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Biopsy of Liver Target Lesions under Contrast-Enhanced Ultrasound guidance. A multicenter study.

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INTRODUCTION

Contrast-enhanced ultrasound (CEUS) imaging, which is based on utilizing a microbubble contrast agent and real time contrast-specific imaging software and technique, has been performed and investigated in a variety of organs over the past fifteen years [1, 2]. The most successful application of CEUS has been the characterization of focal liver lesions because of its improved specificity in the diagnostic effectiveness compared with baseline gray scale Ultrasound (US) and Doppler US [3,4,5] nearly equivalent to that of computed tomography (CT) and magnetic resonance (MR) imaging [6,7].

The panel of experts recommended in the EFSUMB guidelines the use of CEUS-guided biopsy in deep abdominal organs and mainly in the liver in order to avoid necrotic areas or improve targeting of undetectable or not conspicuous lesion targets on B-mode US [8,9].

These recommendations were based on a limited number of papers using small, old case series [10] or more recent single-center case studies that do not accurately assess the role of the CEUS-guided biopsy procedure in the routine clinical setting [11,12].

The purpose of this retrospective study was to characterize the prevalence and impact of CEUS as guidance system for needle biopsy of abdominal lesions across six interventional US centers over a period of 10-years.

MATERIALS AND METHODS

The Institutional Review Board-approved patient database was collected in each participating center during the period 2005–2016.

All patients who underwent CEUS-guided biopsy of liver target lesions (LTL) with fine or cutting needles at the participating centers were included. The different US equipment used at the participating center over the time study period had dedicated software for contrast specific imaging (Esatune, Technos MPX, and MyLab™Twice, Esaote, Genova, Italy; Aplio XG SSA-790, Toshiba Medical Systems, Tokyo, Japan; Alpha 10 and F75, Aloka, Tokyo, Japan; Arietta V70, Hitachi Medical Systems Europe; Logic 7 GE Healthcare, Milwaukee, WI, USA).

Infusion of contrast agent and the scanning technique were carried out in the different centers according to modalities established since the introduction of US contrast agent in our country: CEUS was performed by using an 8-IL/mL solution of sulfur hexafluoride microbubbles stabilized by a phospholipid shell (SonoVue, Bracco SpA, Milan, Italy) as contrast medium. SonoVue was administered IV as 2.4-mL boluses through the antecubital vein in 2–3 seconds, followed by a 5 mL flush of normal saline (0.9%). Biopsy procedures were carried out in patients with permissive coagulation status (platelets >50.000/mm³, INR < 1.5) using cutting needles (Biomol 18–20g, Hospital Service Italy, Rome, Italy; Full-Opty 20 G Sterylab, Rho, Italia, Surecut 18–19G, TSK, Japan) or fine aspiration needles (Chiba needles 20–22g, Hospital Service Italy, Rome, Italy) by operators with over 15-year-experience in interventional US. Interventional procedures were performed during the vascular phase judged the best one to visualize the target by the single operator. In all cases the biopsy procedures were performed during the portal and late phases, 45–300 seconds after contrast agent injection when target conspicuity was expected to be the highest, i.e. hypoechoic with respect to the surrounding enhanced parenchyma in case of not conspicuous/undetectable targets. The same was true in case of complex targets where the marked difference between necrotic (constantly avascular) and vital areas enabled operators to sample targets just after arterial phase.

In all cases LTL were demonstrated at CT and/or MR examinations and were assessed with CEUS prior to biopsy.

All participating centers were asked to gather demographic data of patients, indications to the CEUS-guided procedure (difficult-to-visualize isoechoic lesions, increase of lesion conspicuity, detection of non-necrotic areas within the target lesion), biopsy modality (use of aspiration vs. cutting needles, needle gauge, free-hand technique or use of biopsy guiding

systems), previous non-diagnostic biopsy carried out under conventional US, diagnostic yield and complication rates of both interventional maneuver and contrast agent delivery, and consumption of contrast agent per single biopsy. Indications to CEUS-guided procedures were defined on the basis of videoclips and/or exam reports review; in addition, the size (in mm) of the target measured during CEUS was considered as target lesion size.

Complications were evaluated according to SIR classification [13].

Each participating center provided the total number of diagnostic biopsies performed during the time period of the study in order to assess the CEUS-guided biopsy rate in field practice. The study data were collected on an Excel spread sheet and descriptive statistics were used to represent continuous and categorical variables, whereby results were expressed as median and range. The chi-squared test and Fisher's Exact test was used to analyze the categorical variables; a P-value < 0.05 was considered to be statistically significant.

RESULTS

Between January 2005 and December 2016 CEUS-guided biopsies were carried out in 103 patients (68 M / 35 F, median age 69 years, range 31–89) with 103 LTL (median size: 20 mm; range 8–55). Most of the target lesions (66%) were located in the right lobe and the 5th segment accounted for 27% of all liver biopsies. Only a small proportion of procedures (1.9%) was carried out in the caudate lobe (Table 1).

The rate of interventional procedures under CEUS guidance on LTL ranged between 0.8% and 7.7% in the 6 participating Centers considered alone. On the whole, it was 2.6% of 3818 biopsies performed during the time study. Twenty-six out of 103 patients (25.2%) had a previous non-diagnostic cyto-histological exam performed under non-enhanced ultrasound US guidance.

Biopsy was carried out with cutting needles in 94 cases (91.2%), 18 gauge being the caliber most frequently used (81%). In 3 cases (3%) the Chiba aspiration needles (22–21g) were used. Combined cytological and histological samples were obtained in the remaining 6 cases (5.8%). Different approaches were used to perform the biopsy: lateral biopsy guiding systems connected to convex probes for standard abdominal examination were used in 63 cases (61.2%), dedicated interventional probes with central holes in 26 patients (25.2%) and a free-hand technique in 14 cases (13.6%). The biopsy sample taken with a cutting needle was considered adequate if a specimen of at least 0.5 cm long was obtained; after needle withdrawal the tissue core was placed in 10% neutral buffered formalin for histologic processing. In the case of aspiration biopsy, performed using Chiba needles, in one Center the sampled material was collected and rinsed in a vial of proprietary preservative solution, capturing virtually the entire cell sample. The specimen was then sent to the laboratory, where an automated processor prepares the slide by dispersing and filtering the sample and then placing a representative sample on the slide for microscopic evaluation (Thin-Prep test). In the other five Centers the sampled material was smeared on a slide and immediately fixed and then stained using the Papanicolaou technique for subsequent microscopic examination. A single needle pass was used in 64 patients (62.1%). Two passes were used in 37 patients

(36%) and 3 passes were needed in the remaining 2 cases (1.9%) so that the total number of needle passes was 144 in 103 target lesions.

Indications to CEUS-guided biopsy were: target lesion detected on contrast enhanced imaging methods such as computed tomography or magnetic resonance imaging and not visible on non-enhanced US (i.e. isoechoic with respect to the surrounding parenchyma) in 28 cases (27.2%) (Fig. 1), improvement of conspicuity of the target lesion in 34 patients (33%), depiction of non-necrotic tissue within a complex target mass including necrotic and non-necrotic areas in 47 cases (39.8%) (Fig. 2). Choice of non-necrotic area within the target lesion was the most frequent indication (44.4%) to CEUS-guided biopsy in the subgroup of 26 patients with previous non-diagnostic biopsy. This rate was lower in the remaining 77 patients without previous biopsy (32.4%) and this difference was statistically significant ($P<0.05$).

Final diagnoses are shown in Table 2. Eighty-two LTL out of 98 cases with a clear-cut diagnosis proved to be malignant (83.6%), and most of them (56%) were secondary liver tumors.

Non-diagnostic samples were obtained in 5 cases: one (negative for malignancy in the sampled material) was a true false negative result in that final diagnosis on surgical specimen was cholangiocarcinoma whereas the other four lesions with inconclusive diagnoses did not show any change in size and US appearance during the available follow-up of these patients (10–24 months). On the whole the diagnostic accuracy of CEUS-guided biopsy 99%. A similar value of diagnostic accuracy (100%) was observed in the subset of 26 patients with a previously non-diagnostic bioptic exam performed under conventional US.

A single standard dose (2,4 ml) of medium contrast was used in 69 patients (67%) whereas two or three doses were necessary in the remaining 34 patients (33%). There were no statistically significant differences ($P=0.5$) between patients having either a single or more than one dose as to diagnostic accuracy (100% vs 97%).

No complications ensued infusion of contrast agent during procedures. One self-limiting intra-hepatic hematoma occurred after liver biopsy : the complications rate was 0.9% per patient and 0.7% per number of needle passes. In the group of patients with malignant disease, there were no cases of needle tract seeding reported during the available follow-up.

DISCUSSION

CEUS is increasingly used not only for characterization of LTL but also as an aid to interventional procedures such as planning of biopsy or ablation procedures [8,9]. The advantages of CEUS before biopsy to improve subsequent targeting of the lesion both in the liver [14], lung [15] and soft tissues [16] have been reported.

In a prospective, randomized study Wu et al. [14] showed that diagnostic accuracy of biopsy for malignancy in patients undergone to CEUS prior to biopsy was greater in about 10% higher than that observed in patients without pre-biopsy CEUS and that the number of needle passes was reduced in the former group.

Although recent guidelines issued by EFSUMB [8,9] recommend the use of CEUS-guided biopsy in case of abdominal target not well depicted or not visible at conventional sonography or with complex US pattern due to the presence of necrotic areas, there is limited clinical data supporting such recommendations. Schlottmann et al.[10] studied only 12 patients with focal liver lesions not visible on B-mode US whereas two single-center prospective studies used different selection criteria to carry out CEUS-guided procedure: Yoon et al. [11] selected only patients with masses inadequately visualized on unenhanced US or with necrotic areas inside the target lesions, whereas Spàrchez et al. [12] randomized to US- or CEUS-guided biopsy 178 patients with lesions clearly detected at conventional sonography. These different enrollment criteria may explain the lower technical success reported in the Korean study [11] that could be due to the inclusion of lesions not confidently visible on B-mode US. On the whole, both studies suggest that CEUS-guided biopsic procedure is a valuable tool to improve the visualization of difficult-to-see liver lesions and to achieve optimal sampling in tumors with necrotic areas.

Our retrospective study evaluates the use of CEUS guided biopsy in centers with high volume of interventional US activity. This methodological approach is more likely to provide a real snapshot of field practice. Indeed, to the best of our knowledge, this is the first study quantifying the use of CEUS-guided biopsy in routine daily activity of some selected interventional centers. In our experience, CEUS-guided biopsy of LTL is not frequent (on average 2.6% of all patients undergoing liver biopsy on focal lesions). This result is quite similar to that of Yoon et al. [11] : in their prospective study including a lower number of LTL the Authors reported that CEUS-guidance was necessary in 44 out of 531 liver lesions referred for biopsy (8.3%).

Our data confirm that the main indication to CEUS-guided biopsy are poorly visualized or undetectable lesions, especially if small (less than or equal to 2 cm) and, independently from size, sampling of solid viable areas in complex target lesions in which basal US exam cannot clearly distinguish viable and necrotic areas. Diagnostic accuracy of CEUS-guided biopsy was as high as 99% since only one true false negative result was found.

In routine activity another scenario where CEUS guidance is useful is repeat biopsy after an unsuccessful attempt performed under conventional US guidance. Approximately 25% of patients in this series had a previously non-diagnostic biopsy and CEUS-guided biopsy allowed to reach a correct diagnosis in 100% of these challenging cases. Such a role of CEUS-guided biopsy of LTL has not been evaluated in the other published series dealing with CEUS as an aid to liver lesions biopsy, particularly in suspected HCC nodules. Indeed, in this subgroup of patients a second US guided biopsy has been reported to yield a correct diagnosis in less than 40% of cases, mainly because of presence of necrotic areas in lesions greater than 4 cm [17, 18]. By targeting vital areas inside complex lesions, CEUS-guided biopsy is likely to improve the diagnostic yield of repeat biopsy, as found in our series.

As far as consumption of SonoVue is concerned, there were no differences as to the number of doses according to different indications.

Similarly to other authors [11,12], we found that the CEUS-guided biopsy proved to be effective and safe as diagnosis could be achieved in 99% of cases and no complications occurred after infusion of the contrast agent; the occurrence of a single minor complication not requiring therapeutic intervention such as intrahepatic hematoma after biopsy is in line with the well known low complication rates of abdominal biopsy procedures [19]. In addition, in more than half of the patients only a single needle pass was necessary to provide diagnostic material.

It was beyond the aim of this study to compare the diagnostic yield of CEUS-guided biopsy versus standard technique and the retrospective nature of the study would weaken any conclusion drawn on this issue as patients were selected for CEUS-guided procedure on the basis of subjective, non-randomized criteria. However, we think that the difference may be not substantial and several factors may explain the limited use of the technique in the participating centers: first of all, experienced operators are trained to take an optimal sample from a LTL, e.g. by avoiding central areas of patent necrosis on real time US and by recognizing the adequacy of the sampled tissue, so as to repeat biopsy immediately if needed. In addition, the more and more frequent use of CEUS in the planning phase of interventional procedures, a current policy in the participating centers, is likely to make operators more confident in performing the biopsy on the target lesions in B-mode modality without resorting to CEUS-guided procedure. Finally, the systematic use of CEUS-guided biopsy would increase the technical difficulty and the cost of the US-guided bioptic procedure which has gained popularity over the last three decades because of its simplicity and cost-saving [19, 20]. Indeed, positioning biopsy needles inside target lesions during CEUS is a more challenging procedure especially if the target lesion is small and/or deeply located.

In recent years, fusion imaging of real-time sonography and prior CT or MR has been applied with high technical success rates (>90%) to guide the biopsy of focal hepatic lesions with poor sonographic conspicuity. However, even in these studies the technique was applied in a very selected population accounting for less than 10% of patients undergoing liver biopsy for focal lesions [21,22].

CEUS-guided biopsy might further decrease the number of patients deserving fusion techniques, especially in lesions located in easily approachable liver segments. Future studies should compare technical feasibility, learning curve and technical success rate of the two guidance systems that, in our opinion, should be regarded as complementary rather than alternative techniques by choosing one or the other according to the features of each liver lesion, the operator's experience and the guidance system availability in each Center.

This study has some limitations. Indeed, this is a retrospective survey with all its inherent limits and some unavoidable selection biases, including the presence of confounding factors in the data. Furthermore, different approaches (free hand, lateral devices, dedicated probes) and different kind of needles were used in the participating Centers to perform the biopsy; however, the diagnostic yield did not differ significantly between Centers (data not shown).

In conclusion, our analysis provides a real snapshot of the use of CEUS as guiding system for liver biopsy on focal lesions in US interventional centers with high volume of activity: although CEUS-guided biopsy is not frequently carried out, it represents a very useful tool to cope with challenging clinical scenarios, e.g. poorly visualized or invisible targets, sampling of non-necrotic areas in the target lesions and repeat biopsy after unsuccessful sampling performed using the standard unenhanced technique.

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Fig. 1.

a No clear-cut parenchymal lesion is seen in intercostal US scanning of the right liver lobe in a 60-year-old man with neoplastic mass of the pancreatic head. **b** Split-screen display of intercostal US shows one small hypovascular lesion (black arrow) with hyperenhancing ring (white arrow) in the right liver at the end of arterial phase, 44 seconds after SonoVue injection (right). **c** CEUS-guided biopsy via intercostal route: an 18-gauge cutting needle is driven to the target lesion during the late phase, 200 seconds after SonoVue injection (black

arrow points to needle tip). Metastasis from pancreatic adenocarcinoma was diagnosed at pathological examination

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Fig. 2.

a Repeat biopsy on a 5-cm complex liver lesion (in the fourth segment) after non-diagnostic biopsy performed under conventional US in a 63-year-old woman with history of ovarian cancer. Split-screen display of right subcostal US: in the arterial phase (left), 22 seconds after SonoVue injection the viable area is identified along the posterior aspect of the lesion in a transverse subcostal scan (white arrows). **b** Through an anterior subcostal route an 18-gauge cutting needle is driven to the target lesion during the venous phase, 231 seconds after SonoVue injection (left image on the split-screen display): white arrows show the needle following the prefixed path (dotted line) just before reaching the viable part. Pathological exam yielded the diagnosis of metastasis from ovarian cancer.

Table 1:

Baseline characteristics of patients studied

No. Patients	103
Male/Female	68 M / 35 F
Median age yrs (range)	69 (31–89)
Liver Targets	
No.	103
Median size mm (range)	20 (8–55)
Segment	No.
1	2
2	5
3	8
4	20
5	26
6	17
7	18
8	7

Table 2:

Final histopathological diagnoses in the targets biopsied under CEUS guidance

No. Cases	98 *
Adenoma	2
FNH	2
Solitary Necrotic Nodule	3
Inflammatory Pseudotumor	1
Post-chemotherapy flogosis	1
Parenchymal flogosis	3
Abscess	1
Dysplastic Nodule in cirrhosis	4
Hemangioendotelioma	2
HCC	16
Gallbladder Carcinoma	3
Cholangiocarcinoma	13
Lymphoma	1
Mertastasis	46

* 5 non-diagnostic results

Abbreviations:

HCC = Hepatocellular Carcinoma

FNH = Focal Nodular Hyperplasia