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Combined 75-150µm M1 and 150-300µm [DC-Beads^{tm}] DEB-TACE for Hepatocellular Carcinoma: Single Center Prospective Results

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1. Abstract

1.1. Purpose: To report first single center long-term, prospective results in treating HCC with a combined embolization protocol of 75-150µm M1 and 100-300µm DC-BeadsTM as well as toxicity profile and identification of prognostic factors associated with better treatment outcome.

1.2. Materials and Methods: Fifty-five naïve nodules in thirty-five patients were prospectively enrolled. The embolization protocol was strictly standardized and the amount of DC-Beads administered per nodule/feeder was recorded. Procedural super selectivity was classified according to a dedicated score [range 0-2]. Embolization results were evaluated according to the mRECIST criteria with MDCT/MRI at 1, 3-6 and 9-12 months. Toxicity profile was assessed with lab-test pre- and post-procedural monitoring; complications were recorded. Statistical analysis was performed to identify correlation between patient/nodule characteristics and response to treatment.

1.3. Results: mRECIST classification at 1 month follow-up was: CR 40%, PR 47.3%, SD 12.7% and OR 87.3%; three-six months follow-up was: CR 40%, PR 25%, SD 5% and PD 30% [OR 65%]; nine-twelve months follow-up was CR 51.5%, PR 18.1%, SD 6.1% and PD 24.2% [OR of 69.6%]. Super selectivity Score 0 was achieved in 23 treatments [41.8%], score 1 in 28 [50.9%] and score 2 in 4 cases [7.3%]. Nodules smaller than 3 cm in CR never required the maximum 100-300µm beads dose; nodules bigger than 3 cm in CR always required 100-300 µm beads administration.

1.4. Conclusion: Our proposed combined DEB-TACE protocol significantly improves procedural outcome over other proposed approaches [>300µm or only <100µm particles], with a reasonably low complication rate.

2. Introduction

Transarterial therapies, in particular Drug Eluted Beads Trans Arterial ChemoEmbolization [DEB-TACE], are the standard of care for early–intermediate hepatocellular carcinoma [HCC] not amenable to curative treatments and also as a bridge to liver transplantation [LT]. [1] Latest evidence on DEB-TACE procedures is controversial. As matter of fact, several trials aimed to demonstrate the superiority of DEB-TACE over conventional lipiodol TACE [lp-TACE] failed. [2-4] Despite the presence of specific technical recommendation

[5], the DEB-TACE protocol employed in many countries differs with regards to particles diameter. Padia et al. [6] demonstrated that patients treated with 100-300 μm particles are favoured over 300-500μm. In light of this evidence and thanks to the availability of smaller [<100 μm] drug-eluting beads, different research in both the pre-clinical [rabbit model] [7] and clinical setting have tested the rationale that: smaller particles could achieve better clinical result by associating increased tumor penetration and devascularization, whilts delivering the same chemiotherapeutic dosage of standard caliber particles. In particular, in vivo, safety and efficacy [8-10] as well as toxicity [11], has been tested but long term results are not available yet. The aim of this prospective single centre study is to report clinical long term results in treating typical hypervascular hepatocellular carcinomas [HCC] with a combined embolization protocol of 75-150 μm DC-Beads™ M1 beads and 100-300 μm DC-Beads™ beads [Biocompatibles, Farnham,Surrey, UK].

3. Material and Methods

This study was approved by the local Institutional Review Board. Between September 2014 and July 2015 fifty-five hypervascular typical HCC nodules in thirty-five consecutive patients [mean age 69.3 ± 9.8 [51-83]; 27 Male] with cirrhosis referred for TACE were prospectively enrolled in the study. Clinical and demographics characteristics are summarized in (Table 1).

Table 1: Population demographics and baseline characteristics according to pre-procedural MDCT.

Patient number	N=35
Number of nodules	N= 55
< 3 cm	N=41
> 3 cm	N=14
Nodules Dimension	
Maximum diameter, mm, (mean value, range)	24.8 (10-47)
Minimum diameter, mm, (mean value, range)	20.3 (10-36)
Nodules volume, cm3 (mean value ± SD, range)	8.3 ± 7.6 (0.87 -67.44)
Age, year (mean value ± SD, range)	69.3 ± 9.8 (51-83)
Sex (M/F)	27-Aug
Child Pugh (N/%)	
A	23 (65.7%)
B	12 (34.3%)
C	0
Etiology (N/%)	
HCV	22 (62.8%)
HBV	2 (5.7%)
alcohol related cirrhosis	8 (22.8%)
NASH	1 (2.8%)
Mixed	2 (5.7%)
BCLC	
A	19 (54.2&)
B	16 (45.8%)
MELD	
<15	26 (74.3%)
= >15	9 (25.7%)
Mono-focal / multi-focal disease (N)	11 (31.4%)/ 24 (68.6%)
Mono-lobar / multi-lobar disease (N)	30 (85.7%)/ 5 (14.3%)
AFP serum level	
< 7 μg/L	12 (34.3%)
7-200 μg/L	21 (60%)
= or > 200 μg/L	2 (5.7%)
Diagnosis	
MRI/MDCT agreement	30 (85.7%)
1 second line imaging + AFP > 200 μg/L	2 (5.7%)
1 second line imaging + biopsy	3 (8.6%)
Indication for treatment	
Palliative	15 (42.9%)
Downstaging	11 (31.4%)
bridging	9 (25.7%)

3.1. Inclusion Criteria

Inclusion criteria for treatment were established by consensus within a multidisciplinary board [composed by transplant surgeon, an interventional radiologist, a body radiologist and hepatologist]. All enrolled patients had a previous diagnosis of typical hypervascular HCC and were considered not eligible for curative treatments [surgical resection or percutaneous ablative treatments] due to both anatomical or disease related [total HCC burden] factors. Diagnosis was performed with second line imaging agreement among MDCT and MRI in 30 patients [85.7%]; with MDCT and an alfafetoprotein [AFP] level greater than 200 μg/L in 2 patients [5.7%]; with MDCT associated with a tru-cut biopsy [definitive for HCC diagnosis] executed prior to multidisciplinary board evaluation in three cases [8.6%]. All lesions enrolled were naïve for any percutaneous, catheter based or systemic treatment.

3.2. DEB-Tace Technique

The entire DEB-TACE protocol was highly standardized prior to patient enrollment in order to reduce potential bias. All procedures were performed by two Interventional Radiologist [9 and 7 years experience] in an angio-suite, equipped with a state of the art angiographer [Artis Zee; Siemens, Erlangen, Germany]. Procedures were performed via the femoral access in all cases. Detailed liver nodule feeders mapping was performed with proper DSA [PA and RAO 25 degree] and dual-phase CBCT after having positioned the 4 Fr catheter within the common or proper hepatic artery. After having identified the nodule's feeder, as well as the eventual presence of parasitic extrahepatic feeders, their catheterization were performed with coaxial technique with a 2.7 Fr microcatheter [Progreat, Terumo, Tokyo, Japan], as selectively as possible. The embolization protocol consisted of 1 vial of 75-150 μm DC-Beads M1 particles [Biocompatibles, Farnham,Surrey, UK] pre-loaded with 50 mg of Epirubicin [Pfizer, Latina, Italy] and 1 vial of 100-300 μm DC-BEADS particles pre-loaded with 50 mg of Epirubicin. Embolization was started with M1 particles in all cases followed by 100-300 particles. The embolization's end point was 5 hearts beats stasis. In cases in whom the nodule was fed by more than one single feeder the dose was splitted in order to embolize the different feeding vessels, according to the same methodology [M1 particles first]. Per feeder dose of both beads [ml] and epirubicin [mg] were recorded. All procedures were performed under continuous ECG, pO_2 saturation and SBP monitoring. Premedication consisted in 4 mg of ondansetron [Ondansetron, Fresenius Kabi, Verona, Italy], and 1 g of paracetamol [Paracetamolo, S.A.L.F. S.p.A., Bergamo, Italy] administered at the beginning of the procedure. No antibiotic prophylaxis was given. Repeat embolization during follow-up followed the "on-demand" scheme. Eventual retreatment sessions were performed only in Partial Response [PR] cases, if the clinical goal was not achieved [e.g: drop-out for residual active tissue treated with ablation]. Follow-up imaging timeline included a pre-procedural MDCT/MRI within 1 month from planned treatment, a MDCT/MRI follow-up at 1, 3-6 and 9-12 month from TACE procedures. Embolization results were evaluated according to the mRECIST criteria per nodule. [12]

3.3. DEB-TACE Scoring

In order to compare the different TACE procedures, they were categorized according a dedicated angiographic score, in which score 0 represents those cases in whom only the HCC feeder was embolized; score 1 represents those cases in whom only 1 sub-segmental non target vessel was impaired during embolization; score 2 represents those cases in whom >1 sub-segmental or segmental non target vessels were impaired during embolization (Figure 1).

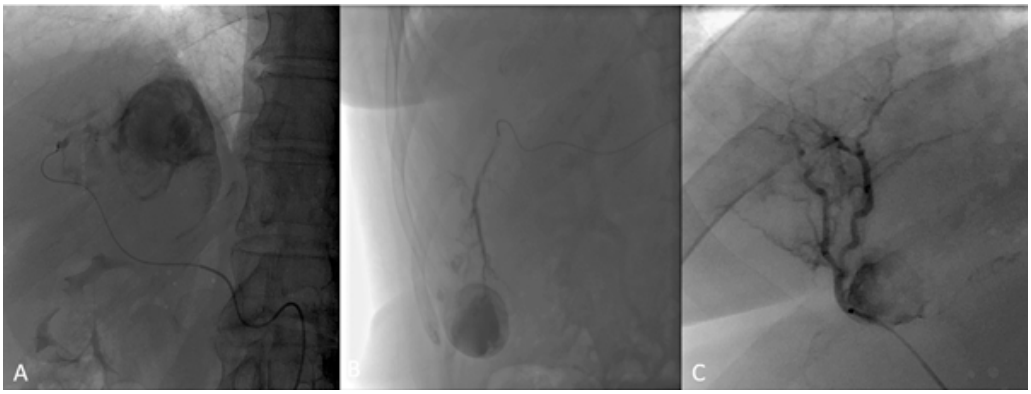

Figure 1: Score of superselectivity: Score 0 superselective tumoral feeder catheterization without extratumoral embolization (A); Score 1 only one sub-segmental non target vessel was embolized (B); Score 2 >1 sub-segmental or segmental non target vessels were embolized (C)

3.4. Study Endpoints

Primary endpoints were [i] tumor response at 1, 3-6 and 9-12 months; [ii] correlation between nodule's dimension, effective administrated dose and mRECIST response at 1 month follow-up; [iii] toxicity. Secondary endpoints were: [*] histological correlation on explanted liver with post-operative mRECIST classification; [**] ability to downstage total tumor burden in order to activate waiting list for LT; [***] identification of patient's prognostic factors correlated with tumor response. In order to assess the safety profile of the DEB-TACE protocol monitoring of liver enzymes was performed pre-procedural [immediately before] and post-procedural [36–48 h] and synchronously with the scheduled imaging follow up visits. Liver function tests included were: serum total and conjugated bilirubin, aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], gamma glutamyl transpeptidase [GGT], and albumin. Prothrombin time [INR] and blood cell counts were also evaluated. All Adverse Events [AEs] recorded were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 [13]. According to the scale, Grade 1 - complications are mild and do not require intervention; Grade 2 - events require bedside medical management and medication; Grade 3 - complications are severe and require additional intervention; Grade 4 - complications are those that are life- threatening and/or result in chronic disability; Grade 5 - complication is death related to the adverse events. Post embolic syndrome [PES] was defined as the onset of post-operative fever and/or nausea and/or pain, greater than a Visual Analogue Score [VAS] of 6. In order to identify prognostic factors associated with better tumor response the entire enrolled cohort was categorized according to several pre- and intra-procedural clinical, radiological imaging, and laboratory characteristics. The clinical characteristics investigated were: age, gender, cirrhosis etiology, Child-Pugh Score, BCLC stage, MELD score; the radiological variables were: number of lesions, lesion segment, monofocal vs multifocal disease, monolobar vs multilobar spread, <3 vs > 3 cm target lesion diameter, target lesion diameter, target lesion volume, capsulate nodule, infiltrative disease. The laboratory analysis evaluated were the same of those assessed to test the safety profile.

3.5. Statistical Analysis

The normality of each continuous variable group was tested using the Kolmogorov-Smirnov Z test. Continuous data were described as the mean value ± SD. Receiver Operating Characteristics [ROC] curve analysis was performed and the model was characterized by the area under the receiver operating characteristics curve [Az] with 95% Confidence Intervals [CIs] [0.5 = no predictive value; 1.0 = perfect prediction]. Mann-Whitney test was used to assess differences between groups whereas the Wilcoxon test was used to identify pair-wise differences before and after therapy. Statistical analysis was performed with the SPSS 13.0 statistical package [SPSS Inc, Chicago, IL]. P values < 0.05 was considered as statistically significant, and all p values were calculated using a two-tailed significance level. Graphics were plotted with MedCalc 8.0 software [MedCalc, Mariakerke, Belgium].

4. Results

Fifty-five nodules were treated in thirty-five patients. Retreatment session was needed for 9/55 nodules [16.4%], respectively four procedures in nodule > 3cm and 5 in nodule < 3 cm, leading to a total number of fourty-four DEB-TACE session. Tumor response rate at 1 months from the DEB-TACE procedure was: Complete Response [CR] 40% [n=22], Partial Response [PR] of 47.3% [n=26], Stable disease [SD] of 12.7% [n=7] leading to an Objective Response [OR] rate [CR+PR] of 87.3% [n=48] and a Disease Control [DC] rate [OR+SD] of 100%. Mean residual volume [RV] of PR patients was 30.8% of the pre-procedural nodule volume [4.4-70%]. Three-six months follow-up was reached by 40 out of 55 nodules demonstrating: CR of 40% [n=16], PR of 25% [n=10], SD of 5% [n=2] and Progressive Disease [PD] of 30% [n=12], thus leading to an OR rate of 65% [n=26] and a DC rate of 70% [n=28]. RV of PR patients was 23.7% [4.4-44.3%]. At this timeline eight patients dropped out from follow-up: for metastatic disease [n=1], worsening of clinical comorbidities [n=2], ablation [n=2], radioembolization [n=3]. The 9-12 months follow-up was reached by 33 out 55 nodules demonstrating a CR of 51.5% [n=17], PR of 18.1% [n=6], SD of 6.6% [n=2] and PD of 24.4% [n=8], thus leading to an OR rate of 69.6% [n=23] and a DC rate of 75.5% [n=25]. RV of PR patients was 25.6% [4.4- 50%]. At this timeline four patients dropped out from follow-up: for orthotopic liver transplantation [n=1], worsening of clinical comorbidities [n=1], ablation [n=1], degradable starch microsphere TACE [DSM-TACE] [n=1]. (Table 2) summarizes tumor response of the entire cohort at the different timepoints and according to the different tumor size [< 3cm vs >3 cm]. Regarding the selectivity of the DEB-TACE procedure, score 0 was achieved in 23 treatments [41.8%] [15 in <3cm nodules and 8 in >3cm nodules], score 1 in 28 [50.9%] [23 in <3cm nodules and 5 in >3cm nodules], score 2 in 4 cases [7.3%] [3 in <3cm nodules and 1 in >3cm nodules]. (Table 3) shows different nodule's mRECIST response at 1 month follow-up after DEB-TACE, stratified for dimension and effective administrated dose. Nodules smaller than 3 cm with a CR never required the maximum 100-300 μm beads dose; nodules bigger than 3 cm with a CR always required 100-300 μm beads administration. Nodules smaller than 3 cm demonstrating OR required the entire dose of 100-300 μm beads only in 3/34 cases [8.8%]; whereas nodules bigger than 3 cm demonstrating OR required the entire dose of 100-300 μm beads in 5/14 cases [35.7%]. With regards to the amount of 100-300 μm beads administred after the entire M1 beads dose, it was appreciable a progressive increase with the increase of the nodule dimension [e.g.: 30% for nodule < 3cm to 42.5% for nodule > 3 cm]. The comparison of pre- and post-procedural [36–48 h] lab tests, demonstrated a statistically significant variation for: AST [55 vs 68, P=0.0001], ALT [44 vs 56, P=0.0002], neutrophil absolute count [2.4 vs 3.71, P=0.0102], neutrophil percentage [54 vs 69, P=0.0001],

only. Among mild [grade 2] clinical adverse events, PES occurred in 6/44 treatment [13.6%] characterized by fever [n=2], nausea [n=4] and pain [n=6] with a median VAS score of 7, all healed with medical therapy, not requiring prolonged hospitalization in any case. The only major adverse event [grade 5] was a case of hepatic abscess, that lead to patient's exitus [18 days from the procedure] due to multi-resistant Klebsiella spp. sepsis. This patient had an history of portal hypertension and primitive biliary cirrhosis. Patient previously underwent transjugular intrahepatic porto-systemic shunt [TIPS] to treat esophageal varices and left biliary drainage and cholangioscopy in order to diagnose the nature of the stenosis causing an indolent [total bilirubine 1,8 mg/dL] left lobe biliary system dilation. These two factors: the flow diversion caused by TIPS and the not sterile biliary tree due to the previous percutaneous procedure, predisposed the patient to complication onset. The procedure was performed because the patient was Child Pugh B7 score and a BCLC B stage so fit for enrollment.

Five patients with nine treated nodules underwent LT. Histological analysis of the explanted liver was concordant with radiological response prior transplantation, in particular: four nodules classified as CR demonstrated an histological necrosis >90% (Figure 2), the remaining 5 nodules [2 PR, 1 SD, 2 PD] demonstrated an active pathological tissue >50%.

In eight patients [18.1%] DEB-TACE protocol allowed downstaging of total tumor burden and listing for LT. Regarding identification of patient's prognostic factors correlated with tumor response, ROC curve analysis of pre- and intra-procedural clinical, radiological imaging and laboratory characteristics demonstrated that: CR at 1 months follow up was related to the amount of M1 beads administered [0.69; p=0.0136]; CR at 12 months was related to higher pre-procedural ALT levels [0.75; p=0.0108]. PR at 1 month follow up was related to lower levels of alphafetoprotein [0.71; p=0.0302]; PR at 6 months follow-up was related to a greater total amount of beads administered [0.8; p=0.0011] and lower age [0.78; p = 0.0069]; PR at 12-months follow-up was related to a lower age [0.77; p=0.0204]; OR at 1 month follow-up was related to the total dose of beads administered [0.83; p =0.0001]; 12 months-OR was related to higher pre-procedural AST [0.78; p=0.0034], ALT [0.75; p=0.0122], ALP [0.79; p=0.414] and alphafetoprotein [0.81; p=0.0302] levels (Supplementary Table).

Table 2: Tumor response classified with mRECIST results (* 8 patients drop-out; ** 4 patients drop-out)

Nodule size	Number of nodules	Follow-up (1 month)				Follow-up *1(3-6 months)				Follow-up*2(9-12 month)			
		CR	PR	SD	PD	CR	PR	SD	PD	CR	PR	SD	PD
< 3 cm	41	17	17	7	0	12	3	2	11	12	2	2	6
≥ 3 cm	14	5	9	0	0	4	7	0	1	5	4	0	2
		22	26	7	0	16	10	2	12	17	6	2	8
Total	55	40%	47.3%	12.7%	0%	40%	25%	5%	30%	51,5%	18.1%	6.1%	24.2%
OR= CR+PR		48 (87.3%)				26 (65%)				23 (69.6%)			
DC=OR+SD		55 (100%)				28 (70%)				25 (75.7%)			

Table 3: Correlation between nodule's dimension, effective administrated dose and mRECIST

	M1 complete administration Nodules (N=)					M1 partial administration Nodules (N=)				
	Entire group	100-300µm Maximum dose	100-300µm Partial dose		100-300µm 0%	Entire group	100-300µm Partial dose		100-300µm 0% M1 partial dose	
	(N=)	(N=)	(N=)	% of beads	(N=)	(N=)	(N=)	% of beads	(N=)	% of beads
	26	7	17	35.4	2	29	10	13	19	42
< 3cm	19	3	14	30	2	22	6	8.4	16	40
≥3cm	7	4	3	42.5	0	7	4	42	3	57.5
CR										
<3cm	9	0	7	33.4	2	8	1	10	7	39.6
≥3cm	4	3	1	17.5	0	1	1	45	0	-
PR										
<3cm	8	3	5	45	0	9	2	11.5	8	35.6
≥3cm	4	2	2	25	0	5	3	38	2	56.2
SD										
<3cm	2	0	2	12	0	4	3	6,6	1	40
≥3cm	0	0	0	-	0	1	0	-	1	60
OR	25	7	15	32.6	2	23	7	10.4	17	38.6

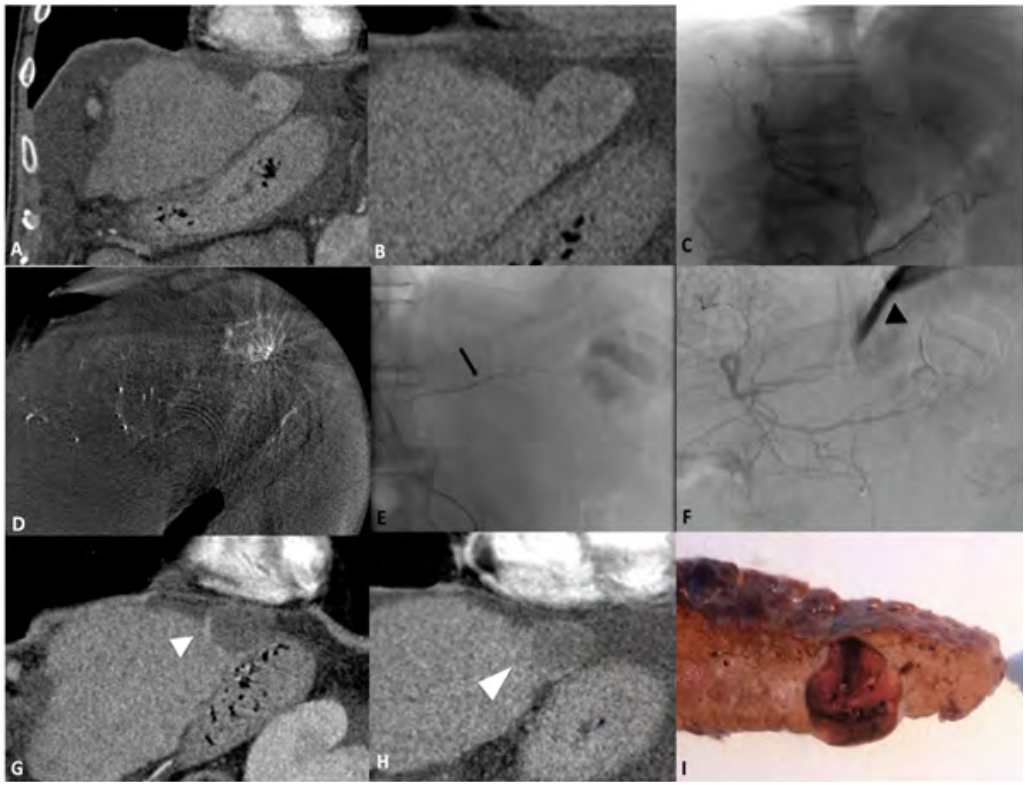

Figure 2: 54 years old patient with HCV related chirrosis (BCLC A; Child-Pugh B8) with a 30x28 mm S2 HCC. Arterial and delayed pre-procedural MDCT (A-B) demonstrate a typical HCC site in S2. Diagnostic left epatic artery injection demonstrate a single feeder (C); CBCT confimrm the lesion and the single feeder (D) that was subsequently superselectively chateterized (score 0). Trough 2,7 Fr microcatheter full dose of M1 beads and 90% of 100-300 μm was administered (E) obtaining an arterial stasis (F). 1 month follow-up CT shows any active tissue with non-patchy (< 2mm) enhancement (G) without wash out in delayed phase (H). Histopathological post-LT examination shows > 90 % of intranodular necrosis.

Supplementary Table: Correlation between pre- and intra-procedural clinical, radiological imaging, and laboratory characteristics with mRecist responce to treatment (statistical significant results in bold).

	CR	CR	CR	PR	PR	PR	OR	OR	OR
	1 month	6 months	12 months	1 month	6 months	12 months	1 month	6 months	12 months
Total Dose of Farmorubicin	0.63 (0.1265)	0.51 (0.9668)	0.59 (0.401)	0.54 (0.6879)	0.8 (0.0011*)	0.72 (0.0748)	0.83 (0.0001*)	0.68 (0.1122)	0.68 (0.1122)
M1 farmorubicin	0.69 (0.0136*)	0.59 (0.3227)	0.53 (0.7657)	0.52 (0.8174)	0.55 (0.6866)	0.54 (0.6866)	0.82 (0.0001*)	0.63 (0.2746)	0.59 (0.321)
100-300	0.53	0.56	0.64	0.54	0.68	0.62	0.54	0.51	0.59
Farmorubicin	(0.6944)	(0.539)	(0.2117)	(0.639)	(0.1789)	(0.4698)	(0.7489)	(0.901)	(0.301)
Tumor volume	0.59 (0.271)	0.51	0.52 (0.846)	0.62 (0.138)	0.5 (0.999)	0.52 (0.822)	0.51 (0.919)	0.53 (0.705)	0.52 (0.915)
Age	0.51 (0.899)	0.62 (0.2397)	0.61 (0.3319)	0.51 (0.8672)	0.78 (0.0069*)	0.77 (0.0204*)	0.53 (0.913)	0.62 (0.3335)	0.57 (0.5989)
Child Pugh Score	0.51 (0.9495)	0.52 (0.8665)	0.52 (0.8365)	0.53 (0.7593)	0.52 (0.8338)	0.57 (0.6642)	0.54 (0.7234)	0.58 (0.5103)	0.56 (0.5534)
MELD	0.58 (0.3611)	0.63 (0.2811)	0.66 (0.1734)	0.61 (0.2072)	0.56 (0.7291)	0.57 (0.7411)	0.51 (0.9202)	0.64 (0.2276)	0.59 (0.4043)
MELD Na	0.58 (0.3448)	0.59 (0.3712)	0.58 (0.4661)	0.65 (0.0792)	0.61 (0.6069)	0.55 (0.8375)	0.69 (0.1541)	0.54 (0.7177)	0.52 (0.8266)
AST	0.54 (0.6372)	0.65 (0.1461)	0.75 (0.0108*)	0.51 (0.8705)	0.64 (0.3209)	0.54 (0.7902)	0.53 (0.8389)	0.62 (0.3795)	0.78 (0.0034*)
ALP	0.51 (0.9691)	0.51 (0.9863)	0.65 (0.2694)	0.61 (0.2855)	0.63 (0.6449)	0.61 (0.5704)	0.63 (0.2524)	0.67 (0.2582)	0.79 (0.0414*)
ALT	0.56 (0.4471)	0.51 (0.9116)	0.61 (0.3992)	0.58 (0.3526)	0.52 (0.9035)	0.6 (0.6023)	0.51 (0.9239)	0.61 (4854)	0.75 (0.0122*)
GGT	0.51 (0.9293)	0.55 (0.6185)	0.61 (0.3648)	0.51 (0.9829)	0.55 (0.7436)	0.55 (0.7774)	0.65 (0.1662)	0.6 (0.4171)	0.61 (0.3525)
Total bilirubine	0.54 (0.6572)	0.51 (0.9521)	0.56 (0.5834)	0.61 (0.1807)	0.54 (0.7639)	0.51 (0.9478)	0.59 (0.7856)	0.58 (0.4870)	0.52 (0.8835)
Direct bilirubine	0.56 (0.6441)	0.54 (0.6871)	0.53 (0.7662)	0.65 (0.0551)	0.54 (0.7412)	0.55 (0.7525)	0.61 (0.5976)	0.51 (0.9545)	0.57 (0.5155)
Albumine	0.51 (0.9762)	0.51 (0.9015)	0.58 (0.4822)	0.59 (0.3051)	0.62 (0.2361)	0.53 (0.8044)	0.53 (0.8629)	0.56 (0.7124)	0.71 (0.2043)
Platelet	0.56 (0.4515)	0.52 (0.8424)	0.56 (0.6003)	0.58 (0.3858)	0.53 (0.8277)	0.61 (0.3711)	0.56 (0.6654)	0.6 (0.3402)	0.65 (01844)
White blood cell (WBC)	0.51 (0.8807)	0.51 (0.9692)	0.51 (0.9435)	0.51 (0.9151)	0.58 (0.5547)	0.55 (0.7303)	0.62 (0.5151)	0.52 (0.8762)	0.53 (0.8752)
Alphafetoprotein	0.63 (0.1395)	0.5 (1)	0.58 (0.5675)	0.71 (0.0302*)	0.63 (0.3311)	0.7 (0.2131)	0.54 (0.8207)	0.72 (0.0894)	0.81 (0.0302*)

5. Discussion

The results of this study report first long-term outcome of a novel combined [DEB-TACE M1 and 100-300 μm particles] embolization protocol, which significantly ameliorate the tumor response rate if compared to previously proposed embolization protocols. This aspect is critical considering that worldwide DEB-TACE protocols employed particles with different diameters. In fact, our protocol leads to a better 6 months tumor control if compared to those of the Precision V study [2] that employed a combination of 300-500 μm and 500-700 μm drug-eluted particles. In particular 6 months CR rate was 40% vs 26.9%, OR rate was 65% vs 51.6% and finally DC rate was 70% vs 63.4%, in our and in the Precision V study, respectively; furthermore, our trend of tumor control was slightly improved at 9-12 months follow-up. These improvements reflect the recent literature evidences that demonstrate an increase of treatment efficacy employing smaller size beads. [6] The rationale underlying this observation is that: only particles smaller than 300 μm permits a deeper penetration of the beads within the tumor network arterioles obtaining a "real" anoxia of the target tissue. As a matter of fact, hypoxia after an incomplete arterial network embolization has been demonstrated to be the most powerful stimulation of Vascular-Endothelial-growing factor [VEGF]; its expression results in recruitment of a new arterial vascularization thus leading to higher percentage of recurrence or lower achievement of complete response. [14-18] Under these evidences, pharmaceutical research was targeted on the production of smaller size [<100 μm] drug-eluting particles that could achieve deeper penetration within the arterioles of the tumor network, thus delivering the highest amount of drug. Several authors investigated the short-term results and the toxicity profile of different <100 μm drug-eluted beads. [7-11]

By analyzing the available literature on clinical series [8, 10] performed with drug-eluted particles smaller than 100 μm emerges that all studies employed these particles only with no combination with 100-300 μm particles. In particular, Spreafico at al. [8], by employing the same small embolic agent [DC BEADS M1] that we used, found a CR in 33,3% with an OR of 77,7% at a median interval of 3 months. Even though data from this study are not comparable, because they are presented as "median time to best response" rather than according to mRECIST at each follow-up time as in our study, Spreafico's results [CR of 50%, OR rate of 67.6% a DC rate of 73.5%] seem to demonstrate a worse trend over local tumor control if compared with our study at 9-12 months. In addition, Malagari et al. [10], by exploring progressive escalation charging dose of doxorubicin on drug-eluted 30-60 μm particles [Hepasphere, Biosphere, Merit], found a lower 1 month CR rate [17.8%] and OR rate [68.9%] if compared to those of the present study of 40% and 87.3%, respectively. In another recent series Greco et al [19] employing Embozene Tandem 40 μm [Boston Scientific, Minneapolis, MA, United States] preloaded with doxorubicin obtained a overall CR rate of 46.6% with a OR of 72,6%. These evidences, support the idea that the better response rate of our protocol compared to the other proposed protocols employing only particles smaller than 100 μm could be due to the combined nature of our modality of embolization [M1 + 100-300 μm particles]. The robustness of our proposed protocol is demonstrated by two additional findings of our study. First, only 4 out of 17 nodules with a CR at 1 month, had a subsequent progression of disease; second the amount of M1 Beads and the total amount of beads are strongly correlated with CR and OR at 1 month, respectively. To strengthen the evidence supporting our proposed embolization protocol is worth to underline the results of the analysis of post-treatment residual tumor volume. The PR mRECIST classification consider that the tumor initial volume [sum of diameters] must be reduced by more than 30%. Thus, the PR category can be very heterogeneous, and it is possible to have a tumor with nearly 70% of initial total diameters that is still vital and another in which only 5% is still vital. To overcome this limit of the mRECIST classification we also assessed all nodule's total volume before and after the procedure, in order to better stratify patient within the PR mRECIST category. According to this evaluation, we found that the mean residual volume of nodules categorized as PR was only 30.8% of the pre-procedural nodule volume. (Figure 3) Therefore the PR patient group, in our series, is a population in which the embolization procedure provided an efficient "debulking" of the total tumor burden allowing activation of transplant waiting list [in eight cases] and the possibility of treating remnant active tissue with curative procedures [RFA in three cases]. Our results are due also to the degree of selectivity of the DEB-TACE procedures. In fact, our dedicated angiographic DEB-TACE score was 0 or 1 in nearly 90% of cases. With regards to the adverse events rate, our series demonstrated a lower incidence than those reported in literature. [10, 11] In particular, we had 13,6% of PES, while Malagari et al [10] reported a PES incidence of 18.5%, in their series embolizations involving one segment in 69.3% of procedures and two segments in the 30.7%. Furthermore Odisio et al. [11] reported an incidence of PES [67,4%] and of Asymptomatic Liver bile duct injuries [ALI] [29.7%] higher than in our study. Their cohort consists in a miscellaneous of pathologies [HCC, colorectal cancer, melanoma, squamous cell carcinoma, leiomyosarcoma] in which the embolization involved an entire liver segment in more than half of procedures, moreover several patients were not naïve for loco-regional treatment, a known risk factor for PES onset. Differently, our study enrolled only patients naïve for locoregional therapies, and all procedures were highly super selective, being score 1 embolization procedure comparable to sub-segmental classification and score 2 comparable to segmental ones. We believe that all these precautions permit to reach such a lower adverse events rate. Despite the proposed embolization protocol determined a statistical significant increase between pre-operatory and post-operatory AST, ALT and neutrophil count, this was not associated with clinical relevant complications. The only reported

major Grade 5 adverse events occurred, reflects in our opinion more than a device-related complications a mistake in patient selection. As matter of fact by retrospectively analyzing the case, even if the patient was fit for enrollment, being Child Pugh B7 score and a BCLC B, we had underestimated the importance of his clinical background [portal hypertension treated with TIPS, previous biliary procedures, double nodules treatment in a single session] Despite every single risk factors reported alone is not to consider an absolute contra-indication for DEB-TACE procedures, their concomitance lead to complication onset. Under the light of these considerations, a more conscientious approach [e.g.: treating only one nodule per session] could have avoided this major complication onset. Regarding the identification of patient's prognostic factors, higher pre-procedural pre-procedural AST, ALT, ALP and alphafetoprotein levels were related with OR at 12 months. These findings are not in line with available literature; in particular a study of Dong-Shen et al [20] demonstrated a statistically significant association between lower Overall Survival rate and higher AST/ALT and alphafetoprotein level. Despite this discrepancy is worth to underline that our study was aimed to identify potential prognostic factors associated to DEB-TACE outcome rather than an association with overall survival, that would have requested a far longer follow-up period considering the mean overall survival reported for similar population.

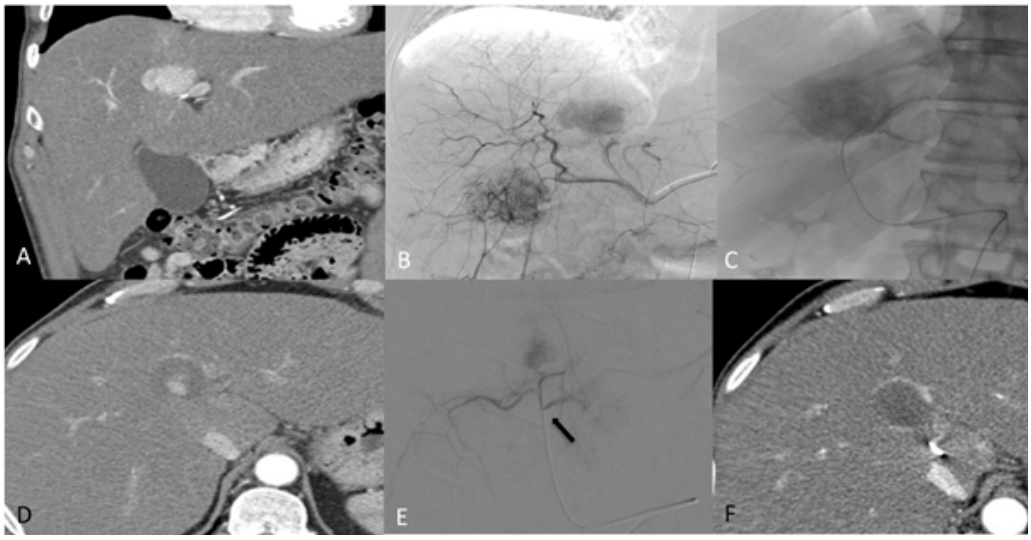

Figure 3: 58 years old male patient with HCV related chirrosis (BCLC B, Child Pugh B7) with a 45x37 mm S4 HCC adjacent to left portal branch (A) and another S5 HCC (B). Proper hepatic artery injection demonstrated a single feeder supply the S4 nodule (B). Superselective catheterization (Score 0) was achieved and 100% of M1 vial and 60% of 100-300 μm were administered (C). 1 month follow-up demonstrate a PR with a residual volume of tissue of 12 % (D). A subsequent retreatment with the administration of 45% of M1 was carried out (F) and a CR response was obtained at 1 month post-retreatment follow-up CT scan.

6. Conclusion

Our proposed combined 75-150μm M1 and 100-300μm DC Beads DEB-TACE protocol significantly ameliorates procedural outcome over other proposed approach [>300μm or only <100μm particles], with a reasonably low complication rate.

7. Conflict of Interest

For all authors there is no potential conflict of interest that could be perceived to bias our work.

Authors had full control of all the data and information presented in this manuscript.

8. Research Ethics and Patient Consent

This study was approved by local Ethics Committee [RIF.CE 5291]. Informed consent was acquired for all patients Included.

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