ORIGINAL ARTICLE

Male Health

Contrast-enhanced ultrasound as a valuable imaging modality for characterizing testicular lesions

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Contrast-enhanced ultrasound (CEUS) is a new form of ultrasound (US) that can dynamically display microvessels in a highly sensitive manner. The purpose of this study was to investigate the efficacy of CEUS for characterizing testicular lesions in comparison with conventional US. Forty-seven patients with testicular lesions were enrolled. The histopathology results revealed that 31 cases were neoplastic (11 cases of seminomas, 8 nonseminomatous germ cell tumors, 8 lymphomas, 2 Leydig cell tumors, and 2 nonspecific tumors), and 16 cases were nonneoplastic (8 cases of infarctions, 3 epidermoid cysts, and 5 inflammation). The indicators of shallow lobulated morphology and cystic-solid echogenicity on conventional US were suggestive of germ cell tumors. More indicators on CEUS were found to be useful for characterizing testicular lesions. All the neoplastic lesions showed hyperenhancement on CEUS. Moreover, germ cell tumors presented with heterogeneous enhancement (73.7%, 14/19), a twisted blood vessel pattern, rapid wash-in and wash-out, and peripheral rim hyperenhancement signs. Lymphoma was characterized by nonbranching linear vessel patterns (87.5%, 7/8), rapid wash-in and slow wash-out. In nonneoplastic lesions, infarction and epidermoid cysts showed no enhancement, and abscesses were observed with marginal irregular enhancement. The sensitivity, specificity, and accuracy of CEUS for differentiating between neoplastic and nonneoplastic lesions were 100%, 93.8%, and 97.9%, respectively, and these values were higher than those for conventional US (90.3%, 62.5%, and 80.9%, respectively). CEUS can sensitively reflect the microvascular perfusion in testicular lesions and offers high accuracy for characterizing them.
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INTRODUCTION

Testicular neoplasms account for only 1% of all solid tumors in males; however, they are the most common solid malignant tumors in males between 15 years and 34 years of age.[1] Orchiectomy is usually the main form of treatment for intratesticular neoplasms.[2] It is vital that these patients can be accurately diagnosed as early as possible in order for timely treatment to be administered.[3] Ultrasound (US) is recommended as the preferred method for evaluating testicular masses. US is relatively easy to perform and can noninvasively display the anatomic changes in real time with no radiation-induced side effects.[4] With the improved resolution of US instruments, conventional US can now sensitively detect testicular lesions even smaller than 5 mm in diameter.[5] Moreover, conventional US can also sensitively identify complete cystic lesions, and therefore, unnecessary surgical resection can be avoided. However, conventional US is associated with certain limitations with regard to distinguishing testicular lesions with solid echogenicity.[1] The characteristics of testicular neoplasms can be similar to those of nonneoplastic lesions on conventional US, thus making diagnosis very difficult.[6]

The emergence of contrast-enhanced ultrasound (CEUS) is considered a revolutionary breakthrough in the history of US. The contrast agents used for CEUS include microbubbles of micron size, which is similar to the size for red blood cells. More importantly, the contrast agents cannot pass through the vascular endothelium into the tissue space, and therefore, they provide good vascular tracers.[7] These contrast agents can be excreted from the body via breathing and have no effect on the liver or kidneys. The incidence of allergic reactions is relatively low, with previously reported incidence rates for severe allergic reactions during CEUS ranging from 0.007% to 0.0086%.[8] CEUS can capture the tiny amounts of low-speed blood flow in the tissues or lesions in real time, in a dynamic and sensitive manner. Some studies have shown that CEUS can identify microvessels with a diameter of 40 μm and can provide useful information for qualitative diagnosis. This technique has been internationally recognized for its efficacy for various abdominal solid organs.[9,10] In recent years, the study of superficial organ US, especially the testis, has become a significant area of focus, although the qualitative diagnostic efficacy of CEUS for testicular lesions remains debatable.[11,12] This retrospective study aimed to evaluate the efficacy of CEUS for the qualitative diagnoses of testicular neoplastic and nonneoplastic lesions.

PATIENTS AND METHODS

Patient data

In total, 47 patients who were admitted to the Department of Urology of Shanghai General Hospital (Shanghai, China) between February

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2013 and November 2020 were included in this retrospective study. This study was approved by the Ethics Committee of Shanghai General Hospital (No. 2013-094). The inclusion criteria were as follows: (1) written informed consent; (2) no testicular biopsy examination before US examination; (3) no contraindications to US contrast agent; (4) clear final pathological results from either surgical resection or puncture; (5) good quality of conventional US and CEUS images, meaning the boundary of the lesion could be clearly detected, the wash-in and wash-out process or nonenhancement of the contrast agent could be clearly visualized, and the background noise interference was mild; and (6) complete clinical information. If anyone of these inclusion criteria was not met, the case was excluded.

Instruments and imaging methods

All patients underwent examinations by grayscale US, color Doppler flow imaging (CDFI), and CEUS. US examinations were carried out using a LOGIQ E9 (GE, Milwaukee, WI, USA), Acuson Sequoia 512 (Siemens Healthcare, Mountain View, CA, USA), Philips IU22 (Philips Healthcare, Best, The Netherlands), or MyLab Twice instrument (Esaote, Genoa, Italy). The linear transducers were separately 9L, 9L4, L9-3, and LA522. The modes of CEUS were separately high-fidelity amplitude modulation imaging, contrast pulse sequence (CPS), pulse-inversion harmonic ultrasonographic imaging (PIH), and contrast-tuned imaging (CnTI).

Each patient was asked to lie supine with the scrotum fully exposed. Conventional US examination was performed first. The ultrasonographic appearance of the lesions and their blood flow signal were observed in grayscale and CDFI modes. The color scale and gain were adjusted to maximally display the blood flow in the lesion and simultaneously avoid random noise. Images from the entire examination were digitally recorded in DICOM format.

After the conventional US examination, CEUS was performed. The largest section on the long axial view was selected for CEUS examination for each lesion. If multiple lesions were present, we chose the largest nodule for CEUS examination. The contrast agent was SonoVue (Bracco SpA, Milan, Italy); this agent was configured in accordance with the methods recommended by the manufacturer. The operators selected a section that showed both the lesion and the surrounding normal tissue to enter the CEUS mode. A 4.5-ml dose of US contrast agent was bolus injected into the forearm vein followed by 5 ml of normal saline. We began recording clips when the contrast agent injection was administered, and the whole observation process lasted for 120 s. The "live-dual" display modality was used, which simultaneously displays CEUS image and grayscale US image on the screen.

Image analyses

On conventional US images, the lesion number, size, morphology, echogenicity, and blood flow signal were analyzed. The Adler method was used to grade the amount of blood flow signal in testicular lesions on CDFI: grade 0 (no vessels in the mass), grade I (a small number of vessels, 1–2 punctate or short rod-shaped vessels), grade II (moderate vessels, 3–4 punctate vessels or 1 longer vessel with a length close to or exceeding the radius of the mass), and grade III (a large number of vessels, more than 5 punctate or 2 longer vessels).[13] Vascularity was classified as hypovascular (grades 0, I, and II) or hypervascular (grade III).

According to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) Guidelines and Recommendations for the Clinical Practice of CEUS in Non-Hepatic Applications, the phases of testicular CEUS can be divided into an arterial phase and a venous phase.[14] The arterial phase is defined as the first 30–50 s after injection of the contrast agent. The venous phase is defined as the time period lasting from 50 s to 2 min after the injection of the contrast agent. According to the enhancement intensity of the surrounding or contralateral normal testicular tissue, the lesions can be characterized by hyperenhancement, isoenhancement, hypoenhancement, or no enhancement. Homogeneous and heterogeneous enhancement was defined in accordance with the distribution of contrast agent in the lesion. When a lesion showed hyperenhancement in both the arterial and venous phases, this was referred to as "rapid wash-in and slow wash-out" mode ($R_{wash-in}$ and $S_{wash-out}$). If the lesion showed hyperenhancement in the arterial phase and hypoenhancement in the venous phase, this was referred to as "rapid wash-in and rapid wash-out" mode ($R_{wash-in}$ and $R_{wash-out}$). According to previous studies, vascular enhanced patterns were classified into twisted vessels, nonbranching linear vessels, random filling vessels, and peripheral irregular vessels.[15,16]

The diagnostic results were divided into five grades, with 1 to 5 corresponding, to definite nonneoplasm, possible nonneoplasm, uncertain, possible neoplasm, and definite neoplasm, respectively. The diagnostic efficacy was calculated for conventional US and CEUS for testicular neoplasm using grade 4 as the boundary. Two senior radiologists (with 5 years and 10 years of experience) were involved in reviewing the images using a double-blind method.

Statistical analyses

Data analysis was carried out using SPSS 26.0 (IBM, Armonk, NY, USA). Quantitative items are reported as mean ± standard deviation (for normally distributed variables) or median and range (for nonnormally distributed variables). Classified variables were compared between groups using the Chi-square or Fisher's exact test, while consecutive variables were compared by Student's *t*-test or Mann–Whitney U test. The Kappa test was used to test intro-observer consistency. $P < 0.05$ was considered to indicate statistical significance. A receiver operating characteristic (ROC) curve was also generated, taking a level 4 diagnosis as the threshold. The area under the curve (AUC) was calculated, and diagnostic efficiency was compared between conventional US and CEUS.

RESULTS

All lesions were confirmed by histopathology. Two nonspecific tumors were referred to as a neuroendocrine tumor and a testicular adrenal rests tumor (TART). There was no significant difference in terms of age between the neoplastic and nonneoplastic groups, while clinical symptoms did significantly differ between these groups ($P = 0.003$; **Supplementary Table 1**).

As shown in **Table 1**, there were significant differences in neoplastic morphology and echogenicity between the groups among the indicators analyzed by conventional US (**Table 1**). A shallow lobulated pattern was more common in germ cell tumors, and cystic-solid echogenicity was more often found in nonseminomatous germ cell tumors (NSGCTs). There was no significant difference between the two groups with regard to color Doppler signal. Rich blood flow signals were not detected in 45.2% (14/31) of testicular neoplasms.

All patients underwent CEUS examination for 5–15 min; none of the patients reported symptoms of discomfort during this examination. CEUS identified significant differences of enhanced intensity, dynamic mode of enhancement, vascular pattern, and peripheral rim hyperenhancement between the two groups (all $P < 0.05$; **Table 2**). All

Table 1: Characteristics of testicular lesions on conventional ultrasound (*n*=47)

Clinical characteristic	Lesion number (single/multiple)	Lesion size (mm), mean±s.d.	Morphology (shallow lobulated/regular)	Heterogeneity of echogenicity (homo/hetero/cystic-solid)	Blood flow signal on CDFI (hyper/hypo)
Neo (*n*=31)					
Seminoma (*n*=11)	8/3	47.0±20.9	9/2	6/5/0	6/5
NSGCT (*n*=8)	8/0	56.4±22.4	5/3	0/3/5	1/7
Lymphoma (*n*=8)	7/1	46.2±19.4	0/8	8/0/0	8/0
Leydig cell tumor (*n*=2)	2/0	7.5±3.5	0/2	2/0/0	1/1
Nonspecific tumor (*n*=2)	1/1	35.5±0.7	0/2	1/1/0	1/1
Nonneo (*n*=16)					
Infarction (*n*=8)	8/0	35.8±17.0	0/8	0/7/1	0/8
Epidermoid cyst (*n*=3)	3/0	21.7±3.8	0/3	0/3/0	0/3
Inflammation (*n*=5)	4/1	32.6±11.2	1/4	1/4/0	5/0
P (neo *vs* nonneo)	0.617	0.028	0.017	<0.001	0.125

NSGCT: nonseminomatous germ cell tumor; homo: homogeneous; hetero: heterogeneous; hypo: hypovascularization; hyper: hypervascularization; neo: neoplasms; nonneo: nonneoplasms; CDFI: color Doppler flow imaging; s.d.: standard deviation

Table 2: Characteristics of testicular lesions on contrast-enhanced ultrasound (*n*=47)

Clinical characteristic	Enhanced intensity in arterial phase (hyper/hypo/none)	Enhanced heterogeneity (homo/hetero)	Dynamic mode of enhancement ($R_{wash-in}$ and $R_{wash-out}/R_{wash-in}$ and $S_{wash-out}$)	Vascular patterns on CEUS (twisted/nonbranching linear/ random/marginal irregular)	Peripheral rim hyperenhancement (yes/none)
Neo (*n*=31)					
Seminoma (*n*=11)	11/0/0	5/6	11/0	10/1/0/0	11/0
NSGCT (*n*=8)	8/0/0	0/8	8/0	8/0/0/0	8/0
Lymphoma (*n*=8)	8/0/0	8/0	0/8	1/7/0/0	1/7
Leydig cell tumor (*n*=2)	2/0/0	2/0	0/2	0/0/2/0	0/2
Nonspecific tumor (*n*=2)	2/0/0	2/0	2/0	0/0/2/0	2/0
Nonneo (*n*=16)					
Infarction (*n*=8)	0/0/8	NA	NA	NA	NA
Epidermoid cyst (*n*=3)	0/0/3	NA	NA	NA	NA
Inflammation (*n*=5)	5/0/0	1/4	0/5	0/0/1/4	0/5
P (neo *vs* nonneo)	<0.001	0.335	0.008	<0.001	0.005

NSGCT: nonseminomatous germ cell tumor; hyper: hyperenhancement; hypo: hypoenhancement; hetero: heterogeneous; homo: homogeneous; neo: neoplasms; nonneo: nonneoplasms; NA: not applicable; CEUS: contrast-enhanced ultrasound

testicular neoplasms showed hyperenhancement during the arterial phase. Most seminomas and mixed germ cell tumors presented with heterogeneous enhancement, $R_{wash-in}$ and $R_{wash-out}$, peripheral rim hyperenhancement, and twisted blood vessels in the lesions (**Figure 1** and **2**). Primary lymphomas showed homogeneous enhancement, $R_{wash-in}$ and $S_{wash-out}$, and nonbranching linear vessels and peripheral rim hyperenhancement (**Figure 3**). One neuroendocrine tumor and TART showed homogeneous enhancement, $R_{wash-in}$ and $R_{wash-out}$, random filling patterns, and peripheral rim hyperenhancement. Leydig cell tumors presented homogeneous hyperenhancement on CEUS, but these lesions were small and showed no signs of peripheral rim hyperenhancement.

A testicular infarction and an epidermoid cyst did not show any enhancement during the CEUS process (**Figure 4**). Testicular inflammation with abscess showed no enhancement inside the lesion, and the margin of the lesions was irregularly hyperenhanced in $R_{wash-in}$ and $S_{wash-out}$ mode (**Supplementary Figure 1**). One case of xanthogranulomatous inflammation (1.5 cm in diameter) appeared homogeneously hyperenhanced in the arterial phase, which was similar to testicular neoplasms.

Good agreement was achieved between the two observers with regard to reviewing the conventional US images and CEUS images (**Supplementary Table 2** and **3**). The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were 90.3%, 62.5%, 80.9%, 82.4%, and 76.9% for conventional US, respectively,

while for CEUS, those were 100%, 93.8%, 97.9%, 96.9%, and 100%, respectively. When level 4 was taken as the threshold for diagnosing testicular neoplasms, the AUCs for CEUS and conventional US were separately 0.969 and 0.764 (**Supplementary Figure 2**).

DISCUSSION
Previous studies have shown that conventional US can distinguish lesions inside and outside the testis and detect intratesticular lesions as small as 1–2 mm in diameter.[17] However, there is still some debate as to whether conventional US is effective for the qualitative diagnosis of testicular neoplasms.[5] Our present results demonstrate that CEUS offers a significantly improved ability for qualitative diagnosis compared with conventional US, especially with regard to differentiating testicular neoplastic and nonneoplastic lesions.

In clinical practice, conventional US is the most important imaging method used to detect testicular neoplasms. In the present study, we found that the morphology and heterogeneity of echogenicity were helpful in identifying germ cell tumors. Germ cell tumors were often shallow lobulated, while most NSGCTs were cystic-solid. However, the morphology of inflammation associated with NSGCTs was similar to that of testicular neoplasms, and this similarity could lead to confusion during diagnosis. Although CDFI can reflect blood flow within lesions, the technique is easily affected by the instrument settings and the experience of the operator. Furthermore, it cannot show small blood flow at low speed, leading
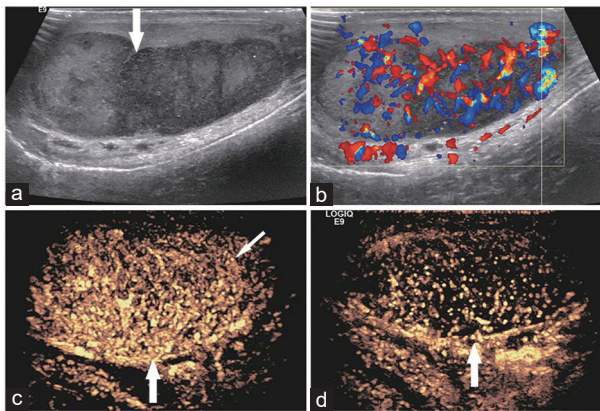
Figure 1: Representative images from a 39-year-old patient with seminoma in the right testis. (**a**) Grayscale ultrasonography demonstrated a heterogeneous echogenicity, with shallow lobulated pattern (thick arrow). (**b**) Color Doppler flow imaging depicted rich vascularity within the lesion. (**c**) Contrast-enhanced ultrasonography demonstrated hyperenhancement of the lesion and twisted blood vessels (thin arrow) in arterial phase, with peripheral rim hyperenhancement sign (thick arrow). (**d**) Contrast-enhanced ultrasonography still showed peripheral rim hyperenhancement sign in venous phase (thick arrow).
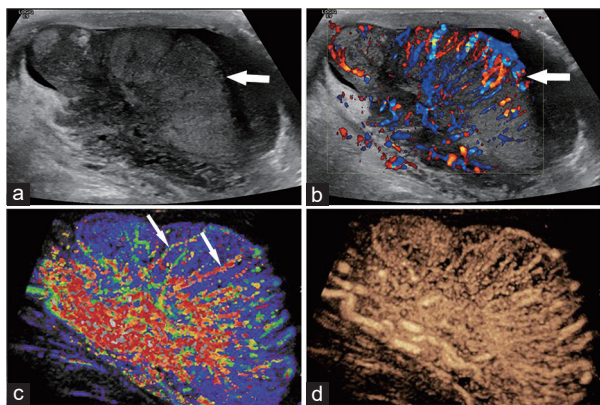

Figure 2: Representative images from a 32-year-old patient with nonseminomatous germ cell tumor. (**a**) Grayscale ultrasonography demonstrated a mixed echogenicity lesion with cystic components and irregular margins (thick arrow). (**b**) Color Doppler flow imaging demonstrated scattered signal in the lesion (thick arrow). (**c**) Contrast-enhanced ultrasonography demonstrated heterogeneous enhancement, twisted blood vessels (thin arrow), and peripheral rim hyperenhancement in arterial phase. (**d**) The corresponding images to **c** in venous phase (thick arrow).

Figure 3: Representative images from a 69-year-old patient after liver transplantation with right testicular lymphoma. (**a**) Grayscale ultrasonography showed a poorly defined hypoechoic lesion (thick arrow). (**b**) Color Doppler flow imaging showed increased vascularity within the lesion (thick arrow). (**c**) On the parametric imaging mode of contrast-enhanced ultrasound, the nonbranching linear patterns were clearly displayed (thin arrows). This mode could display the different perfusion speed of the vessels in different colors; for example, the vessels in red color were perfused sooner, while the ones in blue color were perfused later. (**d**) The corresponding contrast-enhanced ultrasound images for **c**.

Figure 4: Representative images from a 20-year-old patient with left testicular infarction after torsion. (**a**) There was heterogeneous echogenicity, and the border of this lesion was slightly poor (thick arrow). (**b**) No color Doppler signal was detected within the lesion (thick arrow). (**c**) Contrast-enhanced ultrasonography clearly demonstrated the infarcted areas with the absence of contrast agent in arterial phase (thick arrow). (**d**) No contrast agent entered the lesion during venous phase (thick arrow).

to false-negative results. It has been reported that it is difficult to distinguish between lesions with a few or no blood vessels with CDFI, especially for small lesions.[18] In the present study, we could not detect abundant blood flow signals in 45.2% of neoplastic lesions, thus making these cases difficult to diagnose. These are key factors underlying the limited diagnostic ability of conventional US for testicular lesions.

CEUS is very sensitive to microcirculation perfusion in tissues and can make up for the fact that CDFI cannot display low-speed blood flow. Studies have found that CEUS is better than computed tomography (CT) and magnetic resonance imaging (MRI) with contrast agents for detection of small blood vessels.[19,20] Similar to previous reports, all testicular neoplasms in the present study showed the excessive formation of blood vessels; in other words, CEUS
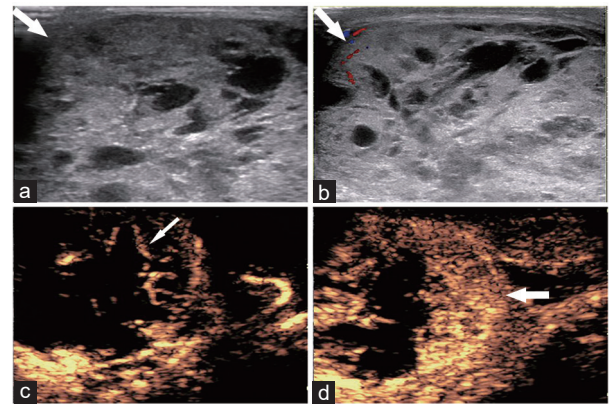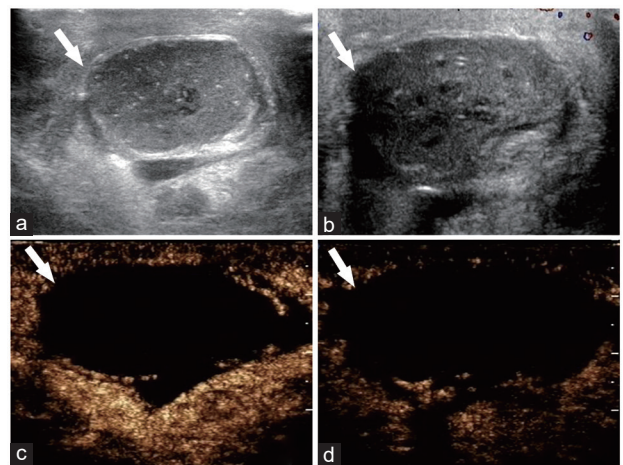
showed hyperenhancement. Furthermore, the nature of the testicular lesions can be diagnosed more accurately by combining enhanced homogeneity, the dynamic mode of enhancement, and vascular patterns.

Germ cell tumors account for 90%–95% of all testicular neoplasms, including seminomas and NSGCTs.[21–23] Testicular germ cell tumors presented hyperenhancement, rapid wash-in and wash-out, heterogeneous enhancement, twisted blood vessels in the margin and interior, and peripheral rim hyperenhancement on CEUS in the arterial phase, and the occurrence rates for those signs in both seminomas and NSGCTs were 100%, 100%, 73.7%, 94.7%, and 100%, respectively. The pathological basis of these imaging manifestations was single morphology and structure of tumor cells with interstitial

lymphocyte infiltration in the seminoma. The texture of this form of tumor involved extensive fibrous septa and flaky necrosis. As a result, seminomas showed heterogeneity and lacked a normal vascular pattern on CEUS. The wrapping of the tunica albuginea around the testis limits the aggressive growth of neoplasms. When a testicular tumor presses on the surrounding tissues, then there is an increase in vascular density, which leads to signs of peripheral rim hyperenhancement.[12,24] However, five seminomas were homogeneously enhanced due to the presence of limited necrosis or hemorrhage in the tumor.

Due to the composition of two or more tumor components, NSGCTs are often associated with hemorrhage, necrosis, and cystic changes, which can present as large plate-like nonenhanced areas or cystic-solid areas on CEUS. Besides the abovementioned specific tumoral manifestations, a highly uneven or cystic-solid CEUS appearance might be suggestive of NSGCTs.

Malignant lymphoma is the most common testicular neoplasm in elderly men; most of these cases involve diffuse large B-cell lymphomas. Testicular lymphoma is often hypervascular and is difficult to distinguish from germ cell tumors and inflammation on conventional US. However, lymphoma has a specific vascular pattern of nonbranching linear vessels, along with rapid wash-in and slow wash-out patterns. Pathological data indicate that these signs are related to the diffuse infiltration and growth of lymphocytes in the neoplasm around the vas deferens. Furthermore, there are still residues within the vas deferens; consequently, the vascular structure is not completely destroyed.[14]

During the course of the present study, we identified two rare testicular neoplasms: TART and a testicular neuroendocrine tumor. The manifestations of these two lesions were similar to those of germ cell tumors on conventional US and CEUS.[25,26] Sex cord-stromal tumors (SCSTs) are rare and usually benign tumors, although the differentiation between stromal cell tumors and germ cell tumors remains controversial. In this study, we found that the CEUS findings for Leydig cell tumors were similar to those for germ cell tumors. However, the diameters of these tumors were less than 2 cm; consequently, there are no signs of regular peripheral rim hyperenhancement.[27]

Unlike testicular neoplasms with hypervascularization, nonneoplastic lesions, such as infarctions and epidermoid cysts, have no blood vessels within the lesion. Although it is sometimes difficult to distinguish these two types of lesions from testicular tumors by conventional US, CEUS can be very effective in differentiating between the two types of tumors.[28] Testicular inflammation is another major disease that needs to be distinguished from testicular neoplasms. Scrotal pain is the first symptom of testicular inflammation. However, symptoms may be atypical in some elderly patients or patients with delayed diagnosis and treatment. After the occurrence of secondary lesions, US findings can be easily confused with neoplastic lesions.[29] The results of the present study showed that there are some similarities between testicular inflammation with abscess and neoplasms on conventional US. After CEUS, inflammation with abscess showed no enhancement in the lesion and irregular peripheral hyperenhancement. This was due to secondary necrosis, liquefaction, or an abscess at the center of the lesion. The congestion and edema around the lesion were the cause of the appearance of marginal hyperenhancement. It is worth noting that when the lesion is small and there are no obvious secondary changes associated with necrosis and liquefaction, the features of inflammation are similar to those of testicular tumors on CEUS. In this study, we identified one case of xanthogranulomatous inflammation (1.5 cm in size) that presented with a rapid wash-in and slow wash-out pattern on CEUS, along with hyperenhancement.

Our results show that the efficacy of CEUS is significantly higher than that of conventional US for the diagnosis of neoplastic and nonneoplastic lesions in the testes, based on a combination of multiple characteristics on CEUS images.[30] In particular, CEUS could significantly improve the blood flow detection in small lesions. This high efficiency for characterizing testicular lesions was conducive to the subsequent selection of treatment options. When CEUS showed signs of neoplasms, surgery would be recommended, while conservative treatment would be preferred if a lesion was considered to be a nonneoplasm on CEUS.

This study has some limitations that need to be considered. First, we had a relatively small number of patients, particularly with regard to the number of patients with Leydig cell tumors. It is therefore impossible to evaluate the true efficacies of US and CEUS for the differential diagnosis of benign and malignant testicular neoplasms. However, based on previous studies and our experience in clinical practice, benign tumors tend to be small and hypervascular with a homogeneous structure. CEUS was able to reveal such characteristics much better than conventional US. Second, most inflammatory lesions in our study were accompanied by secondary changes. When the lesions were small, we still experienced difficulties in the differential diagnosis between testicular inflammation and testicular neoplasms. Third, prognostic assessment is very important for clinicians. However, there are still limitations related to the use of US to evaluate lymph node metastases, distant metastases, or the invasion of tumors by blood vessels. These issues need to be investigated further.

CONCLUSION

In comparison with conventional US, CEUS may offer better diagnostic ability for characterizing testicular lesions based on differences in the perfusion characteristics of microcirculation in neoplasms and nonneoplasms.

AUTHOR CONTRIBUTIONS

JY and XHJ contributed to project and wrote the main body of the manuscript. FL and QJ designed the manuscript. MB, ZJL, QSS, and LFD participated in the clinical study. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

REFERENCES

1 Rocher L, Ramchandani P, Belfield J, Bertolotto M, Derchi LE, *et al.* Incidentally detected non-palpable testicular tumours in adults at scrotal ultrasound: impact of radiological findings on management Radiologic review and recommendations of the ESUR scrotal imaging subcommittee. *Eur Radiol* 2016; 26: 2268–78.

2 Stephenson A, Eggener SE, Bass EB, Chelnick DM, Daneshmand S, *et al.* Diagnosis and treatment of early stage testicular cancer: AUA Guideline. *J Urol* 2019; 202: 272–81.

3 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; 69: 7–34.

4 Smith ZL, Werntz RP, Eggener SE. Testicular cancer: epidemiology, diagnosis, and management. *Med Clin North Am* 2018; 102: 251–64.

5 Rebik K, Wagner JM, Middleton W. Scrotal ultrasound. *Radiol Clin North Am* 2019; 57: 635–48.

6 Badea R, Lucan C, Suciu M, Vasile T, Gersak M. Contrast enhanced harmonic

ultrasonography for the evaluation of acute scrotal pathology. A pictorial essay. *Med Ultrason* 2016; 18: 110–5.

7   Sidhu PS, Cantisani V, Dietrich CF, Gilja OH, Saftoiu A, *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 Med* 2018; 39: e2–44.

8   Piscaglia F, Bolondi L. The safety of Sonovue in abdominal applications: retrospective analysis of 23188 investigations. *Ultrasound Med Biol* 2006; 32: 1369–75.

9   Dietrich CF, Nolsøe CP, Barr RG, Berzigotti A, Burns PN, *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; 46: 2579–604.

10 Ding JM, Zhou Y, Wang YD, Zhou HY, Jing X. Clinical value of contrast enhanced ultrasound in differential diagnosis of early hepatocellular carcinoma and dysplastic nodules. *Adv Ultrasound Diagn Ther* 2017; 1: 14–19.

11 Bertolotto M, Muça M, Currò F, Bucci S, Rocher L, *et al.* Multiparametric US for scrotal diseases. *Abdom Radiol (NY)* 2018; 43: 899–917.

12 Auer T, De Zordo T, Dejaco C, Gruber L, Pichler R, *et al.* Value of multiparametric US in the assessment of intratesticular lesions. *Radiology* 2017; 285: 640–9.

13 Adler DD, Carson PL, Rubin JM, Quinn-Reid D. Doppler ultrasound color flow imaging in the study of breast cancer: preliminary findings. *Ultrasound Med Biol* 1990; 16: 553–9.

14 Sidhu PS, Cantisani V, Dietrich CF, Gilja OH, Saftoiu A, *et al.* The EFSUMB guidelines and recommendations for the clinical practice of contrast-enhanced ultrasound (CEUS) in non-hepatic applications: update 2017 (short version). *Ultraschall Med* 2018; 39: 154–80.

15 Kachramanoglou C, Rafailidis V, Philippidou M, Bertolotto M, Huang DY, *et al.* Multiparametric sonography of hematologic malignancies of the testis: grayscale, color Doppler, and contrast-enhanced ultrasound and strain elastographic appearances with histologic correlation. *J Ultrasound Med* 2017; 36: 409–20.

16 Huang DY, Sidhu PS. Focal testicular lesions: colour Doppler ultrasound, contrast-enhanced ultrasound and tissue elastography as adjuvants to the diagnosis. *Br J Radiol* 2012; 85: S41–53.

17 Luzurier A, Maxwell F, Correas JM, Benoit G, Izard V, *et al.* Qualitative and quantitative contrast-enhanced ultrasonography for the characterisation of non-palpable testicular tumours. *Clin Radiol* 2018; 73: 322.e1–9.

18 Cantisani V, Bertolotto M, Weskott HP, Romanini L, Grazhdani H, *et al.* Growing indications for CEUS: the kidney, testis, lymph nodes, thyroid, prostate, and small bowel. *Eur J Radiol* 2015; 84: 1675–84.

19 Trillaud H, Bruel JM, Valette PJ, Vilgrain V, Schmutz G, *et al.* Characterization of focal liver lesions with SonoVue-enhanced sonography: international multicenter-study in comparison to CT and MRI. *World J Gastroenterol* 2009; 15: 3748–56.

20 Wilson SR, Feinstein SB. Introduction: 4th 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; 46: 3483–4.

21 Barrisford GW, Kreydin EI, Preston MA, Rodriguez D, Harisighani MG, *et al.* Role of imaging in testicular cancer: current and future practice. *Future Oncol* 2015; 11: 2575–86.

22 Cheng L, Albers P, Berney DM, Feldman DR, Daugaard G, *et al.* Testicular cancer. *Nat Rev Dis Primers* 2018; 4: 29.

23 Gilligan T, Lin DW, Aggarwal R, Chism D, Cost N, *et al.* Testicular cancer, version 2.2020, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2019; 17: 1529–54.

24 Drudi FM, Valentino M, Bertolotto M, Malpassini F, Maghella F, *et al.* CEUS time intensity curves in the differentiation between leydig cell carcinoma and seminoma: a multicenter study. *Ultraschall Med* 2016; 37: 201–5.

25 Dumic M, Duspara V, Grubic Z, Oguic SK, Skrabic V, *et al.* Testicular adrenal rest tumors in congenital adrenal hyperplasia-cross-sectional study of 51 Croatian male patients. *Eur J Pediatr* 2017; 176: 1393–404.

26 Herde RF, Kokeny KE, Reddy CB, Akerley WL, Hu N, *et al.* Primary pulmonary carcinoid tumor: a long-term single institution experience. *Am J Clin Oncol* 2018; 41: 24–9.

27 Lock G, Schröder C, Schmidt C, Anheuser P, Loening T, *et al.* Contrast-enhanced ultrasound and real-time elastography for the diagnosis of benign Leydig cell tumors of the testis - a single center report on 13 cases. *Ultraschall Med* 2014; 35: 534–9.

28 Anheuser P, Kranz J, Stolle E, Höflmayer D, Büscheck F, *et al.* Testicular epidermoid cysts: a reevaluation. *BMC Urol* 2019; 19: 52.

29 Bhatt S, Jafri SZ, Wasserman N, Dogra VS. Imaging of non-neoplastic intratesticular masses. *Diagn Interv Radiol* 2011; 17: 52–63.

30 Schröder C, Lock G, Schmidt C, Löning T, Dieckmann KP. Real-time elastography and contrast-enhanced ultrasonography in the evaluation of testicular masses: a comparative prospective study. *Ultrasound Med Biol* 2016; 42: 1807–15.

Supplementary Table 1: Clinical characteristics of 47 patients with testicular lesions

Clinical characteristics (n=47)	Age (median year)	Clinical symptoms			Physical examination	
		None	Pain	Falling distension	Palpable	Nonpalpable
Neo (n=31)						
Seminoma (n=11)	39	7	2	2	8	3
NSGCT (n=8)	33	5	2	1	7	1
Lymphoma (n=8)	66	5	2	1	6	2
Leydig cell tumor (n=2)	57	2	0	0	1	1
Nonspecific tumor (n=2)	26	2	0	0	2	0
Nonneo (n=16)						
Infarction (n=8)	22	1	7	0	6	2
Epidermoid cyst (n=3)	24	3	0	0	2	1
Inflammation (n=5)	72	1	4	0	3	2
P (neo vs nonneo)	0.834	0.003			0.770	

NSGCT: nonseminomatous germ cell tumor; Neo: neoplasms; Nonneo: nonneoplasms

Supplementary Table 2: Reviewing results of conventional ultrasound between Observer I and Observer II

Observer II	Observer I					
	1	2	3	4	5	Total (n=47)
1	2 (4.3)	0	0	0	0	2 (4.3)
2	0	2 (4.3)	0	0	0	2 (4.3)
3	0	0	8 (17.0)	1 (2.1)	0	9 (19.1)
4	0	0	1 (2.1)	17 (36.2)	0	18 (38.3)
5	0	0	1 (2.1)	6 (12.8)	9 (19.1)	16 (34.0)
Total	2 (4.3)	2 (4.3)	10 (21.2)	24 (51.1)	9 (19.1)	47 (100.0)

Chi-Square test value: 62.4; P<0.001; Kappa value: 72.4. Grade 1–5, respectively, represented the diagnosis grading. 1: definite nonneoplasm; 2: possible nonneoplasm; 3: uncertain; 4: possible neoplasm; 5: definite neoplasm

Supplementary Table 3: Reviewing results of contrast-enhanced ultrasound between Observer I and Observer II

Observer II	Observer I					
	1	2	3	4	5	Total (n=47)
1	11 (23.4)	0	0	0	0	11 (23.4)
2	0	1 (2.1)	1 (2.1)	0	0	2 (4.3)
3	0	0	2 (4.3)	0	0	2 (4.3)
4	0	0	0	0	0	0
5	0	0	0	2 (4.3)	30 (63.8)	32 (68.0)
Total	11 (23.4)	1 (2.1)	3 (6.4)	2 (4.3)	30 (63.8)	47 (100.0)

Chi-Square test value: 69.8; P<0.001; Kappa value: 87.4. Grade 1–5, respectively, represented the diagnosis grading. 1: Definite nonneoplasm; 2: Possible nonneoplasm; 3: uncertain; 4: possible neoplasm; 5: definite neoplasm
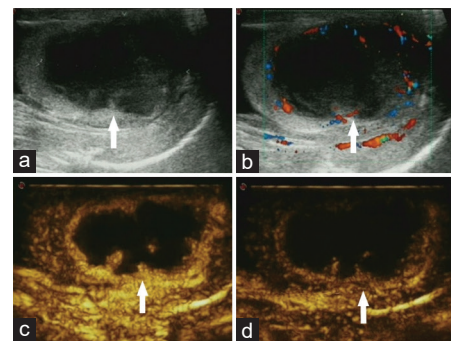
Supplementary Figure 1: Representative images from an 83-year-old man with an abscess on his right side. (**a**) A focal testicular abnormality with hypoechogenicity was noted on grayscale ultrasound (thick arrow). (**b**) On color Doppler flow imaging, a few blood signals were seen at the periphery of the lesion (thick arrow). (**c**) Contrast-enhanced ultrasonography showed no enhancement in the center of the lesion and irregular peripheral hyperenhancement in arterial phase (thick arrow). (**d**) The lesion showed a similar enhancement pattern in venous phase (thick arrow).
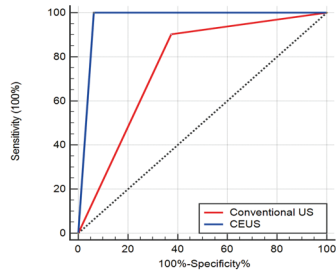
Supplementary Figure 2: Receiver operating characteristic curves for the ability of conventional ultrasound and contrast-enhanced ultrasound to differentiate testicular neoplasms with nonneoplasms, when level 4 was taken as the diagnostic threshold.