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Diagnostic value of qualitative and strain ratio elastography in the differential diagnosis of non-palpable testicular lesions

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SUMMARY

The purpose of this study was to evaluate prospectively the accuracy of qualitative and strain ratio elastography (SE) in the differential diagnosis of non-palpable testicular lesions. The local review board approved the protocol and all patients gave their consent. One hundred and six patients with non-palpable testicular lesions were consecutively enrolled. Baseline ultrasonography (US) and SE were correlated with clinical and histological features and ROC curves developed for diagnostic accuracy. The non-palpable lesions were all ≤1.5 cm; 37/106 (34.9%) were malignant, 38 (35.9%) were benign, and 31 (29.2%) were non-neoplastic. Independent risk factors for malignancy were as follows: size (OR 17.788; $p = 0.002$), microlithiasis (OR 17.673, $p < 0.001$), intralesional vascularization (OR 9.207, $p = 0.006$), and hypoechogenicity (OR, 11.509, $p = 0.036$). Baseline US had 89.2% sensitivity (95% CI 74.6–97.0) and 85.5% specificity (95% CI 75.0–92.8) in identifying malignancies, and 94.6% sensitivity (95% CI 86.9–98.5) and 87.1% specificity (95% CI 70.2–96.4) in discriminating neoplasms from non-neoplastic lesions. An elasticity score (ES) of 3 out of 3 (ES3, maximum hardness) was recorded in 30/37 (81.1%) malignant lesions ($p < 0.001$). An intermediate score of 2 (ES2) was recorded in 19/38 (36.8%) benign neoplastic lesions and in 22/31 (71%) non-neoplastic lesions ($p = 0.005$ and $p = 0.001$ vs. malignancies). None of the non-neoplastic lesions scored ES3. Logistic regression analysis revealed a significant association between ES3 and malignancy ($\chi^2 = 42.212$, $p < 0.001$). ES1 and ES2 were predictors of benignity ($p < 0.01$). Overall, SE was 81.8% sensitive (95% CI 64.8–92.0) and 79.1% specific (95% CI 68.3–88.4) in identifying malignancies, and 58.6% sensitive (95% CI 46.7–69.9) and 100% specific (95% CI 88.8–100) in discriminating non-neoplastic lesions. Strain ratio measurement did not improve the accuracy of qualitative elastography. Strain ratio measurement offers no improvement over elastographic qualitative assessment of testicular lesions; testicular SE may support conventional US in identifying non-neoplastic lesions when findings are controversial, but its added value in clinical practice remains to be proven.

INTRODUCTION

The use of scrotal US has resulted in an increase in incidentally detected non-palpable testicular lesions, with a sensitivity ranging between 90 and 100% (Woodward *et al.*, 2002; Appelbaum *et al.*, 2013). However, US differentiation between benign and malignant testicular neoplasms and, occasionally, between neoplastic and non-neoplastic incidental lesions is limited (Lotti & Maggi, 2015). The ability to characterize testicular lesions could enable the use of conservative surgery for selected cases, especially for bilateral/multiple lesions or in monorchid patients (Isidori *et al.*, 2014). Until recently, all neoplastic lesions were removed because of the assumption that they were mostly malignant (Coret *et al.*, 1995). Recent studies suggest that benign tumors are much more frequent than previously thought (Isidori *et al.*, 2014). Several uro-andrologists have proposed a conservative approach, with close follow-up (Giannarini *et al.*, 2010). However, no large long-term studies are available on the follow-up of non-operated benign testicular tumors (Toren *et al.*, 2010). For this reason, once malignancy has been excluded, it is useful to establish if the lesion is non-neoplastic or is a benign tumor.

Several tools have been proposed to improve the sensitivity and specificity of US. Contrast-enhanced ultrasound (CEUS) has up to 93% diagnostic accuracy in the differential diagnosis of non-palpable tumors (Isidori *et al.*, 2014). Magnetic resonance imaging (MRI) has also recently proved accurate in the characterization of incidental testicular lesions (Gianfrilli *et al.*, 2009;

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Dieckmann *et al.*, 2013; Tsili *et al.*, 2014; Manganaro *et al.*, 2015).

In the last few years, the US techniques strain elastography (SE) and shear wave elastography have gained impetus as supplementary, commonly available add-on tools for various applications (Itoh *et al.*, 2006; Rago *et al.*, 2010; Correas *et al.*, 2013; Cosgrove *et al.*, 2013; Cantisani *et al.*, 2014, 2015). These techniques provide real-time non-invasive tissue characterization performed at the same time and as an adjunct to conventional US imaging. Shear wave elastography uses conventional ultrasound imaging techniques to analyze the shear waves generated inside the human body by an ultrasound burst; the propagation speed of the shear waves directly correlates with tissue stiffness. Strain elastography detects local deformation (strain) under light pressure. Its output include a color-coded representation (qualitative assessment) of the lesion and a semi-quantitative characterization by strain ratio, calculated as the ratio of the surrounding parenchyma to the lesions and providing a measure of lesion stiffness. SE has been successfully used in the evaluation of acute scrotal pathology (Shah *et al.*, 2010; Patel *et al.*, 2014; Yusuf *et al.*, 2015). However, to date, only a few studies (Schurich *et al.*, 2009; Grasso *et al.*, 2010; Aigner *et al.*, 2012; Huang & Sidhu, 2012; Patel *et al.*, 2012; Marsaud *et al.*, 2015) and just one prospective study (Goddi *et al.*, 2012) have explored the role of SE in focal testicular lesions. Furthermore, only one retrospective study has assessed the value of strain ratio measurements in neoplastic testicular lesions (Pastore *et al.*, 2014).

Our purpose was to evaluate the accuracy of qualitative and strain ratio elastography in the differential diagnosis of non-palpable testicular lesions in a prospective study, including its ability to discriminate benign from malignant tumors and its advantages over baseline US.

MATERIALS AND METHODS

Study design and population

The local review board approved the protocol and all patients gave their written informed consent. This prospective study, carried out from September 2009 to September 2014, was conducted at the Sapienza University, using the STARD checklist flow diagram. From the 4615 inpatients and outpatients referred for scrotal ultrasound, 162 were diagnosed with at least one solid testicular lesion (inclusion criterion). Of these, 56 had a palpable lesion and were excluded because of the high pre-test probability of malignancy (exclusion criterion) (Isidori *et al.*, 2014); following surgical exploration, the lesion was confirmed as malignant in 42/56 (75%). The remaining 106 men with incidental, non-palpable testicular lesions were included in the study (Fig. 1).

The diagnostic protocol included routine blood tests, hormonal investigations, and tumor markers [human chorionic gonadotropin (β-HCG), placental alkaline phosphatase (PLAP), alpha-fetoprotein (α-FP), carcinoembryonic antigen (CEA), ferritin, lactate dehydrogenase (LDH)]. Elastography was performed after conventional US. Tissue-sparing surgical enucleation was suggested for histological confirmation of all lesions, as part of the protocol. All patients with solid lesions were sent for enucleation with the awareness that some of them might have turned out to be benign, as current guidelines do not address the management of solid, potentially neoplastic lesions. If the lesion was found malignant on frozen section, the procedure was converted into a radical orchiectomy; otherwise, the testis was left in place (tissue-sparing surgery). Compared to other studies, the current protocol gave a higher number of histologically confirmed benign lesions. Surgeons and pathologists performed the diagnostic procedures independently of the SE results. The primary outcome of the study was to assess the diagnostic accuracy of qualitative and strain ratio elastography in the differential diagnosis of non-palpable testicular lesions. Secondary outcomes included the validation of previously identified risk factors for malignancy at baseline US (Isidori *et al.*, 2014).

Baseline US and strain elastography examination

Baseline US and SE examinations were performed using a Philips IU22 unit (Philips, Bothell, WA, USA) with a 7–15 MHz wideband linear transducer. Standardized protocols with axial and transverse examinations of the lesions were performed (Oyen, 2002, Bhatt *et al.*, 2006; American Institute of Ultrasound in M edicine *et al.* 2011; Lotti *et al.*, 2013). Two experienced sonographers (A.M.I. and C.P., both with >5 years of experience) performed all US and SE examinations and stored the images and loops for subsequent analysis by two other readers (D.G. and V.C., both with >5 years of experience). US features were evaluated according to a predefined protocol, taking into account the most widely accepted risk factors for testicular tumors (Isidori *et al.*, 2014). For each lesion, the size (cm), B-mode, and color-Doppler features were recorded and stored; the elastography settings were then applied and SE images and cine-loops of each lesion were saved for qualitative (color-coded images) and semi-quantitative evaluation of dynamic features (strain ratio on cine-loop). To assess the elasticity score (ES) of the lesion, the physicians operated the transducer with slight pressure, maintaining contact with skin, and perpendicular to the lesion; the testis was held firm and stabilized with sufficient gel to achieve good US visualization of the whole testis and avoid any loss in image quality and artifacts.

A large elastogram region of interest (ROI) box was used to increase the potential number of samples for a strain ratio calculation; when the compression bar visualized on the screen during the examination to assess the quality and validity of the operator's free hand pressure was stable for at least 5–10 sec, the operator acquired 10-sec cine-loops. Images and cine-loops were transferred to our local picture archive system (Merge-eFilm, Milwaukee, WI, USA) for subsequent independent analysis.

Strain elastography qualitative analysis: elasticity score (ES)

A color scale from red through green to blue is used for the elastograms. Red indicates the highest elastic strain (softest tissue) and blue indicate no strain (hardest tissue). Color-coded elastogram images were graded visually on the stiffness of the nodules relative to the surrounding parenchyma, assigning an elasticity score (ES) to each image. In the absence of validated criteria for the testes, we applied the five-point scale according to Itoh *et al.* (2006), Rago *et al.* (2007, 2010), and Hong *et al.* (2009) previously used for qualitative assessment of breast and thyroid nodules. For completeness, sonograms were also interpreted in light of other published scales (Patel *et al.*, 2012) (data not shown). ES score data were reviewed independently for the entire set by two physicians (D.G. and V.C.). The analyses were performed blinded to the conclusion reached by the physician who performed the exam (A.M.I. and C.P.) and blinded to CDU

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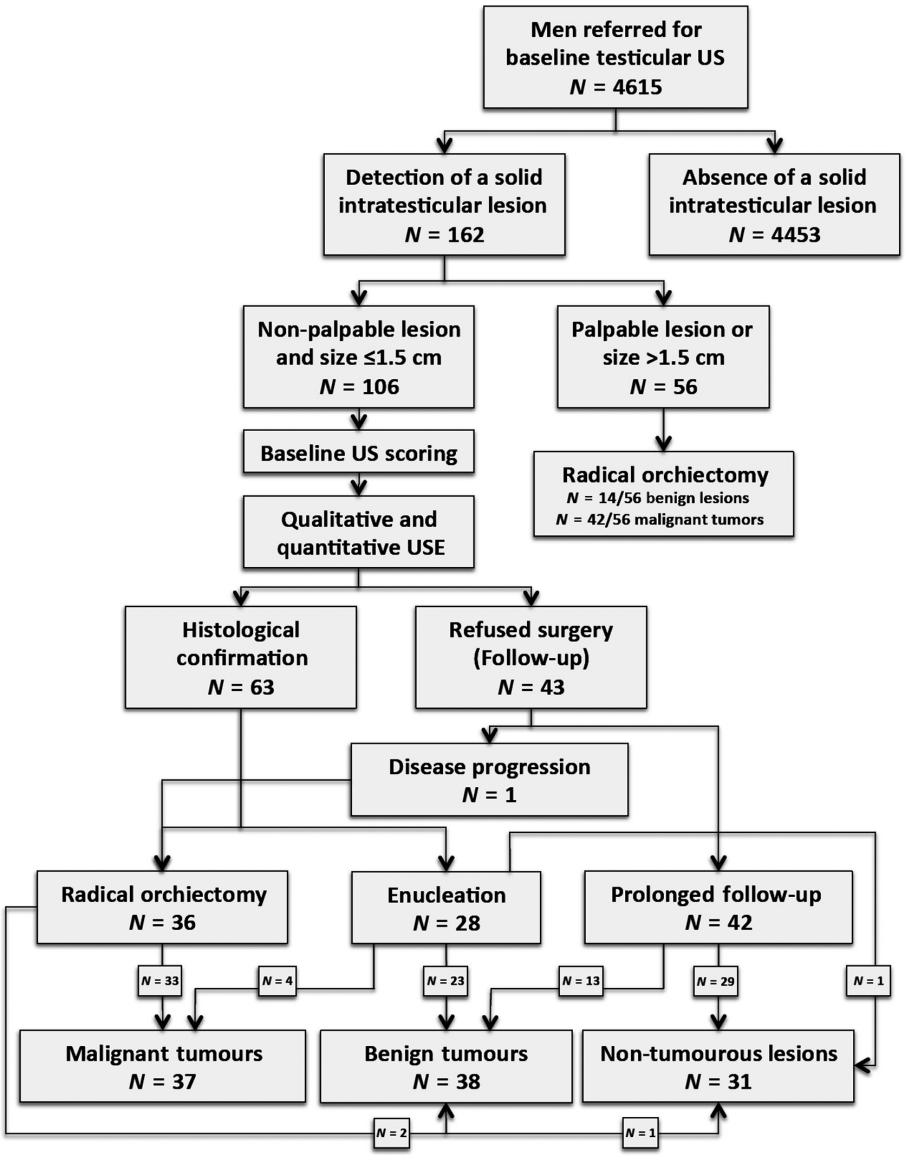

Figure 1 Flow diagram of the study.

features or other clinical features. However, qualitative ES analysis was not entirely blind to simple grayscale because the imaging system used stores side-by-side grayscale US and ES images. The elasticity scores are described in Fig. 2.

Strain elastography semi-quantitative analysis: normal tissue-to-nodule strain ratio (SR)

Elastograms were created using QLab (Philips, Bothell, Wash). Curves were obtained by manually locating a free ROI covering each lesion and an identical ROI on the adjacent parenchyma. The QLab software assesses the degree of distortion of the nodule and surrounding tissue (strain ratio, SR). Two physicians (G.F. and G.B.D.P.) placed the ROI and recorded the parameters: for the entire set, data were collected blinded to the rest of the exam, except for simple grayscale assessment stored simultaneously while acquiring ES images. The cut-off value for strain ratio (SR) was identified using receiver operator curve (ROC) analysis, against the reference diagnosis.

Reference standard: histology and follow-up

Tissue-sparing surgical enucleation was offered for histological confirmation of all solid lesions. When patients declined

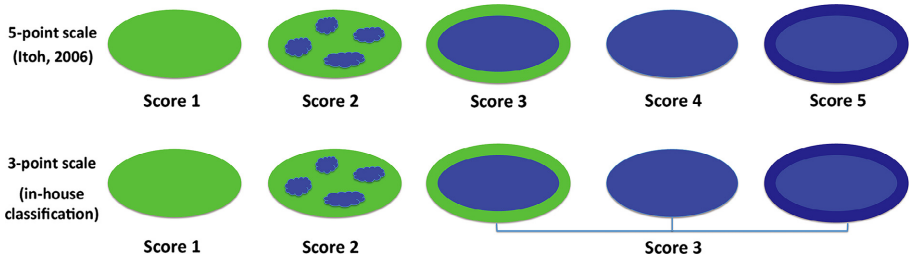

Figure 2 Five-point and three-point elastographic scale.

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surgery and clinical and sonographic findings permitted a 'watchful-waiting' approach, follow-up was with serial US every 3 months for a minimum of 18 months, provided these patients had a previous negative point-of-diagnosis CT or MR examination confirming absent abdominal lymph nodes and a normal chest X-ray or CT.

In the reference diagnosis, we interpreted as non-malignant any lesions histologically confirmed or showing resolution, no growth, no tumor marker rises, or other relevant clinical events at repeated follow-up (stable on ≥6 consecutive scans); among these, we considered all single, entirely solid, hypoechoic lesions with internal vascularization that maintained similar grayscale features and a preserved internal vascularization over time (at least 18 months) as benign neoplasms (stromal tumors). In contrast, any multiple and/or non-vascularized lesions were interpreted as non-neoplastic (Leydig cell hyperplasia, segmental ischemia, fibrosis, cysts) according to a protocol validated by prior histological, CEUS, and MRI published studies (Isidori *et al.*, 2014; Manganaro *et al.*, 2015). Whenever possible, intraoperative frozen section examination (FSE) was performed during surgery (61/64) (Isidori *et al.*, 2014).

Malignant tumors underwent molecular characterization performed by a biologist (F.B., Ph.D. with >6 years' experience in molecular biology). Cytokeratin, beta-human chorionic gonadotropin (β-HCG), placental alkaline phosphatase (PLAP), and alpha-fetoprotein (α-FP) were tested.

Statistical analysis

Independent groups were compared by Mann–Whitney test for continuous variables or odds ratio (OR) confidence intervals for categorical variables. Reliability analysis for qualitative and semi-quantitative parameters was performed by analysis of the intraclass correlation coefficients. Relationships between the qualitative data were examined by chi-square test or Fisher's exact tests. The risk of malignancy was evaluated by binary logistic regression analysis. Receiver operating characteristic (ROC) curve analysis was applied to calculate the cut-off values of SR, through Youden index (J). Sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of qualitative elastography and strain ratio values were calculated using ROC curves with DeLong's method (DeLong *et al.*, 1988). Statistical analyses were performed by SPSS 17.0 (Chicago, IL, USA) using two-tailed significance tests and MEDCALC (12.7.0.0, Ostend, Belgium), with $p \leq 0.05$ considered statistically significant.

RESULTS

Clinical data

Patients were referred for infertility (43/106, 40.6%), screening during andrological prevention campaigns for young men in high schools and universities (21/106, 19.8%), follow-up of a contralateral or ipsilateral tumor (13/106, 12.3%), testicular pain (11/106, 10.4%), varicocele (10/106, 9.4%), Klinefelter's syndrome (5/106, 4.7%), follow-up for microlithiasis (2/106, 1.9%), and detection of elevated levels of alpha-fetoprotein (1/106, 0.9%).

Overall, a histological diagnosis was performed for 64 patients following enucleation ($n = 28$) or total orchiectomy ($n = 36$) performed by a single surgeon (G.F., with >20 years' experience in andrological surgery). Based on the definitive histology and the long-term follow-up of non-operated lesions, 37 of the 106 study subjects had malignant tumors, 38 benign tumors, and 31 non-neoplastic lesions. Leydig cell tumors were the most frequent benign tumors (20/38, 18.9% of the entire set) (Table 1). Malignancies were diagnosed by enucleation in 4/37 cases (three seminomas removed from three monorchid patients for a contralateral tumor plus one atypical Leydig cell tumor), by enucleation followed by orchiectomy in 32 cases, and during follow-up in one case (metastatic embryonal carcinoma). Benign lesions (both neoplastic and non-neoplastic) were diagnosed by enucleation in 24/69 cases, by orchiectomy in three cases (two Leydig cell tumors and one granulomatous orchitis), or because of lack of change during follow-up (42 cases). Malignancies tended to be larger than benign tumors (median, interquartile range: 1.2, 0.6–1.5 vs. 0.5, 0.4–0.7, $p < 0.001$). Groups were not statistically different for age, hormones, or reason for referral. Testicular volumes were in the lower range (right testicular volume: median, interquartile range: 11.2, 8.1–16.5; left testicular volume: median, interquartile range: 11.7, 8.0–15.9).

Tumor markers were normal in all patients except one who was referred because of marginally elevated α-FP levels (which were normal when repeated) and one with embryonal

Table 1 Clinical data (median and interquartile range) of all patients and lesions according to the reference diagnosis

Final diagnosis	n	Age (years)	Diameter (cm)	Testicular volume (mL)	
				R	L
All patients	**106**	**34.5 (28–41.2)**	**0.6 (0.5–1.0)**	**11.2 (8.1–16.5)**	**11.7 (8.0–15.9)**
Malignant tumors	**37**	**35 (29–40.5)**	**1.2 (0.6–1.5)***	**12.0 (9.1–16.7)**	**12.4 (8.1–16.7)**
Pure seminoma	33	36 (29.5–41)	1.2 (0.6–1.5)	11.9 (9.1–16.0)	13.3 (8.1–17.1)
Leydig cell tumor, high grade[a]	1	33	0.5	14	12
Embryonal carcinoma	3	26 (23–26)	1.5 (1.0–1.5)	19.0 (7–19.0)	8.6 (8–8.6)
Benign tumors	**38**	**34.5 (29.7–42)**	**0.5 (0.4–0.7)**	**9.0 (6.7–13.6)**	**9.2 (6.9–14.5)**
Leydig cell tumor	20	39.5 (28.7–42)	0.55 (0.5–0.76)	8.6 (5.2–13.2)	7.9 (4.9–13.8)
Sertoli cell tumor	1	34	0.6	14.3	15.7
Epidermoid cyst	2	21 (19–21)	1.3 (1.3–1.35)	17.2 (14–17.2)	17.6 (14–17.6)
Histology not available	15	34 (30–42)	0.45 (0.4–0.57)	9.0 (6.1–12.6)	9.0 (7.9–13.4)
Non-neoplastic lesions	**31**	**34 (27–44)**	**0.5 (0.4–0.8)**	**12.7 (8.6–18.9)**	**12.0 (9.0–17.2)**
Granulomatous orchitis	1	25	1.5	–	17.5
Histology not available	30	34 (27–44)	0.5 (0.4–0.8)	12.7 (8.6–18.9)	12.0 (9.0–17.2)

*$p < 0.001$ malignant tumors vs. benign tumors. [a]High-grade Leydig cell tumor was included in the malignant neoplastic lesions group because of a higher mitotic index and higher MIB-1 activity (relative to the Leydig cell tumors included in the benign neoplastic lesions group); n, number.

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carcinoma who had normal tumor markers at baseline SE examination, but showed raised α-FP levels three months later.

Baseline ultrasound

This was the first US examination for 93/106 patients. The remaining 13 had undergone previous testicular surgery: eight had undergone previous orchiectomy with the incidental lesion present in the remaining testis, while the remainder ($n = 5$) had undergone enucleation for ipsilateral or contralateral neoplasm. At baseline US, marked hypoechogenicity was found in 36/37 malignant lesions (97.3%), in 28/38 benign tumors (73.7%, $p = 0.018$, OR = 2.554), and in 17/31 of non-neoplastic lesions (54.8%, $p = 0.002$, OR = 3.389). A total of 35.1% (13/37) malignant lesions showed irregular margins, compared to 18.4% (7/38, $p = 0.106$) of benign tumors and 6.5% (2/31, $p = 0.02$) of non-neoplastic lesions. Microlithiasis was significantly associated with malignancy (21/37 vs. 7/69, $p < 0.001$, OR 2.453). Internal vascularization was common in both benign and malignant tumors (28/38 vs. 32/37, $p = 0.172$), but was seen in only 3/31 of the non-neoplastic lesions (9.7%, $p < 0.001$ compared to malignancies). The two atypical epidermoid cysts and 3/37 malignant lesions (two embryonal carcinomas and one seminoma) had intratumoral calcifications (Table S1). Logistic analysis revealed that independent risk factors for malignant tumors were as follows: size (OR 17.788; $p = 0.002$), parenchymal microlithiasis (OR 17.673, $p < 0.001$), intralesional vascularization (OR 9.207, $p = 0.006$), and hypoechogenicity (OR, 11.509, $p = 0.036$). In contrast, when combining benign and malignant tumors (compared to non-neoplastic lesions), parenchymal microlithiasis (OR 4.543, $p = 0.003$) and intralesional vascularization (OR 37.083 $p < 0.001$) were independent risk factors, confirming previous data (Oyen, 2002; Bushby *et al.*, 2007; Isidori *et al.*, 2014; Lock *et al.*, 2014; Maturen, 2015; Richenberg *et al.*, 2015) and validating that the current series was representative.

The diagnostic performance of conventional US is reported in Table 2.

Strain elastography qualitative analysis: elasticity score (ES)

Under the five-point scale developed by Itoh, 39/106 lesions scored IV (36.8%), five scored III (4.7%), 48 scored II (45.3%), and 14 scored I (13.2%). The degree of agreement among measurements from the two operators was high: there was 100% agreement for scores I, II, and V and three discrepancies for scores III and IV (D.G. attributed a score of III to two nodules which were scored IV by V.C. and D.G. attributed a score of IV to one nodule that V.C. scored III). No lesion in our series received a score of V and only 4.7% a score of III, therefore, we simplified Itoh classification to a three-point scale and performed subsequent analysis based on this criteria: ES1 (Fig. 3) and ES2 (Fig. 4) corresponded to Itoh scores I and II, respectively, while ES3 (Fig. 5) aggregated Itoh scores III to V (Fig. 2). Overall, a higher elasticity score was associated with malignant tumors ($\chi^2 = 37.675$ $p < 0.001$) or tumors in general ($\chi^2 = 32.877$ $p < 0.001$); of the 37 malignant nodules, 30 were scored ES3 (81.1%, $\chi^2 = 36.660$ $p < 0.001$ compared to all benign lesions). The remaining malignancies were scored ES2 (7/37, 18.9%, $\chi^2 = 15.945$ $p < 0.001$).

Of the benign neoplastic lesions, 14/38 were scored ES3 (36.8%, $\chi^2 = 15.130$ $p < 0.001$ compared to malignant lesions), 19/38 ES2 (50%, $\chi^2 = 7.996$, $p = 0.005$), and 5/38 ES1 (13.2%, $\chi^2 = 5.216$ $p = 0.054$).

None of the non-neoplastic lesions were scored ES3, 22/31 were scored ES2 (71%, $\chi^2 = 11.665$, $p = 0.001$, compared to all neoplasms), and 9/31 ES1 (29%, $\chi^2 = 11.665$, $p = 0.002$) (Table 3).

When comparing malignant tumors vs. all benign lesions (benign neoplastic and non-neoplastic together), none of the malignant tumors scored ES1, while 7/37 scored ES2 vs. 41/69 benign lesions (18.9% vs. 59.4%, OR = −1.837, $p < 0.001$), indicating that ES1 and ES2 were predictive of benignity. Logistic regression analysis revealed a significant association between ES3 and malignancy ($\chi^2 = 42.212$, $n = 106$, df = 2, $p < 0.001$, Nagelkerke $R^2 = 0.453$), as well as the likelihood of a lesion being neoplastic ($\chi^2 = 43.661$, $n = 106$, df = 2, $p < 0.001$, Nagelkerke $R^2 = 0.481$). Given these results, nodules scored as ES1 or ES2 were classified as probably benign (neoplastic or non-neoplastic), while nodules scored as ES3 were classified as probably malignant. This classification was then compared with the reference diagnosis for calculation of diagnostic accuracy. The diagnostic performance of SE is reported in Table 2.

Strain elastography semi-quantitative analysis: normal tissue-to-nodule strain ratio (SR)

The absolute agreement among SR measurements was 0.961 for average measures (95% CI 0.934 to 0.988). Given the substantial reliability, data obtained by a single operator were used.

Malignant lesions showed higher SR values than benign lesions (median, interquartile ranges 1.48, 1.18–1.84 vs. 1.25, 1.00–1.51) ($p < 0.013$). Neoplasms (median, interquartile ranges 1.37, 1.10–1.70) showed higher SR values than non-neoplastic lesions (1.11, 0.97–1.59) ($p = 0.023$). There was a difference when comparing non-neoplastic lesions with malignancies ($p = 0.008$), but no difference between benign neoplastic lesions with malignancies ($p = 0.089$) or benign tumors and non-neoplastic lesions ($p = 0.175$) (Table 4). Using ROC curve analysis, we estimated an internal, system-specific cut-off for SR of >1.41 for discriminating benign from malignant lesions (AUC 0.631, 95% CI 0.531–0.722, Youden index 0.2613) and >1.19 for

Table 2 Diagnostic performance of plain US, qualitative and strain elastography values. Data are presented as percentage (95% CI)

Qualitative and strain elastography	Benign vs. malignant					Neoplastic vs. non-neoplastic				
	SE	SP	PPV	NPV	AUC	SE	SP	PPV	NPV	AUC
US	89.2 (74.6–97.0)	85.5 (75.0–92.8)	76.7 (61.4–88.2)	93.7 (84.5–98.2)	0.878	94.6 (86.9–98.5)	87.1 (70.2–96.4)	94.7 (86.9–98.5)	87.1 (70.2–96.4)	0.910
ES	81.1 (64.8–92.0)	79.7 (68.3–88.4)	68.2 (52.4–81.4)	88.7 (78–95)	0.804	58.7 (46.7–69.9)	100 (88.8–100)	100 (91.8–100)	50 (32.9–61.5)	0.793
SR	59.4 (42.1–75.2)	66.6 (54.3–77.6)	48.9 (33.7–64.2)	75.4 (62.6–85.6)	0.631	69.3 (57.6–79.5)	61.3 (42.2–78.2)	81.2 (69.5–89.9)	45.2 (29.8–61.3)	0.653

ES score, elasticity score (elasticity score 3 vs. combined elasticity score 1 and 2); SR, strain ratio; SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

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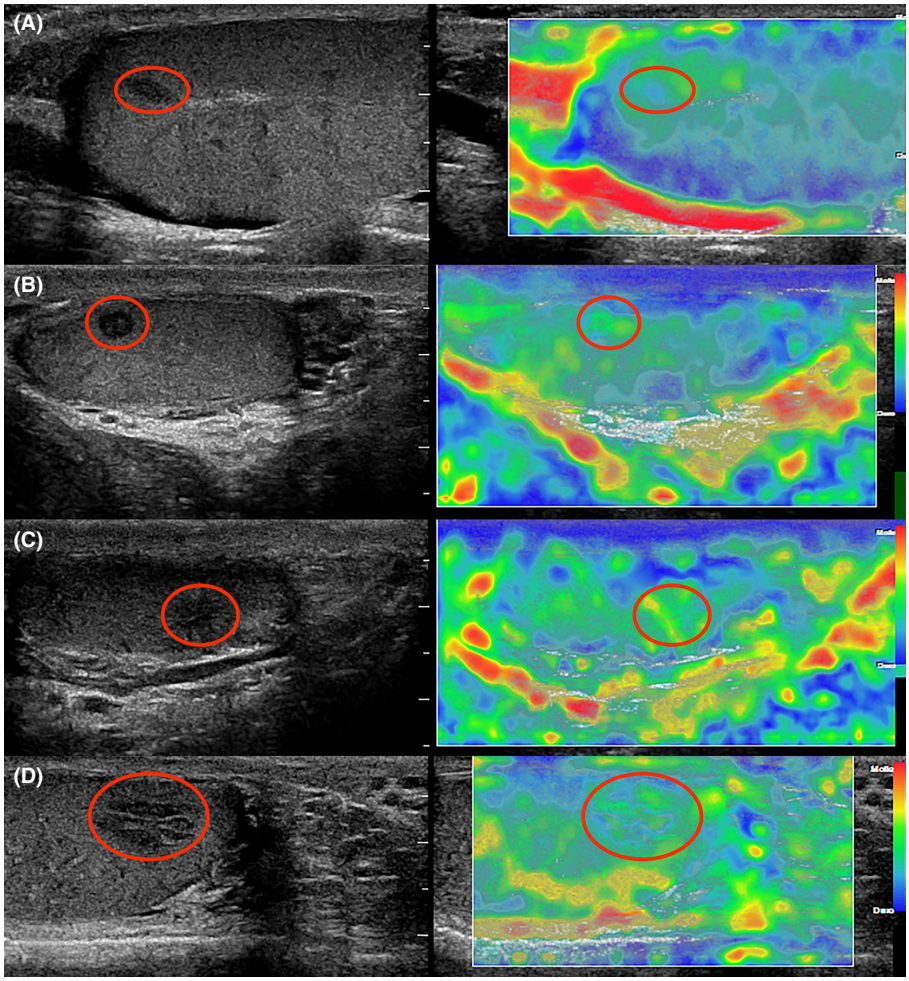

Figure 3 Baseline US and qualitative elastography of soft lesions (ES1). In panel (A) and (D), are described respectively, a Leydig cell hyperplasia (at histology) and a fibrotic lesion (stable at 48-months follow-up); panel (B) and (C) show two hypoechoic lesions, resulted both Leydig cell tumor at histology.

discriminating neoplastic from non-neoplastic lesions (AUC 0.653, 95% CI 0.554–0.743, Youden index 0.3062) (Table 2).

Pairwise comparison of ROC curves showed that strain ratio analysis was significantly worse than qualitative assessment in diagnosing malignancies ($p = 0.003$, ES AUC 0.804, 95% CI 0.716–0.875 vs. SR AUC 0.631, 95% CI 0.531–0.722), as well as in identifying non-neoplastic lesions ($p = 0.011$, ES AUC 0.793, 95% CI 0.704–0.866 vs. SR AUC 0.653, 95% CI 0.554–0.743).

COMPARISON OF IMAGING TECHNIQUES

In the diagnosis of malignant vs. non-malignant lesions, baseline US was 89.2% sensitive and 85.5% specific for malignancy, compared with 81.1% and 79.7% for real-time elastography. Baseline US was therefore more accurate than elastography as a standalone procedure for the diagnosis of malignancies. Analyzing elastography in light of grayscale findings, we found that ES identified as malignant (ES3) three cases that were erroneously considered benign by US (three false negative US findings). Most importantly, ES correctly identified as benign (ES1 and ES2) five lesions that US considered suspicious for malignancies (two Leydig cell tumors, one granulomatous orchitis and two areas of fibrosis; five false-positive US findings). None of the malignant tumors scored ES1. In contrast, elastography misinterpreted nine lesions as potentially malignant (ES3) that were correctly identified as benign by US (two epidermoid cysts, six Leydig cell tumors, one Sertoli cell tumor) and six seminomas as potentially benign (ES2), which, conversely, were correctly identified at US. In summary, a finding of ES1 can be used to increase the specificity of US by excluding cases of suspected malignancy.

In the diagnosis of neoplastic vs. non-neoplastic lesions, none of the non-neoplastic lesions scored ES3 (100% specificity), against 87.1% specificity for conventional US. Analyzing elastography in light of grayscale findings, ES correctly identified as non-neoplastic: one granulomatous orchitis identified as suspicious for malignancy on US as well as two areas of fibrosis and one Leydig cell hyperplasia identified as neoplastic on US (four false positives on US). However, US was much more sensitive (94.6%) than SE (58.7%).

In general, SE offered an advantage in lesions (granulomatous orchitis, areas of fibrosis, burn-out tumors) where US color-Doppler findings were equivocal.

Finally, to test whether ES2 was typically associated with benign tumors, we analyzed the distribution of ES appearance in Leydig cell tumors. We found that five were ES1 (13.2%), 19 were ES2 (50.0%), and 12 were ES3 (36.8%), suggesting no discriminatory value for the identification of Leydig cell tumors.

DISCUSSION

The normal testis demonstrates a homogeneous medium hardness at elastography (Jedrzejewski *et al.*, 2013) (Fig. 6). Focal lesions can often be recognized as harder than the

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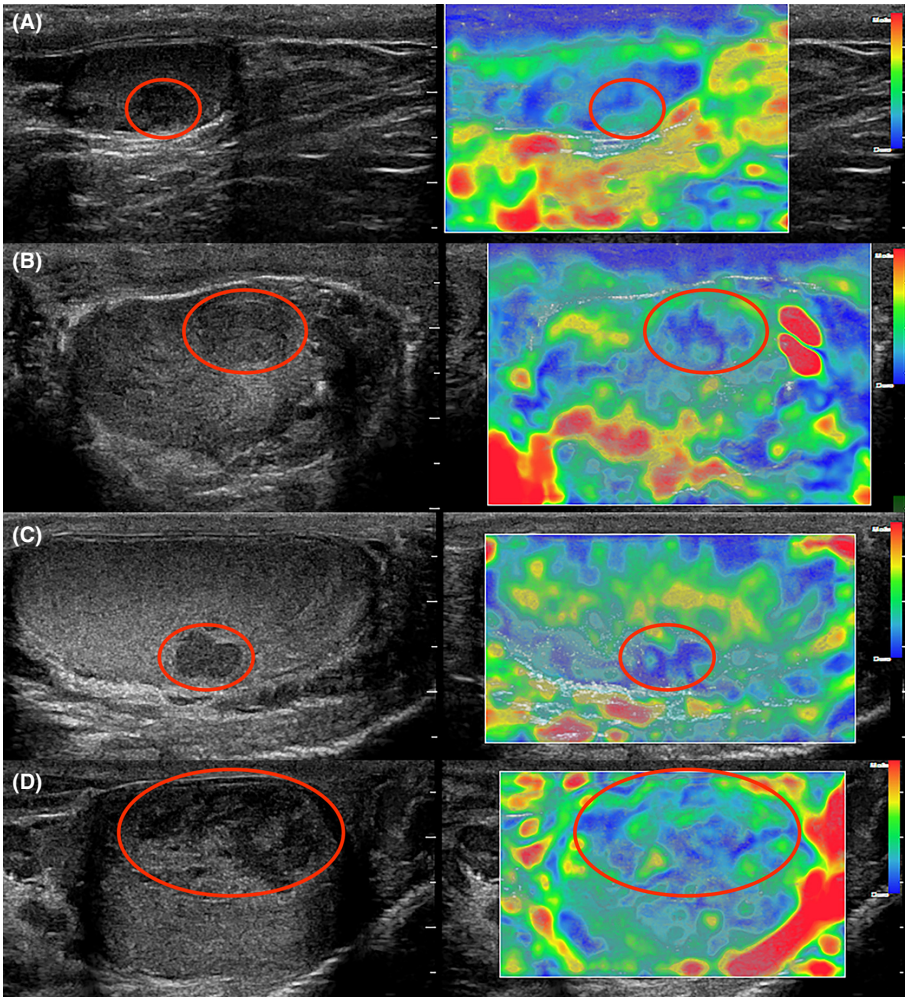

Figure 4 Baseline US and qualitative elastography of intermediate stiff lesions (ES2). Figure 4 shows four lesions, scored ES2 at elastography. Both lesions in panel (A) and (B) resulted Leydig cell tumors at histology, while the lesion in panel (C) resulted a seminoma at histology. In panel (D), the heterogeneous hypoechoic lesion was diagnosed as a testicular hematoma after 18 months of clinical and US follow-up.

surrounding testicular tissue, with some important exceptions reported with hematomas or mixed fluid lesions (Patel *et al.*, 2014; Yusuf *et al.*, 2015). Normal testis parenchyma is hard, but compared with the even harder solid tumorous tissue, it has a relatively softer appearance, displaying areas of green and light blue (Jedrzejewski *et al.*, 2013). Elastography has proven useful for studying focal lesions in various organs (Brock *et al.*, 2012; Cosgrove *et al.*, 2013; Cantisani *et al.*, 2015) and a possible role in the diagnosis of focal testicular lesions was recently advocated. Schurich *et al.*, (2009) described the value of SE in 20 testicular lesions, showing that elastography improved the detection of testicular masses, and reported that all tumors were hard ($n = 15$). Conversely, Grasso *et al.*, (2010) found SE unreliable when used alone to discriminate malignant from benign lesions. Goddi *et al.* (2012) performed the only large prospective study: the authors analyzed 144 lesions in 88 testes using the five-point score suggested by Itoh *et al.* for breast disease (Itoh *et al.*, 2006), and concluded that SE had a diagnostic accuracy of 95.8% in differentiating malignant from all other lesions, reaching 98.8% in lesions smaller than 5 mm. A hard lesion score (ES >4) was observed in 87.5% of malignancies, but the prevalence of histologically proven malignant tumors in their series was low (22%) and the number of very small lesions (<5 mm) was high (Goddi *et al.*, 2012).

In the same year, Aigner *et al.* retrospectively analyzed 50 patients, of whom, 34 had tumors and 16 non-neoplastic lesions (Aigner *et al.*, 2012). They differentiated soft from hard lesions using a two-point score, and found that all 34 testicular tumors were hard on SE (13 seminomas, two embryonal carcinomas, nine mixed germ cell tumors, one teratoma, four Leydig cell tumors, three Sertoli cell tumors, one metastasis, and one epidermoid cyst), demonstrating a sensitivity of 100% and an accuracy of 94% in diagnosing a testicular tumors in general. Marsaud *et al.* found high sensitivity (96%) but low specificity (37.5%) for strain ratio (Marsaud *et al.*, 2015), while Pastore *et al.* suggested that strain ratio measurement may provide additional objective information to support the algorithm used to diagnose testicular lesions (Pastore *et al.*, 2014). It is worth noting that in all these studies, the number of benign stromal neoplasms (Leydig cell or Sertoli cell tumors) was low.

Given the conflicting results, we carried out a prospective study to evaluate the application of qualitative and strain ratio elastography in non-palpable testicular lesions. These are the most challenging lesions, as the larger the lesion, the higher the likelihood of malignancy. In this context, we demonstrated that the color-coded SE classification initially developed for breast lesions and often also used for other endocrine parenchymas (e.g. thyroid), could be simplified into a three-point scale when evaluating the testis. For most other tissues, scores 1 and 2 are generally

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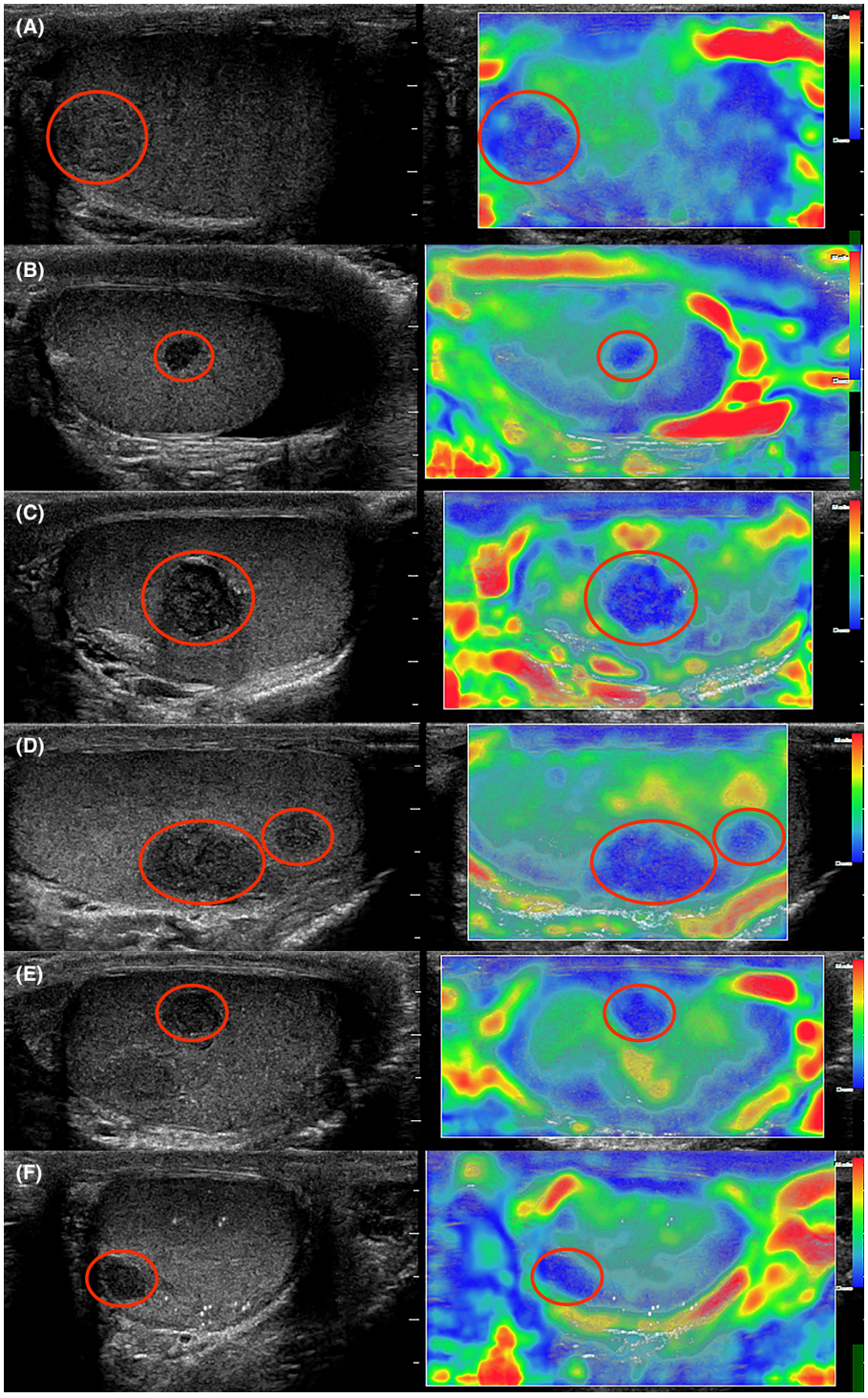

Figure 5 Baseline US and qualitative elastography of hard lesions (ES3). Panel (A–F) show six markedly hypoechoic lesions, scored ES3 at elastography (hard lesions) that resulted Leydig cell tumor (A–C) and seminomas (D–F) at definitive histology.

considered suggestive of benignity, score 3 as an indeterminate/ambiguous lesion, and score 4 and 5 suggestive of malignancy. However, the histological structure of testicular parenchyma is very different from that of breast tissue: it is much more homogeneous, compact, and stable, and therefore easier to assess. In fact, we found that the scores were distributed mainly into three groups. Given this, and in light of the pathology and epidemiological considerations, we thought it appropriate to simplify the scale to a more operator-friendly three-point scale. Score 1 was assigned to features suggestive of benignity (high elasticity), score 2 to ambiguous features (in which color distribution pattern was difficult to interpret), and score 3 to nodules with a neat hard core typical of malignant features (low elasticity).

Most tumorous lesions (benign and malignant) in our series were hard on qualitative elastography (ES3), probably because neoplasms present high cellularity. Half of the benign tumors were scored as 2 ($p = 0.005$, Fig. 5), while malignant tumors were mostly completely hard lesions (ES3, 81.1%, $p < 0.001$). Benign and malignant neoplasms thus show a certain overlap in hardness that does not allow a sufficiently confident differential

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Table 3 Frequencies of elasticity scores (ES). Data are presented as percentages (number)

Final diagnosis	n	Malignant tumors	Benign tumors	M vs. B	All neoplastic lesions (B and M)	Non-neoplastic lesions	N vs. NN	All benign lesions (N and NN)	M vs. ABL
All patients, n	106	37	38		75	31		69	
ES1	14	0.0 (0)	13.2 (5)	0.050	6.7 (5)	29.0 (9)	0.002	20.3 (14)	0.002
ES2	48	18.9 (7)	50.0 (19)	0.005	34.7 (26)	71.0 (22)	0.001	59.4 (41)	<0.001
ES3	44	81.1 (30)	36.8 (14)	<0.001	58.7 (44)	0.0 (0)	<0.001	20.3 (14)	<0.001

ES1, elasticity score 1; ES2, elasticity score 2; ES3, elasticity score 3; n, number; M, malignant tumors; B, benign tumors; N, neoplastic lesions (all tumors); NN, non-neoplastic lesions; ABL, all benign lesions (neoplastic and non-neoplastic).

Table 4 Strain ratio values of elastograms reported in absolute values (median and interquartiles) for malignant vs. benign tumors, neoplastic vs. non-neoplastic lesions, and all benign lesions

Final diagnosis	n	Malignant tumors	Benign tumors	M vs. B	All neoplastic lesions (B and M)	Non-neoplastic lesions	N vs. NN	All benign lesions (N and NN)	M vs. ABL
All patients, n	106	37	38		75	31		69	
SR		1.48 (1.18–1.84)	1.32 (1.07–1.48)	0.089	1.37 (1.10–1.70)	1.11 (0.97–1.59)	0.023	1.25 (1.00–1.51)	0.013

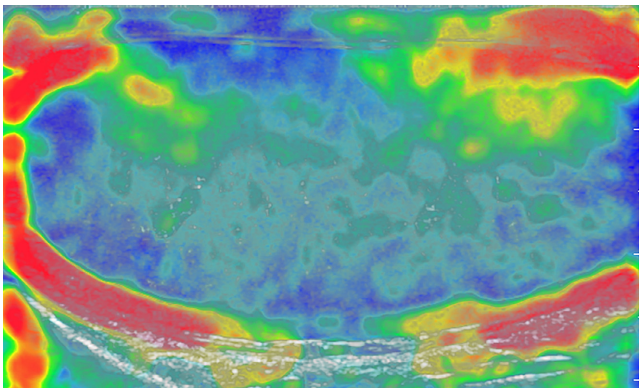
SR, strain ratio; n, number; M, malignant tumors; B, benign tumors; N, neoplastic lesions (all tumors); NN, non-neoplastic lesions; ABL, all benign lesions (neoplastic and non-neoplastic).

diagnosis (e.g. epidermoid cysts) (Patel *et al.*, 2012). In contrast, our three-point score color-coded classification was significantly helpful in discriminating between malignant tumors (ES3) and non-neoplastic lesions (ischemia, abscesses, non-tense cysts), which present as soft (ES1) or intermediate stiff (ES2) lesions. In fact, only neoplastic lesions were scored as ES3, with 100% PPV (against combined ES1 and ES2). Abscesses, partial infarction, Leydig cell hyperplasia, fibrosis and non-tense cysts were all soft on elastography.

Application of elastography to parenchymal cysts remains controversial: even if SE does not confer any benefit in thyroid cyst assessment, it has been found of some advantage in the differential diagnosis of breast cysts (Gheonea *et al.*, 2011). Its application to testicular cysts has not yet been validated, although the inclusion of small, non-tense cysts that were hypo- or anechoic on grayscale US is an advantage of the current series. However, we did not achieve the high sensitivity reported in the previously published articles, and specifically, ES was neither superior to US in diagnosing malignancies (81.1% for ES vs. 89.2% for US, $p = 0.153$) nor in diagnosing neoplasms (58.7% for ES vs. 94.6% for US, $p = 0.009$). Given these results, we are not confident in suggesting the use of elastography as a standalone procedure. However, a maximum hardness score (ES3) was 100% specific in excluding non-neoplastic lesions compared to 87.1% for baseline US. In addition, although a score of ES1 was uncommon (13%) in our series, none of the malignant tumors was ES1; therefore, in doubtful cases, an elastography score of ES1 could allow a careful 'watchful-waiting' approach.

Analysis of individual cases revealed that US accuracy dropped when CDU findings were equivocal. The lack of flow does not eliminate the possibility of neoplasm; for example, in our study, the most challenging cases were burned-out tumors or very rapidly aggressive histotypes, in which internal flow was absent or presented as just a perilesional rim. This appearance, however, is also common to some ischemic/post-traumatic changes, atypical epidermoid cysts, and granulomas. In these cases, ES proved useful. In summary, a score of ES3 could reinforce the US diagnosis in suggesting the neoplastic nature of the lesion, while conversely, ES1 could reasonably exclude a malignant lesion (but not a benign tumor).

Figure 6 Normal testis parenchyma at elastography. Normal testis parenchyma at elastography, shaded in green and blue, showing an intermediate elasticity.



Another novelty of our work was the evaluation of strain ratio obtained by dividing the reference tissue by the lesion strain. Our study is one of the first to investigate the role of SR in discriminating malignancies from benign lesions. Unlike the study by Pastore *et al.* (Pastore *et al.*, 2014), we found that SR measurement did not provide significant information and showed lower sensitivity and specificity in the differential diagnosis of testicular lesions than the score classification system.

Our study has several advantages compared to the previous ones, as it is prospective, large, and with a varied case mix, but it also has some limitations. First, the degree of tissue compression influences the elasticity image and, consequently, the elasticity score; it takes some practice to be able to exert correct pressure to obtain suitable images for elasticity analysis. Second, SR is a measure of relative stiffness between healthy parenchyma and a nodule. The tissue adjacent may not be normal (e.g. in patients with Klinefelter's syndrome), which could theoretically alter the measurements. Third, the strain ratio is dependent on the manufacturer of

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the elastography unit. Our software provides SR measurements automatically, but it is possible that different software on other US systems could provide different values. Fourth, ES was evaluated blinded to CDU and real-time US examination, but not to simultaneous simple grayscale imaging. However, although this might not be a perfect blinding, in real life, ES will always be performed after baseline US, and therefore, we believe that this limitation does not affect the validity of our conclusion, which is to suggest that ES be added on to baseline US. Finally, there could be some selection bias as half of our patients were referred for infertility screening; however, infertile patients are those at highest risk of testicular cancer, so they are the ideal population to test this new diagnostic tool.

In conclusion, the results of our study place SE into context for the evaluation of incidental testicular lesions, highlighting its role, and limitations, and validating a more practical scoring system. We conclude that qualitative assessment in conjunction with a careful clinical history and supplementary diagnostic evaluation could be helpful in differentiating non-palpable testicular malignancies from non-neoplastic lesions in challenging cases, but it cannot be used to discriminate benign from malignant neoplasms. This work supports the need for a multimodality approach combining different techniques (grayscale US, CDU, ES, CEUS, MRI) to achieve the highest diagnostic accuracy in the characterization of testicular masses.

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AUTHORS' CONTRIBUTIONS

Guarantors of integrity of entire study, A.M.I., C.P., D.G.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, A.M.I., C.P., D.G., E.G., G. Fattorini, V.C.; clinical studies, A.M.I., C.P., D.G., P.S.S., G.B.D.P., F.B., C.C., G. Fattorini, G. Franco, V.C; statistical analysis, A.M.I., C.P., D.G., E.G., E.N., F.B.; and manuscript editing, A.M.I., C.P., D.G., V.C., A.L., P.S.S.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Frequencies of qualitative features on B-mode scan. Data are presented as percentages (number).

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