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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