Contrast-enhanced ultrasound in planning thermal ablation of liver metastases: Should the hypervascular halo be included in the ablation volume?☆

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KEYWORDS

Contrast-enhanced
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Abstract *Introduction:* Liver metastases often exhibit a hypervascular halo during the arterial phase of contrast-enhanced ultrasonography (CEUS). This finding has no correlates on baseline gray-scale imaging, and it has never been characterized. The aim of this study was to identify the features of this halo and determine whether it should be included in the ablation volume during thermal ablation procedures.

Materials and methods: We prospectively enrolled 25 patients referred to our department for thermal ablation of liver metastases. Before treatment all patients underwent CEUS, and the maximum diameter of the metastatic lesion was measured before administration of the ultrasound contrast agent and during the arterial and portal venous phases of the contrast contrast-enhanced study. Maximum diameters in the different vascular phases were compared with the Turkey–Kramer test. Two biopsies were obtained from each lesion with a 21-gauge needle: 1) one from the center of the metastasis to confirm the diagnosis and 2) one from the hypervascular peripheral halo identified in the arterial phase at CEUS.

Results: The mean ($\pm$ standard deviation) maximum lesion diameter was 2.67 ± 1.2 cm before contrast agent injection, 3.50 ± 1.4 cm during the arterial phase, and 2.71 ± 1.2 cm during the venous phase. The difference between maximum diameters measured before contrast enhancement and in the arterial phase was highly significant (mean: 0.84 ± 0.45 cm, $p < 0.0001$). Histological examination of halo specimens revealed inflammatory infiltrates with

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no evidence of tumor infiltration in 24/25 (96%) cases and normal hepatic parenchymal tissue in the 25th specimen.

Discussion: The hypervascular halo surrounding liver metastases during the arterial phase of CEUS represents a chronic inflammatory infiltrate, not tumor infiltration. However, since chronic inflammation appears to promote neovascularization and the production of tumoral growth factors, it seems wise to include the hypervascular halo in the intended-to-treat volume when planning the ablation procedure.

Sommario *Introduzione:* All'ecografia con Mezzo di Contrasto (CEUS) dei tumori secondari epatici si evidenzia spesso un alone periferico ipervascolare, che non ha corrispondenza sull'immagine ecografica di base e non è ancora stato caratterizzato istologicamente. Lo scopo dello studio è di caratterizzare tale alone al fine di determinare se sia necessario includerlo nel volume di necrosi pianificato.

Materiali e metodi: Questo studio prospettico ha incluso 25 pazienti inviati per terapia termoblattiva di tumori secondari epatici. Prima del trattamento tutti i pazienti sono stati sottoposti a CEUS. Il diametro maggiore della lesione è stato determinato: pre-contrasto, durante la fase arteriosa e durante la fase venosa portale. I diametri delle lesioni pre e post-contrasto sono stati confrontati mediante il test di Turkey–Kramer. Due campioni biotici sono stati prelevati con ago 21-gauge e analizzati istologicamente: 1) uno dalla zona centrale del tumore e 2) uno dall'alone periferico ipervascolare, identificato alla CEUS solamente nella fase arteriosa.

Risultati: Il diametro massimo medio delle lesioni è risultato ($\pm$ deviazione standard): $2,67 \pm 1,2$ cm pre-contrasto; $3,50 \pm 1,4$ cm in fase arteriosa; $2,71 \pm 1,2$ cm in fase venosa. La differenza media tra il diametro massimo pre-contrasto e in fase arteriosa è risultata di $0,84 \pm 0,45$ cm ($p < 0,0001$). All'esame istologico, 24/25 campioni (96%) hanno dimostrato flogosi senza evidenza di tumore microscopico.

Discussione: L'alone ipervascolare periferico è costituito da infiltrato infiammatorio, non da tessuto tumorale. Esistono evidenze in letteratura che l'infiammazione cronica promuova la neovascularizzazione e la produzione di fattori di crescita per le cellule neoplastiche. Sembra quindi opportuno includere completamente l'alone ipervascolare periferico evidente alla CEUS nel volume di termo-ablazione.

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Introduction

Thermal ablation is a safe and efficacious technique for minimally invasive treatment of liver metastases when surgery is contraindicated (by co-morbidities or the location of the metastases themselves) [1–3].

Contrast-enhanced ultrasonography (CEUS) is one of the diagnostic imaging modalities used for treatment-planning in these cases. It allows evaluation of the vascular pattern of the lesion, its location and size, and possible approaches to be used for percutaneous tumor ablation [4]. Compared with gray-scale ultrasound, CEUS also appears to be more sensitive in the detection of metastatic liver lesions [5,6].

In our experience we observed that during the arterial phase of CEUS, most metastatic liver lesions also present a peripheral hypervascular halo, which has no correspondent on gray-scale imaging. However, a similar halo can be detected with other contrast-enhanced diagnostic imaging modalities, such as CT or MR [7–12]. Preliminary studies with a small number of patients have examined the possible correlations between the enhancing halos visible on CT or MR imaging and the histologic features of these lesions. In one study, the halo area was characterized by a peritumoral desmoplastic reaction, inflammatory infiltration, and vascular proliferation [7]; in another, it appeared to reflect normal hepatic parenchyma [8]. The presence of tumor

cells within the hypervascular halo has never been reported.

The presence of a chronic inflammatory infiltrate at the level of the hyperemic halo is important as there is evidence in the literature that chronic inflammation promotes - via multiple mechanisms - the development and growth of both primary and metastatic tumors [13–15]. The hypervascular halo seen at the periphery of liver metastases at CEUS has never been characterized, and it is not clear whether it represents inflammatory tissue or microscopic tumor infiltration. The aim of this prospective study was to characterize this halo in terms of its thickness and histologic correlates to determine whether it should be included within the intended-to-treat volume when planning the ablation procedure. We also retrospectively evaluated the frequency with which this halo is observed during CEUS examinations of liver metastases.

Patients and methods

Study population

Retrospective frequency study

To evaluate the frequency of the hyperemic halo finding, we retrospectively analyzed all CEUS studies of liver

metastases performed in our department during a 9-month period (May 2009–January 2010).

Prospective histological study

In this prospective pilot study, we consecutively enrolled 25 patients (11 men and 13 women, mean age 70 years) with at least one liver metastasis, who were candidates for either radiofrequency or microwave between December 2008 and December 2009. All 25 provided explicit informed consent to enrolment in the study. The metastatic lesions came from colon-rectal cancers ($n = 13$ patients), breast cancers ($n = 9$ patients), bladder cancers ($n = 1$ patient), pancreatic adenocarcinoma ($n = 1$ patient), and melanoma ($n = 1$ patient).

The only inclusion criterion was the presence of at least one liver metastasis that was well visualized on ultrasound. Exclusion criteria were contraindications to the use of the ultrasound contrast agent (recent acute cardiac events, asthma, allergy), contraindications to liver biopsy (platelet count $<50,000/\text{mm}^3$ and/or prothrombin activity $<50\%$ at the time of enrolment), and absence of a safe percutaneous route insertion of the microwave antenna/radiofrequency electrode.

Imaging technique

Gray-scale ultrasound was used to select the metastatic lesion to be evaluated. We chose an imaging plane that would allow good visualization of the lesion even in the absence of patient collaboration (if possible an intercostal view). CEUS was then performed with either a Siemens Sequoia-512 or Aloka ProSound Alfa 10 (Aloka, Giappone) scanner and a convex multi-frequency transducer (2–5 MHz). Scanners were equipped with contrast-specific software for low mechanical index (range: 0.04–0.1) imaging. A 20-gauge catheter was inserted into a vein of the forearm, and a bolus (2.4–4.8 ml) of ultrasound contrast agent (SonoVue, Bracco, Milan, Italy) was injected followed by a 10-ml saline flush. The entire CEUS examination was recorded to allow measurements after the procedure was completed. The maximum diameter of the metastasis was measured before-contrast injection, during the arterial phase (20–30 s after injection), and during the portal venous phase (>60 s from the injection). To ensure measurement repeatability, all three diameter measurements were made in the same scan plane, verified with the aid of anatomic markers identified on the gray-scale image.

Biopsy technique

Biopsies of each lesion were performed under ultrasound guidance in the same scan plane used for the CEUS study, which allowed good visualization of both the metastasis and the hypervascular halo. We used a microconvex transducer (UST-9133, Aloka, Japan) equipped with a laterally-mounted needle guide with variable inclination (Ultra-Pro II™ Needle Guidance System, CIVCO, USA). Two biopsies were collected from each lesion with a 21-gauge biopsy needle (Biomol, HS Hospital Service, Rome, Italy): one from the central zone of the metastasis, the other from the area included in the peripheral hypervascular halo detected during the arterial phase of the CEUS examination. Patients remained in the hospital for 4 h after the biopsy procedure

and were discharged after an ultrasound examination to exclude the presence of complications.

Biopsy specimens were analyzed by a pathologist to identify the histological features of 1) the metastasis and 2) the tissue corresponding to the hypervascular halo. Each specimen was analyzed after routine hematoxylin-eosin staining. Immunohistochemical typization of the lymphocytes was performed on two biopsy specimens from the periphery of the lesion.

Statistical analysis

For each patient, the difference between maximum diameter of the metastasis measured pre-contrast and in the different vascular phases was calculated. The Turkey–Kramer was used to evaluate the statistical significance ($p < 0.05$) of those differences.

Results

Retrospective analysis of the 43 cases of liver metastases evaluated in our unit with CEUS in the period May 2009–January 2010 revealed 10 cases in which the images recorded were considered insufficient for evaluation of the peripheral halo. In 18 (54%) of the remaining 33 patients, the metastatic lesion exhibited a peripheral hypervascular halo during the arterial phase that had not been visualized on the gray-scale ultrasound examination.

In the population prospectively examined with CEUS and undergoing biopsy, the contrast-enhanced study revealed metastatic lesions that had not been observed on gray-scale ultrasound in 4/25 patients (16%). As for the 25 metastases, one for each patient, analyzed for hypervascular halos, their maximum diameters were (mean $\pm$ standard deviation): 2.67 ± 1.2 cm before injection of the contrast agent, 3.50 ± 1.4 cm during the arterial phase, and 2.71 ± 1.2 cm in the portal-venous phase. The mean difference between the maximum diameters measured during the gray-scale examination and during the arterial phase of CEUS was 0.84 ± 0.45 cm (Fig. 1). The mean difference between maximum diameters measured in the arterial and venous phases of CEUS was 0.79 ± 0.50 cm.

Both differences were statistically significant ($p < 0.001$ for both; 95% confidence intervals [CI]: 0.59–0.90 for arterial phase versus Gy Gy-scale, 0.57–1.2 for arterial versus venous phases). The maximum diameters measured before contrast injection and during the venous phase of CEUS was not statistically significant ($p > 0.5$).

At histological examination, 24/25 (96%) biopsies from the hypervascular halo presented chronic inflammatory infiltrates with no evidence of microscopic tumor (Fig. 2). The other biopsy was negative for tumor and inflammatory cells (normal hepatic tissue). The halo biopsy specimens from two patients subjected to immunohistochemical lymphocyte typing presented positive CD3 reactivity reflecting a predominance of T-lymphocytes in the inflammatory infiltrates.

Discussion

Results from our retrospective study demonstrate that a substantial number of liver metastases (54%) exhibit

Fig. 1 57-year old patient with liver metastasis from colon cancer in segment VIII. Contrast-enhanced ultrasound images, sub-costal scans. (A) Maximum diameter of the lesion at was 18 mm on gray-scale ultrasound and 30 mm during the arterial phase of CEUS (25 s after injection of contrast agent). (B) During the venous phase (106 s), the maximum diameter is once again 18 mm. The difference between the maximum diameters measured in the pre-contrast and arterial phases is 12 mm.

hypervascular peripheral halos during the arterial phase of CEUS, which are not visible on gray-scale ultrasound studies.

Regardless of histotype, a high percentage of metastatic liver lesions display arterial-phase enhancement during CEUS [16]. Preliminary data reported in the literature indicates that a hypervascular peripheral halo in the arterial phase is most frequently observed around metastases from adenocarcinomas of the colon and breast cancers [12].

Before percutaneous thermal ablation treatment, regardless from the specific technique used, the volume of

tissue that needs to be ablated has to be carefully defined. At present, a 5–10 mm safety margin is considered sufficient to ensure complete destruction not only of macroscopic tumor tissue but also of the microscopic tumor infiltration in the normal tissue surrounding the metastasis [17]. The concept of the safety margin derives from surgery and is most important when performing percutaneous tumor ablation of metastasis compared to HCC, due to the different pattern of tumor spread [18–20].

However, the inflammatory reaction at the periphery of the metastatic lesion is not considered in the calculation of

Fig. 2 70 year old patient. (A) Gray-scale ultrasound imaging, intercostal scan: liver metastasis from breast cancer in segment VIII. (B) Contrast-enhanced ultrasound image, arterial phase, intercostal scan. The peripheral enhancing halo is well visualized. (C) Contrast-enhanced ultrasound image, venous phase, intercostal scan. Compared with the arterial phase images, the diameter of the metastasis in the venous phase is clearly smaller. Liver biopsies from the same patient (hematoxylin and eosin): (D) center of the metastasis and (E) the hypervascular halo. Arrowheads show chronic inflammatory infiltrates; arrows indicate areas of hepatic steatosis.

the ablation margin. In recent years, numerous efforts have been made to elucidate the role of chronic inflammation in initiating and promoting tumor growth, and some of the molecular mechanisms linking chronic inflammation and cancerogenesis have now been identified. Chronic inflammation can also increase the proliferation and survival of cancer cells, and it also triggers the “angiogenic switch” by stimulating the release of different cytokines [21,22]. Once the tumor is established, it also sustains the presence of a particular inflammatory infiltrate, whose characteristics vary with the tumor histotype [23].

The data from this pilot study indicate that the hypervascular halo around liver metastases during the arterial phase of CEUS represents a chronic inflammatory infiltrate, and it could play a role in local recurrence after thermal-ablation treatment if a few viable tumor cells were left at the periphery of the ablation area. Since this halo can be visualized and measured on CEUS, the safest approach would be to include its entire thickness in the volume of tissue to be ablated.

One of the limitations of this study is that our needle biopsies sampled only a portion of the inflammatory halo. Histological characterization of the entire lesion (metastasis + inflammatory halo) would require surgical resection. In addition, we investigated the characteristics

of the chronic inflammatory infiltrate present in the hypervascular halo only in two patients.

On the basis of our experience in this study, we can conclude that when treating liver metastasis with thermal ablation, the ablation margin should be more than 5 mm, and if a thicker hypervascular halo is present, the safety margin should be cautiously increased to include the entire inflammation rim in the volume of necrosis.

Conflict of interest statement

The authors have no conflict of interest.

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