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ONCOLOGY

Dynamic contrast-enhanced ultrasonography (D-CEUS) for the early prediction of bevacizumab efficacy in patients with metastatic colorectal cancer

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Abstract

Objectives To investigate early changes in tumour perfusion parameters by dynamic contrast-enhanced ultrasonography (D-CEUS) and to identify any correlation with survival and tumour response in patients with metastatic colorectal cancer (CRC) treated with bevacizumab (B).

Methods Thirty-seven patients randomized to either chemotherapy (C) plus B or C alone were considered for this study. D-CEUS was performed at baseline and after the first treatment cycle (day 15). Four D-CEUS perfusion parameters were considered: derived peak intensity (DPI), area under the curve (AUC), slope of wash-in (A) and time to peak intensity (TPI).

Results In patients treated with C plus B, a ≥22.5 % reduction in DPI, ≥20 % increase in TPI and ≥10 % reduction in AUC were correlated with higher progression-free survival in the C+B arm (p = 0.048, 0.024 and 0.010, respectively) but not in the C arm. None of the evaluated parameter modifications had a correlation with tumour response or overall survival.

Conclusions D-CEUS could be useful for detecting and quantifying dynamic changes in tumour vascularity as early as 15 days after the start of B-based therapy. Although these changes may be predictive of progression-free survival, no correlation with response or overall survival was found.

Key Points

- *D-CEUS showed early changes in liver metastasis perfusion in colorectal cancer.*
- *A decrease in tumour perfusion was associated with longer progression-free survival.*
- *The decrease in perfusion was not correlated with higher overall survival.*

Keywords Metastatic colorectal cancer · Bevacizumab · Dynamic contrast-enhanced ultrasonography · Tumour angiogenesis · Early prediction

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Abbreviations

AIFA	Italian Medicines Agency
AUC	Area under the curve
A	Slope of wash-in
B	Bevacizumab
C	Chemotherapy
CI	Confidence interval
CRC	Colorectal cancer
CT	Computed tomography
dB	Decibel
DCE-CT	Dynamic contrast-enhanced computed tomography

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DCE-MRI	Dynamic contrast-enhanced magnetic resonance imaging
D-CEUS	Dynamic contrast-enhanced ultrasonography
DPI	Derived peak intensity
ECOG	Eastern Cooperative Oncology Group
EFSUMB	European Federation of Societies for Ultrasound in Medicine and Biology
FOLFOX	Folinic acid, 5-fluorouracil and oxaliplatin
FOLFIRI	Folinic acid, 5-fluorouracil and irinotecan
5-FU	5-Fluorouracil
ITACa	Italian Trial in Advanced Colorectal Cancer
KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
LV	Leucovorin
MI	Mechanical index
MTT	Mean transit time
OS	Overall survival
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression-free survival
PS	Performance status
rBF	Relative blood flow
rBV	Relative blood volume
RECIST	Response Evaluation Criteria in Solid Tumors
ROC	Receiver operating characteristic
ROI	Region of interest
TPI	Time to peak intensity
V_0	Initial intensity value
VEGF	Vascular endothelial growth factor
V_{max}	Maximal intensity value
XELIRI	Capecitabine and irinotecan
XELOX	Capecitabine and oxaliplatin

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer death worldwide [1]. About 25 % of CRC patients present with metastatic disease at diagnosis and show a median overall survival (OS) of about 6 months without specific treatments. The combination of a fluoropyrimidine with either oxaliplatin (FOLFOX, XELOX) or irinotecan (FOLFIRI, XELIRI) has been widely accepted as the standard regimen for the first- and second-line treatment of metastatic CRC [2]. The addition of targeted agents has further improved treatment outcomes. In particular, bevacizumab (B), a humanized antivascular endothelial growth factor (VEGF) recombinant monoclonal antibody that inhibits angiogenesis by preventing the binding of VEGF to its cellular receptors, has been shown to improve survival when added to first-line chemotherapy (C) [3–5]. Although B is normally fairly well tolerated, a small number of patients develop severe adverse events and show no survival benefit. Early functional evaluation of therapeutic response is essential to identify those who are more likely to respond, thus sparing non-responders from unnecessary toxicity and lowering overall treatment costs [6].

In recent years some studies have suggested that antiangiogenic drugs induce changes in tumour structure (e.g. decreased tumour perfusion or necrosis), resulting in a therapeutic response before a change in tumour size is observed [7, 8]. Adequate early evaluation of efficacy and response to these treatments is difficult because traditional response criteria are mainly based on a reduction in tumour size (Response Evaluation Criteria in Solid Tumors, RECIST) estimated with imaging techniques [9, 10].

Several dynamic imaging modalities and mathematical models have been used to quantify changes in liver tumour perfusion at an early time-point after the start of treatment. Various functional imaging tools, including contrast-enhanced ultrasound (D-CEUS), dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), dynamic contrast-enhanced computed tomography (DCE-CT) and positron emission tomography (PET), have been proposed to evaluate the antiangiogenic effects of cancer drugs [11–14].

D-CEUS is emerging as a non-invasive and repeatable technique to measure perfusion and therefore as an attractive tool for monitoring antiangiogenic treatment response [5, 15]. D-CEUS has numerous advantages over other functional imaging techniques: the ultrasound contrast agent is appropriate for this type of analysis as it is intravascular and enables perfusion to be studied in real-time. It is also a safe (patients are not exposed to ionizing radiation) and inexpensive method. Semiquantitative perfusion and tumour vascular density such as relative blood volume (rBV) and relative blood flow (rBF) can be determined from the time-concentration curve constructed by perfusion software which represents the kinetics of microbubble contrast agent flow through the tumour [16, 17]. Although the accuracy of D-CEUS in microvascular perfusion assessment and in the evaluation of changes in tumour perfusion after therapy has been documented in both experimental models [18–20] and patients with a wide range of cancers [6, 21, 22], its impact on clinical practice remains modest.

The aim of this monocentre, prospective, controlled clinical trial was to investigate whether significant early changes in tumour perfusion parameters can be assessed by D-CEUS in patients with liver metastases from CRC treated with B-based chemotherapy. The secondary aim was to correlate potential changes in functional parameters with survival and tumour response assessed by RECIST 1.1. The first 37 eligible patients from the ITACa trial were prospectively enrolled into the present study.

Materials and methods

This was a monocentre, prospective, non-pharmacological, controlled clinical trial in CRC patients recruited from the ITACa study. The primary aim was to investigate whether significant early changes in tumour perfusion parameters can be assessed by D-CEUS. The secondary aim was to correlate changes in functional parameters with survival and tumour response.

Patients

Thirty-seven consecutive patients enrolled onto the ITACa (Italian Trial in Advanced Colorectal Cancer) trial (EudraCT no. 2007-004539-44 and ClinicalTrials.gov NCT01878422) from 2009 to 2013 were considered for this study [23]. Inclusion criteria were histologically or cytologically confirmed CRC and one or more non-resectable liver metastases; age ≥ 18 years and < 70 years; Performance Status (ECOG) ≤ 2; measurable disease according to RECIST criteria; estimated life expectancy of at least 12 weeks;

adequate haematological, hepatic and renal function, as follows: haemoglobin ≥ 9 g/dl, absolute neutrophil count ≥ 1,500/μl, platelets ≥ 100,000/μl, total bilirubin ≤ 1.5 × upper limit of normal (ULN), alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 × ULN (≤ 5 × ULN in the presence of liver metastases), serum creatinine ≤ 1.5 × ULN or calculated creatinine clearance > 50 ml/min (calculated on the basis of the standard Cockcroft-Gault formula). Exclusion criteria comprised contraindications to the use of the ultrasound contrast agent; clinically significant cardiovascular or peripheral vascular disease; uncontrolled hypertension; bleeding diathesis or coagulopathy; pulmonary embolism or any arterial or venous embolism; chronic use of aspirin (> 325 mg/day), other antiplatelet agents or anticoagulants; proteinuria (if protein > 30 mg/dl or +1, patients must have ≤ 1 g of protein/24 h) and known central nervous system metastases.

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. All patients were required to give written informed consent before enrolment. The study was approved by the Ethics Committee of IRST and the Wide Catchment Area of Romagna (Area Vasta Romagna).

Treatment

All eligible patients were randomized to receive either C plus B (arm A) or C alone (arm B). The chosen regimens were FOLFIRI or FOLFOX4, at the discretion of the clinician, for both arms. FOLFIRI consisted of irinotecan 180 mg/m^2 given as a 90-min infusion on day 1 added to a standard 5-fluorouracil (5-FU) + leucovorin (LV) regimen: LV 100 mg/m^2 given as a 2-h infusion followed by bolus 5-FU 400 mg/m^2 and a 22-h infusion of 5-FU 600 mg/m^2 on days 1–2 every 2 weeks. FOLFOX4 consisted of the same 5-FU+LV regimen, with the addition of oxaliplatin 85 mg/m^2 as a 2-h infusion on day 1. B was administered as a 30- to 90-min intravenous infusion at a dose of 5 mg/kg on day 1 of each 2-week cycle. Treatment continued until disease progression (PD), withdrawal of consent or unacceptable toxicity, whichever came first. All patients were followed until death.

Dynamic contrast-enhanced ultrasonography

D-CEUS scans were carried out at baseline (day 0) and after the first cycle of treatment (day 15). Two different radiologists (with more than 5 years' experience in D-CEUS) performed the ultrasound independently using the iU22 vision 2008 medical ultrasound system (Philips Healthcare, Best, The Netherlands) and a convex-array abdominal C5-2-MHz transducer for deep target lesions or a 12-MHz linear-array probe for shallow targets. D-CEUS was carried out in two phases. A single target lesion selected on the basis of size (largest) and site (best acoustic window for data acquisition) was studied in each patient.

A morphological study was initially made in B-mode sonography to identify the best scanning plane and, in patients with multiple liver metastases, the largest lesion detectable ('target lesion'). The second phase began after 2.4 ml of a second-generation ultrasound contrast agent (SonoVue, Bracco, Milan, Italy) was injected as a peripheral intravenous bolus and immediately flushed with 5–10 ml of 0.9 % NaCl solution.

The study of the whole vascular phase was performed in accordance with the guidelines of the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) [24]. In particular, a low mechanical index-technique (MI ≤ .08) and adjusting gain, depth and frame rates (7–14 Hz) were used to obtain a real-time digital video of 3 min showing all the steps of contrast enhancement of intratumour vascularization. Patients were required to maintain respiratory apnoea for the entire acquisition time or, when lesions were superficial, to breathe lightly. Each CEUS scan, including preparation and execution, took around 20 min.

Assessment of tumour vascularity

A quantification of contrast uptake in the target liver lesion was performed by advanced ultrasound quantitative analysis software package QLAB, version 7.0 (Philips Healthcare). QLAB plug-in enables the mean, median and standard deviation of pixel intensity to be analysed for each frame of the sequence of images in a specific region of interest (ROI). In all instances, the ROI was drawn around the entire lesion to be studied. Exported data contained multiple frames and the means of pixel intensities were processed in a curve of time/intensity data, which is related to microbubble concentration.

Motion artifacts (e.g. breathing) were deleted by automatic technical readjustments (with a real-time motion compensation algorithm) or when necessary, manually, and by removing sampled frames in the post-processing analysis.

The following perfusion parameters provided by Q-lab time-enhancement intensity curves were used: DPI = derived peak intensity in decibels (dB), related to maximum curve intensity value; AUC = area under the curve, which is proportional to regional 'blood volume'; A = slope of wash-in (in dB s^{-1}); and TPI = time to peak intensity (in seconds), related to regional 'blood flow' (Fig. 1) [24]. The post-processing analyses were performed by a radiologist with more than 3 years' experience in D-CEUS.

Assessment of efficacy

Response to treatment was evaluated according to RECIST 1.1 criteria on CT scans (Philips 256-Slice Multi-Detector) performed before treatment and every 2 months of therapy thereafter until progression [9, 25]. Patients were classified as responders (partial or complete response confirmed) or non-responders (stable or progressive disease confirmed) on the basis of changes in target lesion diameter). Target lesions chosen for CT scan evaluation were not necessarily the same as those used for CEUS as both assessments were performed by independent and blinded radiologists.

Statistical analysis

This was a monocentre, prospective, non-pharmacological, controlled clinical trial of CRC patients who took part in the ITACa study. The first 37 eligible patients from the ITACa trial were prospectively enrolled onto the present study.

Nominal or ordinal variables were presented as frequencies and percentages; mean, standard deviation, median and 95 % confidence intervals (CIs) were calculated for continuous variables. The association between baseline patient characteristics and treatment was evaluated using the Chi-square and Fisher's test (for categorical variables) or the Wilcoxon median test (for continuous variables). Changes in tumour perfusion parameters were calculated as percent changes between the value of the parameter evaluated on day 15 and the value on day 0. A non-parametric median test (Wilcoxon) was used to compare median values and changes in tumour perfusion parameters between the two treatment arms. Two categories of patients were defined for each tumour perfusion parameter according to the best parameter variation cut-off determined using the ROC curve.

Progression-free survival (PFS) was defined as the time form random assignment to the first documentation of progressive disease (per investigator assessment), or death from any cause or the date of the last tumour evaluation. Overall survival (OS) was defined as the time interval between random assignment and death or last follow-up visit. PFS and OS were estimated by the Kaplan-Meier method and compared by the log-rank test (at a significant level of 5 %).

All p values were based on two-sided testing and statistical analyses were performed using SAS Statistical software version 9.4 (SAS Inc., Cary, NC, USA).

Results

Patient characteristics

Thirty-seven consecutive and eligible patients enrolled at our institute in the ITACa trial were included in the present study. Twenty had been randomized to the C plus B arm and 17 to the C arm. Patient baseline characteristics are shown in Table 1. Patient groups according to treatment arm were all comparable for age, gender, histology, ECOG Performance Status, tumour localization, stage at diagnosis, C regimen, KRAS status and prior cancer therapy. The majority of patients had ECOG PS 0 (75.7 %), primary tumour localization in the colon (70.3 %), stage IV disease at diagnosis (83.8 %) and a KRAS mutation (59.5 %).

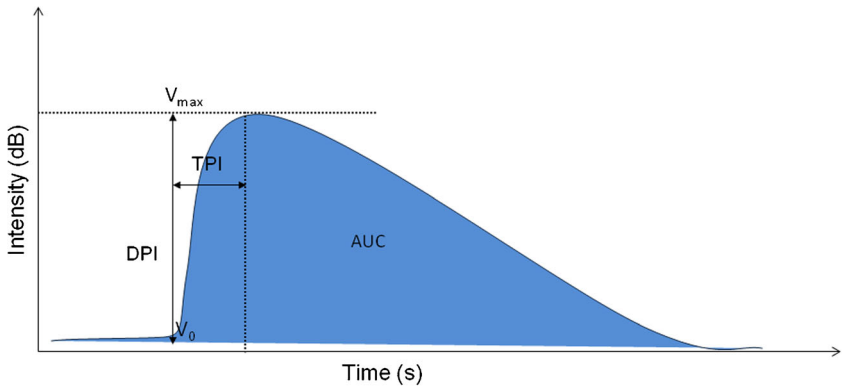

Fig. 1 Quantitative features of contrast-enhanced ultrasonography. Contrast agent uptake curves constructed from raw linear data and after automatic modeling. *dB* decibels, V_{max} maximal intensity value, *TPI* time to peak intensity, *DPI* derived peak intensity, V_0 initial intensity value, *AU* area under the curve

Table 1. Patient characteristics

	Total (n = 37) No. (%)	C+B (n = 20) No. (%)	C (n = 17) No. (%)	p
Median age, years (range)	69 (37–83)	71 (37–83)	68 (37–81)	0.460
Gender				
Male	21 (56.8)	11 (55.0)	10 (58.8)	
Female	16 (43.2)	9 (45.0)	7 (41.2)	0.817
ECOG PS				
0	28 (75.7)	16 (80.0)	12 (70.6)	
1–2	9 (24.3)	4 (20.0)	5 (29.4)	0.512
Tumour localization				
Rectum	11 (29.7)	7 (35.0)	4 (23.5)	
Colon	26 (70.3)	13 (65.0)	13 (76.5)	0.453
Stage at diagnosis				
I–III	6 (16.2)	2 (10.0)	4 (23.5)	
IV	31 (83.8)	18 (90.0)	13 (76.5)	0.272
Chemotherapy regimen				
FOLFIRI	12 (32.4)	7 (35.0)	5 (29.4)	
FOLFOX4	25 (67.6)	13 (65.0)	12 (70.6)	0.721
KRAS status				
Wild type	15 (40.5)	7 (35.0)	8 (47.1)	
Mutated	22 (59.5)	13 (65.0)	9 (52.9)	0.463
Prior cancer therapy				
Surgery	24 (64.9)	14 (70.0)	10 (58.8)	0.484
Radiotherapy	3 (8.1)	1 (5.0)	2 (11.8)	0.459
Adjuvant chemotherapy	2 (5.4)	0	2 (11.8)	0.552

C chemotherapy, *B* bevacizumab, *ECOG PS*, Eastern Cooperative Oncology Group performance status, *FOLFIRI* folinic acid, 5-fluorouracil and irinotecan, *FOLFOX4* folinic acid, 5-fluorouracil and oxaliplatin

Dynamic contrast-enhanced ultrasound

D-CEUS parameters by treatment arm (DPI, TPI, AUC and A) are listed in Table 2. Median values of all evaluated parameters at both baseline and on day 15 showed a similar distribution in both arms. The percent change between the median values of three of the four evaluated parameters differed significantly (DPI decrease, p = 0.041; TPI increase, p = 0.006; and AUC, p = 0.038).

The distribution of D-CEUS parameters and their modification in responders and non-responders is reported in Table 3. No differences in the distribution of median values of the parameters between baseline and day 15 emerged in either arm. Moreover, none of the evaluated parameter ratios showed a correlation with tumour response.

The correlation between D-CEUS parameter modification and PFS is shown in Table 4. A ≥ 22.5 % reduction in DPI, a ≥ 20 % increase in TPI and a ≥ 10 % reduction in AUC were associated with higher PFS in the C plus B arm (p = 0.048, 0.024 and 0.010, respectively) but not in the C alone arm. Variations of A were not correlated with PFS in either treatment arm. None of the evaluated parameter modifications had an impact on tumour response assessed by CT or survival. Although OS was twofold higher in C+B patients showing a ≥ 20 % increase in TPI and a ≥ 10 % decreased in AUC, the finding was not significant (p = 0.097 and 0.231, respectively) (Table 5).

Discussion

Over the past few years, efforts have been intensified to identify surrogate markers that predict a response in patients undergoing antiangiogenic treatments. In the present study, a decrease in tumour vascularization of liver metastases was observed after only one treatment cycle (15 days after the start of treatment in CRC patients treated with B; Fig. 2). A decrease in perfusion was interpreted as a significant reduction of rBV and rBF in the median percent change between the value of DPI on day 15 and day 0, a significant decrease in the median percent change between AUC2 and AUC1, and a significant increase in the median percent change between TPI2 and TPI1. This finding was further confirmed by a

Table 2. Median value of D-CEUS parameters by treatment arm

	C+B (n = 20) Median value (95 % CI)	C (n = 17) Median value (95 % CI)	p
DP1 (day 0)	11.86 (9.61–16.78)	9.22 (5.48–13.66)	0.181
DP2 (day 15)	8.43 (6.90–14.65)	9.06 (5.11–13.57)	0.957
Percent change	-24.03 (-38.02–-1.00)	0.00 (-52.33–99.48)	0.041
TPI1 (day 0)	10.74 (5.94–16.04)	13.56 (7.14–25.53)	0.291
TPI2 (day 15)	12.95 (9.77–18.95)	16.22 (4.81–22.15)	0.899
Percent change	22.22 (1.00–45.82)	-19.80 (-50.71–13.14)	0.006
AUC1 (day 0)	274.57 (183.57–548.30)	176.69 (51.75–530.79)	0.260
AUC2 (day 15)	242.71 (166.94–456.28)	159.33 (45.54–433.52)	0.342
Percent change	-14.09 (-35.14–-1.00)	-6.18 (-33.01–41.34)	0.038
A1 (day 0)	10.46 (5.21–569.32)	3.40 (1.45–15.29)	0.181
A2 (day 15)	4.37 (2.13–437.77)	4.01 (2.12–7.17)	0.505
Percent change	-22.79 (-47.79–0.00)	-10.74 (-59.04–182.00)	0.099

D-CEUS contrast-enhanced ultrasonography, *C* chemotherapy, *B* bevacizumab, *DPI* derived peak intensity, *TPI* time to peak intensity, *AUC* area under the curve, *A* slope of wash-in, *CI* confidence interval

reversal of these median percent changes in the control group (Table 2, Fig. 3).

Our findings suggest that D-CEUS could be used for the quantitative assessment of tumour vascularization in patients undergoing treatment with antiangiogenic agents according to guidelines of the European Federation of Societies for Ultrasound in Medicine and Biology for the clinical practice of contrast-enhanced ultrasound [26–29]. The early assessment of tumour perfusion would help to optimize tailored treatment, especially in non-responders, sparing patients who are not likely to respond from unnecessary toxicity and lowering nonessential costs [5]. Tumour response criteria such as RECIST 1.1 (assessed by CT) have proven limited in assessing response to antiangiogenic drugs [27, 30], while PFS and OS, often used to assess treatment efficacy, require long-term observation.

We found that a change in AUC, DPI and TPI from baseline to day 15 was only related to improved PFS in C+B patients. At baseline, there was no significant association between any of the D-CEUS criteria values and PFS. Of the four parameters assessed, TPI proved to be the most valuable because it showed an opposite trend in the control group. The optimal cut-off value for TPI was an increase of 20 % with respect to baseline.

Table 3. Median value of D-CEUS parameters by treatment arm and responder status

	C+B			C		
	Reponders (n = 11) Median value (95 % CI)	Non responders (n = 9)	p	Reponders (n = 11) Median value (95 % CI)	Non responders (n = 6)	p
DPI1 (day 0)	11.37 (9.61 to17.78)	16.78 (14.86–18.85)	0.269	10.52 (8.25–13.66)	8.30 (5.48–13.53)	0.730
DPI2 (day 15)	7.28 (6.90–8.65)	10.40 (7.19–14.65)	0.654	7.53 (5.11–12.35)	7.84 (5.43–13.57)	0.882
Percent change	-14.59 (-16.88–-1.00)	-29.11 (-38.02–-12.03)	0.575	-12.28 (-52.33–22.81)	18.42 (-15.14–99.48)	0.657
TPI1 (day 0)	9.16 (5.94–13.61)	11.35 (6.14–16.04)	0.708	13.93 (7.14–15.92)	9.96 (12.23–25.53)	0.227
TPI2 (day 15)	15.21 (9.77–22.61)	12.86 (5.42–20.54)	0.822	18.81 (6.64–22.15)	13.01 (4.81–21.35)	0.265
Percent change	23.74 (6.79–45.82)	7.05 (1.00–28.01)	0.318	-19.80 (-37.44–13.14)	-24.06 (-50.71–11.99)	1.000
AUC1 (day 0)	257.38 (183.57–400.23)	302.85 (99.88–548.30)	0.822	262.15 (122.54–530.79)	170.45 (51.75–432.17)	0.265
AUC2 (day 15)	250.31 (166.94–300.41)	235.05 (50.23–456.28)	0.708	175.60 (91.57–433.52)	65.59 (45.54–102.04)	0.405
Percent change	-13.80 (-21.87–-1.00)	-20.97 (-35.14–-8.22)	0.793	-7.54 (-33.01–12.56)	8.53 (-9.77–41.34)	0.462
A1 (day 0)	234.92 (47.18–569.32)	7.32 (5.21–48.43)	0.212	4.05 (1.45–8.28)	5.88 (6.27–15.29)	0.805
A2 (day 15)	182.19 (15.44–437.77)	4.37 (2.13–16.23)	0.318	3.04 (2.12–5.02)	4.85 (3.65–7.17)	0.805
Percent change	-22.45 (-35.36–0.00)	-23.14 (-47.79–-2.05)	0.851	-10.74 (-59.04–22.97)	-8.86 (-19.31–182.00)	0.805

D-CEUS dynamic contrast-enhanced ultrasonography, *C* chemotherapy, *B* bevacizumab, *DPI* derived peak intensity, *TPI* time to peak intensity, *AUC* area under the curve, *A* slope of wash-in, *CI* confidence interval

Table 4. Progression-free survival according to D-CEUS parameter changes by treatment arm

	C+B				C			
	No. of patients	No. of events	Median PFS (months) (95 % CI)	*p*	No. of patients	No. of events	Median PFS (months) (95 % CI)	*p*
Overall	20	20	8.8 (6.2–10.4)	–	17	14	9.5 (4.3–11.9)	–
DPI reduction								
<–22.5 %	9	9	7.9 (4.9–9.1)		12	9	9.5 (1.0–11.9)	
≥–22.5 %	11	11	9.7 (3.5–11.9)	0.048	5	5	10.4 (4.3–18.7)	0.588
TPI increase								
<+20.0 %	9	9	7.9 (2.5–9.1)		15	12	9.5 (4.3–11.9)	
≥+20.0 %	11	11	9.7 (6.2–11.9)	0.024	2	2	6.2 (1.0–11.4)	0.413
AUC reduction								
<–10.0 %	7	7	7.9 (4.9–8.6)		11	9	9.5 (1.0–11.9)	
≥–10.0 %	13	13	9.7 (7.2–11.6)	0.010	6	5	10.4 (4.3–18.6)	0.469
A reduction								
<–23.0 %	11	11	7.9 (4.9–9.1)		10	8	9.5 (0.6–11.9)	
≥–23.0 %	9	9	9.7 (2.5–11.9)	0.381	7	6	10.4 (2.0–18.6)	0.853

C chemotherapy, *B* bevacizumab, *DPI* derived peak intensity, *TPI* time to peak intensity, *AUC* area under the time-intensity curve, *A* slope of wash-in, *PFS* progression-free survival,

CI confidence interval

A recent study by Lassau et al. investigated the role of some parameters related to rBF as TPI and mean transit time (MTT) in predicting outcome in metastatic cancer patients treated with B [31]. Furthermore, a large multicentre cohort study published by the same group in 2014 reported that a change in AUC 30 days from baseline was the best predictive criterion for PFS in patients undergoing antiangiogenic therapy [32]. Similar results were described by Zocco et al. [6]. More recently, CEUS with VEGFR2-targeted microbubbles (BR55) was used to investigate tumour perfusion changes in rats with colorectal carcinoma xenografts treated with regorafenib. A substantial decrease in AUC was reported in the treatment group, with no significant changes in the control group. CEUS parameters were also correlated with

Table 5 Overall survival according to D–CEUS parameter changes by treatment arm

	C+B				C			
	No. of patients	No. of events	Median OS (months) (95 % CI)	*p*	No. of patients	No. of events	Median OS (months) (95 % CI)	*p*
Overall	20	17	24.5 (13.1–35.7)	–	17	14	14.4–29.2)	–
DPI								
<–22.5 %	9	9	28.0 (9.0–36.6)		12	9	25.4 (1.0–42.9)	
≥–22.5 %	11	8	24.5 (10.2–42.9)	0.898	5	5	24.5 (14.4–37.3)	0.743
TPI								
<+20.0 %	9	9	14.5 (4.7–29.6)		15	11	26.8 (14.4–29.2)	
≥+20.0 %	11	8	35.7 (10.2–49.5)	0.097	2	2	12.5 (1.0–24.0)	0.119
AUC								
<–10.0 %	7	7	14.5 (9.0–29.6)		11	10	23.1 (1.0–29.2)	
≥–10.0 %	13	10	28.8 (13.1–42.9)	0.231	6	5	26.8 (14.4–37.3)	0.666
A								
<–23.0 %	11	10	24.5 (9.0–29.6)		10	8	26.8 (0.6–42.9)	
≥–23.0 %	9	7	35.7 (4.7–49.5)	0.302	7	6	24.0 (4.3–37.3)	0.800

C chemotherapy, *B* bevacizumab, *DPI* derived peak intensity, *TPI* time to peak intensity, *AUC* area under the time-intensity curve, *A* slope of wash-in, *OS* overall survival, *CI* confidence interval

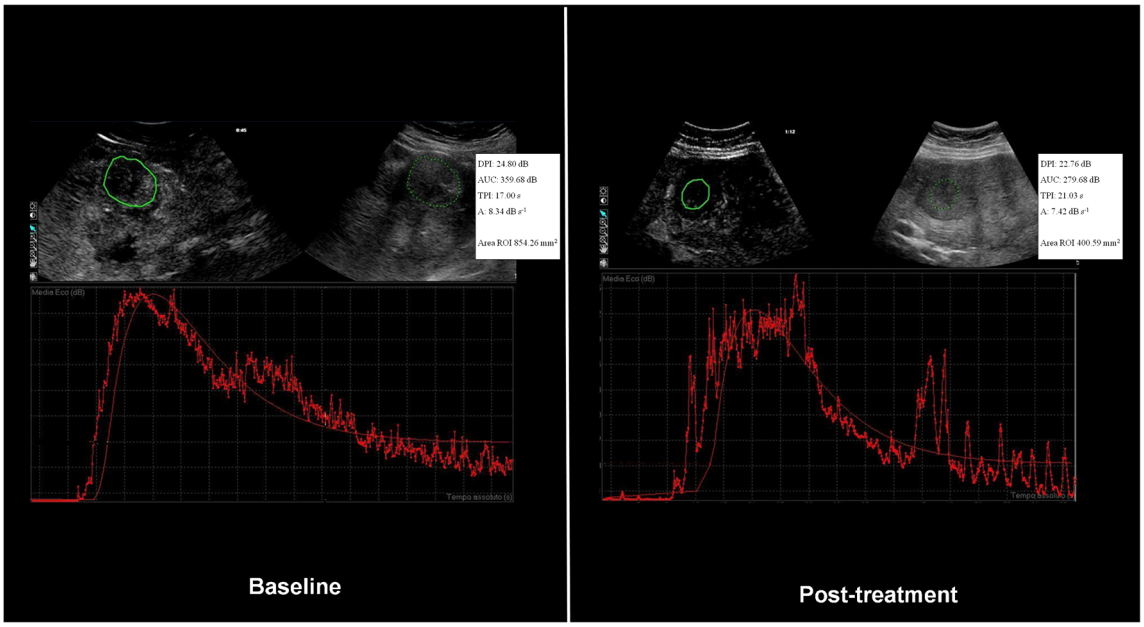

Fig. 2 Representative images of a decrease in tumour vascularity in a responder patient before (baseline) and after (post-treatment) one cycle of chemotherapy plus bevacizumab. *DPI* derived peak intensity, *AUC* area under the curve, *TPI* time to peak intensity, *A* slope of wash-in, *ROI* region of interest (shown as green circles), *s* seconds, *dB* decibels

dynamic contrast-enhanced MRI (DCE-MRI) parameters and immunohistochemistry (VEGFR2, CD31, Ki-67 and TUNEL staining) [20]. In our study, the optimal cut-off value corresponded to a 10 % decrease in the AUC baseline value. No significant correlations were found between perfusion parameters and OS or tumour response assessed by CT.

The main limitations of our study were the small number of patients considered, the lack of a control ROI in normal liver parenchyma, the fact that we were only able to scan one index liver lesion per patient rather than different liver metastases, and the failure to use 3D-CEUS. Of note, it has recently come to light that dynamic 3D-CEUS is superior to 2D-CEUS in visualizing the spatial relationship, vascularity and perfusion patterns of focal liver lesions [33]. Generally speaking, the use of various methodologies for contrast quantification based on different software programs has led to disparities in trial

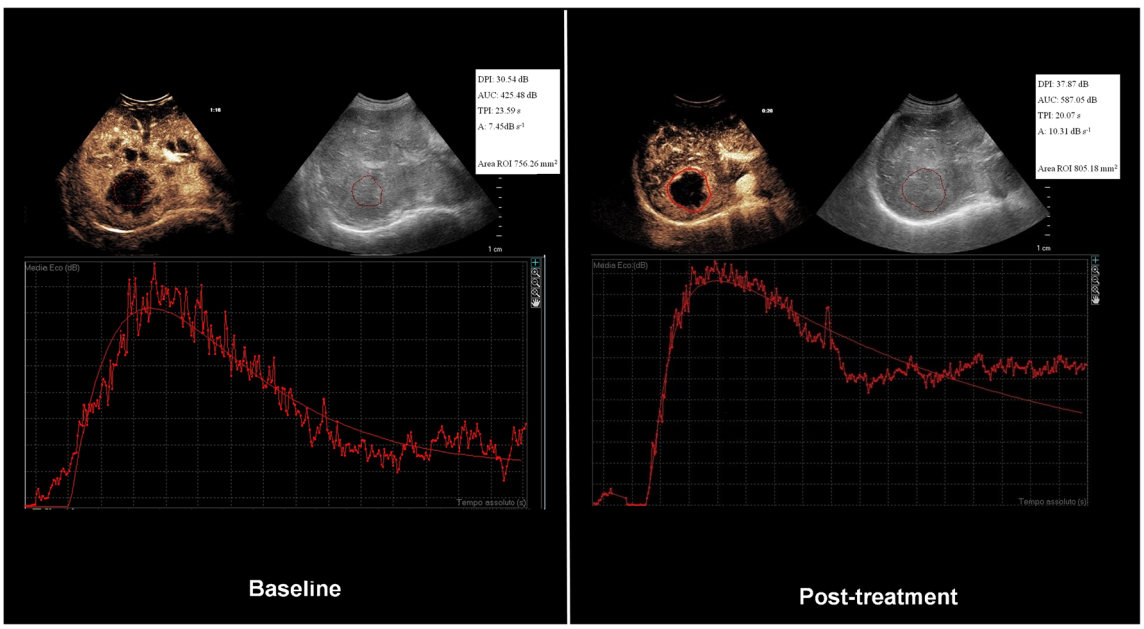

Fig. 3 Representative images of unvaried tumour vascularity in a non-responder before (baseline) and after (post-treatment) one cycle of chemotherapy alone. *DPI* derived peak intensity, *AUC* area under the curve, *TPI* time to peak intensity, *A* slope of wash-in, *ROI* region of interest (shown as red circles), *s* seconds, *dB* decibels

results, indicating that caution is needed when implementing findings into clinical practice [34]. Adequate operator experience in D-CEUS quantification is also essential, together with a preliminary assessment and validation of equipment and software used [34].

In conclusion, dynamic ultrasonography could be potentially useful for detecting and quantifying functional changes in tumour vascularity as early as 15 days after the start of B therapy in patients with metastatic CRC. These early changes in tumour perfusion may be predictive of PFS and could become surrogate measures of the performance of antiangiogenic agents. Further research on a larger sample size is needed to confirm our findings.

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Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Alessandro Passardi.

Conflict of interest The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Statistics and biometry The Corresponding Author, Emanuela Scarpi, is an expert in statistics.

Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Study subjects or cohorts overlap Some study subjects or cohorts have been previously reported in the ITACa trial paper published in Annals of Oncology in 2015 (Passardi et al, Ann Oncol 2015;26(6):1201-7).

Methodology
- Prospective
- Case-control study/randomized controlled trial
- Performed at one institution

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