

Original Article

Shear wave elastography (SWE) is reliable method for testicular spermatogenesis evaluation after torsion

Xiaoying Zhang^{1,2}, Faqing Lv², Jie Tang²

¹Department of Ultrasonography, Aviation General Hospital, Beijing 100012, China; ²Department of Ultrasonography, Chinese PLA General Hospital, Beijing 100853, China

Received December 25, 2014; Accepted April 18, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: This study aims to investigate effect of torsion on testicular stiffness alteration in affected and concomitant testis using improved ultrasound method of shear wave elastography (SWE). We compared the morphology of the testicular spermatogenesis assessed with Johansen's scale on histology specimens with a mean stiffness measured by SWE. A total of 18 New Zealand white male rabbits were divided into two groups (group A and group B), animals from group A were subjected to operation of right testicle torsion while left testicle remained intact. In group B both testicles were normal and right testicle was subjected to sham operation. The protocol of measurement for mean stiffness value was calculated from three elastographic images obtained from each testicle. Significant difference in mean stiffness value and Johnsen's scaling was observed in both groups (A and B), as well as for normal and torted testicle in group A. The mean stiffness positively correlated with histologic grade on both sided testicles in group B, and left sided testicles in group A ($P=0.045$, $r=0.43$; group B; $P=0.001$, $r=0.98$), while histologic grade negatively correlated with mean stiffness in the group A, torted testicle (torsion $P=0.012$, $r=-0.76$). In this study testicular torsion, with consequently higher mean stiffness value determined by SWE, has qualitatively and quantitatively decreased spermatogenesis. Gradual morphology change in testicle unaffected by torsion has not been previously reported. This study confirmed that quantitative change in testicular tissue stiffness as well as change in testicular spermatogenesis can be reliably evaluated with SWE.

Keywords: Testicular torsion, shear wave elastography, ultrasound, spermatogenesis

Introduction

Testicular torsion is one of the most common emergencies in all, prepubertal and young adult, males. The principle treatment would be accurate diagnosis followed by rapid reposition otherwise semi-castration is inevitable [5]. In the clinical situation, non-reperfused, and during the course of time infarcted, testicle is conventionally cut while testicle with achieved reperfusion is retained in scrotum. It still remains unresolved issue whether the retained testicle upon unilateral testicular torsion effects the spermatogenesis of the contralateral testis or overall spermatogenesis quality and whether the orchiepididymectomy of twisted testicle could prevent induced spermatogenesis quality changes [6-9].

Various pathologic conditions can be precisely characterized in tissue upon determination of its biomechanical properties. The elasticity

which is often equivalent of tissue tactile hardness has important medical implications [1]. Diagnostic non-invasive method like shear wave elastography (SWE) which combines B-mode image with color-coded generating a quantitative image SWE (kPa) of the tissue stiffness has potential to exhibit different hardness among different tissue regions in real-time conditions. By use of SWE, different elasticity values dependent on testicular volume and functional properties have been demonstrated [2]. Schurich et al. have also recognized that it is feasible to evaluate both testicle and correlate observed changes with elastogram [3]. In their studies close correlation between testicular spermatogenesis and stiffness was shown. SWE produces more reproducible results than other forms of sonoelastography. Transformation and change in tissue hardness should be therefore confidently documented by SWE. Since, the tissue echogenicity and stiffness are caused by unrelated mechanisms and are thus

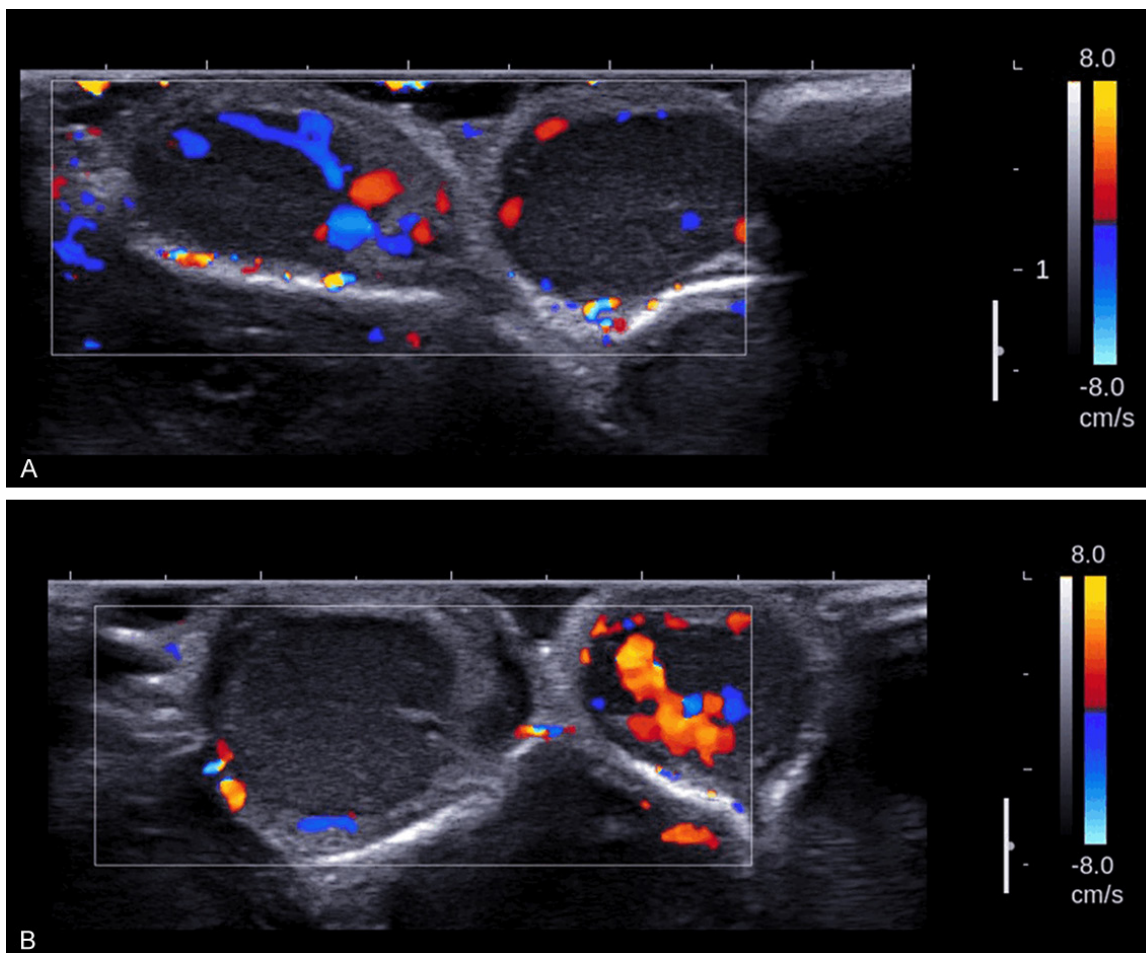


Figure 1. Relation of color flow finding in testicle pre- and post-torsion. A. The color flow US image before right spermatic cord torsion. B. Corresponding color flow US image after right spermatic cord torsion.

uncorrelated with each other, it was expected that the tissue stiffness shown by image will provide new information related to tissue morphology and structure [4].

The aim of the present study was to test assertiveness of SWE and determine the mean testicular stiffness for the twisted and untwisted testicle. Comparison of histologic prognostic features data in twisted and untwisted testicle. Further, we investigated whether the difference in the stiffness can be reliably determined by SWE only, and finally does it impact spermatogenesis of unaffected testicle.

Materials and methods

Animals

Male New Zealand white (NZW) rabbits from the Experiment Animal Center of Medical Department, Beijing University (Beijing, China)

at 12 weeks (weight 2.6-3.2 kg) were used for this experiment. The animals were randomly divided into two groups of nine (group A and group B). Rabbits were maintained in individual cages under conditions with temperature- and light-dark cycle-controlled.

All animal studies and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The experiments were approved by the Committee on the Ethics of Animal Experiments at PLA General Hospital, China. For terminal experiments, animals were sacrificed with 100 mg/kg body weight pentobarbital intravenously and all efforts were made to minimize suffering.

Study protocol

Rabbits in the group B underwent a sham operation similar to the protocol described above

SWE in testicular spermatogenesis

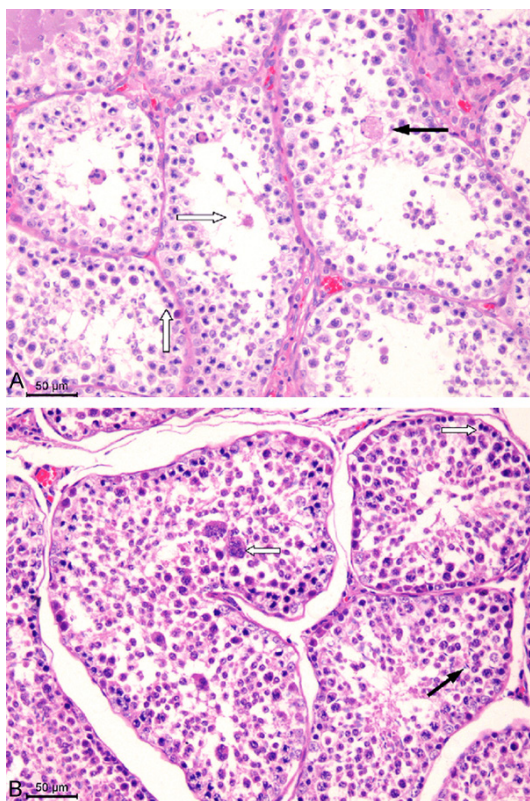


Figure 2. Histology findings in post-torsion period (HE, 400×). A. The left testicle (Johansen's scale 9 grades). The integrity of the seminiferous tubules in basement membrane and the lumens with germ cells in order, a lot of spermatozoon, but with degenerated necrotic anucleated and exfoliated spermatocyte and spermatids (white arrows), multinucleate activity of the leukocytes (black arrow), thinning of the seminiferous tubules epithelium and with decrement of germ cells even aspermia. B. The twisted right testicle (Johansen's scale 6 grades). A tubules still with the normal shape, thinning of the seminiferous tubules epithelium, their lumens with degenerated necrotic anucleated and exfoliated cells (white arrows) and activity of the leukocytes, aspermia, accumulation of protein rich drops in the lumen (black arrow).

(torsion of the right spermatic cord). Intravenous anesthesia, median scrotal incision, delivery of the right testis, and fixation of the testis in the scrotum were achieved without twisting the testicle.

Except for in the group B that underwent the sham operation and both testes were untorsed, in the Group A, right testis was torsed for 24 hours and de-torsed for one week and left testis was normal. Animals were anesthetized intravenously with 3% phenobarbital sodium (1 ml/kg). In sterile conditions, the right scrotum was incised from the dorsal side down

to the tunica vaginalis testis. The right testis was released, twisted clockwise around the spermatic cord to 720-2520° (depending on the dramatic diversity in the length of spermatic cords), confirmed by absence of color blood flow for 24 hours and fixed to the scrotum with 3/0 silk suture after which the incisions were sutured. Generally, the testicle became purple early post-operatively and after 24 hours of torsion, the testis was counter-rotated to the natural position, and reinserted into the scrotum. All animals were regularly monitored after surgery in every 4 hours.

Ultrasound (B-mode, color Doppler flow and shear wave elastography) was performed by one experienced investigator. For ultrasound image acquisition Aixplorer ultrasound equipment (SuperSonic Imagine, Axien-Provence, France) was used with the linear probe that transmits and receives center frequencies of 13.0 MHz. On preoperation, during operation, on post-operation 24 h of torsion and after one week of detorsion respectively, B-mode and color Doppler flow findings were recorded. SWE obtained as three separate images of the each testicle quantitatively analyzed.

Image acquisition

The experimental system was equipped with default software pre-sets for the small imaging body parts (breast, scrotum etc.). Gain and sensitivity time control were set at optimal levels, and the focal point was adjusted to the middle level of the image. The instrumental settings were kept identical for all the studies. During SWE mode, gel was applied on the testicular capsule to prevent side effect on the SWE imaging features. The "Q-Box™" known as the system's quantification tool, was kept by the same size which defined a 4 mm and/or 5 mm diameters region of interest (ROI) positioned over the both testicles and the same depth where ROIs over both sides and placed to allow consistent measurement of the tissue stiffness during procedure. Preoperatively and postoperatively seventh day respectively, all testicles were scanned by use of the elasticity technique after anesthesia. During a few seconds of the SWE image's stabilization, the SWE images were frozen and saved. The average time spent on each examination was about 10-20 minutes. Three elastographic images of transverse planes were obtained for each testicle by holding the probe still, without pressure applied and

SWE in testicular spermatogenesis

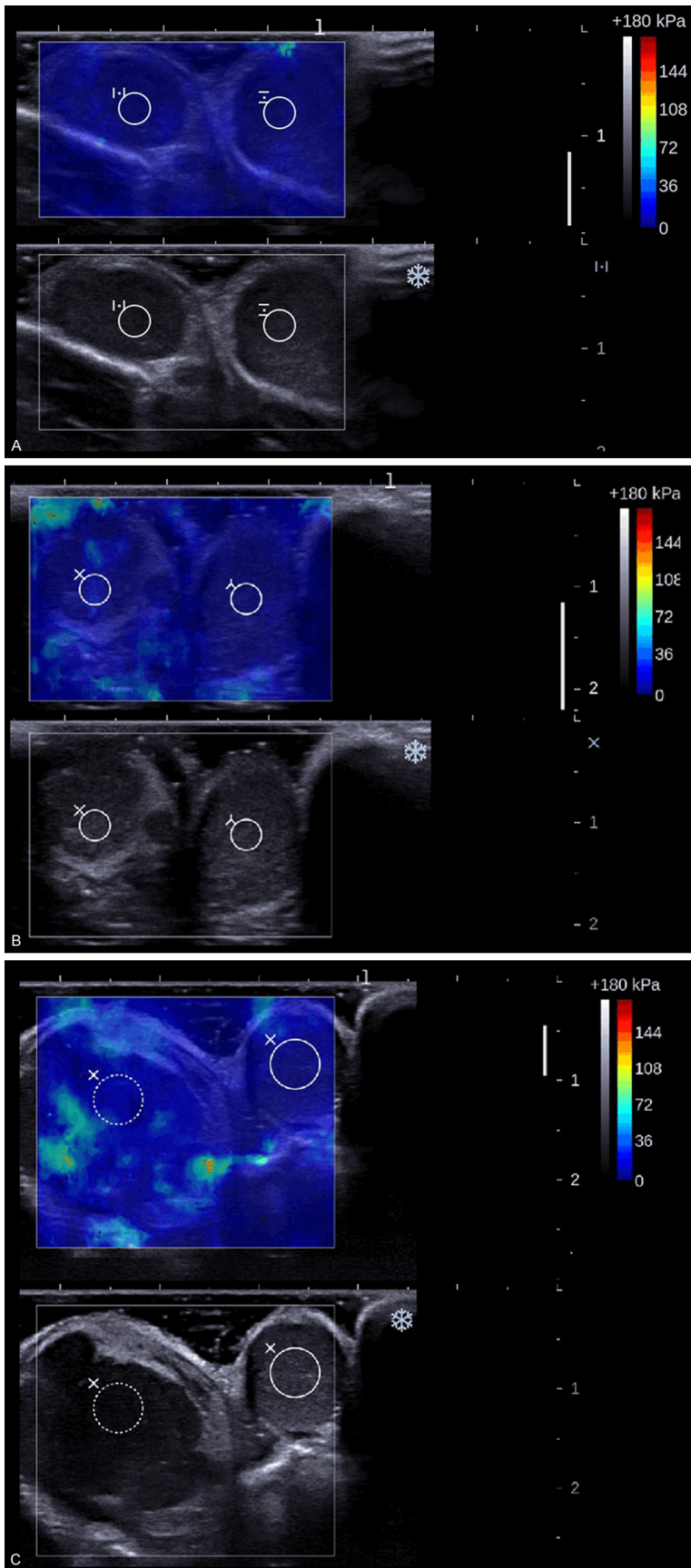


Figure 3. Elastographic findings in twisted testicle. A. Shear-wave elastographic (top) and gray-scale (bottom) images in testicle before torsion (mean stiffness averaged from three images from: the top level, the middle level to the bottom level). B. Shear wave elastographic (top) and gray-scale (bottom) images in testes after right spermatic cord torsion immediately (mean stiffness averaged from three images from: the top level, the middle level to the bottom level). C. Shear wave elastographic (top) and gray-scale (bottom) images in testes one week after right spermatic cord detorsion (mean stiffness averaged from three images from: the top level, the middle level to the bottom level).

allowing the image to form over about an average of 10 seconds. The elasticity measurements were performed by moving a delineated region of interest (ROI) over the color map. As the ROI moved, the figures changed in real time so the ROI can be moved from the upper pole to the lower pole of the testicle on both testicles. The stiffness images displayed in speed transformation with different colors. Tissue elasticity was visible as a semitransparent color overlay of conventional B-mode image with a range from dark blue, implying the lowest stiffness (at just over 0 m/s), up to red, implying the highest stiffness (got at 7.7 m/s for the examination) (as presented on **Figure 1**). The ROI did not include the scrotal capsule and vascular to avoid misinterpretation due to their artifact effects. Upon completion, the elasticity values served as the minimum (E-min), maximum (E-max), and mean (E-mean) stiffness's in kPa or m/s within both testicles. With the

SWE in testicular spermatogenesis

Table 1. Results of volume values in group A at different time

	V1 (cm ³)	V2(cm ³)	V3(cm ³)
R (torsion)	2.57 ± 0.46	3.26 ± 0.59 ^a	2.23 ± 0.49
L (normal)	2.43 ± 0.36	2.47 ± 0.39	2.71 ± 0.83

V was volume as calculated by the formula = length × width × height × 0.71. V1 was volume as calculated by the formula at before torsion. V2 was volume as calculated by the formula at after 24 h of torsion. V3 was volume as calculated by the formula at after 1 w of detorsion. ^a $P < 0.05$ when R-V1 compared with those R-V2.

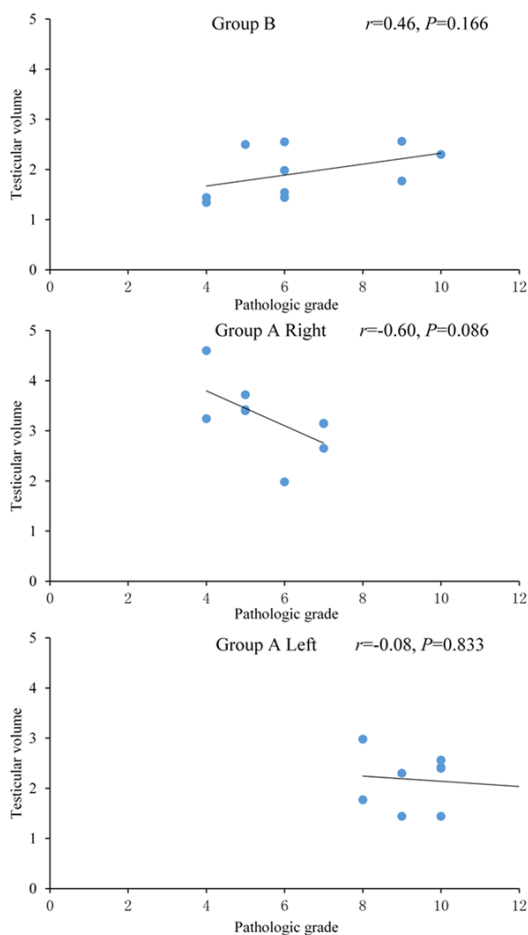


Figure 4. The correlations between pathologic grade and testicular volume in groups A and B.

SuperSonic Imagine equipment, a measurement fails when no/little signals are achieved in the Q-Box™ for all of the acquisitions. The elasticity readings in all examined testicular tissue were obtained.

Orchiectomy

The animals were subjected to the removal of the testicle for histopathological evaluation

immediately after examination in both groups. After intravenously anesthesia, the scrotum was incised and sutures fixing the testis were incised; the spermatic cord was transected, and ligated with 3/0 silk sutures; and the removed testis was placed in Bouin's fixative solution.

Pathohistology evaluation

Upon fixation in Bouin's solution for 48 hours, testicle samples were processed through graded alcohols (70% to 100%) for dehydration, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) stain. The histologic parameters were examined using light microscopy at 200× and 400× magnifications. The pathologist was blinded for information of the torsion duration. According to pathohistological indications specimen, the spermatogenesis evaluation was graded by Johnsen's method and considered as a part of the standardly examined histologic features. The course of spermatogenesis is completed in the epithelium. Therefore, the status of spermatogenesis is reflected on the amount and collocation of all levels of germ cells. The study is used with Johnsen's ten-level grade on account of the epidermis quality. The premise according method is that testicular damage results in the corresponding disappearance of a majority of mature cells, and seminiferous tubular epithelium degeneration, firstly mature sperm cells, and then spermatids, at last spermatogonia. The criterion for the judgment is the quality of the most mature cell, the range of scale is from 1 to 10. Up to now, the mean is adopted as qualitative scaling of pathologic transformation in testicles [10].

Statistical analysis

The statistical analysis was performed with commercial software (SPSS V13.0, SPSS Inc., Chicago, IL, USA). The data are presented as means ± standard deviations. A Student-Newman Keuls test was used to assess the statistical significance of the independent variables of the two groups, and to compare the degrees of the clinical outcomes of the two groups. Relationships between the mean stiffness readings and pathologic variables and between volumes and pathologic variables were investigated by Pearson rank correlation, respectively. A P value of less than 0.05 was considered to indicate a significant difference.

Table 2. Relationships between the mean stiffness at the different twisted time and 1 week after detorsion and postoperative pathologic features comparison the right sides with the left sides or the same sides in the group A.

	Right (torsion)	Left (normal)	P-value
E-mean before torsion (kPa)	7.32 ± 1.5	6.82 ± 1.03	>0.05
E-mean immediately after torsion (kPa)	13.95±2.64 ^a	7.36±0.68	<0.01
E-mean at 24 h after torsion (kPa)	23.16±0.49 ^{a,b}	13.29±0.35 ^{a,b}	<0.01
E-mean at 1 week after detorsion (kPa)	47.38±16.11 ^{a,b}	6.75±1.01	<0.01
Pathologic grade	5.5±0.5	9.5±0.5	<0.01

E-mean, the mean value in the Q-box of the testis as calculated by the system. ^{a)} P<0.01 when E-mean immediately after torsion, 24 h after torsion, and 1 week after detorsion were compared with E-mean before torsion. ^{b)} P<0.01 when E-mean at 24 h after torsion was compared with E-mean immediately after torsion, and when E-mean at 1 week after detorsion was compared with E-mean at 24 h after torsion.

Table 3. Relationships between mean stiffness and pathologic features in group A and group B after 1 week

	Group B right	Group left	Group A right (torsion)	Group A left (normal)
E-mean (kPa)	7.56 ± 1.11 ^b	7.53 ± 1.45	47.38 ± 16.11 ^a	6.75 ± 1.01 ^b
Pathologic grade	10 ± 0.5 ^b	10 ± 0.5	5.5 ± 0.5 ^a	9.5 ± 0.5 ^b

^{a)} P<.01 in comparison with the same side of group B.

Results

Pathohistology evaluation

Pathohistology evaluation included evaluation and analysis of several features; the integrity of the seminiferous tubules in basement membrane, thickening of the germ epithelium, the arrangement of the germ cells, later one being preserved in group B. Numerous sperm cells were arranged in a circle along luminal border. The intraepithelial vacuoles were visible in few germ epithelial segments, while the exfoliated germ cells were present in lumen followed with decreased spermatozoa number (Figure 2A). Generally, similarities between the groups were observed. Focally in the region of group A, some germ cell epithelium showed vacuolization, architectural disorder, exfoliation in the lumina, even multinuclear giant cells (polykaryocytes) were seldom found. The number of germ cells obviously decreased in the same group with presence of different degrees of edema and congestion of the interstitial vessels (Figure 2B).

In right sided testicles from group A, basement membranethickening and degeneration, necrosis and exfoliation of the germ cell epithelium was observed. A small number of polykaryo-

cytes were formed while semen was not observed. The accumulation of the protein rich fluid in the lumen and extravasated blood in the interstitial space were present.

Sonographic readings

In the group A, the right testis was twisted and displayed featuresof complete ischemia, two-dimension images showed reduction of the echogenicity, absence of uniformity; on color Doppler flow imaging was without signal. Regarding elasticity qualitative and quantitatively change was present among twisted and untwisted testicle. Change in coloration; more cyanotic (bluish) comparatively

high difference on the scale of darkness and brightness; and increment of the mean stiffness values (Figure 3A-C).

Comparison of testicular volume and its relationships between with pathologic variables

The significant difference in testicular volume was observed, since after 24 h of torsion, the volume of twisted testicle increased when compared with the volume in period before torsion and 24 h after torsion. No difference was observed when we compared the volume of the left testicles at different torsion or detorsion times with those before torsion, as shown in the Table 1. In the group B, pathologic grade positively correlated with testicular volume, r=0.6. In the group A, volume data demonstrated positive correlation with the pathologic grade atuntwisted testicles, respectively, r= 0.78; r=0.83 (Figure 4).

Comparison of the mean stiffness readings at the different tersed or de-tersed time in group A or with those in group B and Johnsen’s scaling variables

In group A, comparison of the E-mean with those in the same sides at the different time

SWE in testicular spermatogenesis

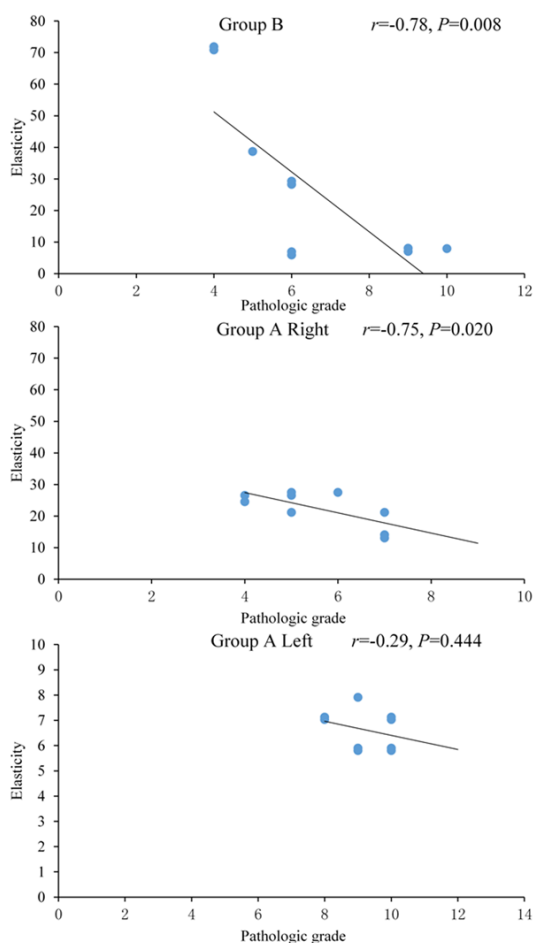


Figure 5. The correlations between pathologic grade and testicular elasticity in groups A and B.

points, except those in left side at immediately after torsion and after 1 week of detorsion, there was statistical difference between the normal testis and/or the torsed testicle for the mean stiffness values ($P > 0.01$). Comparison of the E-mean in the right sides with those in the left sides at the different time, except those at pre-torsion, significant difference was also observed at other different time.

Meanwhile, significant difference was found at comparison of the pathologic grade in the right sides with those in the left sides (**Table 2**). No difference was observed in group B between the mean stiffness findings and pathologic scaling of the left and the right testes. ($P = 0.93$, the mean stiffness; $P = 0.99$, Johnsen's grade). The mean stiffness values and the pathologic grades between group A and group B, except in the right side after 1 week of detorsion, were without significant difference. The pathologic

grades between group A and group B showed significant difference in the right sides only as shown in **Table 3**.

Relationships between mean stiffness readings and pathologic variables

In the Group B, elasticity data showed the positive correlation with the pathologic grade, $r = 0.43$; in the group A, elasticity data demonstrated negative correlation with the pathologic grade of torsion side and the positive correlation with the pathologic grade on non-torsion side, respectively $r = -0.76$; $r = 0.97$ (**Figure 5**).

Discussion

In this study, accent was on assessment of relationship between testicular stiffness measured by SWE and degree of histologically verified damage determined by Johnsen's scale. The use of SWE in routine clinical application in the assessment of testicular spermatogenesis has not been extensively reviewed to the best of our knowledge. Some studies have proved that real time elastography is capable of depicting testicular cancers as lesions with an increased stiffness [11, 12]. This imaging modality allows higher confidence in defining unclear testicular lesions at gray-scale and color and/or power Doppler US. Elastography detects and the characteristics of the focal lesions.

Testicular lesions such as testicular microlithiasis, azoospermia were investigated with the use of sonoelastography technique as strain ratio for clinical application [13-16]. The results showed increased testicular stiffness, due to either the increased seminiferous tubules hardness or the decreased vascular flow, as result of the incrustation and attenuation of the flow through the arterial wall which supplies the seminiferous tubules. In conclusion, increase in testicular stiffness in azoospermic patients was reported with use of elastographic modality.

Since SWE is an acoustic pressure wave method unlike the transient elastography, shear wave's generated by the external vibration devices and propagate in the testis parenchyma, without retention on the testicular surface. Shear wave tissue propagation velocity depends on its consistency (slower propaga-

tion is through softer tissue, faster through harder tissue).

Sakamoto et al. demonstrated strong correlation between testicular volume and its function perservance [17]. Our results are in consistency with their findings. In view of **Table 1** and **Figure 4**, during torsion (24 hours) the organ volume increased (due to interstitial edema and congestion) compared with volume values obtained before torsion ($P < 0.05$). The difference was present in the pathologic grading values within right side before torsion and after 1 week of de-torsion but either testicular volume correlated with Johansen score. After detorsion, the testicular volume decreased due to ischemic necrosis. The testicular volume consists of 70-80% of seminiferous tubules; hence, the volume reflects the spermatogenesis to a certain extent.

Real-time shear wave elastography used as tool to evaluate the tissue stiffness, is resolute in distinction of testicular volume and function, that testicle exhibits by change of hardness [13].

We utilized SWE to evaluate the testicular elasticity in the normal testicle and after torsion of 24 h and to compare data with the postoperative pathology after 1 week in two groups. So far, there is no available reported data on this particular issue.

The choice of protocols in clinical practice directly influence the modality of testicular torsion treatment. Up to now, some studies indicated that there is inconsistency with the impact on the function of the normal testicle of unaffected side caused by the testicular torsion weather tersed testicle kept or not [18-20].

No matter how from the self-comparison or contrast with both groups, actually, SWE in real time quantitatively assess the testicular stiffness development after torsion and detorsion. In the tersed sides after 24 h testicular torsion and 1 week detorsion, the pathophysiologic process occurred across the testicular tissue: congestion, oedema, infiltration of inflammatory cells, engorgement of the blood vessel and degeneration, necrosis and exfoliation of germ cells so interrupted spermatogenesis so the pathologic transformation of the tissue leads to the change of the tissue elastic modulus. Previous imaging research focused on the diag-

nosis of whether the testis is irreversibly damaged and therefore should it be spared, not on whether an effect it had on the spermatogenesis [21, 22].

We explored the utility of SWE in evaluation of the testicular hardness on both the reservation of the tersed lateral and the contralateral sides 1 week after extirpation of torsion side and then compare the E-mean with the histopathology findings. Future investigations need to study whether the tersed testicle elimination has an important role in the contralateral spermatogenesis after detorsion or not at diverse time points.

As any study this one has its limitations too, primarily we find research time frame to be too short and investigated on limited number of animals. Since the period of germ cell maturation is in average 45 days, in future investigations special attention should be paid considering whether sperm cell circle has been effected or not.

Conclusions

We can draw the conclusion that SWE can be used to qualitatively and quantitatively assess the testicular tissue hardness, and thus provides us possibility to explore, investigate and evaluate various aspects of the spermatogenesis alteration. Till now there have been no studies which would confirm gradual morphological change in testicle unaffected by torsion. In this study we have confirmed change in testicular spermatogenesis which can be reliably detected with SWE. The SWE is therefore a reliable method for measuring semi qualitative and quantitative stiffness change of testicular tissue and can be proposed for further clinical application evaluation. Finally, in this study promising data were provided which give credit to this procedure and strong argument for future studies in the field.

Acknowledgements

The study was supported by the National Natural Science Foundation of China (8107-1279). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

SWE in testicular spermatogenesis

Address correspondence to: Dr. Jie Tang, Department of Ultrasonography, Chinese PLA General Hospital, No. 28, Fuxing Road, Haidian District, Beijing 100853, China. Tel: +86-10-66939511; Fax: +86-10-66939511; E-mail: txiner@vip.sina.com

References

- [1] Sarvazyan AP, Rudenko OV, Swanson SD, Fowlkes JB, Emelianov SY. Shear wave elasticity imaging: a new ultrasonic technology of medical diagnostics. *Ultrasound Med Biol* 1998; 24: 1419-1435.
- [2] De Zordo T, Stronegger D, Pallwein-Prettner L, Harvey CJ, Pinggera G, Jaschke W, Aigner F, Frauscher F. Multiparametric ultrasonography of the testicles. *Nat Rev Urol* 2013; 10: 135-148.
- [3] Schurich M, Aigner F, Frauscher F, Pallwein L. The role of ultrasound in assessment of male fertility. *Euro J Obste Gynecol Reprod Biol* 2009; 144 Suppl 1: S192-8.
- [4] Gao L, Parker KJ, Lerner RM, Levinson SF. Imaging of the elastic properties of tissue-A review. *Ultrasound Med Biol* 1996; 22: 959-977.
- [5] Chang TP, Lee GH, Kulendran M, Warusavitarne J. General surgery registrars cross-covering urology on-call: A survey of current training and experience in the management of cases of suspected testicular torsion. *Int J Surg* 2013; 11: 695.
- [6] Cosentino MJ, Nishida M, Rabinowitz R, Cockett AT. Histological changes occurring in the contralateral testes of prepubertal rats subjected to various durations of unilateral spermatic cord torsion. *J Urol* 1985; 133: 906-911.
- [7] Lievano G, Nguyen L, Radhakrishnan J, Fornell L, John E. New animal model to evaluate testicular blood flow during testicular torsion. *J Pediatr Surg* 1999; 34: 1004-1006.
- [8] Krarup T. The testes after torsion. *Br J Urol* 1978; 50: 43-46.
- [9] Arap MA, Vicentini FC, Cocuzza M, Hallak J, Athayde K, Lucon AM, Arap S, Srougi M. Late hormonal levels, semen parameters, and presence of antisperm antibodies in patients treated for testicular torsion. *J Androl* 2008; 28: 528-532.
- [10] Johnsen SG. Testicular biopsy score count: a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones* 1970; 1: 2-25.
- [11] Pallwein L, Steiner H, Akkad T, Bartsch G, Frauscher F. Real-time elastography for evaluation of testicular masses: initial experience. *Eur Urol Suppl* 2006; 5: 96.
- [12] Aigner F, De Zordo T, Pallwein-Prettner L, Junker D, Schäfer G, Pichler R, Leonhartsberger N, Pinggera G, Dogra VS, Frauscher F. Real-time sonoelastography for the evaluation of testicular lesions. *Radiol* 2012; 263: 584-589.
- [13] Sakamoto H, Ogawa Y, Yoshida H. Relationship between testicular volume and testicular function: comparison of the Prader orchidometric and ultrasonographic measurements in patients with infertility. *Asian J Androl* 2008; 10: 319-324.
- [14] Li M, Li FH, Du J, Wang ZQ, Zheng JF, Li Z, Ping P. [Quantitative analysis by real-time elastasonography for the differential diagnosis of azoospermia: preliminary application]. *Zhonghua Nan Ke Xue* 2012; 18: 35-38.
- [15] Aigner F, De Zordo T, Pallwein-Prettner L, Junker D, Schäfer G, Pichler R, Leonhartsberger N, Pinggera G, Dogra VS, Frauscher F. Real-time sonoelastography for the evaluation of testicular lesions. *Radiology* 2012; 263: 584-589.
- [16] Goddi A, Sacchi A, Magistretti G, Almolla J, Salvatore M. Real-time tissue elastography for testicular lesion assessment. *Eur Radiol* 2012; 22: 721-730.
- [17] Grasso M, Blanco S, Raber M, Nespoli L. Elastasonography of the testis: preliminary experience. *Arch Ital Urol Androl* 2010; 82: 160-163.
- [18] Turner TT, Brown KJ. Spermatic cord torsion: loss of spermatogenesis despite return of blood flow. *Biol Reprod* 1993; 49: 401-407.
- [19] Wang SP, Zhai YH, Xie KL, Sun LJ, Li YJ, Wang XS. [Improving effect of Shengjingsan on spermatogenic function following testicular torsion/detorsion in rats and its mechanism]. *Zhonghua Nan Ke Xue* 2013; 19: 346-349.
- [20] Woodruff DY, Horwitz G, Weigel J, Nangia AK. Fertility preservation following torsion and severe ischemic injury of a solitary testis. *Fertil Steril* 2010; 94: 352. e4-5.
- [21] Lorenzini F, Tambara Filho R, Gomes RP, Martino-Andrade AJ, Erdmann TR, Matias JE. Long-term effects of the testicular torsion on the spermatogenesis of the contralateral testis and the preventive value of the twisted testis orchiopididymectomy. *Acta Cir Bras* 2012; 27: 388-395.
- [22] Ali Rizvi SA, Ahmad I, Azfar Siddiqui M, Zaheer S, Ahmad K. Role of color Doppler ultrasonography in evaluation of scrotal swellings: pattern of disease in 120 patients with review of literature. *Urol J* 2011; 8: 60-65.
- [23] Dogra VS, Gottlieb RH, Oka M, Rubens DJ. Sonography of the scrotum. *Radiology* 2003; 227: 18-36.