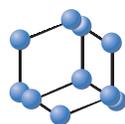


RESEARCH ARTICLE

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Repeated Transarterial Chemoembolization with Degradable Starch Microspheres (DSMs-TACE) of Unresectable Hepatocellular Carcinoma: A Prospective Pilot Study



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Abstract: Objective: The aims of this study were to: a) evaluate tumor response rates using modified-Response-evaluation-criteria-in-solid-tumors (mRecist) criteria, b) evaluate safety of Degradable Starch Microspheres Trans-arterial-chemo-embolization (DSMs-TACE) for unresectable hepatocellular-carcinoma (HCC) treatment.

Materials and Methods: We prospectively enrolled 24 HCC cirrhotic patients (21/3 M/F, mean age 66.3 years) to be treated with repeated DSMs-TACE procedures, performed at 4-6 week intervals on the basis of tumor response and patients tolerance. Clinical and biochemical evaluations were performed before and after each procedure. Treatment response was also assessed by Computed-tomography (CT) or Magnetic-resonance-imaging (MRI)-scan 4-6 weeks following each procedure.

Results: In our experience, DSMs-TACE was both safe and effective. A total of 53 DSMs-TACE procedures were performed (2.2 per patient). No procedure-related death was observed. Complete Response (CR) was observed in 5/24 (20.8%), 4/17 (23.5%) and 5/12 (41.6%) patients after the first, second and third procedure, respectively. At the end of each treatment, all patients experienced at least a partial response. At the end of the repeated procedures, no differences between mono- or bi-lobar disease were observed in patients with CR (64.2% vs 50%; p=ns). In most cases, treatment discontinuation was due to worsening liver function.

Conclusion: DSMs-TACE is a valid, well-tolerated alternative treatment to Lipiodol-TACE or DEB-TACE, as it has demonstrated to achieve a relatively high percentage of complete tumor necrosis.

CR rates were similar between patients with mono- or bi-lobar disease indicating the possibility of carrying-out repeated procedure in a safe and effective way in both types of patients.

Keywords: Hepatocellular carcinoma, transcatheter arterial chemoembolization, degradable starch microspheres, recurrence-free survival, locoregional therapies, HCC cirrhotic patients.

1. INTRODUCTION

Trans-arterial Chemoembolization (TACE), according to the Barcelona Clinic Liver Cancer (BCLC) algorithm, is recommended for unresectable, single or multinodular, Hepatocellular Carcinoma (HCC) in patients with preserved liver function and no evidence of vascular invasion or extra hepatic spread of disease [1].

Conventional-TACE (cTACE) (using chemotherapeutic drugs with or without Lipiodol and the administration of

embolic agents) is the most widespread technique and allows both to increase intratumoral retention of chemotherapy agent and to induce ischemic tumor necrosis through a transient occlusion of tumor feeding arteries [2].

The introduction of Drug Eluting Beads (DEB) has provided an attractive alternative [3]. Many studies have shown that DEB loaded with doxorubicin has a safe pharmacokinetic profile with lower systemic drug exposure and significantly reduced liver toxicity compared with cTACE [4-6].

The long-term survival of patients treated with cTACE is not fully satisfactory due to the phenomenon of cancer recurrence [7].

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There are several factors that could explain this problem, including differences in patient's selection or in tumor characteristics.

cTACE and DEB-TACE, causing interruption of blood flow to the tumor with possible extended ischemia also in normal liver, may precipitate new tumor vessel induction *via* Vascular-endothelial-growth-factor (VEGF) stimulation [8], which could represent a further mechanism causing recurrence after TACE [9-14].

To avoid VEGF overexpression, transient occlusion of tumor feeding arteries using Degradable Starch Microspheres (DSMs) was proposed [15].

These particles provide a temporary vessels occlusion (half-life 35-50 min) [16]. However, until now, the clinical usefulness of DSMs in association with chemotherapeutic agents has been investigated only in few studies in patients with unresectable HCC, with highly contradictory results [16-22] due to the lack of a standardized protocol or to the excessive variability within the therapeutic protocols themselves.

DSMs embolization has the advantage that it could be repeated within a short period of time with limited damage to the non-humoral hepatic tissue. Pieper *et al.*, in a recent study, [16] have shown that temporary embolization of the hepatic artery using DSMs is feasible with complete reperfusion after 30 min in pigs. Even after complete arterial blood flow stasis, no extensive tissue damage to the embolized liver parenchyma was observed at histologic examinations.

Through a transient occlusion tumor feeding vessels, DSMs-TACE is able to highlight the role of intra-arterial chemotherapy infusion and thus a repeated treatment protocol can be adopted to maximize the effects of chemotherapy directly on the neoplastic tissue.

In this prospective pilot study, we report our initial experience with repeated DSMs-TACE, in patients with unresectable HCC.

The primary efficacy end-point was 1 month tumor response rate of repeated DSMs-TACE according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) criteria [23]; The primary safety endpoint was the occurrence of procedures-related adverse events within 30 days of a treatment procedure. Secondary safety outcomes included the incidence and severity of adverse events, liver function parameters, laboratory abnormalities.

2. MATERIALS AND METHODS

2.1. Patients

This study was approved by the local institutional ethics committee. From January to September 2014, we prospectively enrolled a group of cirrhotic patients with unresectable HCC. Diagnosis of HCC was carried out in accordance with the guidelines of the European Association for the Study of the Liver (EASL) [24]. The choice of loco-regional ablative treatment was made through the evaluation of a multidisciplinary team of hepatologists, surgeons and interventional radiologists who assessed each case singularly [25]. To be enrolled in our repeated DSMs-TACE protocol all patients were required to fit the criteria reported in Table 1. Before every treatment patients provided written informed consent to participate to the study.

2.2. Pre-treatment Work-up

Before the first treatment, at baseline condition, all patients underwent a clinical and biochemical examination. All patients underwent a dynamic multislices Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) at least two weeks before each treatment, to stage or restage diseases [26-29].

2.3. DSMs-TACE Procedure Design

All procedures were performed in an operating dedicated angio-suite room, by the same interventional radiologists.

All patients were pre-medicated with a proton-pump inhibitor (Pantoprazole 80 mg i.v.), with a prokinetic drug (Metoclopramide 10 mg i.v.) and an analgesic drug (Ketorolac-Tromethamine 20 mg i.v.); if requested and not contraindicated, conscious sedation was performed during the procedure.

Under local anesthesia, using the Seldinger technique, a 4-French (Fr) sheath introducer was placed in the right common femoral artery. Superior mesenteric artery, celiac and hepatic angiographies were performed in all patients using a 4-Fr catheter. When necessary, a super-selective (segmental or sub-segmental) approach was used by means of a microcatheter. We hence performed a lobar embolization paying particularly attention to no embolized cystic, gastric or splenic anomalous branches. Embolizations were performed as selectively as possible, treating individual segmental arteries when feasible.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Age >18 years	Evidence of severe liver function deterioration
Mono or Multifocal measurable HCC nodules	Complete thrombosis of the main portal vein
Total Bilirubin <3 mg/dl,	Platelet count < 50.000/mm ³
No evidence of extrahepatic metastasis	Serum creatinine levels > 2.0 mg/dL
Prior surgical or loco-regional treatment only if performed more than 16 weeks before the study Eastern Cooperative Oncology Group (ECOG) performance status of 0-1 [25]	Any preexisting medical conditions of sufficient severity to prevent full compliance with the study.

The lobar technique was used in case of multiple (>3) HCC in the same lobe or when the selective catheterization of the feeding artery was not technically feasible.

DSMs were mixed with non-ionic contrast medium. 6 mL of nonionic contrast was used per 4 mL of DSMs prior to injection. Doxorubicin at a dose of 50 mg/m² was diluted in 5 mL of normal saline. No dose adjustment was made for bilirubin concentration or body surface area. An appropriate suspension of DSMs, contrast medium and Doxorubicin was obtained before delivery. The mixture in the syringes was constantly agitated to avoid sedimentation and disaggregation of the microspheres, then slowly injected at the proper site. DSMs and Doxorubicin were injected until initial stasis of this mixture was observed on tumor feeding vessels. As a result, DSMs alone was slowly and continuously injected until a complete embolization was obtained. We did not use DynaCT before embolization. We used only un-enhanced Cone Beam CT acquired directly after DSMs-TACE to assess deposition in hepatocellular carcinoma of temporary embolization agent. If lesions were not satisfactorily embolized we continued to embolize lesion also looking even additional feeders vessels.

We tried to perform repeated DSMs-TACE at 4-6 week intervals in order to obtain maximum tumor response rates unless there was the onset of complications associated with procedure.

2.4. Outcome Assessment

Patients were evaluated with contrast enhanced CT or dynamic MRI at time of enrolment and 25-30 days after each DSMs-TACE procedure in order to identify any morphological and vascular changes, as compared to baseline features. To assess response after all treatment, we used mRECIST criteria on lesions by lesions analysis [23] (Table 2).

At baseline and after 6 h, 24 h and after one week from each procedure patients underwent a thorough clinical and biochemical evaluation. Disease severity was evaluated by means of the Child-Pugh-Turcotte (CPT) and the Model for End Stage-Disease (MELD) scores.

The development of a Post-embolization Syndrome (PES), defined as fever, nausea, vomiting and abdominal pain referred to the shoulder occurring during the first 48h after treatment was investigated and an ultrasound abdominal examination was performed when necessary. Intra- and

post-procedural complications were evaluated according to the classification of the International Society of Radiology (SIR) which distinguishes between major and minor complications [29].

2.5. Statistical Analysis

Data were analyzed using IBM SPSS Statistic release 22.0. Results were expressed as the mean and Standard Deviation (SD) for continuous variables and as values and percentage for categorical variables. Statistical comparison by subgroups was performed using Chi-squared test or Student’s t-test, when appropriate *p*-values less than .05 were considered significant in all statistical analyses. Univariate and multivariate Cox proportional hazards regression models were used to assess the relative prognostic significance of different variables in predicting dropout rate and tumor response rate, as Model for End-Stage Liver Disease (MELD), Child-Pugh score, unilobar or bilobar tumour disease.

3. RESULTS

We enrolled a total of 24 cirrhotic patients with unresectable HCC (21/3 M/F, mean age 66.3years, range 47-82 years). Baseline patients and tumors characteristics are displayed in Table 3. According to the BCLC classification, 21 (88%) patients were identified as stage B, while 3 (12%) as stage C.

58.3% of patients had unilobar tumour disease (41.7% bilobar) (Fig. 1a-d).

3.1. Response to Therapy

A total of 53 DSMs-TACE procedure were performed (2.2 procedures per patient). All procedures were technically completed successfully. We treated a total of 75 nodules (3,12±1,51 lesions/patients) (Fig. 2 and 3).

After the first procedure, 5/24 (20.8%) and 16/24(66.6%) patients achieved a Complete Response (CR) and Partial Response (PR), respectively; only 2 patient showed a Stable Disease (SD) while 1 patients showed PD (Table 4). 17 patients were treated with additional DSMs-TACE and after one month to second procedure, 4/17 patients (23.5%) showed CR, 9 (52,9) PR, 4 (23,5) and no cases of PD were observed. Third DSMs-TACE was performed in 12 patients obtaining a CR in 5 (41,6) patients, PR in 6 (50) patients and only 1 (8,3) SD and no cases of PD.

Table 2. mRecist Criteria (22).

mRecistCriteria	
Complete Response (CR)	Disappearance of any intratumoral arterial enhancement in all target lesions
Partial Response (PR)	At least a 30% decrease in the sum of diameters of viable (enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions
Stable Disease (SD)	Any cases that do not qualify for either partial response or progressive disease
Progressive Disease (PD)	Increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started

Table 3. Baseline patient characteristics (n=24).

Characteristics	Tot pts 24
Gender [Male] n (%)	21 (87.5%)
Mean Age (years) \pm SD	66.3 \pm 10.5
Mean AST levels (UI/L) \pm SD	70.9 \pm 50.8
Mean ALT levels (UI/L) \pm SD	63.7 \pm 63.9
Mean GGT levels (UI/L) \pm SD	184.7 \pm 246
Mean albumin levels (g/dl)	3.2 \pm 0,6
Mean total Bilirubin levels (mg/dl)	1.5 \pm 1.1
Mean Creatinine levels (mg/dl)	0.88 \pm 0.25
Mean INR	1.2 \pm 0.2
Mean PLT levels ($10^3/\mu$ l) \pm SD	92700 \pm 51300
Mean AFP (UI/mL) SD	43.2 \pm 130
Mean MELD	10.6 \pm 3.4
Child Pugh n (%)	
A	15 (60%)
B	6 (28%)
C	3 (12%)
Child Pugh score (Mean \pm SD)	6.6 \pm 1.7
Aetiology:	
HCV	11 (45.8%)
HBV	2 (8.3%)
Alcohol	7 (29.2%)
Steatohepatitis+/-virus	4 (16.7%)
Previous HCC treatment n (%)	16 (66.7%)
HCC side (monolobarvsbilobar)	14 vs. 10 (58.3% vs. 41.7%)
MeanNodulesnumber \pm SD	3.1 \pm 1.5
Mean diameter biggest nodule (mm) \pm SD	27 \pm 12.1
Mean nodules diameter (mm) \pm SD	21.4 \pm 11.6

AFP: Alfa fetoprotein; ALT: Mean Alanine transaminase; AST: Mean Aspartate Aminotransferase; GGT: Gamma-Glutamyl-Transpeptidase; HCV: Hepatitis-C-Virus; HBV: Hepatitis-B-Virus; INR: International Normalized Ratio; MELD: Model for End stage Liver Disease; PLT: Platelets; SD: Standard Deviation.



Fig. (1). a-d. Axial contrast enhanced CT images (a,b,c) show multiple enhanced nodules in patient with multinodular HCC (arrows); hepatic arteriography (d), performed during a DSM TACE procedure, demonstrates multiple areas of non specific arterial enhancement the liver.

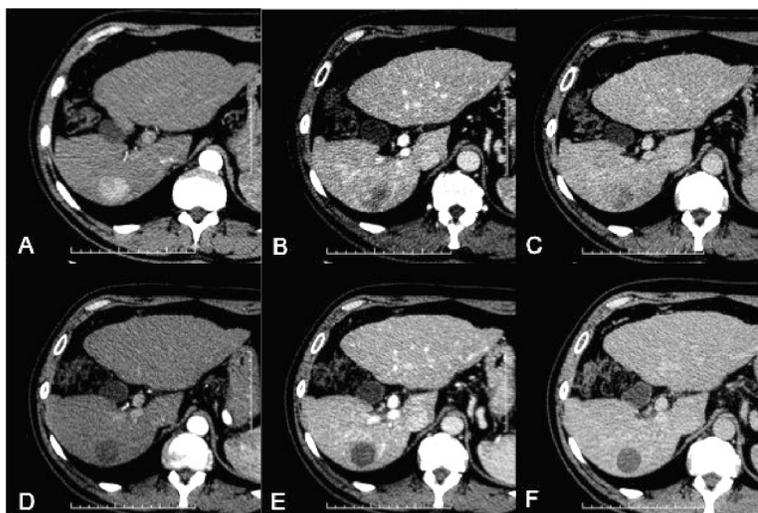


Fig. (2). a-f. Axial dynamic contrast enhanced-CT images show a hypervascular lesion during arterial phase (a) with rapid wash-out during portal (b) and delayed phase (c), localized on VI segment (diameter 32 mm). After two repeated procedures of DMSs-TACE dynamic contrast enhanced-CT (d,e,f) demonstrated a complete response.

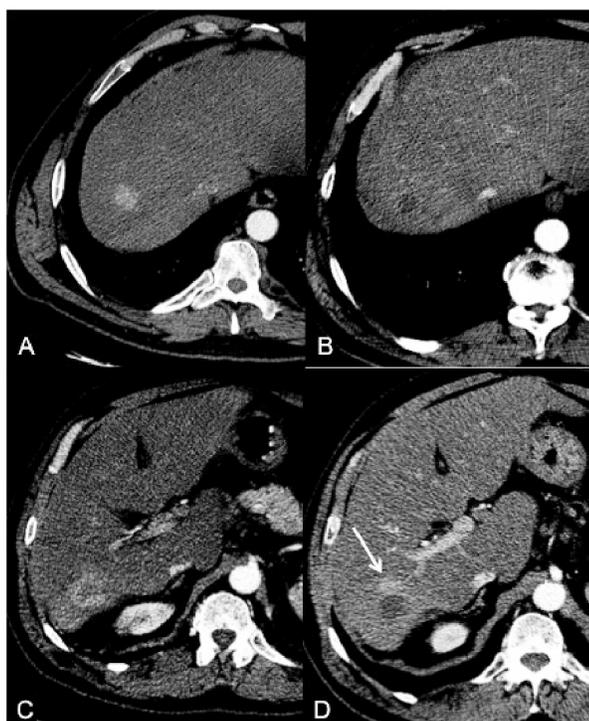


Fig. (3). a-d. Axial dynamic contrast enhanced-CT images show two hypervascular lesions during arterial phase localized on VII segment (a) and on VI segment (c). After one month by a single session of DMSs-TACE dynamic ce-CT shows the absence of contrast enhancement of the lesion on segment VII (b) and persistence of partial contrast enhancement on the anterior margin of the lesion on segment VI (arrow) (d).

Table 4. Treatment response according to mRECIST criteria.

N° of DSMS-TACE	N° OF PATIENTS	CR n°(%)	PR n°(%)	SD n°(%)	PD n°(%)	Stop Repeated DSMS-TACE for OLT or Worsening Liver Function (n° of Pts)
First	24	5 (20,8)	16 (66,6)	2 (8,3)	1 (4,1)	2
Second	17	4 (23,5)	9 (52,9)	4 (23,5)	0	1
Third	12	5 (41,6)	6 (50)	1 (8,3)	0	1

DSMS-TACE: Degradable Starch Microspheres-Transarterial Chemoembolization; CR, Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease; OLT, orthotopic liver transplantation.

Following the first procedure, according to HCC localization (one lobe or two liver lobes), we found that the 5 of 14 patients with single lobe disease achieved a CR, with respect to none of 10 patients affected by bilobar HCC ($p=0.017$).

However, at the end of repeated procedures the CR percentage did not differ between the two groups (9/14 vs. 5/10; $p>0.05$). By means of univariate and multivariate analyses, we did not find any variable that influenced tumor response rate.

3.2. Toxicity and Adverse Events

Only one case of major complications directly related to procedures was recorded: One patient in fact had non-surgical cholecystitis that resolved with medical care. Some patients discontinued treatments cycles because they experienced a progression of liver disease.

At the time of the first DSMs-TACE cycle, only the number and location of lesions were significantly associated with clinical outcome. Four of the total cohort of patients withdrew from the study (drop-out rate 16.7%): 2 patients after the first treatment (1 of whom for worsened liver function, 1 who received liver transplant) and 1 patient after the second treatment (for worsened liver function). PES was observed in 9 patients (37.5%); while 4 subjects (16.6%) required a 24-hours extended stay. When we compared patients treated with multiple repeated treatment with those who had to discontinue the therapeutic cycle, we found that the drop-out group had more severe liver disease as assessed by the Child-Turcotte-Pugh (CPT) and the MELD scores (mean CPT 6 ± 1.3 vs. 7.8 ± 1.9 ; $p=0.01$ and MELD 9.1 ± 2.7 vs. 13.5 ± 3.1 ; $p=0.003$) and more advanced cancer disease (mean nodules diameter 17.5 ± 4.9 mm vs. 30.5 ± 17.6 mm; $p=0.009$). Indeed using univariate analysis, the factors that significantly influenced dropout rate were the liver disease and cancer disease stage. Transient increases in serum aspartate Aminotransferase/Alanine Transaminase (AST/ALT) and Gamma-glutamyl-transpeptidase (GGT) concentration were observed in most patients within 24h from the procedures. The transaminase levels, as well as liver and renal function, did not worsen in patients who completed repeated TACE compared to baseline (Table 5).

4. DISCUSSION

In this study, we report our preliminary experience with the use of repeated DSMs-TACE for the treatment of patients with unresectable HCC. It is well known that repeated cTACE sessions are needed to prolong disease free time survival for unresectable HCC. However the optimal time-point to assess treatment response reflecting long-term clinical prognosis during repeated cTACE sessions has been controversial and it sometimes fails to show clinical efficacy because of the way the antitumor drugs are delivered to the tumor. DEB TACE allows to embolize and release antitumor drugs gradually and locally in order to maximize local ischemia and tumor necrosis. Although DEB-TACE may not lead to longer Overall Survival (OS) than cTACE, it appears to be associated with better objective tumor response and lower toxicity. Ischemia clearly plays a role in the treatment effect seen after embolization, but the benefit of added chemotherapy in the embolization mixture has yet to be defined.

Performing DSMs-TACE the role of chemotherapeutic drug is emphasized theoretically isolating the effect that could be ascribed to embolization (*i.e.* DSMs: 30 min).

Our aim was to treat 24 patients by performing a repeated DSMs-TACE and we were able to perform a total of 53 repeated DSMs-TACE (a mean of 2.2 procedures per patients). After third treatment we found that more than half of patients enrolled showed a CR (14/24; 58.3%) while no patients showed PD. Overall we report a good tumor response and satisfactory patient's tolerability to the procedures. In particular we were able to show that, already after the first treatment, a good number of patients reached a CR (20.8%), even if exclusively in those affected by one lobe liver disease. At present, there is no conclusive data on the comparative efficacy of different retreatment strategies and standard recommendations regarding the policy for retreatment is lacking.

Such methodological approach allowed us to obtain a higher significant percentage of overall CR (58.3%) compared with the previous published data [17-19]. However, to our knowledge, there are no randomized controlled studies currently investigating the benefit of DSM use in TACE procedures.

Table 5. MELD and Child Pugh scores, AST, ALT, GGT, total bilirubin and creatinine levels, in patients submitted to 3 procedures.

	Baseline (n=16)	3st DSMs-TACE (n=12)	<i>p</i>
CPT (mean ± SD)	6.1 ± 1.4	6.1 ± 1.2	ns
MELD (mean ± SD)	9.1 ± 3	9.4 ± 2.4	ns
AST levels (UI/L) (mean ± SD)	77 ± 63	77 ± 52	ns
ALT levels (UI/L) (mean ± SD)	77 ± 80	64.2 ± 38	0.02
GGT levels (UI/L) (mean ± SD)	209 ± 258	278 ± 365	0.000
Total Bilirubin levels (mg/dl) (mean ± SD)	1 ± 0.5	1.2 ± 0.7	ns
Creatinine (mg/dl) (mean ± SD)	0.87 ± 0.28	0.87 ± 0.30	ns

ALT: Mean Alanine transaminase; AST: Mean Aspartate Aminotransferase; CPT: Child-Turcotte-Pugh score; GGT: Gamma-Glutamyl-Transpeptidase; MELD: Model for End stage Liver Disease; SD: Standard Deviation.

Furuse *et al* [17] published encouraging results on a group of seventeen patients suffering multifocal HCC, on whom they performed repeated DSMs-TACE, obtaining an overall response rate of 52.9%. Authors also reported a reduction of adverse effects of DSMs-TACE on liver function compared to Lipiodol-TACE, though they did not show whether DSMs could improve the survival rate.

Data published by Kirchhoff *et al.* [18], demonstrated that the tumour response rate did not differ between patients with advanced HCC treated with DSMs-TACE or Transarterial-Chemoperfusion (TACP) (a doxorubicin and cisplatin mixture was used in both cases, DSMs-TACE and TACP). However, there was a tendency towards the highest tumour response rates in the DSMs TACE arm (26% *vs.* 9%), in a subgroup of patients taken into consideration. In 2007, the same research group published a retrospective analysis of 112 DSM+Lipiodol TACE procedures on 47 HCC patients. No one showed a CR and only 36% of the population obtained a PR. Although the rate of complication they reported was low, it was still found to be higher in comparison to that described in DSMs monotherapy TACE. In fact, the mortality rate was 2.1% (one patient died, presumably from liver failure) and the major complications described were 5.4% [19].

Recently, Yamasaki *et al.* [20] performed a prospective randomized trial in 45 cirrhotic patients with HCC. Authors divided the patients into 3 groups before the angiography: Transcatheter Arterial Infusion chemotherapy (TAI) using lipiodol, TAI using DSMs, and TAI using Lipiodol and DSMs. The study showed a superior trend of higher tumor response rate in the Lipiodol+DSMs-TAI group (CR=40%) compared to the two monotherapy groups (Lipiodol-TAI: CR=26.7% and DSM-TAI: CR=26.7%, respectively). Moreover, the progression-free survival was higher in the Lipiodol+DSM-TAI group compared with DSM-TAI (377 days *vs.* 287 days; $p=0.02$) or Lipiodol-TAI (377 days *vs.* 177 days; $p=0.03$) groups. The overall survival rate did not differ between groups.

Advanced stages of liver disease, bilobar tumor localization and repeated treatments with conventional TACE are associated with higher complications and a greater mortality risk [30, 31].

Chan *et al.* reported an acute hepatic failure rate of 20 % after cTACE with 3% of irreversible liver failure [32].

The efficacy of a transcatheter arterial chemoembolization on hepatocellular carcinoma probably varies according to the tumor stage at diagnosis, the functional status of the uninvolved liver and the chemoembolization timing. Transcatheter arterial chemoembolization was effective on the treatment of unresectable hepatocellular carcinoma in several uncontrolled studies.

DSMs could be used for minimally invasive treatment of liver tumors, due to their transient non-permanent vascular occlusion properties [33]. In our experience, patients who discontinued repeated DSMs-TACE protocol showed more severe liver disease and more advanced cancer disease compared to those who completed repeated DSMs-TACE cycles.

Pieper *et al.* [16], recently, demonstrated that a complete temporary arterial embolization of normal liver tissue in pigs was safe, and did not lead to immediate alterations of the embolized tissue.

In this prospective, DSMs could prove to be effective in preserving the non-tumoral cirrhotic parenchyma, protecting it as possible from ischemic injury. In fact, due to their temporary occluding properties, the of DSMs could limit the ischemic damage to cirrhotic parenchyma, and prevent the paradoxical angiogenetic effects caused by tissue hypoxia, thus maintaining the benefits of intra-arterial infusion chemotherapy.

To date, very few studies have evaluated the efficacy and safety of DSMs-TACE in HCC treatment, and the small patients' population as well as the non-homogeneous therapeutic protocols of the different experimental designs has made it difficult to define the optimal treatment timing.

In our prospective pilot study, we were also interested in assessing the tolerability of consecutive DSMs-TACE at 4-6 week intervals in patients with unresectable uni- or multifocal HCC with an unimpaired liver function. Overall, repeated DSMs-TACE procedures seemed to reduce the risk of liver failure, yet the positive response to treatment in most likely related to the reduction of overexpression of VEGF; this aspect, in turn, may highlight the cytotoxic effect of chemotherapy drugs.

The short-term occlusion obtained using DSMs, which therefore implies rapid tissue reperfusion (DSMs half-life: 35-50 minutes) shortly after embolization, could reduce hypoxia and acidosis, and the consequent VEGF over production [8-16]. The latter is so far described as to be related with rebound neovascularization, tumor re-growth and cancer recurrence after radical (resection or liver transplant) or palliative (TACE) HCC treatments, in patients with incomplete response [34]. Furthermore, the sparing of non-cancer parenchyma from ischemic injury could allow extending treatment to patients with more severe liver disease. Repeated chemotherapy infusion, applied directly to the neoplastic tissue at periodical intervals, belongs to well-known oncological protocols that we have adopted, in an attempt to maximize the effect of chemotherapy, and destroy the highly replicating cancer cells.

However, chemotherapy of tumors is always accompanied by damage to normal tissues, which is a major clinical problem [35].

In this sense, the potential vascular changes in the hepatic arterial branch following repeated hepatic artery chemoembolization, is likely to reduce treatment efficacy [36]. Prolonged contact of cytotoxic agents with the vascular endothelium can certainly result in vascular changes, as has been seen in patients treated with hepatic arterial infusion of chemotherapeutic agents [37]. Thus, performing chemoembolization by administering the chemotherapeutic agent in combination with the DSMs embolic agent, we may preserve antegrade flow and minimize the stasis of the chemotherapeutic agent.

In terms of adverse effects or events, although PES was present in more than one third of patients, we do not report major complications. In addition, patients did not show liver

enzyme alterations compared to baseline values, except for a significant increase in GGT. In most cases, the reason for withdrawal was due to worsening of liver function, mainly in patients with more advanced liver disease, which occurred even before starting treatment.

Our study does, however, present several limitations. Firstly, we studied a relatively small sample of patients within a non-randomized study, and therefore it is of fundamental scientific relevance to confirm our preliminary data in a larger population, while also including a suitable control group. Secondly, our current follow-up is too short; a longer period of follow-up, combined to further assessment of clinical outcomes, is required to calculate the impact on Overall Survival (OS) rates and to obtain a more accurate evaluation of how many patients with HCC can actually be considered cured. However it is well debated that achievement of treatment response at an early time point is still the most robust predictor for favourable outcomes. Kim Bk *et al.* [38] recently demonstrated that objective responders as the initial response had the longest OS, followed by patients who subsequently achieved objective response after at least two sessions and those who did not achieve objective response during treatment course eventually. Surely, we reserve to analyze carefully the survival of these patients, which in this work we did because it was a preliminary experience.

Finally, it would be important to investigate whether a sequential DSMs-TACE protocol is extendable to patients with more advanced liver disease, and if so, to recruit a larger and more varied pool of subjects in future studies.

CONCLUSION

In selected patients with unresectable HCC, DSMs-TACE is a valid, well-tolerated alternative treatment to Lipiodol-TACE or DEB-TACE, as it has demonstrated to achieve a relatively high percentage of complete tumor necrosis. In patients who failed to obtain a CR, DSMs-TACE was still able to limit tumor disease, offering a satisfactory PR also in those who experienced deterioration of liver function and were therefore forced to quit treatment before completion of the protocol.

A very important aspect to consider is the selection of patients, in order to avoid liver failure in those with border-line liver compensation, and limit the number of subjects having to pull out of the study due to intra- or post-procedural complications.

In conclusion, to this date several studies have investigated the efficacy and safety of DSMs-TACE in the treatment of HCCs, yet the limited number of patients tested, combined with the non-homogeneous therapeutic protocols used, has made it difficult to define the optimal standards of treatment. In this prospective, further studies addressing these issues are definitely required, especially to validate the use of this alternative treatment, and to evaluate the impact on overall prolongation of patient survival.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved the institutional ethics committee of University Hospital Tor Vergata.

HUMAN AND ANIMAL RIGHTS

No animal were used in this study, Reported experiments on humans were in accordance with the ethical standards of the committee responsible for human experimentation (institutional national), and with the Helsinki Declaration of 1975, as revised in 2008 (<http://www.wma.net/en/20activities/10ethics/10helsinki/>).

CONSENT FOR PUBLICATION

Before every treatment patients provided written informed consent to participate to the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

REFERENCES

- [1] Bruix J, Sherman, M. Management of hepatocellular carcinoma: An update. *Hepatology* 2011; 53: 1020-2.
- [2] Chegai F, Orlacchio A, Merolla S, Monti S, Mannelli L. Intermediate hepatocellular carcinoma: The role of transarterial therapy. *Hepat Oncol* 2015; 2(4): 399-408.
- [3] Lencioni, R. Loco-regional treatment of hepatocellular carcinoma. *Hepatology* 2010; 52: 762-73.
- [4] Varela M, Real MI, Burrel M, *et al.* Chemoembolization of hepatocellular carcinoma with drug eluting beads: Efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007; 46: 474-81.
- [5] Lammer J, Malagari K, Vogl T, *et al.* Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: Results of the PRECISION V study. *Cardiovasc Intervent Radiol* 2010; 33: 41-52.
- [6] Xie ZB, Wang XB, Peng YC, *et al.* Systematic review comparing the safety and efficacy of conventional and drug-eluting bead transarterial chemoembolization for inoperable hepatocellular carcinoma. *Hepatol Res* 2015; 45(2): 190-200.
- [7] Lencioni R. Management of hepatocellular carcinoma with transarterial chemoembolization in the era of systemic targeted therapy. *Crit Rev Oncol Hematol* 2012; 83: 216-24.
- [8] Lencioni R, Petruzzi P, Crocetti L. Chemoembolization of hepatocellular carcinoma. *Semin Intervent Radiol* 2013; 30: 3-11.
- [9] Zhu AX, Duda DG, Sahani DV, Jain RK. HCC and angiogenesis: Possible targets and future directions. *Nat Rev Clin Oncol* 2011; 8: 292-301.
- [10] Sun HC, Tang ZY. Angiogenesis in hepatocellular carcinoma: The retrospectives and perspectives. *J Cancer Res Clin Oncol* 2004; 130: 307-19.
- [11] Wu XZ, Xie GR, Chen D. Hypoxia and hepatocellular carcinoma: The therapeutic target for hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; 22: 1178-82.
- [12] Minata M, Harada KH, Kudo M, Ikai I, Nishida N. The prognostic value of vascular endothelial growth factor in hepatocellular carcinoma for predicting metastasis after curative resection. *Oncology* 2013; 84 Suppl 1: 75-81.
- [13] Zhong C, Wei W, Su XK, Li HD, Xu FB, Guo RP. Serum and tissue vascular endothelial growth factor predicts prognosis in hepatocellular carcinoma patients after partial liver resection. *Hepatogastroenterology* 2012; 59: 93-7.
- [14] Poon RT, Lau C, Pang R, Ng KK, Yuen J, Fan ST. High serum vascular endothelial growth factor levels predict poor prognosis after radiofrequency ablation of hepatocellular carcinoma: Importance of tumor biomarker in ablative therapies. *Ann Surg Oncol* 2007; 14: 1835-45.

- [15] Taguchi T. Chemo-occlusion for the treatment of liver cancer. A new technique using degradable starch microspheres. *Clin Pharmacokinet* 1994; 26: 275-91.
- [16] Pieper CC, Meyer C, Vollmar B, Hauenstein K, Schild HH, Willheim KE. Temporary arterial embolization of liver parenchyma with degradable starch microspheres (EmboCept[®]S) in a swine model. *Cardiovasc Intervent Radiol* 2015; 38: 435-41.
- [17] Furuse J, Ishii H, Satake M, *et al.* Pilot study of transcatheter arterial chemoembolization with degradable starch microspheres in patients with hepatocellular carcinoma. *Am J Clin Oncol* 2003; 26: 159-64.
- [18] Kirchhoff TD, Rudolph KL, Layer G, *et al.* Chemoocclusion vs chemoperfusion for treatment of advanced hepatocellular carcinoma: A randomised trial. *Eur J Surg Oncol* 2006; 32: 201-7.
- [19] Kirchhoff TD, Bleck JS, Dettmer A, *et al.* Transarterial chemoembolization using degradable starch microspheres and iodized oil in the treatment of advanced hepatocellular carcinoma: Evaluation of tumor response, toxicity, and survival. *Hepatobiliary Pancreat Dis Int* 2007; 6: 259-66.
- [20] Yamasaki T, Hamabe S, Saeki I, *et al.* A novel transcatheter arterial infusion chemotherapy using iodized oil and degradable starch microspheres for hepatocellular carcinoma: A prospective randomized trial. *J Gastroenterol* 2011; 46: 359-66.
- [21] Niessen C, Unterpainner E, Goessmann H, *et al.* Degradable starch microspheres versus ethiodol and doxorubicin in transarterial chemoembolization of hepatocellular carcinoma. *J Vasc Interv Radiol* 2014; 25: 240-7.
- [22] Orlacchio F, Chegai S, Merolla S *et al.* Downstaging disease in patients with hepatocellular carcinoma outside up-to-seven criteria: Strategies using degradable starch microspheres transcatheter arterial chemo-embolization. *World J Hepatol* 2015; 7(12): 1694-1700.
- [23] Lencioni R, Llovet J. Modified RECIST (mRECIST) Assessment for Hepatocellular Carcinoma. *Semin Liver Dis* 2010; 30: 52-60.
- [24] Bruix J, Sherman M, Llovet JM, *et al.* Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; 35: 421-30.
- [25] Gish RG, Lencioni R, Di Bisceglie AM, Raoul JL, Mazzaferro V. Role of the multidisciplinary team in the diagnosis and treatment of hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol* 2012; 6: 173-85.
- [26] Oken MM, Creech RH, Tormey DC, *et al.* Toxicity and response criteria of the eastern cooperative oncology group. *Am J Clin Oncol* 1982; 5: 649-55.
- [27] Lastoria S, Piccirillo MC, Caracò C, *et al.* Early PET/CT scan is more effective than RECIST in predicting outcome of patients with liver metastases from colorectal cancer treated with preoperative chemotherapy plus bevacizumab. *J Nucl Med* 2013; 54: 2062-9.
- [28] Sacco R, Faggioni L, Bargellini I, Ginanni B, Battaglia V, Romano A, *et al.* Assessment of response to sorafenib in advanced hepatocellular carcinoma using perfusion computed tomography: Results of a pilot study. *Dig Liver Dis* 2013; 45: 776-81.
- [29] Chegai F, Merolla S, Greco L, *et al.* Re: Baseline and early MR apparent diffusion coefficient quantification as a predictor of response of unresectable hepatocellular carcinoma to doxorubicin drug-eluting bead chemoembolization. *J Vasc Interv Radiol* 2016; 27(9): 1456-8.
- [30] Brown DB, Nikolic B, Covey AM, *et al.* Quality improvement guidelines for transhepatic arterial chemoembolization, embolization, and chemotherapeutic infusion for hepatic malignancy. *J Vasc Interv Radiol* 2012; 23: 287-94.
- [31] Lladó L, Virgili J, Figueras J, *et al.* A prognostic index of the survival of patients with unresectable hepatocellular carcinoma after transcatheter arterial chemoembolization. *Cancer* 2000; 88: 50-7.
- [32] Chan AO, Yuen MF, Hui CK, Tso WK, Lai CL. A prospective study regarding the complications of transcatheter intraarterial lipiodol chemoembolization in patients with hepatocellular carcinoma. *Cancer* 2002; 94: 1747-52.
- [33] Nishiofuku H, Tanaka T, Matsuoka M, *et al.* Transcatheter arterial chemoembolization using cisplatin powder mixed with degradable starch microspheres for colorectal liver metastases after FOLFOX failure: Results of a phase I/II study. *J Vasc Interv Radiol* 2013; 24: 56-65.
- [34] Sergio A, Cristofori C, Cardin R, *et al.* Transcatheter Arterial Chemoembolization (TACE) in Hepatocellular Carcinoma (HCC): The role of angiogenesis and invasiveness. *Am J Gastroenterol* 2008; 103: 914-21.
- [35] Kwiecień I, Michalska M, Włodek L. The selective effect of cystathionine on doxorubicin hepatotoxicity in tumor-bearing mice. *Eur J Pharmacol* 2006; 550: 39-46.
- [36] Lewandowski RJ, Wang D, Gehl J, *et al.* A comparison of chemoembolization endpoints using angiographic versus transcatheter intraarterial perfusion/MR imaging monitoring. *J Vasc Interv Radiol* 2007; 18: 1249-57.
- [37] Charnsangavej C, Kirk IR, Dubrow RA, *et al.* Arterial complications from long-term hepatic artery chemoinfusion catheters: evaluation with CT. *AJR Am J Roentgenol* 1993; 160: 859-64.
- [38] Kim BK, Kim SU, Kim K *et al.* Complete response at first chemoembolization is still the most robust predictor for favorable outcome in hepatocellular carcinoma. *J Hepatol* 2015; 62(6): 1304-10.