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ORIGINAL ARTICLE

Male Reproduction

Multiparametric ultrasound for the assessment of testicular lesions with negative tumoral markers

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The purpose of this study was to evaluate the diagnostic performance of multiparametric ultrasound (mpUS; grayscale US, color Doppler US, strain elastography, and contrast-enhanced US) in the assessment of testicular lesions with negative tumoral markers. MpUS imaging data, patient age, serum tumor markers, scrotal pain, cryptorchidism, and related clinical information were retrospectively collected for patients who underwent mpUS examination between January 2013 and December 2019. Histologic results or follow-up examinations were used as the reference standard. In total, 83 lesions from 79 patients were included in the analysis. Fifty-six patients were finally diagnosed with benign tumors, and 23 patients were ultimately diagnosed with malignant tumors. Chi-square tests or Fisher's exact tests were used to assess the difference between the two groups. Stepwise multivariate logistic regression analysis showed that lesion diameter (odds ratio [OR] = 1.072, $P = 0.005$), vascularization on color Doppler US (OR = 4.066, $P = 0.001$), and hyperenhancement during the early phase (OR = 6.465, $P = 0.047$) were significant independent risk factors for malignancy; however, when compared with neoplastic lesions, pain (OR = 0.136, $P < 0.001$), absence of vascularization on color Doppler US (OR = 1.680, $P = 0.042$), and nonenhancement during the late phase (OR = 3.461, $P = 0.031$) were strongly associated with nonneoplastic lesions. MpUS features are useful for differentiating testicular lesions with negative tumoral markers and improving the preoperative diagnosis, which may avoid inappropriate radical orchiectomy.

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INTRODUCTION

Testicular cancer accounts for approximately 1% of all malignancies in men and is the most common tumor in young men aged 15–35 years.[1] Despite their low incidence, more than 95% of testicular tumors are malignant and mostly occur during the most active sexual function period.[2,3] Orchidectomy is the main treatment modality, but it may have a negative impact on reproductive function.[4] Testis-sparing surgery (TSS) has been advocated in recent years, especially in patients with bilateral and/or multiple lesions or in monorchid patients, and may be attempted in patients with a solitary testis and is often used to preserve fertility and hormonal function.[3–6] Therefore, it is very important to make a distinct differentiation between malignant and benign testicular lesions before surgery and avoid unnecessary total excision. In cases of small or indeterminate testicular masses with negative tumor markers, patients should be offered TSS to avoid the overtreatment of potentially benign lesions and to preserve testicular function.

Conventional ultrasound (US) is essential for the diagnosis of testicular neoplasms. However, differentiation between various testicular malignancies on conventional US remains challenging.[2] Because of the development of modern US techniques, such as high-frequency US, color Doppler US, US elastography, and contrast-enhanced US (CEUS), multiparametric US (mpUS) imaging is considered the preferred imaging technique for testicular diseases.[7,8] The European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) also recommends CEUS to distinguish vascularized from nonvascularized focal testicular lesions, helping to exclude malignancy and discriminate nonviable regions in testicular trauma. CEUS can also identify segmental infarction and abscess formation and infarction in severe epididymo-orchitis.[9] US elastography, on the other hand, provides information about tissue elasticity, whereas grayscale US only provides information about the testicular structure. Increased vascular density and hardness, which can be detected by CEUS and US elastography, respectively, have been reported in malignancies.[10–12] Therefore, familiarity with mpUS will allow practitioners to appropriately use this approach when grayscale US results are not definite and to achieve improved diagnostic accuracy.

For a distinct testicular mass, elevated serum tumor markers may offer assistance to a definitive diagnosis. The most clinically significant tumor markers include alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and lactate dehydrogenase (LDH). They play

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important roles in determining the histologic type of testicular germ cell tumors (TGCTs). An increase in AFP usually occurs in patients with yolk sac components in yolk sac tumors or mixed germ cell tumors.[13] AFP is generally in the normal range in pure seminoma and choriocarcinoma or teratoma. Serum hCG can increase in choriocarcinoma and a small proportion of seminoma cases. Serum LDH is most frequently elevated in testicular tumors and is mainly used to assess the risk of metastasis and treatment outcome.

All three tumor markers discussed above are helpful to determine some malignant pathological types, but not all testicular tumors present with elevated serum tumor markers. Therefore, it is important to find a solution that can be applied for the diagnosis of testicular tumors with negative tumoral markers. Considering the advantages of mpUS, such as high resolution, no radiation, ease of performance, low cost, and multimodal information of lesion vascularity and hardness, we hypothesized that mpUS would aid the diagnosis of such testicular tumors. To confirm this hypothesis, this retrospective study was carried out to evaluate the diagnostic performance of mpUS, including grayscale US, color Doppler US, US elastography, and CEUS, in the assessment of testicular lesions with negative tumoral markers.

PATIENTS AND METHODS

Patients

The institutional review board of Shanghai Tenth People's Hospital of Tongji University (Shanghai, China) approved this retrospective study, and the requirement for informed consent was waived (approval No. SHSY-IEC-5.0/22K128/P01). Between January 2013 and December 2019, 4520 conventional testicular US examinations were performed in 3098 patients at Shanghai Tenth People's Hospital of Tongji University. Patients were included if they met the following criteria: (1) testicular tumors and tumor-like lesions found on mpUS; (2) clinical follow-up (within the first 1–2 weeks, after 3 months, and after 1–2 years) or histologic diagnosis were available and used as the reference standards; and (3) negative for the serum tumor markers (AFP, hCG, and LDH). All patients were evaluated for scrotal pain and cryptorchidism. Patients without sufficient follow-up or histopathology results were excluded.

Grayscale US and color Doppler US

MpUS examinations were performed using a Logic E9 US scanner with a linear probe (6–15 MHz; GE Medical System, Milwaukee, WI, USA). MpUS was performed by the same physician (GX) with more than 8 years of experience in grayscale US and more than 5 years of experience in both US elastography and CEUS. Each lesion was recorded on longitudinal and transverse imaging planes. Standard grayscale presets were used. Color Doppler US (GE Medical System) examination was performed at a low pulse repetition frequency. The signal gain was set as high as possible to maximize the sensitivity to slow blood flow. Grayscale US characteristics, such as lesion diameter, location, shape, margin, echogenicity, presence of cystic degeneration, intralesional calcifications, and parenchymal microlithiasis, were documented. Furthermore, the presence (internal, peripheral, or mixed) or absence of vascularization was recorded by color Doppler US.

US elastography

The planes of maximum diameters were selected for US elastography assessment. Strain elastography (SE) was applied in this study. The region of interest (ROI) included the lesion and enough surrounding normal tissue. If the lesion was too large, contralateral normal testicular tissue was selected for comparison. The pressure on the testis was adjusted according to the visual compression index displayed on the screen. Tissue stiffness was displayed in real time, and the stiffness of the lesion was shown on B-mode images with a color coding overlay. Tissue elasticity was encoded as red (soft), green (intermediate), or blue (hard). As a rule, soft lesions were considered benign, whereas hard lesions were considered malignant.[10–12]

CEUS

The maximum plane, including the whole lesion and surrounding normal tissue, was selected for CEUS. If there was no normal tissue, the contralateral testis was chosen for comparison. The mechanical index was <0.1, and the gain was 100–120 dB. During the examination, the imaging parameters remained the same. SonoVue (Bracco Suisse SA, Milan, Italy) was used as the contrast agent, which was prepared by injecting 5 ml sterile saline before administration. A rapid injection of 2.4 ml SonoVue was then administered via the anterior cubital vein, followed by a 10-ml saline flush. The ROI and surrounding tissue were observed for at least 2 min. During the examination, the selected plane was kept constant, and the probe was stabilized. All the US images were stored on the hard disk in the US machine for subsequent analysis. The image evaluations were evaluated by an independent radiologist (LD) with at least 10 years of experience in testicular lesion diagnosis who was blinded to the patients' clinicopathologic characteristics.

CEUS images included two phases: early phase and late phase. The period from contrast agent injection to 30 s is the early phase, and the period after 30 s is the late phase. The extent of lesional contrast enhancement is classified as hyperenhancement, isoenhancement, hypoenhancement, or nonenhancement in comparison with surrounding testicular tissue (**Supplementary Figure 1**). Hyperenhancement means that the lesion is significantly enhanced before the microbubbles spread to the surrounding parenchyma. Hypoenhancement means enhancement of the lesion is lower than that in the surrounding parenchyma. By comparing the brightness of the lesion after injection of the contrast agent, the homogeneity of lesional contrast enhancement is classified as "heterogeneous" and "homogeneous". Hyperenhancement on CEUS is considered to be a marker of hypervascularization and a standard for neoplasia.

Reference standard

A histologic diagnosis was available for 48 patients who underwent surgical resection. A combination of follow-up US and clinical examination was used as the reference standard in 31 patients. The patients were followed up within the 1st week and then every 3 months for at least 18 months. Testicular lesions were considered benign at the time of final diagnosis if the size and/or structure remained unchanged or the lesions disappeared without other related clinical changes during repeated follow-up. All lesions with histological data were identified by a histopathologist (Xiao Jiang, Department of Pathology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China) with 5 years of experience in the diagnosis of testicular tumors.

Statistical analyses

Quantitative data were reported as the mean ± standard deviation (s.d.). Normally distributed quantitative data were analyzed with an independent samples *t*-test, whereas abnormally distributed data were analyzed by nonparametric tests, such as the Mann–Whitney U test and Wilcoxon's signed rank sum test. Chi-square or Fisher's exact tests were used to compare the frequency distribution of mpUS in different groups. The risk of malignancy was evaluated by multiple logistic regression analysis. Statistical analyses were performed using

SPSS version 22.0 (IBM, Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Clinical data

In total, 83 lesions from 79 patients (age, mean ± s.d.: 45.9 ± 16.1 years, range: 15–75 years) were included in the analysis. All patients with testicular lesions underwent grayscale US scrotal investigation, and 67 underwent SE and CEUS. The levels of serum tumor markers were normal in all patients. Histologic results were available in 48 patients. According to clinical and mpUS follow-up, 31 patients were finally diagnosed as benign. Bilateral lesions were found in four patients. One patient had a bilateral seminoma (left size: 14 mm, and right size: 28 mm), one patient had a bilateral adenocarcinoma that originated from intestinal metastases (left size: 33 mm, and right size: 36 mm), one patient had a bilateral nonspecific benign lesion (left size: 6 mm, and right size: 10 mm), and one patient had a nonspecific benign lesion and hematoma (left size: 9 mm, and right size: 56 mm).

Of the 79 patients, 22 (27.8%) patients had benign tumors, 23 (29.1%) had malignant tumors, and 34 (43.0%) had nonneoplastic lesions. Seminoma was the most frequent malignant tumor type. The diameter (mean±s.d.) was 37.8 ± 18.0 mm (range: 14–103 mm) for malignant tumors, 15.6 ± 12.0 mm (range: 15–56 mm) for benign tumors, and 25.4 ± 15.6 mm (range: 5–68 mm) for nonneoplastic lesions. Malignant lesions tended to be larger than benign lesions ($P < 0.001$). Nonneoplastic lesions were mostly inflammation or infarction (27/34, 79.4%); most patients had testicular pain (22/34, 64.7%). Pain in patients with neoplastic lesions (9/31, 29.0%) was significantly different from that in patients with nonneoplastic lesions (22/31, 71.0%; $P < 0.001$). All cryptorchidism occurred in patients with neoplastic lesion, and the incidence of cryptorchidism significantly differed between patients with neoplastic lesions and those with nonneoplastic lesions ($P = 0.049$). However, there was no significant difference in age or location. The clinical characteristics of the 79 patients are shown in **Table 1**.

Conventional US

The features of all testicular lesions observed using mpUS are shown in **Table 2**. Of the 83 lesions, 15 were irregular in 25 malignant lesions (60.0%), while 27 were irregular in 58 benign lesions (46.6%; $P = 0.005$). Almost all malignant tumors appeared markedly hypoechoic (19/25, 76.0%), whereas a significantly lower number of benign lesions appeared hypoechoic (24/58, 41.4%; $P = 0.029$). Grayscale US revealed that all isoechoic lesions were benign. However, there was no significant difference between neoplastic and nonneoplastic lesions ($P = 0.765$). Internal vascularization and mixed vascularization were both common to malignant lesions (24/25, 96.0%), whereas the absence of vascularization was a typical feature of benign lesions (32/58, 55.2%) or nonneoplastic lesions (19/35, 54.3%; both $P < 0.001$). No parenchymal microlithiasis was found in nonneoplastic lesions. The appearance of margins, cystic degeneration, intralesional calcifications, and parenchymal microlithiasis was not significantly different among the groups (all $P > 0.05$).

US elastography

All (20/20) malignant tumors and 60.5% (23/38) of neoplastic lesions showed an increase in tissue stiffness on SE. The stiffness significantly

Table 1: Clinical data for all patients and lesions with negative tumoral markers in comparison with the reference diagnosis

Final diagnosis	*Patients (n)*	*Age (year)*[a]	*Diameter (mm)*[a]	*Location (n)*			*Cryptorchidism (n)*	*Pain (n)*	*Reference standard (n)*	
				L	*R*	*Bi*			*Histology*	*Follow-up*
All patients	79	45.9±16.1	26.4±17.5	38	37	4	5	31	48	31
Neoplastic lesions	45	46.5±15.5	27.1±18.9	22	20	3	5*	9	31	14
Malignant tumors	23	45.2±16.0	37.8±18.0*	12	9	2	3	4	23	0
Seminoma	14	37.5±9.1	41.8±21.1	8	5	1	1	3	14	0
Lymphoma	2	59.5±13.4	25.0±14.1	0	2	0	0	0	2	0
Leiomyosarcoma	1	48.0	14.0	0	1	0	0	0	1	0
Mixed germ cell tumor	1	68.0	48.0	1	0	0	0	0	1	0
Neuroendocrine tumor (G2)	1	21.0	38.0	0	1	0	0	0	1	0
Adenocarcinoma	2	67.5±1.7	31.7±5.1	1	0	1	1	1	2	0
Myoepithelial carcinoma	1	36.0	35.0	1	0	0	1	0	1	0
Sex cord stromal tumor (low-grade)	1	72.0	45.0	1	0	0	0	0	1	0
Benign tumors	22	47.9±15.3	15.6±12.0	10	11	1	1	5	8	14
Epidermoid cyst	3	32.3±2.1	23.7±10.8	2	1	0	0	1	3	0
Leydig cell tumor	2	69.5±6.4	20.5±10.6	1	1	0	0	2	2	0
Benign spindle cytopathic disease	1	63.0	29.0	1	0	0	0	0	1	0
Cyst	2	56.0±4.2	31.0±35.4	2	0	0	0	1	2	0
Nonspecific benign lesion[b]	14	46.1±14.6	10.3±4.2	4	9	1	1	1	0	14
Nonneoplastic lesions	34	45.1±17.0	25.4±15.6	16	17	1	0	22*	17	17
Partial infarction	7	37.7±19.6	34.4±11.8	5	2	0	0	7	7	0
Orchitis	17	43.2±15.8	18.9±15.0	8	9	0	0	9	2	15
Tuberculosis	2	67.0±4.2	39.5±26.2	1	1	0	0	1	2	0
Abscess	1	43.0	39.0	0	1	0	0	1	1	0
Hematoma	5	47.3±18.4	26.2±15.7	2	2	1	0	4	4	1
Effusion	2	59.0±2.8	26.5±6.4	0	2	0	0	0	1	1

[a]The value is expressed as mean±s.d. when the number of patients is more than 1. [b]Lesions with benign criteria (reduction in lesion size, disappearance of the lesion, or no significant change in 18 months), but not further characterized. *P<0.001 (neoplastic lesions *vs* nonneoplastic lesions). Bi: bilateral; L: left testis; R: right testis; s.d.: standard deviation

differed between malignant tumors and benign tumors or between neoplastic and nonneoplastic lesions (both $P < 0.001$), as shown in **Table 2**.

CEUS

More malignant tumors showed hyperenhancement during the early phase than benign lesions ($P < 0.001$). The general characteristics of nonneoplastic lesions were nonenhancement during the early phase compared with the surrounding parenchyma (14/29, 48.3%) and nonenhancement during the late phase (21/29, 72.4%). All 20 malignant tumors (100.0%) showed iso- or hyperenhancement during the early phase, and 19 (95.0%) showed hyperenhancement during the early phase (**Table 2** and **Figure 1**). Nonenhancement during the late phase was a typical feature of benign lesions (29/47, 61.7%; **Figure 2**), but not for malignant lesions (6/20, 30.0%; $P = 0.005$; **Table 2**). Thirty-one point nine percent (15/47) of benign lesions (*i.e.*, benign tumors and nonneoplastic lesions) showed iso- or hypoenhancement during the early phase compared with the parenchyma, 38.3% (18/47) showed nonenhancement during the early phase, and 29.8% (14/47) showed hyperenhancement during the early phase (**Figure 3**). The enhancement pattern was not significantly different between benign lesions and malignant tumors ($P = 0.798$) or between neoplastic and nonneoplastic lesions ($P = 0.267$).

Multivariate logistic regression analysis

Stepwise multivariate logistic regression analysis showed that lesion diameter (odds ratio [OR] = 1.072, $P = 0.005$), vascularization on color Doppler US (OR = 4.066, $P = 0.001$), and hyperenhancement during the early phase (OR = 6.465, $P = 0.047$) were significant independent risk factors for malignancy; however, when compared with neoplastic lesions, pain (OR = 0.136, $P < 0.001$), absence of vascularization on color Doppler US (OR = 1.680, $P = 0.042$), and nonenhancment during the late phase (OR = 3.461, $P = 0.031$) were strongly associated with nonneoplastic lesions (**Table 3**).

DISCUSSION

Previous studies[7,14] have reported that conventional US could play an important role in characterizing testicular lesions. The detected sensitivity is reported to be almost 100% when a combination of grayscale US and color Doppler US is used. However, the diagnostic sensitivity of conventional US is relatively low since these techniques do not definitely distinguish testicular cancer from benign lesions (*i.e.*, benign tumors or nonneoplastic lesions), such as partial infarction, orchitis, hematoma, tuberculosis, or Leydig cell tumor (LCT).[15] Although conventional US is able to detect most testicular tumors, it has

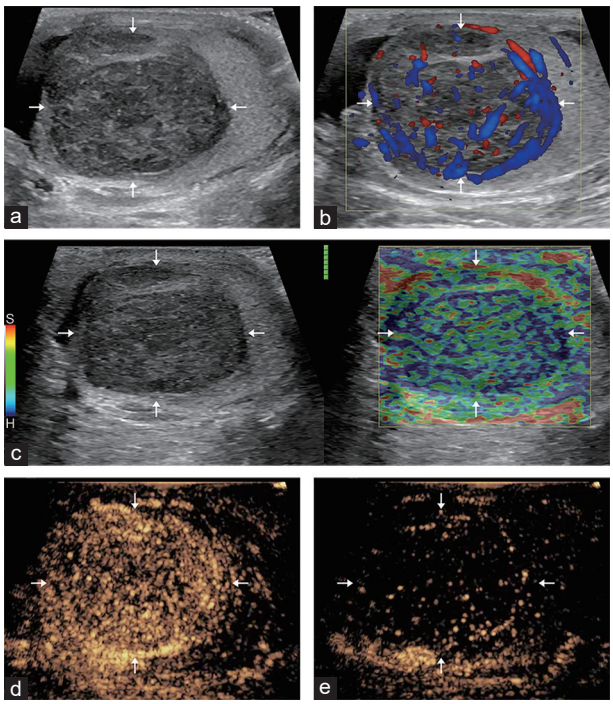

Figure 1: A 38-year-old male with a right testicular seminoma. (**a**) Grayscale US image shows a 38-mm, regular, margin circumscribed, and hypoechoic lesion (arrowheads) in the right testis. (**b**) Color Doppler US image shows mixed (peripheral and central) vascularization. (**c**) Strain elastography image shows a medium-to-hard (encoded blue–green) focal lesion. CEUS image shows (**d**) homogeneous hyperenhancement during the early phase and (**e**) isoenhancement during the late phase relative to the adjacent parenchyma. The arrows indicate testicular lesion seminoma. US: ultrasound; CEUS: contrast-enhanced US; S: soft; H: hard.

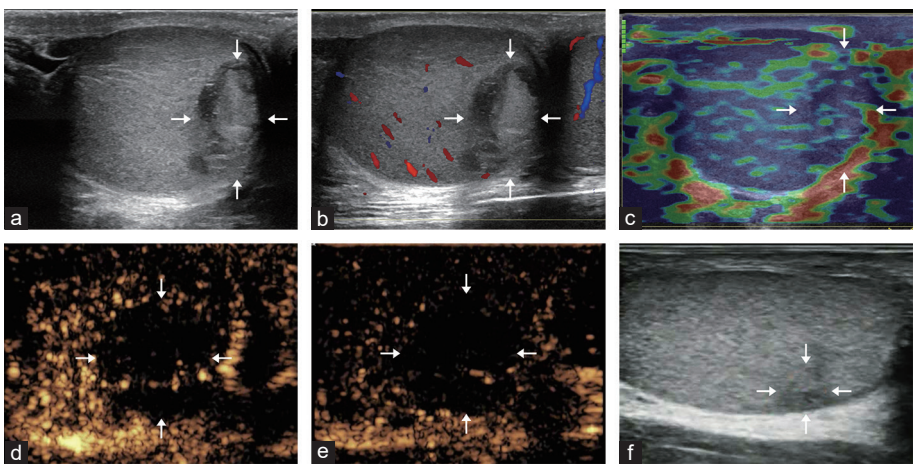

Figure 2: A 31-year-old male with scrotal trauma. (**a**) Grayscale US image shows a 21-mm, irregular, non-circumscribed margin, and hypoechoic lesion (arrowheads) in the right testis. (**b**) Color Doppler US image shows absence of vascularization. (**c**) Strain elastography image shows a medium-to-hard (encoded blue–green) focal lesion. (**d**) Early and (**e**) late phases CEUS show the lesion with nonenhancement. (**f**) The lesion decreases in size significantly one month later. Three months later, the lesion disappears completely. Finally, it is confirmed to be a testicular hematoma. The arrows indicate testicular hematoma. US: ultrasound; CEUS: contrast-enhanced US.

Table 2: Diagnostic performance of multiparametric ultrasound in the differential diagnosis of testicular lesions with negative tumoral markers

Parameter	*Neoplastic lesions, n (%)*		*Nonneoplastic lesions, n (%)*	*P*	
	Malignant tumors	*Benign tumors*		*Benign lesions vs malignant tumors*	*Neoplastic lesions vs nonneoplastic lesions*
Conventional US	25 (30.1)	23 (27.7)	35 (42.2)		
Shape				0.005	0.622
Regular	10 (40.0)	19 (82.6)	12 (34.3)		
Irregular	15 (60.0)	4 (17.4)	23 (65.7)		
Margin				0.443	0.48
Circumscribed	16 (64.0)	19 (82.6)	23 (65.7)		
Noncircumscribed	9 (36.0)	4 (17.4)	12 (34.3)		
Nodule echogenicity				0.029	0.765
Mixed	5 (20.0)	9 (39.2)	14 (40.0)		
Hypoechoic	19 (76.0)	8 (34.8)	16 (45.7)		
Hyperechoic	1 (4.0)	3 (13.0)	3 (8.6)		
Isoechoic	0 (0)	3 (13.0)	2 (5.7)		
Cystic degeneration				0.207	0.768
Absent	23 (92.0)	17 (73.9)	30 (85.7)		
Present	2 (8.0)	6 (26.1)	5 (14.3)		
Vascularization				<0.001	0.006
Absent	0 (0)	13 (56.6)	19 (54.3)		
Internal	13 (52.0)	8 (34.8)	9 (25.7)		
Peripheral	1 (4.0)	1 (4.3)	5 (14.3)		
Mixed (peripheral and internal)	11 (44.0)	1 (4.3)	2 (5.7)		
Intralesional calcifications				0.740	0.637
Absent	23 (92.0)	21 (91.3)	31 (88.6)		
Present	2 (8.0)	2 (8.7)	4 (11.4)		
Parenchymal microlithiasis				0.160	0.132
Absent	23 (92.0)	22 (95.7)	35 (100.0)		
Present	2 (8.0)	1 (4.3)	0 (0)		
US elastography	20 (29.9)	18 (26.9)	29 (43.2)	<0.001	0.035
Soft	0 (0)	15 (83.3)	19 (65.5)		
Hard	20 (100.0)	3 (16.7)	10 (34.5)		
Contrast-enhanced US	20 (29.9)	18 (26.9)	29 (43.2)		
Early phase				<0.001	0.002
Nonenhancement	0 (0)	4 (22.2)	14 (48.3)		
Iso- or hypoenhancement	1 (5.0)	11 (61.1)	4 (13.8)		
Hyperenhancement	19 (95.0)	3 (16.7)	11 (37.9)		
Late phase				0.005	0.010
Nonenhancement	6 (30.0)	8 (44.4)	21 (72.4)		
Iso- or hyperenhancement	11 (55.0)	10 (55.6)	8 (27.6)		
Hypoenhancement	3 (15.0)	0 (0)	0 (0)		
Enhancement pattern				0.798	0.267
Heterogeneous	7 (35.0)	5 (27.8)	13 (44.8)		
Homogeneous	13 (65.0)	13 (72.2)	16 (55.2)		

Neoplastic lesions: benign tumors and malignant tumors; benign lesions: benign tumors and nonneoplastic lesions; US: ultrasound

some limitations,[9,16] especially in small testicular lesions or the absence of elevated tumor markers. For instance, color Doppler US has some technical limitations, such as a poor signal-to-noise ratio and spatial resolution; thus, it may have difficulty in identifying small vessels, small lesions, or lesions with low-velocity blood flow. Microvascular US, the latest development of color Doppler technology, is a noninvasive microvascular imaging technique using low-flow Doppler signal processing.[17] Microvascular US improves the detection of slow and fine vascular flows.[18,19] Yang *et al.*[20] found that a vascular sign of a linear nonbranching pattern on microvascular US provided some help for the early noninvasive diagnosis of primary testicular lymphoma.

CEUS significantly improves the detection of vascular flow in testicular lesions and is more sensitive than color Doppler US.[9] In the present study, we found that CEUS revealed specific properties of testicular tumors that more malignant tumors showed hyperenhancement during the early phase than benign lesions. In 28 cases, the absence of vascular lesion was proven to be benign, as observed by CEUS, and nonenhancement could be interpreted as a strong evidence of a benign lesion. Consistent with previous studies,[16,21] malignant tumors tended to show hyperenhancement during the early phase. This is helpful for the differential diagnosis of testical lesions.[22] CEUS quantification of testicular tumors remains an evaluation tool. Lung *et al.*[23] found that contrast-enhanced time perfusion dynamic is helpful for the differential diagnosis between malignant tumors and

Table 3: Results of multivariate logistic regression analysis

Variable	*Benign lesions vs malignant tumors*			*Neoplastic lesions vs nonneoplastic lesions*		
	B (s.e.)	*OR (95% CI)*	*P*	*B (s.e.)*	*OR (95% CI)*	*P*
Diameter	0.070 (0.025)	1.072 (1.021–1.125)	0.005	–0.007 (0.017)	NS	0.682
Cryptorchidism	1.879 (1.343)	NS	0.162	21.496 (16718.939)	NS	0.999
Pain	–1.758 (0.838)	0.172 (0.033–0.891)	0.036	–1.997 (0.545)	0.136 (0.047–0.395)	<0.001
Conventional US						
Irregular shape	1.096 (0.824)	NS	0.184	–0.105 (0.605)	NS	0.862
Hypoechoic	–1.194 (0.543)	0.303 (0.104–0.879)	0.028	–0.258 (0.299)	NS	0.389
Parenchymal microlithiasis	2.213 (3.077)	NS	0.490	21.611 (19527.624)	NS	0.999
Vascularization	1.403 (0.407)	4.066 (1.831–9.026)	0.001	0.519 (0.255)	1.680 (1.019–2.770)	0.042
US elastography						
Hard	20.664 (6360.043)	NS	0.997	0.677 (0.640)	NS	0.290
Contrast-enhanced US						
Early phase hyperenhancement	1.866 (0.939)	6.465 (1.026–40.728)	0.047	0.315 (0.407)	NS	0.438
Late phase nonenhancement	1.159 (0.745)	NS	0.120	1.242 (0.576)	3.461 (1.120–10.692)	0.031

s.e.: standard error; NS: not significant; OR: odds ratio; CI: confidence interval; US: ultrasound; neoplastic lesions: benign tumors and malignant tumors; benign lesions: benign tumors and nonneoplastic lesions

benign lesions, whereas enhanced intensity is helpful for the differential diagnosis between neoplastic and nonneoplastic lesions. Using time–intensity curves, evaluating the wash-in and wash-out curves may help distinguish malignant from benign tumors. Yu *et al*.[24] found that TGCTs 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 early phase; and the occurrence rates for those signs in both seminomas and nonseminoma germ cell tumors (NSGCTs) were 100.0%, 100.0%, 73.7%, 94.7%, and 100.0%, respectively. Although these results are promising, both qualitative and quantitative CEUS analyses overlap between different histological types.[25] In our study, a mixed germ cell tumor (90% were malignant teratomas, and 10% were seminoma) showed heterogeneous isoenhancement, probably due to intralesional calcification and cystic degeneration. The contrast agent dynamics were different in different histologic groups.

US elastography is used in the diagnosis of various cancers, such as breast and prostate cancer.[26,27] As a rule, most malignant tumors exhibit increased stiffness because they have a higher density of cells and blood vessels than surrounding normal tissues.[28] Therefore, we used SE to identify and characterize testicular lesions with negative tumoral markers. SE revealed increased stiffness in all 20 malignant lesions. It alone had a high sensitivity (20/20, 100.0%) but only moderate specificity (34/47, 72.3%). Some benign lesions, especially nonneoplastic lesions, could also show increased stiffness. In the current study, eight suspected lesions showed increased stiffness but were correctly evaluated as benign lesions after CEUS showed nonenhancement. The diagnostic accuracy can be further improved by combining CEUS with SE.[29] CEUS is superior to other methods in the characterization of testicular lesions. Our study does not support the routine use of SE in differentiating between benign and malignant testicular tumors or in differentiating between TGCTs and non-TGCTs.

In our study, two cases of LCT were misdiagnosed as malignant, of which one was with a diameter of 28 mm in a 74-year-old patient and the other was with a diameter of 13 mm in a 65-year-old patient. Both lesions presented homogeneous hyperenhancement on CEUS. This is thought to be due to the high vascularization of LCT, local estrogen production, and increased levels of endocrine gland vascular endothelial growth factor in Leydig cells.[30] It is difficult to differentiate LCT from nonseminoma. According to previous studies, in comparison with seminoma, LCT showed more sustained enhancement in late phase[31] and more obvious hyperenhancement in the early phase.[32,33] Although these results are promising, both qualitative and quantitative CEUS analyses showed overlap between them. In this study, the two patients were older than 60 years old, with medium lesion size and negative tumor markers. Simple excision of testicular tumors could replace orchiectomy in these patients. Patients who undergo surgery for small lesions should be advised that such lesions are most likely benign. In general, mpUS offers

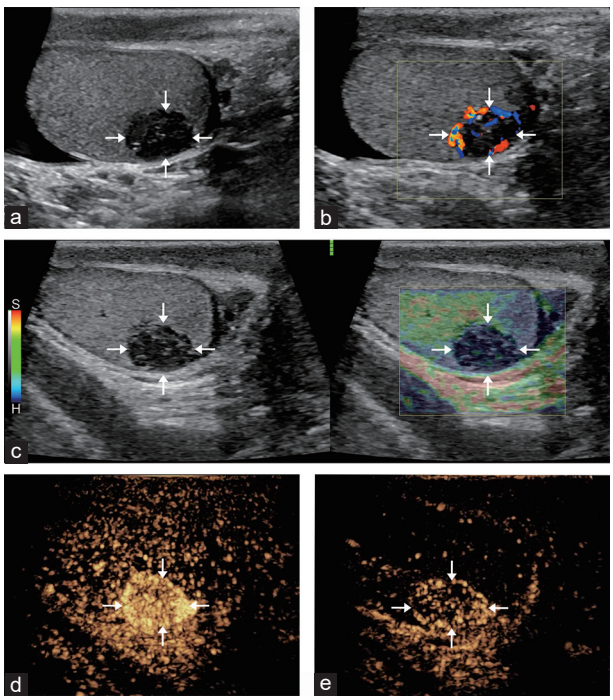

Figure 3: Leydig cell tumor in a 65-year-old male patient. The case is misdiagnosed as malignant prior to surgery. (**a**) Grayscale US image shows a 13-mm, regular, circumscribed margin, and hypoechoic lesion (arrowheads) in the left testis. (**b**) Color Doppler US image shows mixed (peripheral and central) vascularization. (**c**) Strain elastography image shows a mainly hard lesion (encoded blue). CEUS shows (**d**) homogeneous hyperenhancement during the early phase and (**e**) hyperenhancement during the late phase relative to the adjacent parenchyma. The arrows indicate testicular Leydig cell tumor. US: ultrasound; CEUS: contrast-enhanced US; S: soft; H: hard.

additional value over conventional US in clearly assessing the presence (or absence) of blood perfusion in testicular lesions and in providing additional information on tissue stiffness to distinguish abnormal lesion from normal parenchyma. Manganaro *et al*.[34] demonstrated that a low signal intensity on T2-weighted (T2W) images is significantly associated with the benign nature of the lesion. LCT has a typical pattern of hyperenhancement, characterized by homogeneous distribution and rapid and marked wash-in of contrast agent, accompanied by slow and late wash-out. Magnetic resonance imaging (MRI) may provide valuable information for the equivocal results of US.[35] MRI is helpful in the preoperative characterization and staging of TGCTs and may allow the differentiation of benign lesions from TGCTs, thereby allowing reduction of unnecessary surgery. Zhang *et al*.[36] showed that a T2W MRI-based radiomics signature might allow noninvasive differentiation of seminomas from non-TGCTs. Therefore, mpUS combined with MRI may further increase the diagnostic accuracy.

This study had some limitations. First, this was a single-center retrospective study with a small sample size, and only a few types of tumors were included; thus, the results may be biased. Second, owing to the retrospective nature of the study, direct comparative evaluation between various imaging modes could not be carried out, and the evaluation of diagnostic accuracy was qualitative and subjective. Third, mpUS was performed by a single operator, and interobserver agreement could not be evaluated. Fourth, we did not perform quantitative analysis for US elastography or CEUS, such as specific stiffness values, strain ratio, or time–intensity curves, which might provide additional information. Fifth, the lack of comparison with MRI evaluation was a limitation of the current study. Future studies are required to validate our results and strengthen our proposition to avoid unnecessary orchiectomy in some patients.

In summary, mpUS can reliably make a differentiation between benign and malignant testicular lesions with negative tumoral markers and improve the preoperative diagnosis. However, there is still a considerable imaging overlap between different testicular lesions. Therefore, the patient's age, medical history, tumor marker levels, and other clinical information must be combined with a variety of imaging characteristics in the examination process to make a more accurate diagnosis.

AUTHOR CONTRIBUTIONS

HXX, LPS, and HL carried out the study design. LD carried out the data collection, statistical analysis, and data interpretation. HL drafted the manuscript. LHX, GX, JW, YF, SSD, and YJ conceived of the study, participated in its design and coordination, and helped draft the manuscript. HXX and LPS supervised the study. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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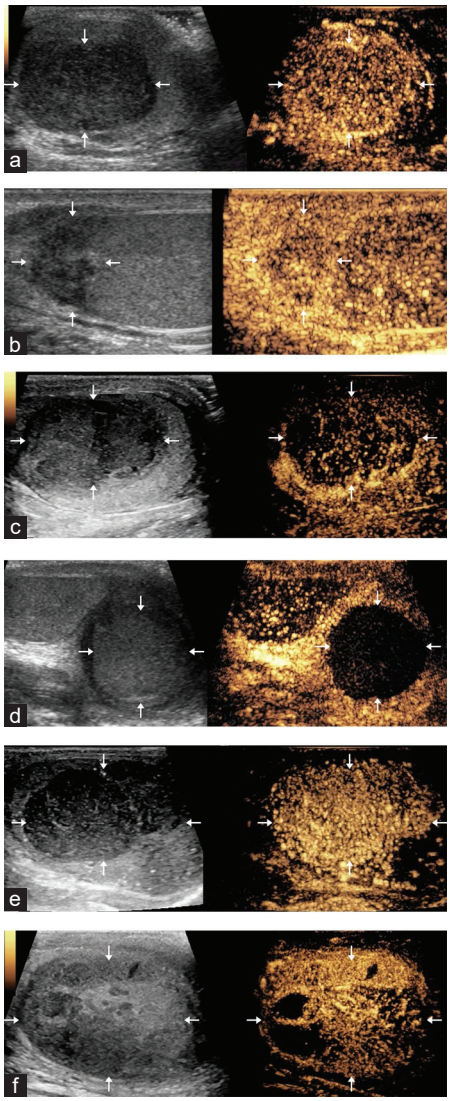

Supplementary Figure 1: The pattern of contrast enhancement on CEUS. (**a**) Hyperenhancement, surgical pathology confirms seminoma; (**b**) isoenhancement, surgical pathology confirms orchitis; (**c**) hypoenhancement, surgical pathology confirms atypical seminoma; (**d**) nonenhancement, surgical pathology confirms testicular torsion and infarction; (**e**) homogeneous, surgical pathology confirms seminoma; (**f**) heterogeneous, surgical pathology confirms testicular tuberculosis. The arrow indicates testicular lesions.