„CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY

BUCUREŞTI

DOCTORAL SCHOOL

FACULTY OF MEDICINE

CONTRAST ENHANCED ULTRASOUND IN THE DIAGNOSTIC ALGORITHM OF SPLENIC LESIONS

PHD THESIS SUMMARY

PhD supervisor:

PROF. UNIV. DR. ILIESCU ELENA LAURA

PhD student:

OZARCHEVICI IOANIȚESCU ELENA IULIA SIMONA

2022

CONTENT

INTRODUCTION

Conventional ultrasound (US) is by far the first imaging method in abdominal cavity and spleen evaluation, due to its advantages: accessibility, non-invasivity, portability and the lack of irradiation, which makes it repeatable whenever necessary during the course of the disease. US can provide very important information for the therapeutic management of the patient in a short time. However, the informations are limited both by the patient's weight status and the experience of the examiner. In tumour assessment, the method is useful mainly for the initial detection of the lesion and solid-liquid differentiation, without the necessary performance for a complete tumour characterization. Tumor characterization and benign-malignant differential diagnosis require the description of the tumor vascular bed at the microcirculation level.

Capillary perfusion data were initially obtained by chromium-51 or Technetium-99-labeled erythrocyte scintigraphy, and later by contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) [1,2].

All these methods have been indispensable until now in the diagnosis of abdominal pathology. Their main disadvantages are the use of ionizing radiation and the toxicity of contrast agents, in addition to reduced accessibility (longer waiting time), lack of portability (the patient must be transported to the examination room) and the duration of the procedure (MRI).

Hence the need to introduce ultrasound contrast agents in clinical practice, which provide information similar to those for CT or MRI, but without their side effects.

Over the last decade, a number of innovations have significantly improved the performance of the ultrasound method, expanding the range of applications. Of these, the introduction of vascular contrast agents (UCAs) probably had the greatest impact on clinical

practice. They increase the intensity of the acoustic signal from the blood vessels, including microcirculation and improve the quality of the vascular image. Thus UCAs highlight the intralesional vascular pattern compared to the normal parenchyma. The second major advantage of contrast-enhanced US (CEUS) is the ability to real time assess the contrast dynamics into the vascular bed of the lesion.

Therefore, CEUS is a real-time ultrasonographic technique that allows the characterization of intralesional microcirculation as contrast-enhanced CT and MRI scans, but maintaining the advantages of conventional US. The examination can be performed immediately after US examination and can be repeated: several contrast administrations can be performed during the same examination, but also CEUS can be performed several times during the course of the disease. The method is easy to be integrated in the diagnostic circuit of a clinic. The equipment does not require specially designed spaces, and can also be used at the patient's bedside.

In the last 10 years, this method has seen a great development, due to the introduction of second-generation UCAs in clinical practice. The accuracy of CEUS in the diagnosis of liver lesions proves to be 85-90%, much higher than B-mode and color Doppler diagnostic accuracy (50-60%) [3,4]. Starting with the characterization of liver nodules, a wide range of applications on other organs have proven or not their usefulness, and others are currently under study.

With the accumulation of data from many studies, a systematization was necessary, which resulted in a series of Guidelines and good clinical practice recommendations for the use of CEUS in liver and non-hepatic applications published by EFSUMB and WFUMB [5–8]. Currently, our country is the only EFSUMB member country that published national Guidelines and good clinical practice recommendations for the clinical practice of CEUS, to which Fundeni Internal Medicine Clinic and the author of this thesis also contributed [9].

With these premises, we chose as general objective of the doctoral thesis the study of the efficiency and diagnostic accuracy of CEUS in the current clinical practice for different types of splenic pathologies. The ultimate goal is to introduce the method in the algorithm for diagnosis and therapeutical screening of splenic lesions, to increase diagnostic performance, while improving patient comfort by avoiding unnecessary invasive procedures.

CURRENT STATE OF KNOWLEDGE

The first chapter of this thesis aims to review the CEUS technique and its applications. US is the first imaging technique used to explore the abdominal cavity. It allows both morphological (B-mode, gray scale examination) and functional (vascular and elastographic modules) visualization of biological structures. Two different physical principles underlie vascular exploration: the Doppler principle and the harmonic principle, each of them with specific features and limitations, which makes them both complementary and partially overlapping.

Unlike Doppler examination, CEUS is based on the emission of harmonic echoes from intravascularly located contrast agent microbubbles in motion, that vibrate under the action of the ultrasound beam. The exploration can be performed on all types of vessels, including small vessels and microcirculation, being independent on the erythrocyte movement, depending only on the intravascular presence of the UCAs. While Doppler mode is currently present on most ultrasound machines, being an important part of the "conventional" US examination, CEUS requires contrast agents, high-performance ultrasound machines with special software that allow examination with a low mechanical index (MI) (0.09 - 0.11) and dedicated, wideband transducers. CEUS highlights certain anatomical structures compared to others, considered as reference, allowing, for example, much faster visualization of tumors. But CEUS itself is primarily a dynamic exploration that takes place over a defined period of time (usually 4-5 minutes) which highlights the transit mode of the circulatory bed of a region of interest (ROI) by the UCAs, thus allowing the differentiation of anatomical structures compared to contrast-enhanced CT or MRI examinations. In contrast to these imaging techniques, by recording the examination, CEUS allows a continuous assessment of the enhancement pattern of ROI. A correct CEUS diagnosis is made based on the analysis of the entire examination and not on a single image interpretation.

The method has undeniable advantages. Being a real time examination technique, it allows a correct assessment of the arterial phase, essential for the benign-malignant differential diagnosis. It can be performed following the conventional US examination, if necessary right at the patient's bedside, and is able to immediately provide important information, which shortens the time required for diagnosis. It does not use ionizing radiation and the UCAs are free of kidney or liver toxicity and rarely have side effects. So, the

procedure can be repeated whenever needed, including in patients with kidney or liver insufficiency or metal implants. But CEUS has the same limitations as US, depending on the best possible ultrasound window, providing little information in case of steatosis, intestinal interposition or in the presence of scars. Like US, CEUS is an operator-dependent technique, requiring an intense training in a tertiary ultrasound center, adequate equipment, contrast agent available and sustained medical practice. Another disadvantage is the lack of overview, being limited to the ROI. This disadvantage can be overcome by performing several successive examinations, each focused on a different ROI. CEUS depends on the microbubble lifespan. Their excessive accumulation or destruction can create artifacts, mimicking tumors. UCAs are blood-pool contrast agents and they cannot allow the glomerular filtration or tissue diffusion asessment. The use of a very small amount of UCAs is a great advantage, but it is limited by the short viability of the microbubbles after preparation (a few hours), which forces us to correlate several (2-3) examinations in order to efficiently use the entire AC vial and reduce the cost of the procedure. The method allows for quantitative analyses, but it is difficult to standardize them. An important limitation is to have inappropriate expectations from CEUS. It is very important to keep in mind that there is no independent, isolated CEUS diagnosis and that ultrasonographic diagnosis is the sum of several US techniques, which include CEUS. CEUS information is correlated with informations obtained by other imaging techniques and is integrated into the clinical and biological context, thus reaching the final diagnosis.

UCAs are inert gas-filled microbubbles in a stabilizing shell. Their diameters range between 2 μm and 8 μm, smaller than that of red blood cells, which allows them to cross the pulmonary and systemic capillaries intact and recirculate. The gas has the role to increase the acoustic impedance by up to 20–30 dB, thus improving the quality of the ultrasound image. Gas is responsible for the solubility and for the acoustic properties of microbubbles. The nature of the gas differentiates between first-generation and second-generation UCAs. First generation UCAs contain air or nitrogen, which have good water solubility and high diffusion capacity. That results in low microbubble stability and a short circulation time. Second generation UACs contain perfluorobutane, octafluoropropane, perfluorocarbons, sulfur hexafluoride or a combination thereof [10,11]. These gases have high molecular weight, low solubility in the blood, low diffusion capacity and a good biocompatibility. Due to these properties, they are not absorbed into the systemic circulation and they are rapidly removed by exhalation. Therefore, microbubbles have a lifetime of several minutes, long enough for diagnostic examinations [12,13].

The shell protects the microbubbles, increases their lifetime, gives them mechanical rigidity and reduces the compressibility of the gas. The type of shell and its thickness control the elasticity of the bubble and thus the acoustic properties of microbubbles. Thus, there are two types of microbubbles, with rigid and elastic shell. Microbubbles with elastic shell are the most used, because they have superior oscillating properties when exposed to ultrasound beam with low MI. The most commonly used shell materials are phospholipids and surfactants. Phospholipid UCAs include Definity®/Luminity® (perflutren gas) and SonoVue®/Lumason® (sulfur hexafluoride gas). Surfactant-stabilized products are Imagent®, Levovist®, Echogen® [12]. It is very important to note that intact microbubbles are exclusively intravascular contrast agents, which do not cross the endothelium and do not diffuse into the interstitial space, which significantly differentiates them from contrast agents for CT and MRI. The only UCA approved for use in the European Union (so also in Romania) is SonoVue® [14,15]. Studies have shown that UCAs (of which the most widely used in clinical practice is SonoVue®) have a very good safety profile, with a low incidence of side effects [16–20]. According to the American College of Radiology (ACR) Guidelines, the severity of adverse reactions to SonoVue® has been classified as [20,21] : a. mild, usually solved without specific treatment: mild nausea or vomiting, flushing, pruritus, mild urticaria, headache; b. moderate, requiring medical attention with specific treatment: marked urticaria, severe vomiting, bronchospasm, facial edema, laryngeal edema, vasovagal reaction, mild hypotension; c. severe, life-threatening reactions, usually representing a progression of the moderate symptoms: anaphylactic shock, severe laryngeal edema, respiratory arrest, cardiac arrest, pulmonary edema, confusion, convulsions, coma, death. Most often, the adverse events were mild and spontaneously resolved in a short time, without sequelae. The most common adverse reactions reported were headache (2.1%), nausea (0.9%), chest pain (0.8%) and chest discomfort (0.5%). All the other side effects had a less than 0.5% frequency [22]. Most cases of allergic reactions and hypotension occurred within several minutes after UCA injection. The severe or even fatal side effects rate is extremely low, with a reported incidence of major anaphylactoid reactions of 0.0086% - 0.04%. [16,19,23–25], significantly lower compared to the rate of anaphylactoid reactions found in iodine-based contrast agents (0.035% 0.095%) [26] or gadolinium-based contrast agents (6.3%) [27]. Remarkably, no deaths were reported in a study of more than 23,000 patients published in 2006 [28].

With the introduction of the second generation UCAs in clinical practice and the spread of CEUS worldwide, a systematization of the terminology and examination technique in order to increase the diagnostic performance of the method was necessary. Good practice

recommendations have been published in all EFSUMB and WFUMB CEUS Guidelines, as well as by the ACR CEUS LI-RADS experts group [5,8,29–32]. All these recommendations were gathered in 2018 in a review entitled „How to perform CEUS", which standardizes the CEUS examination technique based on published evidence and on the personal experience of EFSUMB, WFUMB and the ACR CEUS LI-RADS working group experts [18].

During CEUS examination, the wash-in and wash-out phases are real time assessed for several minutes. These vascular phases are conventionaly set [29,31].

Techniques with low mechanical index (IM <0.2) are currently used. These techniques minimize the amount of destroyed microbubbles, extending the continuous examination period for more than 5 minutes [31]. The image is created by summing the non-linear signals from the microbubbles and canceling the linear signals produced by the tissues [33]. These techniques require broadband transducers in order to collect a wide range of harmonic frequencies [34].

Most ultrasound machines show a dual image, which simultaneously displays the CEUS image and the B-mode image. The CEUS image must be almost black, receiving only a few acoustic signals from the intensely reflectogenic structures. It should be noted that, on most ultrasound machines, the quality of the B-mode image is inferior than the B-mode image obtained with the same settings in non-contrast mode [18]. A single image display, with the CEUS image overlapping the B-mode image can also be used [31].

In order to obtain a quality CEUS image, we must consider two factors: machine settings and UCAs signal strength. The first factor is influenced by the tissue suppression capacity (depending on the MI value) and the sensitivity of the transducers to receive the harmonic signal in broadband. The second factor is directly proportional to the contrast dose and the degree of perfusion of the tissue, as well as to the attenuation and destruction of microbubbles.

As mentioned earlier, the first applications of CEUS were in cardiology, followed by focal liver pathology. From there, the technique has continuously diversified, its range of applications reaching almost all fields in which conventional ultrasonography is used.

The second chapter of this thesis is a broad presentation of the anatomical, structural and functional features of the spleen, as well as the splenic pathologies in which CEUS has proven useful. The spleen, the largest organ of the lymphatic system, is often involved in vascular, neoplastic, or traumatic conditions, while its own pathology is very rare. Often, organ damage occurs in complex pathological contexts, with systemic alterations. The follow up of the spleen can have an important significance in monitoring these mainly

chronic diseases. Many lesions can be asymptomatic or have mild symptoms, although their evolution might be severe. Their analysis includes functional-biochemical tests, imaging techniques, histological examination when necessary, all correlated with the clinical status of the patient.

The spleen is a parenchymal organ, intraperitoneally located in the left hypochondrium, under the diaphragm, in direct anatomical relationship with the ribs 9-11, the stomach, the splenic flexure of the colon, the tail of the pancreas and the left kidney. It has an oblique position, with the upper pole facing back and the lower pole facing forward. The vascular hilum is located on the medial face of the spleen.

The spleen has an unique vascularization supply through the splenic artery. This is the longest and most tortuous branch of the celiac trunk. Usually, the artery has a trajectory along the postero-superior versant of the pancreas and enters the hilum through the spleno-renal ligament. It presents multiple anatomical variants related to the origin, trajectory, terminal ramifications and relations with the pancreas and splenic vein [35]. In parallel with the ramification of the splenic artery into primary and secondary branches, the splenic parenchyma is divided into lobes and segments. Most segmental arterial branches do not show anastomoses, the splenic circulation being a terminal circulation. Intersegmental anastomoses were observed in studies only in 14% of cases [35,36,45–47,37–44].

The venous drainage of the spleen is made through the splenic vein, resulting from the confluence - outside the hilum - of 2-3 venous trunks. The splenic vein has a traject parallel to the artery, and, unlike it, follows a straight path, posterior to the body and caudal segments of the pancreas. Near the pancreatic neck it joins the superior mesenteric vein, forming the portal vein. Unlike the artery, the vein has no abnormalities, so far only one case of congenital malformation has been published, with the splenic vein located anterior to the pancreas [48]. Its tributary veins are the short gastric veins, the left gastroepiploic vein, the inferior mesenteric vein and the pancreatic veins.

Lymphatic vessels have their traject parallel to the arteries and their branches. At the hilum level, they anastomose with lymphatic vessels that drain the gastric fornix and the pancreatic tail, later heading towards the pancreaticolienal lymph nodes, located along the upper edge of the pancreas and further towards the celiac lymph nodes. The innervation of the spleen comes from the splenic plexus (lienal), composed by fibers originating in the celiac plexus, the left celiac ganglion and the right vagus nerve. The nerves reach the splenic trabeculae, where they will innervate the vessels and smooth muscle fibers. Splenic nerves have a role in splenocontraction.

The spleen has an outside capsule composed of dense fibrous tissue, elastic fibers and smooth muscles. It consists in 4 major components: the supporting tissue, the white pulp, the red pulp and the vascular system. The spleen is organized like a "tree" of branched arterial vessels [49]. There are two types of circulation inside the spleen: a slow circulation ("open" circulation) that functions as a filter for erythrocytes, in which the terminal arterioles open into the reticular network of the red pulp, the blood then being collected in the splenic sinusoids, and a rapid circulation ("closed" circulation), in which the arterioles are directly connected to the splenic venous sinuses, avoiding the reticular network. Up to 90% of the total splenic blood flow goes through the adjacent venous sinuses, bypassing the reticular network of the red pulp [50]. These specific features of microvascularization are visible in contrast imaging examinations, including CEUS exploration.

In routine clinical practice, most sonographers measure only the maximum longitudinal diameter, because a study published by Lamb in 2002 showed a good correlation between the length of the spleen measured in right lateral decubitus and the volume, measured by computed tomography [51]. The same study concluded that measuring splenic length in routine clinical practice is a very good indicator of the spleen size [51]. In addition to the spleen length, it was suggested that the largest area should be calculated. Another study, which describes portal hypertension in patients with cirrhosis, defined a normal spleen as having an area < 45 cm^2, a moderately enlarged spleen with an area between 45-65 cm^2 and a marked splenomegaly with an area > 65 cm^2 [52].

Ultrasonographic examination of the spleen is performed after a 6-8 hours fasting, with the patient on right or lateral decubitus, in a left intercostal coronal approach at the level of the ninth intercostal space. A 2-5 MHz broadband convex transducer with specific CEUS-optimized settings is usually used to examine the spleen [18]. It is very important to examine the entire spleen in order to detect small lesions. In this case, for the detection of diffuse focal microlesions, as in hematological pathologies or suspected fungal microabscesses, linear transducers with higher transmission frequencies (usually 5-12 MHz) are recommended. They allow the visualization of superficial planes at a higher spatial resolution. If the use of linear probes in the CEUS examination is subsequently chosen, the UCAs dosage should be increased, usually doubled, due to the faster destruction rate of the microbubbles under a high-frequency ultrasound beam.

Normally, a compromise must be reached between the dose of UCA and the specific settings of the ultrasound machine, especially MI and the transmit frequency of the ultrasound beam. This creates a balance between signal strength, penetrability and

microbubble stability [18]. A higher MI results in a stronger acoustic signal and better penetration, but also increases microbubbles destruction. The correct contrast agent dosage must provide the proper contrast enhancement intensity in the early phase (avoiding the over saturation of structures and posterior shadowing) and the contrast enhancement duration (enough contrast agent concentration in the late phase of examination) [18].

On US, a region of interest in the spleen can be localized or diffuse. The ROI (being also a CEUS indication) might be: a. any clearly or ill defined splenic structural abnormality that raises the suspicion of a possible tumor (CEUS is recommended for better vizualization and characterization of the lesion); b. an area with circulatory abnormalities highlighted by color Doppler (suspected vascular malformation, vascular tumor or acquired arterio-venous fistula); c. unclear structural changes in lymphoproliferative or myeloproliferative systemic diseases (with suspected cell infiltrate or parenchymal infarction); d. hypo/hyperechoic structural changes with the base oriented towards the splenic capsule (to identify a specific circulatory pattern and confirm the infarction diagnosis); e. structural abnormalities appeared after strong abdominal trauma (with the suspicion of parenchyma rupture); e. posttraumatic subcapsular fluid accumulation (suggesting a subcapsular hematoma); f. persistent pain syndrome located in the left hypochondrium, associated with significant splenomegaly (a parenchymal infarction will be considered); g. septic condition associated with splenomegaly and radiating pain in the left shoulder (with suspicion of splenic or left subphrenic abscess).

The UCAs are rapidly seen in the splenic arteries, up to 10-12 seconds after i.v. injection. Normal arterial enhancement is inhomogeneous due to the different blood flow rates in the white pulp and red pulp, creating the so-called "zebra pattern", typical for the vascular dynamics of the spleen, which is also found in contrast-enhanced CT and MRI. The spleen becomes homogeneous 50-60 seconds after injection, and this homogeneous contrast enhancement persists for up to 5-7 minutes. The arterial phase lasts 10-35 seconds after injection and is followed by the venous phase, lasting up to 2 minutes. After 2 minutes, the UCAs disappear from the splenic veins, but remain sequestered in the splenic sinusoids more than in the other organs. The veins appear transonic and can mimic lacerations [53,54]. It is important that this error factor to be known by the practitioner, and in unclear cases a new contrast injection should be performed to reanalyze the suspect area [54]. As in other organs, the arterial phase will be centered on the ROI. Its examination will be continuous during the arterial phase, then intermittent, to avoid the rapid destruction of microbubbles. Arterial and late phases are the most important for diagnosis [6]. The arterial phase is important for the

characterization of the focal lesion, and the parenchymal phase allows its clear delineation and permits the assessment of the wash-out.

Next, the main splenic pathologies are presented, highlighting the role of CEUS in the diagnosis or follow-up of those pathologies. Among the pathologies presented in this chapter are: diffuse splenic pathology, accessory spleen or splenosis, splenic trauma, splenic infarction, bacterial or fungal abscesses, splenic cysts, lymphangiomas, peliosis, solid splenic nodules, splenic granulomas, hemangiomas and hamartomas, benign, malignant or borderline tumors (lymphomas, splenic metastases, angiosarcoma, epithelioid vascular tumors, littoral cell angioma), splenic vein thrombosis, splenic artery aneurysm and arterio-venous fistula.

PERSONAL CONTRIBUTIONS

General objectives and working hypothesis

The aim of our study is to prove the usefulness of CEUS in current clinical practice by evaluating the different contrast enhancement patterns of splenic lesions and the diagnostic accuracy of CEUS in the positive and differential diagnosis of different types of splenic lesions compared to reference standards (RS).

The specific objectives are:

- The presentation of the different lesion types that can be found in a routine ultrasound examination and are suitable for CEUS analysis;
- The description of the CEUS appearance of splenic vascular lesions, in which this examination may establish a positive diagnosis;
- The description of the CEUS appearance of the ectopic splenic tissue, in which this examination may also set a positive diagnosis;
- Description of the CEUS appearance of uncertain splenic lesions and analysis of UCAs wash in and wash out time intervals as well as the time interval between wash in start and wash out start moments, in correlation with other imaging or histopathological assessments, highlighting the prognostic value of CEUS for the final diagnosis.

Materials and methods

We performed an observational, retrospective, cohort study, that included 189 patients who underwent CEUS to assess splenic lesions in 5 university clinics in Romania (Bucharest, Cluj-Napoca, Timișoara and Craiova) between January 2016- December 2021.

Inclusion criteria used included:

- the detection of at least one splenic lesion or inhomogeneity on conventional abdominal US followed by CEUS examination with a dedicated protocol for the detected splenic lesions;
- or CEUS performed to detect splenic lesions or vascular thrombosis.

Patients with splenic lesions without CEUS or incomplete CEUS data, or patients without final diagnosis were excluded.

Data was collected from patients' electronic files as well as by evaluating US recordings. Demographic data was collected, recording the age and sex of the patients. Also, anamnesis data was collected regarding the comorbidities of the patients, the presence of suggestive symptoms for a pathology with splenic involvement, the incidental identification of a splenic lesion.

Data on conventional US was collected according to a protocol agreed by all examiners. The aim was:

- initial evaluation of the abdominal cavity, to identify US elements related to the patient's current pathology (lymphadenopathy, ascites, tumor masses, etc.);

- evaluation of the spleen size, noting the bipolar diameter and using the standard classification for splenomegaly;

- general appearance of the spleen (homogeneous /inhomogeneous);

- the presence of pathological areas or nodules or vascular lesions (arterial or venous thrombosis);

- evaluation of the splenic lesions, noting the location, number, dimensions, US appearance, presence or absence of the Doppler signal, permeability of the splenic artery and vein.

Finally, the analysis of the US images allowed a diagnostic hypothesis for the type of lesion: vascular, uncertain, possible benign lesion, possible malignant lesion. The images were evaluated in interdisciplinary sessions, analyzing the US appearance (determining a diagnostic hypothesis), as well as the paraclinical data and the comorbidities of the patients.

On CEUS, the enhancement pattern of the lesions in the arterial, venous and late phases, recording the hypo, iso, hyper or non-enhacement of the lesions were registered. The location of the lesions, their size, contour and ecostructure were also noted to compare the CEUS appearance with data obtained by conventional US. In selected patients, the time interval between wash in start and wash out start moments was calculated in order to correlate with the type of the lesion. The exams in which these time intervals could be set after records examination were selected.

We noted if CEUS was conclusive for diagnosis, if CEUS lead to a correct diagnosis (malignant versus benign splenic lesions) and if CEUS influenced the therapeutic attitude.

The evolution of patients over time, as well as a control CEUS examination and the final diagnosis of patients were also noted.

After collecting all the data, a worksheet was prepared for each patient. The data was then entered into a Microsoft Excel 2019™ file and statistically analyzed using SPSS version 26 (SPSS Inc., Chicago, 91 IL, USA).

Results

189 patients aged 14 to 89 years, with a mean age of 57.62 +/- 37 years were included into the study. We noticed a slight predominance of females (96 patients, representing 50.79%) over males (93 patients, 49.21%).

Analyzing the medical history, most of the patients had chronic comorbidities - liver cirrhosis, chronic HBV or HCV infection, chronic kidney disease (44.44% of patients). 33 patients (17.46%) had no significant medical history. A total of 50 patients, representing 26.45%, had a history of neoplasia, either hematological (9.52%) (including Hodgkin's and non-Hodgkin's lymphomas, chronic lymphocytic leukemias), or solid neoplasms (16.93%), including kidney, colon, or liver tumors.

All patients underwent abdominal US before the CEUS examination, and a total of 202 splenic lesions were found. Most patients had normal spleen size, with a bipolar diameter less than 13 cm (44.97%). In the case of 3 splenectomized patients, intra-abdominal lesions were detected, raising the suspicion of ectopic spleen tissue. Also, most of the evaluated patients had a homogeneous appearance of the spleen, with the presence of an uncertain lesion (73.01%). In 25.39% of patients the inhomogeneity of the spleen associated with the presence of a lesion was noticed, and in 1.58% of the patients US could not exactly establish the presence of a lesion.

US-detected splenic lesions were characterized as nodular lesions (80.95% of patients), inhomogeneous area (14.28% of patients) or vascular lesions (4.76% of patients). Of the 202 lesions detected by conventional US, in 15 lesions, the location was not exactly specified. 64 lesions had a diffuse distribution (31.68%), 19.30% of them were located at the lower pole, 19.30% were centrally located, 13.86% were located at the upper pole. Most patients (115 patients, representing 56.93%) had a single lesion, while 14.85% had less than 5 lesions and 21.78% had more than 5 splenic lesions. In 13 patients (6.43%) no lesions were found. In terms of size, most of the lesions detected by conventional US had a maximum diameter of more than 2 cm (45.04%). Only 19.80% had a maximum diameter less than 1 cm, and in 8.91% of cases on conventional US the exact diameter was not measured or there were no lesions.

Most detected lesions had a well defined contour (68.31%), while 14.35% of the lesions had an ill defined contour. Most of the detected lesions had inhomogeneous US appearance (49.50%), and 36.63% had a homogeneous US appearance. The US appearance was not characterized in 13.86% of patients. Comparing the US appearance of the lesions with the US appearance of the normal spleen, we found that 49.50% of the lesions were hypoechoic and 21.28% were hyperechoic. 11.88% isoechoic lesions, 5.44% transonic lesions and 1.98% heterogeneous lesions were also described. At the Doppler examination, 24.25% lesions with absent Doppler signal and 21.28% lesions with Doppler signal were detected. In 54.45% of the lesions, the Doppler evaluation did not bring significant information, due to technical reasons (too small or hypoperfused lesions).

After conventional US, most splenic lesions US characteristics were uncertain (59.40%). Only 5 lesions had clearly benign features (2.47%) and another 29 lesions had features suggesting benignity (14.35%). 6.93% of the lesions were suggestive for neoplasia, while in 16.83% the US appearance suggested vascular lesions.

On CEUS evaluation, in the arterial phase, most of the lesions showed non-enhancement (26.73%) or isoenhancement (23.76%). During the venous phase, 26.73% of the lesions that showed non-enhancement were noticed. 16.33% of the lesions showed no wash out. Compared to the arterial phase, one lesion remained hypoenhanced, with central non-enhancement. 18 lesions (8.91%) remained isoenhanced and 14 lesions (6.93%) remained hypoenhanced, with a similar appearance as in the arterial phase. During late phase examination, the hypoenhanced lesion with central non-enhancement maintained its appearance. 27.22% of the lesions showed non-enhancement and 12.87% showed no wash out. Multiple diffuse lesions were discovered on CEUS in 30.19% of cases. Compared to the

conventional US, no splenic lesions were seen in 20 patients (10,58% of patients) in which CEUS had the role to detect the presence of nodules. In 60,84% of patients, a single lesion was identified, in 15.34% of cases less than 5 lesions were identified, and in 20,1% of cases more than 5 splenic lesions were detected. CEUS specified the exact contour of the lesions in most cases (86.13%). The CEUS assessment found 66.83% homogeneous splenic lesions and 17.32% inhomogeneous lesions.

When the wash out was analysed, several patterns were encountered. Thus, 17.82% of the lesions had arterial enhancement, without venous or late wash out, 8.91% had moderate wash out, 12.87% had discrete late wash out, 18.81% had early and rapid wash out, 31.69% did not have a different wash in or wash out than the normal splenic parenchyma. In 9.90% of cases (10.58% of patients without visible lesions) no pattern was described.

The diagnosis of vascular lesion (vascular permeability/hematoma/splenic infarction) was set in 21.28% of the detected splenic lesions. 38.11% of the lesions had definite benign pattern and only 1.48% had definite malignant pattern. 29.23% of the encountered lesions remained uncertain after CEUS examination, and in 9.90% of cases (10.58% of patients) the CEUS examination excluded the presence of splenic lesions.

Out of the 182 lesions confirmed by CEUS, in 145 (79.67%) of cases the lesions were incidentally discovered. 82 lesions were found in symptomatic patients (for underlying pathology). 37 lesions were not incidentally found, and the majority of patients with these lesions (89.18%) had symptoms of underlying pathology.

We found that CEUS set the positive diagnosis in 70.79% of cases. In 3.96% of cases the diagnosis was based on laboratory data and in 0.9% of cases it was necessary to follow up the patient to establish the final diagnosis. The final diagnosis was set on CT imaging in 5.94% of cases, on MRI in 5.44% of cases and on histopathological examination in 12.87% of cases (Figure 1).

In 72.77% of cases, CEUS was considered conclusive for diagnosis. Moreover, in 81.18% of cases it was considered that CEUS influenced the therapeutic attitude. CEUS set the positive diagnosis in 100% of cases with vascular pathology. CEUS failed to establish the diagnosis in 30 cases (90.90% of malignant lesions in the study group). Also, in 29 cases the benign etiology of the splenic lesions could not be determined on CEUS (27.35% of the benign lesions) (Figure 2).

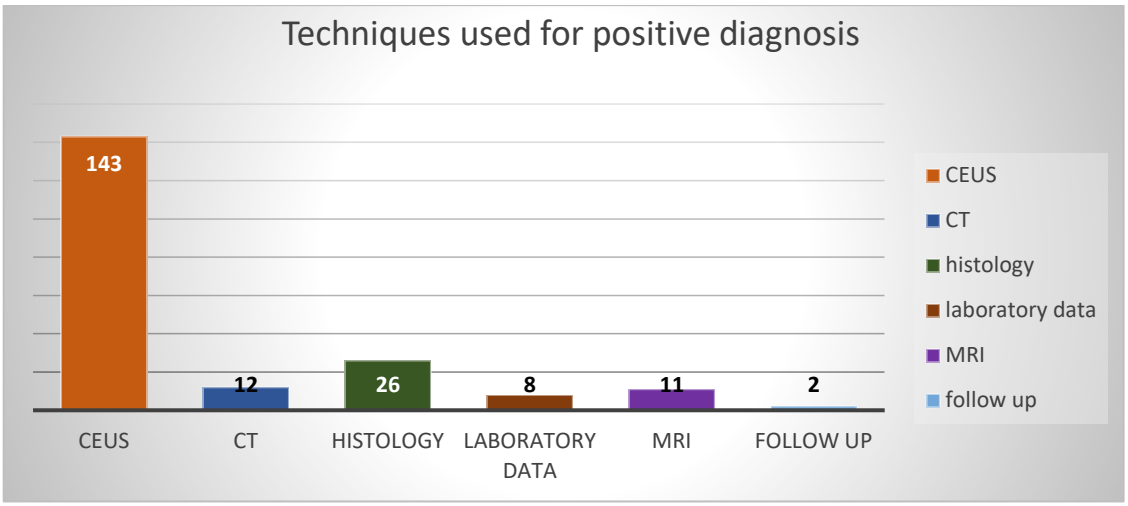

Figure 1. Techniques used for positive diagnosis of splenic lesions in the study group.

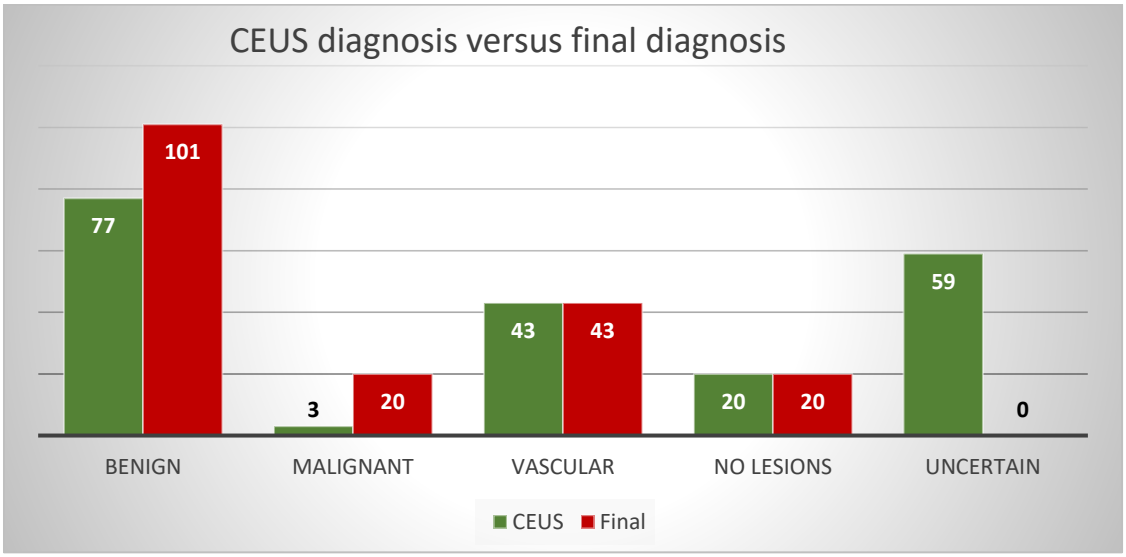

Figure 2. Comparison between CEUS diagnosis and final diagnosis.

Of the total evaluated lesions, the final diagnosis, confirmed by clinical, imaging and/or histological data, was benign in 106 lesions (52.47%) found in 100 patients. The mean age of the patients was 56.3 ± 13.7 years. In our study, there is a slight predominance of female sex, without statistical significance. Regarding the patient's medical history, there is a predominance of patients with chronic infections or inflammatory conditions, such as hepatitis virus infections, liver cirrhosis (46%), autoimmune or acute infectious diseases (12%). It should be noted that 17% of this group of patients had a history of solid or hematological neoplasms. 22% of patients did not have a significant medical history. Of the 106 benign splenic lesions diagnosed, 83.96% were incidentally found on US. Also, 55.66% of lesions were detected in asymptomatic patients. Most asymptomatic patients had as

medical history infections or chronic inflammatory conditions (hepatitis, liver cirrhosis) or did not have a known medical history.

Benign lesions were found mainly in patients with normal-sized spleen (52.83%) or mild splenomegaly (23.58%). In three cases (2.83%), the lesions were detected in splenectomized patients – splenosis.

In most cases with benign lesions (84.90%) the ecostructure of the spleen was homogeneous, with a visible node. The benign splenic lesions appearance on conventional US was mainly nodular (98.11%), only two cases showing an inhomogeneous area appearance.

The benign splenic lesions were located among the central and polar areas of the spleen in almost equal proportions (19.21%, 25.27% and 22.64%, respectively). There were mainly single focal splenic lesions (61.98%), multiple lesions above or below 5 lesions being found in approximately equal proportions (15.09% and 13.20%, respectively). Most benign splenic lesions were larger than 2 cm (43.39%) or between 1 and 2 cm (34.90%). Only 21.69% of lesions had less than 1 cm in size. Most benign splenic lesions had a well defined contour on conventional US (87.73%). In 4 lesions (3.77% of cases), the contour was not specified on conventional US. There was a slightly higher proportion of inhomogeneous focal lesions compared to homogeneous ones (53.77% versus 50%). We noted the predominance of hypoechoic lesions (45.28%) among benign splenic lesions.

After conventional US, definite benign features were found in only 4.71% of lesions. 25.47% of lesions had benign features but the exact type of lesion could not be indicated and most lesions (60.37%) remain uncertain. The CEUS indication in patients with benign lesions was mainly the incidental discovery of a splenic lesion during conventional abdominal US (66.98%). Two other common indications were the splenic lesions characterization in patients with history of neoplasia (13.20%) or in patients with acute infectious disease (7.54%).

CEUS data showed no statistically significant differences from conventional US in terms of the location and the number of the splenic lesions. The results were also similar, both in the case of single nodules and in the case of multiple nodules. CEUS examination showed a more accurate assessment of the contour of the splenic focal lesions. CEUS reveals homogeneous ecostructure in most cases of benign lesions (70.75%).

During the arterial phase, most benign lesions showed isoenhancement to the surrounding parenchyma (32.07%) or non-enhancement (15.09%). On the venous phase, the most lesions did not show washing out (26.41%) or had discrete wash out (20.75%). The

isoenhancement was maintained in 14 of the 34 patients with isoenhancement in the arterial phase. Also, the non-enhancement was maintained. It should be noted that 8 lesions (7.55%) showed early, rapid and pronounced wash out and 5 lesions (4.7%) showed progressive wash out. In the late phase, most benign lesions showed discrete wash out (24.52%). The 16 non-enhancing lesions maintained their appearance even in the late phase (15.09%). Only 11 lesions (10.37% of benign lesions) showed pronounced wash out in the late phase, and in 9 lesions (8.5%) progressive, continuous washing out was noted. Among the benign lesions, the assessment of the transit time interval of the lesion by the UCA was successfully calculated in 59 lesions (55.66%). Most benign splenic lesions had a prolonged transit time interval for more than 120 seconds (42.38% of lesions), while 35.6% of the lesions had a transit time interval between 0 and 40 seconds. Of note, for 20.33% of lesions, this time interval ranged from 0-20 seconds.

CEUS established the positive diagnosis of benign lesions in 74.52% of cases. 12.26% of cases required additional imaging (CT or MRI) while in 7.54% of cases the diagnosis was based on histological data obtained by splenectomy or splenic biopsy. In the remaining cases, the final diagnosis was based on laboratory data or imaging follow-up.

CEUS was considered conclusive in 74.52% of cases. Moreover, in 84.90% of cases, CEUS was considered to provide additional relevant information and to influence the therapeutic attitude in benign splenic lesions.

In our study, 43 (20.87%) vascular lesions were identified. The mean age of the patients was 56.25 years, ranging between 14 and 80 years. The predominance of male patients is noticeable (58.13%). Regarding the underlying pathology, there is a preponderance of patients with history of chronic liver pathology (44.18%), which is consistent with the profile of the clinics that participated in this study. In most patients, splenic vascular lesions were incidentally detected (72.09%). Most patients (74.41%) had symptoms suggestive for the underlying disease.

Vascular lesions were found in patients with normal-sized spleen (37.29%) or mild splenomegaly (25.58%). Most patients had a homogeneous (53.48%) or inhomogeneous US appearance of the spleen (41.86%), with evidence of a splenic lesion; only in two cases (4.65%) conventional US could not distinguish a splenic lesion. In only 20.9% of cases the lesions were located in the vascular lumen, most lesions (32.55%) being diffuse intraparenchymal located. Otherwise, a relatively equal proportion was observed in the lower or central polar distribution of the lesions. The lesions were mainly of the aria type (51.16%). Most patients had a single splenic lesion (62.79%) or less than 5 lesions (23.25%). In 67.44%

of patients, lesions were larger than 2 cm on US, while only 6.97% were less than 1 cm. Conventional US revealed an ill defined contour of vascular lesions in 46.51% of cases and a regular, well-defined contour in 44.18% of cases. Most of the lesions had an inhomogeneous US appearance - 72.09%. Most vascular lesions were hypoechoic to the adjacent splenic parenchyma (53.48%), and in 18.60% lesions were isoechoic compared to the surrounding parenchyma. No Doppler signal could be identified for most lesions. After conventional US, 72.09% of the lesions were highly suggestive for vascular lesions, while the diagnosis of 27.90% of the lesions remained uncertain.

There were no significant differences in location and number of detected lesions after CEUS compared to conventional US. CEUS allowed for more accurate delineation and more accurate measurement of vascular lesion sizes. At the same time, at CEUS, all the detected lesions had a well defined contour, compared to only 44.18% of cases on conventional US.

On CEUS, in the arterial phase, most lesions were non-enhancing (88.37%), higly suggestive for ischemic or necrotic lesions. This aspect was also maintained in the venous and late phases for all non-enhancing lesions (88.37%). There were also three particular cases of hypoenhancement throughout the entire examination, suggestive aspect for splenic contusions.

In 100% of cases, CEUS was able to set correct diagnosis regarding vascular lesions (splenic infarction, splenic vein thrombosis, post-embolization splenic necrosis). Diagnoses were confirmed by alternative methods in 81.39% of cases. In the remaining cases it was considered that no confirmation was necessary.

Malignant splenic lesions diagnosis has been certified in 32 cases. The mean age of the patients was 60.8 ± 13.7 years. There was a predominance of female sex in this subgroup of patients (62.5%). As underlying pathology, most patients had chronic liver disease (34.37%), parenchymal neoplasms (25%) or no relevant medical history (25%). Half of the malignant lesions were incidentally detected, but 96.87% of patients had symptoms.

Conventional US showed increased spleen size in patients with malignant lesions. 31.25% of them had severe splenomegaly. In most cases, suspicious lesions were clearly visible on conventional US, in association with a homogeneous (46.87%) or inhomogeneous (46.87%) spleen. Most of the lesions were nodules - 90.62%. At the same time, most of the lesions were diffuse distributed (62.5%). Most patients had more than 5 splenic lesions (56.25%), while 31.25% had single lesions. Most malignant lesions (65.62%) had a well-defined, regular contour. The preponderance of inhomogeneous lesions (56.25%) is noticeable; most lesions were hypoechoic. After conventional US, in 84.37% of the lesions

the ultrasound appearance was uncertain, while 15.62% had some data suggesting malignancy.

We note that most lesions were incidentally found (13 lesions - 40.62%). 15 lesions (46.87%) were identified in patients with history of malignancy, of which 6 (18.75%) were known with malignant hematological pathology, and 9 (28.12%) had solid malignancy. We also noted that the CEUS indications referred mainly to the characterization of splenic nodules (29 lesions - 90.62%), only in 2 cases (6.25%) it was about the characterization of a splenic area.

CEUS examination did not reveal any differences in location, number and size of lesions detected by conventional US, with 2 exceptions. CEUS succeeded in more accurately characterizing a polynodular splenic lesion, seen as unique nodule in US assessment. Also, in one case, the dimensions by conventional US were overestimated compared to CEUS. CEUS allowed a much clearer delineation of the contour of the splenic lesions. In the arterial phase of CEUS, various patterns of contrast enhancement could be seen, but in the venous phase of CEUS, rapid and early wash out (40.62%) and progressive wash out (28.12%) predominated. Also, in the late phase, rapid and pronounced (56.25%) or progressive (18.75%) wash out persist. 6 hypoenhancing lesions in arterial phase maintained their appearance in the venous and late phase. In 17 lesions, the transit time interval was calculated, the average value being 14,16 seconds. Most of the lesions (14 lesions - 82.35%) had a transit time interval of 0-20 seconds, 2 lesions (11.76%) had a transit time interval of 20-40 seconds, and for a single lesion, a very rare case of primary splenic leiomyosarcoma [55], the transit interval was between 40-120 seconds.

After CEUS, the diagnosis remained uncertain in 84.37% of cases. In only 4 cases (12.5%) it was a definite or possible malignant lesion. In most cases, the positive diagnosis was established based on histopathological examination (56.25%). CEUS was considered conclusive in 34.37% of cases and influenced the therapeutic attitude in 75% of cases.

Using the SPSS student test application, we looked for significant differences in demographic data, data obtained by conventional US and data obtained by CEUS, in patients with benign and malignant splenic lesions.

There were no significant differences between the two groups of patients in terms of the age of the patients. The incidental discovery of splenic lesions was rather suggestive for their benign etiology, while the presence of symptoms raised the suspicion for neoplastic etiology; both of these associations had statistical significance ($p < 0.05$).

On conventional US, we looked for a correlation between the size of the spleen, the number, the contour, the ecostructure and the Doppler signal from the lesions with the malignant or benign character of the splenic lesions. It is noted that normal or slightly increased size of the spleen correlates with the presence of benign lesions, while significant splenomegaly occurs in association with malignant lesions. At the same time, the presence of unique nodule suggests benign lesion, while the presence of more than 5 nodules suggests malignant lesions.

On CEUS, there were positive associations between the number of nodules, the venous and late wash out of the assessed lesions, the washing out pattern and CEUS indication with the benign and malignant final diagnosis (Table 1).

Table 1. Correlations between the CEUS patterns and the malignant or benign character of the lesions.

	Benign	**Malignant**	**p-value**
CEUS			
Lesion size			0.33
< 1 cm	22 (21%)	9 (29%)	
1-2 cm	38 (36.2%)	7 (22.6%)	
> 2 cm	45 (42.9%)	15 (48.4%)	
Lesion contour			0.22
Well defined	100 (100%)	28 (96.6%)	
Ill defined	0 (0%)	1 (3.4%)	
Number of lesions			**<0.001**
Unique	75 (71.4%)	9 (29%)	
Multiple < 5	14 (13.3%)	4 (12.9%)	
Multiple > 5	16 (15.2%)	18 (58.1%)	
Lesion echostructure			0.11
Homogeneous	74 (70.5%)	17 (54.7%)	
Inhomogeneous	23 (21.9%)	10 (32.3%)	
NA	8 (7.6%)	4 (3.8%)	
Arterial enhancement			0.10
homogeneous complete hyperenhancement	5 (4.8%)	1 (3.2%)	
inhomogeneous complete hyperenhancement	4 (3.8%)	0 (0%)	

peripheral ring hyperenhancement, central hypoenhancement	7 (6.7%)	2 (6.5%)	
isoenhancement	33 (31.4%)	6 (19.4%)	
homogeneous hypoenhancement	9 (8.6%)	6 (19.4%)	
inhomogeneous hypoenhancement	4 (3.8%)	3 (9.7%)	
non-enhancement	16 (15.2%)	0 (0%)	
peripheral non-enhancement, central hyperenhancement	1 (1%)	0 (0%)	
wall hyperenhancement, central non-enhancement	4 (3.8%)	1 (3.2%)	
complete hyperenhancement from the periphery	7 (6.7%)	2 (6.5%)	
hypoenhancement	3 (2.9%)	3 (9.7%)	
discrete hypoenhancement	5 (4.8%)	1 (3.2%)	
hyperenhancement	1 (1%)	2 (6.5%)	
inhomogeneous hyperenhancement, necrotic areas inside	2 (1.9%)	4 (12.9%)	
hypoenhancement, central non-enhancement	1 (1%)	0 (0%)	
spoke-wheel hyperenhancement, from the center and periphery	2 (1.9%)	0 (0%)	
wall hypoenhancement, central non-enhancement	1 (1%)	0 (0%)	
Venous wash out			**<0.001**
no wash out	27 (25.7%)	0 (0%)	
early, rapid, pronounced wash out	8 (7.6%)	13 (40.6%)	
discrete wash out	22 (21%)	2 (6.3%)	
non-enhancement	16 (15.2%)	0 (0%)	
progresive wash out	5 (4.8%)	9 (28.1%)	
discrete, rapid wash out	1 (1%)	0 (0%)	
progressive wash out of the wall, non-enhancement inside	1 (1%)	0 (0%)	
no wash out on the wall, non-enhancement inside	3 (2.9%)	1 (3.1%)	

hypoenhancement	7 (6.7%)	6 (18.8%)	
isoenhancement	14 (13.3%)	0 (0%)	
hypoenhancement, central non-enhancement	1 (1%)	0 (0%)	
Late phase wash out			**<0.001**
no wash out	21 (20%)	0 (0%)	
pronounced wash out	11 (10.5%)	18 (56.3%)	
discrete wash out	26 (24.8%)	0 (0%)	
non-enhancement	17 (16.2%)	0 (0%)	
progresive wash out	9 (8.6%)	6 (18.8%)	
progresive wash out on the wall, non-enhancement inside	1 (1%)	0 (0%)	
no wash out on the wall, non-enhancement inside	3 (2.9%)	1 (3.1%)	
hypoenhancement	7 (6.7%)	6 (18.8%)	
isoenhancement	9 (8.6%)	0 (0%)	
hypoenhancement, central non-enhancement	1 (1%)	0 (0%)	
CEUS indication			**<0.001**
1. inhomogeneous spleen - suspected splenic lesion	2 (1.9%)	2 (6.3%)	
2. incidentally discovered splenic nodule / thrombus in the splenic vessels	71 (67.6%)	13 (40.6%)	
3. characterization of a splenic lesion in a cancer patient	13 (12.4%)	9 (28.1%)	
4. characterization of a splenic lesion in a hematological patient	1 (1%)	6 (18.8%)	
5. characterization of a splenic lesion in a patient with infectious disease	8 (7.6%)	1 (3.1%)	
6. post-embolization or post-surgical follow up of the splenic artery	1 (1%)	0 (0%)	
7. ectopic spleen /splenosis suspicion	7 (6.7%)	0 (0%)	
8. checking large vessel permeability or intraparenchymal necrosis detection	0 (0%)	0 (0%)	

9. splenic metastasis detection in a cancer patient	0 (0%)	0 (0%)	
10. posttraumatic spleen assessment	1 (1%)	0 (0%)	
11. detection of splenic lesions in haematological/infectious patients	1 (1%)	1 (3.1%)	
Wash out pattern			**<0.001**
Non-enhancement	25 (24%)	1 (3.2%)	
Early, rapid wash out	11 (10.6%)	27 (87.1%)	
Late, discrete wash out	25 (24%)	0 (0%)	
Moderate wash out	15 (14.4%)	3 (9.7%)	
Enhancement, no wash out	28 (26.9%)	0 (0%)	
Transit time interval	32 (0- 225)	13 (9- 16)	0.07

As in conventional US, CEUS detection of a single splenic lesion is associated with benignity, while the detection of more than 5 lesions is associated with malignancy.

The wash out of the lesions in the venous phase may suggest malignancy. Thus, lesions with early, pronounced, or progressive wash out are more likely to be neoplastic, while non- or iso-enhancing lesions are more likely to be benign. Also, the late phase wash out on CEUS suggests the etiology of splenic lesions, similar to the venous phase. Thus, the pronounced wash out of the lesions is an important feature for malignancy. It is found that the arterial time of CEUS is not enough to set a diagnostic suspicion, while the wash out may indicate a neoplastic lesion.

We tried to correlate the transit time interval and benignity/malignancy but the statistical significance limit was not reached ($p = 0.07$).

Table 2 shows the sensitivity, specificity, positive and negative predictive value for the studied variables. Thus, we find that the normal sized spleen predicts a benign lesion with an accuracy of 56.9%, and severe splenomegaly has an accuracy of 76.6% in predicting malignancies. The large number of splenic nodules predicts their malignancy with an accuracy of 77% in conventional US and 78% in CEUS. The wash out pattern has the highest sensitivity in the characterization of malignant lesions, up to 100%. Incidental discovery has a high specificity for benign lesions. In contrast, the presence of symptoms is specific for malignant nodules (92.4% and 96.8%, respectively).

Table 2. Predictive values for malignant or benign lesions.

	Prediction	Se (95%IC)	Sp (95%IC)	PPV (95%IC)	NPV (95%IC)	Accuracy (95%IC)
Spleen size						
Normal sized spleen	**Benign**	**52.4%** (42.4-62.2%)	**71.9%** (53.2-6.2%)	**85.9%** (77.3-91.6%)	**31.5%** (25.5-38.2%)	**56.9%** (48.2-65.3%)
Severe Splenomegaly	**Malignant**	**31.2%** (16.1-50%)	**90.5%** (83.2-5.3%)	**50%** (31.4-68.6%)	**81.2%** (77.2-84.6%)	**76.6%** (68.7-83.4%)
Number of nodules > 5 (US)	**Malignant**	**54.8%** (36-72.7%)	**83.8%** (75.3-90.3%)	**50%** (36.8-63.2%)	**86.3%** (80.9-90.3%)	**77.2%** (69.2-83.9%)
Number of nodules > 5 (CEUS)	**Malignant**	**58%** (39.1-75.5%)	**84.8%** (76.4-91%)	**52.9%** (39.6-65.9%)	**87.2%** (81.8-91.3%)	**78.7%** (70.8-85-2%)
Venous pattern (CEUS)						
Non-enhancement	**Benign**	**15.2%** (9-23.6%)	**100%** (88.8-100%)	**100%** (98-100%)	**25.8%** (24.3-27.4%)	**34.5%** (26.6-43.2%)
Progressive wash out	**Malignant**	**29%** (14.2-48%)	**95.2%** (89.2-98.4%)	**64.3%** (39.4-83.3%)	**81.9%** (78.3-85.1%)	**80.1%** (72.4-86.5%)
Early, rapid, pronounced wash out	**Malignant**	**59%** (36.3-79.3%)	**92.4%** (85.5-96.6%)	**61.9%** (43.4-77.5%)	**91.5%** (86.7-94.7%)	**86.6%** (79.4-92%)
Late pattern						
Pronounced wash out	**Malignant**	**58%** (39-75.5%)	**89.5%** (82-94.6%)	**62%** (46.5-75.5%)	**87.8%** (82.6-91.7%)	**82.3%** (74.9-88.3%)

Wash out pattern **Enhancement, no wash out**	**Benign**	**26.7%** (18.5-36.2%)	**100%** (88.8-100%)	**100%** (98-100%)	**28.7%** (26.4-31.1%)	**43.4%** (34.9-52.2%)
Early, rapid wash out	**Malignant**	**87.1%** (70.2-96.4%)	**89.5%** (82-94-6%)	**71%** (58-81.3%)	**95.9%** (90.4-98.3%)	**89%** (82.5-93.7%)
Incidentaloma	**Benign**	**92.4%** (85.5-96-6%)	**48.4%** (30.2-66.9%)	**85.8%** (81.1-89.5%)	**65.2%** (46.7-80%)	**82.3%** (75-88.4%)
Simptomatology	**Malignant**	**96.8%** (83.3%-99.9%)	**51.4%** (41.5-61.3%)	**37%** (32.3-41.9%)	**98.2%** (88.6-99.7%	**61.8%** (53-69.9%)

* Se= sensitivity, Sp= specificity, PPV= positive predictive value, NPV= negative predictive value.

Discussions

Our multicenter study aimed to demonstrate the diagnostic accuracy of CEUS for the diagnosis of splenic lesions in current clinical practice at the level of tertiary medicine, in 5 university centers with internal medicine and gastroenterology profile. In our study, CEUS proved to be a very useful method for splenic lesions diagnosis, with an excellent safety profile of UCAs. No adverse reactions were reported. 189 patients with 202 splenic lesions were included in the study. The CEUS examination showed the absence of focal lesions in 20 cases (10.58% of patients), in which the examination was used for nodules detection.

Overall, CEUS set the positive diagnosis in 70.79% of cases. Except for 31 cases, splenic infarcts or nodules with definite benignity, in which the diagnosis was considered certain and no further confirmation was required, the rest of the lesions benefited from imaging, histological, biological confirmation or by imaging follow up. CEUS examination ruled out the presence of splenic lesions in 20 cases where conventional US was inconclusive, which led to a rapid diagnosis and no further more expensive assessments were necessary (CT, MRI).

When separately analyzing the categories of lesions, we notice that 43 (20.87%) were vascular lesions. This category included splenic infarctions or ischemias, hematomas,

lacerations or vascular thrombosis diagnosed per primam or during follow up after postembolization or post-surgery. Also included here were the control of splenic artery or vein permeability post interventional maneuvers or as part of the diagnostic algorithm in acute pancreatitis.

Most vascular parenchymal lesions (72%) were incidentally found in symptomatic patients for the underlying disease (74.41%). More than 60% occurred on normal spleens or mild splenomegaly. In more than 50% of cases they appeared on conventional US as areas and less in pseudonodular form, more unique than multiple, most inhomogeneous, larger than 2 cm. It is known that the B-mode US echostructure varies with the age of the infarcted area or the hematoma. In our study, most lesions were hypoechoic. On CEUS, infarcted areas, necrosis, lacerations, hematomas and benign thrombosis were non-enhancing during the entire exploration, while contusions appeared with varying degrees of hypoenhancement. Permeable vessels have been described by isoenhancing. If conventional US suspected a vascular lesion in 72.1% of cases (the rest remaining uncertain), CEUS set a correct diagnosis in 100% of parenchymal vascular lesions and determined with certainty the vascular permeability and type of thrombosis (benign or malignant). Moreover, it allowed a more precise delineation and size of infarcted areas, necrosis, hematomas and ischemic areas. It should be noted that some of the cases did not require confirmation of the diagnosis as those for which CEUS verified vascular permeability.

Another situation in which CEUS proved 100% diagnostic accuracy was the diagnosis of ectopic splenic tissue (accessory spleen or splenosis). In our study there were 7 cases (3.46%) in which CEUS made the differential diagnosis between ectopic spleen tissue or abdominal masses (tumors or lymphadenopathy). In 3 cases patients had a history of post-traumatic splenectomy, in the remaining 4 cases CEUS was performed for accessory splines with atypical location or for the differential diagnosis with lymphadenopathy in neoplastic patients. On CEUS, the characteristic feature for ectopic spleen tissue was the intranodular persistence of UCA for more than 5 minutes, as in the normal spleen parenchyma. As opposed, in lymphadenopathy or malignant neoplasms, the wash out occurs earlier than 2 minutes [5,56].

In our study, benign lesions predominated (106 lesions per 100 patients), 52.47%, compared to malignant lesions (32 lesions). Statistically, there was a slight age difference between the groups, the benign lesions being discovered in younger patients. We note that the malignant lesions appeared mainly after the age of 50 (87.5%). We also note the preponderance of women (62%) in the group of malignant patients, while benign lesions had

a quite symmetrical distribution between the sexes. In 17% of cases, benign nodules were diagnosed in patients with a history of malignancy or present malignancy, which confirms that splenic metastases are not the rule in neoplastic patients. 83.96% of benign lesions were incidentally found, and 55.66% of patients were asymptomatic at the diagnosis moment. In comparison, 50% of malignant lesions were incidentally found, but 96.87% of patients were symptomatic at the time of diagnosis.

On conventional US, 73.5% of benign lesions were found in patients with normal spleen or mild splenomegaly, while 71.87% of patients with malignant lesions had splenomegaly, of which 31% had severe splenomegaly. Benign nodules were mainly unique (61.98%), while single lesion was found in only 31.25% of malignant nodules. Both categories of nodules were mainly larger than 1 cm and had a well defined contour, inhomogeneity being more common in malignant lesions. The ecostructure of the benign lesions was mainly hypoechoic in 45.28% of cases and hyperechoic in 31.13% of cases. The percentage of hyperechoic and isoechoic lesions was 40.57%. In contrast, the malignant lesions were mostly hypoechoic in a much higher percentage (75%).

After conventional US, the diagnosis remained uncertain in 60.37% of benign lesions and in most malignant lesions.

Analyzing the arterial phase of CEUS, we notice the different patterns of enhancement, both in benign and malignant lesions. We note for benign lesions no less than 18 different patterns of enhancement, of which the most common were isoenhancement in 34 cases and non-enhancement in 16 cases. Lesions with different patterns of hyperenhancement sum 32 lesions, and the hypoenhancing ones, 23 lesions. The same different pattern of contrast enhancement was found in malignant lesions, of which non-enhancing lesions are missing. In the venous phase, we notice the lack of wash out, isoenhancement or discreet wash out in 60.32% of benign lesions. Rapid wash out, progressive wash out or hypoenhancement patterns were found in 18.9% of cases. In the late phase, the rapid, progressive wash out pattern or hypoenhancement were found in a quarter of benign lesions (25.5%). In contrast, all malignant lesions showed rapid and progressive wash out in most cases in the venous and late phase, in two cases of metastasis the wash out was less intense in the venous phase, but became pronounced in the late phase, and a lesion, apparently a cystic mass, did not show wash out of the wall neither in the venous phase, nor late phase. The analysis of the transit time interval of the lesions could be performed only for 59 benign and 17 malignant lesions. The benign lesions for which the transit time was between 0 and 20 seconds were: in 6 cases granulomatous lesions, 2 multiple lesions

appeared in the context of acute infection (lesions quickly disappeared under treatment), a case of hemosiderotic nodules, a case of lymphangioma lesions, one hamartoma, one atypical hemangioma. The benign lesions for which the transit time was between 20 and 40 seconds were: 4 cases of granulomatous lesions, 2 hemangiomas, one case of hemosiderotic nodules, 2 cases of benign nodules, but with uncertain etiology, one of them being discovered in a patient with a possible traumatic history. All lesions in which the transit time interval exceeded 40 seconds were hemangioma/hamartoma lesions. Regarding malignant lesions, the lesion with a transit time interval between 40 and 120 seconds was a case of primary splenic leiomyosarcoma [55], and 2 cases with metastatic lesions had transit time interval between 20 and 40 seconds. The rest of the lesions, with transit time interval ranging from 0-20 seconds, were mainly hematological lesions.

Following the statistical analysis, we found several significant correlations. An incidentally identified splenic lesion is more likely to be benign, especially if it is detected in an asymptomatic patient. The correlation between incidental detection and benign nature of the lesion has a sensitivity (Se) of 92.4% (85.5-96-6%), but a specificity (Sp) of only 48.4% (30.2-66.9%), with a positive predictive value (VPP) of 85.8% (81.1- 89.5%), a negative predictive value (NPV) of 65.2% (46.7-80%) and a diagnostic accuracy of 82.3% (75-88.4%). Symptoms were found in most patients with malignant lesions, with an increased Se of 96.8% (83.3% -99.9%), with a Sp of 51.4% (41.5-61.3%) and a VPP of only 37% (32.3-41.9%), but a VPN of 98.2% (88.6-99.7%) and a diagnostic accuracy of 61.8% (53-69.9%) for the diagnosis of malignancy.

On conventional US, we identified statistically significant correlations between benign or malignant type of lesions with spleen size and number of nodules. Thus, a normal size or slightly enlarged spleen is correlated with benignity, while splenomegaly, especially severe, is correlated with malignancy.

There are some patterns highly suggestive for benignity or malignancy. The non-enhancement of a lesion during the entire CEUS examination is highly suggestive for benignity, with a 100% (88.8-100%) Sp and a 100% (98-100%) VPP, but has only a 15.2% (9-23.6%) Se, a 25.8% (24.3-27.4%) VPN and a diagnostic accuracy of 34.5% (26.6-43.2%), because this pattern is also typical for vascular lesions, such as splenic infarctions, hematomas or vascular thrombosis. The contrast enhancement pattern, without wash out in the venous or late phase, also has a 100% Sp (88.8-100%) and a 100% VPP (98-100%) for benignity. The early, intense and rapid wash out pattern has an 87.1% (70.2-96.4%) Se, a

89.5% (82-94-6%) Sp, a 71% (58-81.3%) VPP, a 95.9% (90.4-98.3%) VPN and a diagnostic accuracy of 89% (82.5-93.7%) for the malignancy diagnosis.

A recent study evaluated the US pattern in malignant splenic lesions and the defining aspects found were: hypoechoic lesions, with ill defined contour, without cystic or necrotic areas, associating splenomegaly. On CEUS, arterial hypoenhancement, early wash out, and the presence of intralesional vascularization were found. In the multivariate logistic regression analysis, the association between the hypoechoic pattern, hypoenhancement and the presence of the vascularization proved to be independent factors for malignancy, with a specificity of 100%, but with a sensitivity of only 55% [57]. The data is conclusive with the results of the present study, but the calculation of the transit time interval led to an increase in the sensitivity for detection of malignant lesions.

Another study that evaluated 50 patients with splenic lesions also noted the importance of CEUS in differentiating malignant from benign lesions, with a sensitivity of 100%, specificity of 98%, positive predictive value of 83% and negative predictive value of 100%, using, as in the study presented in this paper, the correlation of the CEUS data with the histological diagnosis and the follow up of patients [58].

Regarding the correlation between incidental discovery, lack of symptoms and benignity, this was first reported by WFUMB experts in an article published in 2021, but no statistical data was published [59].

Counting the existing data in the literature, consistent with those obtained from our research, and associating the mentioned US features, we propose the following algorithm for evaluating splenic lesions, for differential benign-malignant diagnosis, an algorithm that aims to increase diagnostic efficiency and prioritize the access of the patients to other invasive investigations (Figure 3).

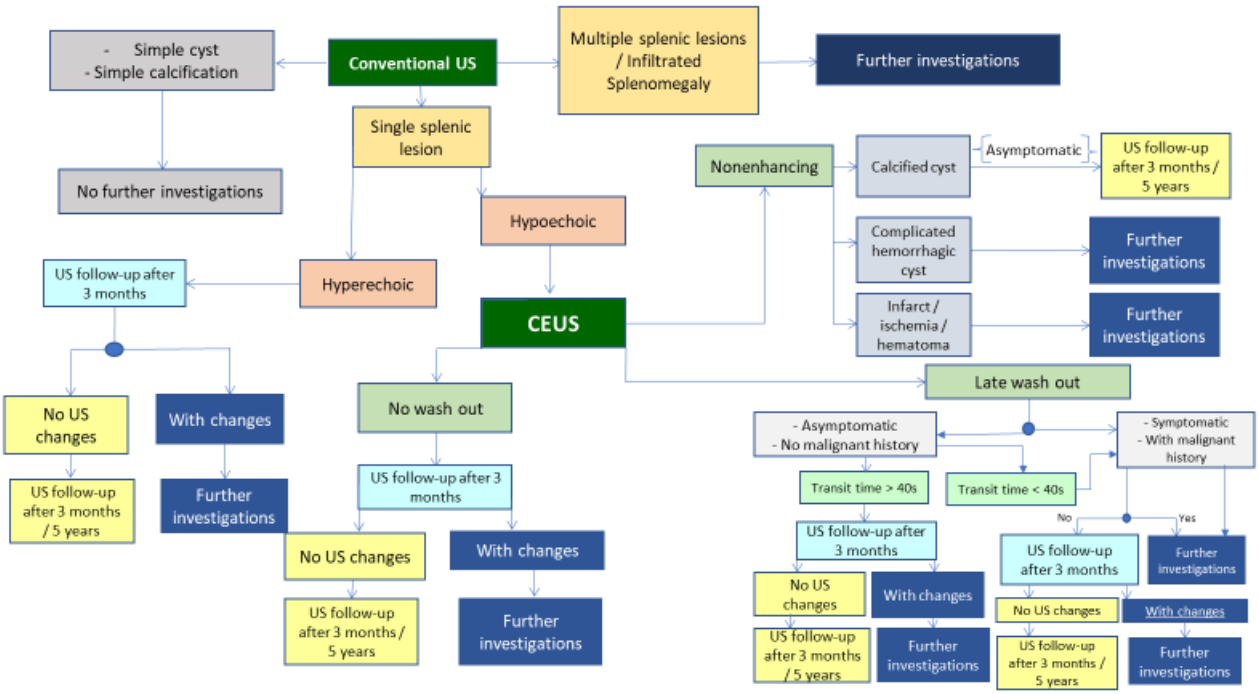

Figure 3. The proposed algorithm for the diagnosis of splenic lesions, based on the CEUS particularities.

Conclusions

CEUS has proven to be a valuable imaging technique in the management of patients with splenic lesions, being more accessible and benefiting from lower costs compared to other imaging methods. It can be performed following (naturally) a conventional US examination, shortening the time required for diagnosis, if necessary right at the patient's bedside. It is a non-irradiating procedure, and UCAs are liver, kidney and iodine-free toxicity and can be used in patients with chronic kidney failure. The risk of side effects is significantly lower compared to other CAs.

In our study, CEUS was able to set a positive diagnosis in over 70% of cases, quickly guiding the therapeutic attitude and the need for further investigation. Vascular lesions such as splenic infarction, hematomas, necrotic areas or vascular thrombosis, benign splenic nodules or ectopic splenic tissue were correctly diagnosed on CEUS. The method allowed the selection of patients who required other investigations, including histological diagnosis.

Recalling the proposed objectives at the beginning of the research, we proved the efficiency of CEUS in the detection of vascular splenic lesions. At the same time, counting the arterial enhancement and the wash out patterns as well as, in particular, the transit time

interval, we were able to develop an easy algorithm in order to increase the accuracy of CEUS in the diagnosis of splenic lesions.

An important limitation of the study is the relatively small number of patients evaluated, with various splenic lesions, which prevented the possibility of accurately describing the typical appearance for each type of lesion, with statistical significance. Also, despite the efforts made, there were inconsistent or incomplete data in the ultrasound evaluation of patients (especially the CEUS evaluation time intervals); this is an explanation for the lack of statistical significance of some parameters, whose graphical trend is obvious.

In the future, I will propose the evaluation of the proposed algorithm for the diagnosis of splenic lesions in order to clinically validate it and to encourage the use of CEUS in many centers for various pathologies, counting on the undeniable advantages of this method. The validation of the algorithm would allow the completion of the current guide on splenic lesions, which is the basis for a fast and efficient diagnosis, as well as for an efficient cost-risk management. Establishing a national (or university, for starter) database that combines ultrasound imaging with other diagnostic methods - paraclinical, histological, as well as clinical patient data can be an important opportunity for the dissemination and standardization of CEUS use for splenic lesions diagnosis in current practice. Exhaustive analysis of such a database would allow the development of new diagnostic and therapeutic protocols in a variety of pathologies that directly or indirectly affect the spleen.

BIBLIOGRAPHY

1. Hall JT, Kim EE, Charnsangavej C, Wallace S, Haynie TP. Variable patterns of technetium-99m MAA perfusion in the therapeutically embolized liver. J Nucl Med. 1989 Jun;30(6):1012–7.
2. Groshar D, Embon OM, Frenkel A, Front D. Renal function and technetium-99m-dimercaptosuccinic acid uptake in single kidneys: The value of in vivo SPECT quantitation. J Nucl Med. 1991;32(5):766–8.
3. Honda T, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Comparison of contrast-enhanced harmonic ultrasonography and power Doppler ultrasonography for depicting vascularity of hepatocellular carcinoma identified by angiography-assisted CT. Hepatol Res. 2003 Dec;27(4):315–22.
4. Quaia E, Calliada F, Bertolotto M, Rossi S, Garioni L, Rosa L, et al. Characterization of focal liver lesions with contrast-specific US modes and a sulfur hexafluoride-filled microbubble contrast agent: diagnostic performance and confidence. Radiology. 2004 Aug;232(2):420–30.
5. Piscaglia F, Nolsøe C, Dietrich CF, Cosgrove DO, Gilja OH, Bachmann Nielsen M, et al. The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): update 2011 on non-hepatic applications. Ultraschall Med. 2012 Feb;33(1):33–59.
6. Sidhu PS, Cantisani V, Dietrich CF, Gilja OH, Saftoiu A, Bartels E, et al. The EFSUMB guidelines and recommendations for the clinical practice of contrast-enhanced ultrasound (CEUS) in Non-Hepatic Applications: Update 2017 (Long Version). Ultraschall der Medizin. 2018;39(2):e2–44.
7. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, Nolsøe CP, et al. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver--update 2012: a WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. Ultraschall Med. 2013 Feb;34(1):11–29.
8. Dietrich CF, Nolsøe CP, Barr RG, Berzigotti A, Burns PN, Cantisani V, et al. Guidelines and Good Clinical Practice Recommendations for Contrast-Enhanced Ultrasound (CEUS) in the Liver-Update 2020 WFUMB in Cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS. Ultrasound Med Biol. 2020 Oct;46(10):2579–604.

9. Sporea I, Badea R, Brisc C, Ioanițescu S, Moga T, Popescu A, et al. Romanian National Guidelines on Contrast Enhanced Ultrasound in clinical practice. Med Ultrason. 2017 Nov;19(4):401–15.
10. Correas JM, Bridal L, Lesavre A, Méjean A, Claudon M, Hélénon O. Ultrasound contrast agents: Properties, principles of action, tolerance, and artifacts. Eur Radiol. 2001;11(8):1316–28.
11. Ignee A, Atkinson NSS, Schuessler G, Dietrich CF. Ultrasound contrast agents. Endosc ultrasound. 2016;5(6):355–62.
12. Paefgen V, Doleschel D, Kiessling F. Evolution of contrast agents for ultrasound imaging and ultrasound-mediated drug delivery. Front Pharmacol [Internet]. 2015;6. Available from: https://www.frontiersin.org/article/10.3389/fphar.2015.00197
13. Marshall G, Sykes A, Berry J, Jonker L. The "humble" bubble: Contrast-enhanced ultrasound. Radiography [Internet]. 2011;17(4):345–9. Available from: https://www.sciencedirect.com/science/article/pii/S1078817411000538
14. Lim AKP, Patel N, Eckersley RJ, Taylor-Robinson SD, Cosgrove DO, Blomley MJK. Evidence for spleen-specific uptake of a microbubble contrast agent: a quantitative study in healthy volunteers. Radiology. 2004 Jun;231(3):785–8.
15. Forsberg F, Goldberg BB, Liu JB, Merton DA, Rawool NM, Shi WT. Tissue-specific US contrast agent for evaluation of hepatic and splenic parenchyma. Radiology. 1999 Jan;210(1):125–32.
16. Piscaglia F, Bolondi L, Aiani L, Luigi Angeli M, Arienti V, Barozzi L, et al. The safety of Sonovue® in abdominal applications: Retrospective analysis of 23188 investigations. Ultrasound Med Biol. 2006;32(9):1369–75.
17. Geleijnse ML, Nemes A, Vletter WB, Michels M, Soliman OII, Caliskan K, et al. Adverse reactions after the use of sulphur hexafluoride (SonoVue) echo contrast agent. J Cardiovasc Med (Hagerstown). 2009 Jan;10(1):75–7.
18. Dietrich C, Averkiou M, Bachmann Nielsen M, Barr R, Burns P, Calliada F, et al. How to perform Contrast-Enhanced Ultrasound (CEUS) Affiliations An introduction to terminology. Ultrasound Int Open. 2017;3:E2–15.
19. Tang C, Fang K, Guo Y, Li R, Fan X, Chen P, et al. Safety of Sulfur Hexafluoride Microbubbles in Sonography of Abdominal and Superficial Organs: Retrospective Analysis of 30,222 Cases. J ultrasound Med Off J Am Inst Ultrasound Med. 2017 Mar;36(3):531–8.
20. Main ML. Ultrasound Contrast Agent Safety. From Anecdote to Evidence. JACC

Cardiovasc Imaging [Internet]. 2009;2(9):1057–9. Available from: http://dx.doi.org/10.1016/j.jcmg.2009.05.008

21. Hu C, Feng Y, Huang P, Jin J. Adverse reactions after the use of SonoVue contrast agent: Characteristics and nursing care experience. Medicine (Baltimore). 2019 Nov;98(44):e17745.
22. Authority R. Revised safety information for SonoVue issued in Europe. Reactions. 2014;1527:6–15.
23. Fischer C, Kunz P, Strauch M, Weber M-A, Doll J. Safety Profile of Musculoskeletal Contrast-Enhanced Ultrasound with Sulfur Hexafluoride Contrast Agent. Ther Clin Risk Manag. 2020;16:269–80.
24. Kitzman DW, Goldman ME, Gillam LD, Cohen JL, Aurigemma GP, Gottdiener JS. Efficacy and safety of the novel ultrasound contrast agent perflutren (definity) in patients with suboptimal baseline left ventricular echocardiographic images. Am J Cardiol. 2000 Sep;86(6):669–74.
25. Wilson SR, Burns PN. Microbubble-enhanced US in body imaging: what role? Radiology. 2010 Oct;257(1):24–39.
26. Cochran ST, Bomyea K, Sayre JW. Trends in adverse events after IV administration of contrast media. AJR Am J Roentgenol. 2001 Jun;176(6):1385–8.
27. Hunt CH, Hartman RP, Hesley GK. Frequency and severity of adverse effects of iodinated and gadolinium contrast materials: retrospective review of 456,930 doses. AJR Am J Roentgenol. 2009 Oct;193(4):1124–7.
28. Piscaglia F, Bolondi L. The safety of Sonovue in abdominal applications: retrospective analysis of 23188 investigations. Ultrasound Med Biol. 2006 Sep;32(9):1369–75.
29. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, Nolsoe CP, et al. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver - Update 2012. Ultraschall der Medizin. 2013;34(1):11–29.
30. Albrecht T, Blomley M, Bolondi L, Claudon M, Correas JM, Cosgrove D, et al. Guidelines for the use of contrast agents in ultrasound: January 2004. Ultraschall der Medizin. 2004;25(4):249–56.
31. Paul Sidhu AS, Cantisani V, Dietrich CF, Helge Gilja O, Saftoiu A, Bartels E, et al. The EFSUMB Guidelines and Recommendations for the Clinical Practice of Contrast-Enhanced Ultrasound (CEUS) in Non-Hepatic Applications: Update 2017

(Long Version) Die EFSUMB-Leitlinien und Empfehlungen für den klinischen Einsatz des kontrastverstärkten Ul. EFSUMB Guidel Ultraschall Med [Internet]. 2018;39:2–44. Available from: https://doi.org/10.1055/a-0586-1107

32. ACR. CEUS LI-RADS® v2017 CORE 2017 [cited 2018 28.01.]. In. Available from: https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/LI-RADS/CEUS-LI-RADS-v2017.
33. Simpson DH, Chin CT, Burns PN. Pulse inversion Doppler: a new method for detecting nonlinear echoes from microbubble contrast agents. IEEE Trans Ultrason Ferroelectr Freq Control. 1999;46(2):372–82.
34. Whittingham TA. Broadband transducers. Eur Radiol. 1999;9 Suppl 3:S298-303.
35. Manatakis DK, Piagkou M, Loukas M, Tsiaoussis J, Delis SG, Antonopoulos I, et al. A systematic review of splenic artery variants based on cadaveric studies. Surg Radiol Anat. 2021 Aug;43(8):1337–47.
36. Bhivate V, Suresh R, Kharate R, Pandey N. Study of diameter, length, tortuosity of splenic artery and its branches with its clinical implications. J Res Med Dent Sci. 2014;2(4):22.
37. Rajasekhar P, Hemasankar C. IJCRR STUDY ON ORIGIN , COURSE , BRANCHING PATTERN AND MORPHOMETRY OF SPLENIC ARTERY AND ITS BRANCHES SUPPLYING THE SPLEEN-A CADAVERIC STUDY. 2014;6(18).
38. García-Porrero JA, Lemes A. Arterial segmentation and subsegmentation in the human spleen. Acta Anat (Basel). 1988;131(4):276–83.
39. Gupta CD, Gupta SC, Arora AK, Singh PJ. Vascular segments in the human spleen. J Anat. 1976 Jul;121(Pt 3):613–6.
40. Ignjatovic D, Stimec B, Zivanovic V. The basis for splenic segmental dearterialization: A post-mortem study. Surg Radiol Anat. 2005;27(1):15–8.
41. Katritsis E, Parashos A, Papadopoulos N. Arterial segmentation of the human spleen by post-mortem angiograms and corrosion-casts. Angiology. 1982 Nov;33(11):720–7.
42. Liu DL, Xia S, Xu W, Ye Q, Gao Y, Qian J. Anatomy of vasculature of 850 spleen specimens and its application in partial splenectomy. Surgery. 1996 Jan;119(1):27–33.
43. Rendina E, Pescarmona E, Marinozzi G. Comparative Study of the Arterial and Venous Segmental Circulation in the Human Spleen. Vasc Surg [Internet]. 1984 Sep

1;18(5):283–8. Available from: https://doi.org/10.1177/153857448401800504

44. Silva LFA, Silveira LMA, Timbó PS, Pinheiro SR, Barros LV, da Silva Filho AR. Morfometric study of arterial branching of the spleen compared to radiological study. Rev Col Bras Cir. 2011;38(3):181–5.
45. Sow ML, Dia A, Ouedraogo T. Anatomic basis for conservative surgery of the spleen. Surg Radiol Anat. 1991;13(2):81–7.
46. Swamy VL, D S, D JS, R ZB, N PM, Chaitanya K. Study of prehilar branches of splenic artery by dissection method. Int J Med Res Heal Sci. 2013;2(3):620.
47. Dixon JA, Miller F, McCloskey D, Siddoway J. Anatomy and techniques in segmental splenectomy. Surg Gynecol Obstet. 1980 Apr;150(4):516–20.
48. Lal H, Boruah DK, Yadav A, Samal A. Anteriorly Placed Splenic Vein: a Case Report. Indian J Surg. 2015;77(April):135–6.
49. Mebius RE, Kraal G. STRUCTURE AND FUNCTION OF THE SPLEEN. 2005;(June):606–16.
50. Schmidt EE, MacDonald IC, Groom AC. Comparative aspects of splenic microcirculatory pathways in mammals: The region bordering the white pulp. Scanning Microsc. 1993;7(2):613–28.
51. Lamb PM, Lund A, Kanagasabay RR, Martin A, Webb JAW, Reznek RH. Spleen size: How well do linear ultrasound measurements correlate with three-dimensional CT volume assessments? Br J Radiol. 2002;75(895):573–7.
52. Gaiani S, Gramantieri L, Venturoli N, Piscaglia F, Siringo S, D'Errico A, et al. What is the criterion for differentiating chronic hepatitis from compensated cirrhosis? A prospective study comparing ultrasonography and percutaneous liver biopsy. J Hepatol. 1997 Dec;27(6):979–85.
53. Miele V, Piccolo CL, Galluzzo M, Ianniello S, Sessa B, Trinci M. Contrast-enhanced ultrasound (CEUS) in blunt abdominal trauma. Br J Radiol. 2016;89(1061).
54. Valentino M, Serra C, Pavlica P, Barozzi L. Contrast-Enhanced Ultrasound for Blunt Abdominal Trauma. Semin Ultrasound, CT MRI. 2007;28(2):130–40.
55. Ioanițescu ES, Grasu M, Toma L. Primary splenic leiomyosarcoma - Case report and literature review. Med Ultrason. 2022;24(1):114–6.
56. Ioanitescu ES, Copaci I, Mindrut E, Motoi O, Stanciu AM, Toma L, et al. Various aspects of Contrast-enhanced Ultrasonography in splenic lesions - a pictorial essay. Med Ultrason. 2020;22(3):2521.

57. Yang R, Lu Q, Xu J, Huang J, Gao B, Zhang H, et al. Value of Contrast-Enhanced Ultrasound in the Differential Diagnosis of Focal Splenic Lesions. Cancer Manag Res. 2021;13:2947–58.
58. Lerchbaumer MH, Kleemann T, Jung E-M, Nagel S, Hamm B, Fischer T. Vascular pattern and diagnostic accuracy of contrast-enhanced ultrasound (CEUS) in spleen alterations. Clin Hemorheol Microcirc. 2020;75(2):177–88.
59. Trenker C, Görg C, Freeman S, Jenssen C, Dong Y, Caraiani C, et al. WFUMB Position Paper—Incidental Findings, How to Manage: Spleen. Ultrasound Med Biol. 2021;00(00).

LIST OF PUBLISHED SCIENTIFIC PAPERS

1. **Elena Simona Ioanitescu**, Ionel Copaci, Eugenia Mindrut, Otilia Motoi, Adriana Mercan Stanciu, Letitia Toma, Elena Laura Iliescu. Various aspects of Contrast-enhanced Ultrasonography in splenic lesions – a pictorial essay. Medical Ultrasonography 2020, Volum 22, No 3, pg 356-363, DOI: 10.11152/mu-2521
   https://www.medultrason.ro/medultrason/index.php/medultrason/article/view/2521/1639
   Impact factor 1.611

2. **Elena Simona Ioanițescu**, Mugur Grasu, Letiția Toma. Primary splenic leiomyosarcoma – case report and literature review. Medical Ultrasonography 2022, Volum 24, No 1, pg 114-116, DOI: 10.11152/mu-3325
   https://medultrason.ro/medultrason/index.php/medultrason/article/view/3325
   Impact factor 1.611

3. Corinna Trenker, Christian Görg, Simon Freeman, Christian Jenssen, Yi Dong, Cosmin Caraiani, **Elena Simona Ioanițescu**, Christoph F. Dietrich. WFUMB POSITION PAPER—INCIDENTAL FINDINGS, HOW TO MANAGE: SPLEEN. Ultrasound in Medicine and Biology 2021, Volum 47, No 8, 2017-2032.
   doi.org/10.1016/j.ultrasmedbio.2021.03.032
   https://www.sciencedirect.com/science/article/pii/S0301562921001538
   Impact factor 2.998

4. Ioan Sporea, Radu Badea, Ciprian Brisc**, Simona Ioanițescu**, Tudor Moga, Alina Popescu, Adrian Săftoiu, Larisa Săndulescu, Zeno Spârchez, Roxana Șirli. Romanian National Guidelines on Contrast Enhanced Ultrasound in clinical practice. Medical Ultrasonography 2017, Volum 19, No 4, pg 401-415. DOI: 10.11152/mu-1349
   https://www.medultrason.ro/medultrason/index.php/medultrason/article/view/1349/1075
   Impact factor 1.611