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Role of liver biopsy in hepatocellular carcinoma

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Abstract

The role of liver biopsy in the diagnosis of hepatocellular carcinoma (HCC) has been challenged over time by the ability of imaging techniques to characterize liver lesions in patients with known cirrhosis. In fact, in the diagnostic algorithm for this tumor, histology is currently relegated to controversial cases. Furthermore, the risk of complications, such as tumor seeding and bleeding, as well as inadequate sampling have further limited the use of liver biopsy for HCC management. However, there is growing evidence of prognostic and therapeutic information available from microscopic and molecular analysis of HCC and, as the information content of the tissue sample increases, the advantages of liver biopsy might modify the current risk/benefit ratio. We herein review the role and potentiality of liver biopsy in the diagnosis and management of HCC. As the potentiality of precision medicine comes to the management of HCC, it will be crucial to have rapid pathways to define prognosis, and even treatment, by identifying the patients who could most benefit from target-driven therapies. All of the above reasons suggest that the current role of liver biopsy in the management of HCC needs substantial reconsideration.

Key words: Hepatocellular carcinoma; Liver biopsy; Prognostic factors; Liver cancer; Recurrence; Liquid biopsy

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Core tip: The role of liver biopsy in the diagnosis of hepatocellular carcinoma (HCC) has been challenged over time by the ability of imaging techniques to characterize liver lesions in patients with known cirrhosis; indeed, in the diagnostic algorithm for this tumor histology is currently relegated to controversial cases. However, increasing knowledge of the phenotypical and molecular characteristics of HCC is leading to a new scenario in which biopsy may play a decisive role. As the information content of the tissue sample increases, the advantages of liver biopsy may modify the current risk/benefit balance.

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INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for approximately 90% of primary liver cancers and, with a rapidly increasing incidence in the last two decades^[1], constitutes a major global health problem^[2]. Importantly, HCC mainly develops in the settings of chronic liver injury or cirrhosis^[3,4]. Indeed, the high rate of HCC in certain risk groups makes surveillance a cost-effective route to reducing mortality^[5] and international societies, including the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD), recommend six-month interval ultrasounds (US), with or without alpha-fetoprotein (AFP) levels for cirrhotic patients^[6,7].

However, a confident diagnosis of HCC of a liver nodule detected by ultrasonography, in the screening setting, represents a major clinical challenge, and, according to the diagnostic algorithm proposed by Forner *et al*^[8], it is almost impossible with current techniques for nodules with a diameter of less than 1 cm^[9]. On the other hand, the diagnosis of HCC can be confidently established using imaging techniques if a nodule larger than 1 cm displays a specific imaging pattern^[7,10]. The hallmark features of HCC on dynamic computed tomography (CT) scan and magnetic resonance imaging (MRI) are an early wash-in combined with late wash-out of contrast agents^[10], that, nonetheless, only occur in a minority of patients with small tumors^[11]. Moreover, the high specificity and positive predictive value of this pattern in larger lesions have been prospectively validated for the diagnosis of HCC only in cirrhotic livers^[10,12-14].

Innovations in cross-sectional imaging, aside from the trend towards a less invasive medical practice, would result in a larger subgroup of patients who can avoid undergoing a liver biopsy for the diagnosis of HCC. On the other hand, the avoidance of biopsies might hamper both the understanding of biological features and the development of targeted therapies for HCC, and the risk of misdiagnosis must always be taken into account. The role of liver biopsy for hepatic nodules thus remains a challenging issue even in the contemporary era. In the present review, we aim to summarize the current clinical guidelines as well as the pros and cons of using liver biopsies in the clinical management of HCC.

DIAGNOSIS OF HCC

Non-invasive diagnosis of HCC, in the setting of liver cirrhosis, is based on a typical imaging diagnostic pattern^[4,15] that relies on the peculiar vascular derangement occurring during hepatic carcinogenesis and it is strongly endorsed by EASL, AASLD, and the Asian Pacific Association for the Study of the Liver^[6,7,16]. All societies concordantly state that the diagnosis of HCC in cirrhotic patients should be based on non-invasive criteria with a strong grade of recommendation and a high level of evidence. Because of their higher sensitivity and ability to analyze the whole liver, CT or MRI should be used first. However, non-invasive criteria can only be applied to

cirrhotic patients for liver lesions above 1 cm of diameter, in light of the high pre-test probability, regardless of which imaging modality is utilized and it is not improved by assessing other MRI parameters^[17,18]. Iavarone *et al* demonstrated that tumor grade might influence the accuracy of dynamic contrast techniques in the diagnosis of small HCC^[19]. Furthermore, non-invasive criteria have not been validated in non-cirrhotic livers.

Of note, HCC in non-cirrhotic patients is likely to be larger at diagnosis^[20], since patients are not enrolled in surveillance programs. Yet, the specificity of the imaging diagnostic hallmarks for HCC is lower in the non-cirrhotic liver, as alternative diagnoses are seen more commonly (*e.g.*, hepatocellular adenoma and metastases). Non-invasive diagnostic criteria for HCC have only been validated in patients with cirrhosis who are followed up with, six-month interval, US. Further, only 50% of HCC occurs in cirrhotic patients where HBV infection is endemic^[21-23]. Therefore, despite a moderate grade of evidence, EASL strongly recommends that the diagnosis of HCC in non-cirrhotic livers is confirmed using a liver biopsy^[6].

Histological diagnosis via liver biopsy may, therefore, be necessary if HCC develops in a non-cirrhotic patient, and if imaging studies are inconclusive for being compatible with HCC. The AASLD does not recommend biopsy for lesions bigger than 1 cm if two different imaging studies yield concordant findings^[7]. Liver biopsy is done under CT or US guidance with varying degrees of sensitivity (66%-93% based on tumor size, operator experience, and needle size) and 100% specificity and positive predictive value^[24]. Furthermore, a liver biopsy may be needed in patients who are not candidates for curative resection, to establish a diagnosis for the purpose of systemic therapy or transplantation.

PATHOLOGICAL DIAGNOSIS OF HCC AND PROGNOSTIC MARKERS

Tissue-biopsy warrants a simple key-hole view of the lesion under examination. The pathological data obtained at the morphological, phenotypical and molecular level from these tiny fragments may be incomplete or only partially representative. However, it still represents the best option to get information from the lesion itself. Thus, every single diagnostic, prognostic and predictive information represented in the tissue is searched for and reported in the pathological report, to the point that grossing material is saved for this purpose.

Tissue-biopsy is mainly obtained for diagnostic purposes if a conclusive diagnosis of HCC cannot be rendered on imaging. In this setting the differential diagnosis takes into consideration two lesions staying close along the process of hepatocarcinogenesis such as High-Grade Dysplastic Nodule (HGDN) and early HCC^[25] and should always be supported by the results of a panel of markers, namely glypican 3 (GPC3), heat shock protein 70 (HSP70), and glutamine synthetase (GS) used in combination^[26,27]. Indeed, this panel warrants 100% specificity and 72% of sensitivity, while the use of single markers alone can be misleading. GPC3 immunoreactivity can be observed in a few cirrhotic cells and lesions showing up to 10% of immunoreactive cells can also be HGDN^[26]. HSP70 can be observed in apoptotic hepatocytes, isolated periseptal hepatocytes, and stellate cells. GS immunoreactivity merits an even greater attention since it is observed in a number of different lesions including: (1) Normal perivenular and periseptal hepatocytes; (2) Focal Nodular Hyperplasia, with a map-like distribution; (3) Exon 7/8 β -catenin mutated hepatocellular adenoma with a faint/focal, patchy immunoreactivity; (4) Exon 3 β -catenin mutated HA, with a strong/diffuse positivity, and (5) HCC^[28]. Other diagnostic markers have been investigated but never endorsed in guidelines^[29,30].

A progressively larger use of the tissue-biopsy is observed to enroll HCC patient in clinical trials. Even if this revitalization of HCC biopsy does not reflect a real change of attitude toward it, the current situation could be of help to renovate its role. A large amount of information can be searched for in the neoplastic tissue and surrounding parenchyma, and reported in the histopathological diagnosis. These include HCC histotype and grade, microscopic vascular invasion, morpho-molecular type, and the expression of phenotypic markers of prognostic impact such as CK 19 and VETC. In addition, if non-neoplastic liver tissue is available, the degree of fibrosis should be reported as well.

The following HCC histotypes are listed by the upcoming WHO classification: steatohepatitic, clear cells, macrotrabecular massive, scirrhous, chromophobe, fibrolamellar, neutrophil-rich and lymphocyte-like^[31]. The correct definition of the histotype enriches the pathological report with prognostic and/or predictive information. The recently reported macrotrabecular massive histotype, which

represents 5%-10% of all HCC, has a poorer outcome^[32,33]; in contrast, the lymphocyte-rich HCC, a rare histotype showing better prognosis^[31], is sustained by the presence of an active immune infiltrate^[34]. It may be argued that several histological features can be represented in a single HCC, especially those of larger size. Available data shows that a median of four architectural patterns/cytological variants might coexist within the same HCC^[32]. A tissue-biopsy documents only a part of this heterogeneity and, as mentioned above, this may underestimate tumor biology. Tumor size, and therefore tumor heterogeneity, also affects the reliability of HCC grading in the biopsy. The concordance between grading in pre-operative biopsy and the surgical specimen is good/excellent in HCC below 4 cm^[19,35] but low in HCC above 7 cm^[36]. It is well known that the worse a grade drives the prognosis^[37], and therefore a clinically meaningful pathological report might indicate both the predominant as well as the worst grade (in line with what is done for prostatic biopsy). A recent meta-analysis confirmed that histological grading of HCC has an important prognostic role in both liver resection or transplantation; however, it also showed a large heterogeneity on the microscopic assessment of HCC pathology urging standardization^[38].

Microscopic vascular invasion (MVI) is a major prognostic feature of HCC and is associated with advanced tumor stage, distant metastasis and adverse outcome^[39-41]. MVI occurs at the rates of 25%, 40%, 55% and 63% in HCC smaller 3, 3-5, 5-6.5, and bigger 6.5 cm, respectively^[42]. Nonetheless, the detection of MVI in a biopsy is fundamentally a chance opportunity. Accordingly, surrogate markers of MVI are intensively investigated. A study on the combination of PIVKA-II with H4K20me2 showed very high specificity and PPV for prediction of MVI in HCC needle biopsies^[43,44]. VETC is a peculiar vascular phenotype, originally described by Fang *et al*^[45]. We recently confirmed its strong impact on the prognosis of resectable HCC^[46]. Moreover we observed the close correlation of VETC with several ominous prognostic features such as MVI. Of interest Feng *et al*^[47] recently showed that VETC+ HCC are those more suitable to sorafenib, prefiguring VETC as a prognostic and predictive marker.

During the last two decades, an increasing understanding of the most abundant molecular alterations of HCC was developed but never translated into daily practice to improve prognostic assessment or therapeutic decision. A recent study firstly demonstrated a strong relationship between molecular and pathological features in HCC^[31] and highlighted the existence of two distinct HCC phenotypes sustained by the mutually exclusive CTNNB1 and TP53 mutations. In the first group, HCC presents as well-differentiated tumors with cholestasis and microtrabecular and pseudoglandular patterns of growth; in the second, HCC is mostly poorly differentiated with frequent vascular invasion. Using the corresponding immunohistochemical markers (p53, β -catenin, and GS) we have recently confirmed the clinical-pathological correlations of these two subclasses in the daily clinical practice^[46]; however, none of these categories proved to be of prognostic relevance^[31,48]. Interestingly, the subgroup of HCC correlated to β -catenin pathway activation was recently associated with an exhausted immune infiltrate^[49]. This finding, possibly explaining the resistance to Immune Checkpoint Inhibitors, prefigures a morpho-molecular classification of HCC as having a predictive role^[50,51].

The use of stemness-related biomarkers represents the field where the translation of molecular information on clinical practice is more advanced. Several stemness-related markers have been identified and intensively investigated (CK19, EpCAM, CD133, SALL4, NCAM, OV6, CD90, nestin, CD44) and almost all were associated with a more aggressive clinical behavior. In particular, HCCs with CK19 immunostaining in more than 5% of tumor cells show higher recurrence rates and higher rates of lymph node metastasis^[52].

A tissue biopsy can be done to distinguish HCC from other primary and secondary malignancies of the liver. Some of these cases may present as poorly differentiated, solid growing carcinomas, in particular, those lesions that previously underwent different lines of treatment. In these cases, the final diagnosis of HCC can hardly be rendered on morphology alone and should be supported by the evaluation of specific markers indicative of hepatocellular differentiation. Those currently used are HepPar-1, Arginase-1, CD10, pCEA, GPC3 and BSEP. In a recent study, Lagana *et al*^[53] investigated their efficiency and showed that BSEP, CD10, and pCEA showed 100% specificity as compared to 97% of GPC3 and HepPar-1 and 94% of Arginase. BSEP and Arginase showed the highest sensitivity (90%).

USE OF LIVER BIOPSY IN CLINICAL MANAGEMENT OF HCC: PROS AND CONS

The main reason for limiting liver biopsies in HCC is the risk of adverse events, possibly impacting on the diagnostic and/or therapeutic pathway. Liver biopsy techniques and characteristics of an optimal liver specimen have been described in detail^[54,55]. The most common complication of liver biopsy is pain that, including mild discomfort, is reported by up to 84% of patients^[56]. Severe complications correlated to liver biopsies, including perforation of gallbladder, bile peritonitis, haemobilia, pneumothorax or hemothorax, are extremely rare^[57]. Severe bleeding is usually evident within 2-4 hours and occurs in 1 out of 2500-10000 biopsies; nevertheless, late hemorrhage, most likely due to clot dissolution, cannot be neglected^[58]. Less severe bleeding, defined as that sufficient to cause pain or reduced blood pressure or tachycardia, but not requiring transfusion or intervention, occurs in approximately 1 out of 500 biopsies^[59-65]. Considering that severe hemorrhages are mostly arteriolar, US guidance is not expected to reduce the risk of bleeding, although it reduces the overall amount of complications^[66]. Even if the risk of mortality is very uncommon after percutaneous biopsy (1 on 10000), it is usually related to severe hemorrhage, mostly after biopsy of malignant lesions^[21,22,24]. Importantly, patient's perspective should be largely considered and informed consent properly acquired.

Furthermore, inserting a needle into a neoplastic lesion could modify the oncologic prognosis of the patient entailing the release of neoplastic cells along the needle path, even if the responsible mechanisms and the real risk of seeding are unclear^[67,68]. The most quoted study about seeding risk is a meta-analysis that showed a rate of 2.7% in 1340 biopsies^[69]. Nevertheless, adding three more recent series to this meta-analysis we would obtain much lower rates of seeding, even less than 1%^[70-72]. Moreover, in most of the reported cases of seeding, its clinical impact is mitigated by the observation that it was usually treated successfully by resective or ablative treatments and did not cause relevant morbidity or mortality^[72,73].

As stated above, advances in imaging tools have led to a decrease in requiring biopsy for liver nodules^[74,75]. However, the risk of misdiagnosis remains a discussed issue; although possibly influenced by the limited sensibility of the available imaging at the time, Freeman *et al*^[76] retrospectively showed that 20% of 789 patients who underwent liver transplantation for HCC had benign nodules. Furthermore, non-invasive parameters are highly influenced by lesion size^[9,11,13].

Finally, it has been recently reported that intrahepatic cholangiocarcinoma (iCCA) can be misdiagnosed as typical HCC in 4% of cases. iCCA is the second most common primary liver cancer worldwide^[77]; given its rising incidence^[78,79] and poor prognosis, close attention is needed to differentiate iCCA from HCC. Risk factors for iCCA are known to be similar to those for HCC^[80,81] and cirrhosis seems to play a pivotal role. Prevalent imaging features of iCCA is a progressive contrast uptake at CT-scan and MRI^[48,82-89] but iCCA have been found to be hypervascular in 4% of cases and the enhancement pattern may be similar to HCC at CT-scan and MRI^[89-92]. To explain this unusual pattern of iCCA, Huang *et al*^[93] demonstrated the presence of erythrocytes and microvessels, detected by immune-histochemical staining, in all the iCCA presenting with HCC-like contrast enhancement features.

Although histological characteristics may have possible use in both prognostic stratification and detection of the therapeutic target, currently they have no leading role in treatment decisions^[7,10]. In particular, having the histological confirmation of HCC in those patients that are deemed to be resectable is not indicated. In these patient, the final histological diagnosis may be properly done on the surgical specimen. Notably, this especially applies when accurate multidisciplinary case discussion is preoperatively performed.

Further, the first-line systemic treatment for HCC with multikinase inhibitors such as sorafenib, is widely prescribed without liver biopsy. Unfortunately, no validated targets are available and liver biopsy remains merely diagnostic with no role in prognostic stratification. Importantly, this practice might have largely limited the identification of therapeutic targets, and eventually has contributed to poor stratification in patients. Nevertheless, molecular markers have been explored in the latest years aiming to identify prognostic markers and to improve patient selection for novel treatments in advanced/unresectable HCC^[94-98]. In particular, genome profiling of both neoplastic tissue and surrounding liver tissue has been assessed, demonstrating that both the tumor and the non-tumor expression signature predicted tumor recurrence^[95]. On the other hand, whereas biomarker-driven enrichments are pursued in most study protocols testing novel anticancer agents, similar approaches in the HCC field have been implemented only in the frame of few clinical trials. Two of them, namely the METIV-HCC^[99] and the JET-HCC^[100] trials of tivantinib versus

placebo, attempted to demonstrate a survival benefit from an investigational MET inhibitor (tivantinib) in patients with elevated MET expression levels, as determined by immunohistochemical analysis. Disappointingly, due to several reasons previously discussed by Rimassa *et al*^[99], both studies eventually failed their respective primary endpoints. Despite the clear frustration that followed these results, the quest for individualized approaches, that may render a conceptual frame for precision medicine in HCC, is still ongoing.

In contrast to other solid tumors such as *BRAF*-mutated melanomas or lung cancers harboring *ALK* fusion rearrangements, no driver (or “trunk”) mutation leading to oncogenic addiction in HCC is thus far deemed actionable^[101]. Conversely, recent investigations suggest that some genomic alterations could lead to the identification of additional molecular targets^[102,103]. In a recent study by Schulze *et al*, using whole exome sequencing, genetic alterations potentially targetable by already approved drugs were identified in 28% of HCC^[104]. Taking advantage from a next-generation sequencing platform, similar data were reported also by Harding and colleagues^[51], who found that 24% of patients in their series had at least one potentially actionable mutation that could be the target for currently available Food and Drug Administration-approved drugs. These data therefore highlight the potential usefulness of HCC genotyping with respect to patient care, despite a relatively lower abundance of targetable alterations, as compared to melanoma or lung cancer^[105]. Further studies investigated the genome-wide profiling of HCC lesions highlighting new possible target areas of chromatin remodeling^[106-108]. Indeed, cancers are more complex than their own genome and a complete molecular assessment should theoretically involve transcriptional profiling and micro-environmental characteristics^[109-111].

LIQUID BIOPSY IN HCC

Identifying patients that could benefit from having a liver lesion biopsied, is a challenging effort. At the same time, we aim to obtain critical information in a less invasive way. A liquid biopsy, which entails the analysis of tumor components released into the bloodstream^[112], is a minimally invasive procedure and decreases the financial costs and potential complications of tissue biopsies. Liquid biopsies are also easy to repeat during follow-up. Even if an effective liquid biopsy in HCC has not been developed yet, different liquid biopsy markers for HCC early detection and precision medicine have been proposed including circulating tumor cells (CTCs), circulating cell-free DNA (cfDNA) integrity, somatic mutations, circulating cell-free tumor DNA methylation, and circulating RNA. Most of the studies exploring CTCs in HCC have shown a direct correlation between higher CTC number and poor clinical outcomes^[113]. Interestingly, D’Avola and colleagues recently described a method that sequentially combines image flow cytometry and high-density single-cell mRNA sequencing to identify CTCs in HCC patients^[114]. Furthermore, a study by von Felden *et al* showed the possible role of circulating DNA methylation markers in the diagnosis, surveillance, and prognosis of HCC^[115]. Advances in the field of liquid biopsy hold great promise in improving early detection of HCC, advancing patient prognosis, and ultimately increasing patient survival rates (Table 1). In addition, liquid biopsies could provide a valuable tool to overcome tumor heterogeneity, which is particularly pronounced in multifocal and advanced HCC, both at genomic and transcriptional levels^[116].

CONCLUSION

Evaluating the pros and cons of extending or reducing liver nodules biopsy indications, the role of a multidisciplinary case by case evaluation has been highlighted. In our opinion, this approach is going to allow avoiding more biopsies than those which will be added. Thus, coming back to the nowadays, the off-label decision to biopsy a “typical” nodule could only be related to clinical features of higher risk of misdiagnoses such as the increase of atypical markers (*i.e.*, Ca19.9) with normal AFP or the presence of iCCA risk factors (*i.e.*, PSC). Importantly, the decision should be taken after discussion in a multidisciplinary tumor board including radiologists, surgeons, oncologists, pathologists and, hepatologists.

Table 1 Advantages and disadvantages of liquid biopsy

	Liquid biopsy	Liver biopsy
Cons	Lack of large-scale validation studies Expensive (will most likely improve in the near future)	Risk of seeding Potential complications
Pros	Minimally invasive Easy to repeat during follow-up. Provides detailed, dynamic information about tumor biology (overcome tumor heterogeneity in multifocal and advanced HCC)	Avoid risk of misdiagnosis Assesment of microscopic vascular invasion Low cost Reproducibility Possibility of review over time
Future prospectives	Improved diagnosis of lesions below than 1-2 cm in diameter Correlation with clinical outcomes Prediction of response to treatment	Prognostic stratification and detection of the therapeutic target (systemic therapy)

REFERENCES

- Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913-2921 [PMID: 24840647 DOI: 10.1158/0008-5472.CAN-14-0155]
- Global Burden of Disease Liver Cancer Collaboration; Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi JY, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MS, Fischer F, Fürst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaeian A, Khader Y, Khang YH, Kumar A, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topór-Mądry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. *JAMA Oncol* 2017; **3**: 1683-1691 [PMID: 28983565 DOI: 10.1001/jamaoncol.2017.3055]
- Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD; HALT-C Trial Group. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; **136**: 138-148 [PMID: 18848939 DOI: 10.1053/j.gastro.2008.09.014]
- Bruix J, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
- Andersson KL, Salomon JA, Goldie SJ, Chung RT. Cost effectiveness of alternative surveillance strategies for hepatocellular carcinoma in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2008; **6**: 1418-1424 [PMID: 18848905 DOI: 10.1016/j.cgh.2008.08.005]
- European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]
- Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**: 358-380 [PMID: 28130846 DOI: 10.1002/hep.29086]
- Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]
- Forner A, Vilana R, Ayuso C, Bianchi L, Solé M, Ayuso JR, Boix L, Sala M, Varela M, Llovet JM, Brú C, Bruix J. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: Prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology* 2008; **47**: 97-104 [PMID: 18069697 DOI: 10.1002/hep.21966]
- European Association for the Study of the Liver; European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- Bolondi L, Gaiani S, Celli N, Golfieri R, Grigioni WF, Leoni S, Venturi AM, Piscaglia F. Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. *Hepatology* 2005; **42**: 27-34 [PMID: 15954118 DOI: 10.1002/hep.20728]
- Sangiovanni A, Manini MA, Iavarone M, Romeo R, Forzenigo LV, Fraquelli M, Massironi S, Della Corte C, Ronchi G, Rumi MG, Biondetti P, Colombo M. The diagnostic and economic impact of contrast imaging techniques in the diagnosis of small hepatocellular carcinoma in cirrhosis. *Gut* 2010; **59**: 638-644 [PMID: 19951909 DOI: 10.1136/gut.2009.187286]
- Leoni S, Piscaglia F, Golfieri R, Camaggi V, Vidili G, Pini P, Bolondi L. The impact of vascular and nonvascular findings on the noninvasive diagnosis of small hepatocellular carcinoma based on the EASL and AASLD criteria. *Am J Gastroenterol* 2010; **105**: 599-609 [PMID: 19935786 DOI: 10.1038/ajg.2009.654]
- Khalili K, Kim TK, Jang HJ, Haider MA, Khan L, Guindi M, Sherman M. Optimization of imaging

- diagnosis of 1-2 cm hepatocellular carcinoma: an analysis of diagnostic performance and resource utilization. *J Hepatol* 2011; **54**: 723-728 [PMID: 21156219 DOI: 10.1016/j.jhep.2010.07.025]
- 15 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J, EASL Panel of Experts on HCC. 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 [PMID: 11592607 DOI: 10.1016/s0168-8278(01)00130-1]
- 16 **Omata M**, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK, Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017; **11**: 317-370 [PMID: 28620797 DOI: 10.1007/s12072-017-9799-9]
- 17 **Rimola J**, Forner A, Tremosini S, Reig M, Vilana R, Bianchi L, Rodríguez-Lope C, Solé M, Ayuso C, Bruix J. Non-invasive diagnosis of hepatocellular carcinoma \leq 2 cm in cirrhosis. Diagnostic accuracy assessing fat, capsule and signal intensity at dynamic MRI. *J Hepatol* 2012; **56**: 1317-1323 [PMID: 22314420 DOI: 10.1016/j.jhep.2012.01.004]
- 18 **Piana G**, Trinquart L, Meskine N, Barrau V, Beers BV, Vilgrain V. New MR imaging criteria with a diffusion-weighted sequence for the diagnosis of hepatocellular carcinoma in chronic liver diseases. *J Hepatol* 2011; **55**: 126-132 [PMID: 21145857 DOI: 10.1016/j.jhep.2010.10.023]
- 19 **Iavarone M**, Sangiovanni A, Forzenigo LV, Massironi S, Fraquelli M, Aghemo A, Ronchi G, Biondetti P, Roncalli M, Colombo M. Diagnosis of hepatocellular carcinoma in cirrhosis by dynamic contrast imaging: the importance of tumor cell differentiation. *Hepatology* 2010; **52**: 1723-1730 [PMID: 20842697 DOI: 10.1002/hep.23903]
- 20 **Schütte K**, Schulz C, Poranzke J, Antweiler K, Bornschein J, Bretschneider T, Arend J, Ricke J, Malfertheiner P. Characterization and prognosis of patients with hepatocellular carcinoma (HCC) in the non-cirrhotic liver. *BMC Gastroenterol* 2014; **14**: 117 [PMID: 24990270 DOI: 10.1186/1471-230X-14-117]
- 21 **Wong GL**, Chan HL, Chan HY, Tse PC, Tse YK, Mak CW, Lee SK, Ip ZM, Lam AT, Iu HW, Leung JM, Wong VW. Accuracy of risk scores for patients with chronic hepatitis B receiving entecavir treatment. *Gastroenterology* 2013; **144**: 933-944 [PMID: 23415803 DOI: 10.1053/j.gastro.2013.02.002]
- 22 **Takano S**, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology* 1995; **21**: 650-655 [PMID: 7875662 DOI: 10.1002/hep.1840210308]
- 23 **Tsukuma H**, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797-1801 [PMID: 7684822 DOI: 10.1056/NEJM199306243282501]
- 24 **Caturelli E**, Solmi L, Anti M, Fusilli S, Roselli P, Andriulli A, Fornari F, Del Vecchio Blanco C, de Sio I. Ultrasound guided fine needle biopsy of early hepatocellular carcinoma complicating liver cirrhosis: a multicentre study. *Gut* 2004; **53**: 1356-1362 [PMID: 15306600 DOI: 10.1136/gut.2003.032359]
- 25 **International Consensus Group for Hepatocellular Neoplasia**. The pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009; **49**: 658-664 [PMID: 19177576 DOI: 10.1002/hep.22709]
- 26 **Di Tommaso L**, Franchi G, Park YN, Fiamengo B, Destro A, Morengi E, Montorsi M, Torzilli G, Tommasini M, Terracciano L, Tornillo L, Vecchione R, Roncalli M. Diagnostic value of HSP70, glypican 3, and glutamine synthetase in hepatocellular nodules in cirrhosis. *Hepatology* 2007; **45**: 725-734 [PMID: 17326147 DOI: 10.1002/hep.21531]
- 27 **Di Tommaso L**, Destro A, Seok JY, Balladore E, Terracciano L, Sangiovanni A, Iavarone M, Colombo M, Jang JJ, Yu E, Jin SY, Morengi E, Park YN, Roncalli M. The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. *J Hepatol* 2009; **50**: 746-754 [PMID: 19231003 DOI: 10.1016/j.jhep.2008.11.014]
- 28 **Rebouissou S**, Franconi A, Calderaro J, Letouzé E, Imbeaud S, Pilati C, Nault JC, Couchy G, Laurent A, Balabaud C, Bioulac-Sage P, Zucman-Rossi J. Genotype-phenotype correlation of CTNNB1 mutations reveals different β -catenin activity associated with liver tumor progression. *Hepatology* 2016; **64**: 2047-2061 [PMID: 27177928 DOI: 10.1002/hep.28638]
- 29 **Seimiya M**, Tomonaga T, Matsushita K, Sunaga M, Oh-Ishi M, Kodera Y, Maeda T, Takano S, Togawa A, Yoshitomi H, Otsuka M, Yamamoto M, Nakano M, Miyazaki M, Nomura F. Identification of novel immunohistochemical tumor markers for primary hepatocellular carcinoma; clathrin heavy chain and formiminotransferase cyclodeaminase. *Hepatology* 2008; **48**: 519-530 [PMID: 18571811 DOI: 10.1002/hep.22364]
- 30 **Cai MY**, Tong ZT, Zheng F, Liao YJ, Wang Y, Rao HL, Chen YC, Wu QL, Liu YH, Guan XY, Lin MC, Zeng YX, Kung HF, Xie D. EZH2 protein: a promising immunomarker for the detection of hepatocellular carcinomas in liver needle biopsies. *Gut* 2011; **60**: 967-976 [PMID: 21330577 DOI: 10.1136/gut.2010.231993]
- 31 **Fukayama M**, Paradis V, Park Y, Schirmacher P. Tumours of the liver and intrahepatic bile ducts. WHO Classification of Tumours of the Digestive System Fourth Edition, 2019, in press
- 32 **Calderaro J**, Couchy G, Imbeaud S, Amadeo G, Letouzé E, Blanc JF, Laurent C, Hajji Y, Azoulay D, Bioulac-Sage P, Nault JC, Zucman-Rossi J. Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. *J Hepatol* 2017; **67**: 727-738 [PMID: 28532995 DOI: 10.1016/j.jhep.2017.05.014]
- 33 **Ziol M**, Poté N, Amadeo G, Laurent A, Nault JC, Oberti F, Costentin C, Michalak S, Bouattour M, Franco C, Pageaux GP, Ramos J, Decaens T, Luciani A, Guiu B, Vilgrain V, Aubé C, Derman J, Charpy C, Zucman-Rossi J, Barget N, Seror O, Ganne-Carrié N, Paradis V, Calderaro J. Macrotrabecular-massive hepatocellular carcinoma: A distinctive histological subtype with clinical relevance. *Hepatology* 2018; **68**: 103-112 [PMID: 29281854 DOI: 10.1002/hep.29762]
- 34 **Kurebayashi Y**, Ojima H, Tsujikawa H, Kubota N, Maehara J, Abe Y, Kitago M, Shinoda M, Kitagawa Y, Sakamoto M. Landscape of immune microenvironment in hepatocellular carcinoma and its additional impact on histological and molecular classification. *Hepatology* 2018; **68**: 1025-1041 [PMID: 29603348 DOI: 10.1002/hep.29904]
- 35 **Colecchia A**, Scafoli E, Montrone L, Vestito A, Di Biase AR, Pieri M, D'Errico-Grigioni A, Bacchi-Reggiani ML, Ravaioli M, Grazi GL, Festi D. Pre-operative liver biopsy in cirrhotic patients with early hepatocellular carcinoma represents a safe and accurate diagnostic tool for tumour grading assessment. *J Hepatol* 2011; **54**: 300-305 [PMID: 21056498 DOI: 10.1016/j.jhep.2010.06.037]

- 36 **Pawlik TM**, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy: implications for transplant eligibility. *Ann Surg* 2007; **245**: 435-442 [PMID: 17435551 DOI: 10.1097/01.sla.0000250420.73854.ad]
- 37 **Han DH**, Choi GH, Kim KS, Choi JS, Park YN, Kim SU, Park JY, Ahn SH, Han KH. Prognostic significance of the worst grade in hepatocellular carcinoma with heterogeneous histologic grades of differentiation. *J Gastroenterol Hepatol* 2013; **28**: 1384-1390 [PMID: 23517197 DOI: 10.1111/jgh.12200]
- 38 **Martins-Filho SN**, Paiva C, Azevedo RS, Alves VAF. Histological Grading of Hepatocellular Carcinoma- A Systematic Review of Literature. *Front Med (Lausanne)* 2017; **4**: 193 [PMID: 29209611 DOI: 10.3389/fmed.2017.00193]
- 39 **Lauwers GY**, Terris B, Balis UJ, Batts KP, Regimbeau JM, Chang Y, Graeme-Cook F, Yamabe H, Ikai I, Cleary KR, Fujita S, Flejou JF, Zukerberg LR, Nagorney DM, Belghiti J, Yamaoka Y, Vauthey JN; International Cooperative Study Group on Hepatocellular Carcinoma. Prognostic histologic indicators of curatively resected hepatocellular carcinomas: a multi-institutional analysis of 425 patients with definition of a histologic prognostic index. *Am J Surg Pathol* 2002; **26**: 25-34 [PMID: 11756766 DOI: 10.1097/00000478-200201000-00003]
- 40 **Qin LX**, Tang ZY. The prognostic significance of clinical and pathological features in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 193-199 [PMID: 11925590 DOI: 10.3748/wjg.v8.i2.193]
- 41 **Rodríguez-Perálvarez M**, Luong TV, Andreana L, Meyer T, Dhillon AP, Burroughs AK. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. *Ann Surg Oncol* 2013; **20**: 325-339 [PMID: 23149850 DOI: 10.1245/s10434-012-2513-1]
- 42 **Pawlik TM**, Delman KA, Vauthey JN, Nagorney DM, Ng IO, Ikai I, Yamaoka Y, Belghiti J, Lauwers GY, Poon RT, Abdalla EK. Tumor size predicts vascular invasion and histologic grade: Implications for selection of surgical treatment for hepatocellular carcinoma. *Liver Transpl* 2005; **11**: 1086-1092 [PMID: 16123959 DOI: 10.1002/lt.20472]
- 43 **Poté N**, Cauchy F, Albuquerque M, Voitot H, Belghiti J, Castera L, Puy H, Bedossa P, Paradis V. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. *J Hepatol* 2015; **62**: 848-854 [PMID: 25450201 DOI: 10.1016/j.jhep.2014.11.005]
- 44 **Poté N**, Cauchy F, Albuquerque M, Cros J, Soubrane O, Bedossa P, Paradis V. Contribution of virtual biopsy to the screening of microvascular invasion in hepatocellular carcinoma: A pilot study. *Liver Int* 2018; **38**: 687-694 [PMID: 28872754 DOI: 10.1111/liv.13585]
- 45 **Fang JH**, Zhou HC, Zhang C, Shang LR, Zhang L, Xu J, Zheng L, Yuan Y, Guo RP, Jia WH, Yun JP, Chen MS, Zhang Y, Zhuang SM. A novel vascular pattern promotes metastasis of hepatocellular carcinoma in an epithelial-mesenchymal transition-independent manner. *Hepatology* 2015; **62**: 452-465 [PMID: 25711742 DOI: 10.1002/hep.27760]
- 46 **Renne SL**, Woo HY, Allegra S, Rudini N, Yano H, Donadon M, Viganò L, Akiba J, Lee HS, Rhee H, Park YN, Roncalli M, Di Tommaso L. Vessels Encapsulating Tumor Clusters (VETC) Is a Powerful Predictor of Aggressive Hepatocellular Carcinoma. *Hepatology* 2019 [PMID: 31206715 DOI: 10.1002/hep.30814]
- 47 **Fang JH**, Xu L, Shang LR, Pan CZ, Ding J, Tang YQ, Liu H, Liu CX, Zheng JL, Zhang YJ, Zhou ZG, Xu J, Zheng L, Chen MS, Zhuang SM. Vessels That Encapsulate Tumor Clusters (VETC) Pattern Is a Predictor of Sorafenib Benefit in Patients with Hepatocellular Carcinoma. *Hepatology* 2019; **70**: 824-839 [PMID: 30506570 DOI: 10.1002/hep.30366]
- 48 **Rimola J**, Forner A, Reig M, Vilana R, de Lope CR, Ayuso C, Bruix J. Cholangiocarcinoma in cirrhosis: absence of contrast washout in delayed phases by magnetic resonance imaging avoids misdiagnosis of hepatocellular carcinoma. *Hepatology* 2009; **50**: 791-798 [PMID: 19610049 DOI: 10.1002/hep.23071]
- 49 **Pinyol R**, Sia D, Llovet JM. Immune Exclusion-Wnt/CTNMB1 Class Predicts Resistance to Immunotherapies in HCC. *Clin Cancer Res* 2019; **25**: 2021-2023 [PMID: 30617138 DOI: 10.1158/1078-0432.CCR-18-3778]
- 50 **Spranger S**, Bao R, Gajewski TF. Melanoma-intrinsic β -catenin signalling prevents anti-tumour immunity. *Nature* 2015; **523**: 231-235 [PMID: 25970248 DOI: 10.1038/nature14404]
- 51 **Harding JJ**, Nandakumar S, Armenia J, Khalil DN, Albano M, Ly M, Shia J, Hechtman JF, Kundra R, El Dika I, Do RK, Sun Y, Kingham TP, D'Angelica MI, Berger MF, Hyman DM, Jarnagin W, Klimstra DS, Janjigian YY, Solit DB, Schultz N, Abou-Alfa GK. Prospective Genotyping of Hepatocellular Carcinoma: Clinical Implications of Next-Generation Sequencing for Matching Patients to Targeted and Immune Therapies. *Clin Cancer Res* 2019; **25**: 2116-2126 [PMID: 30373752 DOI: 10.1158/1078-0432.CCR-18-2293]
- 52 **Kim H**, Choi GH, Na DC, Ahn EY, Kim GI, Lee JE, Cho JY, Yoo JE, Choi JS, Park YN. Human hepatocellular carcinomas with "Stemness"-related marker expression: keratin 19 expression and a poor prognosis. *Hepatology* 2011; **54**: 1707-1717 [PMID: 22045674 DOI: 10.1002/hep.24559]
- 53 **Lagana SM**, Salomao M, Remotti HE, Knisely AS, Moreira RK. Bile salt export pump: a sensitive and specific immunohistochemical marker of hepatocellular carcinoma. *Histopathology* 2015; **66**: 598-602 [PMID: 25378077 DOI: 10.1111/his.12601]
- 54 **Rockey DC**, Caldwell SH, Goodman ZD, Nelson RC, Smith AD; American Association for the Study of Liver Diseases. Liver biopsy. *Hepatology* 2009; **49**: 1017-1044 [PMID: 19243014 DOI: 10.1002/hep.22742]
- 55 **Cholongitas E**, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, Dhillon AP, Burroughs AK. A systematic review of the quality of liver biopsy specimens. *Am J Clin Pathol* 2006; **125**: 710-721 [PMID: 16707372 DOI: 10.1309/W3XC-NT4H-KFBN-2G0B]
- 56 **Eisenberg E**, Konopnicki M, Veitsman E, Kramskay R, Gaitini D, Baruch Y. Prevalence and characteristics of pain induced by percutaneous liver biopsy. *Anesth Analg* 2003; **96**: 1392-1396, table of contents [PMID: 12707140 DOI: 10.1213/01.ANE.0000060453.74744.17]
- 57 **Gilmore IT**, Burroughs A, Murray-Lyon IM, Williams R, Jenkins D, Hopkins A. Indications, methods, and outcomes of percutaneous liver biopsy in England and Wales: an audit by the British Society of Gastroenterology and the Royal College of Physicians of London. *Gut* 1995; **36**: 437-441 [PMID: 7698705 DOI: 10.1136/gut.36.3.437]
- 58 **Reichert CM**, Weisenthal LM, Klein HG. Delayed hemorrhage after percutaneous liver biopsy. *J Clin Gastroenterol* 1983; **5**: 263-266 [PMID: 6863883 DOI: 10.1097/00004836-198306000-00014]
- 59 **Janes CH**, Lindor KD. Outcome of patients hospitalized for complications after outpatient liver biopsy. *Ann Intern Med* 1993; **118**: 96-98 [PMID: 8416324 DOI: 10.7326/0003-4819-118-2-199301150-00003]
- 60 **Piccinino F**, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. *J Hepatol* 1986; **2**: 165-173 [PMID: 3958472 DOI: 10.1016/0168-8273(86)90003-9]

- 10.1016/s0168-8278(86)80075-7]
- 61 **Perrault J**, McGill DB, Ott BJ, Taylor WF. Liver biopsy: complications in 1000 inpatients and outpatients. *Gastroenterology* 1978; **74**: 103-106 [PMID: 618417 DOI: 10.1016/0016-5085(78)90364-5]
- 62 **Firpi RJ**, Soldevila-Pico C, Abdelmalek MF, Morelli G, Judah J, Nelson DR. Short recovery time after percutaneous liver biopsy: should we change our current practices? *Clin Gastroenterol Hepatol* 2005; **3**: 926-929 [PMID: 16234032 DOI: 10.1016/S1542-3565(05)00294-6]
- 63 **Lindor KD**, Bru C, Jorgensen RA, Rakela J, Bordas JM, Gross JB, Rodes J, McGill DB, Reading CC, James EM, Charboneau JW, Ludwig J, Batts KP, Zinsmeister AR. The role of ultrasonography and automatic-needle biopsy in outpatient percutaneous liver biopsy. *Hepatology* 1996; **23**: 1079-1083 [PMID: 8621137 DOI: 10.1002/hep.510230522]
- 64 **McGill DB**, Rakela J, Zinsmeister AR, Ott BJ. A 21-year experience with major hemorrhage after percutaneous liver biopsy. *Gastroenterology* 1990; **99**: 1396-1400 [PMID: 2101588 DOI: 10.1016/0016-5085(90)91167-5]
- 65 **Myers RP**, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver Int* 2008; **28**: 705-712 [PMID: 18433397 DOI: 10.1111/j.1478-3231.2008.01691.x]
- 66 **Stone MA**, Mayberry JF. An audit of ultrasound guided liver biopsies: a need for evidence-based practice. *Hepatogastroenterology* 1996; **43**: 432-434 [PMID: 8714240]
- 67 **Mulier S**, Mulier P, Ni Y, Miao Y, Dupas B, Marchal G, De Wever I, Michel L. Complications of radiofrequency coagulation of liver tumours. *Br J Surg* 2002; **89**: 1206-1222 [PMID: 12296886 DOI: 10.1046/j.1365-2168.2002.02168.x]
- 68 **Yu J**, Liang P, Yu XL, Cheng ZG, Han ZY, Dong BW. Needle track seeding after percutaneous microwave ablation of malignant liver tumors under ultrasound guidance: analysis of 14-year experience with 1462 patients at a single center. *Eur J Radiol* 2012; **81**: 2495-2499 [PMID: 22137097 DOI: 10.1016/j.ejrad.2011.10.019]
- 69 **Silva MA**, Hegab B, Hyde C, Guo B, Buckels JA, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut* 2008; **57**: 1592-1596 [PMID: 18669577 DOI: 10.1136/gut.2008.149062]
- 70 **Ahn DW**, Shim JH, Yoon JH, Kim CY, Lee HS, Kim YT, Kim YJ. Treatment and clinical outcome of needle-track seeding from hepatocellular carcinoma. *Korean J Hepatol* 2011; **17**: 106-112 [PMID: 21757981 DOI: 10.3350/kjhep.2011.17.2.106]
- 71 **Chang S**, Kim SH, Lim HK, Lee WJ, Choi D, Lim JH. Needle tract implantation after sonographically guided percutaneous biopsy of hepatocellular carcinoma: evaluation of doubling time, frequency, and features on CT. *AJR Am J Roentgenol* 2005; **185**: 400-405 [PMID: 16037512 DOI: 10.2214/ajr.185.2.01850400]
- 72 **Szpakowski JL**, Drasin TE, Lyon LL. Rate of seeding with biopsies and ablations of hepatocellular carcinoma: A retrospective cohort study. *Hepatol Commun* 2017; **1**: 841-851 [PMID: 29404497 DOI: 10.1002/hep4.1089]
- 73 **Kosugi C**, Furuse J, Ishii H, Maru Y, Yoshino M, Kinoshita T, Konishi M, Nakagohri T, Inoue K, Oda T. Needle tract implantation of hepatocellular carcinoma and pancreatic carcinoma after ultrasound-guided percutaneous puncture: clinical and pathologic characteristics and the treatment of needle tract implantation. *World J Surg* 2004; **28**: 29-32 [PMID: 14648043 DOI: 10.1007/s00268-003-7003-y]
- 74 **Lim JH**, Cho JM, Kim EY, Park CK. Dysplastic nodules in liver cirrhosis: evaluation of hemodynamics with CT during arterial portography and CT hepatic arteriography. *Radiology* 2000; **214**: 869-874 [PMID: 10715060 DOI: 10.1148/radiology.214.3.r00mr12869]
- 75 **Rode A**, Bancel B, Douek P, Chevallier M, Vilgrain V, Picaud G, Henry L, Berger F, Bizollon T, Gaudin JL, Ducerf C. Small nodule detection in cirrhotic livers: evaluation with US, spiral CT, and MRI and correlation with pathologic examination of explanted liver. *J Comput Assist Tomogr* 2001; **25**: 327-336 [PMID: 11351179 DOI: 10.1097/00004728-200105000-00001]
- 76 **Freeman RB**, Mithoefer A, Ruthazer R, Nguyen K, Schore A, Harper A, Edwards E. Optimizing staging for hepatocellular carcinoma before liver transplantation: A retrospective analysis of the UNOS/OPTN database. *Liver Transpl* 2006; **12**: 1504-1511 [PMID: 16952174 DOI: 10.1002/lt.20847]
- 77 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
- 78 **Patel T**. Cholangiocarcinoma—controversies and challenges. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 189-200 [PMID: 21460876 DOI: 10.1038/nrgastro.2011.20]
- 79 **Khan SA**, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. *Lancet* 2005; **366**: 1303-1314 [PMID: 16214602 DOI: 10.1016/S0140-6736(05)67530-7]
- 80 **Peng NF**, Li LQ, Qin X, Guo Y, Peng T, Xiao KY, Chen XG, Yang YF, Su ZX, Chen B, Su M, Qi LN. Evaluation of risk factors and clinicopathologic features for intrahepatic cholangiocarcinoma in Southern China: a possible role of hepatitis B virus. *Ann Surg Oncol* 2011; **18**: 1258-1266 [PMID: 21207172 DOI: 10.1245/s10434-010-1458-5]
- 81 **Zhou H**, Wang H, Zhou D, Wang H, Wang Q, Zou S, Tu Q, Wu M, Hu H. Hepatitis B virus-associated intrahepatic cholangiocarcinoma and hepatocellular carcinoma may hold common disease process for carcinogenesis. *Eur J Cancer* 2010; **46**: 1056-1061 [PMID: 20202823 DOI: 10.1016/j.ejca.2010.02.005]
- 82 **Soyer P**, Bluemke DA, Reichle R, Calhoun PS, Bliss DF, Scherrer A, Fishman EK. Imaging of intrahepatic cholangiocarcinoma: 1. Peripheral cholangiocarcinoma. *AJR Am J Roentgenol* 1995; **165**: 1427-1431 [PMID: 7484579 DOI: 10.2214/ajr.165.6.7484579]
- 83 **Lacomis JM**, Baron RL, Oliver JH, Nalesnik MA, Federle MP. Cholangiocarcinoma: delayed CT contrast enhancement patterns. *Radiology* 1997; **203**: 98-104 [PMID: 9122423 DOI: 10.1148/radiology.203.1.9122423]
- 84 **Kim TK**, Choi BI, Han JK, Jang HJ, Cho SG, Han MC. Peripheral cholangiocarcinoma of the liver: two-phase spiral CT findings. *Radiology* 1997; **204**: 539-543 [PMID: 9240550 DOI: 10.1148/radiology.204.2.9240550]
- 85 **Loyer EM**, Chin H, DuBrow RA, David CL, Eftekhari F, Charnsangavej C. Hepatocellular carcinoma and intrahepatic peripheral cholangiocarcinoma: enhancement patterns with quadruple phase helical CT—a comparative study. *Radiology* 1999; **212**: 866-875 [PMID: 10478259 DOI: 10.1148/radiology.212.3.r99se32866]
- 86 **Maetani Y**, Itoh K, Watanabe C, Shibata T, Ametani F, Yamabe H, Konishi J. MR imaging of intrahepatic cholangiocarcinoma with pathologic correlation. *AJR Am J Roentgenol* 2001; **176**: 1499-1507 [PMID:

- 11373220 DOI: [10.2214/ajr.176.6.1761499](https://doi.org/10.2214/ajr.176.6.1761499)]
- 87 **Kim SA**, Lee JM, Lee KB, Kim SH, Yoon SH, Han JK, Choi BI. Intrahepatic mass-forming cholangiocarcinomas: enhancement patterns at multiphasic CT, with special emphasis on arterial enhancement pattern--correlation with clinicopathologic findings. *Radiology* 2011; **260**: 148-157 [PMID: [21474703](https://pubmed.ncbi.nlm.nih.gov/21474703/)] DOI: [10.1148/radiol.11101777](https://doi.org/10.1148/radiol.11101777)]
- 88 **Kang Y**, Lee JM, Kim SH, Han JK, Choi BI. Intrahepatic mass-forming cholangiocarcinoma: enhancement patterns on gadoteric acid-enhanced MR images. *Radiology* 2012; **264**: 751-760 [PMID: [22798225](https://pubmed.ncbi.nlm.nih.gov/22798225/)] DOI: [10.1148/radiol.12112308](https://doi.org/10.1148/radiol.12112308)]
- 89 **Iavarone M**, Piscaglia F, Vavassori S, Galassi M, Sangiovanni A, Venerandi L, Forzenigo LV, Golfieri R, Bolondi L, Colombo M. Contrast enhanced CT-scan to diagnose intrahepatic cholangiocarcinoma in patients with cirrhosis. *J Hepatol* 2013; **58**: 1188-1193 [PMID: [23485522](https://pubmed.ncbi.nlm.nih.gov/23485522/)] DOI: [10.1016/j.jhep.2013.02.013](https://doi.org/10.1016/j.jhep.2013.02.013)]
- 90 **Xu J**, Igarashi S, Sasaki M, Matsubara T, Yoneda N, Kozaka K, Ikeda H, Kim J, Yu E, Matsui O, Nakanuma Y. Intrahepatic cholangiocarcinomas in cirrhosis are hypervascular in comparison with those in normal livers. *Liver Int* 2012; **32**: 1156-1164 [PMID: [22417172](https://pubmed.ncbi.nlm.nih.gov/22417172/)] DOI: [10.1111/j.1478-3231.2012.02783.x](https://doi.org/10.1111/j.1478-3231.2012.02783.x)]
- 91 **Kim SJ**, Lee JM, Han JK, Kim KH, Lee JY, Choi BI. Peripheral mass-forming cholangiocarcinoma in cirrhotic liver. *AJR Am J Roentgenol* 2007; **189**: 1428-1434 [PMID: [18029881](https://pubmed.ncbi.nlm.nih.gov/18029881/)] DOI: [10.2214/AJR.07.2484](https://doi.org/10.2214/AJR.07.2484)]
- 92 **Ciresa M**, De Gaetano AM, Pompili M, Saviano A, Infante A, Montagna M, Guerra A, Giuga M, Vellone M, Ardito F, De Rose A, Giulianti F, Vecchio FM, Gasbarrini A, Bonomo L. Enhancement patterns of intrahepatic mass-forming cholangiocarcinoma at multiphasic computed tomography and magnetic resonance imaging and correlation with clinicopathologic features. *Eur Rev Med Pharmacol Sci* 2015; **19**: 2786-2797 [PMID: [26241531](https://pubmed.ncbi.nlm.nih.gov/26241531/)]
- 93 **Huang B**, Wu L, Lu XY, Xu F, Liu CF, Shen WF, Jia NY, Cheng HY, Yang YF, Shen F. Small Intrahepatic Cholangiocarcinoma and Hepatocellular Carcinoma in Cirrhotic Livers May Share Similar Enhancement Patterns at Multiphase Dynamic MR Imaging. *Radiology* 2016; **281**: 150-157 [PMID: [27077381](https://pubmed.ncbi.nlm.nih.gov/27077381/)] DOI: [10.1148/radiol.2016151205](https://doi.org/10.1148/radiol.2016151205)]
- 94 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207 [PMID: [12547409](https://pubmed.ncbi.nlm.nih.gov/12547409/)] DOI: [10.1016/S0168-8278\(02\)00360-4](https://doi.org/10.1016/S0168-8278(02)00360-4)]
- 95 **Hoshida Y**, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Wrobel MJ, Lerner J, Reich M, Chan JA, Glickman JN, Ikeda K, Hashimoto M, Watanabe G, Daidone MG, Roayaie S, Schwartz M, Thung S, Salvesen HB, Gabriel S, Mazzaferro V, Bruix J, Friedman SL, Kumada H, Llovet JM, Golub TR. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 1995-2004 [PMID: [18923165](https://pubmed.ncbi.nlm.nih.gov/18923165/)] DOI: [10.1056/NEJMoa0804525](https://doi.org/10.1056/NEJMoa0804525)]
- 96 **von Felden J**, Heim D, Schulze K, Krech T, Ewald F, Nashan B, Lohse AW, Wege H. High expression of micro RNA-135A in hepatocellular carcinoma is associated with recurrence within 12 months after resection. *BMC Cancer* 2017; **17**: 60 [PMID: [28100188](https://pubmed.ncbi.nlm.nih.gov/28100188/)] DOI: [10.1186/s12885-017-3053-7](https://doi.org/10.1186/s12885-017-3053-7)]
- 97 **Hoshida Y**, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Wrobel MJ, Lerner J, Reich M, Chan JA, Glickman JN, Ikeda K, Hashimoto M, Watanabe G, Daidone MG, Roayaie S, Schwartz M, Thung S, Salvesen HB, Gabriel S, Mazzaferro V, Bruix J, Friedman SL, Kumada H, Llovet JM, Golub TR. Gene Expression in Fixed Tissues and Outcome in Hepatocellular Carcinoma. *New Engl J Med* 2008; **359**: 1995-2004 [PMID: [18923165](https://pubmed.ncbi.nlm.nih.gov/18923165/)] DOI: [10.1056/Nejm0804525](https://doi.org/10.1056/Nejm0804525)]
- 98 **Sia D**, Jiao Y, Martinez-Quetglas I, Kuchuk O, Villacorta-Martin C, Castro de Moura M, Putra J, Camprecios G, Bassaganyas L, Akers N, Losic B, Waxman S, Thung SN, Mazzaferro V, Esteller M, Friedman SL, Schwartz M, Villanueva A, Llovet JM. Identification of an Immune-specific Class of Hepatocellular Carcinoma, Based on Molecular Features. *Gastroenterology* 2017; **153**: 812-826 [PMID: [28624577](https://pubmed.ncbi.nlm.nih.gov/28624577/)] DOI: [10.1053/j.gastro.2017.06.007](https://doi.org/10.1053/j.gastro.2017.06.007)]
- 99 **Rimassa L**, Assenat E, Peck-Radosavljevic M, Pracht M, Zagonel V, Mathurin P, Rota Caremoli E, Porta C, Daniele B, Bolondi L, Mazzaferro V, Harris W, Damjanov N, Pastorelli D, Reig M, Knox J, Negri F, Trojan J, López López C, Personeni N, Decaens T, Dupuy M, Sieghart W, Abbadessa G, Schwartz B, Lamar M, Goldberg T, Shuster D, Santoro A, Bruix J. Tivantinib for second-line treatment of MET-high, advanced hepatocellular carcinoma (METIV-HCC): a final analysis of a phase 3, randomised, placebo-controlled study. *Lancet Oncol* 2018; **19**: 682-693 [PMID: [29625879](https://pubmed.ncbi.nlm.nih.gov/29625879/)] DOI: [10.1016/S1470-2045\(18\)30146-3](https://doi.org/10.1016/S1470-2045(18)30146-3)]
- 100 **Yamasaki M**, Yasuda T, Yano M, Hirao M, Kobayashi K, Fujitani K, Tamura S, Kimura Y, Miyata H, Motoori M, Shiraishi O, Makino T, Satoh T, Mori M, Doki Y. Multicenter randomized phase II study of cisplatin and fluorouracil plus docetaxel (DCF) compared with cisplatin and fluorouracil plus Adriamycin (ACF) as preoperative chemotherapy for resectable esophageal squamous cell carcinoma (OGSG1003). *Ann Oncol* 2017; **28**: 116-120 [PMID: [27687307](https://pubmed.ncbi.nlm.nih.gov/27687307/)] DOI: [10.1093/annonc/mdw439](https://doi.org/10.1093/annonc/mdw439)]
- 101 **Llovet JM**, Montal R, Sia D, Finn RS. Molecular therapies and precision medicine for hepatocellular carcinoma. *Nat Rev Clin Oncol* 2018; **15**: 599-616 [PMID: [30061739](https://pubmed.ncbi.nlm.nih.gov/30061739/)] DOI: [10.1038/s41571-018-0073-4](https://doi.org/10.1038/s41571-018-0073-4)]
- 102 **Bruix J**, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: [24531850](https://pubmed.ncbi.nlm.nih.gov/24531850/)] DOI: [10.1136/gutjnl-2013-306627](https://doi.org/10.1136/gutjnl-2013-306627)]
- 103 **Llovet JM**, Hernandez-Gea V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. *Clin Cancer Res* 2014; **20**: 2072-2079 [PMID: [24589894](https://pubmed.ncbi.nlm.nih.gov/24589894/)] DOI: [10.1158/1078-0432.CCR-13-0547](https://doi.org/10.1158/1078-0432.CCR-13-0547)]
- 104 **Schulze K**, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J, Soysouvanh F, Calatayud AL, Pinyol R, Pelletier L, Balabaud C, Laurent A, Blanc JF, Mazzaferro V, Calvo F, Villanueva A, Nault JC, Bioulac-Sage P, Stratton MR, Llovet JM, Zucman-Rossi J. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015; **47**: 505-511 [PMID: [25822088](https://pubmed.ncbi.nlm.nih.gov/25822088/)] DOI: [10.1038/ng.3252](https://doi.org/10.1038/ng.3252)]
- 105 **Zehir A**, Benayed R, Shah RH, Syed A, Middha S, Kim HR, Srinivasan P, Gao J, Chakravarty D, Devlin SM, Hellmann MD, Barron DA, Schram AM, Hameed M, Dogan S, Ross DS, Hechtman JF, DeLair DF, Yao J, Mandelker DL, Cheng DT, Chandramohan R, Mohanty AS, Ptashkin RN, Jayakumaran G, Prasad M, Syed MH, Rema AB, Liu ZY, Nafa K, Borsu L, Sadowska J, Casanova J, Bacares R, Kiecka IJ, Razumova A, Son JB, Stewart L, Baldi T, Mullaney KA, Al-Ahmadie H, Vakiani E, Abeshouse AA, Penson AV, Jonsson P, Camacho N, Chang MT, Won HH, Gross BE, Kundra R, Heins ZJ, Chen HW, Phillips S, Zhang H, Wang J, Ochoa A, Wills J, Eubank M, Thomas SB, Gardos SM, Reales DN, Galle J, Durany R, Cambria R, Abida W, Cercek A, Feldman DR, Gounder MM, Hakimi AA, Harding JJ, Iyer G,

- Janjigian YY, Jordan EJ, Kelly CM, Lowery MA, Morris LGT, Omuro AM, Raj N, Razavi P, Shoushtari AN, Shukla N, Soumerai TE, Varghese AM, Yaeger R, Coleman J, Bochner B, Riely GJ, Saltz LB, Scher HI, Sabbatini PJ, Robson ME, Klimstra DS, Taylor BS, Baselga J, Schultz N, Hyman DM, Arcila ME, Solit DB, Ladanyi M, Berger MF. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017; **23**: 703-713 [PMID: 28481359 DOI: 10.1038/nm.4333]
- 106 **Guichard C**, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, Clément B, Balabaud C, Chevet E, Laurent A, Couchy G, Letouzé E, Calvo F, Zucman-Rossi J. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet* 2012; **44**: 694-698 [PMID: 22561517 DOI: 10.1038/ng.2256]
- 107 **Fujimoto A**, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, Arai Y, Takahashi H, Shirakihara T, Nagasaki M, Shibuya T, Nakano K, Watanabe-Makino K, Tanaka H, Nakamura H, Kusuda J, Ojima H, Shimada K, Okusaka T, Ueno M, Shigekawa Y, Kawakami Y, Arihiro K, Ohdan H, Gotoh K, Ishikawa O, Ariizumi S, Yamamoto M, Yamada T, Chayama K, Kosuge T, Yamaue H, Kamatani N, Miyano S, Nakagama H, Nakamura Y, Tsunoda T, Shibata T, Nakagawa H. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat Genet* 2012; **44**: 760-764 [PMID: 22634756 DOI: 10.1038/ng.2291]
- 108 **Ferber MJ**, Montoya DP, Yu C, Aderca I, McGee A, Thorland EC, Nagorney DM, Gostout BS, Burgart LJ, Boix L, Bruix J, McMahon BJ, Cheung TH, Chung TK, Wong YF, Smith DI, Roberts LR. Integrations of the hepatitis B virus (HBV) and human papillomavirus (HPV) into the human telomerase reverse transcriptase (hTERT) gene in liver and cervical cancers. *Oncogene* 2003; **22**: 3813-3820 [PMID: 12802289 DOI: 10.1038/sj.onc.1206528]
- 109 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]
- 110 **Hoshida Y**, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. *Semin Liver Dis* 2010; **30**: 35-51 [PMID: 20175032 DOI: 10.1055/s-0030-1247131]
- 111 **Minguez B**, Hoshida Y, Villanueva A, Toffanin S, Cabellos L, Thung S, Mandeli J, Sia D, April C, Fan JB, Lachenmayer A, Savic R, Roayaie S, Mazzaferro V, Bruix J, Schwartz M, Friedman SL, Llovet JM. Gene-expression signature of vascular invasion in hepatocellular carcinoma. *J Hepatol* 2011; **55**: 1325-1331 [PMID: 21703203 DOI: 10.1016/j.jhep.2011.02.034]
- 112 **Crowley E**, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; **10**: 472-484 [PMID: 23836314 DOI: 10.1038/nrclinonc.2013.110]
- 113 **Labgaa I**, Villanueva A. Liquid biopsy in liver cancer. *Discov Med* 2015; **19**: 263-273 [PMID: 25977189]
- 114 **D'Avola D**, Villacorta-Martin C, Martins-Filho SN, Craig A, Labgaa I, von Felden J, Kimaada A, Bonaccorso A, Tabrizian P, Hartmann BM, Sebra R, Schwartz M, Villanueva A. High-density single cell mRNA sequencing to characterize circulating tumor cells in hepatocellular carcinoma. *Sci Rep* 2018; **8**: 11570 [PMID: 30068984 DOI: 10.1038/s41598-018-30047-y]
- 115 **von Felden J**, Schulze K, Krech T, Ewald F, Nashan B, Pantel K, Lohse AW, Riethdorf S, Wege H. Circulating tumor cells as liquid biomarker for high HCC recurrence risk after curative liver resection. *Oncotarget* 2017; **8**: 89978-89987 [PMID: 29163804 DOI: 10.18632/oncotarget.21208]
- 116 **Xu LX**, He MH, Dai ZH, Yu J, Wang JG, Li XC, Jiang BB, Ke ZF, Su TH, Peng ZW, Guo Y, Chen ZB, Chen SL, Peng S, Kuang M. Genomic and transcriptional heterogeneity of multifocal hepatocellular carcinoma. *Ann Oncol* 2019 [PMID: 30916311 DOI: 10.1093/annonc/mdz103]



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