REVIEW

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Predicting recurrence following radiofrequency percutaneous ablation for hepatocellular carcinoma

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Hepatic Oncology

Practice points

- Percutaneous treatment can compete with surgical resection to treat hepatocellular carcinoma (HCC) ≤ 5 cm in diameter developed on cirrhosis.
- Recurrences after percutaneous ablation are roughly divided in early recurrences (< 2 years after ablation) due to tumor metastasis and late recurrences (> 2 years after ablation) due to *de novo* carcinogenesis in cirrhotic parenchyma.
- Local recurrence can result to either from incomplete local treatment or from tumor aggressiveness, while distant recurrence is related to tumor metastasis or *de novo* carcinogenesis.
- Technical breakthrough in percutaneous ablation (multipolar radiofrequency) increases treatment effectiveness and allows to extend indication to difficult-to-treat HCC.
- Tumor features (size and number of nodules, serum biomarkers, histological and immunohistochemical features) as well as severity of liver disease and virological features influence tumor recurrence rate after percutaneous ablation.
- Promising serum and liver biomarkers as molecular signatures and serum metabolites profiling have to be tested in the field of percutaneous ablation of HCC.

SUMMARY Within 5 years after percutaneous ablation of hepatocellular carcinoma, roughly 70% of patients experience tumor recurrence. Relapses beyond curative options affected patients' survival. Ablation shares with resection common predictive factors of recurrence as size of the tumor, multinodularity and presence of vascular invasion. High serum α -fetoprotein level and markers of severity of underlying liver disease have also been found to be associated with recurrence and even survival. However, predictive values for recurrence of technical factors, histopathological and molecular tumors' features have been rarely studied. Few comparative studies have shown that ablation techniques impact recurrence rates. Moreover, although ablation does not allow analysis of the whole tumor, some reports suggest that biopsies allow histopathological and even molecular testing of the risk of recurrence.

KEYWORDS

• cirrhosis • hepatocellular carcinoma • percutaneous ablation • radiofrequency ablation • recurrence

Hepatocellular carcinoma (HCC) represents the most common cause of death among patients with hepatitis B virus (HBV) or hepatitis C virus (HCV)-related cirrhosis. Ultrasonographic surveillance of patients with cirrhosis has led to an increased detection of early-stage (Barcelona Clinic

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Liver Cancer [BCLC]-stage 0/A) HCC eligible for local potentially curative first-line therapy such as percutaneous ablation or resection, with a 40–70% 5-year survival [1]. These two local treatment options are both hampered by a 5-year recurrence rate up to 70% [2–4].

Local treatment has two objectives in order to be curative: removing the whole tumor and its margin, which potentially includes satellite nodules and microvascular invasion; and avoiding reappearance of new tumor related either to metastases or *de novo* carcinogenesis [5]. Whereas the first objective completely relies on the initial treatment procedure, the second objective depends both on tumor's aggressiveness and on the severity of underlying liver disease. Therefore, an adequate analysis of predictive factors for recurrence after local treatment requires distinguishing local relapse, defined as tumor recurrence directly adjacent to the initial treatment zone, from distant recurrence, defined by the emergence of a tumor separated from the treatment zone by nontumor liver (Figure 1). While the tumor's stage, the biology of the tumor and the underlying liver disease could influence both local and distant recurrences, the different technical modalities of ablation should theoretically only impact local recurrence. Classically, distant recurrence occurring early – before 2 years of follow-up – are considered to result from an intrahepatic metastatic process via portal vein or multicentric liver carcinogenesis and late recurrence after 2 years would result only from *de novo* carcinogenesis (Figure 1).

Therefore, local and at distance, early and late recurrences, should be considered as distinct endpoints when analyzing predictive factors for recurrence, taking in account tumor's staging and biological characteristics, together with underlying liver disease and the different techniques of ablation.

The identification of robust factors correlating with the outcome of patients may help to determine the prognosis, identify patients most likely to benefit from specific treatments, monitor response to treatment and therefore could guide clinicians in designing personalized treatment strategies.

Predictive factors of HCC recurrence after local treatment have been mainly assessed after surgical resection. Up to now, beside tumor's characteristics such as size, multinodularity, macroscopic venous invasion, presence of satellite nodules and baseline serum level of

α -fetoprotein (AFP) [6], the main known predictive factors of recurrence are histological parameters retrospectively assessed on the full pathological specimen, such as poor degree of differentiation [7], presence of satellite nodules [8,9] or microvascular invasion [5,9,10]. In addition, composite prognostic models for HCC based on gene expression patterns in tumor and adjacent tissues predict early and overall recurrence and complete factors from clinical and pathological analyses [11,12].

Regarding percutaneous ablation, usual predictive factors of recurrence as tumor size, multinodularity, serum level of AFP and underlying cirrhosis-related parameters have been extensively studied and their predictive value confirmed [13]. The influence of the technical aspect of ablation on recurrence has also been addressed in several randomized trials comparing chemical techniques (mainly ethanol) with radiofrequency ablation (RFA), which has been found clearly superior in achieving complete tumor's destruction [14–16]. However, only few comparative data are available regarding efficacy of different energy-based ablation techniques including RFA technologies [17–19]. In contrast to resection, histopathological predictive factors of recurrence following percutaneous ablation have been rarely studied, due to the absence, in most studies, of a tumor biopsy sample. In line of this, no study investigating the impact of tissues gene expression on HCC outcome after ablation has yet been published to our knowledge.

The aim of this paper is thus to review the predictive factors for recurrence after percutaneous RFA of HCC published in the past years, trying to distinguish the influence of the technical modalities, of the tumor characteristics and of the state of the underlying liver disease.

Table 1 summarizes the main independent risk factors of hepatocellular carcinoma recurrence after local curative treatment that have been described.

Predictive factors of recurrence linked to technologies & techniques of RFA

Local ablation is considered the first-line treatment option for patients at early stages not suitable for surgical therapies. According to guidelines, monopolar percutaneous ablation methods are indicated in curative purpose in patients with compensated cirrhosis and small HCC (i.e., $\leq T2$ in TNM staging or within Milan criteria or less than three nodules, which size is inferior or equal to 3 cm in maximum

Figure 1. Schematic representation of two modalities of recurrences after local treatment of hepatocellular carcinoma. Local relapse, defined as tumor emergence directly at the margin of the initial treatment zone, is linked either to: the stage and the biology of the tumor; the technical and technological conditions of ablation; factors related to underlying liver disease. Distant relapse, defined as separated by nontumor liver, is related either to metastases (usually before the 2-year cut-off time) or *de novo* carcinogenesis (usually after the 2-year cut-off time) and mainly linked to: staging and the biology of the tumor; and factors related to underlying liver disease. HCC: Hepatocellular carcinoma.

Table 1. Independent risk factors of hepatocellular carcinoma recurrence after local curative treatment.

Risk factors of recurrence	Variables	Resection	Radiofrequency ablation
Tumor features			
Tumor number	Radiology/histology	Yes	Yes [23]
Tumor size	Radiology/histology	Yes	Yes [22]
Microvascular invasion	Histology	Yes	NA
Differentiation	Histology	Yes	Yes [22]
Satellites nodules	Radiology/histology	Yes	NA
Serum AFP	Biology	Yes	Yes [27]
Serum AFP-L3	Biology	Yes	Yes [28]
Serum DCP	Biology	Yes	Yes [29]
Serum VEGF	Biology	Yes	Yes [30]
ESM-1	Immunohistochemistry	Yes	Yes [26]
Glutamine synthase	Immunohistochemistry	Yes	Yes [37]
5-gene score	Molecular signature	Yes	NE [59]
G3 subgroup	Molecular signature	Yes	NE [56]
Proliferative subclass	Molecular signature	Yes	NE
Metastasis signature	Molecular signature	Yes	NE
Stem cell features	Immunohistochemistry	Yes	Yes [38,57]
Tumor portal thrombosis	Radiology/histology	Yes	NA
Underlying liver disease – related factors			
Child-Pugh class	Clinic and biology	Yes	Yes [41]
Prothrombin time/INR	Biology	Yes	Yes [39,40]
Bilirubin	Biology	Yes	Yes [26]
Hepatitis C seropositivity	Clinic	Yes	Yes [41]
HBV viral load	Biology	Yes	Yes [39,44]
AgHBs ⁺ and AgHBe ⁻	Biology	Yes	Yes [47]
Low platelet count	Biology	Yes	Yes [41,43]
Hyaluronic acid	Biology	Yes	Yes [44]
Age-platelets index	Clinic/biology	Yes	Yes [45]
186 genes signature	Biology	Yes	NE
Hepatic inflammation	Biology/histology	Yes	NE
Host factors			
Age	Clinic	Yes	Yes [27]
Neutrophil/lymphocyte ratio	Biology	Yes	Yes [52]
High visceral fat area	Clinic/radiology	No	Yes [51]
Treatment-related factors			
Anatomic vs nonanatomic	Treatment	Yes	NA
Surgical margin positive	Treatment	Yes	NA
Subcapsular location	Treatment/location	No	Yes
Adjacent vessel	Treatment/location	No	Yes [11]
Width of resection/ablation margin	Treatment	Yes	Yes [16]
PEI (vs RFA)	Treatment	NA	Yes [2–4]
Perfused electrode (vs internally cooled)	Treatment	NA	Yes [18]

AFP: α -Fetoprotein; DCP: Des- γ -carboxyprothrombin; HBV: Hepatitis B virus; INR: International normalized ratio; NA: Assessment not possible in radiofrequency ablation or resection; NE: Not evaluated in radiofrequency ablation; PEI: Percutaneous ethanol injection; RFA: Radiofrequency ablation.

diameter or with a single nodule inferior or equal to 5 cm in maximum diameter) [1].

• Influence of technical aspects of ablation on local recurrence

Monopolar RFA versus chemical ablation

Several concordant randomized trials have shown that RFA using monopolar technologies achieved a complete response in fewer ablation sessions and with lower local recurrence rate than chemical techniques using injection of ethanol or acetic acid [2–4]. Basically, the energy deposition-based ablation technique like RFA is more reliable than interstitial diffusion of chemical liquid at inducing homogeneous cell death in a given targeted tissue volume. Since its introduction in the end of nineties, monopolar RFA therefore became the most worldwide used technique for percutaneous treatment of liver tumors.

Monopolar RFA & other centrifugal radiating energy ablation techniques

Using monopolar technique, while complete necrosis at imaging 1 month after the procedure up to 97% has been reported in tumors ≤ 2 cm in diameter, it ranges usually from 62 to 83% on liver explants for HCC of < 3 cm in diameter and decreases to $< 50\%$ in tumor > 3 cm in diameter because of the heat loss due to perfusion-mediated tissue cooling within the area ablated. In addition, up to 20% of local recurrence rate has been reported during follow-up [5]. It is probably related to a trend to overestimate the treatment's response with early imaging and the decreased efficiency along tumor radius of centrifugal radiating energy method like RFA due to thermal energy dispersion. Several improvements of monopolar RFA technology have been carried out to push away the limit of maximum diameter that can be induced by applying a single cycle of energy [6]. Although comparative studies conducted in animal models have shown differences in shape and volume of ablation depending on kind of monopolar RFA device used [7], no effect has been demonstrated on the completeness of the ablation in clinical practice. Some operators, using other centrifugal radiating energy methods such as laser and first-generation microwave ablation (MWA) devices obtained similar results in term of local recurrence rate of HCC [8,9]. Recently it has been suggested that advances in MWA technology implemented in second-generation

devices could challenge monopolar RFA [10]. Indeed, the physical properties of MW energy allow higher and faster temperature rise, thus making liver ablation less sensitive to the cooling effect of tissue perfusion in experimental studies [11]. However treating up to T2-stage HCCs with second-generation MWA devices, authors reported in the few available clinical pilot studies a 5–20% local recurrence rate, similar in spite shorter follow up to those observed after monopolar RFA [12].

From centrifugal toward centripetal multibipolar radiating energy ablation techniques

In the rapid moving field of ablation energy-based technologies, data comparing the effectiveness of different techniques are scant. In this setting, liver transplantation performed after ablation gives unique opportunity to assess the completeness of ablation based on histopathological analysis of the whole explant. Rate of complete necrosis lower than 50% has been reported, regardless the type of monopolar RFA technology used for the treatment of tumor ≤ 5 cm [13]. Authors suggested that 3 cm could represent the cut-off diameter above which effectiveness of monopolar RFA declines. Three or even 2 cm is therefore usually regarded as the maximum diameter of the tumor allowing reliable complete destruction by centrifugal radiating energy methods as monopolar RFA because, to be curative, ablation must exceed the macroscopic boundaries of the nodule [14]. Centrifugal radiating energy ablation methods must ideally destroy a 2 cm margin all around the tumor to give patient the best chance of cure [15]. Up to now only no-touch multibipolar RFA, consisting to insert several electrodes outside the tumor then activate sequentially in bipolar mode each possible combination (centripetal radiating energy method), allows a reliable ablation of the nodule and its margins (Figure 2). Comparing effectiveness of monopolar RFA versus no-touch multibipolar RFA for the treatment of tumor ≤ 5 cm in diameter on the basis of histopathological analysis of explanted liver, we found that using this new method the complete necrosis tumor rate climbed from $< 50\%$ to almost 90% [16].

Tumor seeding along needle track

Theoretically, intratumor RFA and tumor biopsy are associated with a risk of neoplastic seeding

Figure 2. Diagrammatic representations of ablation areas induction with multiseparated applicators according the use of centrifugal (monopolar) or centripetal (multibipolar) energy deposition devices. In this instance, for a tumor of roughly 3 cm in diameter, three applicators positioning are required with both devices to achieve complete ablation of the tumor and a minimal 5 mm free margin. On the left, with monopolar centrifugal energy diffusion devices (monopolar radiofrequency ablation (RFA), microwave, cryotherapy and laser), three overlapped single ablation are needed (A). Ablations can be performed sequentially with a single applicator or simultaneously with three applicators. On the right, with multibipolar centripetal energy diffusion devices (multibipolar RFA or electroporation) the same results could be more reliably achieved by single-bloc ablation implying the simultaneous use of three applicators. Each possible pair's combination of applicators is sequentially activated in bipolar mode. As shown in the diagram, below a maximal tumor diameter depending mainly on the technical characteristics of the devices used this type of multibipolar technology allows 'no-touch' ablation consisting to insert applicators outside of the tumor. Thus the achievement of free tumor margin becomes even more reliable because the maximum amount of energy is actively deposited in the margin conversely to monopolar devices which induce ablation of the margin at the end stage of the procedure by passive rapidly decreasing energy diffusion from the center to the periphery. (B–E) : Case of typical complete ablation of small hepatocellular carcinoma (HCC; 3 cm) with proper free margin (1–2 cm) achieved with single no-touch multibipolar RFA. Before treatment, CT shows typical HCC (arrows) with at arterial phase (B, axial view) and washout at portal phase (C, frontal view). One month after treatment there is no more hyperenhancing tumor tissue at arterial phase (D, axial view) but the 'ghost' image of the nodule (arrows) within a large ablation area including a 1 cm minimal free margin even shows at portal phase in orthogonal plan (E, frontal view).

along the needle track especially in case of superficial lesion. However, a recent large study showed that preoperative tumor biopsy does not negatively influence the oncologic course of HCC patients eligible for liver transplantation [17]. Moreover, this risk could be avoided with 'no-touch' RFA.

• Potential influence of RFA technologies & techniques on distant recurrence

Differences in ablative technologies may influence not only the completeness of ablation but also subsequent local recurrence rate. As mentioned above, distant recurrence can either result from *de novo* carcinogenesis or metastatic

process. The more an ablative method allows effective and safe treatment of HCC, the less metastases from residual viable tumor or per operative tumor cells release may occur. Interestingly in a comparative study of two similar cohorts of patients with HCC treated with two different monopolar RFA devices, we found that distant recurrence rate was twice superior in one group whereas the local recurrence rate was similar [18]. In our opinion, the most robust hypothesis to explain this apparently paradoxical result was that one device using saline serum perfused electrode that may induce a dramatic intratumoral pressure promoting release of cells from the periphery of the tumor in blood circulation [19]. In this line, ablation methods avoiding breakage of the tumor, as achieved by no-touch multibipolar RFA, seem theoretically more prone than other centrifugal energy radiating techniques to decrease the recurrent rate.

Influence of imaging options on effectiveness of ablation

Regardless the ablation device used, imaging is mandatory at all steps of the percutaneous procedure, and technical choices and availabilities will impact the therapeutic results. Unfortunately this critical point has not been sufficiently addressed in literature. With the emergence of new imaging technologies as fusion, virtual guidance and specific liver contrast mediums, one can expect that these new tools will stimulate clinical research focused on this topic.

Predictive factors of recurrence linked to the tumor

• Size & number of nodules

The predictive value for recurrence of size and number of nodules have been extensively studied in patients with HCC treated with percutaneous ablation, mainly monopolar RFA. As mentioned above, there is an obvious relationship between size of the tumor and the local recurrence rate after ablation which strongly depends of efficacy of ablative technology used. Thus, beyond 3 cm diameter, the rate of incomplete ablation dramatically increases with monopolar RFA. Combination with intra-arterial chemoembolization has been suggested to palliate the loss of effectiveness of monopolar RFA for HCC above 3 cm in diameter [20,21]. More anecdotally monopolar RFA combined with chemical injection (alcohol, acid acetic) has

been investigated in same purpose. However, the benefit of this combination remains to be assessed when more efficient ablation techniques than monopolar RFA are used like multibipolar RFA. Indeed, by improving ablation technology, the tumor size threshold of efficacy could be increased and make useless resort to such combined therapy. In our experience with multibipolar RFA for the treatment of HCC up to 5 cm in diameter, the size of tumor was no more an independent predictive factor for incomplete necrosis, according to an explanted liver histopathological study [16]. Interestingly, we found in 108 patients treated with multibipolar RFA for HCC ≤ 5 cm in diameter that size of the tumor was positively associated with increasing incidence rate of distant recurrence whereas the 2- and 5-year local recurrence rates were 0 and 4%, respectively [SEROR O, UNPUBLISHED DATA]. The fact is that larger the HCC, higher is the probability of extra nodular spread out of tumor as microsatellite nodules or microvascular invasion [22].

As expected, multinodularity defined as more than two nodules showing typical characteristics of HCC at diagnosis appears as a robust independent predictor of distant recurrence after ablation [23]. On the other hand, it must be pointed out that ablation of multinodular tumor, in spite of higher incidence of distant recurrence, is not necessarily associated with a significantly worse survival [23,24]. The fact that high percentages of recurrences (local and distant) are eligible for repeated ablation procedures (35% in our experience) may account for that [23].

If size and number of HCC are associated with higher risk of recurrence, these parameters appeared too rough to assess aggressiveness of a given tumor and subsequently tailor a global treatment strategy including the choice of the ablation procedure. As for other treatments more accurate predictors are needed.

• Serum biomarkers pretherapeutic level

Serum AFP is one of the most useful and powerful independent predictive factor of HCC recurrence, but only in AFP-producing tumors [23,25,26]. As AFP is linked both to the tumor's aggressivity and to the inflammatory activity of the underlying chronic liver disease, AFP is theoretically associated either to local or distant recurrence of HCC. Besides baseline serum AFP level, incomplete decrease of serum level after RFA is an independent risk factor

significantly associated with tumor recurrence [27]. High serum levels of AFP-L3, an isoform of AFP, and of des- γ -carboxyprothrombin have been linked with a high risk of tumor recurrence in Asian studies including HCC developed on both cirrhotic and noncirrhotic liver treated by RFA [28,29]. However, the prognostic value of these two markers needs to be validated in homogeneous cohorts of cirrhotic patients with HCC in western countries.

A wide spectrum of other potential prognostic serum biomarkers remains to be validated after ablative therapy of HCC. The independent prognostic value of high pretherapeutic serum level of VEGF, demonstrated in 120 patients with HCC undergoing RFA, highlights the importance of tumor biomarkers as a prognostic predictors in the setting of ablative therapies [30]. Other serum biomarkers linked to HCC recurrence after surgical resection, such as osteopontin and periostin [31], remain to be assessed in patients with HCC treated with percutaneous ablation.

• Histo- & immuno-histopathological findings within the tumor

After resection of HCC, several tissular markers such as keratin-19 or EpCAM, indicating a biliary differentiation or a progenitor cell state [32,33], as well as osteopontin [34], have been related to a poor prognosis. However, the most powerful histological prognostic factor remains the presence of satellite nodules and microvascular invasion [35].

Percutaneous ablation allows taking a needle tumor biopsy sample, performed before ablation for diagnosis or during ablative procedure for prognosis. However examination of a small biopsy sample is not fully reliable for the detection of microvascular invasion or satellite nodules. Therefore, as developed in other non-surgically resected cancers or in neoadjuvant therapies, prognostic and predictive factors for response to therapy need to be specifically assessed in biopsy samples. As illustrated in **Figure 3**, two biopsies can be performed using the same needle track, and touch imprints can be immediately realized by apposing the sample on glass slides. One sample is fixed and paraffin embedded for histological, immunohistological and potential *in situ* hybridization analysis, and the other sample is frozen for subsequent transcriptomic and genomic analysis. Touch imprints allows to assess the percentage of tumor tissue in the frozen sample [36].

To our knowledge, only three studies have addressed prognostic factors from biopsy samples of patients who underwent RFA for HCC [26,37,38] and have reported scattered findings. In our study [26], including 150 patients with uninodular early-stage (BCLC 0/A) HCC, the expression of ESM-1 or endocan by endothelial stromal cells lining tumor cells (**Figure 4**) was independently related to recurrence after RFA, together with serum AFP level, while Edmondson grade and other markers such as keratin-19, EpCAM, β -catenin and glutamine synthase were not neither in univariate nor in multivariate analysis. Additionally, we have shown that ESM-1 expression was highly correlated to microvascular invasion in resected tumor samples. Thus, if validated in further studies, this microenvironment marker, reflecting an endothelial stromal cell switch to a more aggressive tumor, could offer hope for stratifying patients and help the decision-making process. Another study performed in Italy [37] investigated retrospectively the prognosis of 207 patients with HCC and available tumor biopsies who underwent RFA. By multivariate analysis, glutamine synthase expression by tumor tissue, that reflects an activation of the Wnt pathway, was correlated with reduced recurrence and specific mortality rates, together with post-treatment serum AFP level and number of nodules. Edmondson grade was not related to recurrence or survival. The third Japanese study [38] assessed keratin-19 expression in biopsy samples of 246 patients with HCC within Milan criteria and found ten patients (4%) with a positive immunostaining. Keratin-19 immunostaining was related to early recurrence (<1 year) and represented an independent risk factor for recurrent tumor status outside Milan criteria after RFA. In aggregate, if current histological Edmondson grade does not seem to represent an adequate prognostic factor in HCC biopsy samples of patients treated by RFA, several tissular markers such as ESM-1, glutamine synthase and keratin-19 have been shown to provide independent prognostic value that can be added to clinical, imaging and biological criteria. Larger multicentric studies of patients treated by RFA with preprocedural biopsies, are needed to precise more accurately the best combination of both tumor and microenvironment markers that will independently help to predict early recurrence.

Figure 3. Touch imprints of hepatocellular carcinoma biopsy samples. One tumor biopsy sample is fixed in formalin (1), paraffin embedded (2) and sections are used for histological and immunohistochemical analysis (3). A second tumor biopsy sample (5) is apposed on sterile glass slides (4) before being snap frozen in liquid nitrogen (5). After staining, cytological analysis of the touched imprints (4) allows to affirm the presence of tumor in the frozen sample.

Predictive factors of recurrence unrelated to HCC

Several factors unrelated to the tumor itself are independently associated with the distant recurrences of HCC and *de novo* carcinogenesis after percutaneous ablation.

• Factors linked to the underlying chronic liver disease

Most tumor-independent factors that correlate with higher HCC recurrence rate are related to the severity of the underlying chronic liver disease, which is already involved in first HCC occurrence in patients with uncomplicated cirrhosis, reinforcing the importance of the liver. Prolonged international normalized ratio over 3 s (hazard ratio [HR]: 3.39; 1.52–5.78; $p = 0.003$) [39,40] and Child-Pugh class B or C (HR: 1.23; 1.03–1.45; $p = 0.02$) [41] related to liver failure, and low platelet count $<100,000/\text{mm}^3$ (HR: 1.36; 1.12–1.64; $p = 0.002$) [27,41–43] related to portal hypertension, are independently associated with overall and distant recurrence of HCC after local treatment by ablative therapies. Moreover, high serum hyaluronic acid level (HR: 5.85; 1.37–21.69; $p = 0.01$) [44] and age-platelet

index (HR: 1.19; 1.01–1.39; $p = 0.03$) [45] were identified as independent predictors of intrahepatic distant or local recurrences, respectively, suggesting that underlying liver fibrosis is an important determinant of liver carcinogenesis.

In addition, the higher distant recurrence rate in patients with high serum level of alanine aminotransferase (HR: 1.01; 1.01–1.02; $p = 0.004$) [46] suggests that the inflammatory activity of the underlying chronic liver disease is involved in *de novo* carcinogenesis. Accordingly, a high serum AFP level, not only observed in tumors of high-grade malignancy but also in active chronic hepatitis without HCC, is a well-known risk factor of HCC occurrence in cirrhotic liver and is associated with distant recurrence after RFA and *de novo* carcinogenesis after ablative treatment (HR: 1.015; 1.014–1.016; $p = 0.015$) [23,25,26]. These facts highlight the potential benefit to control the cause of the underlying chronic liver disease after local treatment of HCC.

• Virological factors linked to the cause of underlying chronic liver disease

Beside factors related to the severity of the underlying chronic liver disease, Asian studies,

Figure 4. ESM-1 or Endocan immunostaining in hepatocellular carcinoma. (A) Several cells lining tumor hepatocytes showed a strong cytoplasmic staining (arrows). (B) At a higher magnification, the elongated shape of the cells along with their sinusoidal localization and small fusiform nuclei allow identifying positive cells as tumor stromal endothelial cells. Tumor cells were negative. (C & D) In our experience, no staining at all was observed in 60% of early-stage uninodular hepatocellular carcinoma.

including mainly HBV infected patients, showed the prognostic value for HCC recurrence after ablation of negativity for serum HBe antigen (HR: 0.47; $p = 0.026$) [47] and high preprocedural serum viral load ≥ 2000 UI/ml (HR: 2.67; 1.16–6.14; $p = 0.021$) [39,44,48]. The link between HBV replication and the recurrence of HCC after RFA suggests that secondary chemoprevention with nucleos(t)ide analogs could improve the prognosis following percutaneous ablation as it was already demonstrated for liver resection [49].

Conversely, very few data are available regarding patients with HCV infection after ablative therapy of HCC. In a large cohort of 1170 patients treated by RFA and prospectively followed up during a mean period of 38.2 months, Shiina *et al.* identified a positive status for serum anti-HCV antibodies as an independent factor associated with distant recurrence [41]. To our knowledge, no link between HCV

replication and HCC recurrence following percutaneous ablation has been established until now. However, we observed that sustained viral response to antiviral treatment was associated with a dramatic decrease of tumor recurrence rate and a high survival rate in the subset of patients with HCC and HCV-related cirrhosis [23]. Thus, as demonstrated after resection [50], eradication of HCV should improve the outcome of patients after ablative percutaneous treatment of HCC.

• Host factors

Age >65 years [27] and high visceral fat accumulation [51] are the main host-related factors independently associated with HCC recurrence after RFA. Lately, postoperative neutrophil-to-lymphocyte ratio change which was reported to predict survival of patients with small HCC undergoing RFA, could also be involved in tumor recurrences [52].

• Perspectives

Beside classical clinical and histological risk factors, analysis of biological profile from both tumor and nontumor tissues have emerged as strong predictors of tumor recurrence in patients with HCC treated by liver resection.

• Molecular signatures of the tumor & the nontumor liver

More than 20 molecular signatures from tumors have been identified in the last 10 years, including proliferative subclasses, G3 subgroups, metastasis signature and HCC with stem cells features [53–58]. Analysis of tumor biology was linked with early recurrence due to intrahepatic tumor metastasis. We recently reported a 5-gene score including *RAN*, *HN1*, *KRT19*, *RAMP3* and *TAF9* that predicts early recurrence and survival rates in 748 patients with HCC treated by liver resection worldwide, whatever the etiology and the underlying liver disease be [59]. This simple molecular signature predicted tumor recurrence and tumor-related death independently of classical clinical and pathological features and predicted prognosis more accurately than several other molecular signatures [59]. Conversely, molecular signatures from the surrounding cirrhotic liver predict late recurrence as a witness to *de novo* carcinogenesis on cirrhosis ('carcinogenic field effect') [60]. A 186-gene signature (also called the 'poor survival' signature) from nontumor formalin-fixed, paraffin-embedded samples of liver parenchyma was linked with late recurrence and survival rates in HCC patients treated by liver resection [54,60]. However, all these molecular signatures have been only reported in HCC patients treated by liver resection and, to our knowledge, their prognostic values in other curative treatment such as RFA or liver transplantation are unknown. In addition, only tumor biopsy will be available in patients treated by percutaneous ablation and the robustness of transcriptomic signature in small amount of material remains to be demonstrated. However supporting the feasibility, a recent study has validated the 186 genes signature in formalin-fixed needle biopsy samples of nontumor cirrhotic liver from patients with HCV-related cirrhosis [61]. The same proof of concept study has to be conducted in tumor biopsy from different clinical settings including percutaneous ablation.

• Metabolomic profiles

A wide spectrum of serum metabolomic biomarkers has been studied as potential

prognostic tools, but their ability to predict the patient's outcome after treatment is up to now not validated [62]. In addition, it has become obvious that the search for a single serum biomarker that can be used in clinical practice to manage patients with HCC is probably utopic. For example, metabolomics is able to identify and quantify metabolic changes within a biological system and, as in many other cancers [63], recent studies have attempted to describe the metabolic phenotype in patients with HCC using ¹H-nuclear magnetic resonance or mass spectroscopy. This has led to the identification of various metabolically impaired pathways in cancer cells, serum and urine [64–66]. Furthermore, more than a single measurement of a given compound in a biological fluid, the analysis of its variations at different time points in longitudinal cohorts after cancer treatment is emerging with interesting results [67]. Serum metabolomic profiling has been shown to correlate with the stage and potential aggressiveness of HCC [68]. Fingerprints associated with advanced HCC reflected the distinct activation or impairment of multiple biological pathways, mainly energetic metabolism involving glutamine/glutamate, ketone bodies and lipids. But more interestingly, the analysis of serum metabolic changes observed in patients with liver cancer eligible for curative procedures revealed particular profiles in specific subgroups that were similar to those of patients with advanced HCC. Noteworthy, longitudinal analysis revealed a worse outcome after RFA procedure in these patients with a higher rate of tumor recurrence and an impaired survival [68].

To conclude, percutaneous ablation represents an alternative to resection for treatment of HCC below to 5 cm in diameter in patients with cirrhosis. From a clinical point of view, further efforts are needed to define the best percutaneous ablation techniques adapted to each tumor type and to high-risk or difficult to treat location in order to improve local control and decrease tumor recurrence. In addition, new avenues for personalized clinical care of patients treated by percutaneous ablation will be opened soon. However, these new strategies and new tools need to be tested in a well-designed prospective fashion. The usefulness of tumor biopsy in this setting has to be reassessed in the future and histological material will be mandatory at least in academic clinical trials [69]. Finally, combining tailored treatment of HCC and of the

underlying liver disease will influence early and late recurrence rates as well as non-HCC liver-related deaths with a probably strong impact on survival.

Conclusion & future perspective

While monopolar RFA is hampered by a higher local recurrence rate compared with hepatic resection, no-touch multibipolar RFA allows a reliable ablation of the nodule and its margins.

Prediction of tumor recurrence using both classical clinical, histological and molecular features from both tumor and nontumor tissues will be useful to tailor treatment to the patient, its tumor and the corresponding cirrhotic liver [70]. Patients with a higher risk of early recurrence will be ideally included in a stratified adjuvant trial. In addition, prediction of tumor recurrence in patients treated in first line by RFA will be useful to guide sequential treatment strategy and proposed a 'de principe' liver transplantation before recurrence. Identification of patients with a higher risk of *de novo* carcinogenesis by analysis of nontumor liver could

advantageously define a subgroup of patients ideally included in a secondary chemoprevention trial [71]. The identification of patients with a higher risk of early and late recurrences will be particularly useful to design adjuvant and chemoprevention trial including a lower number of patients with a higher risk of clinical events. Stratification of patients and exclusion of low-risk patients will improve the ability to demonstrate a clinical meaningful difference in these trials [72]. Importantly, these patients with a low risk of tumor relapse will avoid the adverse events of a useless treatment.

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