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Contrast-Enhanced Ultrasonography (CEUS) of Liver Masses – Principles, Clinical Applications, Drawbacks

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Additional information is available at the end of the chapter

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1. Introduction

Ultrasonography (US) is one of the most widely used imaging procedures. The main advantages of this investigation over others include it being non-invasive and safe, with no radiation exposure. In addition, its images are provided in “real time”, delivering dynamic images with both anatomical and functional information. The images acquired by ultrasonography are sectional and easy to understand. The method is accurate, without distortion. The major disadvantage of ultrasonography is that it is operator dependent, requiring a large number of examinations performed personally in order to reach a high level of proficiency [1]. Additional issues include poorer performance if gas, adipose tissue or bony structures come between the transducer and the region of interest. A number of artifacts, such as reverberations and false reflections, may interfere with the acquisition of the ultrasound image, and need to be recognized and avoided.

Ultrasonography is practiced on a daily basis, both by physicians and specialized personal. Now it is widely accepted that a good correlation of the ultrasonographic data with clinical information is necessary in order to achieve an optimal performance [2]. The proposal that access to US should become generalized and be practiced by every physician within their specialty as a way of completing the clinical examination is increasingly voiced [3]. Liver ultrasonography has been recognized for a long time as an application of US efficient in detecting diffuse liver conditions, hepatic tumors, vascular abnormalities, post-traumatic lesions as well as in guiding interventional procedures [4, 5, 6, 7, 8, 9].

Ultrasonography is a multimodal examination, meaning that the final report and basis for the diagnosis, is a complex one, obtained through multiple procedures. Each procedure brings specific data in relation to the underlying principle of operation. The main US component is bidimensional ultrasonography (“grey scale” ultrasound). It consists of sectional images,

presented on a grey scale, with each shade of grey representing a density (in fact, an acoustic impedance). "Grey scale" ultrasonography is a dynamic examination that allows the overall evaluation of the liver, with measurements of the hepatic lobes, identification of liver segments, as well as characterization of texture and echogenicity (in relation with the degree of fat infiltration and fibrosis). Grey scale US also enables the detection and evaluation of normal anatomical structures (bile ducts, vessels) and of pathological elements (mainly liver masses). Elastography, an additional component, realizes a quantitative and qualitative (using color coding) characterization of the elasticity of the liver parenchyma [10, 11, 12]. The Doppler procedure represents the basis for evaluating the flow within liver vessels. There is also the spectral version of this application which provides quantitative data (direction of flow, flow velocities, debits, etc) and a color coded version that gives information regarding the presence and the direction of blood flow in the region of interest [13, 14]. Ultrasonography using intravenous (i.v.) contrast agents (CEUS) is a procedure that observes the blood flow in a reference region by revealing the harmonic echoes [15]. Recently, a number of other applications have been developed, many of them based on the mathematical processing of the image. These are meant to optimize the information, enabling quantification, for example, and even allow an automatization of the US diagnosis [16]. Generally speaking, ultrasonography is often the first imaging exploration performed after the clinical exam. As a method, it has proven its value in many respects, including in the detection of liver tumors. In its standard form, however, it is not sensitive enough to characterize and establish the nature of these lesions, despite the many technical advances that have been made [17].

2. The principles and physics of contrast enhanced ultrasonography

The use of contrast in ultrasonography represents a huge advance for this investigation. Even though Doppler ultrasound has a certified role and is the recognized technique for detecting vascular abnormalities in large vessels, it is only after the introduction of contrast agents that we can talk about an exact and reproducible evaluation of microcirculation with the help of ultrasound.

Contrast enhanced ultrasonography (CEUS) consists of injecting gas 'microbubbles' into the systemic circulation. The contrast agent (CA) used is made of small bubbles close in size to red blood cell. These microbubbles contain low soluble gases encapsulated into a biocompatible membrane which may have variable composition – lipids, proteins or biopolymers. The membrane can be either rigid or flexible with a thickness between 10-200 nm [18]. Basically, like in any other contrast based imaging procedure, the CEUS exam consists of a "bolus" administration of the contrast media through a superficial peripheral vein. Due to their extremely small size, the microbubbles pass through the pulmonary circulation and then disseminate into the systemic circulation through the arterial blood stream. The contrast agent remains in the blood stream for about 4-5 minutes. There is also a parenchymal phase at the level of the liver and spleen because the contrast agent is captured by the reticuloendothelial system and/or it becomes adherent to the hepatic sinusoids [19]. Unlike the contrast agents used for CT or MRI, the gas used for CEUS is eliminated through the air-ways 10-15 minutes

after administration, while the substances that make up the membrane are eliminated through the kidney or metabolized by the liver. The contrast media used in ultrasonography have no toxicity and the technique is less harmful to patients when compared with other investigations.

The contrast enhanced examination is based on the emission of harmonic echoes by the CA when this passes through an ultrasound beam that has a mechanical index of 0.09 – 0.11. The mechanical index (MI) represents a value that is directly correlated with the biological effect of the ultrasounds upon the tissues. This index is variable depending on the ultrasound machine, but it is basically conditioned by the acoustic power of the ultrasounds beam. The acoustic power (AP), measured in Pascals, represents the energy of the sound beam acting on a target, for example a group of red cells or the contrast agent inside the blood stream. Usually, at high values of the AP, up to MegaPa, the micro bubbles are “broken” and an irregular, non-linear signal is generated. At low values of the AP (30-70 kPa) the microbubbles vibrate in a particular, non-linear manner, producing alternating contractions and relaxations, thus generating harmonic echoes.

The ultrasound equipment produces a separation of the harmonic echoes generated by the CA within the blood stream from the echoes generated by the surrounding tissues. This separation may be realized by modulating the phase and the amplitude of the US beam. There are multiple US generating techniques within the transducer that generate harmonic echoes within the CA. Techniques of pulse inversion (“Pulse Inversion”, “Power Pulse Inversion”, “Cadence Contrast Pulse Sequencing”) perform a “subtraction” of the tissue echoes by alternative emission of pulses found in an inversed phase. A “Vascular Recognition Imaging” technique introduces the Doppler principle in the analysis of the returned signals, allowing a color coding of the red cells in relation to their direction of flow.

Practically, CEUS consists of an injection of a contrast agent, prepared at the time of use, into a cubital vein. This is followed by a bolus of 10 cc saline solution. The region of interest, previously identified during the “grey scale” exam, is continuously observed on the monitor. The monitor may be divided into two identical, real-time images, one using fundamental, “grey scale”, echoes and the other one using harmonic echoes obtained by exposure to a sound beam with a low mechanical index (0.09 – 0.11). The examination continues for 90 seconds. After this time the exploration may be intermittent. CEUS is focused on a single region of interest, usually a mass. The exploration of another mass requires either a “breaking” of the bubbles inside the mass, performed with special software or using the CFM technique, or a repetition of the CEUS exam after another contrast administration focused on the second lesion. There are no risks for the patient, so the injection of the CA can be repeated as often as needed.

Using CEUS in the exploration of the liver has special features that arise from the double vascularity of this organ – through the portal vein (two thirds) and through the arterial system (one third). The sequence of blood entering the liver is first arterial (up to 30 seconds) and then portal (30 to 90 seconds, with little variation). This vascular discrimination (similar to the one obtained by contrast CT or MRI) allows for gathering information regarding the circulatory bed (types of feeding vessels, tumor circulatory volume) of a tumor. The presence of arterio-venous communications is characteristic for the neoplastic circulation and in CEUS is expressed by the “wash-out” process. This phenomenon begins at the end of the arterial phase

and/or during the venous phase, is persistent and is characteristic for neoplastic processes in 90 % of cases [20]. There are studies that correlate the wash-out speed of the tumor with its aggressiveness, attributing CEUS a prognostic value.

An important component of the CEUS exam is represented by the quantitative analysis of the data. This consists of a representation in time of the acoustic impedance variation in one or more predetermined regions of interest. A graphic is obtained that can be correlated with hemodynamic parameters, like the time of maximum systolic ascent in the region of interest, the volume of the circulatory bed, presence and scale of the arteriovenous shunts, etc. All these elements are indicators for the quality of a specific circulatory bed [21].

The advantages of the CEUS technique are summarized in Table 1 and include the lack of ionizing radiation exposure, the non invasive character of the method, as well as very good spatial and temporal resolution [22]. The method is safe for patients, with very few cases of anaphylaxis having been reported (about 0.001% of the total number of investigations). It is therefore recommended in some centres as the firstline procedure for assessment of liver nodules. Contrast CT not only exposes the patient to ionizing radiation, but iodine based contrast agents can be toxic and produce allergic reactions. CT scan imaging may also be less sensitive, "losing" or failing to capture the early arterial phase in highly vascular tumors.

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- very good spatial resolution;
 - high temporal resolution – it is a "real time" examination;
 - it reveals very slow blood flow or stagnant blood streams;
 - non-ionizing examination;
 - lack of anaphylaxis;
 - the contrast agent is eliminated through the air-ways;
 - can be repeated as often as needed
-

Table 1. Advantages of the CEUS examination

It should also be recognized that CEUS exploration has a number of limitations, as summarized in Table 2. It is dependent on expensive and sophisticated equipment that raises the cost of the investigation. The harmonic image is depending on good quality 2D image. Deeper lesions are harder to visualize and attenuation may represent a limit in detecting tumors located further from the transducer. Last, but not least the investigation is operator dependent and often the information that is obtained must be correlated with the clinical data and biochemical functional information.

-
- expensive and sophisticated equipment;
 - high cost of the exploration compared with the standard investigation;
 - operator dependent;
 - it depends on a good quality 2D image;
 - low quality information in case of attenuation like in liver steatosis;
 - it investigates a single region of interest;
-

Table 2. Limitations of CEUS examination

3. CEUS and the assessment of benign liver tumors

The imaging/ultrasonographic contribution to the detection of benign liver tumors is not insignificant. Benign lesions are numerous, affecting about 20% of the population [23]. They are frequently detected following ultrasonography, which is widely available and represents a common investigation in any abdominal complaint. CEUS exploration can distinguish between benign and malignant tumors and consequently can halt the diagnostic algorithm when a mass detected by 2D ultrasonography is characterized by CEUS as being benign. In this way the numbers of investigations is optimal and the patient's discomfort is significantly reduced. There are multiple benign liver masses, however, and not all of them have CEUS characteristic features. Their ultrasonographic aspect is often similar and their discrimination may require additional criteria [24]. Among the lesions that present specific circulation patterns which can be defined when analyzed by CEUS are cysts, hemangiomas, adenoma and benign focal hyperplasia (table 3).

Tumor	Arterial phase	Portal phase	Delayed phase	2D feature
Cyst	No uptake	No uptake	No uptake	Transsonic
Hemangioma	"Ring-like" peripheral uptake	Centripetal enhancement resembling "buds"	Complete uptake	Hyperechoic Well-defined Compressibility "Mirror" effect
Focal nodular hyperplasia	Central enhancement with a "spoked wheel" distribution of the CA	Complete enhancement with an isoechoic appearance compared with liver parenchyma	Isoechoic aspect when compared with the liver parenchyma	Echoic scar in the centre of the lesion Arterial signal in the centre of the tumor
Adenoma	Inhomogeneous uptake	Discrete wash-out Iso or hypoechoic aspect compared to liver parenchyma	Discrete wash-out, Iso or hypoechoic aspect compared to liver parenchyma	Hypoechoic nodule Non-cirrhotic liver

(*) The information presented in the table is referring to typical situations. In practice, there are variations of the 2D or CEUS aspects that require further investigations

Table 3. CEUS and 2D ultrasonographic features in cases of benign liver lesions. (*)

Liver hemangioma. It is the most frequently encountered benign tumor of the liver. It is most often found in women and has a prevalence of about 0.4 – 7.4 % in the population [25]. The mass consists of a vascular, capillary or venous, bundle, rich in fibrotic bands and with no capsule. It may be frequently associated with other benign tumors like cysts or adenomas. Hemangiomas are usually asymptomatic (in very rare cases, when extremely large they may cause a distension of the liver capsule and thrombocytopenia) and have a slow, self-limiting

development. In most cases there is just one lesion, but there is also a multicentric type. The grey scale US appearance of hemangiomas is fairly characteristic: a well circumscribed, hyperechoic liver mass, with a slightly hypoechoic centre or periphery (figure 1).



Figure 1. Liver hemangioma. The lesion is found within the left lobe (asterisk) and has a characteristic appearance on “grey scale” ultrasound: well circumscribed, hyperechoic nodule with irregular margins.

Hemangiomas do not cause vascular or biliary ducts invasion. But they may produce an effect of posterior acoustic enhancement. On closer inspection the operator can observe this effect by changing the aspect of the transducer in combination with profound compression. When the hemangioma is located in contact with the diaphragm it can generate a “mirror” effect that leads to a reproduction of the image in the lung parenchyma. Hemangiomas do not usually show signal on the Doppler investigation as flow velocities inside them are very low. Additionally, in many cases intratumoral ischemia or fibrotic scars will develop. Often, the ultrasonographic diagnostic criteria are clear enough and in most situations the conventional ultrasound which first detects the nodule, is sufficient to characterize the lesion. But there are circumstances when 2D ultrasound is not sufficient for tumor characterization and thus CEUS is needed. In the presence of severe steatosis as well as in patients undergoing chemotherapy for various neoplastic conditions, the appearance of a hemangioma may alter and become “atypical”. Also in patients with liver cirrhosis, a hemangioma may be misinterpreted for a hepatocellular carcinoma or a large regeneration nodule. Extremely large hemangiomas may have a heterogeneous structure due to possible hemorrhage or ischemia developed inside the lesion, which can alter their appearance. Last but not least, CEUS can be very useful in reassuring both operator and patient that a nodule accidentally found during an ultrasound exam is benign.

The appearance of a hemangioma on CEUS is characterized by a peripheral, ring-like, clear enhancement during the arterial phase (figure 2a). This process is continuous and slow and is followed the appearance of contrast “buds” inwardly oriented (figure 2b). In the end, after several minutes of observation, a complete enhancement of the hemangioma is observed (figure 2c). Therefore a pattern of progressive and centripetal enhancement of a nodule is the characteristic feature for the diagnosis of hemangioma [26].

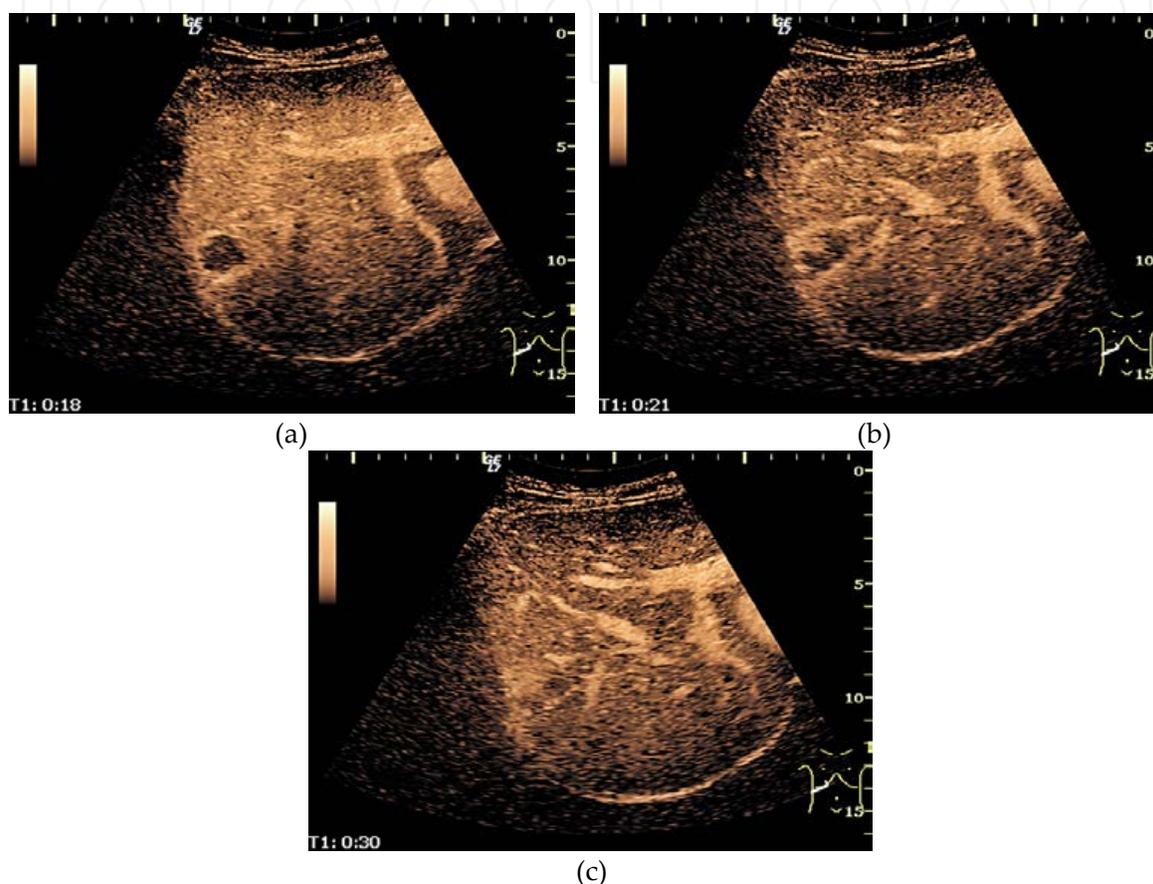


Figure 2. Liver hemangioma. a. CEUS performed during the arterial phase (18 seconds since contrast media injection) shows a well defined “ring” around the nodule. b. The appearance of the contrast “buds” suggests the centripetal character of CA progression. c. At the end of the arterial phase there is complete enhancement of the lesion with contrast agent.

The uptake may take variable amounts of time, from tens of seconds to several minutes (even tens of minutes), depending on the size of the lesion and the type of circulatory bed (figure 3a; figure 3b; figure 3c).

A particular type of hemangioma is the arterialized type. It is characterized by accelerated, complete uptake during the arterial phase. It corresponds to circulatory alterations characterized by an important arterial flow. In this situations a differential diagnosis with hypervascular metastases or HCC is difficult and thus a correlation with other imaging techniques is mandatory [27].

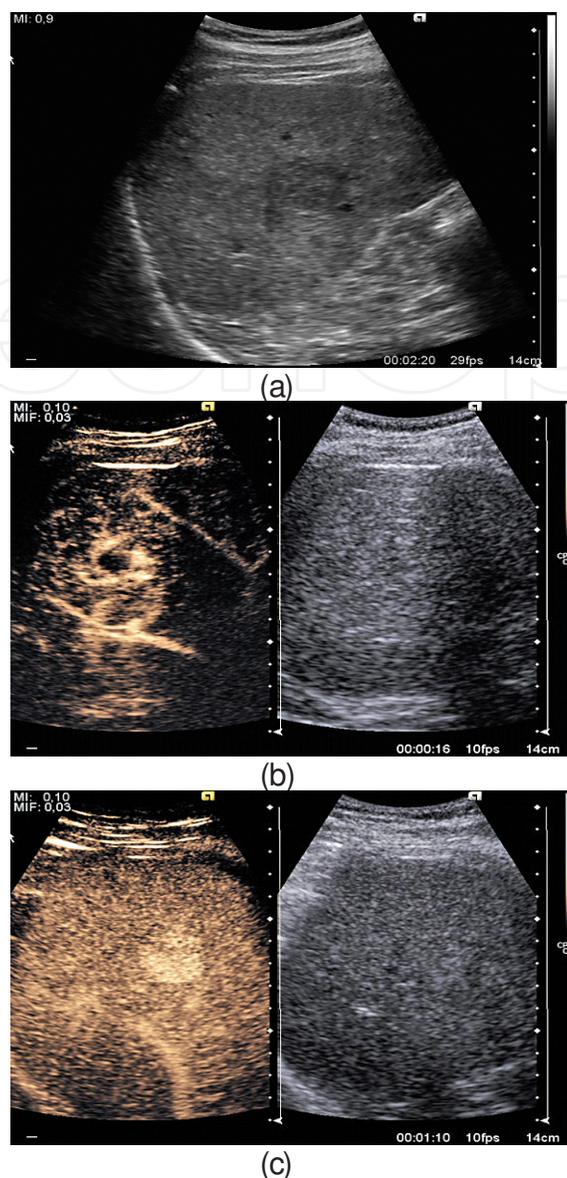


Figure 3. a. “Atypical” liver hemangioma. This is the case of a female patient undergoing chemotherapy for breast cancer. There is increased echogenicity of the liver suggesting therapy induced dystrophy. In the middle of the right lobe there is a hypoechoic, solid lesion that raises the possibility of a liver metastasis. b. CEUS exam shows a “ring” enhancement of the lesion during the arterial phase (16th second). c. CEUS exam reveals a complete enhancement of the nodule at the end of the portal phase (70th second). The enhancement process was centripetal (from periphery towards the centre). The diagnosis is certainly of hemangioma. The case demonstrates the role of the CEUS in patients undergoing oncologic treatments who present liver nodules.

Liver cysts. Liver cysts are serous collection circumscribed by cuboidal epithelium. They are frequently encountered during 2D ultrasound, especially in women. Usually they measure less than 20 mm and can present as single or multiple lesions. An involvement of the entire liver is rare. The grey scale US aspect of cysts is that of a well defined, transsonic lesion with posterior acoustic enhancement. The walls of a cyst are very thin and often difficult to distinguish. Inside the cyst there may be thin septa and sometimes deposits. Cysts show no signal on the CFM (color flow mode) interrogation. A hydatid cyst additionally presents

daughter cysts and abundant deposits inside. The size of a hydatid cyst is usually larger and the 2D pattern may present in one of several ways, including organization as a solid mass. In such cases the differential diagnosis includes a malignant tumor. In addition to these cases, a CEUS examination is indicated in cases of hypoechoic cysts identified in patients with liver steatosis or cirrhosis, when identifying a vascular signal inside the lesion is determinant in establishing the differential diagnosis (figure 4a; figure 4b). During the arterial phase cysts are highlighted even at sizes of 2 mm, due to their transsonic appearance that contrasts the arterialized surrounding parenchyma.

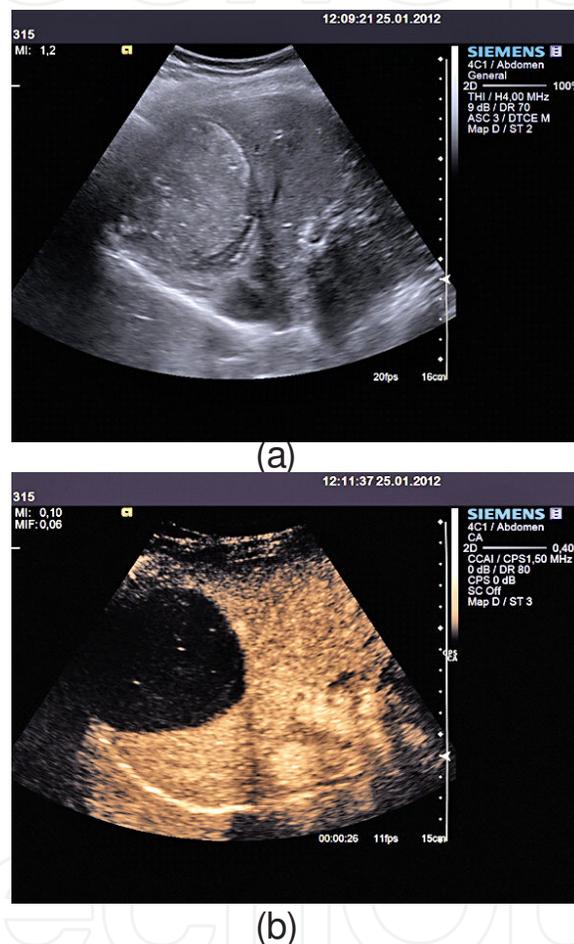


Figure 4. a. Inactive, solid, organized hydatid cyst of the liver (Gharbi classification, 1981). b. Organized hydatid cyst. CEUS exam demonstrates the lack of vascularity within the tumor and thus contributes to the decisive exclusion of a malignant liver tumor.

Focal nodular hyperplasia (FNH). FNH is a pseudotumor characterized by an area of normal liver cells proliferated around an arterialized scar. Focal nodular hyperplasia has an abundant portal circulatory bed. It is more frequent in women and its development may be linked with the use of oral contraceptives [28]. There is no risk of malignant transformation or spontaneous rupture. The lesion may be unique or there can be multiple lesions. The grey scale ultrasonographic aspect is that of a solid lesion with no capsule of its own (figure 5).

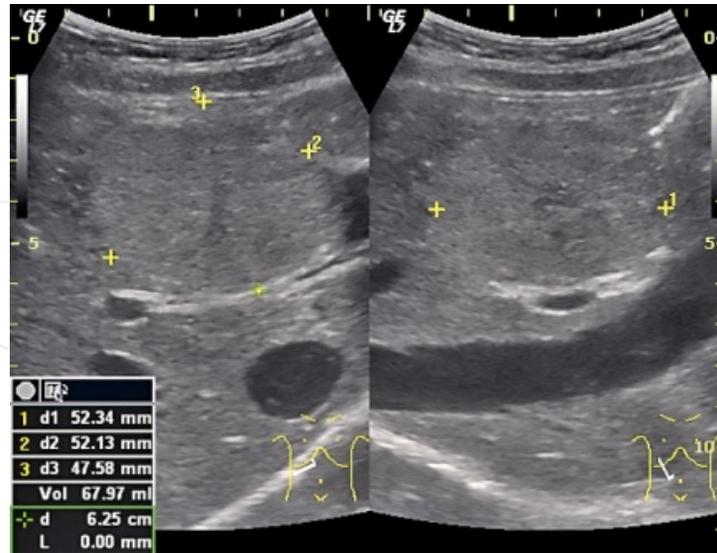


Figure 5. Focal nodular hyperplasia. “Grey scale” ultrasound examination. Perpendicular view through the left lobe. The lesion is solid and well-defined. In the centre of the lesion there is a linear structure that belongs to the typical, central scar (asterisk).

The central scar is more obvious in larger lesions and it presents as a linear, echoic structure. The CFM examination reveals a vascular signal in this area, while pulsed Doppler ultrasound detects arterial flow (figure 6a; figure 6b).

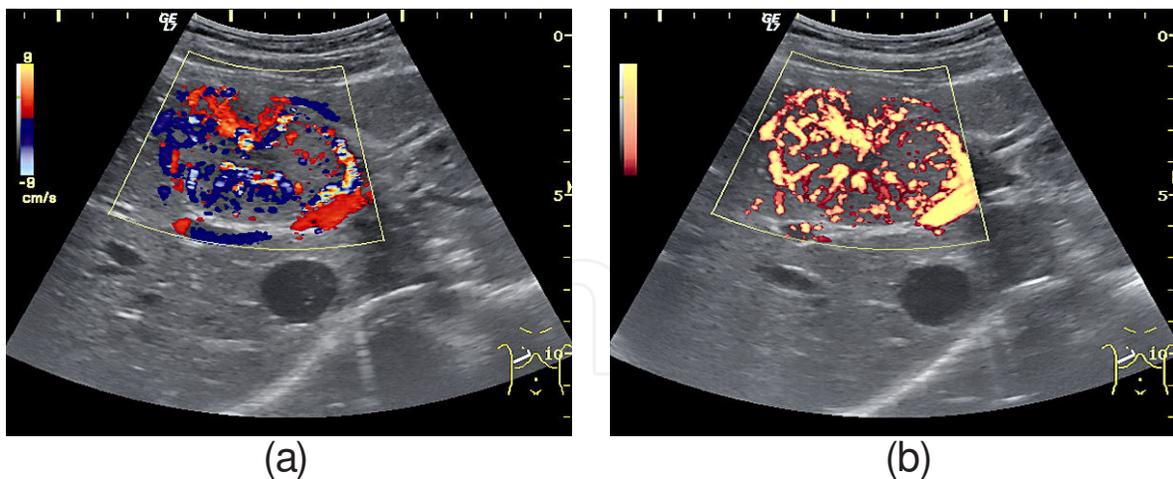


Figure 6. Focal nodular hyperplasia. a. CFM examination. The lesion has a high vascular signal. The vessels have “spoked wheel” spatial distribution. b. “Power mode” exam. “Spoked wheel” vascular pattern.

The CEUS behavior of FNH is characterized by an accelerated uptake in the centre of the lesion during the arterial phase, with a radial distribution, creating a “spoked wheel” appearance (figure 7a). The use of image post-processing procedures allows the identification of the vessels that make up the lesion, as well as their spatial distribution (figure 7b).

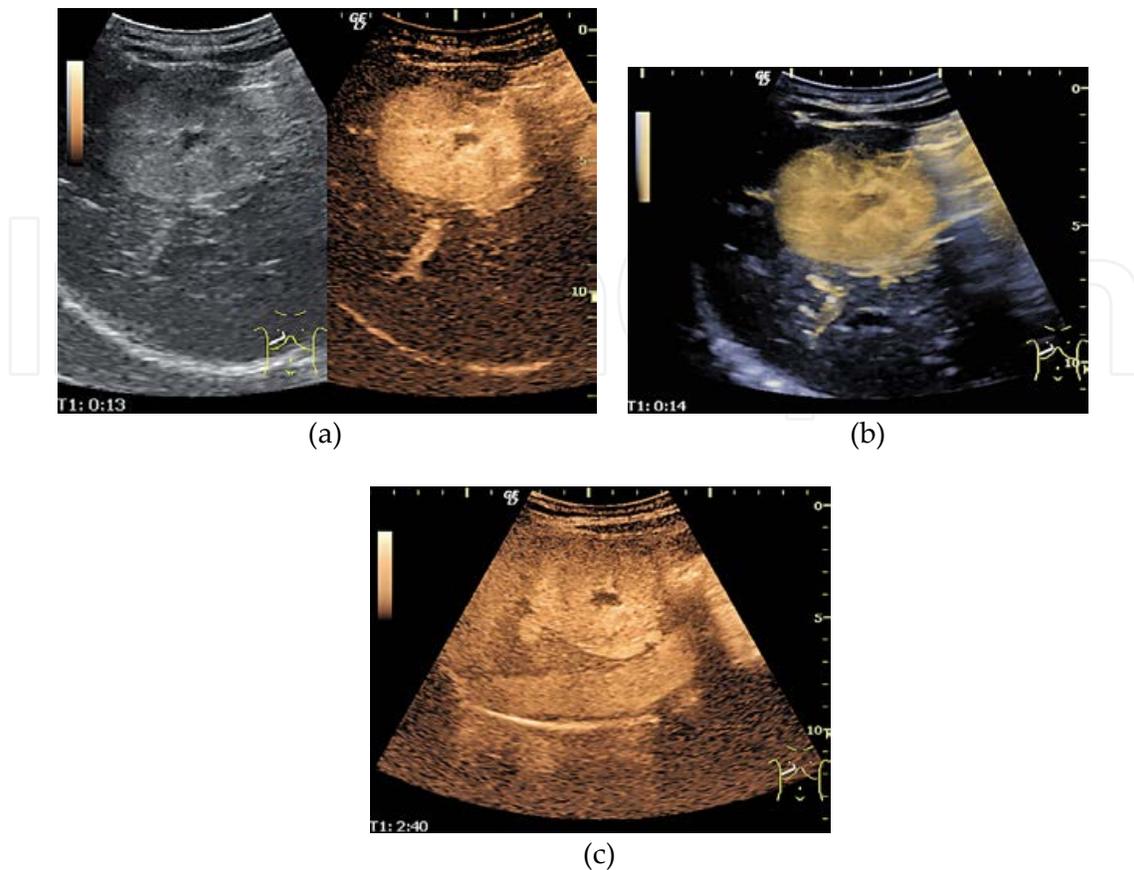


Figure 7. a. Focal nodular hyperplasia. CEUS exam during arterial phase. In the 13th second after contrast administration there is complete enhancement of the lesion. In the centre of the lesion there is a hypoechoic structure (the tumoral scar). The tumor pedicle can also be visualized. b. Focal nodular hyperplasia. Combined CEUS examination, using image post-processing techniques, that allows visualization of the spatial distribution of the vessels, which present a radiant orientation. c. Focal nodular hyperplasia (same case as figures 5, 6a, 6b). The CEUS examination performed after 160 seconds from contrast media injection reveals a similar aspect of the lesion. There is no contrast wash-out, thus excluding malignancy.

Liver adenoma. A liver adenoma is an accumulation of hepatic cells, with no biliary structures or Kupffer cells. Its development can be induced by the use of oral contraceptives. Adenomas may also arise in patients with metabolic diseases such as type I glycogen storage disease, as well as in long term administration of anabolic androgenic hormones. Adenomas are arterIALIZED but they do not contain portal vessels. They may be very large in size and thus become symptomatic through pain and intratumoral bleeding. In 5% of the cases adenomas may undergo malignant transformation [30]. Risk of malignant transformation as well as the risk of rupture within the peritoneal cavity can make detected and characterized adenomas indications for surgery. The grey scale ultrasound aspect is that of a well-circumscribed, hypoechoic, solid tumor. The vascular signal evaluated by CFM is non characteristic. After contrast media administration, during the arterial phase there is an irregular enhancement (due to intratumoral bleeding) (figure 8a, figure 8b). During the portal venous phase there is moderate wash-out which makes the tumor look iso or hypoechoic compared with the surrounding liver parenchyma. This behavior is seen in the delayed phase as well. The slow

wash-out, the hypoechoic aspect during the portal and delayed phases as well as the moderate and inhomogeneous uptake in the arterial phase are elements that may cause misinterpretation of adenomas for malignant tumors.

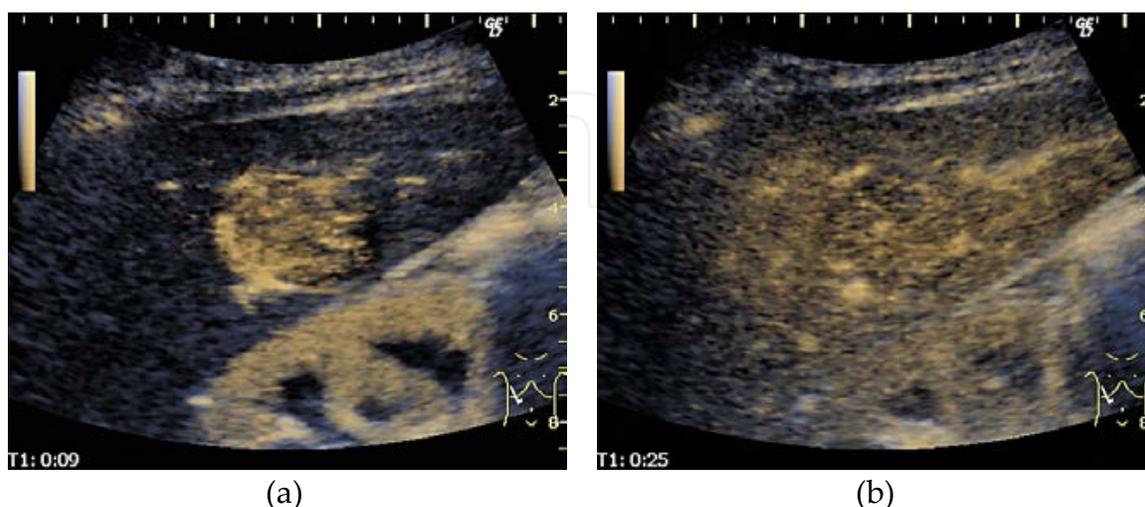


Figure 8. Liver adenoma, CEUS examination. a. during the arterial phase the tumor presents inhomogeneous uptake. b. CEUS shows moderate contrast wash-out at the end of the arterial phase.

The discrimination of adenomas from HCC is often based on the appearance of the liver on which it developed being normal or dystrophic. As HCC can arise in the absence of chronic liver disease, if there is any cause for doubt additional imaging techniques and/or liver biopsy should be considered. Similarly, distinguishing an adenoma from a metastasis is difficult and the lesion may need additional investigation to complete the diagnosis.

4. CEUS and the assessment of malignant liver tumors

Malignant tumors of the liver represent the main application of ultrasonography. The method can detect these masses within an evocative clinical context (a typical example is that of an oncologic patient who presents with liver nodules or that of a patient with cirrhosis that during follow-up develops a liver nodule) or by chance, during a routine ultrasound examination. The spatial resolution of ultrasonography is sufficient to allow the detection of nodules as small as 10 mm (table 4).

The CEUS examination has a high, but not absolute, specificity! The method contributes to the consolidation of the clinical and grey scale ultrasonographic diagnosis of malignant tumor. The final diagnosis (tumor characterization) is based on an accumulation of criteria, among which is the character of the liver on which a nodule develops (cirrhotic or non-cirrhotic liver), the clinical presentation and the biochemical and functional data of the patient. The intensity of intratumoral echogenicity as the CA crosses the nodule is compared with that of the neighboring liver parenchyma during the same vascular phase.

Tumor	Arterial phase	Portal venous phase	Delayed phase	"Grey scale" ultrasound (2D)
Hepatocellular carcinoma	Intense enhancement Hyperechoic aspect	Moderate/Intense wash-out Hypoechoic or isoechoic aspect	Moderate/Intense wash-out Hypoechoic aspect	Solid tumor Inhomogeneous structure "Basket-like" appearance of the CFM vascular pattern Arterialized circulation Portal invasion
Cholangiocarcinoma	Moderate, inhomogeneous uptake Hyperechoic / Isoechoic aspect	Moderate wash-out Hypoechoic aspect	Moderate or intense wash-out Hypoechoic aspect	Solid tumor located in the hilum or subcapsulary Bile ducts dilations oriented towards the tumor
Hypovascular metastases	Peripheral uptake Hypoechoic aspect	Peripheral wash-out Hypoechoic aspect	Intense wash-out Hypoechoic aspect	Multiple, solid masses
Hypervascular metastases	Intense uptake Hyperechoic aspect	Moderate wash-out Hypoechoic aspect	Intense wash-out Hypoechoic aspect	Involvement of all liver lobes

Table 4. CEUS and 2D ultrasonographic features of malignant liver masses

5. Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma is the most frequent primary tumor of the liver [31]. In the vast majority of the cases (over 80%) HCC develops on a liver already affected by cirrhosis. Liver cirrhosis is defined in morphological terms as a process of fibrosis and reorganization. The reorganization of the liver parenchyma leads to the development of variable size, even millimetric, nodules with an ubiquitous distribution involving the entire liver and representing the origin of hepatocellular carcinoma. HCC develops from one or more cirrhosis nodules. Many prospective and retrospective studies have demonstrated this continuity. The development of high resolution imaging techniques that can reliably distinguish between regenerative and cancerous nodules necessitates the accomplishment of practical systematic and validated approaches for their clinically relevant application. In addition to a role in the characterization of such nodules in the diagnosis of cancer, some US characteristics are thought to identify individuals at high risk of developing a cancer. The ultrasonographic pattern of a "restless liver", for example, is recognized as a "risk" model for the development of hepatocellular carcinoma [32].

The work group of the World Gastroenterology Congress in 1994 agreed upon an anatomical and clinical systematization of the nodules that considers both the presence of the nodules (at

the moment of their imaging detection) as well as the dynamics of their development. Regeneration nodules, low dysplastic nodules, high dysplastic nodules with outbreaks of hepatocellular carcinoma, incipient hepatocellular carcinoma (< 2 cm) and typical hepatocellular carcinoma can be identified in the cirrhotic liver [33]. This systematization also considers the existence of a vascular dynamics inside the nodules which is considered a key element of carcinogenesis [34, 35]. Inside the dysplastic nodules there is a progressive reduction – until disappearance – of the portal vessels and a proportional growth of the arterial vessels during the multiplication process of the neoplastic cells. The tumoral circulatory bed is made of arterial vessels with a disorganized, chaotic spatial distribution, arteriovenous shunts and the precapillary sphincter of the arterioles is missing. The vascular characteristics of the neoplastic nodules are considered to be determinant for their echogenicity in contrast with that of the surrounding liver parenchyma. In conclusion, high dysplastic nodules are usually hypoechoic, while incipient nodules of highly differentiated hepatocellular carcinoma are usually isoechoic [36].

The Liver Cancer Study Group of Japan defined the following types of nodules:

- a. a small nodule with ill defined margins, size under 20 mm, consisting of well differentiated cells and portal vessels (about 85% of the cases). It may contain areas of low differentiated cells with a different potential of multiplication, realizing a “nodule in nodule” pattern;
- b. a small nodule with ill defined margins, round shape and non-tumoral capsule. In most situations it is made of well differentiated cells (about 75%) and sometimes (about 20% of cases) it may present histological signs of portal invasion;
- c. a tumor nodule with extratumoral buds consisting in most cases of low differentiated cells;
- d. a multinodular pattern made of several nodules in contact with each other, realizing an irregular delineation. They consist of moderate or low differentiated cells;
- e. an infiltrative pattern characterized by a vague, irregular delineation. In most situations it is made of low differentiated cells and/or transitional hepatocytes and cholangiocytes, generating a mixed tumor between the two cellular types.

On the “grey scale” ultrasound exploration a 10 – 20 mm incipient HCC has the aspect of a heterogenous nodule with hypoechoic and hyperechoic areas inside, an appearance that is influenced by the fat content and the degree of cellular differentiation [37]. The Doppler exploration shows a continuous, portal vascular signal oriented towards the tumor [38]. The undulating character of the flow draws attention to a more significant arterial component, which often correlates with poorly differentiated tumors.

The advanced form of HCC may have the following characteristics: demarcation by a halo and lateral shadow (produced by the fibrous capsule of the tumor); inhomogeneous structure (generated by fibrotic and vascular bands which alternate with areas of intact and necrotic tumoral tissue); irregular, ill defined margins (suggesting an invasion of the surrounding liver parenchyma and of the portal vascular bed); posterior acoustic shadowing (generated by the softer consistency of the tumor compared with the normal liver parenchyma). The distinctive vascular aspect, obtained by using the color coded technique of the blood flow (CFM), is that of a basket pattern, characterized by the presence of arterial vessels that circumscribe the tumor

and feed it from outside [39]. The spectral exploration demonstrates accelerated flow velocities and altered impedance indexes, since the vascular resistance of the circulatory bed is lower in the absence of precapillary sphincters. On CEUS the aspect of the HCC is typical and is the consequence of its vascular features described earlier (Figure 9). It is characterized by accelerated uptake during the arterial phase, contrast wash-out during the portal venous phase and a hypoechoic appearance in the delayed phase. The wash-out speed is conditioned by the degree of cellular differentiation of the tumor, the lower the differentiation the faster the wash-out (figure 10a; figure 10b;).



Figure 9. Multicentric, large hepatocellular carcinoma. Two large lesions are visualized during the arterial phase. One of the lesions presents an important feeding artery.

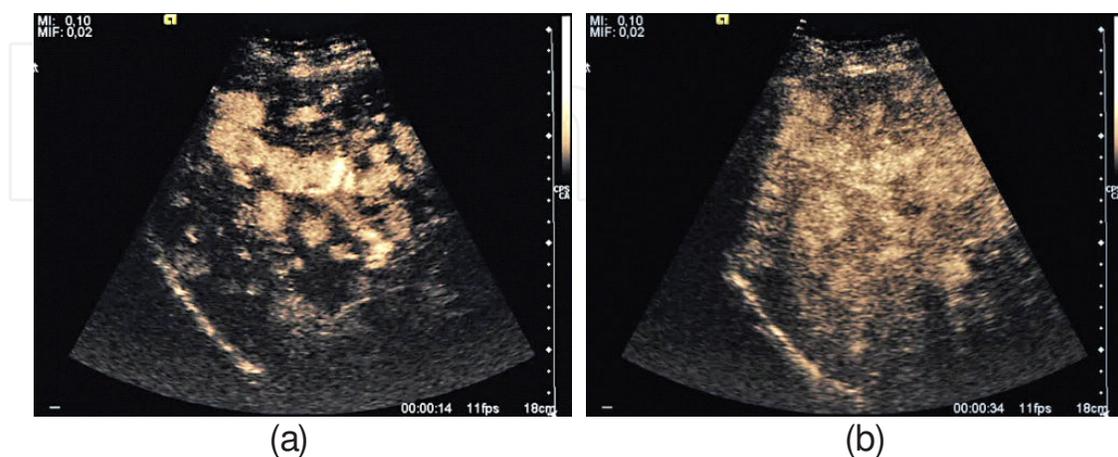


Figure 10. Multicentric, large hepatocellular carcinoma. a. CEUS examination (arterial phase) demonstrates the presence of highly enhancing, numerous nodules. b. The hypoechoic appearance of the lesions can be visualized (34th second), suggesting their malignant nature and the low differentiating grade of the tumor.

The exploration allows the discrimination of a malignant invasion of the portal vein from thrombosis based on the behavior during the arterial phase: in case of tumor invasion, the signal is simultaneous with the one inside the hepatic artery, while during the portal phase it loses its signal and becomes obvious (Figure 11) [40].

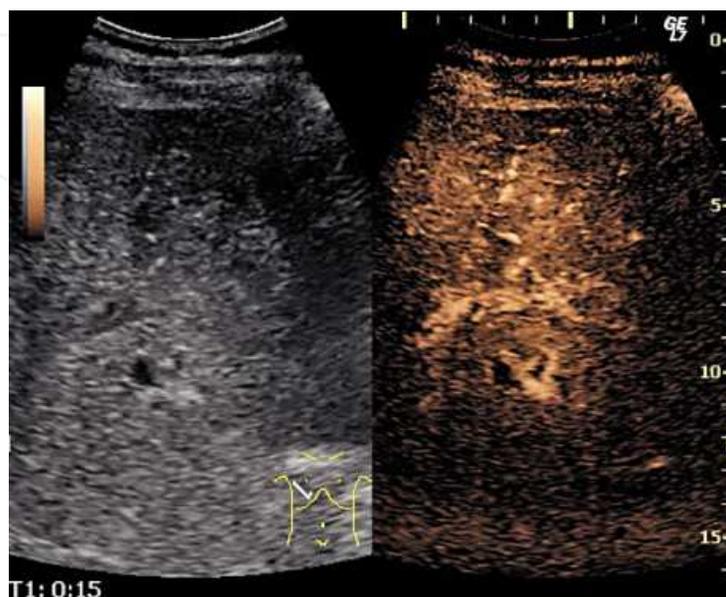


Figure 11. Tumor invasion of the portal vein. Dual harmonics examination with i.v. contrast, that shows a filling with echoes of the right portal vein on the left and contrast media uptake within the portal lumen during the arterial phase (15th second).

6. CEUS assessment of cholangiocarcinoma (CCC)

Cholangiocarcinoma is a rare primary malignancy (3–7% of all malignant liver tumors) which usually develops in a histologically normal liver [41]. There are conditions, however, that present higher risks for developing CCC. These included primary sclerosing cholangitis, choledochal cysts, Caroli disease or intrahepatic biliary lithiasis [30]. The origin of CCC is in the small biliary ducts and tumour development is frequently associated with an early appearance of jaundice. The tumor may present a nodular pattern (most cases), an intraductal circumscribed pattern, or a periductal infiltrative type. The nodular type is well-circumscribed, with a fibrotic structure and moderate or poorly differentiated cells. It is frequently associated with metastases in the surrounding parenchyma (developed by contiguity as well as through portal veins) as well as lymph nodes in the hepatic hilum [42]. On the grey scale ultrasound examination, an early CCC is undetectable. In this situation the diagnosis is based on indirect signs, the main one being the dilatation of the intrahepatic bile ducts. Biochemical serum tests, especially those elevated and reflecting abnormal biliary function such as CA 19–9 and CEA, may be useful for diagnosis [28]. When the tumor is advanced stage the diagnosis is based on the presence of a solid mass, well delineated, but without a capsule, adjacent to the bile ducts

(often in the hilum). The bile ducts may be well dilated. The vascular signal detected upon CFM exploration is weaker than that detected in HCC. The vessels are arterial and have a chaotic spatial distribution. The tumor may be unique or multicentric and it may have a subcapsular localization. An indirect element that sustains the diagnosis is the development of the tumor in a normal liver. The CEUS exam may present an inhomogeneous uptake during the arterial phase, the behavior being uncharacteristic. During the portal venous phase the appearance is hypoechoic and it persists this way in the delayed phase [43].

7. CEUS in the assessment of liver metastases

The liver represents the second site for malignant secondary tumors in oncology. There are usually multiple lesions and rarely single. The common sites of origin are mainly represented by the digestive tract, lungs, breast and pancreatic head [44]. They have fewer vessels than primary malignancies of the liver. During their evolution they may develop hemorrhage, areas of ischemia and necrosis, as well as areas of fibrosis and calcifications. These features contribute to imaging appearances that are extremely diverse and uncharacteristic for their origin when assessed by US. In essence, in a patient with a known neoplastic condition, or in the situation of weight loss in the context of a malignancy, the presence of multiple nodules, measuring more than 10 mm, involving the whole liver, is suggestive for the presence of liver metastases. The CFM exploration does not bring significant information. The CEUS exam is based on demonstrating a hypoechoic aspect during the portal venous phase and delayed phase, which is highly suspicious for malignancy. During the arterial phase metastases may present either a hypo or a hypervascular pattern (figure 12; figure 13a, figure 13b).



Figure 12. Liver metastasis (breast neoplasm). CEUS exploration shows a vascular mass 23 seconds after contrast media injection.

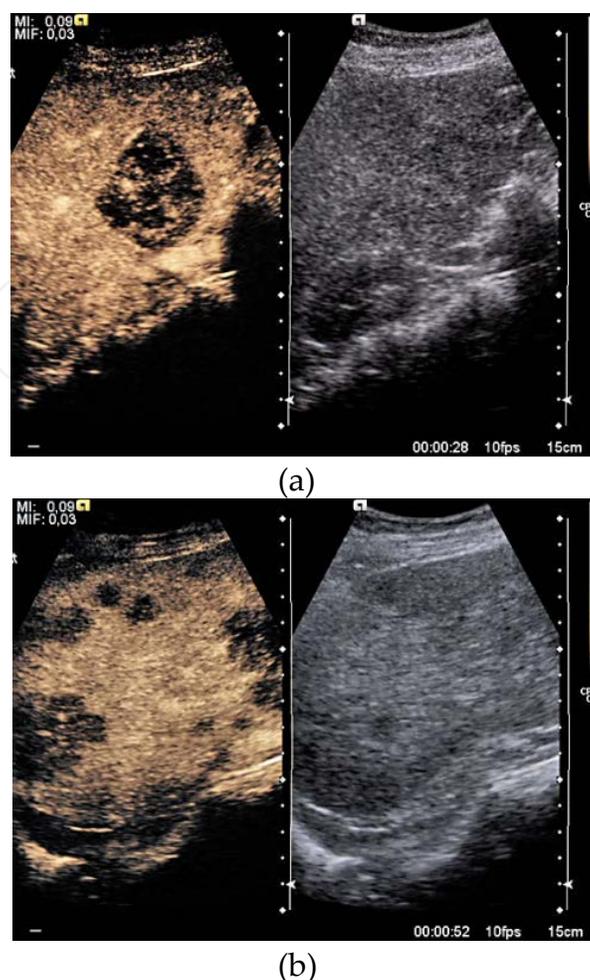


Figure 13. a. Liver metastasis (colon neoplasm). CEUS examination reveals a hypoechoic mass 28 seconds after injection. b. Liver metastasis (colon neoplasm). CEUS examination shows multiple hypoechoic masses at 52 seconds after injection (portal venous phase). Typical appearance.

Hypervascular mets may be associated to carcinoid tumors, melanomas, sarcomas and thyroid or renal tumors. The role of CEUS in the evaluation of liver metastases is focused on at least two applications: a. detection of metastases smaller than 10 mm and counting them. The method significantly increases the sensitivity of ultrasound and gets it closer to that of i.v. contrast-enhanced CT [45]. b. assessment of therapy efficiency (chemotherapy and chemoembolization). In this regard the method can document the disappearance of the circulatory bed and suggests an efficient treatment.

8. CEUS in the assessment of other types of liver masses

This category includes non neoplastic liver masses that are quite frequently encountered. Ultrasonography often detects such lesions. The US exam must clarify whether there are evolving features or not and if there are special risks for the patient (table 5).

Liver mass	Arterial phase	Portal venous phase	Delayed phase	"Grey scale" US
Liver abscess	Peripheral uptake and liver parenchyma uptake Hypoechoic/Transsonic aspect	Echoic appearance of the liver parenchyma Hypoechoic aspect	Echoic appearance of the liver parenchyma Hypoechoic aspect	Hypoechoic or transsonic lesion Semifluid content Intratatumoral gas
Focal liver steatosis	Simultaneous uptake with the liver parenchyma	Isoechoic aspect	Isoechoic aspect	Hypoechoic area "Shining" appearance of the liver parenchyma
Regeneration nodule	Weak uptake Hypoechoic aspect	Isoechoic aspect	Isoechoic aspect	Solid nodule, d = 10 – 20 mm Inhomogeneous liver

Table 5. CEUS and "grey scale" US criteria for the diagnosis of non neoplastic masses of the liver.

Liver abscess. Liver abscesses may arise in various circumstances: primary abscesses (in immune deficiency cases) or secondary abscesses in postoperative patients, or associated with sepsis, abdominal abscesses, post traumatic conditions, acute angiocholitis or acute pancreatitis etc. The "grey scale" ultrasound aspect is variable in relation to the number and size of the lesions (figure 14). In general it presents itself as a unique, large mass, well circumscribed, but irregular, with a semi-fluid content and with echoic tissue elements and air inside. It can also present as multiple, smaller size lesions, a situation when their texture may mimic liver metastases. The CFM exploration reveals that the vessels are displaced by the mass and that the abscess has no vascular signal inside.



Figure 14. Liver abscess. Hypoechoic mass, with semifluid features, visualized in the right lobe of the liver. There is also right pleural fluid collection.

The CEUS exam shows a progressive uptake in the periphery of the abscess during the arterial phase. This enhancement happens simultaneously with that of the surrounding liver parenchyma which is congested (figure 15a). This aspect is persistent during the portal venous phase as well. During the delayed phase the fluid areas inside the abscess/abscesses are highlighted due to the enhancement of the liver sinusoids and the reticuloendothelial system of the liver (figure 15b) [46].

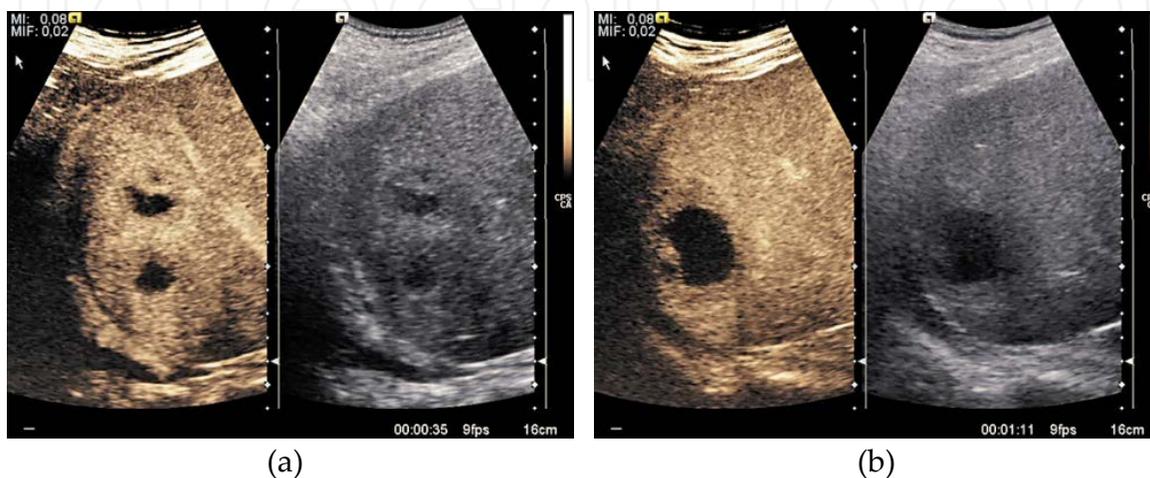


Figure 15. Liver abscess (same case as figure 14). a. Dual image showing on the left an increased echogenicity of the liver parenchyma during arterial phase. The aspect suggests liver congestion and represents an indirect sign that supports the diagnosis of a liver abscess. b. Portal-venous phase. A decrease of liver echogenicity is noted while the infected collection is emphasized.

Focal liver steatosis. Focal steatosis develops in the context of metabolic conditions, chemotherapy or excessive alcohol consumption. The liver is intensely echogenic and presents hypoechoic, rather well circumscribed “islands” or “areas” inside. The Doppler exploration does not reveal additional vessels. The CEUS exam reveals an isoechoic aspect of the liver during the arterial phase. During the portal venous phase and the delayed phase the region of interest is identical with the surrounding liver and thus excludes the presence of a mass.

Regeneration nodules. Regeneration nodules appear as nodular, well circumscribed, hypoechoic masses, usually measuring less than 20 mm. They are often numerous and have a uniform, ubiquitous distribution, involving all the segments of the liver. They may come together as a cluster and form large pseudotumors. This aspect is characteristic for viral liver cirrhosis. These nodules do not present Doppler signals. CEUS exploration is indicated when a certain nodule is larger than the majority of the liver nodules. An increase in size, demonstrated by measurements performed at 6 – 8 weeks, represents an additional indication. Characteristically, the vascular signal is weak or absent during the arterial phase, while in the portal and delayed phases the lesion is persistently isoechoic (figure 16). CEUS is useful in order to exclude an active lesion at the moment of the examination, but it has no prognosis value and that is why the patient must be reexamined at short time intervals [8]. A correlation with the clinical findings and with the values of AFP is mandatory.

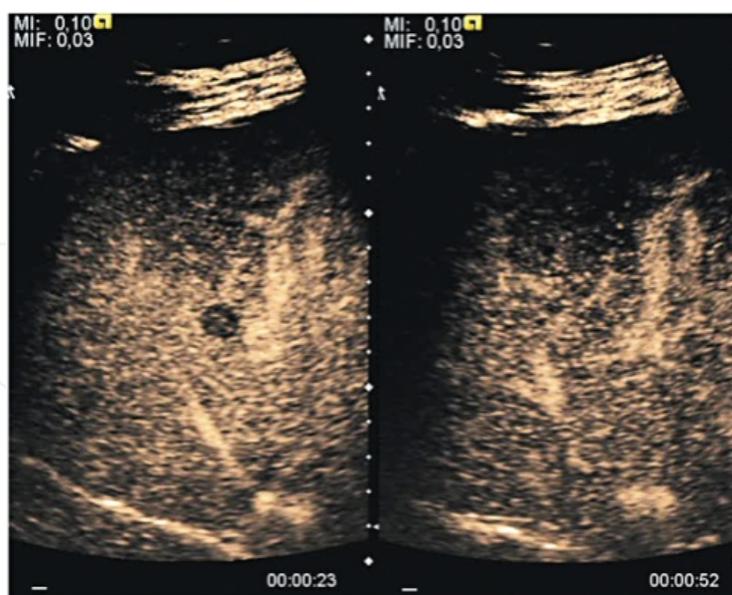


Figure 16. Regeneration nodule. CEUS reveals low irrigation during the arterial phase (23th second) and lack of contrast wash-out during the portal venous phase (52th second).

9. Final remarks

Contrast-enhanced ultrasonography is a noninvasive imaging procedure that allows the gathering of information regarding the dynamics of circulation and the features of the circulatory bed in the region of interest [47]. The investigation is based on the identification of the circulatory phases within the liver – arterial, portal and delayed phase – which are overlapping those identified at contrast CT or MRI. The dynamic analysis of the image in relation with these phases allows the detection and characterization of tumors with similar accuracy to that of CT and MRI [48, 49]. The risks and side effects of the method are insignificant and that is why CEUS must be considered a safe procedure for the patient. This investigation should be commonly used for the characterization of liver nodules developed on liver cirrhosis and suspicion of HCC, regardless of the stage, as well as for the detection of small size metastases. Low price of the procedure, large accessibility and lack of the radiation, are among the most prominent advantages of CEUS that should be taken into consideration. Real time information and clinical character are among them also. These characteristics make CEUS a good and reliable procedure for the detection of liver tumors and follow-up under treatment. However, CEUS is restricted and with low performance in every case where 2D is limited e.g. attenuation, intense liver steatosis, deep region of interest. The procedure is limited to a single lesion and should be repeated to each other one when multiple tumors are detected. Finally, CEUS is operator and equipment dependent. These are reasons because CEUS cannot replace CT or MRI. In every case when the investigation is considered inconclusive, which happens in about 10 % of the cases, other sectional explorations must be considered, like the CT or MRI, and/or liver biopsy [48].

Abbreviations

US = ultrasonography; CEUS = contrast-enhanced ultrasonography; CA = contrast agent; HCC = hepatocellular carcinoma; CCC = cholangiocarcinoma; AP = acoustic power;

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