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● *Original Contribution*

MULTI-PLANAR DYNAMIC CONTRAST-ENHANCED ULTRASOUND ASSESSMENT OF BLOOD FLOW IN A RABBIT MODEL OF TESTICULAR TORSION

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Abstract—To assess correlation between multi-planar, dynamic contrast-enhanced ultrasound (US) blood flow measurements and radiolabeled microsphere blood flow measurements, five groups of six rabbits underwent unilateral testicular torsion of 0°, 180°, 360°, 540° or 720°. Five US measurements per testis (three transverse/two longitudinal) were obtained pre-operatively and immediately and 4 and 8 h post-operatively using linear transducers (7–4 MHz/center frequency 4.5 MHz/10 rabbits; 9–3 MHz/center frequency 5.5 MHz/20 rabbits). Björck's linear least-squares method fit the rise phase of mean pixel intensity over a 7-s period for each time curve. Slope of fit and intervention/control US pixel intensity ratios were calculated. Means of transverse, longitudinal and combined transverse/longitudinal US ratios as a function of torsion degree were compared with radiolabeled microsphere ratios using Pearson's correlation coefficient, ρ. There was high correlation between the two sets of ratios ($\rho \geq 0.88$, $p \leq 0.05$), except for the transverse US ratio in the immediate post-operative period ($\rho = 0.79$, $p = 0.11$). These results hold promise for future clinical applications. (E-mail: harriet.paltiel@childrens.harvard.edu)

Key Words: **Blood flow, Ultrasound, Ultrasound contrast, Radiolabeled microspheres, Testicular torsion, Experimental studies.**

INTRODUCTION

Testicular torsion is a disorder that occurs most often in the pediatric population, where venous outflow from and arterial inflow to the testis are impaired as a consequence of twisting of the spermatic cord. Because of the risk of infarction, testicular torsion must be immediately diagnosed to identify patients who require urgent surgical intervention (Mansbach et al. 2005). Color Doppler ultrasound (US) is the accepted "gold standard" test for diagnosis of torsion. It relies on a qualitative assessment of relative bilateral testicular arterial blood flow, with an absence or marked diminution in flow considered diagnostic of torsion. However, this technique has persistent limitations, especially in the pediatric population in whom testicular arterial blood flow is low; in cases of partial torsion, where there may be preservation of arterial flow; and in the setting of intermittent torsion, where hyperemia may occur during transient detorsion (Kalfa et al. 2004; Karmazyn et al. 2005; Nussbaum Blask et al. 2002).

Dynamic contrast-enhanced US is a technique potentially applicable to the clinical investigation of a wide variety of blood flow abnormalities (Abramowicz 2005; Charnley et al. 2009; Eyding et al. 2002; Kaufmann et al. 2007; Klauser et al. 2005; Lassau et al. 2012; Lencioni et al. 2007; Pallwein et al. 2007; Weber 2009), including acute scrotal disorders (Valentino et al. 2011). Valentino et al. (2011) found contrast-enhanced US useful in providing an accurate and definitive diagnosis in patients with acute scrotal disease when conventional gray-scale and color Doppler US findings were uncertain. In their series of 50 patients, sensitivity and specificity were 76% and 45%, respectively, for conventional gray-scale and color Doppler US, compared with 96% and 100%, respectively, for contrast-enhanced US. Studies

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were performed using a bolus injection of contrast material, and no quantitative analyses were performed.

A number of animal studies using US contrast agents for the diagnosis of testicular ischemia and torsion have been published (Brown et al. 1997; Coley et al. 1996; Metzger-Rose et al. 1997; O'Hara et al. 1996). These investigations used a bolus technique for intravenous contrast injection and qualitative methods of analysis. More recently, attempts have been made to provide quantitative measures of testicular blood flow using a bolus technique of contrast administration (Caretta et al. 2010; Chen et al. 2009) and disruption-replenishment data during US microbubble infusion (Paltiel et al. 2006; Thierman et al. 2006). Flow data based on consecutive disruption-replenishment measurements in the same plane during US microbubble infusion have superior reproducibility compared with data derived from repeated bolus injections. Infusion of microbubbles also permits multiple independent measurements to be made with a single dose of contrast agent (Williams et al. 2011).

Paltiel et al. (2006) compared qualitative visual assessment of blood flow in a rabbit model of testicular ischemia by two independent readers blinded to the side of the experimental and control testes to calculated blood flow ratios derived from disruption-replenishment infusion of microbubbles. Flow data were derived from manually placed right and left testicular regions of interest (ROIs) acquired from a single transverse imaging plane through the scrotum. These data, in turn, were compared with radiolabeled microsphere-derived blood flow ratios. The percentage of times a testis classified as having definite blood flow had greater blood flow as measured with radiolabeled microspheres than a testis classified as having no blood flow or possible blood flow was higher with contrast-enhanced US imaging than with color Doppler US (85%–98% versus 72%–89%). Identification of the testis with less blood flow was better with quantitative methods than with qualitative assessment of images by the readers (75%–79% versus 34%–60%).

The quantitative studies of Paltiel et al. (2006), Thierman et al. (2006), Chen et al. (2009) and Caretta et al. (2010) are limited by the fact that dynamic blood flow data (*i.e.,* data acquired over a particular period) were obtained from a single tissue plane that is unlikely to be representative of the entire organ. A volumetric acquisition would be expected to more accurately reflect blood flow within the testis as a whole. Currently, US devices used in clinical examination of the scrotum do not permit simultaneous acquisition of time-intensity data from multiple tissue planes. However, it is possible that averaging of data obtained from two or more planes over a short interval would result in an improved estimate of blood flow to the entire testicular volume (Paltiel et al. 2006; Su et al. 2009; Thierman et al. 2006; Wei et al. 1998, 2001). These considerations provided the impetus for the current investigation: to assess the correlation between multi-planar, dynamic contrast-enhanced US-derived measurements of blood flow in a rabbit model of testicular torsion and radiolabeled microsphere measurements, an experimental "gold standard."

METHODS

Animal selection

The study was approved by the Animal Care and Use Committee of our hospital, and conformed to guidelines issued by the National Institutes of Health for care of laboratory animals and the Guiding Principles for Research Involving Animals and Human Beings as adopted by the American Physiological Society. A rabbit model of testicular torsion was developed as rabbit testes are similar in size and shape to human pediatric testes. In humans, mean testicular volume at 1 y of age is approximately 0.48 mL, at 10 years 0.97 mL and at 12 years 2.0 mL. After 12 y of age, the testes grow rapidly, with a mean volume of about 14 mL at the age of 18 y (Goede et al. 2011). Clinically, a boy is considered to have reached puberty once the testis reaches a volume of 4 mL (Kulin 1993; Wu et al. 1993). Rabbit testes have been successfully studied with a variety of US techniques (Brown et al. 1997; Coley et al. 1996; Frush et al. 1995; O'Hara et al. 1996; Paltiel et al. 2006, 2011; Thierman et al. 2006). Thirty adult male New Zealand white rabbits (Millbrook Breeding Labs, Amherst, MA, USA) with a mean weight of 4.1 kg and a mean testicular volume of 3.57 mL were evaluated.

Animal preparation

General anesthesia was induced with glycopyrrolate (American Reagent Laboratories, Shirley, NY, USA) 0.04 mg/kg intramuscularly, followed by ketamine (Fort Dodge Animal Health, Overland Park, KS, USA) 10 mg/kg IV and acepromazine maleate (Fort Dodge Animal Health) 0.5 mg/kg IV. A catheter was inserted into an ear vein for purposes of US contrast agent administration (BD Insyte Autoguard, BD Medical, Sandy, UT, USA). Straight catheters were placed into the ascending aorta just above the aortic valve for radiolabeled microsphere injection (3-F Morpheus CT PICC, AngioDynamics, Queensbury, NY, USA), and into one femoral artery for blood pressure monitoring and to obtain reference blood samples (3-F; Cook, Bloomington, IN, USA).

Between experiments, the animals were recovered and observed every 15 min until ambulatory, then every 30 min. They were placed on soft bedding with a warming blanket and monitored for signs of discomfort. A dose of

ketoprofen (1 mg/kg) was administered intramuscularly as necessary for pain control. For a more complete description, please refer to Paltiel et al. (2011).

Surgical procedure

The rabbit fur overlying the scrotum was removed using a commercially available topical depilation agent (Nair or Veet).

After the baseline US studies (see below), bilateral medial, ventral scrotal incisions were made under sterile conditions, and the scrotal layers were dissected to the tunica vaginalis. The testes were exposed, and unilateral torsion with contralateral orchiopexy was performed. Orchiopexy is a surgical procedure in which the testis is anchored to the scrotal wall. The testes were then secured in place, and the overlying scrotum was closed. Rabbits underwent unilateral 0° (sham surgery, n = 6), 180° (n = 6), 360° (n = 6), 540° (n = 6) or 720° (n = 6) of spermatic cord torsion after which the post-operative US studies were performed.

In the 720° torsion group, torsion of the right testis was performed in two rabbits, and torsion of the left testis was performed in four rabbits. In all of the remaining experimental groups, torsion of the right testis was performed in three rabbits, and torsion of the left testis was performed in three rabbits. The intra-aortic catheter was always placed through the groin opposite the torsive testis. In the sham surgery group, the intra-aortic catheter was placed through the right groin in two rabbits and through the left groin in four rabbits.

Contrast agent administration

The US contrast agent Definity (Lantheus Medical Imaging, Billerica, MA, USA) was used in the study. Definity consists of perflutren lipid microspheres made of octafluoropropane encapsulated in an outer lipid shell. The mean diameter of the microspheres ranges from 1.1 to 3.3 μm. One milliliter of contrast agent suspension contains a maximum of 1.2×10^{10} perflutren microspheres; 0.6 mL of contrast agent suspension was reconstituted with normal saline (0.9% NaCl solution) to yield a 20-mL solution.

The contrast agent solution was placed in a plastic syringe that was connected to the ear vein catheter by an infusion pump that was oriented horizontally on a horizontal surface (Model 55–1111, Harvard Apparatus, South Natick, MA, USA). Because the contrast agent had a tendency to separate from the suspending fluid over time, re-suspension was achieved immediately before infusion by gently rotating and inverting the syringe. Definity was intermittently infused intravenously at the rate of 160 mL/h for 3 to 4 min for both baseline and post-operative studies, resulting in a dose of between 0.24 and 0.32 mL of Definity per study, and a total dose of between 0.96 and 1.28 mL of Definity per rabbit.

Image acquisition

Dynamic contrast-enhanced US was performed by the first author using pulse inversion imaging. Studies were done pre-operatively and immediately and 4 and 8 h post-operatively. Of the 30 rabbits in the study, the first 10 were investigated with a Model HDI 5000 machine (Philips Ultrasound, Bothell, WA, USA) and a 7- to 4-MHz linear transducer with a center frequency of 4.5 MHz (0° torsion in 2, 180° in 2, 360° in 2, 540° in 1, 720° in 3). While the study was in progress, the HDI 5000 machine was replaced with a Model iU22 machine (Philips Ultrasound); thus, the remaining 20 rabbits were investigated with the iU22 unit and a 9- to 3-MHz linear transducer with a center frequency of 5.5 MHz (0° torsion in 4, 180° in 4, 360° in 4, 540° in 5, 720° in 3). Although there are potential concerns regarding the effects of the machine and transducer changes on the resulting data, we believe that the effects were minimized, as detailed under Quantitative Image Analysis.

Ultrasound images were obtained using a technique based on the destruction-reperfusion principle (Wei et al. 1998, 2001). At each time point, an intravenous infusion of the contrast agent was established, followed by intermittent pulse inversion imaging at a low mechanical index (MI) of 0.07 and a frame rate of at least 18 Hz (range: 18–100 Hz) for each US machine to ensure adequate time sampling of data, until the blood concentration of the microbubbles reached equilibrium. Equilibrium was visually determined by the radiologist who performed the US examination, and was generally achieved within 90 to 120 s.

Next, continuous pulse inversion imaging at a MI of 0.07 for each machine was performed for several seconds, followed by delivery of a burst of high-energy US pulses into the scrotum (MI = 1.0 for each machine) and, finally, by reversion to low-MI imaging. Delivery of high-energy US pulses into the tissues caused microbubble destruction within the imaging plane (Chomas et al. 2001; Wei et al. 1998). Of note, three high-energy pulses were delivered to the first animal in the series; the remaining 29 animals received five high-energy pulses. Low-MI US scanning performed immediately after delivery of the high-energy US pulses permitted imaging of a progressive increase in tissue US pixel intensity caused by replenishment of the microbubbles *via* arterial flow (Wei et al. 1998). Images were acquired uniformly over time for an average of 13.1 s (standard deviation = 2.2 s), during which contrast agent replenishment to a steady-state level within the imaging plane was achieved.

At each time point, a scan sequence was performed that consisted of seven disruption-replenishment acquisitions: three with the US probe oriented transversely over the scrotum so as to include both testes in the field-of-view, and four with the US probe oriented along the longitudinal axes of the right and left testes, respectively (two longitudinal acquisitions per testis). For each acquisition, the US probe was held as still as possible to prevent out-of plane motion and the mis-registration of data on subsequent image analysis. The transverse acquisitions were manually chosen to include the upper, mid- and lower testicular planes, respectively, whereas each longitudinal acquisition was performed through a manually chosen mid-sagittal plane and included the entire testis within the field-of-view. Persistence and other post-processing features were disabled to minimize spatial and temporal averaging. At each time point, the time-gain controls were aligned centrally, followed by optimization of image depth, transmit focus, receiver gain and dynamic range, and were not altered after contrast administration.

Digital cine loops of all imaging sequences recorded from the time of initiation of continuous low-MI imaging through microbubble destruction and subsequent full contrast agent replenishment were transferred to a personal computer for image processing and analysis.

Regional blood flow measurements

Reference testicular blood flow measurements were obtained using a radiolabeled microsphere technique (Heymann et al. 1977; Matsumoto et al. 1982). Immediately after US evaluation, radiolabeled microspheres were injected into the proximal ascending aorta, and a reference arterial blood sample was obtained for subsequent determination of true testicular blood flow. A different radioisotope was used for each blood flow determination: cerium 141 (baseline), ruthenium 103 (immediately post-operatively), niobium 95 (4 h post-operatively) and scandium 46 (8 h post-operatively) (PerkinElmer Life and Analytical Sciences, Billerica, MA, USA). Each radioisotope has unique photon energy and can therefore be measured independently. For every blood flow measurement, 0.2 mL of a solution containing 10 μCi (0.74 MBq) of 15-μm-diameter radiolabeled microspheres was injected into the ascending aortic catheter and flushed with approximately 5 mL of saline solution over a period of about 20 s. A reference blood sample was simultaneously removed from the femoral artery with a 5-mL syringe and withdrawal pump (Model 55-1143, Harvard Apparatus, Holliston, MA, USA) at the rate of 2.0 mL/min for 1.5 min. The withdrawal interval was measured with an electronic timer. Each rabbit was then sacrificed with an intravenous dose of pentobarbital (1 mL/4.5 kg). The testes were removed and immediately sectioned for regional blood flow determination (Paltiel et al. 2006, 2011).

The right testes ranged in volume from 2.32 to 4.82 cm^3 (mean = 3.57 cm^3), and the left testes, 2.34 to 5.0 cm^3 (mean = 3.57 cm^3). Each testis was used in its entirety for blood flow measurement by sectioning it longitudinally into four strips. The tissue and reference blood samples were weighed on a balance (Model AB204, Mettler-Toledo, Greifensee, Switzerland). Radioactivity of the tissue and of the reference blood samples was measured in a deep-well gamma counter (Packard, Downers Grove, IL, USA). Blood flow at each time point was determined by comparing counts for the appropriate radioisotope in the testicular tissue samples with counts in the reference blood samples. Regional blood flow measurements (expressed as mL/g/min testicular tissue) for each tissue sample were determined. The blood flow measurements for the four individual tissue strips were averaged to obtain mean baseline and post-operative blood flow measurements for each testis (Paltiel et al. 2006, 2011).

Image processing

In the initial phases of the study, time history of the US images was recorded in uncompressed (full frame) AVI, but later changed to DICOM. In both cases, images were stored such that 8-bit gray-scale values were provided over a spatial grid of 640 $\times$ 480 for each time frame. No significant differences in the data from the two acquisition types were observed, and so processing was identical for both storage formats.

Twenty-eight time-history data sets per rabbit were collected, resulting in a total of 840 data sets. To facilitate the manipulation of this relatively large amount of data (approximately 192 Gb uncompressed), a batch code was programmed to parse the files and process each file frame-by-frame. The data were initially processed as described by Thierman et al. (2006). Each image in a time series was filtered by subtracting background noise. The noise level was determined by calculating the mean pixel value over a subset of each image that was not coincident with the location of the testes. Any pixel equal to less than 1.1 times the mean of the noise (background pixels) was not considered. Likewise, pixels greater than 1.3 times the mean of the noise were taken to indicate large vessels and were also eliminated. The selection of threshold values was made by empirical observation, where it was determined that signals in the lower cut-off were largely indicative of the region outside the testes and signals above the cut-off were primarily within large vessels. The remaining pixels were assumed to represent the US signal from the testicular microvasculature, and their pixel values were left unchanged. For images acquired in a transverse orientation, the image

was divided to isolate the response from the right and left testes. Each half-image was then considered the ROI for the included testis. For images acquired in a longitudinal orientation, the entire image constituted the ROI. This approach to selection of the ROI accomplished the goals of automating image processing by removing the subjective element inherent in an arbitrary selection of a smaller ROI (or multiple ROIs) within each testis. In addition, by analyzing multiple tissue planes, we increased the chances of detecting focal zones of decreased blood flow (Fig. 1). Cine loops corresponding to Figures 1(a–c) are depicted in Supplementary Video 1(a–c).

Quantitative image analysis

An established method for estimating relative blood flow involves fitting the time history of the US signal intensity over the replenishment stage within a given region to the function

$$S(t) = A\left(1 - e^{\beta t}\right) \quad (1)$$

where β is proportional to regional mean flow, and A is proportional to blood volume (Wei et al. 1998). Although this model is incomplete (Hudson et al. 2009), it has been reported to yield reasonable results *in vivo* for measuring blood flow (Kogan et al. 2011; Thierman et al. 2006). A drawback of this empirical approach is that it necessitates calibration between subjects. In practice, this is problematic, because in addition to non-linear bubble oscillation, pixel intensity can vary with anatomy, acoustic beam profile, system settings and other factors. The analysis used in the present study was designed to at least partially offset some of this subjectivity. It is first assumed that background signal can be subtracted such that eqn (1) holds and S is zero at time $t = 0$. We next examine modification of eqn (1) under the assumption that remaining unknown factors are time

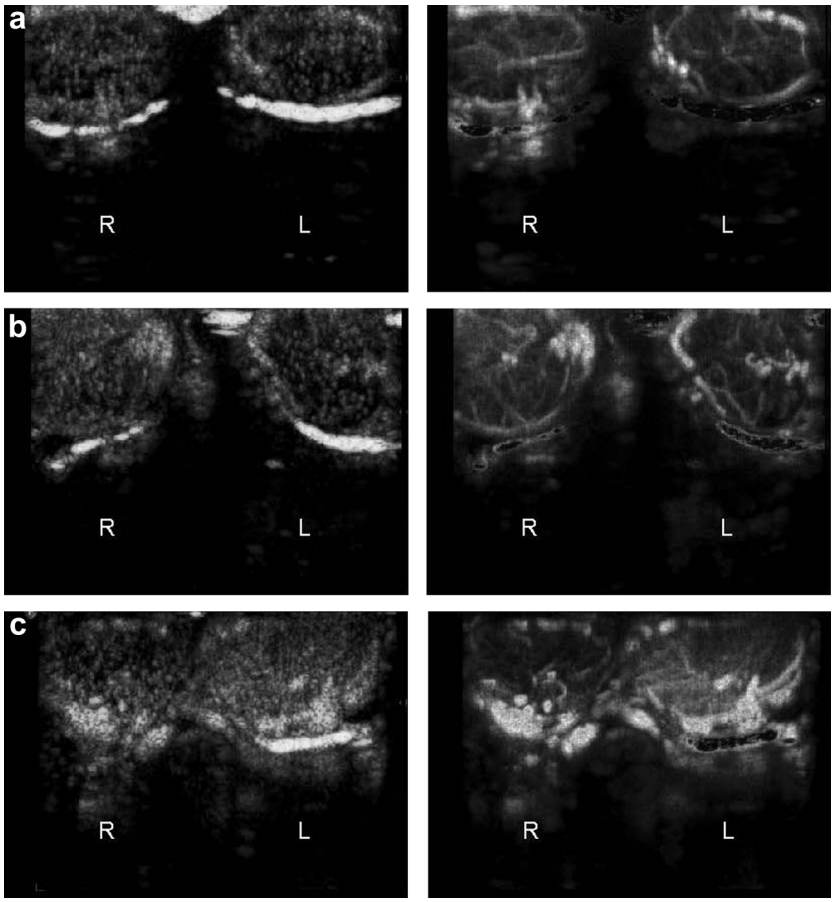

Fig. 1. Output of analysis program in which the radiolabeled microsphere testicular blood flow ratio was (a) 0.9 L/R, (b) 1.35 R/L and (c) 2.43 L/R. Images were acquired with the ultrasound probe oriented transversely over the scrotum so that both testes appeared in the field-of-view. Left: Final frame of a cine loop without additional processing. Right: Same post-processed frame depicting the mean rate of change (slope) in pixel intensity averaged over the refill stage. Echogenic, linear branching structures are visible in right-sided frames, presumably representing flow within the testicular microvasculature. Cine loops corresponding to the experiments depicted in images (a)–(c) are available in the Supplementary Video 1(a–c). R = right testis, L = left testis.

independent and can be represented by a factor α independent of blood flow:

$$S(t) = \alpha A\left(1-e^{ßt}\right) \quad (2)$$

Blood flow (volume of blood per unit time) per unit mass of tissue would be proportional to the product $A\beta$, so long as the tissue mass within the volume of interest (VOI) is constant (Wei et al. 1998). To extract a value proportional to this quantity, the signal is examined at its half-peak value of $A/2$, which occurs at time $t_0 = -\ln(0.5)/\beta$. A first-order Taylor expansion of eqn (2) about this time point yields

$$S(t) = \alpha A/2(ßt-\ln(0.5)/ß) \quad (3)$$

The subjective value α is removed by examining a nearby control volume S_0. It is assumed that this volume possesses an α value identical to the VOI. It is further assumed that the two volumes, functioning normally, would have similar signal response (*i.e.,* blood flow in the VOI and that in the control are ideally identical). Noting that the time derivative of eqn (3) is proportional to $\alpha A\beta$, the ratio

$$Q = (dS/dt)/(dS_0/dt) \quad (4)$$

yields a value proportional to blood flow.

Time-varying values assigned in the US images were assumed to be solely a result of bubble response; that is, the tissue response to the incident US beam was linear. For each time step, pixel values were summed and then divided by the total number of analyzed pixels as a function of time to obtain a mean value. The processed time history was then stored in a database. The linear least-squares method (Björck 1996) was used to fit the rise phase of the mean signal over a 7-s period about its midpoint. The midpoint was assumed to be the maximum of the first derivative of the curve as a function of time. The slope of the fit was determined, and the intervention/control (I/C) ratio was calculated (Paltiel et al. 2011), providing an experimental approximation to eqn (4). The standard deviation of the residuals was used to quantify the error in the fit. In this process, the uncertainty in the curve was determined by calculating the maximum and minimum slopes that fit within one standard deviation from the curve using only vertical offsets.

Individual US pixel intensity ratios were first averaged over the ROI for each transverse, longitudinal and combined acquisition data set, respectively. For each experimental time point in each animal (*i.e.,* pre-operatively, immediately and 4 h and 8 h post-operatively), these ratios were then separately averaged over the three sets of transverse measurements, two sets of longitudinal measurements and combined transverse and longitudinal measurements (five data sets per testis). Finally, the ratios per experimental time point were averaged over all six animals in each of the five torsion groups (*i.e.,* 0°, 180°, 360°, 540° and 720°). For each experimental time point (pre-operatively and immediately and 4 and 8 h post-operatively), each averaged US I/C data set (transverse, longitudinal and combined transverse/longitudinal ratios) for each torsion group was then compared with the corresponding radiolabeled microsphere I/C blood flow ratio.

Post-operative US and radiolabeled microsphere I/C ratios, as a function of torsion degree, were compared using Pearson's correlation coefficient, ρ. Based on the normal distribution of the radiolabeled microsphere ratios, and scatter plots between radiolabeled microspheres and US ratios, which in most cases exhibited an approximately linear relationship between the two sets of ratios, Pearson's correlation coefficient was selected as the most appropriate measure of correlation between I/C ratios at distinct degrees of torsion. All reported p-values were from two-sided tests, assuming a statistical significance level $\alpha = 0.05$.

RESULTS

The combined US data and the radiolabeled microsphere data provided calculated measurements expressed, respectively, as the mean I/C testicular signal intensity ratio (±standard deviation) and the mean radiolabeled microsphere testicular blood flow ratio (±standard deviation) (Table 1).

After unilateral testicular torsion, the post-operative US I/C pixel intensity ratios decreased as torsion increased above 360° (Table 2). However, at each degree of torsion, there were relatively small fluctuations in the ratios over time except for the combined 4-h post-operative value in the 540° torsion group compared with the immediate post-operative and 8-h post-operative values (0.61 vs.

Table 1. Comparison of baseline intervention/control testicular mean ultrasound pixel intensity ratios with baseline intervention/control testicular mean radiolabeled microsphere-based blood flow ratios*

Number of animals	Imaging plane	Intervention/control ultrasound ratio	Intervention/control radiolabeled microsphere ratio
30	Transverse	0.98 ± 0.21	0.98 ± 0.05
	Longitudinal	1.00 ± 0.26	
	Combined	0.98 ± 0.13	

* Data were acquired with the HDI 5000 and iU22 ultrasound systems (Philips Ultrasound, Bothell, WA, USA) at a mechanical index of 0.07. Ratios were estimated at baseline before any treatment. Data have been averaged over pixels within the region of interest; over number of acquisitions for transverse, longitudinal and combined imaging planes; over all 30 animals; and over both ultrasound systems. Values are means ± SD.

Table 2. Postoperative intervention/control testicular mean ultrasound pixel intensity ratios stratified by degree of torsion and time after surgery*

Torsion	Number of animals	Imaging plane	Ultrasound intervention/control ratio		
			Immediately postop	4 h postop	8 h postop
0°(sham)	6	Transverse	1.15 ± 0.60	1.01 ± 0.15	1.05 ± 0.52
		Longitudinal	1.02 ± 0.29	0.99 ± 0.33	1.08 ± 0.25
		Combined	0.98 ± 0.36	1.01 ± 0.24	1.10 ± 0.34
180°	6	Transverse	0.97 ± 0.33	0.88 ± 0.44	1.00 ± 0.55
		Longitudinal	1.14 ± 0.25	1.01 ± 0.37	0.88 ± 0.29
		Combined	1.07 ± 0.36	1.03 ± 0.39	0.93 ± 0.48
360°	6	Transverse	1.43 ± 0.34	1.05 ± 0.33	0.95 ± 0.31
		Longitudinal	0.95 ± 0.54	0.64 ± 0.41	0.81 ± 0.18
		Combined	1.09 ± 0.52	0.85 ± 0.44	0.94 ± 0.32
540°	6	Transverse	0.44 ± 0.45	0.51 ± 0.39	0.56 ± 0.52
		Longitudinal	0.41 ± 0.34	0.28 ± 0.25	0.43 ± 0.34
		Combined	0.40 ± 0.36	0.61 ± 0.58	0.45 ± 0.41
720°	6	Transverse	0.44 ± 0.09	0.50 ± 0.34	0.41 ± 0.24
		Longitudinal	0.35 ± 0.30	0.57 ± 0.35	0.33 ± 0.21
		Combined	0.41 ± 0.16	0.48 ± 0.29	0.35 ± 0.19

* Data were acquired with the HDI 5000 and iU22 ultrasound systems (Philips Ultrasound, Bothell, WA, USA) at mechanical index = 0.07. Data have been averaged over pixels within the region of interest; over number of acquisitions for transverse, longitudinal and combined imaging planes; over all six animals per torsion group; and over both ultrasound systems. Values are means ± SD.

0.40 and 0.45, respectively). The post-operative radiolabeled microsphere I/C blood flow ratios decreased as torsion increased above 180° (Table 3). At each degree of torsion, there was also little change in the ratios over time except for the immediate post-operative value in the sham surgery group, where there was a transient increase in the ratio over baseline, and the 4-h post-operative value in the 720° torsion group, where there was a transient increase in the ratio compared with the immediate post-operative value.

Comparison of US I/C testicular mean US pixel intensity ratios with radiolabeled microsphere I/C testicular mean blood flow ratios immediately and 4 and 8 h post-operatively revealed overall high correlation ($\rho \geq 0.88$, $p \leq 0.05$) between the averaged transverse, averaged longitudinal and combined averaged transverse and longitudinal planar data, with the exception of the transverse US ratio in the immediate post-operative period ($\rho = 0.79$, $p = 0.11$) (Fig. 2).

Table 3. Postoperative radiolabeled microsphere-based intervention/control testicular mean blood flow ratios stratified by degree of torsion and time after surgery

Torsion	Number of animals	Intervention/control radiolabeled microsphere ratio		
		Immediately postop	4 h postop	8 h postop
0°(sham)	6	1.44 ± 0.63	0.91 ± 0.12	1.03 ± 0.21
180°	6	1.07 ± 0.38	1.03 ± 0.34	1.05 ± 0.53
360°	6	0.80 ± 0.41	0.78 ± 0.43	0.80 ± 0.32
540°	6	0.04 ± 0.06	0.03 ± 0.03	0.06 ± 0.05
720°	6	0.03 ± 0.04	0.16 ± 0.24	0.06 ± 0.05

Values are means ± SD.

DISCUSSION

In the clinical setting of acute scrotal pain, a reliable assessment of testicular blood flow must be made to diagnose those patients with torsion who require urgent surgery to prevent testicular infarction. Conventional color Doppler US can usually differentiate between relatively increased flow to an affected testis caused by inflammatory conditions and diminished flow resulting from torsion. However, normal blood flow to the small testes of pre-pubertal children is frequently difficult to depict, as are minor differences in flow between the symptomatic and asymptomatic testes that often occur in patients with partial or intermittent torsion (Cassar et al. 2008; Kalfa et al. 2004; Karmazyn et al. 2005; Nussbaum Blask et al. 2002).

Our prior work indicated that both qualitative and quantitative analyses of relative US pixel intensity derived from 2-D contrast-enhanced pulse inversion imaging in a rabbit model of unilateral testicular ischemia are superior to qualitative analyses of conventional color and power Doppler imaging (Paltiel et al. 2006; Thierman et al. 2006). The present study builds on our earlier experience through the development of a rabbit model of testicular torsion that more closely mimics the anatomic and physiologic features of testicular torsion in humans than does the prior rabbit model of testicular ischemia (Paltiel et al. 2006; Thierman et al. 2006). We extended the time frame of the current investigation to assess relative changes in blood flow over an 8-h period, because testicular salvage in humans is time dependent, with irreversible ischemic injury occurring as soon as 4 h after spermatic cord occlusion (Bartsch et al. 1980).

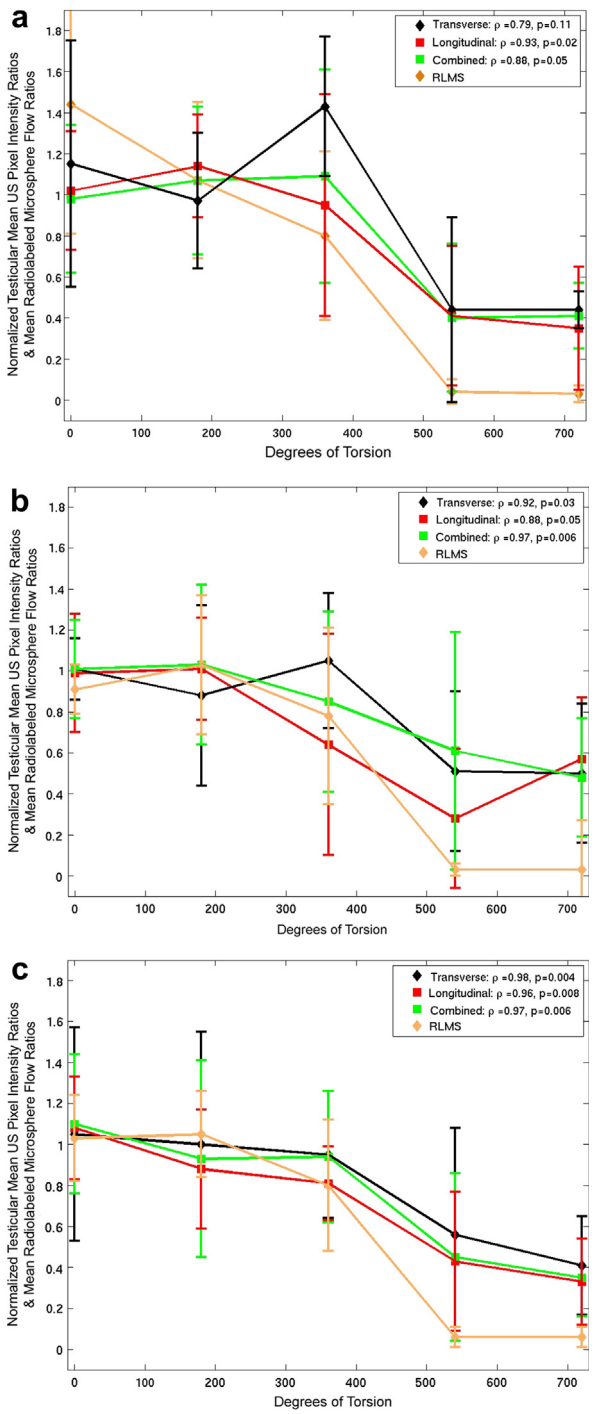

Fig. 2. Mean ultrasound (US) pixel intensity ratios immediately (a) and 4 h (b) and 8 h (c) after surgery follow a trend resembling that of the mean radiolabeled microsphere (RLMS) blood flow ratios at each degree of torsion. Data were acquired with the HDI 5000 and iU22 US systems (Philips Ultrasound, Bothell, WA, USA) at a mechanical index of 0.07 in transverse, longitudinal and combined transverse/longitudinal planes. Data have been averaged over pixels within the region of interest; over number of acquisitions for transverse, longitudinal and combined imaging planes; over six animals for each degree of torsion; and over both US systems. The reported correlation coefficients were estimated on the basis of comparisons of RLMS ratios (a vector of ratios for all degrees of torsion) and US ratios in different planes.

Data were obtained from five different tissue planes per testis in an attempt to better characterize flow throughout the testicular volume.

A determination of blood flow to an organ, tissue or tumor would ideally involve acquisition and analysis of a 4-D data set. Conventional US technology currently limits dynamic contrast-enhanced imaging to a single tissue plane so that only a small portion of a VOI is imaged at any one time. However, repetitive disruption-replenishment measurements can be made in rapid succession during a single examination using a continuous intravenous infusion of contrast material, thereby permitting a sampling of the entire volume.

Our preliminary results with a multi-planar approach to blood flow assessment are promising, with overall high correlation between US pixel intensity and radiolabeled microsphere blood flow I/C ratios for the averaged transverse planes, averaged longitudinal planes and averaged combined transverse and longitudinal planes. Interestingly, there was no consistent improvement in correlation when data from the transverse and longitudinal planes were combined over those obtained from separate analysis of data from the averaged transverse planes and averaged longitudinal planes, although the weakest correlation was seen with US data derived from the averaged transverse planes alone in the immediate post-operative period. One possible explanation for the overall lack of difference between the averaged US data derived from the transverse and longitudinal scan planes is that blood flow throughout the testis is relatively uniform: a single capsular arterial vessel gives rise to parenchymal branches that supply the entire testicular volume. Proximal occlusion of this vessel in the setting of testicular torsion should therefore affect all portions of the parenchyma to a comparable degree. In different clinical situations where disease processes have a differential effect on the arterial supply of an organ or tissue, uniformity of blood flow to the entire organ would not be anticipated.

The technique described in this article was developed in an experimental model of testicular torsion and is potentially applicable to the evaluation of acute scrotal pain. It could also be employed more widely to assess blood flow to many organs and tissues, including the liver, kidneys and ovaries; visceral transplants; and tumors.

There were several limitations to this study. First, it was performed using two different US machines with US probes of different imaging frequencies. These technical differences could theoretically have affected

the results, as the behavior of the US microbubbles is determined by the insonating frequencies. However, the center frequencies of the two US probes are similar (4.5 MHz vs. 5.5 MHz). Furthermore, our study analyzed the ratios of US pixel intensities, not absolute values; thus any differences in pixel intensity resulting from differences in insonating frequency should apply to both testes equally, and pixel intensity ratios should not be affected. Absolute measures of blood flow are not currently possible using the 2-D planar data available with conventional US instruments. Absolute measurements of blood flow should be possible in the future using matrix array US transducers. Three- and four-dimensional data acquisition will permit quantification of US pixel intensity from multiple tissue planes simultaneously and an analysis of blood flow inhomogeneity (Hoyt et al. 2012; Leen et al. 2012; Paltiel et al. 2011). Another potential limitation was the tendency of the US contrast material to settle out of solution over time. Destruction-reperfusion imaging requires continuous infusion of microbubbles over several minutes, and relies on the assumption of a constant concentration of contrast agent in the blood pool to make quantifiable measurements. In this investigation, the microbubbles were resuspended immediately before infusion by rotating and inverting the syringe containing the contrast material and orienting the infusion pump horizontally, thereby minimizing contrast separation (Kaya et al. 2009). Selection of US imaging planes was subjective and, therefore, a potential source of error when analyzing and comparing data from acquisition to acquisition. However, by acquiring data from multiple tissue planes that, in aggregate, included all portions of each testis, we believe that we increased the chances of obtaining a representative sampling of bilateral testicular blood flow. Finally, the sample size for each degree of torsion was small (n = 6). Thus, these results need to be validated in a larger study.

CONCLUSIONS

Preliminary investigation of multi-planar dynamic contrast-enhanced ultrasound assessment of blood flow in a rabbit model of testicular torsion revealed overall high correlation with radiolabeled microsphere blood flow measurements. This technique holds promise for future clinical applications, particularly for diagnosis of testicular torsion in the pediatric population.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ultrasmedbio.2013.08.017.

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