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REVIEW ARTICLE

Historical trends for the standards in scrotal ultrasonography: What was, what is and what will be normal

Francesco Lotti[1] | Michele Bertolotto[2] | Mario Maggi[3]

[1] Andrology, Female Endocrinology and Gender Incongruence Unit, Department of Experimental, Clinical and Biomedical Sciences, University of Florence, Florence, Italy

[2] Department of Radiology, Cattinara Hospital, University of Trieste, Trieste, Italy

[3] Endocrinology Unit, Department of Experimental, Clinical and Biomedical Sciences, University of Florence, Florence, Italy

Correspondence
Francesco Lotti, Andrology, Female Endocrinology and Gender Incongruence Unit, Department of Experimental, Clinical and Biomedical Sciences, University of Florence, Viale Gaetano Pieraccini 6, 50139 Florence, Italy.
Email: francesco.lotti@unifi.it; flottimd@yahoo.it

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Abstract

Background: Ultrasonography is the gold standard imaging method for scrotal investigation. Colour Doppler ultrasonography, contrast-enhanced ultrasonography and sonoelastography allow sonographers to assess size, echotexture, vascular features and stiffness of the scrotal organs and abnormalities. Scrotal ultrasonography has been used to investigate male reproductive health, scrotal pain, masses and trauma. However, ultrasonography thresholds/classifications used to distinguish normal and pathologic features of the scrotal organs have changed over time, and have not yet been fully standardized.

Objectives: To evaluate historical trends for the standards in scrotal ultrasonography: what was, what is and what will be normal.

Methods: An extensive Medline search was performed identifying the most relevant studies in the English language from inception to date.

Results: We provide here (i) a brief overview of the history of ultrasonography, (ii) current indications for scrotal ultrasonography and (iii) previous and current normal values, cut-offs and classifications of the main colour-Doppler ultrasonography parameters/characteristics of the scrotal organs, as derived from recent guidelines and evidence-based studies. In addition, we report recommendations and the clinical utility of contrast-enhanced ultrasonography and sonoelastography. Finally, we discuss critical issues needing further evidence and future directions to fill in the current gaps.

Discussion: Several studies on scrotal ultrasonography are available. However, guidelines/recommendations dealing with specific ultrasonography applications have been published only in recent years. More recently, the European Academy of Andrology published evidence-based scrotal colour-Doppler ultrasonography reference ranges/normative parameters derived from a cohort of healthy, fertile men. In addition, a standardization of the methodology to evaluate qualitative and quantitative colour-Doppler ultrasonography parameters was reported. Other international societies reported indications, methodological standards, clinical utility and limitations of contrast-enhanced ultrasonography and sonoelastography.

Conclusions: To date, colour-Doppler ultrasonography normative values for the scrotal organs are available. However, a wide international consensus on assessment and

classification of several ultrasonography parameters is still lacking. An alignment of the world societies on these issues is advocated.

KEYWORDS
colour-Doppler ultrasound, contrast-enhanced ultrasound, scrotal ultrasound, sonoelastography, testicular ultrasound, varicocoele

1 | INTRODUCTION

Currently, ultrasonography (US) represents the gold standard imaging method for scrotal investigation.[1] Using high-frequency sound waves, US is a simple, rapid and harmless diagnostic tool able to provide live images of the scrotal content and, among imaging techniques, it is the least expensive. The high-resolution greyscale mode associated with colour and power Doppler examination allows sonographers to investigate the size, echotexture and vascular features of the scrotal organs and abnormalities.[1] More recently, the use of contrast-enhanced US (CEUS) and sonoelastography (SE) has led to further improvements in the differential diagnosis of scrotal diseases.[2,3]

So far, scrotal US has shown a relevant impact both on reproductive and general male health.[1–3] In fact, US has been used to assess scrotal features related to (i) reproductive health, (ii) scrotal pain, (iii) masses and (iv) trauma.[1–3] (i) Regarding reproductive health,[1–3] US can detect alterations in size, echotexture and vascularisation of the testes, which are associated with sperm abnormalities and, frequently, with low testosterone levels. In addition, scrotal US provides information on epididymal and deferential abnormalities, possibly associated with semen quality impairment, or on their bilateral absence, causing obstructive azoospermia. Finally, scrotal colour-Doppler US (CDUS) is able to detect and stage varicocoele, which may exert a negative role on sperm parameters. (ii) Regarding scrotal pain,[1–3] CDUS can detect testicular or epididymal size and echopattern abnormalities as well as hypervascularisation, suggesting inflammation (i.e. orchitis and epididymitis), or an absent vascularisation, indicating spermatic cord/testicular torsion. Furthermore, CDUS can assess severe varicocoele or inguinal/scrotal hernias, often associated with mild discomfort and even overt pain. (iii) Moreover, US plays a key role in investigating testicular or extra-testicular masses,[1–3] characterizing them as benign or malignant with fair accuracy, although without providing diagnostic certainty. In addition, US can assess testicular microlithiasis (TML),[1–3] which, when associated with additional risk factors (see below), might underlie a coexisting or developing testicular malignancy. (iv) Finally, US plays a crucial role in the evaluation of scrotal trauma.[2,3]

Although US has been widely used to explore the scrotal organs, until very recent years the method used to assess several qualitative and quantitative US parameters had not been standardized.[1] Furthermore, in recent decades, normative parameters and thresholds to distinguish normal from pathologic features of the scrotal organs were often not evidence based.[1] Finally, the possible impact of several US findings on male reproductive and general heath is still unclear.[1]

We here evaluate the standards in scrotal US used in the past and the current ones, as derived from recent guidelines and evidence-based studies. In addition, critical issues needing further evidence will be discussed, and future directions to fill in the current gaps will be considered.

2 | METHODS

An extensive Medline search was performed with no restrictions regarding date of publication (i.e. from inception date until March 2021) including the following words: ('scrotally'[All Fields] OR 'scrotum'[MeSH Terms] OR 'scrotum'[All Fields] OR 'scrotal'[All Fields] OR 'testicular'[All Fields]) AND ('diagnostic imaging'[MeSH Subheading]) OR ('diagnostic'[All Fields] AND 'imaging'[All Fields]) OR ('diagnostic imaging'[All Fields] OR 'ultrasound'[All Fields] OR 'ultrasonography'[MeSH Terms] OR 'ultrasonography'[All Fields] OR 'ultrasonics'[MeSH Terms] OR 'ultrasonics'[All Fields] OR 'ultrasounds'[All Fields] OR 'ultrasound s'[All Fields]) OR ('contrast-enhanced'[All Fields] AND 'diagnostic imaging'[All Fields] OR 'ultrasound'[All Fields] OR 'ultrasonography'[MeSH Terms]) OR ('elasticity imaging techniques'[MeSH Terms] OR ('elasticity'[All Fields] AND 'imaging'[All Fields] AND 'techniques'[All Fields]) OR ('elasticity imaging techniques'[All Fields] OR 'sonoelastography'[All Fields]). The identification of relevant studies in the English language was performed independently by all the authors. In addition, a 'pearl growing' strategy was employed, whereby, after obtaining the full text articles, the reference lists of all included studies were reviewed for additional publications that could be used in this manuscript. An analysis of previous reports in US textbooks and updated online guidelines including scrotal US was also performed.

3 | HISTORICAL MILESTONES

Prior to the introduction of US, diagnosis of most scrotal abnormalities required surgical exploration.[4] The first studies using US to investigate the scrotal organs date back to the seventies. Initially, scrotal examination was performed through the contact B-scan technique, requiring extensive experience on the part of the operators.[4] In the late seventies, Miskin et al.[5] and Gottesman and Sample's team[7,8] reported the use of a new greyscale instrumentation to diagnose scrotal abnormalities. Subsequently, Leopold et al.[8] introduced the high-resolution (10 MHz) real-time US to characterize testicular and extra-testicular

lesions. With the development of US equipment characterized by an increasing performance, scanning techniques and image resolution have improved significantly over time. Today, the greyscale evaluation associated with colour/power-Doppler examination with spectral Doppler analysis allows sonographers to investigate the structural and vascular characteristics of scrotal organs and lesions and to detect and stage varicocoele.[1] More recently, the introduction of CEUS and SE has led to further improvements in the differential diagnosis of testicular abnormalities.[2,3]

CEUS is a relatively new technique based on the use of microbubble contrast agents to demonstrate microvascular organization and parenchymal perfusion.[2,3] Its application in scrotal imaging was published for the first time by Coley et al.[9] in an animal model (rabbit) to investigate acute testicular torsion. Subsequently, Catalano et al.[10] reported the first pilot experience with CEUS in human males evaluating traumatic and non-traumatic emergencies. In 2004, Greis[11] published an overview on SonoVue (sulphur hexafluoride microbubbles), a second-generation US contrast agent containing an inert lipophilic gas with very low solubility in blood, avoiding early leakage of the gas and making the microbubbles much more stable. Subsequently, the introduction of the Vuebox, a toolbox/software application for quantifying tissue perfusion using Dynamic CEUS (DCE-US) DICOM clips, led from qualitative to quantitative analysis of linear data obtained with a wide range of US systems.[12] In 2011, Bertolotto et al.[13] reported the use of SonoVue in evaluating acute segmental testicular infarction, whereas Lock et al.[14] published an early experience with SonoVue in the diagnosis of testicular masses. Thereafter, further studies and guidelines have been published, until the most recently updated guidelines (see below).

SE is an imaging modality that maps the elastic properties and stiffness of soft tissues.[15] The SE rationale is that whether the tissue is hard or soft offers diagnostic information about the presence or status of a disease (e.g. cancer will often be harder than the surrounding tissue).[15] The first report using SE to evaluate human testes is relatively recent,[16] reporting a preliminary experience in 41 patients with scrotal pain, scrotal painless enlargement, testicular nodules or infertility. Afterward, SE has been mainly used to better characterize testicular nodules; however, it is more of an ancillary technique compared to CDUS and CEUS.[2,3] Some studies evaluated also a possible SE application in investigating infertility,[17] TML,[18] undescended testis[19] and varicocoele effect on testicular structure and function,[20] with preliminary results. Updated SE guidelines indicate SE use and criticisms in scrotal imaging (see below).

4 | GUIDELINES AND RECOMMENDATIONS

Several studies on scrotal US are available[1]; however, only in recent years have guidelines/recommendations dealing with specific US applications been published. In particular, in 2014 the Italian Society of Urology (SIU) in collaboration with the Italian Society of Ultrasound in Urology, Andrology and Nephrology (SIEUN) published practical recommendations for performing US scanning in the uro-andrological field.[21] In 2015, a collaboration among the American Institute of Ultrasound in Medicine (AIUM), the American College of Radiology (ACR), the Society for Pediatric Radiology (SPR) and the Society of Radiologists in Ultrasound (SRU) led to the AIUM Practice Guideline for the Performance of Scrotal Ultrasound Examinations.[22] The aforementioned documents mainly focused on the indications and methodology of scrotal US. From 2000, the American College of Radiology (ACR) has published several documents, including the 2019 updated version[23] on CDUS appropriateness criteria in evaluating acute scrotum. In 2021, the American Urological Association (AUA) updated urologic (including scrotal) trauma guidelines,[24] and more recently the European Society of Urogenital Radiology (ESUR) published a more accurate position statement on the appropriate use of multiparametric US, along with other imaging modalities, in the evaluation of scrotal trauma.[25] In addition, in 2015 the ESUR published TML imaging and follow-up guidelines,[26] and, in 2020, guidelines and recommendations for the detection, classification and grading of varicocoele.[27,28] Furthermore, the European Association of Urology (EAU) reported recommendations on the utility of scrotal US in imaging of infertile men[29] and testicular neoplasms,[30] whereas AUA/ASRM, in its guideline on the diagnosis and treatment of infertility in men,[31] provided a few indications on when to perform US. Finally, in March 2021, the European Academy of Andrology (EAA) published an international multicentre study reporting, in an evidence-based manner, scrotal CDUS reference ranges/normative parameters as derived from a cohort of 248 healthy, fertile men studied exhaustively.[32,33] In the same study, a standardization of the methodology used to evaluate qualitative and quantitative CDUS parameters was reported, according to the consensus of 11 EAA Centers.[32,33] Regarding CEUS application in scrotum investigation, in 2018 the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) published updated guidelines and recommendations.[34,35] In 2019, the same federation published updated guidelines and recommendations on SE application in testicular investigation.[36]

5 | SCROTAL US: INDICATIONS

Indications for scrotal CDUS have not changed significantly over time, although methodology and sonographic equipment evaluating different features/abnormalities of the scrotal organs have improved and been refined with the newer advances in technology. Conversely, the indications for CEUS and SE application in scrotal investigation have increased over time. The indications for scrotal US are reported in Table 1.

6 | SCROTAL US: METHODOLOGICAL STANDARDS

6.1 | Scrotal CDUS

The standardization of the methodology used to assess scrotal CDUS is relatively new. Practical recommendations for performing scrotal

TABLE 1 Current indications for scrotal US (adapted from AIUM,[22] ESUR[25–28] and EFSUMB[34–36] guidelines)

Greyscale and colour-Doppler US (CDUS)
Reproductive health
Evaluation of male infertility
- Evaluation of TV (especially when physical examination is unreliable[a])
- Evaluation of TI (and TML)
- Evaluation of testicular nodules
- Localization (when possible) and evaluation of non-palpable testes
- Evaluation of cryptorchid testis after orchiopexy and contralateral descended testes
- Evaluation of epididymal dilation (and echotexture) suggesting proximal or distal sub-obstruction
- Evaluation of vas deferens presence or absence
- Detection/evaluation of varicocoeles
Scrotal pain or discomfort
- Evaluation of infectious or inflammatory scrotal disease (e.g. orchitis, epididymitis), testicular ischemia/torsion or trauma
- Evaluation of palpable testicular, intra-scrotal or inguinal masses
- Evaluation of scrotal asymmetry, swelling, enlargement or potential intra-scrotal hernias
- Detection/evaluation of varicocoeles
- Exclusion of scrotal causes for acute scrotal pain
Scrotal masses/oncologic risk
- Evaluation of palpable testicular, intra-scrotal or inguinal masses
- Evaluation and follow-up of small non-palpable testicular nodules
- Evaluation of scrotal asymmetry, swelling, enlargement or potential intra-scrotal hernias
- Localization and evaluation of non-palpable testes
- Evaluation and follow-up of cryptorchid testis after orchiopexy and contralateral descended testes
- Follow-up of testicular TML
- Follow-up of prior indeterminate scrotal US findings
- Detection of occult primary tumours in patients with metastatic germ cell tumours or unexplained retroperitoneal adenopathy
- Follow-up of patients with prior primary testicular neoplasms, leukaemia or lymphoma
- Evaluation of abnormalities noted on other imaging studies (including but not limited to computed tomography, magnetic resonance imaging and positron emission tomography).
Scrotal trauma
- Assessment of tunica albuginea integrity
- Assessment of testicular parenchyma viability
- Identification and follow-up of hematomas and fluid collections
- Detection of the testis in post-traumatic dislocation
Evaluation of a disorder of sexual development
Contrast-enhanced US (CEUS)[b]
- Differentiation between neoplastic and non-neoplastic lesions
- Differentiation between avascular and poorly vascularized lesions
Contrast-enhanced US (CEUS)[b]
- Identification of segmental infarction
- Discrimination of non-viable regions in testicular trauma
- Identification of abscess formation and infarction in severe epididymo-orchitis
- Evaluation of the integrity of the tunica albuginea
- Evaluation of parenchymal testicular vascularisation
Sonoelastography (SE)[b]
- Adjunctive role in discriminating between neoplastic and non-neoplastic lesions

Abbreviations: TI, testicular inhomogeneity; TML, testicular microlithiasis; TV, testicular volume.

[a]TV evaluation at physical examination may be unreliable in case of large hydrocele or large varicocoele, inguinal testis, epididymal enlargement or fibrosis, thickened scrotal skin, and obesity.[1,31.]

[b]CEUS and SE are used in equivocal cases at CDUS.

CDUS were reported by the SIU/SIEUN collaboration[21] in 2014 and in the AIUM Practice Guideline[22] in 2015. In 2015, Lotti and Maggi published a systematic review[1] dealing with the measurement and assessment (as well as clinical significance) of male genital tract quantitative and qualitative parameters, respectively. In particular, regarding scrotal US, the authors reported how, in previous studies, each organ/segment (e.g. testis, epididymal head, body, tail, vas deferens pampiniform plexus) of the scrotal sac had been measured, and the classifications that were used to stratify each qualitative feature's (e.g. testis inhomogeneity) severity. In addition, the authors reported the thresholds suggested in previous studies to distinguish normal from pathologic features, in an effort to align them. However, the authors concluded that, for several parameters, sonographic imaging of the male genital tract was suffering from a lack of standardization, often leading to subjective and vague diagnoses. For this reason, the EAA promoted an international multicentre study (see at https://www.andrologyacademy.net/eaa-studies) aimed at defining the male genital tract CDUS reference ranges and characteristics as derived from a cohort of healthy, fertile men, in order to obtain normative parameters. The development and methodology of the 'EAA US study' have been reported in a 2020 study.[32] A detailed description of the Standard Operating Procedures (SOPs) to evaluate scrotal quantitative and qualitative parameters, and assessment of the CDUS intra- and inter-operator comparability, has been reported in a further study.[33] In our opinion, following the CDUS SOPs proposed by the EAA US consortium[32,33] in clinical practice will help in reducing the operator-dependent differences among sonographers. Table 2 summarizes the EAA-proposed SOPs[33] to assess scrotal CDUS.

6.2 | Contrasted-enhanced US

The methodological standards for the clinical practice of CEUS in non-hepatic applications, including scrotum investigation, have been

TABLE 2 EAA Standard Operating Procedures (SOPs) to assess scrotal CDUS

Testis
Testicular volume (TV)
Evaluate the three maximum diameters of each testis (anterior–posterior [height] and transverse [width] diameters in transverse scan; longitudinal diameter [length] in longitudinal scan)
Calculate TV using the ellipsoid formula (length × height × width × 0.52)
Testicular homogeneity/inhomogenety (TI)
Use a 4-point Likert scale: 0 = *homogeneity* 1 = *mild (grade 1) inhomogeneity (presence of small hypoechoic foci/thin hypoechoic striae)* 2 = *moderate (grade 2) inhomogeneity (presence of thick hypoechoic striae)* 3 = *severe (grade 3) inhomogeneity (diffuse inhomogeneity with 'netting'/'geographical map' appearance)*
Testicular echogenicity
Use a 3-point Likert scale: 0 = *normoechoic* 1 = *mainly hypoechoic* 2 = *mainly hyperechoic*
Calcifications and microlithiasis
Macrocalcifications: calcifications with a size >3 mm
Microcalcifications: small (1–3 mm) bright echogenic foci with no acoustic shadowing
TML: presence of ≥5 microcalcifications in a single US scan, classified as (1) limited, (2) 'clusters' or (3) diffuse ('starry sky' appearance). Report localization in the upper, middle and lower third of the testis
Testicular nodules
Evaluate the three diameters and characteristics (0 = *cystic*; 1 = *mixed*; 2 = *solid*), shape (0 = *regular*; 1 = *irregular*), homogeneity (0 = *homogeneous*; 1 = *inhomogeneous*), echogenicity (0 = *normal echogenicity*; 1 = *mainly hypoechoic*; 2 = *mainly hyperechoic*), calcifications and/or cysts (0 = *absent*; 1 = *present*) and vascularisation (0 = *absent*; 1 = *peripheral*; 2 = *intra-nodular*)
Testicular vascularisation
Qualitative assessment: normal, reduced, enhanced (in the entire testis and/or focal areas); compare the two testes
Quantitative assessment: evaluate arterial PSV, acceleration, RI and PI in the testicular artery – in the spermatic cord, 2 cm before the gonadal hilum – and the intratesticular arteries (recurrent rami of the centripetal arteries).
Other findings
Evaluate and measure dilated rete testis (three diameters).
Evaluate and measure parenchymal cysts (major diameter).
Evaluate and measure testis appendices (longitudinal diameter).
Evaluate and measure (major diameter) extra-testicular calcifications (including scrotoliths).
Evaluate and measure hydrocele (three diameters and volume); use convex probe when bulky.
Epididymis and vas deferens
Evaluate the CDUS features of the three epididymal segments (head, body and tail) and vas deferens

(Continues)

TABLE 2 (Continued)

Epididymis and vas deferens
Size (diameters)
Head: measure the longitudinal diameter from the top to the base of the triangle
Body and tail: measure the anterior-posterior diameters in a single longitudinal scan (if possible, including the proximal vas deferens)
Vas deferens: evaluate presence or absence. Measure the anterior-posterior diameter (if possible, in the same longitudinal scan with epididymal body and tail)
Homogeneity/inhomogeneity
Report it as a dummy variable (0 = *homogeneous*; 1 = *inhomogeneous*)
Echogenicity
Use a 3-point Likert scale (0 = *normal echogenicity*; 1 = *mainly hypoechoic*; 2 = *mainly hyperechoic*)
Vascularisation
Qualitative assessment: normal, reduced, enhanced; compare the two epididymides
Quantitative assessment: evaluate arterial PSV, acceleration, RI and PI at the level of the head (branch of the testicular artery) and of the tail (branch of the deferential artery)
Other findings
Evaluate the presence of nodules (in the same way of 'testicular nodules')
Evaluate the presence and number of cysts and the three diameters of the major cyst for each segment
Evaluate and measure epididymal calcifications (major diameter).
Evaluate and measure epididymal appendices (longitudinal diameter).
Pampiniform plexus/varicocoele
Measure the largest vein, irrespective of location[a], with the patient standing, at rest, bilaterally.
Evaluate the extension of the largest vein to the funicular region, upper or lower pole of the testis.
Evaluate the presence of a retrograde venous flow the patient standing, at rest, using CDUS, and classify it as a dummy variable (0 = *absent or intermittent/fluctuating during spontaneous breath*; 1 = *continuous*[b]). Then evaluate the presence of a retrograde venous flow during Valsalva manoeuvre.
CDUS varicocoele is defined in presence of venous vessels >3 mm at rest, with retrograde venous flow detected at least during Valsalva manoeuvre.
Use Sarteschi et al./Liguori et al. classifications for grading varicocoele[c].
'Severe' varicocoele: venous vessels dilation (>3 mm) characterized by a continuous venous reflux at rest, increasing or not during a Valsalva manoeuvre (consistent with grade 4 and 5 of Sarteschi et al.[194]/Liguori et al.[195] classifications)
Subclinical varicocoele: venous reflux detected by CDUS but not clinically evident[27,28,33]

(Continues)

TABLE 2 (Continued)

Pampiniform plexus/varicocoele
EAA classification of varicocoele[c] - Grade 1: Venous vessels dilation (>3 mm) at rest at the funicular region with retrograde venous flow absent/intermittent at rest and enhanced during Valsalva manoeuvre. - Grade 2: Venous vessels dilation (>3 mm) at rest at the upper pole of the testis with retrograde venous flow absent/intermittent at rest and enhanced during Valsalva manoeuvre. - Grade 3: Venous vessels dilation (>3 mm) at rest at the lower pole of the testis with retrograde venous flow absent/intermittent at rest and enhanced during Valsalva manoeuvre. - Grade 4: Venous vessels dilation (>3 mm) at rest (irrespective of location, but usually extending to the peritesticular region) with retrograde venous flow 'continuous' at rest and enhanced during Valsalva manoeuvre. Possible testicular hypotrophy. - Grade 5: venous vessels dilation (>3 mm) at rest (irrespective of location, but usually extending to the peritesticular region) with retrograde venous flow 'continuous' at rest and not increasing during Valsalva manoeuvre. Possible intratesticular varices and/or testicular hypotrophy.

Note: The EAA SOPs are derived from the EAA scrotal US study.[33]

Abbreviations: PI, pulsatility index; PSV, peak systolic velocity; RI, resistive index.

[a]Eventually evaluate the maximum diameter of the internal spermatic vein between the inguinal ligament and upper pole of the testis[33,196] in order to assess a straight vein instead of/avoiding the convoluted vessels below.

[b]Use power Doppler with spectral Doppler analysis and angle correction.[33]

[c]The EAA classification of varicocoele has been derived from ESUR[27,28]/Sarteschi et al.[194]/Liguori et al.[195] classifications of varicocoele modified according to SOPs used in the EAA scrotal US study.[33]

reported by the EFSUMB Guidelines and Recommendations from 2012[37] and in the 2017 updated version.[34,35] According to the EFSUMB,[34,35] US equipment based on contrast-specific US modes is needed for examinations, based on the separation between a non-linear response induced by microbubble US contrast agent oscillations and a linear US signal reflected by tissues. In order to decrease the non-linear harmonic US signals generated by the tissues, a low acoustic pressure is generally used, based on a low mechanical index (at least below 0.3, and often 0.08 or 0.05). Each examined lesion should be described in terms of enhancement, taking into account the temporal behaviour, degree of enhancement as compared with the surrounding tissues (non-enhanced, hypo-enhanced, iso-enhanced or hyper-enhanced), as well as the contrast distribution (homogeneity or inhomogeneity). Two phases are described for organs that have a single arterial blood supply, including the testis: (a) the arterial phase starts from ~10–20 s until ~35–40 s after contrast injection, showing a progressive degree of enhancement; (b) the venous phase starts from ~30 to 45 s after contrast injection, showing a plateau and then a progressive decrease. A B-mode and CDUS examination of the lesion with linear high-frequency transducers should be performed to relate to the subsequent CEUS findings. A higher US contrast agent concentration is required to examine the scrotal contents, typically 4.8 ml of SonoVue™ (Bracco SpA, Milan). The arterial phase in CEUS is the most important aspect of the examination. The testis and epididymis enhance rapidly but the arrival time varies between individuals. The arteries enhance first, followed within seconds by complete parenchymal enhancement. There is no accumulation of the US contrast agent in the parenchyma of the testis and the enhancement declines over a variable period of time such that there is minimal residual enhancement after 3 min.

Thanks to these methodological standards, the assessment of some pathological conditions using CEUS have improved.[34,35] Using time-intensity curves, evaluating the wash-in and wash-out curves may help distinguish malignant from benign tumours, although CEUS analyses still overlap between different histological types.[34,35] In addition, CEUS can discriminate non-viable regions in testicular trauma and can identify segmental testicular infarction.[34,35]

6.3 | Sonoelastography

The methodological standards for the clinical practice of SE in non-hepatic applications, including testicular investigation, have been reported by the EFSUMB Guidelines and Recommendations in the 2019 updated version.[36] So far, strain elastography and shear wave elastography, which includes acoustic radiation force impulse-based techniques, and transient elastography are available. The basic principles of SE have been extensively described in previous EFSUMB guidelines,[15] whereas methodological standardization for different organs, including the testis, is reported in the updated EFSUMB guidelines.[36] An adequate knowledge and training in US and elastographic methods is required. From a methodological point of view, SE use to investigate focal testicular lesions can only be recommended in conjunction with other US techniques, as there is overlap between benign and malignant neoplasms.[36] Measurements using shear wave elastography between the centre and peripheral zones differ and the point of measurement still requires standardization. SE assessing overall background parenchyma has been used to investigate infertility,[17] TML[18] and undescended testis.[19] Currently, however, these specific applications remain in the research field.

7 | STANDARDS IN SCROTAL US

We here evaluate and discuss the standards in scrotal US used in the past and to date, focusing on 'what was' and 'what is' normal at CDUS, CEUS and SE (see below). Table 3 shows normal values and cut-offs of the main US parameters as well as US classifications used previously and currently in evaluating scrotal organs at greyscale and colour-Doppler US. Figures 1–4 show some paradigmatic normal and abnormal findings at multiparametric US.

7.1 | Testis

US is useful in evaluating several testicular characteristics, including volume, echotexture, vascularisations, abnormalities/lesions and

TABLE 3 Previous and current normal values, cut-off and classifications of the main CDUS parameters/characteristics of the scrotal organs

	Previous normal values, cut-off and classifications at CDUS	Current[a] normal values, cut-off and classifications at CDUS
Testis		
Mean TV (ellipsoid)	14–19 ml[a]	17 ml
Right TV hypotrophy	<12 ml[b]	<12 ml
Left TV hypotrophy	<12 ml[b]	<11 ml
TI classification	Lenz et al.[42]	EAA US study[33]
	1 = *very uniform pattern*	0. Homogeneity
	2 = *slightly irregular pattern*	1 = *mild inhomogeneity (presence of small hypoechoic foci/thin hypoechoic striae)*
	3 = *moderately irregular pattern or small echogenic points*	2 = *moderate inhomogeneity (presence of thick hypoechoic striae)*
	4 = *very irregular pattern or bright echogenic spots*	3 = *severe inhomogeneity (diffuse TI with 'netting'/'geographical map' appearance)*
	5 = *tumour suspected (demarcated area)*	
	Westlander et al.[82]	
	1 = *homogeneous*	
	2 = *homogeneous with some hyperechogenic foci*	
	3 = *heterogeneous with spread hyperechogenicity*	
	4 = *heterogeneous with both hyperechogenic and cystic (hypoechogenic) parenchyma*	
	5 = *post-operative intratesticular lesion*	
TML (most used definitions)	≥5 microcalcifications per field of view	≥5 microcalcifications per field of view[b]
	≥5 microcalcifications in the whole testis	
Vascularisation	Normal, reduced or enhanced	Testicular artery (TA) PSV[g]: 3–11 cm/s
		Intratesticular arteries PSV[g]: 3.7–7 cm/s
Epididymis and vas deferens		
Epididymis head diameter	≤12 mm[c]	≤11.5 mm
Epididymis body diameter	≤4 mm[d]	≤5 mm
Epididymis tail diameter	≤6 mm[e]	≤6 mm
Vas deferens diameter	Not reported	≤4.5 mm
Epididymis inhomogeneity	Homogeneous or inhomogeneous	EAA US study (Lotti et al.)[33]
		0 = *homogeneity*
		1 = *inhomogeneity*
Epididymis echogenicity	Normoechoic, hypoechoic, hyperechoic	EAA US study (Lotti et al.)[33]
		0 = *normal echogenicity*
		1 = *mainly hypoechoic*
		2 = *mainly hyperechoic*
Epididymis vascularisation	Normal or enhanced	Head artery (TA branch) PSV[g]: 3.1–4.6 cm/s
		Tail artery (deferential) PSV[g]: 1.8–8.0 cm/s

(Continues)

TABLE 3 (Continued)

	Previous normal values, cut-off and classifications at CDUS	Current[a] normal values, cut-off and classifications at CDUS
Varicocoele	Several classifications[f]	Venous vessels >3 mm at rest[c], irrespective of location, with retrograde venous flow detected at least during Valsalva manoeuvre, with grading according to Sarteschi et al./Liguori et al. classifications[b]

[a]Current normative parameters are mainly derived from the EAA US study,[33] and, in part, from the ESUR guidelines/recommendations.[26–28] Of note, we here report the main findings of the EAA US study, which, in the original article,[33] reports extensively normative values/reference ranges of all the scrotal organs CDUS parameters. For a detailed description of 'previous normal values' see the main text.
[b]Definitions shared by the EAA and ESUR.
[c]EAA reports 'at rest', ESUR reports 'during Valsalva'.
[a]Different mean or median TV has been reported in different previous studies.[42,56,64–68].
[b]According to references.[23,56,69] Only two studies reported hypotrophy for TV < 10 ml.[48,70].
[c]According to references.[56,177,178,188,201] A single study[189] suggests a value <10.85 mm, higher values indicating obstruction.
[d]According to references.[177,178,201].
[e]According to references.[177,178,188].
[f]See, for review, reference 1.
[g]Along with peak systolic velocity (PSV) reference range evaluated in testicular and epididymal arteries, the EAA US study reports normative values for acceleration, pulsatility and resistive index.[33].
Abbreviations: TI, testicular inhomogeneity; TML, testicular microlithiasis; TV, testicular volume.

location.[1] We discuss here what 'was' and what 'is' normal regarding these issues.

7.1.1 | Testicular volume

Testicular volume (TV) is an essential parameter in clinical practice, reflecting not only the seminal and hormonal status of the subject but also the presence of previous or current testicular or systemic disorders.[1,38–40] TV is usually assessed clinically by Prader's orchidometer.[1,38–40] However, orchidometry overestimates TV when compared to US,[41–45] and US offers a greater accuracy in TV measurement than physical examination.[42,46,47] Previous studies reported that US-estimated TV was positively related to total sperm count,[42,48–51] sperm motility,[48,51] normal sperm morphology[42,51] and testosterone levels[45,48,51] and negatively with luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels.[48,51,52] A negative correlation between US TV and non-conventional sperm parameters (sperm DNA fragmentation[47,53–55,63,64] percentage of spermatozoa with low mitochondrial membrane potential, phosphatidylserine externalization or chromatin compactness[55]) has been also reported.

What was normal

TV US estimation varies according to the mathematical formula applied. In the last decade, no consensus on the best mathematical model to be used has been achieved. Previous studies, as discussed in comprehensive articles,[1,56] reported US-assessed TV using ellipsoid (length × height × width × 0.52),[57] Lambert's (length × height × width × 0.71)[58] or Hansen's (length × width^2 × 0.52)[59–61] formula, making comparisons between different studies complicated. The most commonly applied formula is the ellipsoid formula.[28,56] However, in studies investigating the difference between US and 'real' TV by water displacement,[47,62,63] the empirical Lambert's formula[58] was reported to be superior. In 2014, SIU/SIEUN recommendations[21] supported the use of the ellipsoid formula, whereas in 2015 AIUM guidelines[22] reported that, in paediatric patients, TV could be provided using Lambert's or the ellipsoid formula.

Using the ellipsoid formula, healthy German[56] and Danish[42] men showed a median TV of ~14 ml, young Italian[64] and South Korean[65] men a mean TV of ~15 ml and ~18 ml, respectively, and fertile Italian men[66–68] a mean TV of ~19 ml. The relatively wide range of a 'normal' average TV could depend on the lack of international US SOPs standardization, on the difference between 'mean' and 'median' TV values, on the diverse age range of the subjects studied and on differences between populations studied, belonging to different countries. Although TV difference among ethnic groups and TV variations with age in adult men seem to be modest, available studies[1] on these topics are relatively scanty and, in some cases, conflicting, representing possible confounders. A TV < 12 ml has been proposed by previous studies to indicate testicular hypotrophy at US, using the ellipsoid formula[53,69] or irrespective of the mathematical formula used,[26] although without any evidence base. In addition, a TV < 10 ml using the Lambert's formula was reported to be associated with testicular dysfunction, although only in two studies assessing Japanese men with infertility.[48,70]

What is normal

Recently, the ESUR guidelines on varicocoele[27,28] supported the use of Lambert's formula to calculate TV at US, considered the most accurate according to previous studies,[47,58,63] although without a 'strong' consensus.[27]

Most recently, the EAA US consortium, evaluating a cohort of 248 healthy, fertile men,[32] reported the US refence range of testicular diameters and TV according to the ellipsoid, Lambert's and Hansen's mathematical formulas, providing evidence-based normative parameters.[33] In the EAA study, the US TV calculated with the

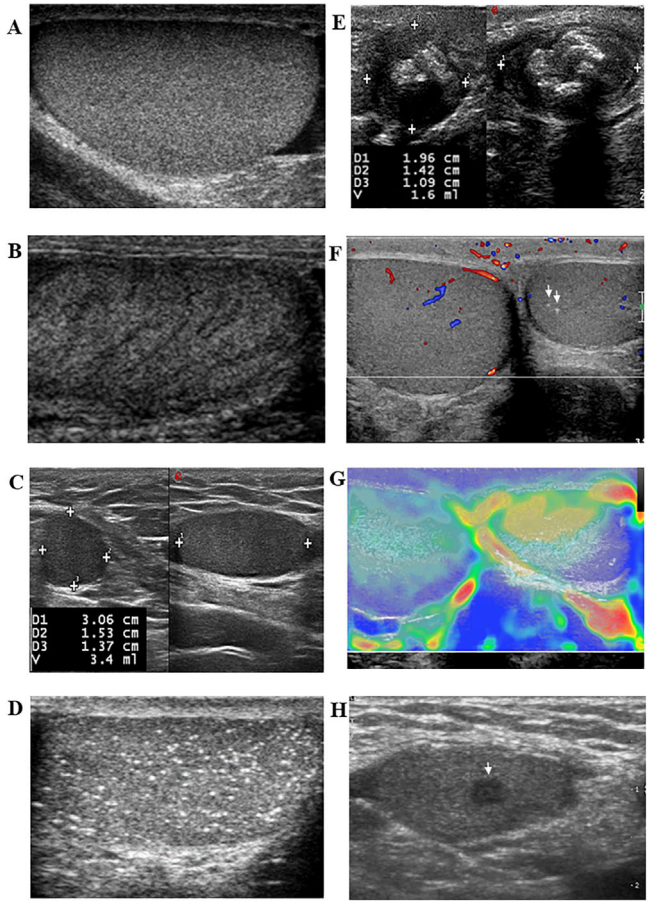

FIGURE 1 Testicular size and echotexture: Normal and abnormal appearance at US. (A) Normal testis, characterized by a homogeneous granular echo-texture, made up of uniformly distributed medium-level echoes (i.e. homogeneous and normoechoic). (B) Testis characterized by echotexture inhomogenicity leading to a 'striated' appearance. (C) Small, hypotrophic undescended testis located in the inguinal canal, with a uniformly hypoechoic appearance. (D) 'Starry sky' testicular microlithiasis appearance. (E) Small, hypotrophic undescended testis with inhomogeneous and hypoechoic appearance and macrocalcifications. (F and G) Previously undescended left testis. Spectacle view of the testes shows a smaller left testis (F) with two microcalcifications (arrows) and lower vascular spots than the contralateral testis. The left testis appears as softer than the contralateral one at strain elastography (G). (H) Small undescended testes retained in the inguinal canal, hypoechoic, with a focal more hypoechoic lesion (arrow) which was histologically an area of Leydig cell hyperplasia

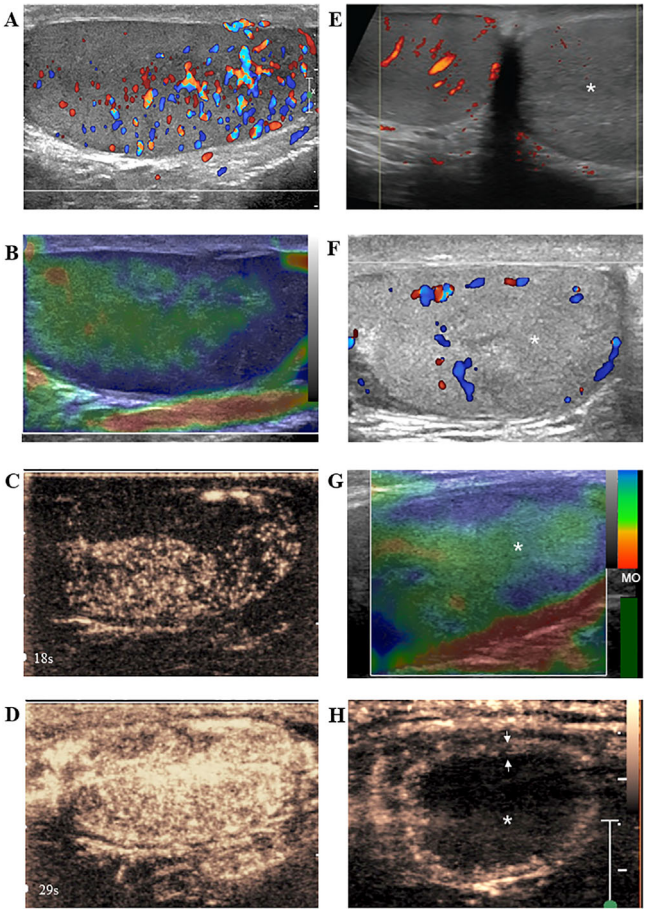

FIGURE 2 Abnormal testicular vascularisation suggesting focal orchitis, testicular torsion and segmental testicular infarction at multiparametric US. (A–D) Focal orchitis involving the lower pole and part of the midportion of the left testis in a patient presenting with left acute scrotal pain. The lower pole and the midportion of the testicular parenchyma show enhanced vascularisation at colour-Doppler US evaluation (A), hard at elastography (B), with early enhancement (18 s) after microbubble contrast injection (C). The testis enhances homogeneously within 29 s after microbubble injection (D). (E) Absent vascularisation of the left testis in a patient presenting with acute scrotal pain diagnosed as testicular torsion. Spectacle view of the testes shows no vascular spots in the left testis (asterisk) and normal vascularisation in the contralateral testis. (F–H) Segmental testicular infarction in a patient presenting with left scrotal pain for 3 days. The lower pole of the left testis (asterisk) is slightly hypoechoic and shows no colour signals at colour-Doppler US (F). The lesion (asterisk) is soft at sonoelastography (G) and avascular at contrast-enhanced US (H). A perilesional enhancing rim is appreciable (arrows in panel H)

ellipsoid formula showed the most accurate correlation with the Prader orchidometer-assessed TV, where Lambert's formula overestimated orchidometry.[33] Hence, EAA supports the use of the ellipsoid formula, considering that it fits better into the clinical (and not experimental) reality.[33] In addition, the ellipsoid formula is easier to use in clinical practice, because it is automatically calculated by most US devices.[28,33] Using the ellipsoid formula, the EAA US consortium reported a mean TV of ~17 ml in healthy, fertile men.[33] Because the EAA study is an international multicentre one, the aforementioned value can be considered the 'normal' mean TV of the European adult population of reproductive age. The multicentre nature of the study and the limited age range (23–53 years) of the subjects investigated avoid confounders related to nationality/ethnicity and aging. The EAA US TV lowest reference limit for right and left testis is 12 and 11 ml, respectively, defining in an evidence-based manner 'testicular hypotrophy' as being below these thresholds.[33]

Regarding the evaluation of TV in the paediatric population, reference curves for mean US-TV are available for boys aged 0–6 years,[71] 0.5 months to 18 years[44] and 6 months to 19 years.[72] Of note, the last

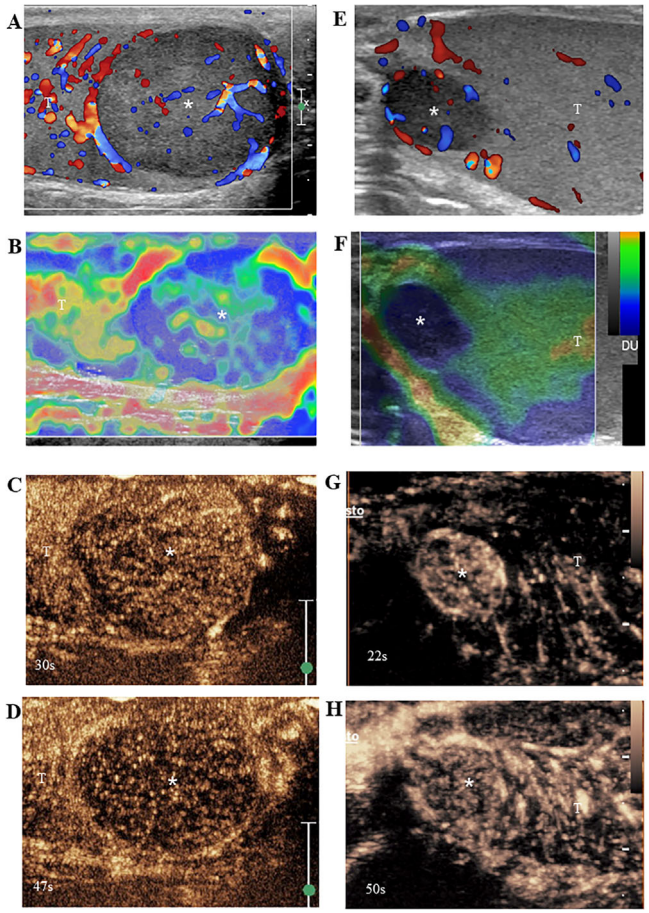

FIGURE 3 Appearance of frequent testicular tumours at multiparametric US. Histologically proven pure seminoma (A–D) and Leydig cell tumour (E–H), investigated with colour-Doppler US (A and E), sonoelastography (B and F) and contrast-enhanced US (C and D, and G and H). Both lesions (asterisks) are hypoechoic, vascularized at colour-Doppler US (A and E), hard at strain elastography (B and F), with marked enhancement at contrast-enhanced US 20–30 s after microbubble injection (C and G), more pronounced in the leydigoma (G). After 47–50 s, wash-out is evident in seminoma (D), whereas the leydigoma is iso-enhancing to testis (H). T = testis

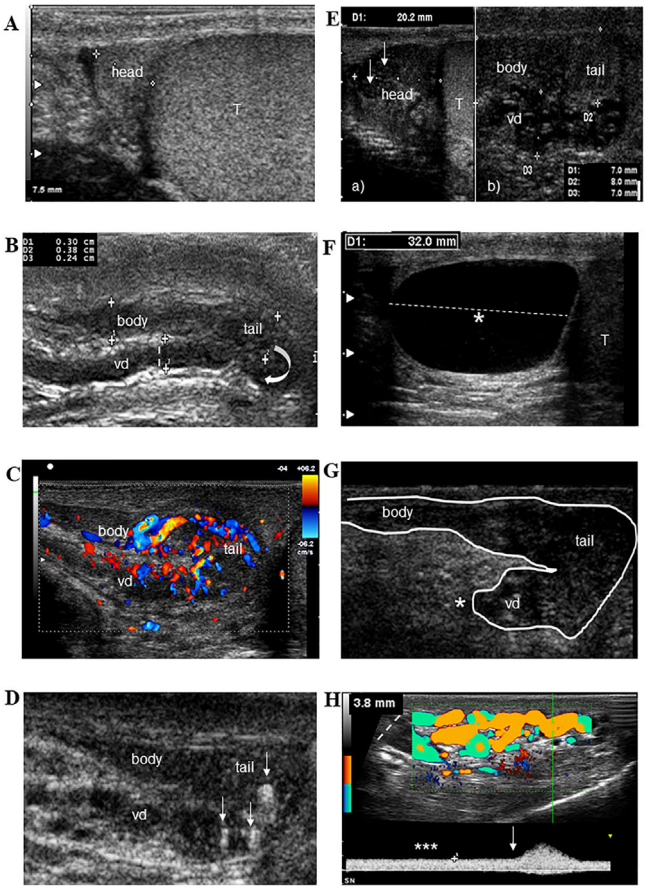

FIGURE 4 Epididymis and vas deferens normal and abnormal findings at greyscale and colour-Doppler US (CDUS), and an example of severe varicocoele. (A) Normal epididymal head with triangular shape in a longitudinal scan, homogeneous, with echogenicity comparable to that of the testis (T). Its length is measured from the top to the base of the triangle. (B) Homogeneous epididymal body and tail and proximal vas deferens (vd) in a longitudinal scan. Their echogenicity is slightly hypoechoic compared with the testis and the epididymal head in panel A. Their diameters are measured and are normal (see the main text). The curve arrow indicates the epididymal–deferential handle. (C) Acute epididymitis. The body and tail of the epididymis are enlarged, inhomogeneous, hypoechoic, and show diffuse and intense enhanced vascularisation. (D) Chronic/previous epididymitis at greyscale US. The body and tail of the epididymis are dilated and inhomogeneous, the tail slightly hyperechoic, and macrocalcifications (arrows) are present in the tail and in the proximal portion of the vas deferens. (E) Epididymal and vas deferens dilation in a subject reporting previous vasectomy. The right side (a) shows a dilated (20.2 mm), inhomogeneous, hypoechoic epididymal head with two spermatoceles (arrows). The left side (b) shows dilated (7, 8 and 7 mm, respectively), inhomogeneous, hypoechoic epididymal body and tail and vas deferens, the latter characterized by a clear tubular dilation. (F) Large, anechoic cyst (asterisk; 32 mm in longitudinal scan) of the epididymal head. (G) Vas deferens agenesis. The body and tail are visible and dilated, whereas only the proximal portion of the vas deferens is detectable, showing an abrupt interruption (asterisk). (H) Severe varicocoele at CDUS. US shows dilated veins (>3 mm) of the pampiniform plexus, with a coloured signal and a continuous venous reflux at rest (***), increasing with Valsalva manoeuvre (arrow), identifying a 'severe' varicocoele at CDUS (see the main text)

study[72] also reported the distribution of TV within the Tanner stages of pubic hair development. However, all these studies have been performed in the Netherlands, hence their results might not apply to different ethnic groups. As a corollary, a Korean study[73] also evaluated changes and ranges of paediatric TV in 0- to 10-year-old boys, although without reporting clear reference curves.

7.1.2 | Testicular homogeneity/inhomogeneity and echogenicity

From the 80s, the normal adult testis has been described as characterized by a homogeneous granular echo-texture, made up of uniformly distributed medium-level echoes (i.e. homogeneous and normoechoic) resembling the echogenicity of the normal thyroid gland[1] (Figure 1A). The occurrence of testicular structural abnormalities is associated with

an alteration of the echo distribution, leading to inhomogenicity and abnormal echogenicity (see below).

What was normal

The image resolution and clinical significance of testicular inhomogeneity (TI) has changed over time. In the 70s, when US was used for the first time to investigate the scrotal organs through contact B-scan technique,[4] and in the 80s, after the introduction in clinical practice of the first high-frequency devices,[8] US image quality and resolution was significantly lower than today. Hence, at first, severe TI was considered as a warning for the presence of a possible testicular malignancy. In this setting, the reported association of very irregular testicular patterns and carcinoma in situ in cryptorchid men[74] or testicular tumour[75] by Lenz et al. is paradigmatic. In 1993, Lenz et al.[42] reported the first classification of TI (Table 3), proposing a 5-point 'testicular echotexture score' ranging from 0 (*regular pattern*) to 5 (*tumour suspected*), which correlated positively with the presence of a carcinoma in situ.[76] In addition, the TI score negatively correlated with normal sperm morphology,[42] sperm count[49] and US-TV.[42,49,76] In 1995, Fournier et al.,[77] comparing surgical findings to those of preoperative scrotal US in 50 patients undergoing surgical exploration for different disorders (including trauma, tumours or benign atraumatic conditions), reported that TI was the most reliable predictor of a pathological condition. In 1996, the 'striated testis' (Figure 2B) was first described,[78] a specific US pattern characterized by the presence of hypoechoic striae within the testicular parenchyma, resembling the black stripes of a zebra's coat.[79] In the aforementioned study,[78] the striated US pattern was associated with testicular fibrosis and not malignancy. Subsequently, the hypoechoic striae were associated with an exaggeration of the normally unapparent interlobular septa as a consequence of seminiferous tubule reduction and interstitial proliferation, and the inhomogeneous, striated testicular pattern was associated with atrophy and fibrosis.[80] Hence, over time, the clinical significance of TI has shifted from a risk factor for the presence of a testicular tumour to an US abnormal pattern associated with other pathological conditions and/or testicular function impairment (see below). In parallel, in view of the advancing US technology, 'suspected tumours' have been described as 'nodular lesions' and no more as 'echotexture abnormalities'. In line, Westlander et al.[81] proposed in 2001 a 4-point scale TI classification, a semi-qualitative score modified from that of Lenz et al. not considering suspected tumours. The classification was modified soon after into a 5-point scale[82] (Table 3) introducing a further category, 'intratesticular lesions observed after testicular sperm aspiration', which resolved after 6–9 months of follow-up.

Regarding testicular echogenicity, no standardized classification has been published in the past, although the description of normoechoic, hypoechoic or hyperechoic testis has been previously reported.[1,83–85] Echogenicity depends on the seminiferous tubules maturation and germ cell representation.[1] Prepubertal testes have been described as slightly more hypoechoic than the adult ones, because seminiferous tubules have not developed a lumen yet.[1,83] During puberty, as a function of lumen development, testis echogenicity progressively increases, up to the average adult level.[1,83] In the case of testicular damage (congenital or acquired), a reduction of testicular parenchyma and/or an increase in interstitium occur, often leading to a hypoechoic echotexture[86] (Figure 1C).

What is normal

Although TI is often observed in the elderly and considered normal, in young subjects it has been associated with several pathological conditions, including ischemia, orchitis, trauma, torsion, exposure to physical or chemical agents, chemo- and radiotherapy or alcohol abuse and Klinefelter's and metabolic syndrome.[1] In addition, TI has been associated with testicular function impairment,[42,87] including hypogonadism, abnormal sperm morphology,[42,88] impaired sperm quality and azoospermia.[89]

Recently, Pozza et al.[51] developed, in a cohort of 2230 men, a semi-quantitative, multiparametric (including bilateral US TV, echotexture, echogenicity and microlithiasis) score, ranging from 0 to 7 and named 'testicular ultrasound (TU) score', that has proven to be significantly more accurate than Lenz's score[42] in predicting impaired spermatogenesis and able to predict hypogonadism. In the TU score,[51] the parameter testicular 'homogeneity' has been considered dichotomously (0 = *homogeneous*; 1 = *inhomogeneous*). Because subjects with a homogeneous testis showed better sperm and hormonal parameters than the rest of the sample, testis homogeneity can be considered 'normal'.

More recently, the EAA US consortium[33] has proposed a new, 4-point scale classification of TI, easy to use in clinical practice and avoiding the term 'suspected tumours' (see Table 3). In a cohort of healthy, fertile men, only very few subjects had TI, always of a mild degree (grade 1).[33] Those subjects showed a lower sperm vitality when compared to the rest of the sample.[33] Hence, it is possible to define as 'normal' the presence of a homogeneous, or at least slightly inhomogeneous (mild TI), testicular pattern, especially from a reproductive point of view.

Regarding testicular echogenicity, the normal adult testis appears normoechoic at US[1,83,85] (see above). Detection of focal abnormalities may underlie several conditions, including tumours,[1,83,84] whereas a diffuse hypoechogenicity may be related to widespread malignancies[1,83,84] (see below, in Section 7.1.5). However, more often, diffuse hypoechogenicity is observed in damaged testes[1,83,84] (e.g. undescended and/or hypotrophic testes; see below), and can be associated with TI at US,[1,83,84] indicating parenchymal reduction and interstitial proliferation.[80] In line with the latter issue, testis hypoechogenicity, as well as TI, has also been associated with increased levels of M540 bodies, round anucleated elements detected by flow cytometry in the semen, considered markers of testicular apoptosis/spermatogenesis derangement.[54] With reference to the echogenicity of the whole testis (and not to focal lesions), the EAA US consortium[33] has recently classified it on a 3-point Likert scale (see Table 3).

7.1.3 | Testicular vascularisation

The vascular anatomy of the testis has been described in detail elsewhere.[1] Pictures of the flow characteristics of the main

testicular arteries and their branches have also been reported elsewhere.[90] Testis vascularisation plays a critical role in the differential diagnosis among orchitis (Figure 2A–D), orchi-epididymitis or some malignant conditions (i.e. leukaemia, lymphoma) (enhanced), testicular torsion (absent) (Figure 2E), infarction (absent or peripheral; Figure 2F–H),[1,91,92] other pathological conditions resulting in testicular ischemia, such as tension idroceles,[93] as well as in the assessment of scrotal trauma, when considered along with other clinical and US features.[25] In the event of acute scrotal pain, a normal scrotal content and testis vascularity should prompt extension of the US investigation to the abdomen, to exclude non-scrotal causes of acute pain.[94] In addition, the vascular pattern of intratesticular lesions may suggest their benign or malignant nature. This is important, in particular, in patients with lymphomas or haematological malignancies,[91,92] in patients with bilateral synchronous tumours[95] and in patients with multiple, synchronous lesions in the same testis, in which the therapeutic approach is determined by the most aggressive histotype.[96] However, so far, the assessment of testis vascularisation with CDUS is still qualitative, with no clear quantitative cut-off distinguishing the aforementioned conditions. CEUS and SE (see below) have improved the capacity to investigate pathological processes, although without any diagnostic certainty.

What was normal

Until 2021, the reference range of testis vascular parameters was lacking. Only one study[97] reported the reference range of a single parameter, the peak systolic velocity of the testicular artery, evaluating 306 healthy Caucasian men aged 18–88 years. Other available studies investigated testis vascularisation to assess different pathological conditions (see above) qualitatively. In addition, some testis vascular parameters have been associated with sperm quality[98,99] or have been suggested to be useful in discriminating obstructive- and non-obstructive azoospermia[100–103] or residual spermatogenic areas in non-obstructive azoospermia.[100] However, at present, these have been investigated only for research purposes, with no impact on the clinical management of azoospermic men.[1] Finally, CDUS could be used to evaluate possible damage occurring during testicular sperm extraction.[104,105]

What is normal

Recently, the EAA US study[33] reported a standardization of the measurement of the testis vascular parameters (see Table 2) and their reference ranges in healthy, fertile subjects (see Table 3). In addition, a recent meta-analysis[106] demonstrated that CDUS represents an effective imaging modality for diagnosing testicular torsion in adult patients with acute scrotal pain. Furthermore, recently the ESUR published a position statement on imaging in scrotal trauma,[25] clearly defining the role of CDUS evaluation of testicular/scrotal vascularisation in different traumatic conditions, and indicating CEUS and SE as advanced techniques that are useful as problem-solving tools in equivocal cases. Brief statements on this topic were also reported in the 2020 AUA urotrauma guidelines.[24]

7.1.4 | Testicular microlithiasis

TML is an US diagnosis. Its association with testicular tumours and infertility has been widely debated. Recent meta-analyses support a significant association between TML and testicular cancer in the general male population,[107] infertile men[108] and children with contributing factors for primary testicular tumour.[109] However, recent reviews[110,111] reported that TML is not an independent risk factor for testicular cancer but is associated with malignancy depending on the co-occurrence of specific risky conditions. No meta-analyses have evaluated the association between TML and sperm parameters. Recent reviews[110,111] have reported that the relationship between TML and male infertility is still debated. Some studies support TML as an additional feature of the 'testicular dysgenesis syndrome[111]' and there is evidence of a genetic predisposition for TML.[112]

TML (see below for definition) is based on the presence of microcalcifications, which are bright echogenic non-shadowing foci less than 3 mm.[1,26] They are made of micro-calcium deposits with surrounding fibrosis.[26] They do not cause symptoms and are impalpable.[26] Microcalcifications must be distinguished from macrocalcifications, which have never worried sonographers or clinicians, and which have been associated with a prior testicular insult (trauma, orchitis, infarction, torsion, chemo/radiotherapy), testicular atrophy or maldescended testis.[1,83,84] However, they can be, albeit rarely, related to a burnt-out tumour.[1,83,84] In these aforementioned cases, the associated pathological condition, and not the presence of the macrocalcification itself, could play a negative role in general and/or reproductive male health.

What was normal

The first sonographic identification of TML was described by Doherty et al.[113] in 1987 as 'innumerable tiny bright echoes diffusely and uniformly scattered throughout the substance of testes'. Thereafter, a large number of varying definitions have been used in the sonographic literature on this topic.[114,115] However, the two main definitions for TML proposed in the past were as follows: ≥5 microcalcifications in the whole testis[116] or ≥5 microcalcifications per field of view.[117] According to a previous version[118] of the EAU guidelines, the presence of TML with no associated risk factors (see below) did not require scrotal US follow-up or biopsy, whereas presence with associated risk factors (infertility, bilateral TML, atrophic testes, history of cryptorchidism or of testicular cancer) was considered an indication for regular scrotal US follow-up, and, eventually, testicular biopsy.

What is normal

Current EAU guidelines[29,30] on TML management have not changed significantly in the last decade. It is still a critical issue that in men with 'TML and additional risk factors' either US follow-up or biopsy is advised, possibly leading to different and non-standardized management of patients. In addition, the timing of the testicular US follow-up has not been suggested. However, in 2015, the ESUR published guidelines[26] on TML imaging and follow-up, recommending that annual follow-up is advised only in patients with 'TML and additional risk

factors' (personal/family history of testicular tumour, maldescent testis, orchidopexy, testicular atrophy) up to age 55, and, eventually, in men with no risk factors but diffuse ('starry sky'; Figure 1D) TML. Annual US follow-up is suggested also for children/adolescents with maldescent testis/post-orchiopexy or with testicular atrophy.[26] In addition, recommendations for men with genetic disorders (including Klinefelter and McCune–Albright syndromes) have been reported, and are the same as those for the general population.[26] Finally, ESUR indication for biopsy in TML men is very limited. In particular, in men who, at orchiectomy for a germ cell tumour, show TML or atrophy of the contralateral testis, a testicular biopsy may be indicated to look for carcinoma in situ.[26]

Regarding the possible relationship between TML and male infertility, several studies have reported that the prevalence of TML in infertile men is higher than in fertile men.[110,111] In addition, in a relatively large cohort of men with fertility intention, those with TML showed worse semen parameters than the rest of the sample.[119] More recently, in a larger cohort of males of infertile couples, men with TML showed lower mean testis volume and sperm concentration and higher FSH levels than those with limited (<5 hyperecogenic spots per sonogram) or no microcalcifications.[120] TML appears to be linked to infertility as an indicator/part of testicular dysgenesis syndrome.[110,111] As a corollary, so far, TML is not considered a risk factor for the production of antisperm antibodies in infertile men.[121]

7.1.5 | Testicular lesions

From the first application of greyscale US to investigations of scrotal content, the main interest of physicians has been to explore scrotal masses.[5,6] Over time, technical US skills have improved, focusing to date on testicular lesions with different approaches, including CDUS, CEUS and SE[1–3,122–132] (Figure 3). What 'was' and what 'is' normal regarding testicular lesions is more related to their management, different for small (millimetric) and large nodules, and to the role of US in their evaluation, than to the capacity of US to definitively discover a malignancy and its histological type. In fact, US is still not accurate enough to define the origin of several lesions, and histology remains the only certain diagnostic tool.[1,122,133–136]

What was normal

Until the advent of CEUS, CDUS was the only way to evaluate testicular and extra-testicular lesions. Sonologists mainly described the characteristics of the lesion, including size, homogeneity, echogeneity, margins and vascularisation.[122,123] However, especially for large lesions, surgery was mandatory. Differential diagnosis was difficult, particularly when, at US, severe TI was detected.[42,74,75] The difference between anechoic and solid lesions was detectable, allowing clinicians to distinguish intratesticular benign cysts from possible malignant lesions.[122,137] However, large solid lesions were considered as likely neoplasms. In addition, differential diagnosis between hypoechoic areas, underlying segmental infarction, post-traumatic or post-inflammatory outcomes, intratesticular hematomas or possible tumours was difficult.[122] The finding of large lesions required compulsory surgery, whereas that of millimetric lesions was managed with strict follow-up, requiring surgery in the event of unstable characteristics over time.

What is normal

So far, with the improvement of US devices' resolution and vascular assessment, greyscale US with power/colour-Doppler is able to evaluate testicular lesions quite well, providing, in some cases, specific diagnosis. Clinical and CDUS patterns of testicular[83,84,122] and extra-testicular[83,84,123,138] lesions have been described in detail elsewhere[83,84,122,123,138] (see also Figure 3). Table 4 summarizes the CDUS characteristics of the main malignant and benign testicular lesions. In addition, new US imaging techniques, such as CEUS and SE, have improved the characterization of testicular abnormalities (see Figure 3), both in adult[2,3,122–131] and pediatric[3,139,140] patients. According to the EFSUMB recommendations,[34,35] CEUS can distinguish vascularized from non-vascularized focal testicular lesions, helping to exclude malignancy. In addition, CEUS can discriminate non-viable regions in testicular trauma and can identify segmental infarction.[34,35,141–143] Finally, CEUS can identify abscess formation and infarction in severe epididymo-orchitis.[34,35,144] As a corollary, recently ESUR published its position statements on imaging in scrotal trauma,[25] reporting standardization, methodology and information derived from CDUS/CEUS/SE application. Regarding SE, according to the EFSUMB recommendations,[36] its use for the evaluation of focal testicular lesions can only be recommended in conjunction with other US techniques, as there is overlap between benign and malignant neoplasms.[145]

Regarding methodology and indications, palpation should be the first step of an US investigation[1,146] of the scrotal content, and, in selected cases, can help to identify scrotal lesions that are not immediately seen at US.[146] According to recent EAU guidelines,[30] high-frequency (>10 MHz) testicular US should be used to confirm a testicular tumour even in the presence of a clinically evident testicular lesion. The use of testicular US can (i) determine whether a mass is intra- or extra-testicular; (ii) determine the volume and anatomical location of the testicular lesion and (iii) be used to characterize the contralateral testicle to exclude other lesions and identify risk factors for carcinoma in situ.[30] Testicular US is also recommended for all men with retroperitoneal or visceral masses and/or without elevated tumour markers in the absence of a palpable testicular mass and for fertility work-up evaluation.[30]

According to the EAU guidelines,[30] every subject with a suspected testicular mass must undergo surgical exploration, with orchiectomy if a malignant tumour is found or testicular biopsy with histological examination if the diagnosis is not clear. Regarding large nodule management, US should be performed even in the presence of a clinically evident testicular mass.[1,30] In this scenario, US frequently plays an adjuvant role, sometimes allowing for differential diagnosis among different clinical conditions (i.e. malignancy, inflammation, cysts) and evaluating the contralateral testis.[1,30] However, currently, the real challenge is represented by the imaging and

TABLE 4 Main clinical and US characteristics malignant and benign lesions of the testis

Main testis lesions	Age peak incidenceand biologic behaviour	Most common US characteristics	Associated abnormalitiesor syndromes
Malignant			
Germ cell tumours (~95%)			
Seminoma (35–55%)	- Typical (85%): 20–40/50 years - Anaplastic (5%–10%): 20–40 years - Spermatocytic (5%–10%): 50–70 years Good prognosis	- Homogeneous, hypoechoic, solid nodules of varies size; round, oval or with polycyclic lobulated margins; often high-flow, low-resistance vascular arborisation; rarely bilateral or in mixed germ cell tumour - Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in and wash-out - Hard at SE	Cryptorchidism Microlithiasis
Non-seminoma - Embryonal cell carcinoma (20%–25%) - Teratoma (5%–10%) - Choriocarcinoma (0.5%) - Yolk sac tumour (<1%) - Mixed (20%–40%)	20–30 years, aggressive <4 years, benign; 20–30 years, malignant 10–30 years, highly malignant <2 and 20–30 years, malignant Mixed: 20–30 years, malignant	- Inhomogeneous, hypoechoic, solid nodules with cystic areas or calcifications within the lesion - Variable vascularisation at CEUS, inhomogeneous enhancement - Coexisting stiff and soft areas at SE	
Stromal (~5%)			
Leydig cell tumour (3%)	Children and 20–50 years (90% benign, 10% malignant)	- Often small, unilateral and solitary with circumferential blood flow - Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in and delayed wash-out - Hard at SE	May secrete oestrogens (30%): - Gynecomastia - Pseudo-pubertas praecox - Azoospermia - Erectile dysfunction - Loss of libido
Sertoli cell tumour (~1%)	Children and 20–30 years ('borderline'; < 20% malignant)	Hypo- or hyperechoic nodules with possible calcifications • With dysplastic syndromes: bilateral and multifocal • Without dysplastic syndrome: unilateral and focal • Large-cell calcifying Sertoli cell tumour (LCCSCT): diffusely heterogeneous pattern, hyperechoic, large calcifications - Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in and delayed wash-out (poorly described, non-applicable in LCCSCT)- Hard at SE	- Rarely secrete oestrogens: gynecomastia. - Klinefelter syndrome - Peutz–Jeghers syndrome - Androgen insensitivity (testicular feminization) syndrome - Carney complex
Others			
Lymphoma	2% or 25% of testicular tumours in subjects with <50 or 50–70 years, respectively. Malignant	- Homogeneous hypoechoic diffuse testis infiltration or unifocal/multifocal hypoechoic lesions of various size; parallel hypoechoic lines radiating peripherally from the mediastinum (blood vessels), high vascularisation; 8%–18% bilateral, synchronous or asynchronous - Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in wash-out, non-branching linear pattern. - Hard at SE	

(Continues)

TABLE 4 (Continued)

Main testis lesions	Age peak incidenceand biologic behaviour	Most common US characteristics	Associated abnormalitiesor syndromes
Leukaemia	Children Malignant, often recurs	- Unilateral or bilateral, diffuse or focal, hypo- or hyper-echoic lesions with longitudinal hypoechoic striae and increased blood flow. - Highly vascularized at CEUS, homogeneous enhancement, rapid wash-in wash-out, non-branching linear pattern - Hard at SE	
Metastases	50–70 years Aggressive	US findings depend on the primary tumour and necrosis degree	Common primary sites: prostate, lung, bowel, melanoma, kidney
Benign			
Intratesticular cysts	Variable	- Usually solitary, can be multiple; often near the mediastinum; well-defined round anechoic lesions; various size - Avascular at CEUS - SE: triple layout pattern; shear wave elastography (SWE): signal defect	
Tunica albuginea cysts	40–60 years	- SWE Small round anechoic peripheral lesions of various size - Avascular at CEUS - Palpable, difficult to evaluate at SE	
Dilation of the rete testis	50–70 years	- Multiple micro- or macro-tubular fluid-filled structures, often near the mediastinum; no vascularisation at CDUS - Single bubbles running in small vessels surrounding the dilated tubules of the rete testis at CEUS - Soft at SE	
Epidermoid cyst	1. ears; benign	- Variable: 1–3 cm; hyperechoic fibrous or calcified rim; sometimes 'onion ring' pattern - Avascular at CEUS - Hard at SE	
Dermoid cyst	Children	Similar to mature teratoma	
Global and segmental ischemia	Variable	- Diffuse or focal, usually segmental, area without blood flow at CDUS in an otherwise normal testicular parenchyma - Avascular at CEUS, perilesional enhancing rim in subacute lesions - Soft at SE, may be harder at the periphery	Results from torsion, epididymal-orchitis or trauma
Hematoma	Variable	- Hypoechoic not vascularized lesions - Avascular at CEUS - Soft early, harder when organized at SE	Trauma
Abscess	Variable	- Complex heterogeneous fluid collection, hypo/anechoic, with irregular walls, occasionally with hypervascular margins - Avascular at CEUS, peripheral rim enhancement - Soft at SE	Usually complication of epididymitis and/or orchitis

(Continues)

TABLE 4 (Continued)

Main testis lesions	Age peak incidenceand biologic behaviour	Most common US characteristics	Associated abnormalitiesor syndromes
Adrenal rest	Neonates, rarely adults. Regression with corticosteroids	Rounded hypoechoic small eccentric solid masses, which may be bilateral or multifocal. Typically, the vessels course through the lesion is not deviated. Variable enhancement at CEUS, variable consistency at elastography	Congenital adrenal hyperplasia.
Sarcoidosis	20–40 years	Hypoechoic, irregular or rounded masses, often unilateral Variable enhancement at CEUS Soft at elastography	
Gummas	20–40 years	Hypoechoic nodule Highly vascularized at CEUS, rapid wash-in and wash-out Hard at elastography. As necrosis progresses, heterogeneous enhancement, coexisting stiff and soft areas at elastography	Syphilis

Note: Data are reported according to references.[83,84,122.]

management of small (millimetric) lesions. Small hypoechoic areas, especially when not vascularized, may be related to spermatoceles, cysts, focal Leydig cell hyperplasia, fibrosis and focal inhomogeneity as a consequence of previous pathologic conditions.[1,83,84] However, they may also indicate small tumours.[1,83,84] Hence, they require careful evaluation and follow-up, with periodic US examination, especially if additional risk factors for malignancy are present (i.e. infertility, bilateral TML, cryptorchidism, testicular atrophy, inhomogeneous parenchyma, history of testicular tumour, contralateral tumour and age <50 years).[29,30,147,147] If a small nodule grows,[148] or additional risk factors for malignancy are present, testicular biopsy/surgery should be considered.[29,30]

Recently, ESUR published recommendations[150] on the incidentally US-detected non-palpable testicular lesions in adults. According to the ESUR, characterization of testicular lesions is primarily based on US examination.[150] Most small non-palpable testicular lesions seen on US are benign simple cysts and require neither follow-up nor surgery.[150] Non-palpable single sporadic solid nodules <5 mm without any microliths are benign in up to 80% of cases, with Leydig cell tumours being the most frequent.[150] US follow-up can be an alternative to orchiectomy in young and/or infertile men if tumour markers are negative.[150] Large (>1 cm), multiple, mixed cystic, heterogeneous or solid vascularized nodules, irregular margins, associated microliths or hypoechoic regions may indicate malignancy.[150] CEUS optimizes enhancement in lesions which are apparently avascular at colour-Doppler. The rate of the wash-in and the wash-out of the contrast agent may help to differentiate malignant from benign tumours.[127,150,151] Leydig cell tumours have been reported to show a prolonged wash-out in one study,[127] and a shorter filling time than germ cell tumours in another.[152] The role of CEUS is evolving; however, only a few studies are available to date, limiting the recommendations for the routine use of CEUS for managing incidental testicular masses.[150] Conversely, the role of SE in differentiating between malignant and benign testicular nodules is still unclear.[36] Accordingly, a recent study[128] reported that strain ratio measurement offers no improvement over elastographic qualitative assessment of testicular lesions, and that SE may support conventional US in identifying non-neoplastic lesions when findings are controversial, but its added value in clinical practice has yet to be proven. More recently, the same authors[129] prospectively evaluated a large cohort of patients with Leydig cell tumours, reporting a good oncological prognosis when recognized early. The authors[129] indicated that tissue-sparing enucleation is curative and should replace orchiectomy and that conservative surgery or active surveillance in compliant patients through clinical and radiological follow-up can be considered safe options.

7.1.6 | Cryptorchidism

The term *cryptorchidism* is derived from the Greek words *kryptos* and *orchis*, literally meaning 'hidden testis'. Cryptorchidism, or undescended testis, is the absence of at least one testicle in the scrotum.[1,153,154] It is the most common birth defect in newborn males, affecting about 3% of full-term and 30% of premature male infants.[1,153,154] Approximately 80% of cryptorchid testes descend by the third month of life, making the true incidence of cryptorchidism ~1%.[1,153,154] Current guidelines recommend orchidopexy between 6 and 12 (American Academy of Pediatrics Section on Urology [AAP SOU],[155] European Society for Paediatric Urology and European Association of Urology [ESPU-EAU][156] and Nordic consensus[157]) or before 18 (AUA[158]) months of age. However, many experts recommend surgery early, at around 6 months, to optimize testicular growth and fertility,[154,159] although a recent meta-analysis suggested that early (<1 year of age) orchiopexy show some evidence only in improving fertility potential.[160]

The undescended testis is commonly unilateral, being bilateral in one out of 10 cases.[1,153] It is associated with an increased risk of infertility[1,153,154,161–164] and testicular malignancy.[1,153,154,162,165,166] The risk of a testicular tumour in cryptorchid men has been estimated as four- to sevenfold higher than in the healthy population.[1,153,154] Cancer commonly occurs in the undescended testis; however, one out of five tumours occur in the contralateral descended testis.[1,153,154] About 80% of undescended testes are located within the inguinal canal, 5%–16% high in the abdomen, whereas rarely the testis can be ectopic from the path of descent or absent/vanishing.[1,153,154] The AUA Guidelines[158] report that in paediatric patients, more than 70% of cryptorchid testes are palpable by physical examination and need no imaging. In the remaining 30% of cases with a non-palpable testis, the challenge is to confirm the absence or presence of the testis and to identify its location.[158] The role of US in paediatric preoperative planning before orchiopexy is controversial and has changed over time. On the other hand, US is useful in adult men with a history of cryptorchidism/orchiopexy.

What was normal

Until the last decade, some paediatricians used to require US to locate a non-palpable undescended testis.[167] In fact, a non-palpable testis may be present in the inguinal–scrotal region or within the abdominal cavity, or it may be absent.[154] Surgical exploration is mandatory to localize the testis when present or confirm an absent testis.[154,167,168] However, accurate presurgical diagnosis of an absent testis would spare a child an unnecessary surgery, whereas the correct localization of the testis could limit the extent of surgery.[167,168] Observational studies performed in paediatric patients until 2011 revealed conflicting diagnostic performance features, and did not rigorously evaluate the clinical utility of US in localizing non-palpable testes.[168–170]

What is normal

In 2011, a systematic review and meta-analysis[168] reported that US does not reliably localize non-palpable testes or rule out an intra-abdominal testis in paediatric patients. Hence, all recent guidelines[156,158,171–174] recommend against the use of US in paediatric patients because it does not change management nor add diagnostic accuracy,[174,175] except in select cases[156,174] (e.g. suspicion of sexual development disorders).

On the other hand, guidelines dealing with US in adult men with a history of cryptorchidism are not available. However, it is well recognized that in adult men who have undergone orchiopexy US plays a key role in cancer detection or in the follow-up of the cryptorchid and contralateral testis.[1] Recommendations on the follow-up timing and duration in men with a history of undescended testis/orchiopexy are not available. Considering that cryptorchidism is a risk factor for testicular cancer more relevant than TML, recommendations given by the ESUR for the follow-up of 'TML with additional risk factors'[26] could be suggested in principle, that is annual follow-up up to age 55. However, cryptorchid men should be educated on prevention with frequent self-examination of the testes, especially in the age range (15–34 years)[1,122] associated with the highest occurrence of testicular malignancy, to identify a lump possibly underlying a growing tumour early.

As a corollary, some men may present to clinicians with a non-palpable testis. Because some authors reported that US can reliably identify a cryptorchid testis lying below the level of the internal inguinal ring,[176] US may be suggested to identify the undescended testis at the high scrotal level or in the inguinal canal.[1] However, if US was unreliable, other imaging investigations or surgical exploration should be recommended.

At US, the cryptorchid testis is often hypotrophic, inhomogeneous, hypoechoic, with or without macro- or micro-calcifications and with normal or reduced vascularisation[1,83,84,153] (Figures 1C and 1D–G). However, especially in cases of early orchidopexy, it could show normal volume and echotexture.[83,84] Echotexture inhomogeneity can be associated with one or more hypoechoic micronodular lesions[83,84] (Figure 1G), often resulting from cryptorchidism- or postoperative-related damage. Nodular lesions should be managed as reported above (see Section 7.1.5) and even more carefully considering the cryptorchidism-related risk of cancer. CEUS can distinguish vascularized from non-vascularized focal testicular lesions, helping to exclude malignancy.[34]

7.2 | Epididymis and vas deferens evaluation

CDUS is useful in evaluating size, echopattern and vascularisation of the epididymis (classically divided into three segments: head, body and tail) and the vas deferens. At US, the normal epididymal head is triangular, with echogenicity comparable to that of the testis and usually slightly more echogenic than the body and tail[1,83,84,177,178] (Figures 4A and 4B). Blood flow is detectable by CDUS in discrete vascular spots in all tracts of the epididymis.[1,83,84,177,178] The vas deferens appears at US as a straight duct, slightly hypoechoic compared with the epididymis, originating from the epididymal tail and extending, along the spermatic cord, toward the inguinal channel[1,83,84] (Figure 4B).

7.2.1 | What was normal

CDUS plays a key role in investigating abnormalities of epididymal size, echopattern and vascularisation, which, alone or combined, can suggest different diagnoses.[1,97,123,138,177,178] In subjects with scrotal pain or prostatitis-like symptoms, the dilation of the whole epididymis or one of its segments (especially head or tail) when associated with hypervascularisation suggests inflammation.[1,97,123,138,177,178] In the acute form, an enlarged hypoechoic inhomogeneous epididymis with enhanced vascularisation is often detected[1,97,123,138,177,178] (Figure 4C), whereas in the chronic form the epididymis is often dilated and may appear hyperechoic (Figure 4D) and vascularisation is only slightly increased.[1,123,138,177] A dilated epididymis associated with echopattern abnormalities (including calcifications) may also represent the outcome of a past infection/inflammation, currently asymptomatic[1,123,138,177–180] (Figure 4D). On the other hand, in subjects with obstructive azoo- or oligospermia, the detection of

epididymal enlargement (Figure 4E) may suggest post-testicular obstruction, which could be (i) at the epididymal level (especially when the downstream vas deferens shows a normal size),[181] (ii) at the vas deferens level,[182] especially in men treated by epididymovasostomy[183] or after vasectomy[184] (Figure 4E) or (iii) at the prostatic level, the latter to be further investigated extending US to the prostate–vesicular region.[185,186] Furthermore, US allows the assessment of epididymal nodules, often perceived at physical examination, frequently represented by cysts (Figure 4F), but possibly underlying benign (including tuberculosis-related granulomatous masses) or, very rarely, malignant lesions.[123,138,177,178] Finally, US is useful in imaging epididymis after scrotal trauma, often showing features mimicking epididymitis.[24,25]

US is also very useful in detecting bilateral absence of vas deferens in men with obstructive azoospermia[1,187] (Figure 4F). In addition, US can detect unilateral absence of vas deferens incidentally or in men with kidney agenesis.[1,187]

Although the aforementioned US abnormalities, as well as their possible significance, are known to experienced sonologists, until 2021 values indicating epididymal dilation have been only suggested empirically, and were lacking for the vas deferens. In addition, normal and enhanced epididymal vascularisations were qualitative and operator-dependent concepts. An epididymal tail >6 mm and/or head >12 mm have been proposed as suggestive of epididymal inflammation,[188] whereas a head approximately >11 mm as indicative of obstruction.[189] After vasectomy, an epididymal head >15 mm[190] or >2 cm has been reported.[191,192] In subjects with unilateral epididymitis, associated with orchitis in almost half of cases, a two- to threefold increase in the peak systolic velocity of the testicular artery of the affected side has been reported compared to the healthy side.[97] However, normal values evaluated in healthy control subjects were lacking.

7.2.2 | What is normal

Recently, the EAA US study[33] reported a standardization of the measurement of the epididymal–deferential parameters (Table 2) and led to the identification of reference ranges and normative thresholds for epididymal segments and vas deferens size and vascular parameters (Table 3). Normal epididymal head, body, tail and vas deferens size have been defined in an evidence-based way as <11.5, 5, 6 and 4.5 mm, respectively.[33] In addition, normal values of different epididymal (and testicular) vascular parameters have been reported.[33]

As a corollary, recently it has been reported that epididymal, besides testicular, US abnormalities (suggestive of chronic epididymal inflammation; e.g. dilation and inhomogeneity) are associated with the occurrence of antisperm antibodies both in infertile and fertile men.[68]

7.3 | Pampiniform plexus and varicocoele

Normal pampiniform plexus is scarcely appreciable during physical examination, whereas CDUS is able to examine it with great accuracy.[1] In normal conditions, the pampiniform plexus appears as a complex network of small vessels converging into the spermatic veins.[1] Conversely, an abnormal dilatation of the pampiniform plexus characterized by retrograde venous flow indicates the presence of a varicocoele.[1] Although the clinical classification of varicocoele has been universally accepted from 1970,[193] the diagnosis and classification of varicocoele with CDUS is one of the most debated topics in andrology/urology.[1,27]

7.3.1 | What was normal

Several classifications have been proposed over time, with differences mainly related to the cut-off diameter to indicate a dilated vein, the indication or not of the vein's extension in the scrotal sac for grading varicocoele, duration of the venous reflux and the presence or not of testicular hypotrophy in the most severe grade.[1,27] In a 2015 systematic review,[1] Lotti and Maggi attempted to align the classifications available until then, supporting, for the severe form, the presence of a continuous venous reflux at rest, increasing or not during a Valsalva manoeuvre. In the aforementioned review,[1] previous varicocoele classifications have been described in detail.

7.3.2 | What is normal

In 2020, the ESUR published guidelines[27,28] for detection, classification and grading of varicocoele. ESUR reported methodological recommendations, supporting a standardised protocol for varicocoele US examination.[27,28] According to the guidelines,[27,28] a greyscale and colour-Doppler examination, with spectral Doppler analysis, should be performed bilaterally with the patient supine and standing, during spontaneous breathing and during the Valsalva manoeuvre. Measurement of the largest vein, irrespective of location, with the patient in the upright position and during the Valsalva manoeuvre is recommended. A maximum venous diameter ≥3 mm can be considered diagnostic for a varicocoele, grading varicocoele according to Sarteschi classification. A reflux in the testicular veins lasting >2 s with the patient standing and during the Valsalva manoeuvre should be considered to be abnormal. TV, evaluated according to Lambert's formula, should be measured in all cases. In patients with subclinical varicocoeles, imaging follow-up is recommended. After varicocoele repair, US can be used to identify early postoperative complications. Sperm analysis forms the basis of follow-up after varicocoele repair, without the routine use of US. Of note, as reported above, the recommendation of using Lambert's formula to evaluate TV has not reached a strong consensus.[27] Similarly, the recommendation against the routine use of US after varicocoele repair has not gained a strong consensus.[27] The latter point depends on the fact that some members of the ESUR-Scrotal and Penile Imaging Working Group (SPIWG) involved in the guidelines' production supported the idea that US after varicocoele repair is necessary, in order to identify varicocoele persistence or recurrence in the short term.

More recently, the EAA US study[33] assessed reference ranges for pampiniform plexus CDUS parameters in healthy, fertile men.[32]

The EAA US consortium reported SOPs for varicocoele evaluation[33] (Table 2), welcoming most, but not all, ESUR recommendations. In particular, the EAA consortium[33] supported the measurement of the largest vein with the patient standing, at rest (and not during Valsalva manoeuvre) in order to avoid the possible confounder of a variable intra-abdominal pressure increase with Valsalva, recommending Valsalva manoeuvre be used for varicocoele grading, to be performed according to Sarteschi et al.[194]/Liguori et al.[195] classifications (essentially overlapping[1]). In addition, the EAA consortium suggested the evaluation also of the maximum diameter of the internal spermatic vein between the inguinal ligament and upper pole of the testis[196] besides the assessment of the convoluted vessels below, supporting the 3 mm threshold to define vein dilation. Finally, the evaluation of TV using the ellipsoid instead of Lambert's formula was suggested (see above).[33] Of note, the EAA consortium defined 'severe' varicocoele as venous vessel dilation (>3 mm) characterized by a continuous (long-lasting, without reporting duration cut-off) venous reflux at rest, increasing or not during a Valsalva maneuvre,[33,197] consistent with grade 4 and 5 varicocoele according to Sarteschi et al.[194]/Liguori et al.[195] classifications (Figure 4G). Table 2 reports the varicocoele classification proposed by the EAA US consortium.

The EAA study reported, in fertile men, a varicocoele prevalence of ~37% (with a severe form in almost one out of five men),[33] similar to that reported in primary infertile men.[27,28] These data suggest that varicocoele may exert a scanty effect on male fertility, and that its surgical correction should be limited to highly selected populations.[32,33] Accordingly, current EAU Guidelines on male infertility[29] support very specific indications for varicocoele treatment both in adults and adolescents.

8 | WHAT WILL BE NORMAL

SOPs and normative parameters derived from the EAA US study in healthy, fertile men[32,33] will help in characterizing their pathologic counterparts, including subjects with testicular damage (e.g. as a consequence of cryptorchidism, torsion, trauma, orchitis), inflammation (orchitis), some malignancies and infertility.

In particular, the comparison between US characteristics of healthy, fertile subjects and findings revealed in men with any pathology will allow for an evidence-based definition of what is 'really' abnormal. Accordingly, US findings that show no clear or blunt association with a pathologic condition will undergo a reshaping of their clinical significance, leading to a change in the current patient management. As an example, a standardized evaluation and stratification of varicocoele will help in understanding which grades exert a real negative impact on fertility and in which patients (different patients have different clinical, seminal and hormonal profiles), avoiding uncritical varicocoelectomy.[32,33] CEUS and SE will have an additional or decisive role in specific cases.

The application of SOPs will help to assess testicular diameters using a standardized approach. A consensus on a single TV mathematical formula as well as of a TI classification and TML definition to be used in clinical practice is advocated. Accordingly, the definition of a TV reference range in adult men, and TV growth curves in the paediatric population, according to the different ethnic groups will have to be defined. Multiparametric US eventually associated with other imaging techniques (e.g. magnetic resonance imaging[198–200]) and the technical advancement of US devices will help to characterize testicular lesions more and more, with the aim of distinguishing benign and malignant lesions with increasing accuracy. Accordingly, the definition of US parameters indicating an early tumour in cryptorchid and/or TML testes will be useful. In addition, recommendations on the timing and duration of follow-up of small, non-palpable nodules and cryptorchid men and studies on nodular growth rate and its clinical significance are advocated. Further studies are advocated to evaluate TI, TML and testicular vascularisation's impact on cancer risk and infertility. The quantitative assessment of testicular and epididymal vascular parameters in pathological conditions is required. Finally, a consensus on a single varicocoele classification, the standardization of its assessment modality and the evaluation of varicocoele's clinical impact on male fertility according to its CDUS grade and patient phenotype will have to be elucidated.

9 | CONCLUSIONS

Standards in ultrasonography have changed over time, thanks to the improvement of ultrasonography technology and to the increase in scientific, clinical and ultrasonography knowledge. However, only in the last decade have international societies published guidelines/recommendations dealing with specific ultrasonography applications.[21–31] In addition, very recently the European Academy of Andrology ultrasonography consortium has provided Standard Operating Procedures to assess qualitative and quantitative colour-Doppler ultrasonography parameters and colour-Doppler ultrasonography reference range/normative parameters of the scrotal organs.[32,33] In parallel, the European Federation of Societies for Ultrasound in Medicine and Biology has reported a methodological standardization and indications for contrast-enhanced ultrasonography and sonoelastography.[34–36] However, a wide international consensus on the best method to measure testicular volume, classification of qualitative features (e.g. testicular inhomogeneity), assessment and classification of varicocoele, follow-up of non-palpable nodular lesions and cryptorchid men is still lacking. In addition, the characterization of testicular lesions with multiparametric ultrasonography must improve, testicular volume reference range in adult men and testicular volume growth curves in the paediatric population according to the different ethnic groups must be defined, and quantitative vascular measurements in pathological conditions must be investigated. Therefore, an effort to align the various world societies and further research and technological improvements in the ultrasonography field are advocated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

F.L. conceived the study. F.L., M.B. and M.M. performed the literature search. F.L. wrote the manuscript with input from all the authors. F.L. provided CDUS pictures. M.B. provided CEUS and SE pictures. M.B. and M.M. provided critical feedback and helped shape the manuscript. All authors have read and agreed to the published version of the manuscript.

ORCID

Francesco Lotti https://orcid.org/0000-0001-8343-1807

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