Plasma vascular endothelial growth factor and serum Alpha Fetoprotein as predictors of response of Hepatocellular carcinoma treated by Radiofrequency ablation

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Received 12 May 2019 Accepted 29 August 2019

Abstract

Aim: to investigate whether plasma vascular endothelial growth factor (VEGF) and serum Alpha-fetoprotein (AFP) could be used to predict short term response of hepatocellular carcinoma (HCC) treated by radiofrequency ablation (RFA). Methods**:** Seventy-two patients with confirmed diagnosis of HCC patients were treated by RFA. Triphasic CT Abdomen in addition to assay of serum AFP and plasma VEGF were done before and six weeks after procedure. **Results**: There is a significant difference between pretreatment and post treatment AFP values among complete responder group with highly significant drop in follow up post treatment AFP in the responder patients (P value 0.009). There is a significant difference between pretreatment and post treatment VEGF value among the non-responder's group with significant rise in VEGF values in post treatment VEGF (P value =0.002). **Conclusion:** Decrease in post RFA AFP value compared to pretreatment value significantly indicates response to treatment while increase in post treatment VEGF value compared to pretreatment value significantly indicates nonresponse to RFA.

Keywords: Vascular endothelium growth factor (VEGF), Percutaneous Radiofrequency Ablation (RFA) , Hepatocellular Carcinoma (HCC).

INTRODUCTION

Primary hepatic carcinoma is the fifth frequent malignancy and the second most common ethology of cancer-related mortality globally (1). It is the most common primary liver tumour and constitutes a major global health problem especially in areas where chronic hepatitis C is endemic like Egypt (2). Treatment approaches for HCC depends mainly on tumour status, liver functions and health performance status. Up till now, surgical removal of the tumour is the first-line definite treatment, but unfortunately it is frequently not possible due to severity of the underlying cirrhosis and portal hypertension (3).

Living donor Liver transplantation (OLT) is a modality which could treat both the tumour and underlying hepatic dysfunction and had shown very promising survival rates in those at an early stage of HCC (4). However, its main limitation is the lack of donor tissue that make the waiting time for transplantation over one year in Europe and the United States (5). Radiofrequency ablation (RFA) had replaced percutaneous ethanol injection therapy (PEIT), for the ablation of small HCC foci. RFA is considered one of the curative lines for early HCC when surgery is Not feasible or contraindicated (6). It is a relatively safe procedure and efficient method with satisfactory results, and we could repeat it in case of local HCC recurrence. The 5-year survival rate after RFA is better than percutaneous ethanol ablation (7-8) Several serological markers were

studied to determine their value in the diagnosis and prognosis of HCC. The most important of them was Vascular endothelial growth factor (VEGF) which have role in HCC angiogenesis and was shown to has a role in tumour progression and microvascular invasion. It showed high sensitivity among the serum tumor markers for detection of HCC (9)

Different factors could influence the response of HCC to RFA, mainly tumour size. One the unmet needs in the field of HCC research are to assess the role of prognostic and predictive markers in interventional therapies within prospective investigations This might help in the selection of patients who will benefit from this treatment modality (10). So, we designed this study to evaluate the role of measurement of plasma VEGF in predicting short term outcome of HCC patients to RFA and if Post treatment VEGF values could replace contrast-based imaging for determination of treatment response.

Patients and Methods

Study design

This study was conducted in Beni-Suef University hospital in the period between February 2017 to October 2018. Seventy-two patients with confirmed diagnosis of HCC were enrolled. Those patients were treated by percutaneous radiofrequency ablation in the Interventional Ultrasonography Unit, Tropical Medicine Department. A written formal consent was taken from each patient before performing the procedure.
All subjects were subjected to full history taking, Complete general and local examination. Liver function tests, kidney function tests, hepatitis markers and PCR for HCV RNA were done.

Diagnosis of Hepatocellular carcinoma: HCC was diagnosed was by Abdominal ultrasound and Triphasic CT abdomen. Typical CT finding was contrast uptake in arterial phase and wash out in venous or delayed venous phase.

Inclusion criteria: 1-Patients with multinodular HCC or single focal lesion more than 3 cm. 2-Child Pugh score A or B liver cirrhosis. 3- INR less than 1.7 and platelet count more than 50,000/cmm. **Exclusion criteria:** 1-HCC with vascular spread (portal vein thrombosis), lymph node metastasis or distant metastasis.2-Subcapsular lesions or lesions with close vicinity to the gall bladder, bowel, or portal vein. 3-Clinically decompensated liver disease (Child Pugh score C liver cirrhosis)

Assay of serum AFP and plasma VEGF: Serum AFP and plasma VEGF were measured for the HCC patients before the procedure and six weeks after. Blood was taken into an EDTA tube for plasma analysis of VEGF and into serum separator tubes or clot-activator tubes for serum analysis of AFP. Samples were centrifuged at 3,000 r/min for 10 min within 30 minutes of collection and samples were stored within 30-60 minutes after being centrifuged at -80 °C until assay. Levels of plasma VEGF were quantified by the Quantikine human VEGF immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). The levels of serum AFP were quantified using calbiotech AFP human ELISA kits.

RFA procedure: Percutaneous RFA was performed guided by real- time ultrasound guidance. For tumors located in the right lobe, an intercostal approach with the patient in the left lateral or supine decubitus generally was performed. For tumors located in the left lobe, a subcostal approach was used most often. Patients were monitored in the inpatient department for 12 hours to detect any procedure related complications then. After discharge, patients were instructed to attend the unit to report any complaints or complications.

Assessment of Treatment Efficacy and Follow-Up. Apart from measurement of serum AFP and plasma VEGF, Abdominal ultrasonography and dynamic CT scan were done six weeks after the date of the last ablation session. Successful ablation was defined as either complete hypoattenuation of the lesion including the surrounding liver parenchyma (Complete response) or at least a 30% decrease in the diameter of previous contrast enhancement in the arterial phase (Partial response). Patients were considered non responders if they developed new lesions or had an increase in the size of the lesion or if the lesion didn't show any decline in the size of the previous arterial enhancement recorded before starting RFA.

Statistical methods: Data was analysed using SPSS 23 and Epicalc 2000 programs, statistics were divided into two parts: A) Descriptive statistics: in which quantitative data was presented in the form of mean ($\bar{X}$), standard deviation (SD), median, range and qualitative data was presented in the form numbers (N) and percentages (%). B) Analytic statistics: tests of significance which were used included: Chi-square test ($\chi 2$): was used to study association between two qualitative variables. Whenever any of the expected cells were less than five, Fischer's Exact test was used. Student t test (t): was used for comparison of quantitative variables between two groups of normal distributed data. Roc curve analysis was used to detect sensitivity and specificity of AFP and VEGF towards the response. P value was considered statistically significant if it is < 0.05.

Results Table (1): Demographic & clinical characteristics of patients

Patient age	Total patient number = 72
40 -49	14 (19.4%)
50-59	33(45.8 %)
60-69	25(34.8%)
Sex	
Male patients	51 (70.8%)
Female patients	21 (29.2%)
Child score	
Child A	64 (88.9%)
Child B	8 (11.1)
BCLC stage	
BCLC stage A	33 (45.8%)
BCLC stage B	39 (54.2%)

80% of the patients are over 50 years. Minimum age:40 years. Mean: 54,9 years. Most of patients were males (70.8%). Regarding severity of liver disease, most of them were classified as a Child score A (88.1%). Only 11% were class B. As regards BCLC staging for HCC, 54.2 % are BCLC -B, while 45.5% were BCLC-A

Table(2): Tumour characteristics in the treated patients

Tumor number	**Patients number**
Single tumor	67 patients (93.1%)
Multiple tumors	5 patients (6.9%)
Tumor size in cm	**Total number of HFLs**
1-3cm 3-5cm	51(64.5%) 28(35.5)

Five patients in our study had multiple tumors , 2 of them had 3 HFLs , all in the right lobe and 3 patients have 2 HFLs , and also all in the right lobe.

Table (3): Pretreatment AFP &VEGF values distribution in the total 72 HCC

AFP values (ng/ml)	**Number of patients(total=72)**
AFP<20ng	34
AFP=20-100ng	15
AFP>100ng	23 patients
VEGF values pg /ml	**Number**
VEGF<100 pg	47
VEGF: 100-200 pg	11
VEGF >200 pg	14

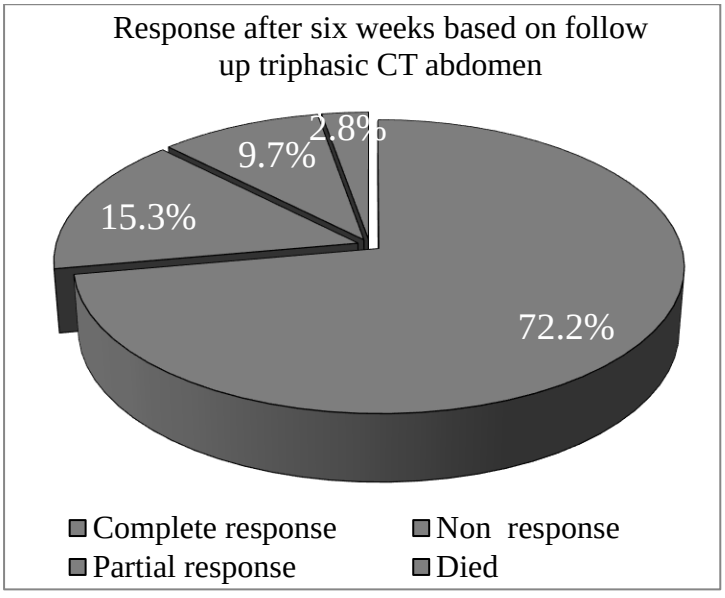

Figure (1): Evaluation of short-term clinical outcome of the 72 HCC patients treated by RFA

As shown in figure 1, Two mortality cases reported in the study, one of them was related to the procedure and died from sever intraperitoneal bleeding. While the second mortality case was unrelated to the procedure (severe uncontrolled hematemesis)

Table (4) : Comparison between pre and post treatment AFP and VEGF in all groups of patients according to degree of response

		Pretreatment	Post treatment	P value	Sig.
Complete response	AFP (ng/ml) Range Mean±SD	0.8-893 83.4±157.4	1.4-250 32.5±56.5	0.009	**HS**
	VEGF (pg/ml) Range Mean±SD	0-555 113.7±113.4	12.2-621 113.4±101.2	0.265	NS
Nonresponse	AFP (ng/ml) Range Mean±SD	5.4-463.3 171.2±135.5	24.2-1006 380.6±389.1	0.202	NS
	VEGF (pg/ml) Range Mean±SD	8-173 63.5±49.0	27.7-367 141.5±98.5	0.002	**HS**
Partial response	AFP (ng/ml) Range Mean±SD	4.8-1029 270.6±366.0	8.4-892 194.4±390.2	0.863	NS
	VEGF (pg/ml) Range Mean±SD	3.5-278.8 99.5±106.7	53.5-278.4 121.4±97.2	0.105	NS

There is a significant difference between pretreatment and post treatment AFP values among complete responder group with highly significant drop in follow up post treatment AFP in the responder patients. There is a significant difference between pre treatment and post treatment VEGF value among the non responders group with significant rise in VEGF values in post treatment VEGF with mean of 141.5±98.5 pg /ml when compared to pre treatment VEGF with mean of 63.5±49.0pg with highly significant P value =0.002.

Table (5): Correlation between AFP and VEGF values with age ,tumor size and platelets count in HCC patients

	AFP (ng/ml)		VEGF (pg/ml)	
	R	P value	r	P value
VEGF (pg/ml)	0.1	0.562	-	-
Age (Year)	0.1	0.248	0.1	0.237
Tumor size	0.1	0.514	-0.03	0.830
Platelets count/cmm	-0.1	0.544	0.3	**0.014***

There is significant positive correlation between VEGF level and platelets count with P value =0.014 which means that when platelet count increase VEGF value significantly increase and vice versa.

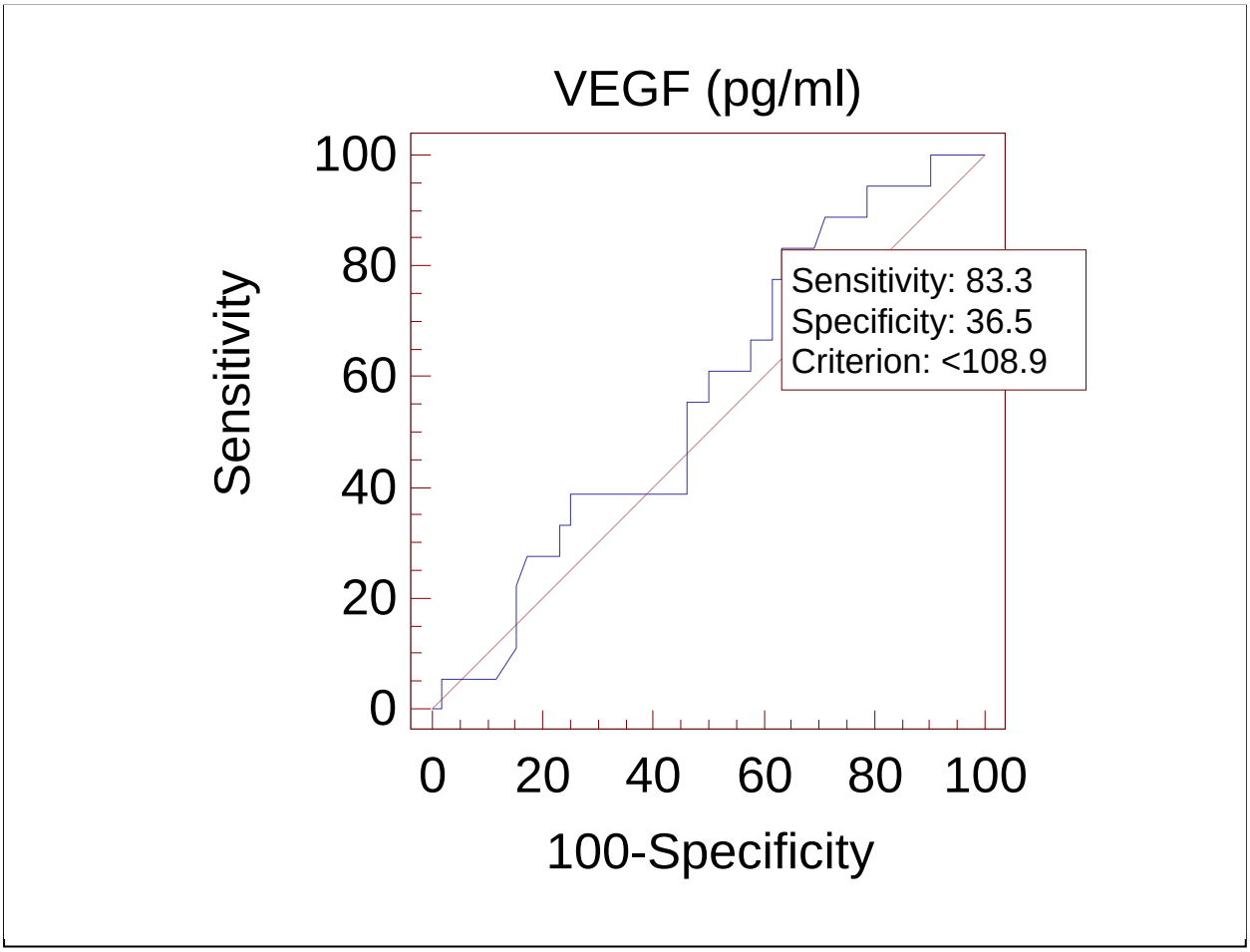

Figure (2): Cut off value , sensitivity and specificity for pretreatment VEGF between complete response and non response groups

Pretreatment VEGF value of less than 108.9 pg in HCC patients who underwent RFA has a sensitivity of 83.3 % of detecting response (true positive rate). While Pretreatment VEGF of $\geq$ 108.9pg in HCC patient who underwent RFA has specificity of 36.5 % of excluding response (true negative rate) .

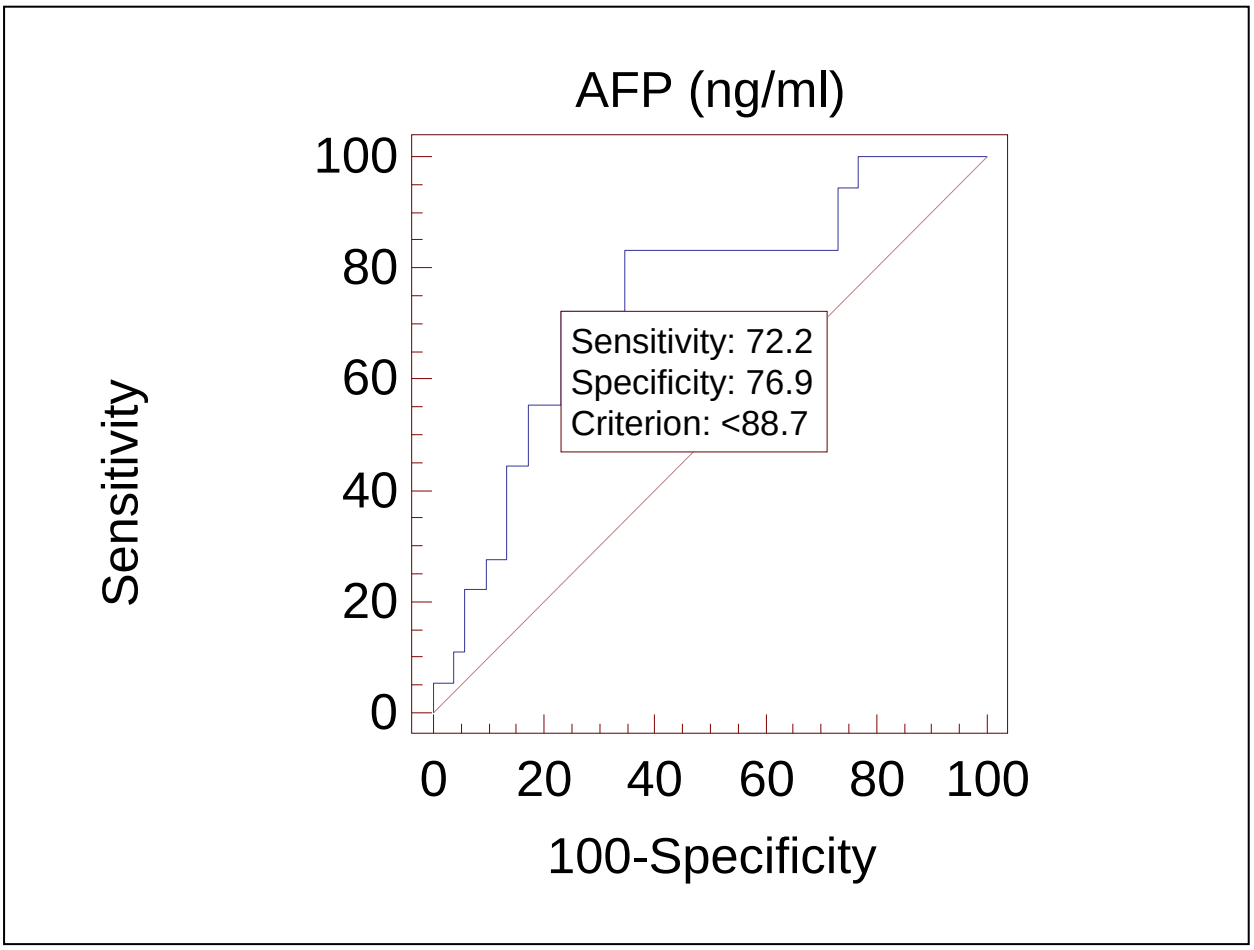

Figure (3) : Cut off value, sensitivity and specificity for pretreatment AFP between complete response and non-response groups

Pretreatment AFP value of <88,7 ng in HCC patients who underwent RFA has a sensitivity of 72.2% of detecting response (true positive rate) . while Pretreatment AFP of ≥ 88.7ng in HCC patients who underwent RFA has a specificity of 76.9 % of detecting nonresponse (true negative rate)

DISCUSSION

Growth and proliferation of liver tumours is dependent on angiogenesis. VEGF was found to be highly expressed in hepatocellular carcinoma and correlated with its metastasis (11). Nevertheless, it has not been reported if the serum VEGF could be used as a prognostic indicator for evaluating the short-term efficacy of RFA, which is a vital curative treatment modality for HCC. In this study, HCC patients were treated by RFA, and level of plasma VEGF were determined before and after therapy then correlated with the treatment response determined by dynamic CT.

When evaluating initial treatment response to RFA in our cohort, successful response including complete and partial response was achieved in 72% and (9,7%) respectively In previous reports, therapeutic efficacy of RFA reached 91% which is near to result of our study (12-14) Other investigators detected an initial complete response rate of 95% which is higher than response rate of our study and this is explained by the smaller median tumor size involved which is a strong predictor of good response to RFA (15-16). Comparable to our study, complete response rate was 94.8% (317 of total 335) by Chen et al in which foci were larger than 3.5cm in diameter (17)

In our study pretreatment AFP value is a significant predictor of response to RFA Similar to our study Choi et al and other researchers reported therapeutic results of treatment of HCC patients by RFA procedures and found that pretreatment AFP is a prognostic factor of response and overall survival (18-20). In contrast to our study**, Nan** et al evaluated AFP value as predictor of initial response among 32 HCC patients treated by RFA and found that neither pre-RFA nor 1-month post-RFA AFP values were associated with short-term outcome (21). This difference in results may be due to the difference in tumor characteristics between the two studies.

We also noticed a significant drop in AFP levels in patients with complete response which is Similar to Kao et al who evaluated the role of post treatment AFP value as predictor of response of 313 HCC tumors treated by 350 RFA session and found that >20% drop in post treatment AFP value one month after procedure compared to pretreatment value indicate good response (15).

When evaluation the role of VEGF values as predictors of response of HCC to RFA in the study, pretreatment VEGF was never an indicator of any type of response but there was a significant difference is present between pretreatment VEGF and post treatment VEGF values one month after procedure among the non-responders' patients with higher post treatment VEGF. on the other hand, Poon et al evaluated the role of VEGF as predictor of response in 120 HCC patient treated by RFA and found that high level of pretreatment VEGF above median of 240 pg was associated with worse response and overall survival (24).

Also, Sedrak et al, evaluated role of VEGF as predictor of response of HCC to percutaneous ethanol injection and found that high level of VEGF before (in contrary to our study) and after therapy (similar to our study) is a significant predictor of non-response to therapy (23). Explanation of this rise is that VEGF involved in neovascularization and infiltration of hepatoma cells into its capsule in patients with HCC, so the plasma levels of VEGF are usually shooting in cases of large or multinodular HCC in comparison with smaller unimodular HCC (24). Another reason for such elevation that, RFA causes coagulative necrosis in the tumour tissue that will in turn causes cancer cell death efficiently. A month after the procedure, tissue hypoxia resulting from RFA drives the process of angiogenesis through the interaction between hypoxia-inducible factor (HIF-1a) and VEGF leading to re-rise again (25-26). Lastly, it was seen by immunostaining that VEGF expression is very intense in patients with large HCC, and its pattern of staining was associated with rapid recurrence and poor survival (27)

In the present study there was a positive significant correlation between plasma VEGF and platelet count which explains the role of platelets as scavenger for VEGF. Similarly, Ronnie et al, demonstrated positive significant correlation between VEGF and platelets count (28). The explanation of that correlation is Vascular injury or invasion is accompanied by platelet activation which harbors and release vast amount of VEGF upon activation that dramatically occurs after vascular injury. Thrombopoietin was cultured in hepatoma cells, is another factor responsible for compensatory thrombocytosis after relative consumption of platelets in the formation of tumor microthrombi. These factors create a vicious circle of VEGF release (29-30)

The present study showed a negative but insignificant correlation between VEGF level and tumor size. In contrary to our study Poon et al , Ronnie et al ,

demonstrated positive significant correlation between high serum VEGF level and tumor size and found that high VEGF is associated with absence of tumor capsule, presence of intrahepatic metastasis, presence of microscopic venous invasion, and advanced stage . such discrepancy may be explained by the difference between the studied population regarding tumor burden, presence of metastasis (28,31).

When discussing procedure related mortality, there is only one case of procedure related mortality in our study who died from sever intra peritoneal bleeding with total mortality rate of 1.3% per treatment and 1.1 % per session. Other investigators noticed major complications in their cohort including cases of intraperitoneal bleeding, bowel perforation and tumor seeding along the needle track (17,20, 32).

On the other side Tateishi et al, didn't report RFA related death but just reported tumor seeding along the needle track, hepatic abscess formation requiring drainage and minimal intraperitoneal hemorrhage (19). This discrepancy in incidence of mortality reflects tumor burden, number of sessions, patient performance status and self-experience of the operator.

Conclusion: Decrease in post RFA AFP value compared to pretreatment value significantly indicates response to treatment while increase in post treatment VEGF value compared to pretreatment value significantly indicates nonresponse to RFA.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: This research did not receive any specific grant from public, commercial or not-for-profit funding agencies.

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