

vascular endothelial growth factor monitoring in advanced hepatocellular carcinoma patients treated with radiofrequency ablation plus octreotide: A single center experience

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Abstract. Local therapies such as radiofrequency ablation (RFA) represent a valuable choice in limited hepatocellular carcinoma (HCC) and are increasingly used also in advanced tumors. Medical treatments generally gave frustrating results in advanced HCC especially if comorbidities exist. Several biologic non-chemotherapeutic drugs are currently tested in HCC and, among them, octreotide was evaluated in single series of HCC patients reporting conflicting results. We have treated a series of 35 patients affected by advanced HCC (26 M and 9 F; age range: 55-85 years, median: 73 years) with RFA followed by octreotide to primarily evaluate the safety of combined treatment and to give preliminary evaluation on its activity. We have also evaluated serum VEGF changes during the study. Child A and Child B represented 60% and about 34% of the cases, respectively. Only two patients with Child C compensated cirrhosis were included in this study. All patients have multiple liver HCC nodules and one had bone metastases. Two complete responses, 3 partial responses and 23 disease stabilization for at least three months were obtained (overall response rate, 14,2%; clinical benefit, 80%). Mean overall survival was 31.4 months. The combined treatment was well tolerated. Statistically significant correlation was found between serum VEGF and tumor progression. In

conclusion, the combination of RFA and octreotide was active in advanced HCC, however, confirmation in a larger series is required.

Introduction

The majority of patients with a diagnosis of hepatocellular carcinoma (HCC) are considered not amenable for resection because of advanced cirrhosis, poor liver function, tumor local or extrahepatic spread and coexistent adverse medical conditions. These patients referred for 'non-operative' management are unlikely candidated for any therapeutic approach. A variety of treatment is used in unresectable HCC, including transcatheter arterial chemoembolization (TACE), percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), chemotherapy and novel medical therapies (1). Efficacy of palliative modalities in terms of lifetime extension has been shown for TACE and PEI (1). Surgery, percutaneous and transarterial interventions are effective in patients with limited disease (1-3 lesions, <5 cm in diameter) and compensated underlying liver disease (cirrhosis Child A). However, at the time of diagnosis >80% patients present with multicentric HCC and advanced liver disease or comorbidities that restrict the therapeutic measures to best supportive care (2). RFA proved to be effective in the treatment of unresectable HCC. RFA can selectively destroy tumor tissues without significant damage to vascular structures in the remaining liver. Due to advances in radiofrequency technology, RFA has been used also to treat patients with more advanced tumors (3). When tumor size exceeds 3 cm and/or the number of nodules is >3, the rate of local treatment success is significantly reduced. However, all local treatments can only destroy tumor nodules, but do not act on the pathogenetic mechanisms underlying cirrhosis such as growth factor release and inflammation. A recent review has analyzed 13 comparative studies, 4 of which were randomized, controlled trials. There did not seem to be any distinct differences in the complication rates between RFA and any of the other procedures for treatment of HCC.

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The local recurrence rate at 2 years showed a statistically significant benefit for RFA over percutaneous ethanol injection for treatment of HCC (6% vs 26%, 1 randomized, controlled trial). Better results can be achieved with laser dissection and surgery when they are possible (4). However, the survival and responses rates in HCC treated with RFA remains low and alternative therapeutic strategies are required.

One of the innovative therapeutic tool investigated in HCC is the Somatostatin (SS) analogue octreotide. *In vivo* and *in vitro* expression of somatostatin receptors has been reported in HCC and especially the subtype 2 (5-7). In a series of 56 HCC a variable receptor expression was detected. The subtype 1 (sst1) was present in 46%, sst2 in 41%, sst3 in 64% and sst5 in 75%, of cases studied, while sst4 was not detected (8). Some authors supposed that only a subset of HCC patients, corresponding to the reported presence of somatostatin receptors (7), can benefit from octreotide (9). Recently all five sst subtypes were shown in cirrhotic liver and HCC at both protein and at mRNA level by immunohistochemistry and RT-PCR, respectively (10). Two studies on cell models revealed a pro-apoptotic effect exerted by octreotide and the SS analogue AN-238 in HCC (11,12). SS analogues act through receptor interaction stopping directly cell growth (by inducing apoptosis or regulating cell cycle) and/or indirectly reducing the release of growth factors, such as IGF-1 or EGF, and inhibiting angiogenesis. In this view, HCC is a hypervascular tumor and angiogenic factors such as vascular endothelial growth factor (VEGF) are overexpressed in HCC tumor cells and the surrounding stroma cells (13).

On the basis of these considerations, octreotide was previously used in HCC patients with conflicting results. While impressive outcome were suggested by some case reports (14,15) and promising results were reported by some trials (6,16,17), other studies report equivocal findings (18), and very recently octreotide appeared to show no benefit in a consistent series of patients as compared to untreated (19). However, in the latter study no evaluation of the quality of life was performed.

On the basis of the low toxicity profile of octreotide and of the potential to favorably interact with RFA reducing growth tumor and vessels, we have explored a combined approach of RFA and octreotide in order to evaluate the tolerability of this treatment regimen in patients with advanced HCC. The activity of this combination in terms of disease control and survival was also assessed. Moreover, given the supposed role of VEGF as a biomarker and the anti-angiogenic activity of octreotide in HCC, serum VEGF was monitored in this series of HCC patients.

Patients and methods

Patient characteristics. Patients enrolled in the study had histologically proven HCC or documented α -FP rise (more than 250 ng/ml) with concomitant nodule in cirrhotic liver, multiple liver lesions not amenable to surgery or local treatment with radical intent or progressive disease after local treatment, disease assessable by radiology (ultrasounds, computed tomography) and/or biochemistry (α -FP), compensated cirrhosis and ECOG Performance Status 0-2. Previous

therapies such as IL-2 and tamoxifen were allowed. Informed consent was obtained from all patients and Local Ethics Committee approved the study. The exclusion criteria were: active encephalopathy, colelithiasis, brain metastases, metabolic disorders or concomitant systemic diseases considered as high-risk conditions for local treatment and any therapies different by supportive care, abnormal liver function: AST >5.0 x normal value; ALT >5.0 x normal value; international normalized ratio (INR) >1.5 x normal value; total bilirubin >3.0 x normal value and basal hemoglobin <10.0 g/dl; WBC <2.0x10⁹/l; platelets <50.0x10⁹/l.

RFA technique. All patients were treated with a radiofrequency equipment (Elektrotom 106 HiTT, Berchtold) using 15-gauge needle electrodes with an exposed active tip 2.5 cm long. Only tumor lesions >4 cm were treated. RFA was done percutaneously and the day after the procedure all patients underwent liver ultrasounds to exclude complications and assess the results obtained.

Treatment protocol. All patients were evaluated jointly by oncologists (SdP), radiologists (VN) and interventional (LT) physicians. All patients underwent one session of RFA targeting nodules <5 cm. A testing dose of 100 μ g on day 1 was given to all patients. The next day (day 2), they received octreotide LAR (Novartis Pharma, Origgio, Italy) at 20 mg as first dose to evaluate treatment side effects and 30 mg thereafter once every 28 days. The next day (day 3) the patients were subjected to RFA. Medical treatment was administered until disease progression.

Study design. A single institution phase II study was prospectively projected according to the Simon's two-stage optimal design. The primary end-point was the objective response rate (CR + PR) at the liver target lesion. According to this design a number (n1) of patients entered the first stage of the trial. The accrual continues to a total of n2 patients only if a specified r1 response rate is achieved in the first series. We have selected as target activity a 10-30% response rate with a lower activity of 5%, with a 0.05 α error and a 0.20 β error. The treatment under investigation should be considered non-active if it produced less than two responses out of 10 consecutive patients in the first series and fewer than 5/29 patients in the overall series.

Vascular endothelial growth factor (VEGF) evaluation. Serum samples were collected prior to first dose of octreotide and prior to RFA, then at each octreotide administration. They were tested by immunoassay Quantikine VEGF (R&D Systems, Inc., Minneapolis, USA), to determine VEGF₁₆₅ levels according to supplier suggested methods. Results obtained for naturally occurring human VEGF and recombinant human VEGF₁₂₁ showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. All samples were processed between 30 and 60 min after collection. Briefly, a serum separator tube was used and, after 30 min to allow clotting, samples were centrifuged (1000 g, 15 min). Serum was removed and stored at -20°C for further analysis. The assay employs the quantitative sandwich enzyme immunoassay

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Tumor response, survival and statistical analysis. The patients were staged at study entry by liver ultrasounds and whole body computed tomography (CT). In absence of signs of clinical progression, liver ultrasounds was repeated every three months and CT was repeated every 6 months. Disease control was evaluated and expressed as following: complete response corresponds to absent or inactive tumor lesions according to two independent observers, partial response was defined as $\geq 50\%$ reduction of the tumor lesions, stable disease was described as an unchanged tumor size; progression of disease as appearance of new lesions or increase of size of existing nodules. Basal cytokine levels were compared using the Wilcoxon's test for non-parametric dependent continuous variables.

The overall survival time was calculated as the period from the date of starting treatment until death from any cause or until the date of the last follow-up, at which point data were censored. Overall survival was determined by Kaplan-Meier product-limit method (20).

The differences in terms of OS according to the presence of VEGF reduction was evaluated by the log-rank test (21). The cut-off point for survival data was December 2006; for safety data, it was March 2005. SPSS software (version 13.0, SPSS, Chicago) was used for statistical analysis. A $p < 0.05$ was considered to indicate statistical significance.

Results

Clinical activity and patient characteristics. At August 2006 35 patients (26 males, 9 females; age range 55-85 years; median 73 years) with non-resectable HCC lesions were enrolled in this study. Comprehensive patient data are described in Table I. In the majority of cases, Hepatitis C virus infection was detected (51%). Hepatitis B virus infection, coinfection by hepatitis B and C virus and ethanol abuse were recognized respectively in 4, 3 and 9 patients, while ethanol plus hepatitis C infection were considered causative in one patient. Most of the patients were classified as Child A and B cirrhosis, respectively 60% and ~34% of the cases. Only two patients with Child C compensated cirrhosis were included in this study. Signs of portal hypertension were present in 40% of patients. Four patients had previous or concomitant tumors: in two cases bladder cancer, in one case jejunal leiomyosarcoma and head and neck tumor. All patients have multiple liver HCC nodules with a maximum diameter of 90 mm. One patients had bone metastasis by HCC.

Two complete responses (5.7%) and 3 partial responses were recorded (14.2%). In 23 patients (57.1%) the combined tailored treatment with octreotide and RFA was able to maintain stable disease for at least 3 months. The overall response rate was 14.2% and the disease control rate was ~80.0%. In three patients tumor progression was documented. Clinical progression of underlying cirrhosis was identified in four patients and appear to condition the outcome, independently from HCC (Table I). Globally, seven PD were recorded in our series independently from the cause of the evolution of their disease. Mean survival was 31.49 months (CI 95% 21.67-41.31) (Fig. 1 and Table II).

Table I. Patients' characteristics.

No. of patients	35
Median age (years)	73
Age range (yrs)	55-85
Sex (male/female)	26/9
Etiology of liver disease	
Hepatitis B	4
Hepatitis C	18
Coinfection B and C	3
Ethanol abuse	9
Ethanol abuse & HCV	1
Child-Pugh grade	
Child-Pugh A	21
Child-Pugh B	12
Child-Pugh C	2
Signs of portal hypertension	
Partial/complete portal vein thrombosis	
Ascites	
Esophageal varices	
Tumor lesion	
Single	0
Multiple	35
Tumor diameter (range, mm)	3-90

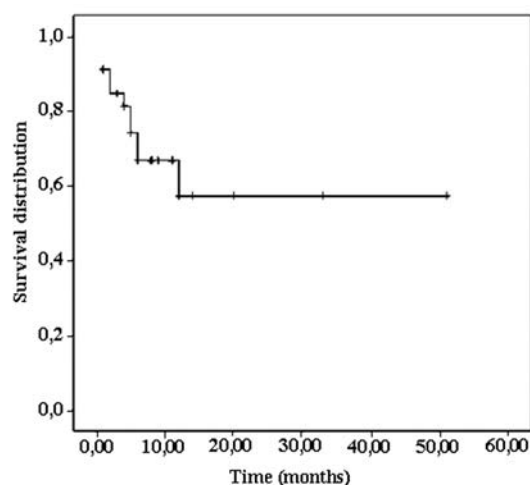


Figure 1. Kaplan-Meier estimate of overall survival of the patients enrolled in the study.

Kaplan-Meier analysis of overall survival showed no significant differences according to sex, age, and HCV versus other causes. Stage was the only factor which seems to significantly condition survival ($p < 0.021$) (Fig. 2).

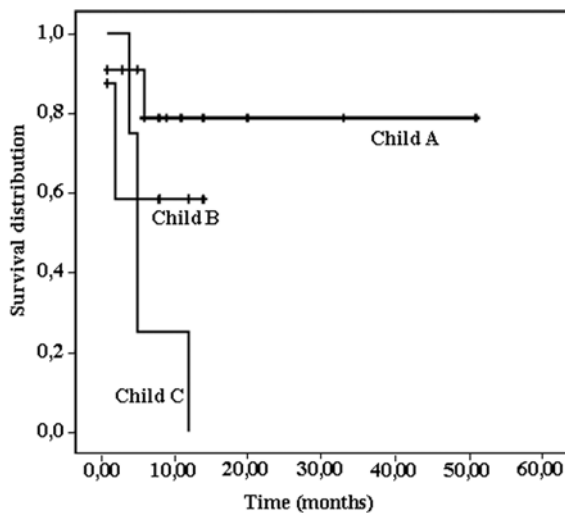


Figure 2. Kaplan-Meier estimate of overall survival of the patients enrolled in the study according to their initial Child grade.

Table II. Response rate to treatment.

Parameter	No. of patients	%
Complete response	2	5.7
Partial response	3	8.5
Objective response (CR+PR)	5	14.2
Stable disease	23	65.7
Overall disease control (CR+PR+SD)	28	79.9
Progressive disease	7	20

Toxicity evaluation. Both medical and local treatments were well tolerated. In particular, as concerns RFA, we registered mild right upper quadrant discomfort in 40% of the patients immediately after the procedure with need for pain medication in 20% of the cases and AST/ALT rise in comparison with

start-up patient values in ~60% of the patients with return to baseline levels within one week. On the other hand, the only reported side-effect related to octreotide in this series of HCC patients was meteorism and dyspepsia in one case which disappeared after one month.

Modulation of serum VEGF. VEGF analysis was performed in all enrolled patients, at baseline and after 3 and 6 cycles of combined treatment. VEGF values showed variations during time, the comparison between first and second measurement revealed a decrease of VEGF levels of at least 2-fold in 13, while an increase of at least 2-fold in 14 patients, and remained unchanged in other 13 treated patients. Interestingly, among the 7 patients that had a disease progression during the treatment, 5 revealed a statistically significant increase of VEGF level ($p < 0.001$). The statistical analysis, performed in our population, did not reveal any other correlation between the VEGF serum levels and the response to treatment after three cycles (Table III), and these results were confirmed after six cycles of therapy (data not shown).

The age, sex, the type of virus infection, the value of α -fetoprotein and the Child score did not reveal any correlation to angiogenetic marker levels course. No correlation was found between VEGF value changes and response or overall survival.

Discussion

Advanced HCC is characterized by an extremely poor prognosis with median survival times ranging between 3 and 6 months in most studies (21). Chemotherapy gave very poor results in HCC. The intrinsic cell resistance to chemotherapeutic agents and the impaired liver metabolism contribute to failure of all chemotherapeutic strategies applied until now. Given the lack of recognized active chemotherapy in HCC, other medical treatments such as tamoxifen have been evaluated (22). Despite the tolerability profile of tamoxifen, no evidence of efficacy emerged from available studies (23,24). New treatment proposals are eagerly awaited in HCC (25,26).

This is the first study evaluating RFA plus long-acting octreotide in a series of advanced HCC. Despite the limited

Table III. Correlation of VEGF expression and response to treatment in patients with HCC treated with radiofrequency ablation plus octreotide.

Parameter	VEGF expression after 3 cycles of treatment			p-value
	No. of patients			
	Increased	Invariated	Decreased	
Complete response	1		1	0.12
Partial response	1	1	1	0.1
Objective response (CR+PR)	2	1	2	0.2
Stable disease	8	8	7	0.13
Progressive disease	5	2	-	0.001



SPANDIDOS PUBLICATIONS f patients enrolled in this series, the mean survival (~31 months) was an interesting result. In a study on

RFA performed on liver lesions by different unresectable and refractory tumors, overall survival was 25.2 months for HCC (27). Median survival of patients with inoperable HCC treated with long-acting octreotide ranges between 4.7 and 15 months, in negative and positive-reporting studies respectively (16,19).

In the present study, survival appears to be not related to age, sex, etiology, but only condition by the stage of cirrhosis by Child.

The combined strategy of medical and local treatment used had the aim to maximize the effect of both and obtain durable disease control. This approach hampered the analysis of the real weight of each therapy. However, the independent evaluation by two observers allowed the assessment of tumor response especially as concerns the remnants with more or less signs of activity. The ORR and clinical benefit reported, 14.2 and 80%, suggest that this treatment strategy is not only able to successfully control disease as previously demonstrated with octreotide only (16), but also to achieve a definite response. In the present study disease control rate was higher than in others (40%) (16). The treatment strategy used was well tolerated by all patients. RFA can be considered relatively safe also in advanced HCC. Moreover, as known, octreotide has a good toxicity profile that respects the poor balance of cirrhotic patients. In this study the combined strategy appeared to be feasible also in patients which can be considered more fragile such as elderly patients. In this series, patients aged >70 years were ~71%.

VEGF evaluation was based on previous data highlighting a positive correlation with prognosis in HCC patients (28,29). Determination of VEGF was performed in HCC patients treated with thalidomide without showing any significant difference between responders and non-responders (13). In a series of HCC patients treated with octreotide alone or in combination with rofecoxib, serum VEGF appeared to be the most significant predictor for tumor progression and survival (30). In the present study we confirmed the involvement of angiogenetic pathway in mechanism of action for this drug; the evaluation of VEGF course revealed that high levels of VEGF were significantly associated with tumor progression, independently from other prognostic factors. This biomarker can represent an important predictor of treatment efficacy. The limited number of patients can conditioned other results reported, and needs a confirmation in a largest trial. However, we believe that VEGF is a powerful biomarker in HCC and warrants evaluation in future studies involving HCC patients and anti-angiogenic drugs.

In conclusion, the results obtained suggest that RFA plus octreotide can be safely used in patients with advanced HCC, to obtain disease control and prolong survival in a significant proportion of cases.

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