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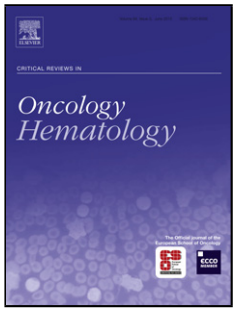


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# Clinical and circulating biomarkers of survival and recurrence after radiofrequency ablation in patients with hepatocellular carcinoma

**Short title: HCC survival and recurrence after radiofrequency ablation**

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## Abstract

Radiofrequency ablation (RFA) is an effective local treatment for curative intent in patients with cirrhosis of the liver and hepatocellular carcinoma (HCC) with diameter < 3 cm. Several meta-analyses have shown that RFA and surgical resection are comparable in terms of their impact on overall survival. The only clinical data available on markers that are predictive of recurrence and survival after RFA treatment are based on retrospective observational studies. Prospective randomized trials are thus needed to further research in this area.

In the present review we analyzed a number of clinical factors that are considered to predict recurrence or survival in HCC patients treated with RFA. We also discussed in detail the circulating biomarkers investigated to date, together with their potential to predict prognosis and recurrence after RFA therapy.

Overall survival rates of patients with HCC are significantly affected by liver function, defined as Child-Pugh class, high baseline serum alpha-fetoprotein levels, and the presence of portosystemic collaterals. However, the development of local tumor progression does not significantly affect overall survival. This result is achieved by the

effective therapies in patients who relapse after treatment with RFA. For this reason there is an urgent need to identify new circulating biomarkers.

**Keywords**

Clinical outcome, radiofrequency ablation, hepatocellular carcinoma, alpha-fetoprotein, microRNAs, PD-L1, nivolumab, tremelimumab, immunotherapy, circulating miRNAs.

## 1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide [1]. Traditionally, hepatic resection and transplantation are considered the treatments of choice for patient care and have led to great improvements in morbidity, mortality and long-term survival [2]. However, radiofrequency ablation (RFA) is emerging as an effective local treatment for curative intent in patients with cirrhosis of the liver and HCC with a diameter < 3 cm [3 4]. Several meta-analyses [5–7] have shown that RFA and surgical resection have a comparable impact on overall (OS) and recurrence-free survival (PFS) when patients have Child-Pugh class A liver function and a lesion < 3 cm [8].

RFA is also used as bridge to liver transplantation, as reported by Lu et al. [9] and Mazzaferro et al. who demonstrated the effectiveness of this clinical approach in two large case series with very low dropout rates (6% and 0%, respectively) [10].The majority of recurrences are distant rather than local. Although several studies have been carried out to identify biomarkers that could be used to predict cancer recurrence, specific pathological patterns remain to be explored.

### 2. Clinical factors of RFA survival

There are still no prospective randomized studies evaluating clinical factors that are potentially predictive of recurrence and survival after RFA treatment, and findings published to date are based on retrospective observational studies. As shown in Table 1, various factors have been considered to predict survival in HCC patients treated with RFA.

With regard to liver function, patients with Child-Pugh B disease [11–14] have an increased risk of death compared to those with Child-Pugh A [3,15–21]. The relative risk between Child Pugh B and A has been reported as around 2.26 and 5.39, respectively [15,18]. The severity of the underlying liver disease may also represent a risk factor for HCC development and recurrence, highlighting the role of liver function in hepatocarcinogenesis. Interestingly, N'Kontchou et al. reported that the response to antiviral treatment in patients with hepatitis C virus (HCV)-derived cirrhosis, which resulted in the restoral of normal hepatic function, was associated with a dramatic decrease in HCC recurrence and a higher survival rate. In a group of 35 HCC patients with HCV-derived cirrhosis given antiviral therapy, only 10 obtained a viral response. All patient that achieved a complete response after RFA treatment had without tumor recurrence.[3]

With regard to liver function, other factors of poorer prognosis include reduced albumin values [3,15,16,22–25], increased bilirubin levels [3,22,25,26], and portal hypertension [15–17,26]. However, data reported are somewhat contradictory [3,13–17,19,21–29], probably due to different methods of patient enrollment in the various studies. In particular, when lesion size is considered as a continuous variable, there is often no evidence of a poorer prognosis if size increases. In studies in which lesion size was dichotomized over 2 cm, results were positive, confirming that RFA is less effective for lesions > 3 cm.

The percentage of patients with non-solitary nodules treated in the various studies is an important factor. From an analysis of negative studies [14,15,19,23,25,29], it emerges that about 15% of enrolled patients had multiple lesions, whereas a higher number of patients with multiple nodules were recruited (> 20%) showing positive results in univariate analyses [3,13,16,17,22,24,30].

Concerning local tumor progression, with the exception of N'Kontchou's study [3] where the hazard ratio (HR) was 0.53, patients enrolled in various studies with local tumor progression all had a poorer prognosis [3,12,15,16]. These findings are undoubtedly correlated with radiofrequency rescue therapies which have a strong impact on survival (orthotopic liver transplantation, surgery, transarterial chemoembolization [TACE]), but are also linked to systemic therapies). Finally, parameters such as sex [3,11–19,21–24,27–30] and hepatitis B virus (HBV) [19,22,24–26,31] or hepatitis C virus (HCV) [21,22,25,26] positivity do not seem to affect post-RFA survival. Moreover, as elucidated above, the response to antiviral treatments led to a lower recurrence rate and better survival.

### 3. Clinical factors of RFA recurrence

As previously mentioned, there are still no randomized clinical trials that have evaluated and validated clinical factors potentially predictive of post-RFA recurrence. Starting from the analysis of various studies (Table 2), the size and number of lesions are crucial parameters for predicting recurrence in the majority of cases, in contrast to what is reported for survival. This is attributable to the fact that, when more than one tumor is observed in the liver, intrahepatic metastases or multicentric HCC are more likely to occur. The few negative studies that considered these 2 parameters had patients with early-stage disease or solitary tumors. In fact, the median tumor size was around 2 cm at baseline. In this patient setting, RFA represents a promising strategy to control local

disease. Parameters such as sex and HBV or HCV positivity do not seem to affect post-treatment recurrence.

## 4. Circulating biomarkers of RFA recurrence

Several investigations have been carried out to identify which biomarkers markers could be useful to predict cancer recurrence, but specific pathological patterns have yet to be elucidated. We will now focus on the circulating biomarkers investigated to date and discuss their potential to predict HCC prognosis and recurrence after RFA therapy. As shown in Table 3, various factors have been considered.

## 5. Alpha-fetoprotein, Des-γ-carboxy prothrombin and Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein

Alpha-fetoprotein (AFP) was one of the first markers to be investigated for the diagnosis and prognosis of HCC and is still widely used in clinical practice because it is a simple and inexpensive test. However, given that AFP also has a clinical significance in patients with non-cancerous hepatic disorders, such as chronic hepatitis, cirrhosis or massive hepatic necrosis, [32–34] it is not considered to be a completely reliable marker for HCC diagnosis. Conversely, it is believed to be predictive of HCC recurrence [35–37] . In a study by Siripongsakun et al., AFP levels in the pre-RFA sera of 146 patients were tested as a diagnostic tool using the same cutoff as that used to predict HCC recurrence (20 ng/mL), revealing a sensitivity of 72.2% and a specificity of 56% [38]. Considering modified criteria of AFP ≥100ng/mL and alanine aminotransferase ≥40 U/L, sensitivity in detecting recurrence reached 100%, with a specificity of 85%. In this setting, the role of AFP as a circulating biomarker of HCC recurrence after RFA treatment has been investigated, showing conflicting results. Pre- and post-RFA serum levels of AFP were

assessed in a large case series of HCC patients undergoing RFA, demonstrating a significant association between low circulating amounts of AFP (≤20 ng/mL) and time to recurrence (HR: 1.995; 95% CI, 1.476-2.697), but not a significant benefit in OS[39]. In another study, AFP levels ≥ 20 ng/mL proved to be a prognostic factor of early recurrence, within 6 months after treatment (HR: 3.02; 95% CI, 1.69–5.38), while an AFP level <20 ng/mL was a marker of favorable OS (HR: 2.90; 95% CI, 1.47–5.74) in patients undergoing RFA after TACE failure [40]. Moreover, a decrease of >20% in AFP serum levels after RFA therapy strongly influenced the 5-year survival rate and tumor recurrence in a subgroup of patients with pre-treatment serum levels of AFP ≥100 ng/mL [41].

Conversely, AFP baseline sera levels did not distinguish between 98 HCC patients who relapsed after RFA treatment and those who did not [42]. Furthermore, another study did not observe a significant correlation between AFP serum levels and short-term outcome despite a sharp decrease in this marker after RFA therapy [43]. The role of AFP in evaluating recurrence after RFA is still subject to debate and at present there are no indications to perform it systematically. Randomized studies are needed to evaluate the real predictive role of AFP.

Des-γ-carboxy prothrombin (DCP) is a form of prothrombin produced by cancerous hepatocytes [44]. As the protein is linked to neoangiogenesis, it is thought to play a role in HCC recurrence. In a case series of 1057 patients treated with RFA, DCP levels >200 mAu/mL proved to be the strongest indicator of vascular invasion at the 1-, 3-, 5-year follow-up (HR: 3.24, 95% CI, 1.90–5.51 ) [45]. In another study on 10-year outcome after RFA, DCP serum levels >400 mAu/mL were found to be an independent factor related to tumor local progression [46]. Of note, a recent study identified pre-ablation serum DCP levels ≥40 mAu/mL as an independent risk factor for OS, while the same levels post-

ablation were a risk factor for both OS (HR: 3.438; 95% CI, 1.331-8.877) and DFS (HR: 4.934; 95% CI, 2.76-8.81) [39]. Another study found that pre-treatment DCP serum levels ≥40mAu/mL were predictors of time to recurrence, as were serum levels of AFP ≥50 ng/mL and a prothrombin time of <70% [47]. These data were later confirmed in other studies in which pre-treatment DCP serum levels <100AU/mL were identified as predictors of prolonged OS (HR: 5.49; 95% CI, 2.23-13.5) time to recurrence (HR: 6.82; 95% CI, 3.49-13.3) [48].

Lens culinaris agglutin-reactive fraction of AFP (AFP-L3) is a fucosylated isoform of AFP with higher binding affinity to lectin Lens culinaris agglutinin [49]. It is still not used in clinical practice, even though it has been shown to improve sensitivity and specificity of diagnostic tools when used in combination with AFP and DCP [50].

With regard to HCC recurrence after RFA treatment, an AFP-L3 serum level >15% both pre- and 2 months post-RFA was a strong indicator of tumor recurrence (HR: 4.25; 95% CI, 1.42–12.74), while pre-ablation and post-ablation levels of >15% and ≤15%, respectively, were not [51]. Another study comparing the clinical values of AFP-L3, AFP and DCP in the serum of 124 patients undergoing RFA showed that AFP-L3 was the most reliable marker for OS and time to recurrence [52]. Serum levels of AFP-L3>10% prior to RFA therapy have also proven to be an independent risk factor for local tumor progression and OS after RFA (HR: 2.94; 95% CI, 1.09–7.94) [53]. Ueno et al. found that high levels (>20 ng/mL) of pre-RFA AFP serum levels were predictive of poorer OS in 160 patients undergoing RFA, but only in association with high levels of Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) and des-γ-carboxy prothrombin (DCP) (OR: 1.78; 95% CI, 1.16-2.72) [54], casting doubt on the accuracy of previous data that showed a significant association between AFP-L3 ≥10% and lower

recurrence-free survival, but not between AFP and recurrence or between the three markers and recurrence [55].

AFP, AFP-L3 and DCP are the most widely investigated circulating biomarkers in hepatocellular carcinoma and have been shown to be correlated with diagnosis and clinical outcome. Conversely, these markers have not proven very reliable in predicting recurrence after treatment for HCC, in particular RFA. Of the 3, DCP has the greatest predictive potential, but still lacks validation and an objective cut-off value.

## 6. Immune response biomarkers

Radiofrequency is recognized as an acute inflammation-inducing treatment because it directly destroys tumor cells, resulting in a release of tumor-associated neo-antigens (TAA). In fact, an increase in circulating markers such as histones, myeloperoxidase (MPO), inflammatory cytokines (IL-1β, IL-6, IL-10, TNF-α), and markers of liver damage such as ALT and AST have been found in the plasma of patients 24 hours post-RFA [56,57]. In this setting, several immune response markers have been investigated as potential prognostic tools. Given the role of heat shock proteins (HSPs) in chaperoning tumor peptides, this class of proteins are known to have an adjuvant effect and to act as an alarm for anti-tumor T cell-mediated immunity. In a study of patients with different malignancies including HCC, pre- and post-RFA serum levels of heat-shock protein-70 (HSP70) were analyzed and correlated with outcome. An increase in HSP70 levels was transiently detectable one day after RFA treatment, and patients with a ≥2-fold increase in HSP70 had better clinical outcome, possibly due to the immune response activated through the tumor peptides carried by HSPs [58].

An *ex-vivo* study investigated TAA T-lymphocyte response after RFA treatment, detecting them in 62.3% of analyzed patients. The number of TAA-specific T-cells

post-ablation was correlated with RFS, whereas those pre-ablation were not [59]. The aspartate aminotransferase-to-platelet ratio index (APRI) >1.38 was recently identified as an independent risk factor for tumor recurrence after RFA treatment (HR: 2.64, CI 95%, 1.488–4.714) [42], while a baseline neutrophil-to-lymphocyte ratio (NLR) > 2.4 was previously associated with poorer OS but was not significantly related to relapse. In contrast, NLR > 2.4 post-RFA was found to be predictive of both OS and time to recurrence [19]. Moreover, an activated phenotype of NK cells was detected in the blood of patients after RFA, with a higher secretion of interferon-γ (IFNγ). Interestingly, when patients were divided into two subgroups, *i.e.* high *vs.* low IFNγ production and cytotoxicity, both parameters were associated with disease-free survival [60]. These data suggest that the immune system response to RFA treatment and tumor-derived neo-antigens may be predictive of recurrence after RFA treatment.

Of note, a recent study associated an increase in plasma C-X-C motif chemokine 10 (CXCL10) with earlier recurrence of HCC, finding a significant correlation between time of recurrence and CXCL10 plasma levels at baseline, 1 and 7 days after RFA[61]. C-reactive protein (CRP), a marker of systemic inflammation related to various diseases including HCC, has also been investigated, Fujiwara et al. observing that patients with CRP pre-treatment circulating levels >0.08mg/dL had early recurrence after RFA and worse OS with respect to patients with CRP circulating levels >0.08mg/dL [62].

A study by Shi et al. evaluated the expression of programmed-death ligand 1 (PD-L1) in patients with liver metastasis from colorectal cancer (CRCLM) before and after RFA treatment prior to surgery. The authors confirmed an increased T-cell infiltration in the tumor microenvironment of patients who underwent RFA compared to those who did not, with a higher CD8/CD4 ratio. Interestingly, they also found increased levels in PD-

L1 expression in both cancer cells and immune cells associated with RFA with respect to the tumor microenvironment of the non-RFA group. In particular, a comparison between pre- and post-ablation levels of PD-L1 in RFA patients revealed an increase in the expression of the protein in cancer cells and immune cells that was correlated with the ablation therapy [63]. PD-L1 expression has been shown to be predictive of response to anti-PD1 antibodies [64] and a phase I/II study highlighted durable responses and good safety profiles of anti-PD-1 Nivolumab monotherapy in HCC patients, including those with HBV and HCV [65]. A pilot study explored the safety and feasibility of Tremelimumab in combination with locoregional therapies aimed at promoting immunogenic tumor cell death in 18 patients (8 underwent TACE and 10 RFA) with advanced-stage HCC after refractoriness/intolerance to sorafenib. This combined therapy proved safe and, of the 10 patients evaluable for response outside of TACE or RFA treated lesions, 4 achieved confirmed partial objective response and post-treatment tumor biopsies showed immune cell infiltration in all evaluable patients. [66] To date, several trials testing safety and efficacy of immunotherapy are ongoing in advanced disease, and we think that in the future, immunotherapy can be a useful treatment in patients underwent RFA (NCT03383458).

Another study investigated clinical response in 62 HCC patients treated with RFA followed by cellular immunotherapy or RFA alone, reporting significant benefits in time to recurrence, OS and risk of local tumor progression (HR=0.136; 95% CI, 0.049–0.379) in the group that underwent the combined therapy [67]. Given that tumor cells are capable of immunomodulating the tumor microenvironment, and that RFA is an immune-related treatment, it might be useful to investigate the role of immune targeted-therapy as an adjuvant to RFA. In fact, apart from local inflammation, RFA results in a release of neo-TAAs into the blood circulation that are recognized by antigen-

presenting cells (APC) and presented in secondary lymphoid organs through major histocompatibility complexes to cytotoxic T lymphocytes for priming and activation. Activated tumor-specific CTLs recognize and attack tumor cells, enhancing the anti-tumor action of RFA (Fig.1). In this setting, combining these therapeutic approaches could enhance the immune response against tumor neo-antigens, blocking the mechanism of tumor immune-editing to obtain a restored and continuous action of the cancer-immunity cycle [68].

## 7. microRNAs

Interesting research has been carried out into microRNAs (miRNAs), small non-coding RNAs involved in several physiological and pathological patterns, including carcinogenesis. Several studies have identified tissue and/or circulating miRNAs for HCC diagnosis, prognosis and recurrence after locoregional treatment [69–72]. Conversely, few significant results are available on the relation between miRNAs and HCC recurrence after RFA treatment. Plasmatic expression of miR-122 (>100) prior to RFA was associated with poorer OS in a cohort of 57 HCC patients [73], while low plasmatic levels of mir26a and 29a were correlated with worse DFS (HR=1.72; 95% CI, 1.04–2.83 and HR=1.75; 95% CI, 1.04–2.94, respectively) in patients treated with surgical resection or RFA [74].

Epithelial-to-mesenchymal transition (EMT) has also been proposed as a resistance mechanism in HCC recurrence. Low expression levels of 200c and miR34a were found in bioptic tissue samples of 10 HCC patients who relapsed after RFA compared to 78 who did not undergo RFA, suggesting that EMT-related markers might be useful to predict poorer prognosis and aggressive local recurrence after RFA treatment [75].

Moreover, a fascinating scenario is that involving the miRNAs as therapeutic targets; miRNA-based therapy takes advantege of two main strategies: the use of miRNA antagonists (antagomiR or antimiR) to block oncogenic miRNAs, or miRNA replacement, to restore lost function of oncosuppressor miRNAs [76]. For example, the therapeutic potential of miR-122, miR26a and miR-124 has been developed in mice, and there is a need to further investigate their potential role as miRNA mimics [77]. Several tissue miRNA profiles have been evaluated in relation to tumorigenesis, cancer development and metastasis [78]. Disrupting the tumor cells, RFA treatment renders evaluable tissue biomarkers at circulating level, and given the rising importance of miRNAs in HCC therapeutics, finding and targeting such biomarkers in the blood circulation to predict recurrence could be very important in the management of disease. Given the acknowledged role of miRNAs in HCC diagnosis and prognosis, it would seem logical to evaluate their potential as a prognostic and therapeutic tool to predict recurrence after RFA.

## 8. Other studied biomarkers

A study by Ma et al. focused on γ-glutamyltranspeptidase (GGT), an enzyme related to extracellular oxidative stress, high circulating levels of which are usually found in HCC patients. Results showed that elevated serum GGT levels before RFA treatment correlated with worse OS and time to recurrence when compared with normal serum GGT levels [79]. Furthermore, pre-RFA levels of serum ferritin <244 ng/mL, a marker of iron accumulation and consequent hepatic damage and carcinogenesis, were found to be predictive of better OS and longer time to recurrence [18]. Although serum levels of vascular endothelial growth factor (VEGF) have not shown increased levels when measured at baseline, 2 or 5 days after radiofrequency ablation [80], pre-RFA circulating

VEGF levels >240 pg/mL have been found to be associated with worse OS in HCC patients and time to recurrence [81]. In this setting, further research into angiogenic factors as predictors of post-RFA recurrence are warranted given that RFA exhibits a lower angiogenic effect than TACE [82].

## 9. Uninvestigated potential biomarkers

To date, no studies have been carried out about the potential of long non-coding RNAs (lncRNAs) to predict recurrence after RFA treatment. It could be interesting to test these markers in patients undergoing RFA treatment, given that the dysregulation of many circulating lncRNAs have been correlated with HCC onset and development. For example, HULC (highly upregulated in liver cancer) has been found up-regulated in liver cancer tissues compared to non-cancerous tissues [83]; high expression of HOTAIR (HOX transcript antisense RNA) has been correlated with poorer prognosis and disease recurrence after surgery [84] in HCC patients and MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) has been related to cell proliferation and migration, and it has been described as a potential diagnostic and prognostic biomarker for HCC patients [85,86].

Moreover, there is a growing interest about the role of circulating microvescicles, that contain a plethora of biomarkers that could be potentially useful in cancer diagnosis, prognosis and therapeutic monitoring. Protected by the vescicle membrane, and less exposed to RNAses, intravescicular miRNAs and RNAs are more stable than circulating miRNAs and coding RNAs themselves, and, as a consequence, more reliable markers. For what concerns HCC, most of studies focused on the transcriptome of the exosomes, a particular class of microvescicles, underlying the clinical significance of several miRNAs [87,88]. In this setting, it could be useful to investigate the potential of circulating

microvescicles as a non-invasive tool to test new biomarkers in monitoring response to therapy and in predicting disease recurrence after RFA treatment. Techniques such as massive parallel sequencing for RNA (RNASeq) could be very useful to this aim.

As a non-invasive and low cost tool, the approach of metabolomic could be an intriguing research field. As most of compounds pass through the liver after intestine absorption, this organ is a key regulator of many circulating metabolites, empowering the possibility to relate their serum levels to liver physiopathology. The small bio-compounds (usually <1kDa molecules) profiles in chronic liver diseases including HCC have been investigated by NMR , but still there are no biomarkers able to predict time to recurrence after locoregional treatments that could be translated to clinical practice [89]. The same potential could be expected by proteomics approaches, *e.g.* mass spectrometry (MS), useful in identifying new circulating biomarkers related to liver physiopathology. Most of studies in HCC highlighted proteomic plasmatic and serum signatures of pathways related to immunity, iron metabolism and homeostasis, apoptosis and cell degeneration/regeneration [90]. These biomarkers profiles could be used in combination to AFP, AFP-L3 and DCP to empower the sensitivity in monitoring tumor progression and recurrence after RFA treatment.

In the field of liquid biopsy, increasing interest is focusing on circulating DNA (ctDNA). The analysis of this biomarker could be carried out with two principal aims: quantitative evaluation, and qualitative changes. The quantitative analyses evaluate changes in ctDNA burden in plasma/serum of patients, and there are data correlating ctDNA quantity with poorer prognosis of patient with HCC [91–93]. Methylation patterns at CpG sites of oncosuppressor genes occurs frequently in HCC, and these qualitative changes are the most investigated at a circulating level[94,95]; in particular, methylation status predicts metastastasis or recurrence of disease[96] and poor prognosis[97]. More

recent studies are focusing on genome-wide methylation technologies for ctDNA, and a study by Wen et al. identified a 4 gene signature for diagnosis of small nodules (<3 cm)[98]. It could be important to test this molecular profile to diagnose also early disease recurrence. Moreover, it has been proved that presence of ctDNA is predictive for portal vein invasion and extra-hepatic metastases in patients undergoing hepatectomy or liver transplantation[99]. Still there are no data on the potential of ctDNA in prediction of disease recurrence of patients with HCC after RFA, and this biomarker could be a non-invasive tool to use in clinical practice to predict prognosis and recurrence in patients with HCC treated with RFA.

## Conclusions

In conclusion, RFA is an effective first-line treatment modality for patients with early-stage HCC, obtaining good overall survival rates. OS is significantly affected by liver function, defined as Child-Pugh class, high baseline serum AFP level, and the presence of portosystemic collaterals, but not by long-term potentiation does New circulating biomarkers are thus urgently needed as non-invasive tools to monitor response to treatment and to detect HCC recurrence after RFA treatment.

**Disclosure Statement**

The authors declare no conflicts of interest.

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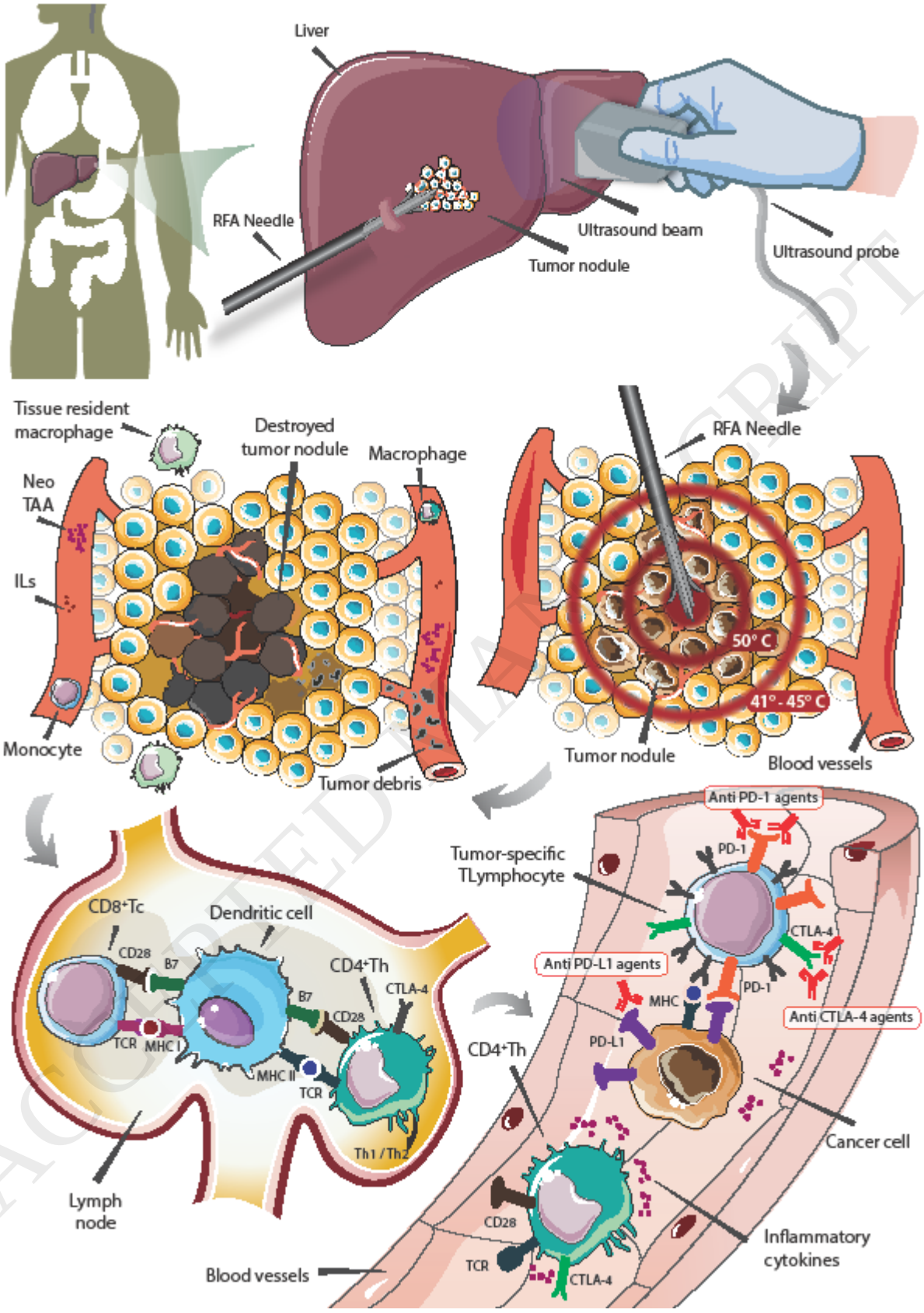
## Figure and table Legends

**Fig.1.** Radiofrequency ablation (RFA)-induced release of neo-tumor-associated antigens (neo-TAAs) and immune system response. **A)** RFA needle is inserted into the tumor mass. **B)** Activation of current generates frictional heating around the electrode. **C)** Destruction of tumor nodule, with subsequent release of neo-TAAs and tumor debris into the local blood circulation where inflammatory infiltrate accumulates (macrophages, natural killers, neutrophils and T lymphocytes). **D)** Neo-TAAs are drained to secondary lymphoid organs through afferent lymphatic vessels, stimulating immature dendritic cells to activate T cells. **E)** Activated tumor-specific T-cells enter the blood circulation and kill tumor cells. Immune co-stimulators and immune checkpoint inhibitors have been proposed as potential adjuvant therapies with curative intent ILs, interleukins.



**Tables:**

Table 1. Graphical aspect on overall survival of HCC patients after RFA. For Material and methods show supplementary materials

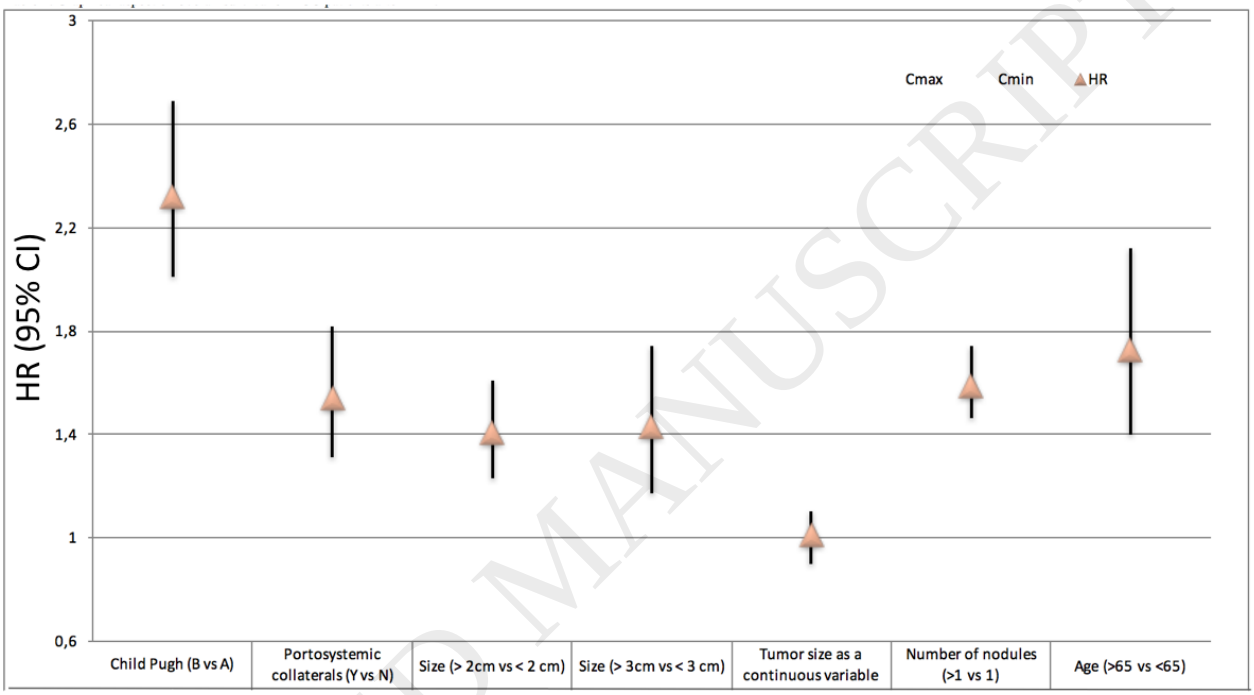


Table 2. Graphical aspect on recurrence free survival of HCC patients after RFA. For Material and methods show supplementary materials

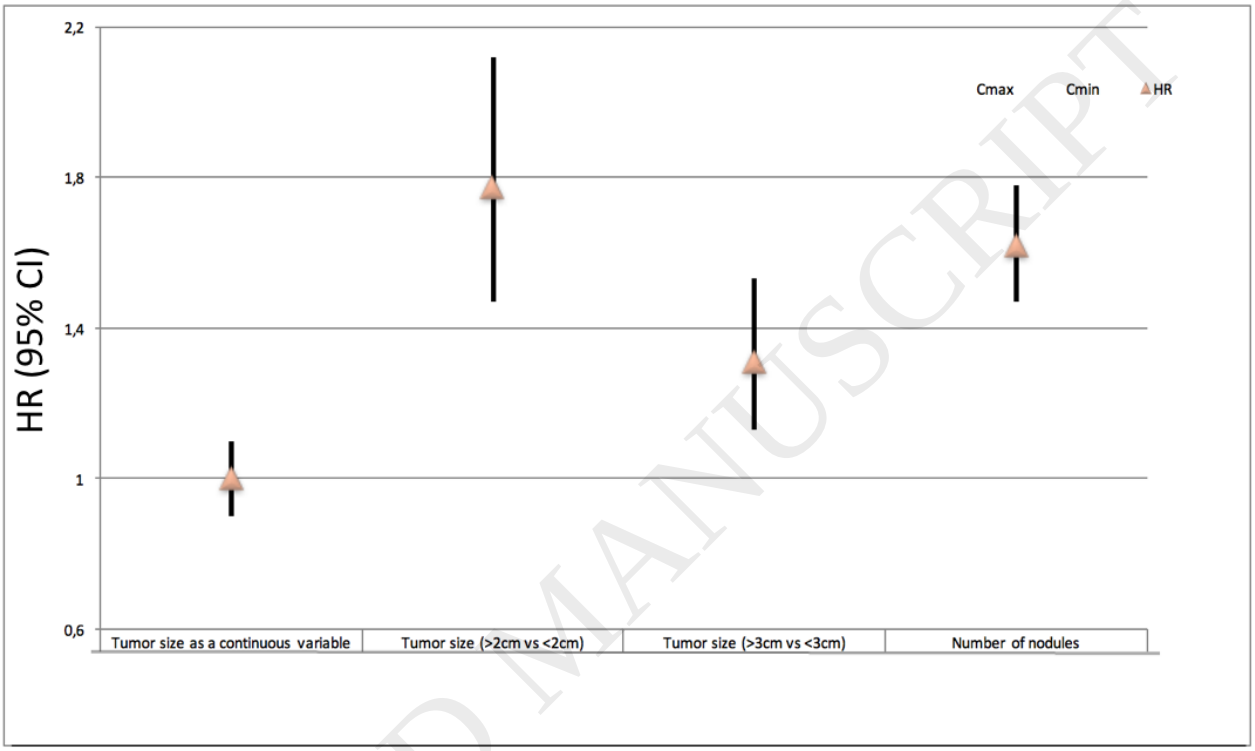


Table 3. Circulating biomarkers investigated to date for HCC recurrence after RFA

| Circulating biomarker | Category | Application | RFA recurrence | Reference |
|---|---|---|---|---|
| AFP | Serum glycoprotein | Diagnosis and prognosis | <20ng/mL post-RFA treatment (P<0.001) | 39 |
| AFP-L3 | Serum glycoprotein | Diagnosis and prognosis | ≥20 ng/mL levels predicted early recurrence (HR 3.02)<br><20ng/mL levels predicted better OS (HR 2.9) | 40 |
| | | | >100 ng/mL levels reached 100% sensitivity and 85% specificity in predicting recurrence | 38 |
| | | | >20% decrease starting by >100ng/mL pre-RFA treatment associated with better DFS and OS | 41 |
| | | | Pre-treatment levels did not discriminate between recurrent and non-recurrent patients | 42 |
| | | | >20ng/mL levels only predicted OS in association with AFP-L3 and DCP | 44 |
| | | | >15% pre- and post-RFA was an indicator of HCC recurrence (HR 4.25) | 48 |
| DCP | Enzyme secreted by cancerous hepatocytes | Diagnosis and prognosis | <40 mAU/mL post-RFA predictor of better OS (P=0.002) and RFS (P<0.001) | 39 |
| | | | More reliable than AFP in predicting DFS and OS | 49 |
| | | | >10% pre-RFA was an indicator of tumor progression and poor survival; HR 2.94 | 50 |

| | | | | |
|---|---|---|---|---|
| | | | >400 mAU/mL levels were predictors of tumor local progression | 53 |
| APRI | Aminotransferase-to-platelet ratio index | | ≥40 mAU/mL levels were predictors for OS and DFS | 39 |
| | | | ≥40 mAU/mL levels were predictive of intrahepatic distant recurrence (p=0.006) | 54 |
| | | | <100 mAU /mL levels were associated with prolonged OS (HR 5.49) and RFS (HR 6.82) | 55 |
| | | | >1.38 was predictive of tumor recurrence (HR 2.64) | 42 |
| NLR | Neutrophil-to-lymphocyte ratio | Tumor inflammation compared to host immunity | >2.4 was a predictor of recurrence (p=0.01) and poorer OS (p=0.006) | 19 |
| GGT | Serum biomarker | Extracellular oxidative stress | OS survival (P=0.001) and recurrence (P=0.001) | 76 |
| Ferritin | Serum biomarker | Iron accumulation and liver damage | <244 ng/mL levels predicted better OS (P<0.001) and a longer time-to-recurrence (P<0.001) | 18 |
| miR122 | Serum miRNA | Related to hepatocarcinogenesis | Expression >100 predicted poorer OS (P=0.042) | 73 |
| mir26a, miR29a | Serum miRNA | Tumor suppressors in carcinogenesis and metastasis | Low expression predicted poorer DFS (HR 1.72, HR 1.75) | 74 |
| miR200c, miR34a | Tissue miRNA | EMT inhibitors | Low expression associated with relapse (P=0.04) | 75 |

HCC, hepatocellular carcinoma; RFA, radiofrequency ablation; HR, hazard ratio; RFS, relapse-free survival; OS, overall survival; DFS, disease-free survival; AFP, alpha-fetoprotein; AFP-L3: lens culinaris agglutinin-reactive fraction of AFP; DCP: des-γ-carboxy prothrombin; GGT, γ-glutamyl transpeptidase; EMT, epithelial-mesenchymal transition