPIC and SCCAIC, respectively. Both of these can be detected in the serum of HCC patients.[54,58,59]

Between 2001 and 2005, 961 consecutive patients with HCC (n = 499) and those with cirrhosis uncomplicated by malignancy (n = 462) were studied in multiple centers in Italy and France.[60]. AFP, SCCA, AFPIC and SCCAIC were measured using ELISA tests in all patients. SCCA levels were inversely correlated with tumor size. The combined use of AFPIC, SCCA and SCCAIC in patients with low levels of AFP (< 20 IU/mL) detected 25.6% of HCCs (186/725). There was no correlation found between AFP and the other markers investigated. The authors suggested, perhaps optimistically, that each marker was related to a different aspect of HCC, so that the use of all of these markers in combination in clinical practice would provide a non-invasive and relatively simple series of tests that could improve the accuracy of HCC diagnosis.[54]. However, it is unlikely that this suggestion will be taken up widely, as many of these tests have restricted availability and there are cost implications that will need further evaluation.

**Golgi protein 73 (GP73, also known as Golph2)**
GP73 is a resident Golgi-specific membrane protein expressed by biliary epithelial cells in normal liver. Hepatocyte expression of GP73 is up-regulated in patients with acute hepatitis, cirrhosis and HCC, while in published studies, there is no considerable difference in biliary epithelial cell expression of this marker.[60,61]

It has been reported that GP73 is superior to AFP for the detection of early HCC in patients with cirrhosis. According to a study of 352 patients, measurement of serum GP73 based on immunoblots revealed that HCC patients had significantly higher levels than patients with cirrhosis.[62]. At the optimal cut-off (10 relative units), the sensitivity and specificity were 69% and 75%, respectively. For the diagnosis of early HCC, this marker had a significantly higher sensitivity (62%) than AFP (25%).[62] Interestingly, serum GP73 levels were elevated in 57% of patients with HCC associated with normal AFP levels.

**Hepatocyte growth factor (HGF)**
HGF is a multi-functional factor that is produced in various body organs and can affect mitogenesis, cell motility, matrix invasion, and epithelial carcinogenesis.[29,53,64]. Increased HGF serum levels have been reported in patients with squamous cell carcinoma of the esophagus[65] and lymphomas, in addition to non-malignant diseases, such as aortic dissection, pulmonary thromboembolism[66], coronary syndrome[67], cerebral infarction[68] and sepsis.[69,70]. In a prospective study, blood samples were collected from 99 patients with chronic hepatitis, cirrhosis, and HCC.[71]. Serum HGF levels were significantly elevated in HCC patients compared to patients with cirrhosis or chronic hepatitis but no malignancy. All patients with a serum HGF concentration of greater than 0.6 ng/mL had HCC, irrespective of the AFP or DCP levels.

HGF has been used as a prognostic marker in HCC. Serum HGF levels greater than or equal to 1.0 ng/mL have been associated with poor survival in HCC patients.[72]. A recent study conducted in HCC patients who underwent hepatic resection associated high HGF levels in peripheral and portal blood with adverse prognosis.[73]. The authors postulated that HGF induces proliferation and invasiveness of HCC cells through expression of its receptor, the c-met receptor. Also, the persistent elevated level of serum HGF with intensive expression of c-met protein after partial hepatectomy were found to predict early tumor recurrence and metastasis.[74]. This may be explained by the role of HGF in initiating proliferation of normal and malignant hepatocytes after partial hepatectomy.
Transforming growth factor-beta 1 (TGF-β1)

TGF-β1, a multifunctional factor, has a vital role in the regulation of growth and differentiation of normal and transformed cells, angiogenesis, extracellular matrix formation, immunosuppression and carcinogenesis. It has been reported that TGF-β1 and TGF-β1 mRNA levels were significantly higher in the serum of patients with HCC compared to patients with non-malignant chronic liver diseases. Using a cut-off level of 1.2 μg/L for the diagnosis of HCC, the sensitivity was 89.5% and the specificity was 94%. Interestingly, expression of TGF-β1 in liver tissues was related to the degree of HCC differentiation. Hence, this biomarker might find a role as a prognostic marker in HCC. Simultaneous detection of TGF-β1 level and serum AFP yielded a higher detection rate of 97.4%.

It has also been reported that TGF-β1 levels might increase in patients with cirrhosis, owing to decreased hepatic clearance in such patients. In addition, this biomarker is up-regulated in extra-hepatic tumors, wound healing, angiogenesis and fibrosis, indicating lack of disease-specificity.

Vascular endothelial growth factor (VEGF)

VEGF is an endothelial cell mitogen that initiates and promotes neovascularization and endothelial cell proliferation, and it was initially identified as a vascular permeability factor. VEGF has a major effect in regulating angiogenesis, and its expression has been shown to correlate with carcinogenesis. In a study by Poon and colleagues, conducted in 108 patients with HCC and 20 healthy controls, serum VEGF levels in HCC patients were significantly higher, compared to control individuals, and was correlated with venous invasion and advanced tumour stage. In this study, a serum VEGF level of 245 pg/mL or more was associated with poor overall survival.

The expression of VEGF in HCC tissues was correlated with AFP, DCP tumor size and histological grade of the tumor. Furthermore, this biomarker was related to invasiveness and metastasis of HCC. The expression of VEGF in HCC patients with microscopic venous invasion was significantly higher than that in HCC patients without microscopic venous invasion.

Serum proteomics

Recently, surface-enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF) has been used to identify specific serum protein fragments. Paradis and colleagues conducted a SELDI-based study in 82 French patients and identified a six-peak panel of the cases that distinguished HCC and non-HCC patients in 90% of the cases. The C-terminal fragment of vitronectin was identified as the highest discriminating peak. Identification of this fragment as a marker for HCC is possible, as it could be generated in vitro by cleavage of the intact vitronectin molecule by a metalloprotease.

A more recent study compared the sensitivity and specificity of SELDI-TOF MS with AFP, AFP-L3, and prothrombin induced by vitamin K absence-II (PIVKA-II) for the detection of established HCC. For AFP, the sensitivity and specificity were 73% and 71%, respectively, using a cut-off level of 20 ng/mL. With AFP-L3, a cut-off level of 10% gave a sensitivity and specificity of 63% and 94%, respectively. Using the PIVKA-II cut-off of 125 mAU, the sensitivity and specificity were 84% and 69%, respectively. The sensitivity and specificity of SELDI-TOF MS were 79% and 86%, respectively. The authors concluded that SELDI-TOF MS analysis is more accurate than other conventional means of biomarker assessment in detecting small tumors.

RADIOLOGICAL DIAGNOSIS OF HCC

(FIGURE 1)

Imaging modalities employed in HCC diagnosis can be divided into two main groups: those routinely used such as ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI); and those that are more invasive, including iodized oil-CT, CT during hepatic arteriography (CTHA), CT arterial portography (CTAP) and conventional hepatic angiography.

Ultrasound scanning (US)

Currently, US is the technique of choice for screening focal hepatic lesions. On US, lesions may appear hyperechoic, hypoechoic or show a ‘target lesion’ appearance, but none of these is specific. Any mass detected on US in a cirrhotic liver is suspicious of HCC, particularly if it is > 1 cm in size. As a screening test, US has a sensitivity of 65%-80% and has a specificity of > 90%. US permits the detection of smaller sized tumors (1 cm) in early carcinogenesis. However, the detection of small HCC in a cirrhotic liver by US is much more difficult than the detection of metastases in a normal liver, owing to disturbed parenchymal architecture.

The ultrasonographic characteristics of HCC depend on nodule characteristics and tumor size. Most small HCC nodules (less than 3 cm) are well defined, homogeneous and hypoechoic without posterior echo-enhancement. These features are non-specific and may be indifferent from the echo pattern of regeneration nodules in cirrhosis. As the tumor grows in size, it becomes non-homogeneous and more hyperechoic or isoechoic, owing to fatty degeneration or coagulation necrosis. Alternatively, it may show a heterogeneous (mosaic) pattern with a star-shaped central hypoechoic area, owing to the presence of fibrous septae. There is another, less common type of nodular HCC that is homogenous and diffusely hyperechoic, owing to fatty changes or dilated sinusoids. These features do not differ as the tumor grows.

In addition, US can be used to assess the vascular structures and the presence of hilar adenopathies associated with advanced tumor stage. The presence
of intra-hepatic venous thrombosis, a mass protruding from the hepatic surface or a dilated intra-hepatic bile duct offer indirect evidence that raise the suspicion of a liver tumor, even in the absence of a definite finding of

Figure 1 There is a large HCC in the left lobe of the liver with a pseudocapsule, hyper-enhancing on arterial phase and showing washout on late phases on MR and CEUS and is iso-intense in portal phases on CT and CEUS. The pseudo-capsule enhances in the portal phase on all modalities. A1: T2-weighted scan showing slightly higher intensity HCC (arrow); A2: T1-weighted scan shows same HCC which is iso-intense (arrow); B1: MultiHance enhanced T1-weighted scan in the arterial phase showing enhancement of the HCC (arrow); B2: MultiHance enhanced T1-weighted scan in the portal phase showing iso-enhancement of the HCC (arrow); B3: MultiHance enhanced T1-weighted scan at 2 min showing contrast wash-out in the HCC (arrow); B4: MultiHance enhanced T1-weighted scan at 40 min showing hypointense HCC (arrow); C1: Baseline ultrasound shows iso-echoic HCC (arrow); C2: SonoVue enhanced ultrasound shows hyper-enhancing HCC (arrow) in the arterial phase; C3: SonoVue enhanced ultrasound shows iso-enhancing HCC (arrow) in the portal phase with enhancement of the pseudocapsule; C4: SonoVue enhanced ultrasound shows wash-out of the HCC (arrow) in the late phase; D1: Contrast-enhanced CT scan shows enhancement of the HCC (arrow) in the arterial phase; D2: Contrast-enhanced CT scan shows iso-enhancement of the HCC (arrow) in the portal phase with enhancement of the pseudocapsule.
a liver mass on US\cite{99}. Tumor size has been found to affect the sensitivity of US in detecting HCC. Kim and colleagues (2001) assessed the performance of grey-scale US in pre-transplant patients. The sensitivity for HCC nodules greater than 2 cm was 38% and for lesions less than 2 cm, it was 30%\cite{93}. Other studies showed the sensitivity for tumors smaller than 1 cm to be about 42%\cite{3,95} compared to 95% for tumors of larger size\cite{92}.

It has been found that US had low sensitivity and high specificity in detecting HCC and DN in patients with end-stage liver disease requiring liver transplantation\cite{80}. According to a retrospective study of 200 patients with liver failure who underwent liver transplantation within 90 d of US scanning, correlating the US findings with explanted livers showed a sensitivity of 75% for large lesions (> 5 cm), but for small lesions (1-5 cm), the sensitivity ranged from 13.6 to 50%\cite{91}.

**Colour Doppler US**

Colour Doppler US gives an approximation of the mean velocity of blood flow within a vessel by color coding the flow and displaying it superimposed on the grey-scale image, while power Doppler assesses the amplitude of signals\cite{89}. The diagnostic performance of US in the identification of tumor portal thrombosis in patients with HCC can be increased when combining Doppler with US: the sensitivity reaches up to 92% and the specificity virtually 100%. When US-Doppler reveals permeability of the portal system, portal thrombosis is ruled out and hepatic arteriography should be avoided\cite{93}.

Patients with tumors treated with percutaneous ethanol injection may develop chemical thrombosis. Such benign thrombi can be differentiated from tumoral thrombosis using US Doppler, based on the presence of blood flow in the thrombus. The presence of a hepatofugal pulsatile flow inside the thrombus confirms the presence of tumoral invasion of portal vessels\cite{105}.

**Contrast enhanced ultrasound (CEUS)**

CEUS using non-linear imaging modes has been used to improve sonographic visualization of hepatic tumor vascularity\cite{84,85}. CEUS can give information about the nature of liver lesions that are not characterized with baseline US, and every lesion detected during US surveillance in patients with chronic liver disease, or in patients with past history of malignancy\cite{96,98}. CEUS is safe and well tolerated. The contrast study can be performed once a focal lesion is detected on standard US, providing immediate information about the vascular characteristics of the nodule\cite{77}.

US contrast agents consist of microbubbles of low-solubility gas surrounded by a protein, lipid or polymer shell. The microbubbles are 1 to 10 μm, which are too large to pass through the vascular endothelium and, as such, they are considered pure blood pool agents\cite{99,100}. In the liver, the microbubbles dissolve several minutes later in the circulation leading to exhalation of the gas and metabolism of the shell\cite{99,101}. The microbubbles change their size when subjected to an US wave. On the other hand, soft tissues express minor changes. The bubbles are highly reflective, even when they are present in a small concentration. Furthermore, the expansion of these bubbles during the rarefaction phase exceeds their contraction during the pressure phase leading to the production of a returning signal (echo) that contains harmonics\cite{99,102}.

The characterization of a hepatic lesion with microbubbles depends on all phases of contrast enhancement, i.e. the hepatic arterial phase (starting from 10-20 s after injection of contrast agent and lasting for about 10-15 s), portal venous phase (up to 120 s post-injection) and late parenchymal phase (up to 4-6 min after injection)\cite{103}. The arterial phase helps in predicting the degree and pattern of vascularity, while the portal and late phases are helpful in determining the nature of a lesion, as most malignant lesions are hypo-enhancing in contrast with the benign lesions which are iso-or hyper-enhancing\cite{83,104}.

The majority of HCCs are characterized by arterial phase enhancement and washout of the contrast during the late phase, so as to appear as a defect. However, well-differentiated HCCs may not show this washout. Moreover, it has been observed that the more differentiated a lesion, the more gradually it is likely to washout\cite{99,103,104}. Recently, it was reported by Pompili and colleagues that CEUS and multidetector row CT have a similar sensitivity (up to 87%), unaffected by nodule size (< 2 cm vs 2-3 cm)\cite{99}. Most hypervascular nodules (85.2%) were exactly identified by both methods, while CT was slightly more sensitive in detecting arterial vascularity. The authors concluded that CEUS is a dependable imaging tool for vascular characterization of small nodules (less than 2 cm) in patients with cirrhosis.

CEUS gives equivalent accuracy to CT and MRI in the characterisation of focal liver lesions\cite{99} and is probably the best alternative when there are contraindications to CT or MRI\cite{77}. However, the performance of CEUS compared with CT or MRI, is highly affected by operator skill and experience, patient-related factors, such as body habitus and cooperativeness, and tumor-related factors, such as nodule location. Also, it is inappropriate for panoramic detection and staging of HCC\cite{104}.

**CT**

Multiphasic helical CT (MPCT) is deemed the imaging technique of choice for the detection and staging of HCC\cite{98,105,106}. MPCT includes four phases: pre-contrast, hepatic arterial, portal venous and delayed phases. A high-speed single detector spiral scanner is used to achieve the images. Images are obtained after contrast injection at a delay of 25 s (arterial phase), 70 s (portal venous phase) and 300 s (equilibrium phase). HCCs appear hypervascular during the hepatic arterial phase, owing to the fact that the hepatic artery provides the main blood supply, and it appears rather hypodense during the delayed phase, which is attributed to the early wash-out of contrast. It was found that delayed phase images can aid in the diagnosis of HCC in 14% of
patients\textsuperscript{88,107}. Typically, HCC lesions are heterogeneous on CT and the appearance of satellite nodules around the lesion is characteristic\textsuperscript{108}. Combining the hepatic arterial and portal venous phases improves the detection of small malignant tumors\textsuperscript{88,109}.

It has been reported that the presence of intraluminal low attenuation with distension of the occluded venous segment would be indicative of tumor thrombus\textsuperscript{88}. Tumor thrombosis can be differentiated from benign thrombosis during the arterial phase. Since tumor thrombosis enhances, such enhancement can be detected either as “diffuse”, which is typical of HCC, or as streaks of tumor vessels inside the thrombus\textsuperscript{88,108}. Several studies have assessed the performance of spiral CT in the diagnosis of HCC. One study on 41 patients who underwent transplantation within 100 d of imaging, revealed a sensitivity and specificity for HCC of 80% and 96%, respectively\textsuperscript{101}.

The diagnostic accuracy of CT is affected by technical factors, such as the injection of contrast, and intrinsic factors related to the tumor, such as tumor size and vascularity. It was reported that the diagnostic efficacy of CT is diminished in small tumors (less than 2 cm) owing to the hypo-vascularization of small-sized tumors\textsuperscript{3,112,113}. The sensitivity of four phase CT in detecting HCC was up to 100% for tumors greater than 2 cm in size, 93% for tumors 1-2 cm in size, and for tumors less than 1 cm in size, it was 60%\textsuperscript{114}. Spiral CT is the standard imaging technique for detecting the response to loco-regional treatment of HCC\textsuperscript{115}. It has been reported to be more effective than combining US scanning and AFP level estimation in the detection of early HCC recurrence after successful treatment\textsuperscript{116}.

More recently, a study to evaluate the diagnostic efficacy of contrast-enhanced helical computed tomography (CECT) and CEUS has been conducted in patients with small hepatic nodules, previously detected by surveillance programs\textsuperscript{117}. The sensitivity, specificity and diagnostic accuracy were 91.1\%, 87.2\%, and 89.3\%, respectively, for CECT, the sensitivity, specificity and diagnostic accuracy were 80.4\%, 97.9\%, and 88.4\%, respectively. The authors found no significant difference between CEUS and CECT in characterising small (1-2 cm) hepatic nodules.

**Multidetector helical CT (MDCT)**

Recently, MDCT has allowed collection of early (18-28 s after injection of contrast) and late or so-called early parenchymal (35-45 s) arterial phase images. The early arterial images illustrate vessels optimally needed for treatment planning in patients who are likely to undergo surgery, while the late arterial phase images demonstrate the lesions better than the early arterial phase\textsuperscript{118}. Evaluation of both early and late arterial phase images results in better sensitivity and positive predictive values\textsuperscript{89,119}. MDCT with two arterial phases carry the risk of increased radiation exposure thus, limiting its use\textsuperscript{89}. MDCT has been shown to have a higher sensitivity in the detection of HCC in cirrhotic liver, owing to the high speed and flexibility leading to achievement of high quality, thin section imaging and three dimensional capabilities\textsuperscript{108}. In addition, vascular tumors can appear hypodense, relative to liver parenchyma during the equilibrium phase (3-5 min post-injection). It is reported that tumors measuring less than 2 cm can be best detected in this phase, owing to the more rapid washout of contrast from the tumor than from the normal liver parenchyma\textsuperscript{89,124}.

Recently, it was reported that MDCT scanning is useful in early detection and the effective treatment of small HCCs, especially during follow-up of patients with chronic hepatitis and cirrhosis. A study compared the efficiency of gadolinium-enhanced multiphase dynamic MRI with MDCT scanning in the detection of small HCC\textsuperscript{125}. The detection rate of small HCC on MDCT was 97.5\%-97.6\% and it was 90.7\%-94.7\% on MRI, according to tumor size. For very small HCC \( \leq 1 \) cm, the sensitivity of detection on MDCT was higher compared to MRI (90.0\%-95.0\% and 70.0\%-85.0\%, respectively). The authors concluded that MDCT scanning was better than MRI for early detection of small HCC during the follow-up of patients with chronic hepatitis and cirrhosis\textsuperscript{121}.

**MRI**

MRI has been used to improve detection and characterization of hepatic malignant lesions\textsuperscript{11}. HCC appears hyper-intense on T\(_1\)-weighted images with variable signal intensity on T\(_2\)-weighted images. Usually, there is no signal drop-out on the in- and out-of-phase images, because of a low incidence of fatty change. With dynamic gadolinium-enhanced imaging, the lesion enhances in the arterial phase then becomes isointense in the portal phase then becomes hypo-intense in the delayed phase\textsuperscript{86,122}.

MRI was found to be more accurate than CT or US in detecting HCC and estimating the actual tumor size\textsuperscript{123}. Moreover, MRI was more effective than spiral CT in detecting HCC and dysplastic nodules in patients with cirrhotic liver. The sensitivity of MRI for characterising HCC was 61\% while the sensitivity of CT was 52\%\textsuperscript{124}.

The sensitivity of MRI in detecting HCC depends on tumor size. It is about 95\% in tumors larger than 2 cm, while in tumors less than 2 cm the sensitivity is reduced to 50\%\textsuperscript{125}. MRI is also very good at delineating the internal architecture of the tumor, the tumor margins and intrahepatic vascular invasion\textsuperscript{126}. Hence, MRI is deemed the best tool in differentiating HCC from hepatic hemangioma\textsuperscript{114,115}. It has also been suggested that liver function may affect hepatic parenchymal signal intensity leading to the appearance of observed liver-to-lesion contrast on delayed images\textsuperscript{89,126}.

**Angiography**

Owing to the hypervascular nature of HCC, the arterial supply to the tumor is often dilated, tortuous, distorted and displaced. An intense tumor stain, vascular lakes and venous pools are commonly observed\textsuperscript{88}. It is thought that the diagnostic efficacy of hepatic arteriography
is related to tumor size and vascularization\textsuperscript{[112]}. The sensitivity, specificity and diagnostic accuracy of angiography in the detection of HCC smaller than 5 cm has been reported as 82%-93%, 73% and 89%, respectively. When tumor size was smaller than 2 cm, these values were reduced\textsuperscript{[3,127,128]}. Currently, angiography is often used to delineate hepatic anatomy before resection or as guidance for transarterial chemoembolization of HCC lesions\textsuperscript{[109]}.

**Histological diagnosis of HCC**

Cytological examination of a suspected lesion can be achieved by fine needle aspiration biopsy (FNAB). FNAB diagnostic efficacy varies from 60% to 90% according to the size of the lesion, the diameter of the puncturing needle and level of operator training. The specificity and positive predictive value of this technique are from 90% to 100%. It is a safe technique with minimal risk of complications\textsuperscript{[3,129,130]}.

Histopathological examination is considered the chief method for a sure diagnosis of HCC. It is mandatory to study non-tumoral liver tissue to exclude or to confirm the presence of liver cirrhosis, which affects the treatment modality\textsuperscript{[3,129]}. Complications associated with liver biopsy are low with mortality rates of 0.006%-0.3%. The risk of tumor seeding along the needle tract has been estimated at up to 3%\textsuperscript{[108,129,134]}. A combination of the two pathological techniques can improve diagnostic performance\textsuperscript{[3,129]}. One study reported that the sensitivity of cytological and histological examination was about 80% for each one separately. When combining the two methods, sensitivity reached 89%\textsuperscript{[130]}.

Microscopically, HCC cells have an elevated nuclear to cytoplasmatic ratio, trabecular architecture, atypical naked nuclei, and peripheral endothelial wrapping\textsuperscript{[108,133]}.

Pathological findings range from almost normal-appearing hepatocytes in well differentiated tumors to the largely anaplastic multinucleate giant cells in poorly differentiated HCC\textsuperscript{[108,134]}.

**DIAGNOSTIC APPROACH**

The European Association for the Study of the Liver (EASL) has formulated a consensus statement to regulate the diagnostic approach in HCC patients, based on histological and radiological criteria for identifying HCC in patients with cirrhosis. Recommendations were considered based on the size of the lesion (Table 2)\textsuperscript{[115,135]}. HCC lesions of greater than 2 cm in diameter can be diagnosed non-invasively, based on radiographic criteria in patients with cirrhosis\textsuperscript{[115,135]}. Detection of nodules with arterial hypervascularization in two imaging modalities, or in only a single imaging modality associated with an AFP level $\geq$ 400 ng/mL in the cirrhotic liver, is considered diagnostic of HCC. EASL recommended evaluating the vascularity of hepatic nodules using US, contrast-enhanced CT or MRI, with formal angiography used in cases of diagnostic uncertainty. Histological confirmation by biopsy was not mandatory owing to the excellent diagnostic accuracy of imaging criteria and the 10%-20% false-negative rate from histological samples\textsuperscript{[116-130]}.

Focal hepatic lesions of less than 1 cm in size were found to be non-malignant in 50% of cases\textsuperscript{[135,141-143]}. The EASL consensus statement recommended repeated ultrasound scanning every 3 mo until the lesion grows to 1 cm in diameter. Nodules from 1-2 cm in size are more likely to be HCC and pathological confirmation was recommended using fine-needle aspiration or biopsy or both for the diagnosis of these nodules. However, it carries a hazard for tumor seeding with a 30%-40% false-negative diagnostic rate\textsuperscript{[115,135,139]}.

Recently, the American Association for the Study of Liver Disease (AASLD, 2005) issued guidelines which also proposed a diagnostic approach for HCC (Table 3). AFP of 200 ng/mL should lead to diagnostic suspicion of HCC requiring further investigation. In terms of imaging, nodules less than 1 cm in diameter should be repeatedly imaged for up to 2 years, due to uncertainty in the current diagnostic techniques in establishing a firm diagnosis. Nodules between 1 and 2 cm should be investigated with two dynamic imaging techniques such as CT scan, CE US or MRI\textsuperscript{[139]}. If they show hypervascularization with washout in the portal venous phase, the lesion can be diagnosed as HCC. Nodules greater than 2 cm in size that reveal typical features of HCC on dynamic profile (arterial hypervascularization with wash-out in the early or delayed venous phase) can be diagnosed as HCC by using a single imaging modality. Histological diagnosis was recommended if the vascular pattern is not characteristic for HCC on imaging modalities, to establish the diagnosis\textsuperscript{[84,139]}.

**CONCLUSION**

The incidence of HCC is increasing around the world.

**Table 2** EASL consensus diagnostic criteria for HCC (adapted from Bruix et al\textsuperscript{[115]} , 2001)

| Cyto-histological criteria |
|---------------------------|
| Non-invasive criteria (restricted to patients with cirrhosis) |
| Radiological criteria: two coincident imaging techniques |
| Focal lesion \( \geq \) 2 cm with arterial hypervascularisation |
| Combined criteria: one imaging technique associated with elevated serum AFP levels |
| Focal lesion \( \geq \) 2 cm with arterial hypervascularisation |
| AFP levels $\geq$ 400 ng/mL |

**Table 3** Diagnostic criteria for HCC (adapted from Bruix et al\textsuperscript{[135]} , 2006)

| Cyto-histological criteria |
|---------------------------|
| Non-invasive criteria (cirrhotic patients) |
| Focal lesion \( \leq \) 2 cm. Two imaging techniques with arterial hypervascularisation and venous washout |
| Focal lesion \( \geq \) 2 cm. One imaging technique with arterial hypervascularisation and venous washout |
Although international consensus exists on a diagnostic pathway, there is no ideal screening modality. AFP serum level is the most commonly used serum test, while US is the most commonly used imaging test. Future research may delineate more specific and sensitive markers using proteomic or metabolomic approaches to screening blood or other biofluids such as urine.

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