Early diagnosis of primary liver cancer: imaging versus genetics

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ABSTRACT

Early diagnosis of hepatocellular carcinoma (HCC) in nodules smaller than 2 cm detected by screening ultrasounds becomes essential given that, at that stage, no vascular invasion is usually detected and treatment is associated with a high rate of long-term survival. Improvements in imaging techniques in the last few years have allowed a conclusive diagnosis of HCC in these small nodules without invasive procedures. However, a conclusive diagnosis of HCC by imaging is not always possible and, in more than half of cases, biopsy is needed. On the other hand, histological confirmation of HCC in such tiny nodules is very complex, and in most cases impossible because of the limited sample obtained. In addition, serum tumor markers currently available show low accuracy and are useless for early diagnosis. Progress in the knowledge of molecular mechanisms associated with malignant transformation will allow the use of new techniques that will facilitate diagnosis for HCC in very early stages.

Key words: Hepatocellular carcinoma. Early diagnosis. Imaging techniques. Tumor markers. Immunohistochemistry. PCR.

INTRODUCTION

Hepatocellular carcinoma (HCC) has shown a significantly increased incidence in the last few decades (1), and currently is the third most common cause of cancer-related mortality (2). This neoplasm rather develops in cirrhotic livers and is the primary cause of death in these patients (3). Currently available curative treatments (surgery, liver transplant, ablation) only apply for early stage HCC identified in symptom-free patients, which warrants HCC screening using abdominal US every 6 months.
for cirrhotic patients who may benefit from early diagnosis (4,5). Various pathology studies in explants obtained after surgical resection or liver transplantation have described an early stage consisting of nodules smaller than 20 mm in size, with mixed portal and arterial vascularization, that most often exhibit no vascular or adjacent parenchymal invasion, thus corresponding to in situ carcinoma as described for other organs (6). The use of radiological measures for these single tumors smaller than 20 mm in size is associated with a high response rate and 5-year survivals superior to 75% (7-9). Thus, these tumors are categorized as a distinct prognostic stage that is designated “very early” (stage 0 in BCLC classification, Fig. 1) (10).

The excellent prognosis of HCC in this stage accounts for the interest in diagnosing HCC earlier. Traditionally, diagnosing HCC in this very early stage was only feasible with histological confirmation. However, percutaneous biopsy has a low yield in the adequate characterization of such small nodules, not only because of potential sampling errors but also in view of the difficulties entailed by differentially diagnosing a dysplastic nodule from early HCC using a usually scant sample (11). On these grounds, and given the continual technological advances experienced by imaging techniques, non-invasive diagnostic criteria have been temptatively established based on the identification of a specific vascular pattern allowing for a conclusive diagnosis of early HCC with no need for histological confirmation. However, these criteria may only be applied to a small percentage of patients, hence biopsy remains necessary in many cases. Knowledge of molecular mechanisms responsible for malignant transformation has significantly advanced during the last few years, which has led to the evaluation of various gene expression patterns potentially leading to early HCC diagnosis in patients with an inconclusive pathological study.

NON-INVASIVE DIAGNOSIS USING IMAGING TECHNIQUES

HCC has a typical, exclusively arterial vascularization in contrast to the remaining liver parenchyma, which exhibits a mixed, both portal and arterial vascularature. This phenomenon determines a specific vascular pattern consisting of intense contrast uptake on the arterial phase followed by rapid contrast washout during the portal or late venous phase (Fig. 2). Several studies comparing the results of various dynamic imaging techniques to those of pathology analysis on explants or surgical resection speci-
imens have shown the specificity of this vascular pattern in patients with liver cirrhosis (12,13).

At the EASL consensus meeting held in Barcelona in 2000 non-invasive diagnostic criteria were proposed, which referred to the coincidental detection of a hypervascular nodule in a cirrhotic liver by two imaging techniques (4). However, these criteria were only applicable to nodules greater than 2 cm in size. Therefore, the only way to diagnose HCC in nodules smaller than 2 cm was by using liver biopsy. While these non-invasive imaging criteria have been successfully used in the clinical setting and proven useful, particularly in the diagnosis of big tumors, until recently no prospective validation was undertaken. Furthermore, the description of anecdotal hypervascular nodules other than HCC in cirrhotic livers (e.g., atypical hemangioma, cholangiocarcinoma) led to revise these non-invasive criteria (14). Thus, the recent clinical guidelines published by AASLD in 2005 (5), as well as the conclusions from the EASL consensus meeting held in Barcelona in 2005 (unpublished data), incorporated a need to detect, in addition to hypervascularization on the arterial phase, early washout during the venous phase in a dynamic imaging technique (contrast-enhanced US, MR, or contrast-enhanced CT). In nodules between 1 and 2 cm, this specific pattern should be coincidentally demonstrated in two imaging techniques to prevent potential false positive diagnoses based on a wrong reading of a single imaging test. If these criteria were unmet, a definitive diagnosis will be based on a biopsy study. Lastly, close follow-up with quarterly US scans is recommended for nodules smaller than 1 cm aiming at early growth detection, given their low malignant potential and the difficulties of appropriate characterization (Fig. 3).

While many authors have tried to validate these non-invasive diagnosis guidelines, studies were based on retrospective reviews of radiographic reports from patients with a diagnostic HCC biopsy (16) or assessments of dynamic CT and contrast-enhanced US, with HCC diagnosis being confirmed by pathology only for nodules displaying no coincidental hypervascularization (17), which has not allowed an appropriate assessment of diagnostic accuracy for non-invasive criteria.

With the goal of assessing the diagnostic yield of contrast-enhanced ultrasound (CEUS) and gadolinium dynamic MRI, and of adequately validating diagnostic recommendations as suggested by AASLD, we performed in our Unit a prospective study where including asymptomatic cirrhotic patients with preserved liver in whom a newly nodule between 5 and 20 mm in size was detected by screening US (18). They underwent gadolinium MRI, contrast-enhanced US (SonoVue®), and aspiration/biopsy testing; histology results were considered the gold standard, and biopsy was repeated for initial negative results. Patients where a diagnosis with HCC failed to be reached after two biopsies were followed up with quarterly contrast-enhanced ultrasounds, and half-yearly MRI scans.

Fig. 2. Dynamic MRI scan showing the vascular pattern specific for HCC. Figure A reveals a 20-mm nodule with intense contrast uptake on the arterial phase. Figure B shows early washout during the venous phase, and the presence of a HCC-specific pseudocapsule.
and puncturing was repeated in all cases where growth or hypervascularization was detected. Eighty-nine patients were included during 3 years. Upon study completion a total of 60 nodules were finally diagnosed as HCC; in one patient the diagnosis was cholangiocarcinoma, and the remaining 28 nodules were benign conditions where a overlooked HCC diagnosis was ruled out after a median 2 years’ follow-up. Thirteen nodules were smaller than 10 mm in diameter (14.6%), 44 were 11-15 mm (49.4%), and 32 were 16-20 mm in diameter (36%). Most importantly, a diagnosis of HCC was reached in only 2 of 13 nodules smaller than 10 mm (15.3%); however, 29 out of 32 nodules (90.6%) 16-20 mm in size were diagnosed as HCC. Therefore, the low prevalence of HCC in nodules smaller than 10 mm would justify the recommendation of performing no tests but only a closer follow-up of patients.

Finally we analyzed the diagnostic yield of CEUS and MRI taking two vascular patterns into consideration – one based on EASL recommendations, consisting of increased arterial uptake regardless of the presence of washout on the venous phase (suspicion of HCC), and one based on AASLD guidelines, which require not only hypervascularization but also washout (conclusive of HCC). Results obtained are summarized in table I. The sensitivity of both tests including suspect diagnosis was very high (79% and 85% with CEUS and MRI, respectively), but was associ-ated with a non-negligible number of false-positive diagnoses with HCC (4 for CEUS, 3 for MRI). The use of conclusive criteria was associated with decreased sensitivity (52% and 62% for CEUS and MRI, respectively) but improved specificity (93.1% and 96.6% for CEUS and MRI, respectively). However, both techniques had false positive results (one cholangiocarcinoma and one angiomma for CEUS, and one regeneration nodule for MRI) despite the highly experienced team of radiologists taking part in the study, and the use of state-of-the-art imaging techniques. When both imaging techniques were combined sensitivity decreased significantly (down to 33% if both tests were required to be conclusive for HCC) but specificity and positive predictive value reached 100%. Therefore, these results prospectively validate non-invasive criteria as proposed by AASLD for the diagnosis of HCC (18).

**TUMOR MARKERS**

The most widely used tumor marker as a diagnostic tool for HCC is alpha-fetoprotein (AFP). Its usefulness to this end has been assessed by multiple studies, but most were retrospective case-control analyses including patients with advanced HCC, hence its applicability for early HCC diagnosis has not been adequately assessed to this day (19). In addition, the diagnostic yield obtained by various studies...
was very low, with a variable sensitivity and specificity depending on cut-off value. For example, the study reported by Trevisani in 2001, whose best cut-off following a ROC curve was 16 ng/mL, showed a sensitivity of just 62.4% and a specificity nearing 90% despite the fact that a significant proportion of patients had advanced HCC (20). When in the above study we prospectively assessed AFP levels in the diagnosis of nodules smaller than 2 cm, we acknowledged their scarce utility; no significant differences in AFP were seen between patients ultimately diagnosed with HCC versus patients with benign nodules (8.5 vs. 5 ng/mL, respectively), and a cut-off at 20 ng/mL only showed a sensitivity of 24% with a specificity approaching 80%, which confirms the scant usefulness of AFP for early HCC diagnosis (18).

In the last years, other tumor markers have been suggested for the diagnosis of HCC (21). These include PIVKA (also known as de-gamma carboxy-prothrombin) (22), fraction L3–AFP (23), HGF (human hepatocyte growth factor), IGF-1 (insulin-like growth factor-1) and glypican-3 (24). While preliminary results are encouraging, no single marker has been adequately validated for use in the clinical setting, and in most cases these markers were evaluated in patients with advanced HCC, hence their role in early HCC diagnosis remains unknown.

MOLECULAR DIAGNOSIS

In the last few years we have witnessed tremendous advances in the understanding of molecular pathways determinant for HCC development, promotion, and progression (25,26). From a theoretical standpoint, the identification with laboratory techniques of the various genes associated with hepatocarcinogenesis would allow an early diagnosis of HCC with no regard to whether nodules showed the typical vascularization enabling non-invasive diagnosis or even before the presence of a conclusive histological pattern. This is why several studies have been recently reported that have examined the role of various gene expression patterns in the early diagnosis of HCC (27). Paradis and colleagues evaluated gene expression in healthy liver tissue (n = 5), cirrhosis (n = 12), regenerative nodules (n = 24), dysplastic nodules, both low- (n = 8) and high-grade (n = 3), very early HCC (n = 10), and advanced HCC (n = 16) using real-time PCR. The most relevant result was the identification of an HCC-related index consisting of 13 genes (TERT, IGF2, GJB2, TEF, TIAM1, CXCL12, TOP2A, A2M, PLG, ARF, PDGFRA, MKI67, and THBS1), which allowed the diagnosis of HCC with a high sensitivity and a specificity of 100%. Therefore, molecular analysis was shown to be a valuable tool for diagnosing HCC (28). Di Tommaso and colleagues evaluated the usefulness of HSP70, glypican 3, and glutamine synthetase detection using immunohistochemistry for the diagnosis of HCC. They assessed a total of 105 nodules obtained following surgical resection, which comprised regenerative nodules (n = 15), both low-grade (n = 15) and high-grade (n = 22) dysplastic nodules, and HCC (n = 53), with 43 of the latter cases being < 3 cm. The most interesting result was that positivity for at least two markers showed a specificity and positive predictive value nearing 100%, with a sensitivity that might approach 59% (29). The team from Mount Sinai assessed the expression of 55 genes previously described as biomarkers or involved in hepatocarcinogenesis in a sample of 17 dysplastic nodules and 20 HCC smaller than 2 cm from patients with HCV-related liver cirrhosis using real-time quantitative PCR; candidate genes were contrasted in 10 healthy liver samples, 10 cirrhosis samples, and 20 advanced HCC samples. Twelve genes were expressed differently in initial HCC versus dysplastic nodules: 5 were overexpressed (TERT, glypican-3, gankyrin, survivin, TOP2A) and 7 were underexpressed (LYVE1, E-cadherin, IGFBP3, PDGFRA, TGFA, cyclin D1, HGF). A subsequent regression analysis categorized genes according to their best ROC curve-determined cut-offs, and obtained various models, with the

| Table I. Diagnostic yield of contrast-enhanced ultrasonography (CEUS) and magnetic resonance imaging for the diagnosis of HCC in nodules smaller than 20 mm |
|-----------------|----------------|-----------------|-----------------|----------------|
|                | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
| **CEUS**       |             |              |                           |                             |
| Suspicious     | 78.3%       | 86.2%        | 92.2%                      | 71.4%                       |
| Conclusive     | 51.7%       | 93.1%        | 93.9%                      | 50.9%                       |
| **MRI**        |             |              |                           |                             |
| Suspicious     | 85%         | 89.7%        | 94.4%                      | 74.3%                       |
| Conclusive     | 61.7%       | 96.6%        | 97.4%                      | 54.9%                       |
| **Both techniques** | |              |                           |                             |
| Suspicious     | 66.7%       | 100%         | 100%                       | 59.2%                       |
| Conclusive     | 33.3%       | 100%         | 100%                       | 42%                         |

Imaging findings were categorized as suspicious for HCC when nodules showed increased uptake on the arterial phase regardless of the presence or absence of venous-phase washout (criterion suggested by EASL in 2000), or as conclusive when nodules showed increased uptake on the arterial phase followed by venous-phase washout (criterion proposed by AASLD and EASL in 2005).
LYVE1, glypican 3, and survivin combination exhibiting a greater diagnostic accuracy with a sensitivity of 95%, a specificity of 94%, and a positive predictive value of 95%. Other models with a diagnostic yield included the following combinations: LYVE1, survivin, and E-cadherin; glypican 3 and survivin, and glypican 3 and TERT. The expression of the five genes present in these high-yield models was evaluated in an independent sample including 10 dysplastic nodules and 19 early HCCs, which validated the above results. Lastly, the expression of glypican 3 and survivin was examined using immunohistochemistry in 36 paired samples of cirrhotic tissue and 14 dysplastic nodules, 22 HCC (9 early, 13 advanced), and 3 healthy controls; glypican 3 was positive in all nodules with HCC, and negative in all dysplastic nodules and cirrhotic tissue (30).

While these results are encouraging, their use in standard clinical practice has not been validated yet. A drawback of studies assessing molecular markers and gene expression patterns to facilitate the early diagnosis of HCC is result heterogeneity among different papers, partly accounted for by differing methodologies, particularly regarding patient selection according to the underlying liver condition’s etiology and lesion’s nature, which leads to endorse these results cautiously. In addition, all these studies were performed using histological material obtained by surgical resection. However, the actual diagnostic value of these markers has not been assessed to date in samples prospectively obtained by percutaneous puncture aspiration, where samples are small and potentially “contaminated” by underlying cirrhotic liver tissue. Therefore, the actual value of these techniques in early HCC diagnosis remains unknown, as the typical phenotype allowing a non-invasive diagnosis remains undeveloped.

CONCLUSIONS

The early diagnosis of HCC is a major goal for professionals involved in the follow-up of patients with liver cirrhosis. This is why HCC screening is justifiable and should be performed with half-yearly abdominal US scans performed by expert radiologists. However, the correct diagnosis of nodules identified by screening ultrasonography remains a challenge, especially for nodules smaller than 2 cm. An early diagnosis of HCC in such small nodules was previously feasible by histological confirmation alone. However, the diagnostic yield of FNA in this setting was very low and not exempt of complications. In the last few years technological advances in the field of imaging diagnosis, particularly the emergence of contrast-enhanced US, improved MRI techniques, and the development of multislice CT, which is able to explore the entire liver parenchyma in a few seconds, has allowed an early diagnosis of HCC in nodules smaller than 2 cm. In this regard, AASLD and EASL favor a diagnosis of HCC using selected imaging criteria particularly based on the finding of a characteristic vascular pattern consisting of intense contrast uptake on the arterial phase followed by washout on the venous stage. For nodules smaller than 2 cm, and in order to prevent false diagnoses, two coincidental imaging tests are required. These criteria, based on the analysis of a number of retrospective case-control studies, have been recently validated prospectively. Unfortunately, a non-invasive diagnosis is only feasible in one third of patients with nodules smaller than 2 cm, hence biopsy plays a primary role in the early diagnosis of HCC. However, a high rate of false negative results must be considered, which may be of up to 30%, this being why a negative biopsy will not rule out a diagnosis with HCC. Currently available tumor markers are not useful for the early diagnosis of HCC given their unacceptable low sensitivity.

In recent years we have witnessed an increasing understanding of molecular pathways associated with hepatocarcinogenesis. Among other things, this has allowed an evaluation of several gene expression patterns to reach a conclusive differential diagnosis between HCC and the various preneoplastic stages. Different genes have been studied, particularly TERT, glypican 3, survivin, LYVE1, HSP70, and IGF-associated genes, with promising results. However, their use in daily practice has not been assessed, and prospective studies are needed to confirm the usefulness of these molecular biology techniques.

REFERENCES