

## Contemporary role of liver biopsy in hepatocellular carcinoma

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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 five years ago. As time has passed, the development of high-throughput molecular technologies has helped reveal 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 subtypes have prognostic impact. In the context of personalized treatment, the role of LB will be carefully reconsidered. Until then, it is mandatory to know the various techniques of LB, their performances, complications and limitations. 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 long from now, the role of LB will be reconsidered. It is possible that we will go back in time and once again use biopsy for HCC diagnosis. Then again, we may move back to the future to try to improve the use of liquid biopsy in the follow-up of HCC patients after various treatment modalities.

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

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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 have been described using both molecular and histological characterization. In the context of histological HCC sub-classes, each with distinct molecular patterns and prognostic impacts, the need for liver biopsy in HCC management becomes a necessity. In this era of personalized medicine, knowing the strengths of each sampling technique is of the utmost importance.

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## INTRODUCTION

The correct identification, either malignant or benign, of focal liver lesions is one of the most important issues in cirrhotic patients. Nodular lesions are frequently discovered during an ultrasound screening 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 ultrasound (US), providing a simple, easy-to-perform and immediately available dynamic imaging tool<sup>[1]</sup>. The use of CEUS might therefore shorten the diagnostic and therapeutic work-up of hepatocellular carcinoma (HCC) patients. The large applicability of CEUS for the diagnosis of HCC in cirrhosis was questioned due to the risk of false-positive diagnoses in cases of cholangiocarcinoma. This has caused 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<sup>®[2]</sup>. 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 of misdiagnosing pure cholangiocarcinoma<sup>[3]</sup>.

Despite all the latest improvements in liver imaging, correctly identifying these lesions remains a challenge, especially when dealing with small focal lesions.

According to the AASLD and EASL guidelines, the images in certain situations may not be characteristic, or the results from two imaging techniques may be conflicting (a liver biopsy (LB) is required in these cases)<sup>[4]</sup>. In addition, the information from tumor tissue may provide prognostic data that are useful in the selection of therapy.

## TECHNIQUES, PERFORMANCE, COMPLICATIONS

The invasive techniques used for the morphological diagnosis of 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. Cytology sensitivity varies between 69%-95% across different studies (consistently lower in well-differentiated HCC), while specificity varies between 70%-100%<sup>[5-11]</sup>. The diagnostic accuracy of the method is lower in lesions < 3 cm (50%-83%) vs large ones (85%-95%)<sup>[7,12]</sup>. The smear cytology technique using Papanicolaou's method will decrease the number of both required passes and 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, which is obtainable only with 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 tumor tissue and facilitating certain special staining techniques. These advantages are, however, counterbalanced 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: the small size of the target (smaller lesions are harder to approach in liver with significant fibrosis), the 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 within the tumor<sup>[11]</sup>. Additionally, lesions that are poorly visible or invisible using conventional ultrasound are another cause of liver biopsy failure.

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

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<sup>[7,9,11,17]</sup>. In addition, micro-histology has a high accuracy (89.6%) in diagnosing nodules < 2 cm and varies 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<sup>[17]</sup>.

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<sup>[7,9]</sup>.

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, particularly in the case of large tumors that often display central necrosis<sup>[18]</sup>; and (2) the nodules that are poorly visible or invisible on conventional ultrasound, which become clearly visible after contrast injection in both the arterial or late phase<sup>[18,19]</sup>.

The negative predictive value of liver biopsy remains low, and malignancy cannot be excluded from 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 a 35% increase in positive diagnosis<sup>[20]</sup>. If a tumor was not found in the first biopsy, the chances of success are higher (50%) than in cases with a non-diagnostic result (necrosis) (25%)<sup>[20]</sup>. 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%)<sup>[11,21]</sup>. Risk factors for bleeding include: hemostatic abnormalities, the degree of liver failure, age, the presence of ascites, or the technique used. The risk is generally considered to increase with each additional pass, with a larger needle diameter, and with a smaller area of interposed parenchyma<sup>[22]</sup>. The actual recommendations are to use a needle < 1.2 mm in diameter for a maximum of two passes, and an oblique approach, which would allow at least 1 cm between the lesion and the liver capsule<sup>[15,23]</sup>.

The incidence of needle-tract seeding varies in the 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%<sup>[24]</sup>. Apparently, the larger the needle diameter and the number of passes, or the lower the degree of tumor differentiation, 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 is not reduced by using the coaxial technique<sup>[25]</sup>. 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 either the primary tumor or cirrhosis<sup>[26]</sup>.

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 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%)<sup>[24,25, 27-37]</sup>.

## CURRENT INDICATIONS OF LB IN THE DIAGNOSIS OF HCC

Presently, the indications of performing LB in patients with liver cirrhosis and HCC are highly regulated. The two extreme perspectives that include 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 that indicate, adjust or limit the use of biopsy in HCC are presented in Table 2.

In the following paragraphs, we will make a critical appraisal of the indicators of LB in the diagnosis of HCC for 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<sup>[38,39]</sup>, 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 washout in the portal/parenchymal phase using two imaging techniques)<sup>[38]</sup>. It follows that 50%-70% of patients will require a biopsy in order to receive an exact diagnosis<sup>[38,39]</sup>. US-guided LB may not be justified in patients with decompensated cirrhosis, despite the nature of the nodule, and 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 rates higher than those of biopsy itself<sup>[14]</sup>. It is difficult to assess 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<sup>[40]</sup>. Compared to LB, new imaging techniques such as Gd-EOB-DTPA magnetic resonance imaging (MRI) might be more accurate in the differential diagnosis between early HCC and dysplastic nodules. Hyperintensity at diffusion-weighted imaging (DWI) was shown

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

Ref.	Year	Lesion	Needle	No. of biopsies	No. of seeding	%
Yamashita <i>et al.</i> <sup>[35]</sup>	1995	HCC	0.8-1.2 mm Bard	125	1	0.80
Huang <i>et al.</i> <sup>[11]</sup>	1996	HCC	1.4-2 mm	455	9	2
Kanematsu <i>et al.</i> <sup>[36]</sup>	1997	HCC	FNB 0.8 mm	50	2	4
Ch Yu <i>et al.</i> <sup>[15]</sup>	1997	HCC	1.2 mm gun	139	0	0
Chapoutot <i>et al.</i> <sup>[37]</sup>	1999	HCC	1.0-1.2 mm	150	4	2.66
Kim <i>et al.</i> <sup>[28]</sup>	2000	HCC	1.1 mm gun	205	7	3.40
Takamori <i>et al.</i> <sup>[27]</sup>	2000	HCC	FNB	59	3	5
Durand <i>et al.</i> <sup>[14]</sup>	2001	HCC	1.2 mm	137	2	1.60
Kosugi <i>et al.</i> <sup>[29]</sup>	2004	HCC	n.a	372	6	1.61
Ng <i>et al.</i> <sup>[30]</sup>	2004	HCC	FNA	91	1	1.09
Shuto <i>et al.</i> <sup>[31]</sup>	2004	HCC	n.a	480	5	1.04
Wang <i>et al.</i> <sup>[32]</sup>	2005	HCC	FNA	90	0	0
Saborido <i>et al.</i> <sup>[33]</sup>	2005	HCC	FNA	26	2	7.69
Maturen <i>et al.</i> <sup>[25]</sup>	2006	HCC	1.2 mm, coaxial	128	0	0
Colecchia <i>et al.</i> <sup>[34]</sup>	2012	HCC	0.95 mm	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, which are more severe in patients with cirrhosis and coagulopathy
3 Inadequate sampling of HCC lesions, especially in cases with 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 the allocation of therapy, and is based mainly on pathology data
5 Modern therapies sometimes have 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.

to be a useful feature for differentiating hypovascular early HCC from dysplastic nodules, which appear as hypointense nodules in Gd-EOB-DTPA MRI<sup>[41]</sup>. A more recent study reported a sensitivity of 94.7% and a 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<sup>[42]</sup>. More importantly, the benign nodules appeared hyperintense in the HB phase, and HCC rarely develops from hyperintense hepatic nodules in the HB phase. This suggests that these type of nodules do not require treatment or more intensive follow-up<sup>[43]</sup>.

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

Identifying the exact nature of the cirrhotic nodules gains additional importance in the context of liver transplantation. Several situations where LB may play

a central role can be defined. For instance, identifying an HCC in a patient already on the transplant list using imaging studies will increase his or her priority score. In the first years of using the MELD score, 7%-31% of stage 1 HCC patients who received transplants were found to have no HCC in the explanted liver<sup>[44,45]</sup>. Secondly, although HCC is the most frequent tumor type to develop in cirrhotic liver, other tumors are also possible (especially cholangiocarcinoma). It is currently believed that  $\leq 20\%$  of nodules developing in a cirrhotic liver, with imaging behavior typical for HCC, will actually have another histological structure<sup>[16]</sup>. The incidence of cholangiocarcinoma has increased considerably in the past years, and the imaging appearance of small peripheral lesions is very similar (or 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<sup>[16]</sup>. Thirdly, HCC may sometimes occur 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 hy-

pervascular, 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 HCC tumor differentiation is strongly correlated to survival, both after resection and transplantation. The risk of recurrence is higher for poorly- or moderately-differentiated vs well-differentiated tumors<sup>[16,46]</sup>. This is also applicable for tumors outside of the Milan criteria, but within the Up-to-seven criteria. This means that the patients with well-differentiated HCC, and without vascular invasion, have a very good prognosis (1- and 3-year survival rates of 84.2% and 67.4%, respectively)<sup>[47]</sup>.

Vascular micro-invasion is difficult to ascertain by liver biopsy, and its risk can only be estimated at best. For instance, for a poorly differentiated tumor > 4 cm, the risk of vascular micro-invasion is 61%<sup>[46]</sup>. For well-differentiated tumors, the size and extent of vascular invasion do not appear to influence prognosis<sup>[46]</sup>. 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 MRI, provided a sensitivity of 93.5% and a specificity of 72.2% for the prediction of micro-vascular invasion during the preoperative evaluation of HCC<sup>[48]</sup>. Consequently, knowing the exact type of tumor appears to be very important for optimizing patient selection for transplantation<sup>[46,47]</sup>.

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, therefore avoiding the granting of 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 the most from transplantation<sup>[44,49]</sup>.

An argument for the use of LB before resection concerns a poor correlation (sometimes below 50% for large biopsied tumors) between the degree of differentiation found on biopsy and on the resected tumor<sup>[34,50]</sup>. This can be explained by the high heterogeneity of larger tumors, which relates to their varying degrees 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%)<sup>[51]</sup>. However, some authors claim that fine-needle aspiration before resection does not affect either mortality or survival rates<sup>[30]</sup>.

Thirdly, we must not ignore the risk of complications

(seeding, bleeding), as well as the contraindications and limitations 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<sup>[52]</sup>. The current approach states that LB should be indicated and performed only in tertiary centers that are equipped with state-of-the-art imaging techniques, high imaging expertise, interventional techniques, and pathology labs<sup>[47]</sup>. In other conditions, performing liver biopsy before resection should be avoided, except in cases where the biopsy result is expected to substantially alter the therapy<sup>[53]</sup>.

### **BCLC intermediate and advanced stages**

In each of 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 not only 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<sup>[54]</sup>; (2) diagnosing a portal thrombus as benign using liver biopsy may suggest the need for liver transplantation or resection for a patient in an advanced stage; and (3) Considering the poor efficacy of current antiangiogenic therapies (Sorafenib), which can be attributed to its severe adverse effects and high cost, it is essential to exclude other tumors that may form in a cirrhotic liver (cholangiocarcinoma, mixed types - hepatocholangiocarcinoma) and that would require a different therapy<sup>[49]</sup>. The lack of histological confirmation in the Sharp studies, as well other similar ones, raises the question of whether or not some cases of hepatocholangiocarcinoma may have been wrongly diagnosed as HCC in the study groups.

Molecular testing is a staple nowadays in oncology. The selection of systemic treatments is made by considering the tumor molecular biology (as in breast or lung cancer). The concept of non-invasive diagnosis of HCC (which is the only tumor that does not require 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, considering that certain studies fail to prove the efficacy of other systemic therapies in HCC<sup>[55]</sup>. In the future, the multitude of studies performed on systemic therapy for 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<sup>[49,53]</sup>. Current progress in molecular biology will soon allow for guided treatment based on the expression of tumor genes<sup>[53]</sup>. At present, molecular genetic

tests are costly, and their widespread use is limited by their ongoing validation and standardization, as well as by the lack of consensus in their guidelines<sup>[53]</sup>.

## 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<sup>[56,57]</sup>. Over the last decade, the emergence of high-throughput molecular technologies has provided the ability to interrogate the main molecular mechanism involved in HCC development and progression. HCC is best considered a highly heterogeneous entity that is composed of distinct transcriptomic subgroups with various genetic alterations<sup>[58,59]</sup>. 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<sup>[60]</sup>. Furthermore, the chromophobe subtype shows a distinct morphology, while also utilizing a specific molecular mechanism to overcome replicative senescence, which is in contrast to the telomerase activation seen in most HCCs<sup>[61]</sup>. Several histological subtypes, which feature distinctive and recognizable morphological features, have also been reported, such as the steatohepatitic, cirrhotic, lymphoepithelioma-like, and inflammatory HCCs<sup>[61-63]</sup>. Indeed, the molecular mechanism behind these histological subtypes awaits clarification, however this is only a matter of time considering the rapid advancement of molecular technologies. It is estimated that 20%-30% of HCCs belong to a recognizable morphological/molecular subtype<sup>[57]</sup>. A recent paper, published in *Hepatology*, described another HCC subtype that displays distinct histological and molecular features<sup>[64]</sup>. The macrotrabecular-massive HCC (MTM-HCC) was identified in 12% of the total cohort (16% of surgically-resected samples, 8.5% of liver biopsy samples). In 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)<sup>[65]</sup>. Bi-specific, anti-angiopoietin 2 and anti-VEGFA antibodies may represent potent treatments for this subclass of HCC.

Taking into account this 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 exist<sup>[66]</sup>. Tumor heterogeneity will not be fully reflected in all liver biopsies, however many HCCs can be sub-classified appropriately. The discovery of different histological subtypes, each with distinct molecular features, is still in its infancy. Until further evidence is revealed, no recommendations can be made regarding how to best treat different subtypes. For the

time being, HCC should instead be considered as one disease. On the contrary, 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 and close-approaching scenario, liver biopsy will allow for the correct diagnosis of HCC subtypes, the corresponding upregulated signaling pathways, and the proper choice of specific molecules. This will ultimately open the path for 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 quantifiable. Common arguments against liver tumor biopsy have included 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 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 must also consider the risks and benefits of treating a non-HCC patient as though they had HCC. What is the benefit of targeting a molecular pathway in a patient with HCC when that targeted pathway is not activated? We do not believe that the current guidelines are wrong, because the data that form the basis of the existing guidelines are against liver biopsy. Nevertheless, due to advancements in molecular biology, more and more molecular and histological classes of HCC have been, and will continue to be, described. We believe that there will come a time when diagnostic biopsies will be commonly performed. This will improve the diagnosis of HCC and increase our ability 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 byproducts known as "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 "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 proper identification in flow cytometry or cell search systems. Similar to conventional biopsies, CTs or MPs can be stained for various surface markers specific for HCC. A detailed description of all cancer

byproducts is beyond the scope of this review and has already been nicely reviewed elsewhere<sup>[67]</sup>. 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, CTC numbers correlated significantly with tumor size, PVT and survival<sup>[68]</sup>. Others have found that patients with preoperative detectable EpCAM<sup>mRNA+</sup> CTCs had significantly shorter TTR (median of 10.9 mo vs not reached) and higher recurrence rates (59.6% vs 25.7%) than those without detectable EpCAM<sup>mRNA+</sup> CTCs<sup>[69]</sup>. Chan *et al.*<sup>[70]</sup> confirmed the existence of typical DNA copy number variations in the peripheral blood of four HCC patients, which primarily disappeared after surgical resection. Circulating miRNA is probably the most studied form of liquid biopsy in HCC. Several miRNAs have been reported to have a role in diagnosis, prognosis and follow-up<sup>[67]</sup>. More recently, another form of liquid biopsy has gained particular attention. Circulating tumor microparticles that are positive for a combination of antigens, particularly AnxinV<sup>+</sup>EpCAM<sup>+</sup>ASGPR1<sup>+</sup>CD133<sup>+</sup>, allowed for 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, AnxinV<sup>+</sup>EpCAM<sup>+</sup>ASGPR1<sup>+</sup> microparticles were increased in liver cancer-bearing patients compared to patients with cirrhosis that lacked any detectable liver malignancy<sup>[71]</sup>.

The term liquid biopsy has only recently been introduced, and the technology for cancer byproduct identification is still in its infancy. Until more and more data becomes available, liquid biopsy cannot be performed in daily practice and should instead be used for research intents. Time will decide the limits of liquid biopsies and whether it can replace conventional biopsies. The reported sensitivity and specificity of liquid biopsy in HCC is rather modest. 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. Continuously improving the detection and characterization of CTCs, circulating free DNA, and MPs is of the utmost importance, since liquid biopsy has several advantages over conventional biopsy: (1) it is a non-invasive procedure; (2) it 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<sup>[72]</sup>.

## CONCLUSION

Presently, morphological examination during HCC diagnosis is very carefully adjusted, as one must consider the availability of non-invasive techniques and, on the other hand, the need for prognostic criteria and individualized 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 recent advances in high-throughput molecular technologies, which have allowed for the identification of novel HCC subclasses with prognostic impact, the role of liver biopsy will gain increasingly more attention and reconsideration.

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