Recent advances in the imaging of hepatocellular carcinoma

From ultrasound to positron emission tomography scan

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ABSTRACT

Recent advances in imaging techniques for hepatocellular carcinoma (HCC) offer the possibility of investigating contrast perfusion of liver nodules in cirrhosis. It is now accepted that a non-invasive diagnosis of HCC can be established based on the vascular pattern obtained with pure blood pool contrast agents. The diagnostic pattern consists of contrast enhancement in the arterial phase, indicative of arterial hypervascularization, followed by contrast wash out in the portal and late phases, which leads the nodule to show the same, or, more specifically, a lower contrast signal than the surrounding parenchyma. Such patterns can be obtained by CT, MRI and, more recently, by real time Contrast Enhanced Ultrasonography with second-generation ultrasound contrast agents. A typical vascular pattern in a nodule perceptible also without contrast is highly specific for HCC, so that non-invasive diagnostic algorithms have been developed and recently updated.

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Hepatocellular carcinoma (HCC) is the most common primary liver cancer. Its incidence is increasing, driven by the burden of hepatitis C virus (HCV) infection and cirrhosis, not only in Japan, but also in Mediterranean, North European and American populations.[1-4] The large majority of HCCs develop in patients with chronic liver disease, usually in a cirrhotic stage. The underlying cirrhosis frequently prevents common oncologic approaches, based on surgery and chemotherapy. In order to overcome such limitations, percutaneous local treatments were developed in the 1980's,[5,6] but, unfortunately, they can be effectively applied only on small tumors (<3-5 cm).[5-8] Liver transplantation is now accepted as the best option for patients with unresectable early stage HCC, as it is curative for both cancer and liver cirrhosis, but a rigorous candidate selection, based on tumor stage, is warranted to avoid high recurrence rate. The detection of HCC at an early stage is therefore, crucial to improve the patient's outcome.[7,9-12] Surveillance programs for cirrhotic patients, mainly based on ultrasonography (US) at intervals of 4-12 months, are widely accepted as effective in detecting HCC when still single and of a small size.[12-16] As a result, an increasing number of small nodular lesions are detected at US and need to be further characterized. To this end, rapidly evolving technology has produced new tools in the investigation of liver nodules in cirrhosis. The present review focuses on the most recent advances in the field of imaging techniques for the diagnosis of HCC and is preceded by a brief background on the development of this malignancy, to which diagnostic features are strictly related.

Multistep carcinogenesis of HCC. A limited number of HCC develop directly from the cirrhotic parenchyma and show malignant features when still microscopic ("de novo" HCC). The large majority of HCCs develop, instead, through a progressive pathway from pre-malignant nodular lesions to HCC in a cirrhotic liver.[17-22] The transition from non-malignant to malignant nodules is gradual and shows a patchy distribution within the lesion. In agreement with this problem, no single classification of non-malignant hepatocellular nodules has achieved a worldwide application. The classification system introduced at the World Congress of Gastroenterology in 1994 includes 4 categories: low-grade dysplastic nodules (LGDN), high-grade dysplastic nodules (HGDN), well-differentiated HCC, and HCC. Unfortunately, even the pathologic distinction of HCC from other non-neoplastic or pre-neoplastic liver nodules is sometimes

difficult, particularly on small tissue specimens from percutaneous biopsy.[22] Important clues to distinguish small well-differentiated HCC from HGDN (such as the invasion of the intranodular portal tracts and the surrounding stroma by the tumor cells,[21-24] may be hardly assessable in small bioptic samples, and some nodules classified as HGDN may contain small foci of HCC not reached by bioptic procedure (sampling error). A precise distinction would not be futile, since early well-differentiated HCC, despite usually only showing local aggressiveness, is reported to determine intrahepatic regional cancer spread in approximately 7% of cases. This figure is significantly lower than that found in overt HCCs (42%), but nonetheless is not negligible.[25] On the contrary, true HGDN have no malignant potential yet, and their ablation leads to the definite arrest of the multistep carcinogenesis for that nodule. In addition to difficulties and pitfalls in interpreting histological findings, percutaneous biopsy is not always feasible (due to the position of the nodule in the liver), needs a skilled operator to correctly target the nodule in the case of small lesions, and involves procedure-related morbidity and mortality risks. Consequently, imaging modalities able to differentiate the various steps of HCC development have been researched. Significant advances were produced in recent years, largely based on the capacity to characterize intranodular vascularization.

Angioarchitecture of liver nodules. The liver has a peculiar blood supply through both the portal system (which accounts for approximately two thirds of the total supply to healthy livers) and the arterial system (providing the remaining third of total hepatic blood volume). Various pathologic[26-28] and imaging studies[29-31] have clarified that the progression of dysplastic nodules to overt HCC is associated with a progressive decrease of blood supply through the portal system and through paired arteries, up to the complete disappearance. Conversely, newly formed, unpaired arteries, take over the total blood supply to nourish overt HCC.[32,33] Newly developed arteries are characterized by structural abnormalities that determine higher arterial flow blood flow volume per unit of tissue than the surrounding non-neoplastic parenchyma. The progressive changes in blood supply during the multistep carcinogenesis has been investigated by Japanese studies based on computed tomography hepatic arteriography (CTHA) and computed tomography arterio-portography (CTAP): such techniques are able to selectively investigate the arterial or portal blood supply alone.[30] Large regenerative nodules (LRN) show the same rate of portal and arterial supply as surrounding parenchyma. The LGDN are characterized by an initial decrease in both arterial and portal supply, but no newly formed unpaired arteries have appeared yet. In HGDN, a more evident decrease in portal and arterial blood supply takes place, and in a few instances, some newly formed abnormal arteries are evident. In established well-differentiated HCC, portal and paired arteries blood supply decrease to a minimum, whereas abnormal arteries become evident. With progression of dedifferentiation, portal and paired arteries inflow completely disappear and abnormal arterial supply increases.[34]

Imaging techniques. The vascular derangement translates into morphologic and functional alterations at imaging techniques, so that a distinction between malignant and benign nodules on cirrhosis can be made by analyzing their vascular pattern. The first technique identifying arterial neovascularization was transfemoral hepatic angiography.[35] The introduction of Doppler US since the end of the 1980s' provided a new non-invasive functional tool to detect arterial neovascularization.[33,36,37] Overt HCC, up to 4 cm in diameter are characterized by high peak flow, high flow impedance (resistance index >0.71) and prolonged systolic acceleration time (>100 msec),[36,38] whereas non-malignant nodules usually do not show such features. However, Doppler US requires experienced operators, good patient-operator cooperation, and high-end US instrumentation, limiting its effectiveness in routine clinical practice. In recent years, many contrast enhanced imaging techniques have been developed that are able to dynamically study the perfusion of hepatic masses, namely, CT, MRI, and more recently contrast enhanced ultrasonography (CEUS). Some features of hepatic nodules on cirrhosis are common in all these techniques: HCC is characterized by contrast enhancement in the arterial phase, corresponding to hypervascularization through abnormal arteries,[39-42] and by a subsequent wash out of the contrast medium in the portal and late phases. Regenerative and dysplastic nodules are generally not visualized after contrast agent injection because their vascularization is quite similar to the surrounding liver. Nevertheless, some HGDN may disclose slight arterial hypervascularization. The presence of contrast uptake in the arterial phase was the first radiological sign that has been considered diagnostic for HCC. More recently, contrast medium wash out in the portal and late phases has been recognized as a specific sign of HCC, capable of increasing the accuracy of non-invasive diagnosis if associated with arterial hypervascularization. The detection of delayed hypointensity by contrast-enhanced MRI in an arterially-enhancing liver mass <2 cm has been reported to have a sensitivity of 89% and a specificity of 96% for HCC.[43] Progression of wash out to lower enhancement than the surrounding liver in the late phase is the most specific finding, but also contrast wash out reaching the same contrast level of liver parenchyma is considered consistent with the diagnosis of HCC.

Computed tomography. The accuracy of CT in HCC diagnosis has greatly improved thanks to the advent of helical CT at the end of the 1990s' and more recently of multidetector helical CT. These technologies allow the scan of the whole liver within a few seconds (even <10 seconds). As a result, a dynamic study of liver masses can be obtained by repeatedly acquiring hepatic parenchyma images during different perfusional phases. The selective visualization of the arterial phase (which lasts in general from 15-35 seconds after contrast medium infusion in a peripheral vein) allows the detection of the typical hypervascularization of HCC, whereas a subsequent scan in the portal venous and late phases, beginning at 55-75 seconds, shows the contrast agent wash out from the lesion (**Figure 1**). Unfortunately, small nodules on cirrhotic liver, whether malignant or not malignant, are often not visible at baseline unenhanced CT scan, but are identified only after contrast medium injection. This represents a limit of CT, as vascular abnormalities not related to a nodule may simulate HCC.[42,44] False positive results are therefore, possible if the diagnosis of HCC relies on a single functional imaging, and particularly on CT. In the United States of America, up to 20% of patients who underwent liver transplantation for HCC (mainly small nodules diagnosed with only one imaging technique) did not show malignancy at pathological analysis of the explanted liver.[45] Parenchymal areas, not corresponding to a mass, that appear hyperdense on the arterial phase and isodense during the portal-venous phase are named transient hepatic attenuation difference (THAD). In cirrhotic livers, they are frequently caused by small fistulae determining arterial-portal shunting.[46] The absence of hypodensity in the portal phase is highly suspect for THAD. Other features of THAD are the wedge shape and the absence of mass effect. Conversely, also HCC may determine THAD due to intratumoral arterial-portal shunts, especially in large tumors. The most accurate approach would be, therefore, to combine morphologic (direct visualization of the nodule) and functional (vascular pattern) aspects to confirm diagnosis of HCC. In recent years, MRI and US underwent technical achievements able to produce completely new diagnostic capacities, including combined dual information, in most cases.

Magnetic resonance imaging. Recent advances in MRI hardware and software provide faster sequences that can be acquired within a breath-hold, decreasing motion and respiratory artifacts, and permitting investigation of the liver within the time frame of the arterial phase alone. Such advancement, combined with use of non-specific extracellular fluid space contrast agents, mainly gadolinium chelates, enables dynamic study of vascularization in liver nodules. Unenhanced MRI, based on T1-T2 weighted, gated and in/out-of-phase patterns,[47,48] already provides important information, since it is able to identify even small nodules (<1 cm) and provides possible information on their nature. In particular, HCC appear hyperintense on T2 weighted scans, whereas benign nodules are isointense to the surrounding parenchyma.[47,49,50] However, a considerable overlap between benign and malignant appearances prevents definitive diagnosis.[47,49] Once again, the combination of morphologic (unenhanced MR) and functional (contrast enhanced MRI) imaging provides the highest accuracy in characterizing liver nodules in cirrhosis. The most specific pattern for the diagnosis of an HCC at intravascular contrast MRI is a nodule that becomes hyperintense during the contrast arterial phase

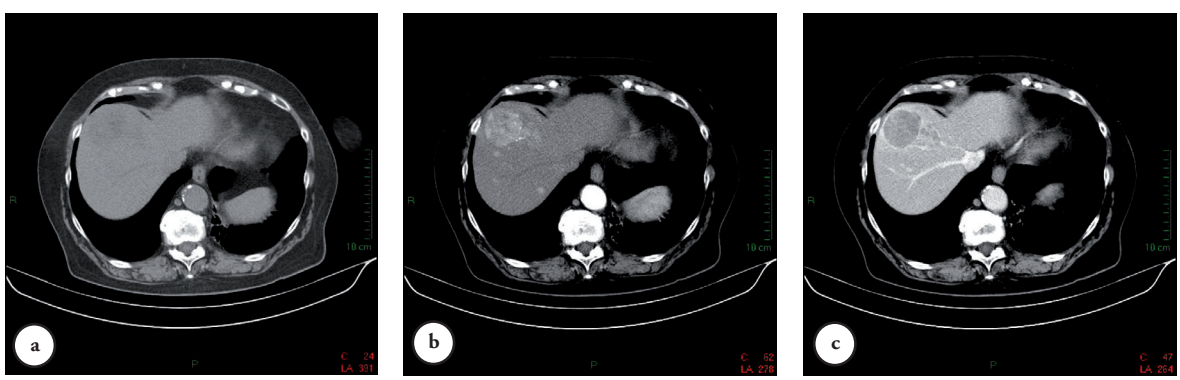

Figure 1 - Helical computed tomography (CT) showing a voluminous hepatocellular carcinoma on cirrhotic liver of the fifth segment, surrounded by small satellite nodules and a small hepatocellular carcinoma in segment 7. **a)** Unenhanced CT: only a slightly hypodense area is detectable. **b**) Arterial phase: after contrast agent injection, a hypervascularized mass (hyperdense) becomes evident, surrounded by other small hypervascular nodules. A further small hepatocellular carcinoma is visible in segment 7 near the liver capsule. **c**) In the portal and late phases, the large mass and the small tumors become hypodense due to contrast wash out.

and changes to hypointense in the late phase.[43,51] As for CT scan, lesions that are visible only in the arterial phase, but not in the delayed phases and on unenhanced scans, are highly suspect for false positive results.[52] The sensitivity of contrast enhanced MRI in the detection of small HCC nodules is particularly high (up to 84% for nodules of 10-20 mm) and significantly better than helical CT's.[50] An MRI has been therefore proposed as the optimal technique for HCC staging before curative treatment, but it is expensive, of limited availability and, despite probably being the most accurate staging method available to date, has limited sensitivity for very small lesions (<1 cm) when challenged with careful analysis of whole explanted livers.[53-55] Consequently, in several countries, MRI is still limited to a small number of cirrhotic patients, whereas helical triphasic CT is routinely used to characterize liver nodules in a recall strategy after nodule detection at US and to stage HCC patients. Other types of MRI contrast agents may provide additional clues to solve differential diagnosis between the malignant and non-malignant nature in controversial cases. In particular, superparamagnetic iron oxide (SPIO) particles are selectively taken up by cells of the reticular-endothelial system (RES), which are poorly, or not at all, represented within malignant tumors. Uptake of such particles leads the liver to appear markedly hypointense. Therefore, lack of uptake by RES in a small nodule, lets it appear hyperintense and suggests the diagnosis of malignancy even when conventional criteria are incomplete.[46,48] The combined use of SPIO and gadolinium chelates was demonstrated to be more accurate than MRI with the use of SPIO or gadolinium-chelates alone.[56,57] At present, MRI with SPIO agents remains limited to equivocal cases or research protocols.

Contrast enhanced ultrasonography. Recently, various technical advances in grey-scale US (such as harmonic imaging, the real-time spatial compounding technique, and real time adaptative imaging processing), allowed an increase in spatial resolution, contrast resolution, and signal-to-noise ratio, enhancing lesion detection.[58] However, the main progress in US has been the introduction of ultrasound contrast agents at the end of 1990s'. Ultrasound contrast agents are gas-filled microbubbles that were initially developed to increase flow signals to be detected with Doppler US.[59,60] Subsequently, the development of dedicated hardware and software made possible the detection of contrast enhancement in liver sinusoids and extracellular space, as it was for CT and MRI contrast agents. First generation US contrast agents are air-filled microbubbles encapsulated in relatively stiff shells of phospholipids.[61] Contrast agent visualization is obtained when microbubbles burst, as after insonation with high acoustic pressure (Mechanical Index >1.0). Microbubbles burst causes strong changes in reflected echoes, that are registered by the US equipment, producing evidence of contrast presence.[62,63] At lower acoustic energy, first generation contrast agents do not burst nor oscillate and produce therefore, the same type of echoes of surrounding tissues. Unfortunately, any single emission of high acoustic ultrasound output causes disruption of all microbubbles in the insonated area of the liver, so that a certain time (from 1-10 seconds) is required before new microbubbles replenish again major vessels and sinusoids allowing a new informative ultrasound scan. Working in the real-time mode with this approach would not produce optimal contrast visualization, since microbubbles are continuously destroyed and do not have time to reach sinusoids in sufficient quantities. This technique was consequently referred to as "intermittent harmonic imaging" ultrasound mode. However, intermittent harmonic imaging is usually cumbersome and difficult to be performed appropriately, especially by not highly skilled operators: microbubble destruction has to be triggered at the appropriate time with a perfectly targeted scan to visualize arterial enhancement. In small nodules, which are the most relevant to be characterized from a clinical point of view, it may be difficult to distinguish between absence of hypervascularization or technical pitfalls. In the last few years, second generation contrast agents licensed for liver investigation became available in Europe. In particular, sulfur exafloride (SonoVue, Bracco, Milan, Italy) was commercialized in 2001. Second generation contrast agents are more resistant to acoustic pressure than first generation ones.[61] When second generation microbubbles are insonated by low acoustic pressure at certain ultrasound frequencies, they respond with repeated elastic compressions and expansions, like a soap bubble, without bursting. Such phenomenon, termed "resonance", produces ultrasound waves at various harmonic frequencies, that are recorded and elaborated by US equipment, tuned at second harmonics. Since harmonic phenomenon takes place without microbubble destruction, US investigation can be performed in real time, allowing continuous monitoring of target lesions throughout all the contrast phases (**Figure 2**). It is therefore possible to detect even slight or short lasting arterial enhancements and to explore any part of a lesion, not only the central part as with intermittent imaging. Real-time contrast enhanced US (CEUS), also termed "perfusional angiosonography", has been reported to be as sensitive as CT to show hypervascularity in HCC of 1-3 cm in size,[24,64] and may sometimes detect arterial hypervascularization missed at CT.[64] Contrast wash-out leads to moderate hypoechogenicity in the late phases, which should be

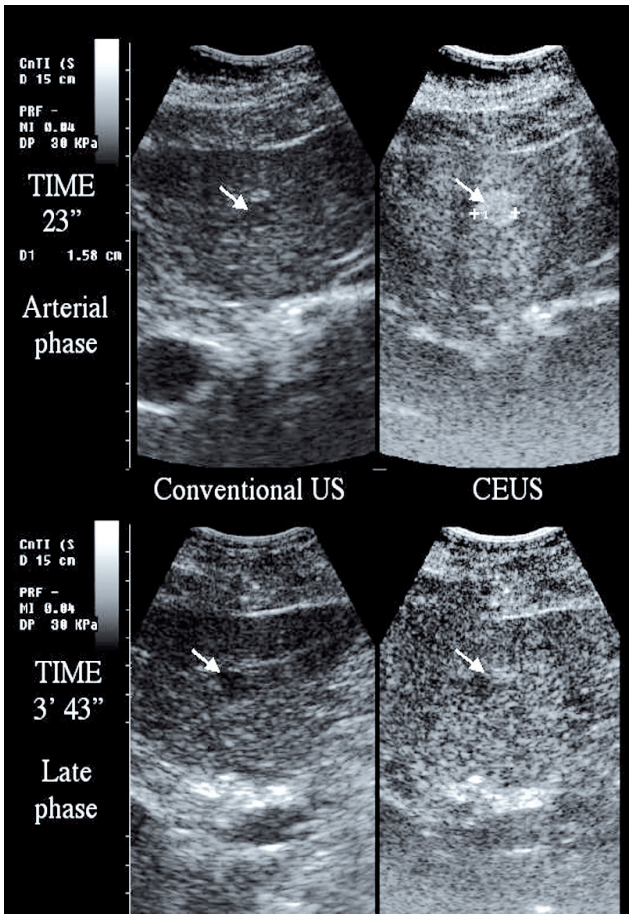

Figure 2 - A small nodule (16 mm in diameter, white arrow) is detected by conventional ultrasonography (US) in a cirrhotic subject during surveillance. Real time contrast enhanced ultrasonography (CEUS) is immediately performed, showing a hypervascular pattern in the arterial phase (upper panel; left frame shows conventional sonography appearance, whereas the right panel depicts the CEUS pattern in the same moment. The nodule appears hyperechoic at CEUS in the arterial phase, 23 seconds from contrast injection, as indicated by the time on the left. A contrast wash out is evident in the late venous phase (lower panel). Such a pattern is suggestive for hepatocellular carcinoma.

regarded as a confirmatory aspect of HCC in cirrhosis, or to isoechogenicity, which is consistent with HCC, but not as specific as hypoecogenicity.[64] Sensitivity of 68-96% and specificity of 74-96% are reported for the diagnosis of HCC.[65,66] As with MRI, CEUS combines morphological aspects (direct visualization of focal lesions at conventional US, **Figure 2**) with functional perfusion aspects, making characterization more accurate and limiting the risk of false positive results. As US is the principal tool in surveillance programs for HCC, CEUS may be immediately performed when a new liver nodule is detected at screening US, without further latency between screening and activation of the recall policy. Unfortunately, CEUS is not the optimal tool for HCC staging, so that TC or MRI are commonly required to exclude additional lesions prior to curative treatments. At last, sulfur exafluoride (SonoVue), is at least as safe as MRI contrast agents,[67] with an incidence of serious adverse events slightly lower than with iodinated radiological contrasts. Based on these early reports, real time CEUS was recently included in the practice guidelines of the American Association for the Study of Liver Disease (AASLD),[68] as one of the imaging techniques able to establish a diagnosis of HCC.

Positron emission tomography. Positron emission tomography (PET) with ^{18}F-fluoro-2-deoxy-D-glocose (^{18}F-FDG) is a noninvasive diagnostic tool for diagnosis, initial staging, and follow up of many malignancies.[69] After intravenous injection, ^{18}F-FDG, a non-physiological glucose analog, tends to accumulate mainly in malignant cells due to their high glucose metabolism. Recently, a whole body combined PET-CT scanner has been developed, that combines high quality PET and CT images in a single scanning session, providing both structural and metabolic information.[70] Metastatic liver tumors generally show high FDG accumulation as they have both high glucose uptake and very low activity of the enzymes that decrease the intracellular FDG levels (especially glucose-6-phosphatase), that in contrast are highly represented in the surrounding normal liver. On the contrary, the overall sensitivity of ^{18}F-FDG-PET in the detection of HCC is low (50-55%).[71] The disappointing results of ^{18}F-FDG-PET can be explained by the wide variability in enzyme activity in the individual HCC: in well-differentiated HCC, ^{18}F-FDG metabolism may be similar to that of the surrounding liver, leading to a false negative result, while higher sensitivity has been reported in poorly differentiated HCC. Consequently, ^{18}F-FDG-PET is not useful in the diagnosis of small, well-differentiated HCC. It has been proposed in the detection of extrahepatic spread and in the differential diagnosis of suspected neoplastic portal thrombosis, as these 2 conditions are generally associated to less differentiated HCC.

Diagnostic algorithms for HCC. A non-invasive diagnosis of HCC based on imaging techniques can be made when abnormal arterial vascularization is documented in a nodule on cirrhotic liver. The first diagnostic algorithm based not only on biopsy, but also on non-invasive procedures was proposed by the European Association for the Study of the Liver (EASL) Conference that took place in Barcelona in 2000.[72] In this algorithm, created as a recall policy for cirrhotic patients with a nodule detected at US during surveillance, diagnosis of HCC is accepted when arterial hypervascularization is confirmed by at least 2 concordant techniques in nodules larger than 2 cm on cirrhotic liver (the techniques being CT, angiography,

Doppler US, or MRI). Wash out was not recognized yet as a specific sign of HCC. In nodules of 1-2 cm in diameter, biopsy was recommended as arterial contrast-enhancement was not considered sufficiently specific and sensible in small nodules. Only follow up with US every 3 months was recommended for nodules <1 cm, due to the difficulty of reaching a definitive diagnosis (even by biopsy) and to the low probability of malignancy in the case of very small lesions. Recently, the AASLD produced a new guideline for HCC management,[68] in which the non-invasive criteria for HCC diagnosis were revisited and their application widened. The diagnostic pattern for HCC is now considered the presence of both contrast enhancement in the arterial phase and wash out in the portal venous phase. As this pattern is more specific than the sole arterial enhancement, its recognition is considered diagnostic even in nodules of 1-2 cm if 2 techniques are concordant in showing it. If the nodule is >2 cm in diameter, a single technique may be sufficient for the diagnosis if the typical pattern is present. For nodules <1 cm, US follow up is still recommended. The techniques now recommended for non-invasive diagnosis are CT, MRI, and CEUS.

The advances in imaging techniques have certainly improved the accuracy in non-invasive diagnosis, but some conditions have to be satisfied to avoid diagnostic errors. This algorithm is applicable only to nodules seen at US during a surveillance program. A nodule diagnosed at a first US without a precedent negative examination, needs more stringent diagnostic criteria, the same as nodules first identified at CT that are not detectable at unenhanced techniques. Nodules greater than one cm, without the non-invasive criteria for HCC diagnosis deserve further considerations. Absence of the typical pattern is primarily consistent with regenerative or dysplastic nodules, but it does not rule out malignancy. The nodule may be in an early stage of malignancy along the multistep carcinogenesis, when the unpaired, abnormal arteries have not developed sufficiently to be disclosed at imaging techniques. Therefore, any such nodule should be further investigated with biopsy, when technically feasible. If histology excludes HCC and HGDN, or if the biopsy cannot be performed, then a follow-up should be started, using US at a 3 month interval, with repetition of enhanced imaging, biopsy, or both when an increase in size is documented, or at latest after one year from initial diagnosis. If HCC is diagnosed at histological examination, this is commonly referred to as hypovascular HCC, at least in Western Countries. This is not a rare finding: in a recent report, hypovascular HCC accounted for 8.3% of HCC >3 cm in diameter.[64] Using only imaging techniques for the diagnostic work up of nodules of 1-2 cm, the diagnosis of HCC would be missed in up to 38% of cases.[64] The definition of hypovascular HCC may be not completely correct, since it includes both HCC that are truly hypovascular (with reduced arterial and portal vascularization) and others that more appropriately are isovascular (in which normal paired arteries are decreased and newly formed abnormal arteries compensate the decrease without exceeding the arterial vascularization of the surrounding parenchyma). Such differentiation could be made only by CT during arterio-portography, but it is not considered so relevant in clinical practice to justify hepatic angiography. In nodules smaller than one cm, a strict follow up protocol is considered appropriate. However, it may also be decided to proceed to imaging studies. If 2 consistent techniques show coincident and specific findings of HCC, at both the morphological and functional (vascular) level, the lesion can be regarded as an HCC even without biopsy, but no conclusive evidence is currently available to recommend this approach. Degree of intensity in the diagnostic approach is to be balanced in all cases, and particularly in the latter of nodules below one cm, with the expected changes in the prognostic assessment and therapeutic strategies.

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