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# contemporary role of liver biopsy in hepatocellular carcinoma

Sparchez Z *et al*. Liver biopsy in HCC

# Zeno Sparchez, Tudor Mocan

**Zeno Sparchez, Tudor Mocan,** 3rd Medical Department, Institute for Gastroenterology and Hepatology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca 400162, Romania

**ORCID number:** Zeno Sparchez (0000-0002-3813-1677); Tudor Mocan (0000-0001-7785-6403)

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**Correspondence to: Tudor Mocan, MD, Associate Specialist, Doctor,** 3rd Medical Department, Institute for Gastroenterology and Hepatology, Iuliu Hatieganu University of Medicine and Pharmacy, Croitorilor st. 19-21, Cluj-Napoca 400162, Romania. mocan\_tudor@yahoo.com

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**Abstract**

A correct diagnosis of hepatocellular carcinoma (HCC) in cirrhotic patients with focal liver lesions is one of the most important issues nowadays. Probably one of the oldest debates in the hepatology community is whether to perform liver biopsy (LB) in all cirrhotic patients with focal liver lesions. We now face a time when oncology is moving towards personalized medicine. According to the current European Association for the study of Liver diseases HCC guidelines, LB has only a minor role in the management of HCC. However, the current recommendations were made more than 5 years ago. The time has passed, and along with it the development of high-throughput molecular technologies has allowed to define the main molecular mechanism involved in HCC development and progression. Several subtypes of HCC with both molecular and histological characterization have been described. Importantly, some of these sub-types, with prognostic impact. In the context of personalized treatment, the role of LB will be definitely reconsidered. Until then, it is mandatory to know the various techniques of LB, their performances, complications and limits. The balance of risk and benefit defines many of the decisions that we make as providers of medical care. In this review, we discuss not only the risks associated with LB but also the benefits of biopsy in various clinical scenarios. Not far from now, the role of LB will be reconsidered. Possibly we will go back in time and use once again biopsy in HCC diagnosis, and then again, back to the future and try to improve the use of liquid biopsy in the follow-up of HCC patients after various treatment modalities.

**Key words:** Liver biopsy; Hepatocellular carcinoma; Molecular classification; Bleeding; Seeding

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**Core tip:** We now face a time when oncology is moving towards personalized medicine. The development of high-throughput molecular technologies has allowed us to define the main molecular mechanism involved in hepatocellular carcinoma (HCC) development and progression. Several subtypes of HCC with both molecular and histological characterization have been described. In the context of histological sub-classes of HCC each with a distinct molecular pattern and some of them with prognostic impact the need for liver biopsy in HCC management becomes a necessity. Knowing the strengths of each sampling techniques in the era of personalized medicine is of outmost importance.

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# Introduction

The correct identification, either malignant or benign, of the nature of focal liver lesions is one of the most important issues in cirrhotic patients. Nodular lesions are frequently discovered during an ultrasound screening program of these patients. Recent progress in ultrasound has led to an earlier discovery of these lesions. Moreover, the application of contrast agents has gained more and more attention. Compared to other imaging modalities, contrast enhanced ultrasound (CEUS) can be performed immediately after conventional US, being a simple, easy to perform and immediately available dynamic imaging tool[1]. The use of CEUS might therefore, shorten the diagnostic and therapeutic work-up of HCC patients. The large applicability of CEUS for the diagnosis of HCC in cirrhosis was questioned because of the risk of a false positive diagnosis in case of cholangiocarcinoma. This has determined the American College of Radiology to release a diagnostic scheme for the characterization of focal liver lesions in patients at risk for HCC named CEUS LI-RADS®[2]. In a multicenter Italian study, the use of CEUS LI-RADS in small HCC showed that the LR-5 category was 98.5 predictive of HCC with no risk for misdiagnosis for pure cholangiocarcinoma[3].

Despite all the latest improvements in liver imaging, the correct identification of these lesions is still challenging, especially when dealing with small focal lesions.

According to the AASLD and EASL guidelines, in certain situations the image may not be characteristic or the results of 2 imaging techniques may be conflicting: a liver biopsy (LB) is required in these cases[4]. In addition, the information offered by the tumoral tissue may provide prognostic data useful in the selection of therapy.

# Techniques, performance, complications

The invasive techniques used for the morphological diagnosis of hepatocellular carcinoma (HCC) are ultrasound-guided fine-needle aspiration (FNA) and needle-core biopsy. The performance of these techniques is somewhat similar in the morphological diagnosis of HCC. The sensitivity of cytology varies between 69%-95% in different studies, lower in well-differentiated HCC, while specificity varies between 70%-100%[5–11]. The diagnostic accuracy of the method is lower in lesions smaller than 3 cm (50%-83%) than in large ones (85%–95%)[7,12]. The smear cytology technique using Papanicolaou`s methodwill decrease the number of required passes and of inadequate fragments. Flow cytometry and the various immunohistochemical techniques are extremely helpful in the characterization of neoplastic cells.

The difficulty of a correct differential diagnosis between a regenerative nodule and a well-differentiated HCC can only be overcome by using a relatively large tissue sample, obtainable only by the use of thick needles.

Core biopsy performed with large needles (1.1-1.6 mm outer diameter) ensures the recovery of an adequate tissue fragment; it also allows for a better preservation of tissue architecture, providing more information on the tumoral tissue and facilitating certain special staining techniques. These advantages are however counter-balanced by the high risk of complications.

The rate of successful sampling using large needles is 85%-98.5%. It may be diminished by certain factors: small size of the target (small lesions are harder to approach, in a liver with significant fibrosis), location of the lesion in deeper segments (posterior and superior segments such as segments IV B, VII and VIII) and the presence of necrotic areas in the tumor[11]. Poorly visible or invisible lesions on conventional ultrasound are another cause of liver biopsy failure.

The sensitivity of core needle biopsy for HCC diagnosis is 86%-96%, higher in case of multiple passes[11,13–16]. The specificity ranges between 95%-100%, especially when sampling also from an extra-nodular area (non-neoplastic neighboring parenchyma)[9,12]. The accuracy of the method varies between 85% and 91%[11,13,14,16].

Micro-histology combines the safety profile of fine-needle aspiration with a higher quality of tissue samples (similar to that provided by core needle biopsy); it has a higher sensitivity and specificity than conventional cytology: 92.6% and 100% *vs* 81.3% and 97.6%, respectively[7,9,11,17]. In addition, micro-histology has a high accuracy (89.6%) in diagnosing nodules smaller than 2 cm, varying according to size: 88.6% for nodules ≤ 10 mm, 86.2% for nodules between 11–15 mm and 91.3% for nodules between 16–20 mm in diameter[17].

Some needles (Histocut) allow the recovery of tissue fragments for both cytology and micro-histology during the same pass. The cytology-micro-histology combination increases the sensitivity of HCC diagnosis: 89.8%-90% *vs* 80%-85.6% for cytology and 61%- 66.1% for micro-histology[7,9].

Using real time contrast-enhanced harmonic ultrasound (SonoVue) to guide the biopsy will increase its diagnostic sensitivity by targeting: (1) the enhanced, vascular areas of the tumor in the arterial phase, in case of large tumors which often display central necrosis[18]; (2) the poorly visible or invisible nodules on conventional ultrasound which become clearly visible after contrast injection in both arterial or late phase[18,19].

The negative predictive value of liver biopsy remains low, and malignancy cannot be excluded after one negative result alone. The management of these patients includes long-term imaging follow-up and re-biopsy. If a re-biopsy is taken into consideration, it is imperative to recall its low chance of success when performed immediately after the first biopsy, with only 35% increase in positive diagnosis[20]. If the first biopsy did not find a tumorthe chances of success are higher (50%) than in cases of a non-diagnostic result (necrosis) (25%)[20]. In these cases, especially in nodules <2 cm, imaging follow-up is recommended. Performing liver biopsy for the diagnosis of HCC is not without risks**.** Hemorrhage is more frequent when using thick needles (1.1% *vs* 0.5% for fine needle biopsy) and when sampling an HCC (2.5%)[11,21]. Risk factors for bleeding are hemostatic abnormalities, the degree of liver failure, age, the presence of ascites or the technique used. It is generally considered that the risk is higher with each additional pass, with a larger needle diameter and with a smaller area of interposed parenchyma[22]. The actual recommendations are to use a needle smaller than 1.2 mm in diameter, for a maximum of 2 passes and an oblique approach, which would allow at least 1 cm between the lesion and the liver capsule[15,23].

The incidence of needle-tract seedingvaries in literature between 0% and 7.69%, with a mean of 3.16% and a median of 2.66% (Table 1);this value is lower, (1.43%), when considering the global incidence. A meta-analysis published in 2007 has established the median incidence of tumor cell seeding to be 2.7%[24]. Apparently, the larger the needle diameter and the number of passes or the lower the degree of tumordifferentiation, the higher the risk of seeding; there are no studies, however, to confirm this supposition. Seeding can occur in the thoraco-abdominal wall or intraperitoneally, sometimes several years after the biopsy and even after performing liver transplantation. The risk of seeding may be reduced to 0 by using the coaxial technique[25]. The treatment of needle-tract seeding, especially if parietal, is surgical; after surgery, most patients experience no recurrences. The occurrence of seeding does not alter global survival rates, which only depend on the progression of the primary tumor or of cirrhosis[26].

Liver cells are generally found in the blood after both liver biopsy and liver resection, as attested by the presence of mRNA AFP in the serum. It is not exactly known whether these cells are normal or tumor cells. No association between this phenomenon and tumor cell seeding has been demonstrated to date.

Mortality after biopsy is higher when using thick (0.15%-0.19%) *vs* fine needles (0.008%)[24,25, 27–37].

# Current indications of LB in the diagnosis of HCC

The indications of performing LB in patients with liver cirrhosis and HCC are highly regulated at present. The 2 extreme perspectives recommending either biopsy in all cases (as was the norm before the introduction of non-invasive criteria) or the avoidance of biopsy at all costs when having good diagnostic imaging studies have both been abandoned. The main factors indicating, adjusting or limiting the use of biopsy in HCC are presented in Table 2.

In the following paragraphs, we will make a critical appraisal of the indications of LB in the diagnosis of HCC in each of the BCLC stages.

# *BCLC stages 0 and A (very early and early HCC)*

# Correlation with imaging techniques. Nodules measuring between 1 and 2 cm are difficult to characterize using non-invasive methods[38,39], since up to 33% are benign, while HCC nodules frequently have no distinctive pattern of behavior. Only 33% of HCC nodules meet the precise diagnostic criteria recommended by the AASLD (hypervascularization in the arterial phase and wash out in the portal/parenchymal phase in 2 imaging techniques)[38]. It follows that 50%-70% of patients will require a biopsy in order to receive an exact diagnosis[38,39]. US guided LB may not be justified in patients with decompensated cirrhosis in whom whatever the nature the nodule, liver transplantation might be considered. In contrast, in patients with a small nodule and compensated cirrhosis US guided LB should be performed before surgical resection which carries morbidity and mortality higher than those of biopsy itself[14]. It is difficult to perform the differential between a well-differentiated HCC and a dysplastic nodule when using a fragment sampled by LB. The use of molecular markers (GPC3, HSP70, and GS) will identify the exact nature of nodules with 57% sensitivity and 100% specificity[40]. Compared to LB, new imaging techniques such as Gd-EOB-DTPA MRI might be more accurate in the differential diagnosis between early HCC and dysplastic nodules. Hyper-intensity at diffusion-weighted imaging (DWI) was shown to be a useful feature for differentiating hypovascular early HCC from dysplastic nodules which appear as hypointense nodules at Gd-EOB-DTPA MRI[41].A more recent study, reported a sensitivity of 94.7% and specificity of 99.3% in classifying high grade dysplastic nodules which appear hypointense in the hepatobiliary (HB) phase without arterial phase hyperintensity and without DWI restriction[42]. More importantly, the benign nodules appeared hyperintense in the HB phase, and HCC rarely develops from hyperintense hepatic nodules in the HB phase suggesting that this type of nodules require neither treatment nor more intensive follow-up[43].

The degree of tumor differentiation in nodules measuring 1-2 cm can be identified with 60% accuracy, but the sensitivity of the histological examination especially after fine-needle biopsy in assessing vascular micro-invasion is low[34]. Since vascular micro-invasion defines the prognosis in patients allocated to various therapies, its estimation (using nodule size and the degree of differentiation) is of the outmost importance[34].

Identifying the exact nature of the cirrhotic nodules gains additional importance in the context of liver transplantation. Several situations where LB can play a central role can be defined. For instance, identifying an HCC on imaging studies in a patient already on the transplant list will increase his or her priority score. In the first years of using the MELD score, 7%-31% of patients transplanted for stage 1 HCC were found to have no HCC in the explanted liver[44,45]. Secondly, although HCC is the most frequent tumor to develop in a cirrhotic liver, other tumors are also possible (especially cholangiocarcinoma). It is currently believed that up to 20% of nodules developing in a cirrhotic liver and with imaging behavior typical for HCC will actually have another histological structure[16]. The incidence of cholangiocarcinoma has increased considerably in the past years and the imaging appearance of small peripheral lesions is very similar, even identical with that of HCC. Since the risk of recurrence after transplant is much higher for these tumors than for HCC, other patient selection criteria are required, as well as a more aggressive pre-transplant treatment[16]. Thirdly, HCC may occur sometimes in patients with chronic liver disease prior to the development of cirrhosis. The risk for HCC development is lower in these patients, and consequently any newly discovered nodule, even if hypervascular, should be biopsied.

The fourth situation when a pre-transplant liver biopsy is warranted is related to the importance of assessing the degree of tumor differentiation and vascular invasion. It has been clearly proven that in HCC tumor differentiation is strongly correlated to survival, both after resection and transplantation. The risk of recurrence is higher for poorly or moderately differentiated than for well-differentiated tumors[16,46]. This is applicable also for tumors outside of the Milan criteria but within the Up-to-seven criteria, meaning that the patients with well-differentiated HCC and without vascular invasion have a very good prognosis (1- and 3-year survival of 84.2% and 67.4%, respectively)[47].

Vascular micro-invasion is difficult to ascertain by liver biopsy, and its risk can at best be estimated. For instance, for a poorly differentiated tumor larger than 4 cm the risk of vascular micro-invasion is 61%[46]. For well-differentiated tumors, the size and vascular invasion do not appear to influence prognosis[46]. In situations where vascular micro-invasion cannot be estimated the use of imagistic methods might be of real importance. Diffusion-weighted imaging (DWI) an emerging technique in hepatic magnetic resonance imaging (MRI) provided o sensitivity of 93.5% and a specificity of 72.2% for the prediction of micro-vascular invasion during the preoperative evaluation of HCC[48].Consequently, knowing the exact type of tumor appears to be very important for a better patient selection for transplantation[46,47].

In conclusion, choosing to perform a pre-transplant biopsy in patients with liver cirrhosis and HCC depends on the tumor stage and the severity of cirrhosis. For instance, in patients with compensated cirrhosis and HCC diagnosed with the Milan criteria, LB should be performed in order to correctly confirm or exclude an HCC and therefore avoid granting additional MELD points. In patients with decompensated cirrhosis, liver biopsy is not indicated since transplantation is already an immediate necessity. For patients, outside of the Milan but within the Up-to-seven criteria, liver biopsy is very useful in selecting patients with well-differentiated tumors who would benefit most from transplantation[44,49].

Arguments for the use of LB before resection*.* A poor correlation (sometimes below 50%) has been found in large biopsied tumors between the degree of differentiation found on biopsy and on the resected tumor[34,50]. It can be explained by the high heterogeneity of larger tumors in what concerns the degree of differentiation. Secondly, performing a biopsy before a resection will expose the patient to a higher risk of peritoneal metastases (12.5% *vs* 1.6%) and will decrease 5-year disease-free survival (24% *vs* 52%)[51]. However, some authors consider that fine-needle aspiration before resection does not affect mortality and survival rates[30].

Thirdly, we must not ignore the risk of complications (seeding, bleeding) as well as the contraindications and limits of LB (ascites, coagulopathy or isoechoic nodules). The negative predictive value of LB does not reach 100%, and a new biopsy or imaging follow-up is recommended in the case of negative results. This approach will prolong the time to resection and will expose the patient to additional risks[52]. The current approach states LB should be indicated and performed only in tertiary centers equipped with state-of-the-art imaging techniques, high imaging expertise, interventional techniques and pathology lab[47]. In other conditions, performing liver biopsy before resection should be avoided, excepting the cases where the biopsy result is expected to substantially alter the therapy[53].

# *BCLC intermediate and advanced stages*

In these stages the indication to perform LB is made based on the following issues: (1) choosing the optimal therapy from a variety of possible treatment courses. For instance, patients in the intermediate stage may benefit from chemoembolization but also from curative options such as resection, percutaneous ablation or liver transplantation. Curative treatment is indicated in the presence of favorable prognostic factors, such as well-differentiated HCC or the lack of vascular micro-invasion[54]; (2) diagnosing a portal thrombus as benign through liver biopsy mayrecommendlivertransplantationorresectionforapatientintheadvancedstage; and (3) Considering the poor efficacy of current antiangiogenic therapy (Sorafenib), its severe adverse effects as well as their high cost, it is essential to exclude other tumors which may occur in a cirrhotic liver (cholangiocarcinoma, mixed types - hepatocholangiocarcinoma) and which would require a different therapy[49]. The lack of histological confirmation in the Sharp and other similar studies raises the question whether or not some cases of hepatocholangiocarcinoma may have been wrongly diagnosed as HCC in the study groups.

Molecular testing is nowadays a staple in oncology; the selection of systemic treatments is made considering the tumor molecular biology (as in breast or lung cancer). The concept of non-invasive diagnosis in HCC (which is the only tumornot requiring morphological examination) was established before the introduction of new therapeutic agents. Several authors speculate whether this lack of histological data may explain the limited efficacy of Sorafenib and the fact that certain studies fail to prove the efficacy of other systemic therapies in HCC[55]. In the future, the multitude of studies performed on the systemic therapy in HCC will have to make use of pathological, molecular and genetic information provided by the tissue fragment in order to accurately establish the prognosis and to individualize the therapy[49,53]. The progress in molecular biology will soon allow guided treatment based on the expression of tumor genes[53]. At present, the molecular genetic tests are costly and their widespread use is limited by their on-going validation and standardization as well as by the lack of consensus guidelines[53].

# Liver biopsy in the context of personalized medicine

The role of liver biopsy for the management of patients with HCC is one of the most active debates in the liver cancer community[56,57]. Over the last decade, the emergence of high-throughput molecular technologies has allowed to define the main molecular mechanism involved in HCC development and progression. HCC is best considered a highly heterogeneous entity composed of distinct transcriptomic subgroups with various genetic alterations[58,59]. Importantly, a high degree of heterogeneity can also be observed at the histological level. For instance, fibrolamellar carcinoma is already a well-accepted morphological and molecular subtype of HCC[60]. Furthermore, the chromophobe subtype shows a distinct morphology as well as a specific molecular mechanism to overcome replicative senescence, in contrast to telomerase activation seen in most HCCs[61]. Several histological subtypes, which feature distinctive and recognizable morphological features, have also been reported such as the steatohepatitic, cirrhotic, lymphoepithelioma-like, and inflammatory HCCs[61–63]. Indeed, the molecular mechanism behind these histological subtypes awaits clarification, but considering the rapid advancement of molecular technologies this is only a matter of time. It is estimated that 20%-30% of HCCs belong to a recognizable morphological/molecular subtype[57]. A recent paper, published in Hepatology, described another subtype of HCC with both histological and molecular distinct features[64]. The macrotrabecullar-massive HCC (MTM-HCC) was identified in 12% of the whole cohort (16% of surgically resected samples, 8.5% of liver biopsy samples). On multivariate analysis, the MTM-HCC subtype was an independent predictor of early and overall recurrence. From the molecular point of view, MTM- HCC was characterized by high expression of angiopoietin 2 and vascular endothelial growth factor A (VEGFA)[65]. Bi-specific, anti-angiopoietin 2 and anti VEGFA antibodies might represent a potent treatment of this subclass of HCC.

Taking into account the new recently described, MTM-HCC subclass we now have an estimated 36%-46% of HCCs that belong to a recognizable morphological or molecular subtype. For the remaining HCCs, molecular subtypes likely exists[66]. Tumor heterogeneity will not be fully reflected in all liver biopsies, but many HCCs can be sub-classified appropriately. The discovery of different histological subtypes each with distinct molecular features is still in its infancy and until further evidence, no recommendations can be made on how to treat best different subtypes. For the time being HCC should rather be considered as one disease. On contrary, in the future once all the signaling pathways for each HCC subtype have been described liver biopsy will indeed be necessary for the correct identification of such signaling pathways. Moreover, the identification of distinct signaling pathways for different subtypes of HCC will allow for the development of new treatments. In this ideal but not far from now scenario, liver biopsy will allow for the correct diagnosis of HCC subtype, the corresponding up-regulated signaling pathways, and the proper choice of specific molecule and ultimately will open the path for a personalized medicine.

The balance of risk and benefit defines many of the decisions that we make as providers of medical care. With respect to the use of liver biopsy in diagnosing HCC, the risks are well-defined and can be quantified. Common arguments against liver tumor biopsy have been the risk of bleeding and tumor seeding (Table 1). Up to 20% of focal liver lesions developed on a background of liver cirrhosis are not HCC (14) and almost 46% of the HCCs have a distinct histological or molecular signature that might benefit from targeted therapies. We are all afraid of the invasive nature of liver biopsy but what are the risks and benefits of treating a non-HCC patient as being a HCC? What is the benefit of targeting a molecular pathway in a patient with HCC in which the targeted pathway is not activated? We do not believe that the current guidelines are wrong, because the data which formed the basis of the existing guidelines was against liver biopsy. However, due to the advancement in molecular biology more and more molecular and histological classes of HCC have been and will be described. We consider that there will come a time where diagnostic biopsies will be commonly performed. This will improve diagnosis of HCC and increase our chance to provide better patient care in the future.

# Liquid biopsy: The future of liver biopsy

In the past, few decades several studies have demonstrated the utility of circulating cancer by-products called “liquid biopsy”, which could provide accessible, accurate, and dynamic information to evaluate tumor progression. Circulating tumor cells (CTCs), circulating cell-free DNA, circulating miRNA, and circulating tumor associated microparticles (MPs) can all be united under the term of liquid biopsy**.** Compared to liver biopsy, liquid biopsy is a noninvasive method used for the identification of CTCs, circulating MPs or circulating miRNA/DNA in the blood of patients with HCC. Moreover, it is well accepted by the patients since only 1 ml of blood is enough for the proper identification using flow cytometry or cell search system. Similar to conventional biopsies CTSs or MPs can be stained for various surface markers specific for HCC. A detailed description of all cancer by-products is beyond the purpose of this review and has already been nicely reviewed elsewhere[67]. We will only provide some brief examples.

CTCs were detected in blood samples from 45 out of 69 HCC patients compared to 0 out of 31 controls. Moreover, CTCs number correlated significantly with tumor size, PVT and survival[68]. Others, have found that, patients with preoperative detectable EpCAMmRNA+ CTCs had significantly shorter TTR (median, 10.9 mo *vs* not reached) and higher recurrence rates (59.6% *vs* 25.7%) than those without detectable EpCAMmRNA+ CTCs[69]. Chan et al[70] confirmed the existence of typical DNA copy number variations in the peripheral blood of 4 HCC patients and they almost all disappeared after surgical resection. Circulating miRNA is probably the most studied form liquid biopsy in HCC. Several miRNAs have been reported to have a role in the diagnosis, prognosis and follow-up[67]. More recently, another form of liquid biopsy has gained particular attention. Circulating tumor microparticles positive for a combination of antigens, particularly AnexinV+EpCAM+ASGPR1+CD133+ microparticles allowed the distinction of liver malignancies (HCC or CCA) and cirrhosis from tumor-free individuals and, more importantly, from patients carrying other non-liver cancers. In addition, AnexinV+EpCAM+ASGPR1+ microparticles were increased in liver cancer- bearing patients compared to patients with cirrhosis that lacked any detectable liver malignancy[71].

The term liquid biopsy has been only recently introduced and the technology for cancer by-products identification is still in its infancy. Until more and more data becomes available liquid biopsy cannot be performed in daily practice and should rather be used for research intents.Time will decide the limits of liquid biopsies and whether it can replace or not conventional biopsies. The reported sensitivity and specificity of liquid biopsy in HCC is rather modest than high. Better performance was reported for liquid biopsy as a tool to monitor treatment outcomes. Indeed, a lot of work must be done in this field before we can draw any conclusions. The continuous improvement of CTCs, circulating free DNA, MPs, detection and characterization is of the utmost importance since liquid biopsy has several advantages over conventional biopsy: (1) it is a non-invasive procedure; (2) can be easily repeated over time, which offers a more complete portrait of the disease; (3) it could better reveal the genetic complexity of a highly heterogeneous tumor; and (4) it is much faster[72].

# Conclusion

The indications of the morphological examination in the diagnosis of HCC are at present very carefully adjusted, because they must consider the availability of non- invasive techniques and on the other hand the need for criteria for prognosis and the individualization of therapy. Improving the biopsy technique (higher needle performance, more accurate guidance in the active, hypervascular areas of the tumor and the use of techniques with a lower seeding risk) will increase the sensitivity of the procedure and decrease the complication rate. With the recent advances in high- throughput molecular technologies, which allowed for identification of novel HCC subclasses with prognostic impact, the role of liver biopsy will gain more and more attention and reconsideration.

**References**

1 **Giorgio A**, Montesarchio L, Gatti P, Amendola F, Matteucci P, Santoro B, Merola MG, Merola F, Coppola C, Giorgio V. Contrast-Enhanced Ultrasound: a Simple and Effective Tool in Defining a Rapid Diagnostic Work-up for Small Nodules Detected in Cirrhotic Patients during Surveillance. *J Gastrointestin Liver Dis* 2016; **25**: 205-211 [PMID: 27308652 DOI: 10.15403/jgld.2014.1121.252.chu]

2 **Kono Y**, Lyshchik A, Cosgrove D, Dietrich CF, Jang HJ, Kim TK, Piscaglia F, Willmann JK, Wilson SR, Santillan C, Kambadakone A, Mitchell D, Vezeridis A, Sirlin CB. Contrast Enhanced Ultrasound (CEUS) Liver Imaging Reporting and Data System (LI-RADS®): the official version by the American College of Radiology (ACR). *Ultraschall Med* 2017; **38**: 85-86 [PMID: 28249328 DOI: 10.1055/s-0042-124369]

3 **Terzi E**, Iavarone M, Pompili M, Veronese L, Cabibbo G, Fraquelli M, Riccardi L, De Bonis L, Sangiovanni A, Leoni S, Zocco MA, Rossi S, Alessi N, Wilson SR, Piscaglia F; CEUS LI-RADS Italy study group collaborators:. Contrast ultrasound LI-RADS LR-5 identifies hepatocellular carcinoma in cirrhosis in a multicenter restropective study of 1,006 nodules. *J Hepatol* 2018; **68**: 485-492 [PMID: 29133247 DOI: 10.1016/j.jhep.2017.11.007]

4 **Ye SL**, Chen RX. [Comments on management of hepatocellular carcinoma: an update]. *Zhonghua Gan Zang Bing Za Zhi* 2011; **19**: 251-253 [PMID: 21805733 DOI: 10.1002/hep.24199]

5 **Bru C**, Maroto A, Bruix J, Faus R, Bianchi L, Calvet X, Ayuso C, Vilana R, Gilabert R, Rodés J. Diagnostic accuracy of fine-needle aspiration biopsy in patients with hepatocellular carcinoma. *Dig Dis Sci* 1989; **34**: 1765-1769 [PMID: 2555123 DOI: 10.1007/BF01540056]

6 **Bolondi L**, Gaiani S, Benzi G, Zironi G, Rigamonti A, Fusconi F, Barbara L. Ultrasonography and guided biopsy in the diagnosis of hepatocellular carcinoma. *Ital J Gastroenterol* 1992; **24**: 46-49 [PMID: 1315177]

7 **Fornari F**, Filice C, Rapaccini GL, Caturelli E, Cavanna L, Civardi G, Di Stasi M, Buscarini E, Buscarini L. Small (< or = 3 cm) hepatic lesions. Results of sonographically guided fine-needle biopsy in 385 patients. *Dig Dis Sci* 1994; **39**: 2267-2275 [PMID: 7924754 DOI: 10.1007/BF02090383]

8 **Duysburgh I**, Michielsen P, Fierens H, Van Marck E, Pelckmans P. Fine needle trucut biopsy of focal liver lesions: a new technique. *Dig Dis Sci* 1997; **42**: 2077-2081 [PMID: 9365138 DOI: 10.1023/A:1018870501882]

9 **Caturelli E**, Bisceglia M, Fusilli S, Squillante MM, Castelvetere M, Siena DA. Cytological vs microhistological diagnosis of hepatocellular carcinoma: comparative accuracies in the same fine-needle biopsy specimen. *Dig Dis Sci* 1996; **41**: 2326-2331 [PMID: 9011437 DOI: 10.1007/BF02100122]

10 **Livraghi T**, Sangalli G, Giordano F, Vettori C. Fine aspiration versus fine cutting needle, and comparison between smear cytology, inclusion cytology and microhistology in abdominal lesions. *Tumori* 1988; **74**: 361-364 [PMID: 3041658]

11 **Huang GT**, Sheu JC, Yang PM, Lee HS, Wang TH, Chen DS. Ultrasound-guided cutting biopsy for the diagnosis of hepatocellular carcinoma--a study based on 420 patients. *J Hepatol* 1996; **25**: 334-338 [PMID: 8895013]

12 **Borzio M**, Borzio F, Macchi R, Croce AM, Bruno S, Ferrari A, Servida E. The evaluation of fine-needle procedures for the diagnosis of focal liver lesions in cirrhosis. *J Hepatol* 1994; **20**: 117-121 [PMID: 8201212 DOI: 10.1016/S0168-8278(05)80477-5]

13 **Radu B, Zeno S.** Biopsia leziunilor focale pe ficatul cirotic: indicaţii, tehnică, performanţe, complicaţii.*Med Ultrason* 2001; **3**: 21–25

14 **Durand F**, Regimbeau JM, Belghiti J, Sauvanet A, Vilgrain V, Terris B, Moutardier V, Farges O, Valla D. Assessment of the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. *J Hepatol* 2001; **35**: 254-258 [PMID: 11580148 DOI: 10.1016/S0168-8278(01)00108-8]

15 **Ch Yu S**, Metreweli C, Lau WY, Leung WT, Liew CT, Leung NW. Safety of percutaneous biopsy of hepatocellular carcinoma with an 18 gauge automated needle. *Clin Radiol* 1997; **52**: 907-911 [PMID: 9413963]

16 **Durand F,** Belghiti J, Paradis V. Liver transplantation for hepatocellular carcinoma: Role of biopsy. *Liver Transplant* 2007; **13**: S17-23 [PMID: 17969095 DOI: 10.1002/lt.21326]

17 **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]

18 **Schlottmann K**, Klebl F, Zorger N, Feuerbach S, Schölmerich J. Contrast-enhanced ultrasound allows for interventions of hepatic lesions which are invisible on convential B-mode. *Z Gastroenterol* 2004; **42**: 303-310 [PMID: 15095120 DOI: 10.1055/s-2004-812712]

19 **Sparchez Z**, Radu P, Zaharia T, Kacso G, Grigorescu I, Botis G, Badea R. Usefulness of contrast enhanced ultrasound guidance in percutaneous biopsies of liver tumors. *J Gastrointestin Liver Dis* 2011; **20**: 191-196 [PMID: 21725517]

20 **Caturelli E**, Biasini E, Bartolucci F, Facciorusso D, Decembrino F, Attino V, Bisceglia M. Diagnosis of hepatocellular carcinoma complicating liver cirrhosis: utility of repeat ultrasound-guided biopsy after unsuccessful first sampling. *Cardiovasc Intervent Radiol* 2002; **25**: 295-299 [PMID: 12324817 DOI: 10.1007/s00270-001-0123-6]

21 **Souto E**, Gores GJ. When should a liver mass suspected of being a hepatocellular carcinoma be biopsied? *Liver Transpl* 2000; **6**: 73-75 [PMID: 10648581 DOI: 10.1002/lt.500060108]

22 **Little AF**, Ferris JV, Dodd GD 3rd, Baron RL. Image-guided percutaneous hepatic biopsy: effect of ascites on the complication rate. *Radiology* 1996; **199**: 79-83 [PMID: 8633176 DOI: 10.1148/radiology.199.1.8633176]

23 **Blanc JF**. Traitement du carcinome hépatocellulaire: Un tournant? *Hepatogastroenterology* 2016; **23**: 79-85 [10.1684/hpg.2015.1244]

24 **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]

25 **Maturen KE**, Nghiem HV, Marrero JA, Hussain HK, Higgins EG, Fox GA, Francis IR. Lack of tumor seeding of hepatocellular carcinoma after percutaneous needle biopsy using coaxial cutting needle technique. *AJR Am J Roentgenol* 2006; **187**: 1184-1187 [PMID: 17056903 DOI: 10.2214/AJR.05.1347]

26 **Torzilli G**, Minagawa M, Takayama T, Inoue K, Hui AM, Kubota K, Ohtomo K, Makuuchi M. Accurate preoperative evaluation of liver mass lesions without fine-needle biopsy. *Hepatology* 1999; **30**: 889-893 [PMID: 10498639 DOI: 10.1002/hep.510300411]

27 **Takamori R**, Wong LL, Dang C, Wong L. Needle-tract implantation from hepatocellular cancer: is needle biopsy of the liver always necessary? *Liver Transpl* 2000; **6**: 67-72 [PMID: 10648580 DOI: 10.1002/lt.500060103]

28 **Kim SH**, Lim HK, Lee WJ, Cho JM, Jang HJ. Needle-tract implantation in hepatocellular carcinoma: frequency and CT findings after biopsy with a 19.5-gauge automated biopsy gun. *Abdom Imaging* 2000; **25**: 246-250 [PMID: 10823443 DOI: 10.1007/s002610000025]

29 **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]

30 **Ng KK**, Poon RT, Lo CM, Liu CL, Lam CM, Ng IO, Fan ST. Impact of preoperative fine-needle aspiration cytologic examination on clinical outcome in patients with hepatocellular carcinoma in a tertiary referral center. *Arch Surg* 2004; **139**: 193-200 [PMID: 14769580 DOI: 10.1001/archsurg.139.2.193]

31 **Shuto T**, Yamamoto T, Tanaka S, Kanazawa A, Takemura S, Tanaka H, Kubo S, Hirohashi K, Sakaguchi H, Seki S. Resection of needle-tract implantation after percutaneous puncture for hepatocellular carcinoma. *J Gastroenterol* 2004; **39**: 907-908 [PMID: 15565415 DOI: 10.1007/s00535-003-1411-5]

32 **Wang CW**, Lin ZY, Chuang WL, Wang LY, Yu ML, Chen SC, Hsieh MY, Tsai JF, Chang WY. Safety of fine-needle aspiration in patients with small hepatocellular carcinoma. *Hepatol Res* 2005; **31**: 31-35 [PMID: 15652468 DOI: 10.1016/j.hepres.2004.11.002]

33 **Saborido BP**, Díaz JC, de Los Galanes SJ, Segurola CL, de Usera MA, Garrido MD, Elola-Olaso AM, Sánz RG, Romero CJ, Garcia García I, González EM. Does preoperative fine needle aspiration-biopsy produce tumor recurrence in patients following liver transplantation for hepatocellular carcinoma? *Transplant Proc* 2005; **37**: 3874-3877 [PMID: 16386569 DOI: 10.1016/j.transproceed.2005.09.169]

34 **Colecchia A**, Scaioli 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]

35 **Yamashita Y**, Matsukawa T, Arakawa A, Hatanaka Y, Urata J, Takahashi M. US-guided liver biopsy: predicting the effect of interventional treatment of hepatocellular carcinoma. *Radiology* 1995; **196**: 799-804 [PMID: 7644646 DOI: 10.1148/radiology.196.3.7644646]

36 **Kanematsu M**, Hoshi H, Takao H, Sugiyama Y. Abdominal wall tumor seeding at sonographically guided needle-core aspiration biopsy of hepatocellular carcinoma. *AJR Am J Roentgenol* 1997; **169**: 1198-1199 [PMID: 9308498 DOI: 10.2214/ajr.169.4.9308498]

37 **Chapoutot C**, Perney P, Fabre D, Taourel P, Bruel JM, Larrey D, Domergue J, Ciurana AJ, Blanc F. [Needle-tract seeding after ultrasound-guided puncture of hepatocellular carcinoma. A study of 150 patients]. *Gastroenterol Clin Biol* 1999; **23**: 552-556 [PMID: 10429862]

38 **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]

39 **Sersté T**, Barrau V, Ozenne V, Vullierme MP, Bedossa P, Farges O, Valla DC, Vilgrain V, Paradis V, Degos F. Accuracy and disagreement of computed tomography and magnetic resonance imaging for the diagnosis of small hepatocellular carcinoma and dysplastic nodules: role of biopsy. *Hepatology* 2012; **55**: 800-806 [PMID: 22006503 DOI: 10.1002/hep.24746]

40 **Tremosini S**, Forner A, Boix L, Vilana R, Bianchi L, Reig M, Rimola J, Rodríguez-Lope C, Ayuso C, Solé M, Bruix J. Prospective validation of an immunohistochemical panel (glypican 3, heat shock protein 70 and glutamine synthetase) in liver biopsies for diagnosis of very early hepatocellular carcinoma. *Gut* 2012; **61**: 1481-1487 [PMID: 22287594 DOI: 10.1136/gutjnl-2011-301862]

41 **Hwang J**, Kim YK, Jeong WK, Choi D, Rhim H, Lee WJ. Nonhypervascular Hypointense Nodules at Gadoxetic Acid-enhanced MR Imaging in Chronic Liver Disease: Diffusion-weighted Imaging for Characterization. *Radiology* 2015; **276**: 137-146 [PMID: 25734551 DOI: 10.1148/radiol.15141350]

42 **Renzulli M**, Biselli M, Brocchi S, Granito A, Vasuri F, Tovoli F, Sessagesimi E, Piscaglia F, D'Errico A, Bolondi L, Golfieri R. New hallmark of hepatocellular carcinoma, early hepatocellular carcinoma and high-grade dysplastic nodules on Gd-EOB-DTPA MRI in patients with cirrhosis: a new diagnostic algorithm. *Gut* 2018 [PMID: 29437912 DOI: 10.1136/gutjnl-2017-315384]

43 **Sano K**, Ichikawa T, Motosugi U, Ichikawa S, Morisaka H, Enomoto N, Matsuda M, Fujii H. Outcome of hypovascular hepatic nodules with positive uptake of gadoxetic acid in patients with cirrhosis. *Eur Radiol* 2017; **27**: 518-525 [PMID: 27255397 DOI: 10.1007/s00330-016-4423-2]

44 **Marsh JW**, Dvorchik I. Should we biopsy each liver mass suspicious for hepatocellular carcinoma before liver transplantation?--yes. *J Hepatol* 2005; **43**: 558-562 [PMID: 16112246 DOI: 10.1016/j.jhep.2005.07.014]

45 **Hayashi PH**, Trotter JF, Forman L, Kugelmas M, Steinberg T, Russ P, Wachs M, Bak T, Kam I, Everson GT. Impact of pretransplant diagnosis of hepatocellular carcinoma on cadveric liver allocation in the era of MELD. *Liver Transpl* 2004; **10**: 42-48 [PMID: 14755776 DOI: 10.1002/lt.20020]

46 **Ramos Rubio E**, Llado Garriga L. [Usefulness of pre-surgical biopsy in selecting patients with hepatocellular carcinoma for liver transplant]. *Cir Esp* 2010; **87**: 133-138 [PMID: 20074710 DOI: 10.1016/j.ciresp.2009.11.026]

47 **Cresswell AB**, Welsh FK, Rees M. A diagnostic paradigm for resectable liver lesions: to biopsy or not to biopsy? *HPB (Oxford)* 2009; **11**: 533-540 [PMID: 20495704 DOI: 10.1111/j.1477-2574.2009.00081.x]

48 **Suh YJ**, Kim MJ, Choi JY, Park MS, Kim KW. Preoperative prediction of the microvascular invasion of hepatocellular carcinoma with diffusion-weighted imaging. *Liver Transpl* 2012; **18**: 1171-1178 [PMID: 22767394 DOI: 10.1002/lt.23502]

49 **Parisi G**. Should a radiological diagnosis of hepatocellular carcinoma be routinely confirmed by a biopsy? Yes. *Eur J Intern Med* 2012; **23**: 34-36 [PMID: 22153528 DOI: 10.1016/j.ejim.2011.10.015]

50 **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]

51 **Young AL**, Malik HZ, Abu-Hilal M, Guthrie JA, Wyatt J, Prasad KR, Toogood GJ, Lodge JP. Large hepatocellular carcinoma: time to stop preoperative biopsy. *J Am Coll Surg* 2007; **205**: 453-462 [PMID: 17765162 DOI: 10.1016/j.jamcollsurg.2007.04.033]

52 **Stigliano R**, Burroughs AK. Should we biopsy each liver mass suspicious for HCC before liver transplantation?--no, please don't. *J Hepatol* 2005; **43**: 563-568 [PMID: 16120469 DOI: 10.1016/j.jhep.2005.07.015]

53 **Heuman DM**, Gilles HS, Solomon C, Bajaj JS. Should a radiological diagnosis of hepatocellular carcinoma be routinely confirmed by a biopsy? No. *Eur J Intern Med* 2012; **23**: 37-39 [PMID: 22153529 DOI: 10.1016/j.ejim.2011.09.014]

54 **Bolondi L**, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, Piscaglia F, Raoul JL, Sangro B. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis* 2012; **32**: 348-359 [PMID: 23397536 DOI: 10.1055/s-0032-1329906]

55 **Schirmacher P**, Bedossa P, Roskams T, Tiniakos DG, Brunt EM, Zucman-Rossi J, Manns MP, Galle PR. Fighting the bushfire in HCC trials. *J Hepatol* 2011; **55**: 276-277 [PMID: 21439335 DOI: 10.1016/j.jhep.2011.03.004]

56 **Sherman M**, Bruix J. Biopsy for liver cancer: how to balance research needs with evidence-based clinical practice. *Hepatology* 2015; **61**: 433-436 [PMID: 25308482 DOI: 10.1002/hep.27563]

57 **Torbenson M,** Schirmacher P. Liver cancer biopsy - back to the future?! *Hepatology* 2015; **2**:431-433 [PMID: 25271144 DOI: 10.1002/hep.27545]

58 **Zucman-Rossi J**, Villanueva A, Nault JC, Llovet JM. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. *Gastroenterology* 2015; **149**: 1226-1239.e4 [PMID: 26099527 DOI: 10.1053/j.gastro.2015.05.061]

59 **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]

60 **Rieber A**. [Fibrolamellar carcinoma]. *Z Gastroenterol* 1994; **32**: 651-653 [PMID: 7886976 DOI: 10.1097/PCR.0000000000000063]

61 **Solinas A**, Calvisi DF. Lessons from rare tumors: hepatic lymphoepithelioma-like carcinomas. *World J Gastroenterol* 2015; **21**: 3472-3479 [PMID: 25834311 DOI: 10.3748/wjg.v21.i12.3472]

62 **Salomao M**, Yu WM, Brown RS Jr, Emond JC, Lefkowitch JH. Steatohepatitic hepatocellular carcinoma (SH-HCC): a distinctive histological variant of HCC in hepatitis C virus-related cirrhosis with associated NAFLD/NASH. *Am J Surg Pathol* 2010; **34**: 1630-1636 [PMID: 20975341 DOI: 10.1097/PAS.0b013e3181f31caa]

63 **Seok JY**, Na DC, Woo HG, Roncalli M, Kwon SM, Yoo JE, Ahn EY, Kim GI, Choi JS, Kim YB, Park YN. A fibrous stromal component in hepatocellular carcinoma reveals a cholangiocarcinoma-like gene expression trait and epithelial-mesenchymal transition. *Hepatology* 2012; **55**: 1776-1786 [PMID: 22234953 DOI: 10.1002/hep.25570]

64 **Ziol M**, Poté N, Amaddeo G, Laurent A, Nault JC, Oberti F, Costentin C, Michalak S, Bouattour M, Francoz 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* 2017 *[Epub ahead of print]* [PMID: 29281854 DOI: 10.1002/hep.29762]

65 **Calderaro J**, Couchy G, Imbeaud S, Amaddeo 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]

66 **Boyault S**, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P, Zucman-Rossi J. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 2007; **45**: 42-52 [PMID: 17187432 DOI: 10.1002/hep.21467]

67 **Yin CQ**, Yuan CH, Qu Z, Guan Q, Chen H, Wang FB. Liquid Biopsy of Hepatocellular Carcinoma: Circulating Tumor-Derived Biomarkers. *Dis Markers* 2016; **2016**: 1427849 [PMID: 27403030 DOI: 10.1155/2016/1427849]

68 **Ogle LF**, Orr JG, Willoughby CE, Hutton C, McPherson S, Plummer R, Boddy AV, Curtin NJ, Jamieson D, Reeves HL. Imagestream detection and characterisation of circulating tumour cells - A liquid biopsy for hepatocellular carcinoma? *J Hepatol* 2016; **65**: 305-313 [PMID: 27132171 DOI: 10.1016/j.jhep.2016.04.014]

69 **Vona G**, Estepa L, Béroud C, Damotte D, Capron F, Nalpas B, Mineur A, Franco D, Lacour B, Pol S, Bréchot C, Paterlini-Bréchot P. Impact of cytomorphological detection of circulating tumor cells in patients with liver cancer. *Hepatology* 2004; **39**: 792-797 [PMID: 14999698 DOI: 10.1002/hep.20091]

70 **Chan KC**, Jiang P, Zheng YW, Liao GJ, Sun H, Wong J, Siu SS, Chan WC, Chan SL, Chan AT, Lai PB, Chiu RW, Lo YM. Cancer genome scanning in plasma: detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. *Clin Chem* 2013; **59**: 211-224 [PMID: 23065472 DOI: 10.1373/clinchem.2012.196014]

71 **Julich-Haertel H**, Urban SK, Krawczyk M, Willms A, Jankowski K, Patkowski W, Kruk B, Krasnodębski M, Ligocka J, Schwab R, Richardsen I, Schaaf S, Klein A, Gehlert S, Sänger H, Casper M, Banales JM, Schuppan D, Milkiewicz P, Lammert F, Krawczyk M, Lukacs-Kornek V, Kornek M. Cancer-associated circulating large extracellular vesicles in cholangiocarcinoma and hepatocellular carcinoma. *J Hepatol* 2017; **67**: 282-292 [PMID: 28267620 DOI: 10.1016/j.jhep.2017.02.024]

72 **Friedlander TW**, Premasekharan G, Paris PL. Looking back, to the future of circulating tumor cells. *Pharmacol Ther* 2014; **142**: 271-280 [PMID: 24362084 DOI: 10.1016/j.pharmthera.2013.12.011]

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**Table 1 The incidence of needle-tract seeding after hepatocellular carcinoma biopsy**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Year** | **Lesion** | **Needle** | **No. biopsies** | **No. of**  **seeding** | **%** |
| Yamashita *et al*[35] | 1995 | HCC | 0.8-1.2 mm  Bard | 125 | 1 | 0.80 |
| Huang *et al*[11] | 1996 | HCC | 1.4-2 mm | 455 | 9 | 2 |
| Kanematsu *et al*[36] | 1997 | HCC | FNB 0.8mm | 50 | 2 | 4 |
| Ch Yu *et al*[15] | 1997 | HCC | 1.2 mm gun | 139 | 0 | 0 |
| Chapoutot*et al*[37] | 1999 | HCC | 1.0-1.2mm | 150 | 4 | 2.66 |
| Kim *et al*[28] | 2000 | HCC | 1.1mm gun | 205 | 7 | 3.40 |
| Takamori *et al*[27] | 2000 | HCC | FNB | 59 | 3 | 5 |
| Durand *et al*[14] | 2001 | HCC | 1.2 mm | 137 | 2 | 1.60 |
| Kosugi *et al*[29] | 2004 | HCC | n.a | 372 | 6 | 1.61 |
| Ng *et al*[30] | 2004 | HCC | FNA | 91 | 1 | 1.09 |
| Shuto *et al*[31] | 2004 | HCC | n.a | 480 | 5 | 1.04 |
| Wang *et al*[32] | 2005 | HCC | FNA | 90 | 0 | 0 |
| Saborido *et al*[33] | 2005 | HCC | FNA | 26 | 2 | 7.69 |
| Maturen *et al*[25] | 2006 | HCC | 1.2 mm,  coaxial | 128 | 0 | 0 |
| Colecchia *et al*[34] | 2012 | HCC | 0.95mm | 81 | 0 | 0 |
| Total | | | | 2588 | 42 | 1.62 |

n.a: not available; HCC: Hepatocellular carcinoma; %: Percent; FNA: Fine needle aspiration.

**Table 2 Factors influencing the use of liver biopsy in hepatocellular carcinoma**

|  |
| --- |
| 1. Poor accuracy of contrast-enhanced methods in the diagnosis of HCC; especially in  small lesions. |
| 2. The risks of LB, more severe in patients with cirrhosis and coagulopathy |
| 3. Inadequate sampling of HCC lesions, especially in case of very small or very large  ones |
| 4. The complex system of staging, treatment and patient allocation to various therapy regimens (BCLC); the correct assessment of prognosis is important in allocation to  therapy and is based mainly on pathology data. |
| 5. Modern therapies have sometimes limited applicability (transplantation), cost and effectiveness (systemic treatment); information resulting from histological analysis is  necessary in order to increase effectiveness and personalize treatment. |

LB: Liver biopsy; HCC: Hepatocellular carcinoma; BCLC: Barcelona clinic liver cancer staging*.*