Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.1301

World J Gastroenterol 2009 March 21; 15(11): 1301-1314
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Dr. Shahid A Khan, *Series Editor*

Diagnosis of hepatocellular carcinoma

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

Asmaa I Gomaa, Shahid A Khan, Simon D Taylor-Robinson, Department of Hepatology and Gastroenterology, Division of Medicine, Imperial College London, St Mary's Hospital Campus, South Wharf Road, London W2 1NY, United Kingdom
Edward LS Leen, Imaging Sciences Department, MRC Clinical Sciences Centre, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London W12 0HS, United Kingdom
Asmaa I Gomaa, Imam Waked, National Liver Institute, Menoufiya University, Shebeen ElKom, 32111, Egypt
Author contributions: All authors generated the ideas and contributed to the writing of this manuscript; Gomaa AI and Khan SA performed the original literature search and collated the data; Leen ELS was the main contributor to the imaging section; The whole manuscript was reviewed and verified by Waked I and Taylor-Robinson SD.
Supported by The NIHR Biomedical Research Centre funding scheme and the following for authors' funding and support: AIG is funded by a scholarship from the Egyptian Government; SAK is supported by a grant from the Higher Education Funding Council for England (HEFCE); SDTR is funded by grants from the British Medical Research Council (MRC), London, United Kingdom; The British Engineering, Physics and Science Research Council (EPSRC), Swindon, United Kingdom; The Alan Morement Memorial Fund AMMF, Essex, United Kingdom; Broad Foundation, Los Angeles, United States; Pfizer Global Research and Development Inc, Sandwich, United Kingdom and GlaxoSmithKline, Ware, United Kingdom
Correspondence to: Shahid A Khan, Department of Hepatology and Gastroenterology, Division of Medicine, Imperial College London, St Mary's Hospital Campus, South Wharf Road, London W2 1NY, United Kingdom. shahid.khan@imperial.ac.uk
Telephone: +44-207-8866454 Fax: +44-207-7249369
Received: November 14, 2008 Revised: December 5, 2008
Accepted: December 12, 2008
Published online: March 21, 2009

Abstract

Hepatocellular carcinoma (HCC) is one of the commonest cancers worldwide, particularly in parts of the developing world, and is increasing in incidence. This article reviews the current modalities employed for the diagnosis of HCC, including serum markers, radiological techniques and histological evaluation, and summarises international guidelines for the diagnostic approach to HCC.

Key words: Diagnosis; Hepatocellular carcinoma; Imaging; Serum markers

Peer reviewer: Dr. Paolo Del Poggio, Hepatology Unit, Department of Internal Medicine, Treviglio Hospital, Piazza Ospedale 1, Treviglio Bg 24047, Italy

Gomaa AI, Khan SA, Leen ELS, Waked I, Taylor-Robinson SD. Diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 2009; 15(11): 1301-1314 Available from: URL: http://www.wjgnet.com/1007-9327/15/1301.asp DOI: http://dx.doi.org/10.3748/wjg.15.1301

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 vitellin 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

Table 1 Serum markers for HCC

Alpha-1 fetoprotein
Lens Culinaris Agglutinin-Reactive AFP (AFP-L3)
Des-gamma carboxyprothrombin (DCP)
α-L-Fucosidase
Glypican-3
Squamous cell carcinoma antigen (SCCA)
Golgi protein 73 (GP73)
Hepatocyte growth factor (HGF)
Transforming growth factor-b1 (TGFb1)
Vascular endothelial growth factor (VEGF)
Serum proteomics

AFP in the absence of cancer. Also, AFP is elevated in hepatocarcinogenesis, embryonic carcinomas[2-7] and in gastric[8] and lung cancer[9].

AFP has been used as a serum marker for HCC for many years. It was first described by Abelev *et al*[10] in the 1960s. The first quantitative serum assays for AFP were established by Ruoshlati and Seppala[11]. 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 tumour. The test had 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[12]. 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 tumours (< 5 cm) and furthermore, only 30% of HCC patients have levels higher than 100 ng/mL in this context[3,13].

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 without chronic hepatitis B or hepatitis C infection[14]. However, up to 42% of patients with HCC present with serum AFP levels within normal values[3,15].

In another study, conducted in 290 Chinese patients with chronic hepatitis B, 44 patients were found to have an elevated AFP (> 20 ng/mL)[16]. Of these, only 6 (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[17]. Moreover, patients with chronic hepatitis B or C with reactivation were found to have AFP levels > 500 ng/mL[18].

AFP seems to be of prognostic value at the time of tumour diagnosis. A high AFP concentration (≥ 400 ng/mL) in HCC patients is associated with greater tumour size, bilobar involvement, portal vein invasion, and a lower median survival rate[7]. 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%)[19]. This may relate to the finding that well-differentiated tumours express lower levels of AFP[20].

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 chemoembolisation, usually indicates a favourable 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[17].

A large multicentre study, based on both retrospective and prospective data collection, was carried out by Farinati *et al*[21] 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[21].

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[22]. Its sensitivity and specificity ranges from 75% to 96.90% and 90% to 92%, respectively at the cut-off level of 15%[22-24].

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[25]. Elevated levels of AFP-L3 were associated with a shorter tumour 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 as

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 normalisation with treatment[17]. Another study reported that AFP-L3 > 15% is a significant predictor for HCC recurrence[26].

AFP-L3 levels were found to be related to progression from moderately differentiated to poorly differentiated tumours. HCC patients with AFP-L3 > 10% had a higher frequency of poorly differentiated tumours[27]. Thus, this biomarker may be able to predict advanced tumour 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[28], 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[7]. 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[17]. 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[7,32].

DCP is a well-recognized tumour 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[3,7,33]. Conversely, in Europe, studies have not shown these results. These discrepant results were related not only to racial factors but also to different aetiological 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, tumour 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 tumours, although higher than that of AFP for large tumours.

Multiple studies have shown that DCP can be a useful indicator of vascular invasion in HCC patients[27,28,36]. Moreover, it was found to be a helpful marker for monitoring the effectiveness of treatment and the recurrence of HCC after treatment[17,37].

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 tumour 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 tumours, 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[7,22,38], 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[45]. 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 tumour suppressor[29,48,49].

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%[22,51].

A recent study evaluated the level of GPC3 in 49 fine needle aspiration biopsies, using immunocytochemical staining. For the diagnosis of HCC in the cytological material, the sensitivity of GPC3 was 83.3%, the specificity 96%, 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 centres in Italy and France[54]. AFP, SCCA, AFPIC and SCCAIC were measured using ELISA tests in all patients. SCCA levels were inversely correlated with tumour 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[29,63,64]. Increased HGF serum levels have been reported in patients with squamous cell carcinoma of the oesophagus[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 tumour 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[7,75]. 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[7,75,76]. 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%[75].

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 tumours, wound healing, angiogenesis and fibrosis, indicating lack of disease-specificity[29,76].

Vascular endothelial growth factor (VEGF)

VEGF is an endothelial cell mitogen that initiates and promotes neovascularisation 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[7]. In a study by Poon and colleagues, conducted in 108 patients with HCC and 20 healthy controls[77], 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 tumour size and histological grade of the tumour[78]. Furthermore, this biomarker was related to invasiveness and metastasis of HCC[17,79]. The expression of VEGF in HCC patients with microscopic venous invasion was significantly higher than that in HCC patients without microscopic venous invasion[80].

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 that distinguished HCC and non-HCC patients in 90% of the cases[81]. 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[29].

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[82]. 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 tumours.

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[83].

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[84,85]. 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 to 80% and has a specificity of > 90%[84-86]. US permits the detection of smaller sized tumours (1 cm) in early carcinogenesis[3,13,87]. 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[88,89].

The ultrasonographic characteristics of HCC depend on nodule characteristics and tumour 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[88,89]. As the tumour grows in size, it becomes non-homogeneous and more hyperechoic or isoechoic owing to fatty degeneration or coagulation necrosis. Alternatively, it may show a heterogenous (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 homogeneous and diffusely hyperechoic owing to fatty changes or dilated sinusoids. These features do not differ as the tumour grows[88].

In addition, US can be used to assess the vascular structures and the presence of hilar adenopathies associated with advanced tumour stage[3]. The presence

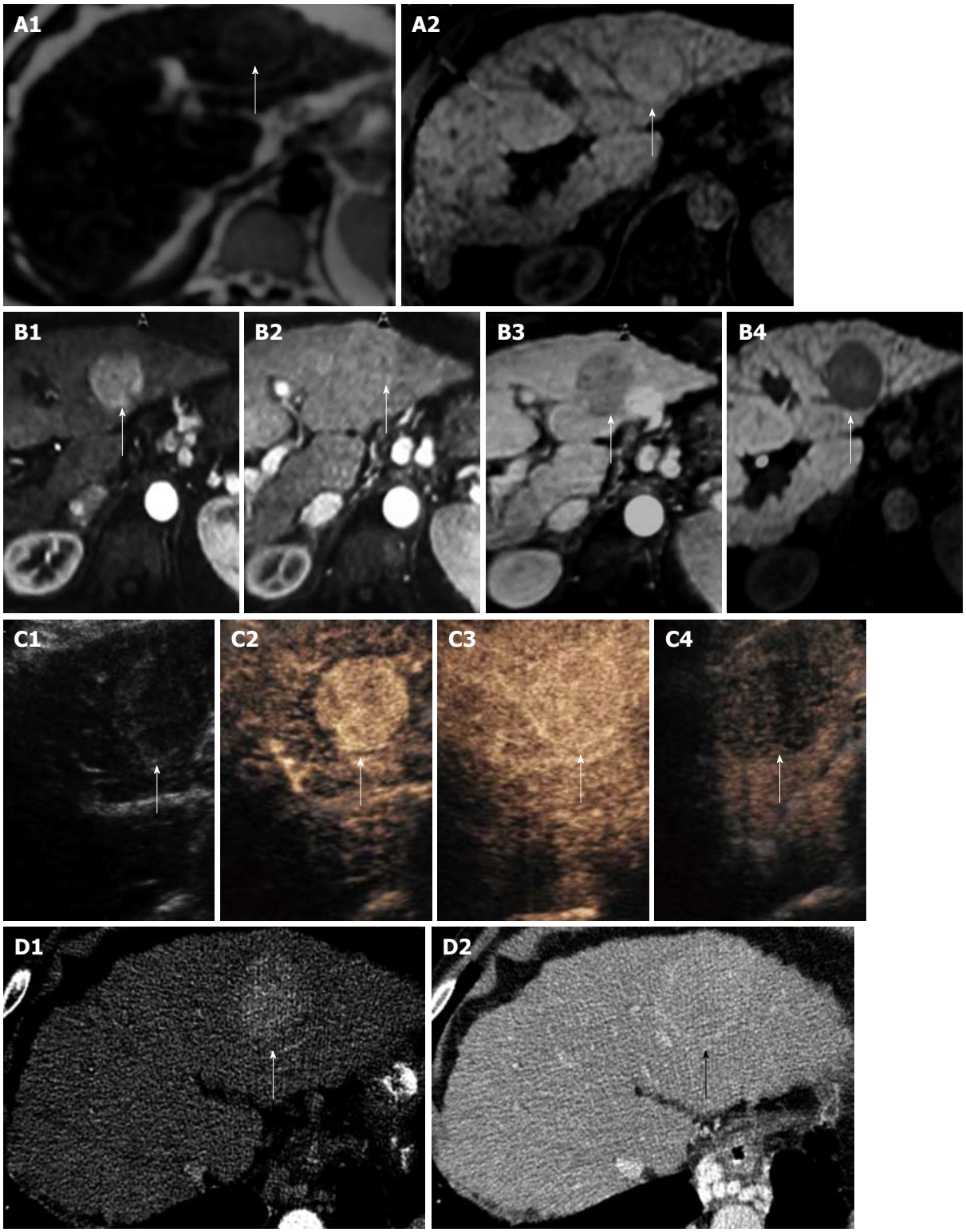

Figure 1 There is a large HCC in the left lobe of the liver with a pseudocapsule, hyper-enhancing on arterial phase and showing washout on late phases on MR and CEUS and is iso-intense in portal phases on CT and CEUS. The pseudo-capsule enhances in the portal phase on all modalities. A1: T2 weighted scan showing slightly higher intensity HCC (arrow); A2: T1 weightted 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 hypoiintense 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.

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 tumour, even in the absence of a definite finding of

a liver mass on US[88].

Tumour 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%[83]. Other studies showed the sensitivity for tumours smaller than 1 cm to be about 42%[3,90,91] compared to 95% for tumours of larger size[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[89]. According to a retrospective study of 200 patients with liver failure who underwent liver transplantation within 90 d of ultrasound 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%[93].

Colour Doppler US

Colour Doppler US gives an approximation of the mean velocity of blood flow within a vessel by colour coding the flow and displaying it superimposed on the grey-scale image, while power Doppler assesses the amplitude of signals[89]. The diagnostic performance of US in the identification of tumour 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[3,94].

Patients with tumours treated with percutaneous ethanol injection may develop chemical thrombosis. Such benign thrombi can be differentiated from tumoural 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 tumoural invasion of portal vessels[3,95].

Contrast enhanced ultrasound (CEUS)

CEUS using non-linear imaging modes has been used to improve sonographic visualization of hepatic tumour vascularity[96,97]. 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[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[97].

Ultrasound 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[99,100]. In the liver, the microbubbles dissolve several minutes later in the circulation leading to exhalation of the gas and metabolisation of the shell[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[99,102].

The characterization of a hepatic lesion with micro-bubbles 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)[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[103].

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[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)[97]. 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[98] and is probably the best alternative when there are contraindications to CT or MRI[97]. 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 tumour–related factors, such as nodule location. Also, it is inappropriate for panoramic detection and staging of HCC[97].

Computed tomography

Multiphasic helical computed tomography (MPCT) is deemed the imaging technique of choice for the detection and staging of HCC[88,105,106]. MPCT includes 4 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[88,107]. Typically, HCC lesions are heterogenous on CT and the appearance of satellite nodules around the lesion is characteristic[108]. Combining the hepatic arterial and portal venous phases improves the detection of small malignant tumours[88,109].

It has been reported that the presence of intraluminal low attenuation with distension of the occluded venous segment would be indicative of tumour thrombus[88]. Tumour thrombosis can be differentiated from benign thrombosis during the arterial phase. Since tumour thrombosis enhances, such enhancement can be detected either as "diffuse", which is typical of HCC, or as streaks of tumour vessels inside the thrombus[89,110]. 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[111].

The diagnostic accuracy of CT is affected by technical factors, such as the injection of contrast, and intrinsic factors related to the tumour, such as tumour size and vascularity. It was reported that the diagnostic efficacy of CT is diminished in small tumours (less than 2 cm) owing to the hypo-vascularisation of small sized tumours[3,112,113]. The sensitivity of four phase CT in detecting HCC was up to 100% for tumours greater than 2 cm in size, 93% for tumours 1-2 cm in size, and for tumours less than 1 cm in size, it was 60%[114].

Spiral CT is the standard imaging technique for detecting the response to loco-regional treatment of HCC[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[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 programmes[117]. The sensitivity, specificity and diagnostic accuracy were 91.1%, 87.2%, and 89.3%, respectively, for CEUS. 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 computed tomography (MDCT)

Recently, MDCT allows 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[118]. Evaluation of both early and late arterial phase images results in better sensitivity and positive predictive values[89,119]. MDCT with two arterial phases carry the risk of increased radiation exposure thus, limiting its use[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[108]. In addition, vascular tumours can appear hypodense, relative to liver parenchyma during the equilibrium phase (3-5 min post-injection). It is reported that tumours measuring less than 2 cm can be best detected in this phase, owing to the more rapid washout of contrast from the tumour than from the normal liver parenchyma[89,120].

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[121]. The detection rate of small HCC on MDCT was 97.5%-97.6% and it was 90.7%-94.7% on MRI, according to tumour size. For very small HCC $\leqslant$ 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[121].

Magnetic resonance imaging (MRI)

MRI has been used to improve detection and characterization of hepatic malignant lesions[3]. HCC appears hyper-intense on T_2-weighted images with variable signal intensity on T_1-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[88,122].

MRI was found to be more accurate than CT or US in detecting HCC and estimating the actual tumour size[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%[124].

The sensitivity of MRI in detecting HCC depends on tumour size. It is about 95% in tumours larger than 2 cm, while in tumours less than 2 cm the sensitivity is reduced to 30%[125]. MRI is also very good at delineating the internal architecture of the tumour, the tumoural margins and intrahepatic vascular invasion[125]. Hence, MRI is deemed the best tool in differentiating HCC from hepatic haemangioma[3,13]. 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[89,126].

Angiography

Owing to the hypervascular nature of HCC, the arterial supply to the tumour is often dilated, tortuous, distorted and displaced. An intense tumour stain, vascular lakes and venous pools are commonly observed[88]. It is thought that

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

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

Table 3 Diagnostic criteria for hepatocellular carcinoma (adapted from Bruix *et al*[85], 2006)

Cyto-histological criteria
Non-invasive criteria (cirrhotic patients)
Focal lesion ≤ 2 cm. Two imaging techniques with arterial hypervascularisation and venous washout
Focal lesion > 2 cm. One imaging technique with arterial hypervascularisation and venous washout

the diagnostic efficacy of hepatic arteriography is related to tumour size and vascularisation[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 tumour size was smaller than 2 cm, these values were reduced[3,127,128]. Currently, angiography is often used to delineate hepatic anatomy before resection or as guidance for transarterial chemoembolization of HCC lesions[108].

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[3,13,129,130].

Histopathological examination is considered the chief method for a sure diagnosis of HCC. It is mandatory to study non-tumoural liver tissue to exclude or to confirm the presence of liver cirrhosis, which affects the treatment modality[3,129]. Complications associated with liver biopsy are low with mortality rates of 0.006%-0.3%. The risk of tumour seeding along the needle tract has been estimated at up to 3%[108,131,140,144].

A combination of the two pathological techniques can improve diagnostic performance[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%[132].

Microscopically, HCC cells have an elevated nuclear to cytoplasmic ratio, trabecular architecture, atypical naked nuclei, and peripheral endothelial wrapping[108,133]. Pathological findings range from almost normal-appearing hepatocytes in well differentiated tumours to the largely anaplastic multinucleate giant cells in poorly differentiated HCC[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)[115,135].

HCC lesions of greater than 2 cm in diameter can be diagnosed non-invasively, based on radiographic criteria in patients with cirrhosis[115,135]. Detection of nodules with arterial hypervascularization in two imaging modalities, or in only a single imaging modality associated with an AFP level ⩾ 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[136-138].

Focal hepatic lesions of less than 1 cm in size were found to be non-malignant in 50% of cases[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 tumour seeding with a 30%-40% false-negative diagnostic rate[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). An 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 two 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, contrast-enhanced US or MRI[85]. If they show hypervascularity 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 hypervascularity 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[84,85].

CONCLUSION

The incidence of HCC is increasing around the world.

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.

ACKNOWLEDGMENTS

We are grateful for support from the NIHR Biomedical Research Centre funding scheme. In addition, we are grateful to the following for their funding and support: AIE is funded by a scholarship from the Egyptian Government; SAK is supported by a grant from the Higher Education Funding Council for England (HEFCE); SDTR is funded by grants from the British Medical Research Council (MRC), London, United Kingdom; the British Engineering, Physics and Science Research Council (EPSRC), Swindon, United Kingdom; the Alan Morement Memorial Fund AMMF, Essex, United Kingdom; Broad Foundation, Los Angeles, United States; Pfizer Global Research and Development Inc, Sandwich, United Kingdom and GlaxoSmithKline, Ware, United Kingdom. We are grateful to Dr. I Jane Cox and Dr. Robert Edwards, Imperial College London for useful discussions.

REFERENCES

1 **World Health Organization.** Mortality database. Available from: URL: http://www.who.int/whosis/en/
2 **Gupta S**, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; **139**: 46-50
3 **França AV**, Elias Junior J, Lima BL, Martinelli AL, Carrilho FJ. Diagnosis, staging and treatment of hepatocellular carcinoma. *Braz J Med Biol Res* 2004; **37**: 1689-1705
4 **Terentiev AA**, Moldogazieva NT. Structural and functional mapping of alpha-fetoprotein. *Biochemistry* (Mosc) 2006; **71**: 120-132
5 **Canick JA**, MacRae AR. Second trimester serum markers. *Semin Perinatol* 2005; **29**: 203-208
6 **Ding X**, Yang LY, Huang GW, Yang JQ, Liu HL, Wang W, Peng JX, Yang JQ, Tao YM, Chang ZG, Ling XS. Role of AFP mRNA expression in peripheral blood as a predictor for postsurgical recurrence of hepatocellular carcinoma: a systematic review and meta-analysis. *World J Gastroenterol* 2005; **11**: 2656-2661
7 **Grizzi F**, Franceschini B, Hamrick C, Frezza EE, Cobos E, Chiriva-Internati M. Usefulness of cancer-testis antigens as biomarkers for the diagnosis and treatment of hepatocellular carcinoma. *J Transl Med* 2007; **5**: 3
8 **Chen J**, Röcken C, Treiber G, Jentsch-Ulrich K, Malfertheiner P, Ebert MP. Clinical implications of alpha-fetoprotein expression in gastric adenocarcinoma. *Dig Dis* 2003; **21**: 357-362
9 **Hiroshima K**, Iyoda A, Toyozaki T, Haga Y, Baba M, Fujisawa T, Ishikura H, Ohwada H. Alpha-fetoprotein-producing lung carcinoma: report of three cases. *Pathol Int* 2002; **52**: 46-53
10 **Abelev GI**, Perova SD, Khramkova NI, Postnikova ZA, Irlin IS. Production of embryonal alpha-globulin by transplantable mouse hepatomas. *Transplantation* 1963; **1**: 174-180
11 **Ruoslahti E**, Seppälä M. Studies of carcino-fetal proteins. 3. Development of a radioimmunoassay for -fetoprotein. Demonstration of -fetoprotein in serum of healthy human adults. *Int J Cancer* 1971; **8**: 374-383
12 **Daniele B**, Bencivenga A, Megna AS, Tinessa V. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S108-S112
13 **Ebara M**, Ohto M, Kondo F. Strategy for early diagnosis of hepatocellular carcinoma (HCC). *Ann Acad Med Singapore* 1989; **18**: 83-89
14 **Trevisani F**, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; **34**: 570-575
15 **Lescano M**, Carneiro M, Elias Junior J, Martinelli A, França A. Experiencia inicial en la evaluación de pacientes with carcinoma hepatocelular em um Hospital terciario. *Gastroenterología y Hepatología* 2002, **25** Suppl 2: I-9-I-43
16 **Lok AS**, Lai CL. alpha-Fetoprotein monitoring in Chinese patients with chronic hepatitis B virus infection: role in the early detection of hepatocellular carcinoma. *Hepatology* 1989; **9**: 110-115
17 **Yuen MF**, Lai CL. Serological markers of liver cancer. *Best Pract Res Clin Gastroenterol* 2005; **19**: 91-99
18 **Yuen MF**, Lai CL. Screening for hepatocellular carcinoma: survival benefit and cost-effectiveness. *Ann Oncol* 2003; **14**: 1463-1467
19 **Sakata J**, Shirai Y, Wakai T, Kaneko K, Nagahashi M, Hatakeyama K. Preoperative predictors of vascular invasion in hepatocellular carcinoma. *Eur J Surg Oncol* 2008; **34**: 900-905
20 **Llovet JM**, Beaugrand M. Hepatocellular carcinoma: present status and future prospects. *J Hepatol* 2003; **38** Suppl 1: S136-S149
21 **Farinati F**, Marino D, De Giorgio M, Baldan A, Cantarini M, Cursaro C, Rapaccini G, Del Poggio P, Di Nolfo MA, Benvegnù L, Zoli M, Borzio F, Bernardi M, Trevisani F. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? *Am J Gastroenterol* 2006; **101**: 524-532
22 **Zhou L**, Liu J, Luo F. Serum tumor markers for detection of hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 1175-1181
23 **Taketa K**, Okada S, Win N, Hlaing NK, Wind KM. Evaluation of tumor markers for the detection of hepatocellular carcinoma in Yangon General Hospital, Myanmar. *Acta Med Okayama* 2002; **56**: 317-320
24 **Khien VV**, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV, Hop TV, Tuan NA, Don LV, Taketa K, Satomura S. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. *Int J Biol Markers* 2001; **16**: 105-111
25 **Kumada T**, Nakano S, Takeda I, Kiriyama S, Sone Y, Hayashi K, Katoh H, Endoh T, Sassa T, Satomura S. Clinical utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: special reference to imaging diagnosis. *J Hepatol* 1999; **30**: 125-130
26 **Tateishi R**, Shiina S, Yoshida H, Teratani T, Obi S, Yamashiki N, Yoshida H, Akamatsu M, Kawabe T, Omata M. Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. *Hepatology* 2006; **44**: 1518-1527
27 **Miyaaki H**, Nakashima O, Kurogi M, Eguchi K, Kojiro M. Lens culinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. *J Gastroenterol* 2007; **42**: 962-968
28 **Hagiwara S**, Kudo M, Kawasaki T, Nagashima M, Minami Y, Chung H, Fukunaga T, Kitano M, Nakatani T. Prognostic factors for portal venous invasion in patients with hepatocellular carcinoma. *J Gastroenterol* 2006; **41**: 1214-1219
29 **Wright LM**, Kreikemeier JT, Fimmel CJ. A concise review of

serum markers for hepatocellular cancer. *Cancer Detect Prev* 2007; **31**: 35-44
30 **Weitz IC**, Liebman HA. Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. *Hepatology* 1993; **18**: 990-997
31 **Liebman HA**, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, Coleman MS, Furie B. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984; **310**: 1427-1431
32 **Marrero JA**, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, Lok AS. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology* 2003; **37**: 1114-1121
33 **Semela D**, Dufour JF. Angiogenesis and hepatocellular carcinoma. *J Hepatol* 2004; **41**: 864-880
34 **Volk ML**, Hernandez JC, Su GL, Lok AS, Marrero JA. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers: a comparison of AFP, DCP, and AFP-L3. *Cancer Biomark* 2007; **3**: 79-87
35 **Nakamura S**, Nouso K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, Toshikuni N, Tanaka H, Miyake Y, Matsumoto E, Shiratori Y. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *Am J Gastroenterol* 2006; **101**: 2038-2043
36 **Shirabe K**, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, Aishima S, Maehara Y. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. *J Surg Oncol* 2007; **95**: 235-240
37 **Kim do Y**, Paik YH, Ahn SH, Youn YJ, Choi JW, Kim JK, Lee KS, Chon CY, Han KH. PIVKA-II is a useful tumor marker for recurrent hepatocellular carcinoma after surgical resection. *Oncology* 2007; **72** Suppl 1: 52-57
38 **Haydon GH**, Hayes PC. Screening for hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 1996; **8**: 856-860
39 **Ishizuka H**, Nakayama T, Matsuoka S, Gotoh I, Ogawa M, Suzuki K, Tanaka N, Tsubaki K, Ohkubo H, Arakawa Y, Okano T. Prediction of the development of hepato-cellular-carcinoma in patients with liver cirrhosis by the serial determinations of serum alpha-L-fucosidase activity. *Intern Med* 1999; **38**: 927-931
40 **Tangkijvanich P**, Tosukhowong P, Bunyongyod P, Lertmaharit S, Hanvivatvong O, Kullavanijaya P, Poovorawan Y. Alpha-L-fucosidase as a serum marker of hepatocellular carcinoma in Thailand. *Southeast Asian J Trop Med Public Health* 1999; **30**: 110-114
41 **Wang JJ**, Cao EH. Rapid kinetic rate assay of the serum alpha-L-fucosidase in patients with hepatocellular carcinoma by using a novel substrate. *Clin Chim Acta* 2004; **347**: 103-109
42 **Bukofzer S**, Stass PM, Kew MC, de Beer M, Groeneveld HT. Alpha-L-fucosidase as a serum marker of hepatocellular carcinoma in southern African blacks. *Br J Cancer* 1989; **59**: 417-420
43 **Ayude D**, Fernández-Rodríguez J, Rodríguez-Berrocal FJ, Martínez-Zorzano VS, de Carlos A, Gil E, Páez de La Cadena M. Value of the serum alpha-L-fucosidase activity in the diagnosis of colorectal cancer. *Oncology* 2000; **59**: 310-316
44 **Abdel-Aleem H**, Ahmed A, Sabra AM, Zakhari M, Soliman M, Hamed H. Serum alpha L-fucosidase enzyme activity in ovarian and other female genital tract tumors. *Int J Gynaecol Obstet* 1996; **55**: 273-279
45 **Bernfield M**, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem* 1999; **68**: 729-777
46 **Filmus J**, Capurro M. Glypican-3 and alphafetoprotein as diagnostic tests for hepatocellular carcinoma. *Mol Diagn* 2004; **8**: 207-212
47 **Nakatsura T**, Nishimura Y. Usefulness of the novel oncofetal antigen glypican-3 for diagnosis of hepatocellular carcinoma and melanoma. *BioDrugs* 2005; **19**: 71-77
48 **Pilia G**, Hughes-Benzie RM, MacKenzie A, Baybayan P, Chen EY, Huber R, Neri G, Cao A, Forabosco A, Schlessinger D. Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. *Nat Genet* 1996; **12**: 241-247
49 **Song HH**, Shi W, Filmus J. OCI-5/rat glypican-3 binds to fibroblast growth factor-2 but not to insulin-like growth factor-2. *J Biol Chem* 1997; **272**: 7574-7577
50 **Capurro M**, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, Filmus J. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003; **125**: 89-97
51 **Hippo Y**, Watanabe K, Watanabe A, Midorikawa Y, Yamamoto S, Ihara S, Tokita S, Iwanari H, Ito Y, Nakano K, Nezu J, Tsunoda H, Yoshino T, Ohizumi I, Tsuchiya M, Ohnishi S, Makuuchi M, Hamakubo T, Kodama T, Aburatani H. Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. *Cancer Res* 2004; **64**: 2418-2423
52 **Ligato S**, Mandich D, Cartun RW. Utility of glypican-3 in differentiating hepatocellular carcinoma from other primary and metastatic lesions in FNA of the liver: an immunocytochemical study. *Mod Pathol* 2008; **21**: 626-631
53 **Cheng W**, Tseng CJ, Lin TT, Cheng I, Pan HW, Hsu HC, Lee YM. Glypican-3-mediated oncogenesis involves the Insulin-like growth factor-signaling pathway. *Carcinogenesis* 2008; **29**: 1319-1326
54 **Giannelli G**, Fransvea E, Trerotoli P, Beaugrand M, Marinosci F, Lupo L, Nkontchou G, Dentico P, Antonaci S. Clinical validation of combined serological biomarkers for improved hepatocellular carcinoma diagnosis in 961 patients. *Clin Chim Acta* 2007; **383**: 147-152
55 **Torre GC**. SCC antigen in malignant and nonmalignant squamous lesions. *Tumour Biol* 1998; **19**: 517-526
56 **Giannelli G**, Marinosci F, Trerotoli P, Volpe A, Quaranta M, Dentico P, Antonaci S. SCCA antigen combined with alpha-fetoprotein as serologic markers of HCC. *Int J Cancer* 2005; **117**: 506-509
57 **Guido M**, Roskams T, Pontisso P, Fassan M, Thung SN, Giacomelli L, Sergio A, Farinati F, Cillo U, Rugge M. Squamous cell carcinoma antigen in human liver carcinogenesis. *J Clin Pathol* 2008; **61**: 445-447
58 **Beneduce L**, Castaldi F, Marino M, Tono N, Gatta A, Pontisso P, Fassina G. Improvement of liver cancer detection with simultaneous assessment of circulating levels of free alpha-fetoprotein (AFP) and AFP-IgM complexes. *Int J Biol Markers* 2004; **19**: 155-159
59 **Beneduce L**, Castaldi F, Marino M, Quarta S, Ruvoletto M, Benvegnù L, Calabrese F, Gatta A, Pontisso P, Fassina G. Squamous cell carcinoma antigen-immunoglobulin M complexes as novel biomarkers for hepatocellular carcinoma. *Cancer* 2005; **103**: 2558-2565
60 **Kladney RD**, Bulla GA, Guo L, Mason AL, Tollefson AE, Simon DJ, Koutoubi Z, Fimmel CJ. GP73, a novel Golgi-localized protein upregulated by viral infection. *Gene* 2000; **249**: 53-65
61 **Kladney RD**, Cui X, Bulla GA, Brunt EM, Fimmel CJ. Expression of GP73, a resident Golgi membrane protein, in viral and nonviral liver disease. *Hepatology* 2002; **35**: 1431-1440
62 **Marrero JA**, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, Comunale MA, D'Amelio A, Lok AS, Block TM. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. *J Hepatol* 2005; **43**: 1007-1012
63 **Hasuike S**, Ido A, Uto H, Moriuchi A, Tahara Y, Numata M, Nagata K, Hori T, Hayashi K, Tsubouchi H. Hepatocyte growth factor accelerates the proliferation of hepatic

oval cells and possibly promotes the differentiation in a 2-acetylaminofluorene/partial hepatectomy model in rats. *J Gastroenterol Hepatol* 2005; **20**: 1753-1761

64 **Jiang WG**, Martin TA, Parr C, Davies G, Matsumoto K, Nakamura T. Hepatocyte growth factor, its receptor, and their potential value in cancer therapies. *Crit Rev Oncol Hematol* 2005; **53**: 35-69

65 **Ren Y**, Cao B, Law S, Xie Y, Lee PY, Cheung L, Chen Y, Huang X, Chan HM, Zhao P, Luk J, Vande Woude G, Wong J. Hepatocyte growth factor promotes cancer cell migration and angiogenic factors expression: a prognostic marker of human esophageal squamous cell carcinomas. *Clin Cancer Res* 2005; **11**: 6190-6197

66 **Giles FJ**, Vose JM, Do KA, Johnson MM, Manshouri T, Bociek G, Bierman PJ, O'Brien SM, Kantarjian HM, Armitage JO, Albitar M. Clinical relevance of circulating angiogenic factors in patients with non-Hodgkin's lymphoma or Hodgkin's lymphoma. *Leuk Res* 2004; **28**: 595-604

67 **Soeki T**, Tamura Y, Shinohara H, Sakabe K, Onose Y, Fukuda N. Serum hepatocyte growth factor predicts ventricular remodeling following myocardial infarction. *Circ J* 2002; **66**: 1003-1007

68 **Matsumori A**, Takano H, Obata JE, Takeda S, Tsuyuguchi N, Ono K, Okada M, Miyamoto T, Ohnishi T, Daikuhara Y, Sasayama S. Circulating hepatocyte growth factor as a diagnostic marker of thrombus formation in patients with cerebral infarction. *Circ J* 2002; **66**: 216-218

69 **Sekine K**, Fujishima S, Aikawa N. Plasma hepatocyte growth factor is increased in early-phase sepsis. *J Infect Chemother* 2004; **10**: 110-114

70 **Hata N**, Matsumori A, Yokoyama S, Ohba T, Shinada T, Yoshida H, Tokuyama K, Imaizumi T, Mizuno K. Hepatocyte growth factor and cardiovascular thrombosis in patients admitted to the intensive care unit. *Circ J* 2004; **68**: 645-649

71 **Yamagamim H**, Moriyama M, Matsumura H, Aoki H, Shimizu T, Saito T, Kaneko M, Shioda A, Tanaka N, Arakawa Y. Serum concentrations of human hepatocyte growth factor is a useful indicator for predicting the occurrence of hepatocellular carcinomas in C-viral chronic liver diseases. *Cancer* 2002; **95**: 824-834

72 **Vejchapipat P**, Tangkijvanich P, Theamboonlers A, Chongsrisawat V, Chittmittrapap S, Poovorawan Y. Association between serum hepatocyte growth factor and survival in untreated hepatocellular carcinoma. *J Gastroenterol* 2004; **39**: 1182-1188

73 **Chau GY**, Lui WY, Chi CW, Chau YP, Li AF, Kao HL, Wu CW. Significance of serum hepatocyte growth factor levels in patients with hepatocellular carcinoma undergoing hepatic resection. *Eur J Surg Oncol* 2008; **34**: 333-338

74 **Wu FS**, Zheng SS, Wu LJ, Ding W, Ma ZM, Wang ZM, Teng LS, Zhao WH. [Study on the prognostic value of hepatocyte growth factor and c-met for patients with hepatocellular carcinoma] *Zhonghua Waike Zazhi* 2006; **44**: 603-608

75 **Dong ZZ**, Yao DF, Zou L, Yao M, Qiu LW, Wu XH, Wu W. [An evaluation of transforming growth factor-beta 1 in diagnosing hepatocellular carcinoma and metastasis] *Zhonghua Ganzangbing Zazhi* 2007; **15**: 503-508

76 **Song BC**, Chung YH, Kim JA, Choi WB, Suh DD, Pyo SI, Shin JW, Lee HC, Lee YS, Suh DJ. Transforming growth factor-beta1 as a useful serologic marker of small hepatocellular carcinoma. *Cancer* 2002; **94**: 175-180

77 **Poon RT**, Ho JW, Tong CS, Lau C, Ng IO, Fan ST. Prognostic significance of serum vascular endothelial growth factor and endostatin in patients with hepatocellular carcinoma. *Br J Surg* 2004; **91**: 1354-1360

78 **Amaoka N**, Osada S, Kanematsu M, Imai H, Tomita H, Tokuyama Y, Sakashita F, Nonaka K, Goshima S, Kondo H, Adachi Y. Clinicopathological features of hepatocellular carcinoma evaluated by vascular endothelial growth factor expression. *J Gastroenterol Hepatol* 2007; **22**: 2202-2207

79 **Li XM**, Tang ZY, Qin LX, Zhou J, Sun HC. Serum vascular endothelial growth factor is a predictor of invasion and metastasis in hepatocellular carcinoma. *J Exp Clin Cancer Res* 1999; **18**: 511-517

80 **Huang GW**, Yang LY, Lu WQ. Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in hepatocellular carcinoma: Impact on neovascularization and survival. *World J Gastroenterol* 2005; **11**: 1705-1708

81 **Paradis V**, Degos F, Dargère D, Pham N, Belghiti J, Degott C, Janeau JL, Bezeaud A, Delforge D, Cubizolles M, Laurendeau I, Bedossa P. Identification of a new marker of hepatocellular carcinoma by serum protein profiling of patients with chronic liver diseases. *Hepatology* 2005; **41**: 40-47

82 **Zinkin NT**, Grall F, Bhaskar K, Otu HH, Spentzos D, Kalmowitz B, Wells M, Guerrero M, Asara JM, Libermann TA, Afdhal NH. Serum proteomics and biomarkers in hepatocellular carcinoma and chronic liver disease. *Clin Cancer Res* 2008; **14**: 470-477

83 **Kim CK**, Lim JH, Lee WJ. Detection of hepatocellular carcinomas and dysplastic nodules in cirrhotic liver: accuracy of ultrasonography in transplant patients. *J Ultrasound Med* 2001; **20**: 99-104

84 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236

85 **Bruix J**, Hessheimer AJ, Forner A, Boix L, Vilana R, Llovet JM. New aspects of diagnosis and therapy of hepatocellular carcinoma. *Oncogene* 2006; **25**: 3848-3856

86 **Bolondi L**, Sofia S, Siringo S, Gaiani S, Casali A, Zironi G, Piscaglia F, Gramantieri L, Zanetti M, Sherman M. Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. *Gut* 2001; **48**: 251-259

87 **Tong MJ**, Blatt LM, Kao VW. Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. *J Gastroenterol Hepatol* 2001; **16**: 553-559

88 **Yu SC**, Yeung DT, So NM. Imaging features of hepatocellular carcinoma. *Clin Radiol* 2004; **59**: 145-156

89 **Saar B**, Kellner-Weldon F. Radiological diagnosis of hepatocellular carcinoma. *Liver Int* 2008; **28**: 189-199

90 **Bizollon T**, Rode A, Bancel B, Gueripel V, Ducerf C, Baulieux J, Trepo C. Diagnostic value and tolerance of Lipiodol-computed tomography for the detection of small hepatocellular carcinoma: correlation with pathologic examination of explanted livers. *J Hepatol* 1998; **28**: 491-496

91 **Dodd GD 3rd**, Miller WJ, Baron RL, Skolnick ML, Campbell WL. Detection of malignant tumors in end-stage cirrhotic livers: efficacy of sonography as a screening technique. *AJR Am J Roentgenol* 1992; **159**: 727-733

92 **Colli A**, Fraquelli M, Casazza G, Massironi S, Colucci A, Conte D, Duca P. Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. *Am J Gastroenterol* 2006; **101**: 513-523

93 **Bennett GL**, Krinsky GA, Abitbol RJ, Kim SY, Theise ND, Teperman LW. Sonographic detection of hepatocellular carcinoma and dysplastic nodules in cirrhosis: correlation of pretransplantation sonography and liver explant pathology in 200 patients. *AJR Am J Roentgenol* 2002; **179**: 75-80

94 **Tanaka K**, Numata K, Okazaki H, Nakamura S, Inoue S, Takamura Y. Diagnosis of portal vein thrombosis in patients with hepatocellular carcinoma: efficacy of color Doppler sonography compared with angiography. *AJR Am J Roentgenol* 1993; **160**: 1279-1283

95 **Vilana R**, Bru C, Bruix J, Castells A, Sole M, Rodes J. Fine-needle aspiration biopsy of portal vein thrombus: value in detecting malignant thrombosis. *AJR Am J Roentgenol* 1993; **160**: 1285-1287

96 **Albrecht T**, Blomley M, Bolondi L, Claudon M, Correas JM, Cosgrove D, Greiner L, Jäger K, Jong ND, Leen E, Lencioni R, Lindsell D, Martegani A, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines for the use of

contrast agents in ultrasound. January 2004. *Ultraschall Med* 2004; **25**: 249-256
97 **Pompili M**, Riccardi L, Semeraro S, Orefice R, Elia F, Barbaro B, Covino M, Grieco A, Gasbarrini G, Rapaccini GL. Contrast-enhanced ultrasound assessment of arterial vascularization of small nodules arising in the cirrhotic liver. *Dig Liver Dis* 2008; **40**: 206-215
98 **Quaia E**. Microbubble ultrasound contrast agents: an update. *Eur Radiol* 2007; **17**: 1995-2008
99 **Morin SH**, Lim AK, Cobbold JF, Taylor-Robinson SD. Use of second generation contrast-enhanced ultrasound in the assessment of focal liver lesions. *World J Gastroenterol* 2007; **13**: 5963-5970
100 **Brannigan M**, Burns PN, Wilson SR. Blood flow patterns in focal liver lesions at microbubble-enhanced US. *Radiographics* 2004; **24**: 921-935
101 **Cosgrove D**, Blomley M. Liver tumors: evaluation with contrast-enhanced ultrasound. *Abdom Imaging* 2004; **29**: 446-454
102 **Uhlendorf V**, Scholle FD, Reinhardt M. Acoustic behaviour of current ultrasound contrast agents. *Ultrasonics* 2000; **38**: 81-86
103 **Claudon M**, Cosgrove D, Albrecht T, Bolondi L, Bosio M, Calliada F, Correas JM, Darge K, Dietrich C, D'Onofrio M, Evans DH, Filice C, Greiner L, Jäger K, Jong N, Leen E, Lencioni R, Lindsell D, Martegani A, Meairs S, Nolsøe C, Piscaglia F, Ricci P, Seidel G, Skjoldbye B, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) - update 2008. *Ultraschall Med* 2008; **29**: 28-44
104 **Fan ZH**, Chen MH, Dai Y, Wang YB, Yan K, Wu W, Yang W, Yin SS. Evaluation of primary malignancies of the liver using contrast-enhanced sonography: correlation with pathology. *AJR Am J Roentgenol* 2006; **186**: 1512-1519
105 **Lopez Hänninen E**, Vogl TJ, Bechstein WO, Guckelberger O, Neuhaus P, Lobeck H, Felix R. Biphasic spiral computed tomography for detection of hepatocellular carcinoma before resection or orthotopic liver transplantation. *Invest Radiol* 1998; **33**: 216-221
106 **Shapiro RS**, Katz R, Mendelson DS, Halton KP, Schwartz ME, Miller CM. Detection of hepatocellular carcinoma in cirrhotic patients: sensitivity of CT and ultrasonography. *J Ultrasound Med* 1996; **15**: 497-502; quiz 503-504
107 **Lim JH**, Choi D, Kim SH, Lee SJ, Lee WJ, Lim HK, Kim S. Detection of hepatocellular carcinoma: value of adding delayed phase imaging to dual-phase helical CT. *AJR Am J Roentgenol* 2002; **179**: 67-73
108 **Bialecki ES**, Di Bisceglie AM. Diagnosis of hepatocellular carcinoma. *HPB* (Oxford) 2005; **7**: 26-34
109 **Hollett MD**, Jeffrey RB Jr, Nino-Murcia M, Jorgensen MJ, Harris DP. Dual-phase helical CT of the liver: value of arterial phase scans in the detection of small (< or = 1.5 cm) malignant hepatic neoplasms. *AJR Am J Roentgenol* 1995; **164**: 879-884
110 **Tublin ME**, Dodd GD 3rd, Baron RL. Benign and malignant portal vein thrombosis: differentiation by CT characteristics. *AJR Am J Roentgenol* 1997; **168**: 719-723
111 **Lim JH**, Kim CK, Lee WJ, Park CK, Koh KC, Paik SW, Joh JW. Detection of hepatocellular carcinomas and dysplastic nodules in cirrhotic livers: accuracy of helical CT in transplant patients. *AJR Am J Roentgenol* 2000; **175**: 693-698
112 **Ikeda K**, Saitoh S, Koida I, Tsubota A, Arase Y, Chayama K, Kumada H. Imaging diagnosis of small hepatocellular carcinoma. *Hepatology* 1994; **20**: 82-87
113 **Ohashi I**, Hanafusa K, Yoshida T. Small hepatocellular carcinomas: two-phase dynamic incremental CT in detection and evaluation. *Radiology* 1993; **189**: 851-855
114 **Kim T**, Murakami T, Takahashi S, Tsuda K, Tomoda K, Narumi Y, Oi H, Sakon M, Nakamura H. Optimal phases of dynamic CT for detecting hepatocellular carcinoma: evaluation of unenhanced and triple-phase images. *Abdom Imaging* 1999; **24**: 473-480
115 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430
116 **Colagrande S**, La Villa G, Bartolucci M, Lanini F, Barletta G, Villari N. Spiral computed tomography versus ultrasound in the follow-up of cirrhotic patients previously treated for hepatocellular carcinoma: a prospective study. *J Hepatol* 2003; **39**: 93-98
117 **Dai Y**, Chen MH, Fan ZH, Yan K, Yin SS, Zhang XP. Diagnosis of small hepatic nodules detected by surveillance ultrasound in patients with cirrhosis: Comparison between contrast-enhanced ultrasound and contrast-enhanced helical computed tomography. *Hepatol Res* 2008; **38**: 281-290
118 **Baron RL**, Brancatelli G. Computed tomographic imaging of hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S133-S143
119 **Murakami T**, Kim T, Takahashi S, Nakamura H. Hepatocellular carcinoma: multidetector row helical CT. *Abdom Imaging* 2002; **27**: 139-146
120 **Mitsuzaki K**, Yamashita Y, Ogata I, Nishiharu T, Urata J, Takahashi M. Multiple-phase helical CT of the liver for detecting small hepatomas in patients with liver cirrhosis: contrast-injection protocol and optimal timing. *AJR Am J Roentgenol* 1996; **167**: 753-757
121 **Zhao H**, Yao JL, Wang Y, Zhou KR. Detection of small hepatocellular carcinoma: comparison of dynamic enhancement magnetic resonance imaging and multiphase multirow-detector helical CT scanning. *World J Gastroenterol* 2007; **13**: 1252-1256
122 **Hussain SM**, Semelka RC, Mitchell DG. MR imaging of hepatocellular carcinoma. *Magn Reson Imaging Clin N Am* 2002; **10**: 31-52, v
123 **Snowberger N**, Chinnakotla S, Lepe RM, Peattie J, Goldstein R, Klintmalm GB, Davis GL. Alpha fetoprotein, ultrasound, computerized tomography and magnetic resonance imaging for detection of hepatocellular carcinoma in patients with advanced cirrhosis. *Aliment Pharmacol Ther* 2007; **26**: 1187-1194
124 **de Lédinghen V**, Laharie D, Lecesne R, Le Bail B, Winnock M, Bernard PH, Saric J, Couzigou P, Balabaud C, Bioulac-Sage P, Drouillard J. Detection of nodules in liver cirrhosis: spiral computed tomography or magnetic resonance imaging? A prospective study of 88 nodules in 34 patients. *Eur J Gastroenterol Hepatol* 2002; **14**: 159-165
125 **Ebara M**, Ohto M, Watanabe Y, Kimura K, Saisho H, Tsuchiya Y, Okuda K, Arimizu N, Kondo F, Ikehira H. Diagnosis of small hepatocellular carcinoma: correlation of MR imaging and tumor histologic studies. *Radiology* 1986; **159**: 371-377
126 **Grazioli L**, Morana G, Caudana R, Benetti A, Portolani N, Talamini G, Colombari R, Pirovano G, Kirchin MA, Spinazzi A. Hepatocellular carcinoma: correlation between gadobenate dimeglumine-enhanced MRI and pathologic findings. *Invest Radiol* 2000; **35**: 25-34
127 **Takayasu K**, Shima Y, Muramatsu Y, Goto H, Moriyama N, Yamada T, Makuuchi M, Yamasaki S, Hasegawa H, Okazaki N. Angiography of small hepatocellular carcinomas: analysis of 105 resected tumors. *AJR Am J Roentgenol* 1986; **147**: 525-529
128 **Sumida M**, Ohto M, Ebara M, Kimura K, Okuda K, Hirooka N. Accuracy of angiography in the diagnosis of small hepatocellular carcinoma. *AJR Am J Roentgenol* 1986; **147**: 531-536
129 **França A**, Giordano H, Trevisan M, Escanhoela C, Seva-Pereira T, Zucoloto S, Martinelli A, Soares E. Fine needle aspiration biopsy improves the diagnostic accuracy of cut needle biopsy of focal liver lesions. *Acta Cytologica* 2003; **47**: 332-336

130 **Ding W**, He XJ. Fine needle aspiration cytology in the diagnosis of liver lesions. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 90-92
131 **Bravo AA**, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001; **344**: 495-500
132 **Caselitz M**, Masche N, Bleck JS, Gebel M, Atay Z, Stern C, Manns MP, Kubicka S. Increasing sensitivity of morphological diagnosis in hepatocellular carcinoma (HCC) by combination of cytological and fine-needle histological examination after ultrasound guided fine needle biopsy. *Z Gastroenterol* 2003; **41**: 559-564
133 **Pitman MB**. Fine needle aspiration biopsy of the liver. Principal diagnostic challenges. *Clin Lab Med* 1998; **18**: 483-506, vi
134 **Robbins S**, Kumar V. Basic pathology. 4th ed. Philadelphia: WB Saunders, 1987: 598
135 **Talwalkar JA**, Gores GJ. Diagnosis and staging of hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S126-S132
136 **Bru C**, Maroto A, Bruix J, Faus R, Bianchi L, Calvet X, Ayuso C, Vilana R, Gilabert R, Rodés J. Diagnostic accuracy of fine-needle aspiration biopsy in patients with hepatocellular carcinoma. *Dig Dis Sci* 1989; **34**: 1765-1769
137 **Longchampt E**, Patriarche C, Fabre M. Accuracy of cytology vs. microbiopsy for the diagnosis of well-differentiated hepatocellular carcinoma and macroregenerative nodule. Definition of standardized criteria from a study of 100 cases. *Acta Cytol* 2000; **44**: 515-523
138 **Huang GT**, Sheu JC, Yang PM, Lee HS, Wang TH, Chen DS. Ultrasound-guided cutting biopsy for the diagnosis of hepatocellular carcinoma--a study based on 420 patients. *J Hepatol* 1996; **25**: 334-338
139 **Durand F**, Regimbeau JM, Belghiti J, Sauvanet A, Vilgrain V, Terris B, Moutardier V, Farges O, Valla D. Assessment of the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. *J Hepatol* 2001; **35**: 254-258
140 **Takamori R**, Wong LL, Dang C, Wong L. Needle-tract implantation from hepatocellular cancer: is needle biopsy of the liver always necessary? *Liver Transpl* 2000; **6**: 67-72
141 **El-Serag HB**. Hepatocellular carcinoma: an epidemiologic view. *J Clin Gastroenterol* 2002; **35**: S72-S78
142 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917
143 **Nakashima T**, Kojiro M. Pathologic characteristics of hepatocellular carcinoma. *Semin Liver Dis* 1986; **6**: 259-266
144 **Chang S**, Kim SH, Lim HK, Kim SH, Lee WJ, Choi D, Kim YS, Rhim H. Needle tract implantation after percutaneous interventional procedures in hepatocellular carcinomas: lessons learned from a 10-year experience. *Korean J Radiol* 2008; **9**: 268-274

S- Editor Li LF **L- Editor** Webster JR **E- Editor** Zheng XM