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Automated sonographic evaluation of testicular perfusion

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Abstract

Contrast-enhanced ultrasound (US) imaging is potentially applicable to the investigation of vascular disorders of the testis. We investigated the ability of two automated computer algorithms to analyse contrast-enhanced pulse inversion US data in a rabbit model of unilateral testicular ischaemia and to correctly determine relative testicular perfusion: nonlinear curve fitting of the US backscatter intensity as a function of time; and spectral analysis of the intensity time trace. We compared (i) five metrics based on the algorithmic data to testicular perfusion ratios obtained with radiolabelled microspheres, a reference standard; (ii) qualitative assessment of the US images by two independent readers blinded to the side of the experimental and control testes to the radiolabelled microsphere perfusion ratios; and (iii) results of the algorithmically-derived metrics to the qualitative assessments of the two readers. For the curve fit method, the algorithmically-derived metrics agreed with the reference standard in 54% to 68% of all cases. For the spectral method, the results agreed in 70% of all cases. The two readers agreed with the reference standard in 40% and 35% of all cases, respectively. These results suggest that automated methods of analysis may provide useful information in the assessment of testicular perfusion.

(Some figures in this article are in colour only in the electronic version)

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1. Introduction

Acute scrotal pain is common, with an estimated risk of development of 1 in 160 males by the age of 25 years, whereas the overall incidence of testicular torsion, potentially the most damaging cause of acute scrotal symptoms, is approximately 1 in 4000 (Williamson 1976). Because of the risk of infarction, testicular torsion must be immediately excluded in any patient who presents with acute scrotal pain. However, clinical history and physical examination are often unreliable, even in the hands of experienced practitioners. Diagnostic studies frequently fail to provide definitive information, and controversy still exists as to which imaging test is most reliable (Paltiel *et al* 1998, Nussbaum Blask *et al* 2002). Testicular torsion occurs in all age groups, but is most common around puberty, with a second but much smaller peak in the first year of life (Williamson 1976). Salvage of the neonatal testis is rare, whereas in older patients, salvage rates decline precipitously within 12–24 h after the onset of symptoms due to prolonged ischaemia. Testicular torsion is not an 'all-or-none' phenomenon. It may be intermittent, and the degree of torsion can vary from 180° to 720° or more (Cass *et al* 1980). Patients with lesser degrees of torsion and/or intermittent testicular torsion pose a particularly difficult diagnostic challenge as residual blood flow may be detectable on imaging studies, thereby confounding or delaying appropriate diagnosis and treatment (Bentley *et al* 2004).

Currently, colour Doppler ultrasound (US) is the imaging test of choice in the evaluation of acute scrotal symptoms (Burks *et al* 1990, Lerner *et al* 1990, Middleton *et al* 1990, Dogra *et al* 2003). US diagnosis of testicular torsion depends on an entirely subjective assessment of relative testicular perfusion. However, in prepubertal boys with testes of small volume, testicular perfusion may be difficult to confirm with colour Doppler US. Conversely, the presence of a Doppler US signal in a patient with clinical manifestations of testicular torsion does not exclude the diagnosis. Thus, occasionally it is not possible to diagnose testicular torsion on the basis of colour Doppler US imaging when blood flow is only slightly decreased (Baker *et al* 2000, Nussbaum Blask *et al* 2002, Bentley *et al* 2004, Kalfa *et al* 2004, Karmazyn *et al* 2005). Short of surgery, there is no clinically available means of determining with certainty whether unilaterally diminished yet detectable testicular perfusion is within the range of normal or due to torsion.

Recently developed contrast-enhanced US harmonic imaging techniques have demonstrated greater sensitivity to flow in small blood vessels than conventional colour Doppler US (Goldberg *et al* 1994, Burns 1996). Experimental studies of testicular torsion have shown improved qualitative depiction of testicular blood flow with US contrast agents compared to unenhanced colour Doppler US imaging methods (Coley *et al* 1996, O'Hara *et al* 1996, Brown *et al* 1997, Metzger-Rose *et al* 1997). Quantification of tissue perfusion is also possible and has been investigated in the brain (Rim *et al* 2001, Seidel *et al* 2001), myocardium (Wei *et al* 1997, 1998, Villanueva *et al* 2002), kidney (Schlosser *et al* 2001), and recently in the testis as well (Paltiel *et al* 2006). However, the quantitative methods used for analysing US perfusion imaging data in all of these studies suffer from alignment uncertainties in that the predicted perfusion level is highly dependent on the particular imaging plane considered as well as the subjective selection of regions of interest (ROIs) for perfusion analysis (Lucidarme *et al* 2003, Paltiel *et al* 2006).

Only recently has the ability of an observer experienced in US diagnosis to discriminate visually between various degrees of perfusion in the setting of acute testicular ischaemia been studied or comparison of a subjective assessment of perfusion with an objective measure of perfusion been attempted (Paltiel *et al* 2006). The aim of this study was to assess the ability of two automated computer algorithms to accurately determine relative testicular perfusion based on quantitative analysis of contrast-enhanced pulse inversion US images in a rabbit model of

unilateral acute testicular ischaemia compared to (i) qualitative assessment of relative testicular perfusion by two readers blinded to experimental conditions, and (ii) radiolabelled microsphere tissue perfusion measurements, a reference standard.

2. Materials and methods

Bristol-Myers Squibb Medical Imaging, Inc. (Billerica, MA) provided the contrast agent Definity™ used in the study (grant CG no. 21129). The authors had control of the data and the information submitted for publication.

An abbreviated description of animal selection, preparation, surgical procedure, contrast agent administration, image acquisition, regional perfusion measurements and qualitative image analysis is given below. For a more complete description please refer to the publication by Paltiel *et al* (2006).

2.1. Selection of animals

The study was performed according to a protocol approved by the Animal Care and Use Committee of Children's Hospital Boston, and conformed to guidelines issued by the National Institutes of Health for care of laboratory animals. Use of the rabbit in experimental models of testicular ischaemia is well established (Frush *et al* 1995, Coley *et al* 1996, O'Hara *et al* 1996, Paltiel *et al* 2006). Thirty-five adult male New Zealand white rabbits were studied.

2.2. Animal preparation

General anaesthesia was induced by administering intramuscular atropine (0.04 mg kg^{-1}), intravenous (IV) ketamine (10 mg kg^{-1}) and acepromazine (0.5 mg kg^{-1}).

2.3. Surgical procedure

Unilateral inguinal incision with exposure of the spermatic cord was performed. Right-sided incision was followed by left-sided incision for consecutive experimental animals. An inflatable pressure cuff (5 mm occluder; *In vivo* Metric, Ukiah, CA) was placed around the spermatic cord followed by cuff inflation with normal saline so as to produce, in succession, mild, moderate, and severe degrees of occlusion of the testicular artery and vein. The degree of occlusion was visually determined by one of the authors in every instance by monitoring flow within the ipsilateral testis with colour Doppler US while increasing pressure within the cuff and comparing the resultant flow to flow within the control testis. The criteria used to categorize perfusion as 'mildy', 'moderately' or 'severely' diminished relative to the control testis consisted of a qualitative judgement comparable to that which would ordinarily be employed in clinical practice.

2.4. Contrast agent administration

The US contrast agent Definity™ was used in the study. Definity™ was intermittently infused IV at a rate of 1.3 mL min^{-1} with a maximal dose of 20 mL per experimental animal. The duration of each infusion was less than 4 min.

2.5. Image acquisition

Pulse inversion US imaging was performed using a model HDI 5000 unit (Philips Medical Systems, Bothell, WA) and a linear transducer at baseline and after mild, moderate and severe degrees of spermatic cord occlusion. Of the 35 experimental animals in the study, 22 were investigated using a high frequency 12–5 MHz transducer, and 13 with a lower frequency 7–4 MHz transducer. Pulse inversion images were obtained using the so-called 'negative bolus technique' which is based on the principle of destruction–reperfusion (Wei *et al* 1997, 1998, Becher and Burns 2000). Imaging was performed with the US probe fixed by means of a mechanical arm over a mid-transverse plane of the scrotum so as to include both testes within the field of view. One or two focal zones were positioned adjacent to the mid-to-lower portions of the testes. Pulse inversion imaging was performed using a 170 dB dynamic range with the persistence turned off. Grey scale gain was adjusted prior to contrast agent administration, and was not altered following contrast administration. At baseline and at each level of spermatic cord occlusion, an IV infusion of Definity^{TM} was established, followed by intermittent pulse inversion imaging at a low mechanical index setting until the blood concentration of the microbubbles reached equilibrium. Equilibrium was visually determined by the radiologist performing the US examination and was generally achieved within 90 s. Continuous pulse inversion imaging at low mechanical index was then performed for several seconds followed first by delivery of seven destructive pulses into the scrotum at maximal power (0.6–0.9 mechanical index) and then by a reversion to low mechanical index imaging.

Digital cineloops of all imaging sequences were acquired uniformly over time from the initiation of continuous low mechanical index scanning through microbubble destruction and subsequent contrast replenishment to a steady-state level within the imaging plane. The cineloop image data represented US signal intensity which was log compressed by the Philips US system software using a standard frequency compounding method to decrease the dynamic range of the intensity data to fit 8-bit, 0–255 pixel values and to decrease speckle noise. Cineloops ranged from 7 to 20 s in duration with a mean length of 14 s from the last high-energy pulse to the end of the imaging sequence. The sampling interval was 0.075 s in 90% of the sequences with a range of 0.0375 to 0.115 s. A scan sequence performed with a low mechanical index setting of 0.16 was immediately followed by a second scan sequence performed with a low mechanical index setting of 0.09. All cineloops were subsequently transferred to a personal computer for image processing and analysis.

2.6. Regional perfusion measurements

Reference testicular perfusion measurements were obtained using a radiolabelled microsphere technique (Heymann *et al* 1977). At the conclusion of the experiment, each rabbit was sacrificed with an IV overdose of pentobarbital (10 mg kg^{-1}; 1 mL/4.5 kg). The testes were then removed and immediately sectioned for regional perfusion determination.

2.7. Image processing

The digital cineloops were converted to AVI files by one of the authors (HJP) and by the author's administrative assistant with manufacturer's software (QLAB, version 1.0, Philips Ultrasound, Bothell, WA) and stripped of all identifying information, including date of the study, animal number and experimental side. Identifying links to each animal were available only to this author and to the author's administrative assistant, and were stored on password-protected computer files. Each image file was assigned a randomly generated index number and exported to a password-protected website for review.

2.8. Quantitative image analysis

For numeric analysis, the AVI files were imported into Matlab® for processing. The data were converted into three-dimensional matrices representing the two spatial dimensions and time. The L7-4 transducer had a spatial resolution ranging from 0.8 to 0.9 mm axially × 1.0 to 1.6 mm laterally for operating depths of 2–6 cm. The L12-5 transducer had a spatial resolution ranging from 0.7 to 0.8 mm axially × 0.9 to 1.3 mm laterally over the same range. The temporal resolution was 100 frames s^{-1} resulting in a Nyquist cut-off frequency of 50 Hz.

Next it was necessary to choose relevant testicular regions of interest (ROIs). In earlier work based on the same data, multiple ROIs within each testis were chosen in a completely subjective manner (Paltiel *et al* 2006). In order to eliminate the subjective element from the analysis and to ensure that no potentially ischaemic regions were ignored, a more objective, quantitative approach was adopted. The noise level for each image in the cineloop was first determined by calculating the mean pixel value over a subset of each image that was not coincident with the location of the testes. The entire image was then processed frame-by-frame and a simple grey scale transformation was applied to eliminate any pixel which was less than 1.1 times the mean of the noise (background pixels) and any pixel which was greater than 1.3 times the mean of the noise (large vessels) by remapping the values of these pixels to 0. The remaining pixels were assumed to represent the US signal from the testicular microvasculature and their pixel values were left unchanged. The selection of thresholds of 1.1 and 1.3 times the noise was made by empirical observation. After eliminating pixels representing background signal and large vessels, the image was divided into half in order to isolate the response from the right and left testes. Justification of this simple method was based on the anatomical separation between the right and left testes, which was located at approximately the midline of each image.

We considered the entire notch-filtered, half-image as the ROI for the right or left testis, respectively. Our algorithm for selecting the ROI accomplished the goals of automating image processing, avoiding large vessels and background noise, and removing the subjective element inherent in an arbitrary selection of a smaller ROI (or multiple ROIs) within each testis. In addition, by assessing the entire testis within each image frame we increased the chances of detecting zones of decreased perfusion. Finally, we summed the US intensity values over the entire ROI for each testis to obtain a mean time-domain US signal for use with the two algorithms described below.

2.8.1. Nonlinear curve fitting algorithm. The curve fit analysis is similar to methods reported in the literature whereby an exponential curve is fitted to the experimental data (Marquardt 1963, Foss 1970, Glass and de Garreta 1971, Stirling 1985, Wei *et al* 1998). We used the Marquardt algorithm (Marquardt 1963) to fit the data to the exponential function

$$p(t) = A\left(1 - e^{\frac{-1}{S}t}\right) \tag{1}$$

where $p(t)$ is the fitted US signal intensity, t is the reperfusion time in seconds from the last destructive high-energy US pulse, S is the time constant, and A is the value of the nonlinear curve as $t \to \infty$. Images were processed as a function of time and fitted to the exponential curve as described by (1) to determine the two parameters, A and S, for each testis in every experiment. These parameters characterize the time dependence of the US response signal and were used to evaluate the relationship between the echogenicity data and perfusion to each testis. We obtained the value of the exponential fit curve at $t = 7$ s (p_7) and the value of the exponential fit curve corresponding to the last frame of each AVI file ($p_$final). We chose the 7 s time interval in an attempt to standardize the data across all imaging sequences since

every cineloop was at least 7 s long (*vide supra*). The final image frame was chosen in order to obtain a measurement based on maximal reperfusion of each testis.

2.8.2. Spectral method algorithm. The spectral method is designed to detect time-variant oscillations in US signal intensity, such as those that might occur in the presence of disordered perfusion caused by restricted arterial inflow and venous outflow. The Fourier transform pair was used to convert data between the time and frequency domains and is given by

$$\begin{aligned} P(\omega) &= \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} p(t)\, \mathrm{e}^{-\mathrm{j}\omega t}\, \mathrm{d}t \\ p(t) &= \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} P(\omega)\, \mathrm{e}^{\mathrm{j}\omega t}\, \mathrm{d}\omega \end{aligned} \tag{2}$$

where $p(t)$ is the US signal intensity, t is the time, $\mathrm{j} = \sqrt{-1}$ is the imaginary number, and $\omega = 2\pi f$ is the angular frequency.

We used the fast Fourier transform function (FFT) in Matlab® to convert the time-domain signal from the US images to extract the frequency content. A preliminary investigation of the FFT of the echogenicity signal revealed no apparent association between the frequency spectrum and the radiolabelled microsphere perfusion values. We then examined the FFT of the derivative of the echogenicity signal with respect to time. The derivative was calculated as the running difference of the US signal between consecutive time points and measured *changes* in signal intensity over time rather than the signal intensity itself, as described below.

The expression of the FFT of the derivative of the function $p(t)$ is given in equation (3).

$$\Im\{\dot{p}(t)\} = \frac{\mathrm{j}}{\sqrt{2\pi}}\omega \int_{-\infty}^{\infty} p(t)\, \mathrm{e}^{-\mathrm{j}\omega t}\, \mathrm{d}t = \mathrm{j}\omega P(\omega). \tag{3}$$

The FFT of the derivative of the time-varying signal can be understood as a frequency-weighted FFT whereby the higher frequencies of the original FFT are amplified as a result of the ω term scaling the original FFT. The result is an expression similar to the frequency spectrum but with higher frequency signal weighted disproportionately to lower frequency signal. We hypothesize that blood flow within the testicular parenchyma will be dampened due to obstructed arterial inflow and venous outflow, resulting in a narrower, lower range of velocities than the normally perfused testis, and should be manifested as a relative loss of high frequency signal.

We calculated the left side of equation (3) by taking the fast Fourier transform of the discrete time derivative of the signal. These discrete calculations introduced a 'dc' component, $p(0)$, which we subsequently subtracted from the function and then plotted the spectrum as parameterized by frequency. This 'dc' component of the spectrum was removed as it is the portion of the spectrum with a frequency of 0, rather than the higher frequency information with which we were concerned. The spectral information was quantified by summing the spectrum over all frequencies below the Nyquist frequency to obtain a single value for each testis that represented the total frequency content of the time derivative of the US signal. This value, which we call F_w for weighted frequency content, was used to evaluate the relationship between the echogenicity data and perfusion to each testis.

2.9. Qualitative image analysis

Qualitative assessment of all original contrast-enhanced images was performed independently by two readers, both radiologists with particular expertise in sonography, who were blinded as to which side was the intervention testis and which the control testis. The readers categorized

Table 1. Number of animals tested at each nominal occlusion level.

Intervention side	Baseline	Mild	Moderate	Severe
Right	17	17	16	14
Left	18	18	16	16
All	35	35	32	30

relative testicular perfusion as 'right greater than left' (R > L), 'right equal to left' (R = L), or 'right less than left' (R < L). The criteria used to categorize relative perfusion as 'greater than', 'less than' or 'equal to' consisted of a qualitative judgement comparable to that which would ordinarily be employed in clinical practice.

2.10. Comparison of quantitative and qualitative analyses

For each experiment the ratio of the intervention/control (I/C) radiolabelled microsphere perfusion measurement was calculated and used as the reference standard to which the I/C ratios derived from qualitative assessment of the US cineloops by the two radiologists and from quantitative measures derived from the two automated algorithms (p_7, p_final, A, S and F_w) were compared. When qualitative assessment of relative testicular perfusion by one of the readers agreed with the radiolabelled microsphere I/C perfusion ratio (i.e., the reader's determination of equal testicular perfusion or identification of the lesser perfused testis agreed with the radiolabelled microsphere-based perfusion ratio) the reader was scored as correct. Similarly, when the I/C ratio derived from the quantitative measures (p_7, p_final, A, S and F_w) agreed with the radiolabelled microsphere perfusion I/C ratio (i.e., both ratios were <1.0, $=1.0$ or > 1.0), the algorithm was scored as correct.

We initially analysed all of the data together, including those experiments performed with both the L12-5 and L7-4 US transducers at mechanical index settings of 0.09 and 0.16. Included in this analysis were the cases where differences in perfusion between the right and left testes were small. Next we considered only those cases with greater disparities in perfusion between the intervention and control testes, namely those instances where the I/C perfusion ratio was (i) <0.60 and (ii) <0.33. We then separately considered the experiments performed with mechanical index settings of 0.09 and 0.16 due to our initial observation that the US signal appeared less noisy in the experiments using the lower mechanical index setting of 0.09. Finally we separately analysed the results obtained with the L12-5 MHz and L7-4 MHz transducers.

The performance of the calculated I/C testicular perfusion ratios based on p_7, p_final, A, S and F_w measurements was compared to the I/C ratios derived from the radiolabelled microsphere regional perfusion measurements by determining nonparametric Spearman correlations. This additional analysis was undertaken in order to determine whether the automated methods could not only determine the lesser-perfused testis, but could also quantify the degree of perfusion abnormality.

3. Results

Of the thirty-five rabbits that were studied, three died during the course of the experiment. Autopsy revealed no obvious cause of death. Technical problems with radioisotope injection and data transfer account for the additional decrease in numbers tested at the 'moderate' and 'severe' occlusion levels (table 1).

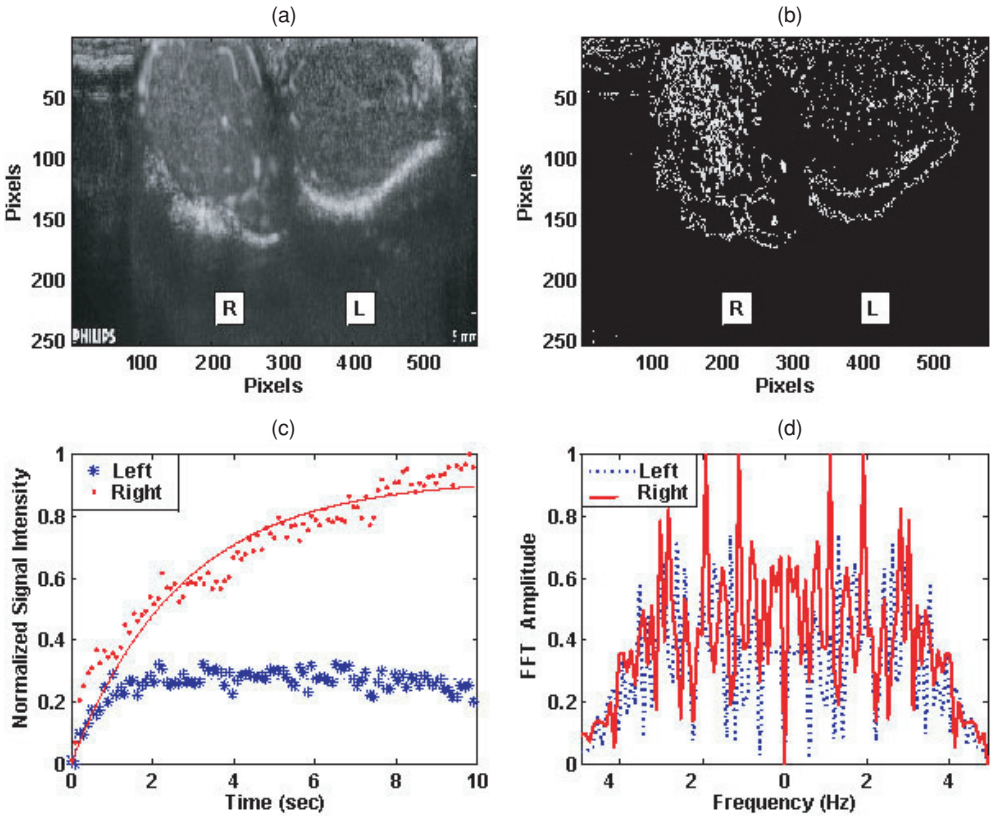

Figure 1. Output of analysis program where the left-to-right radiolabelled microsphere testicular perfusion ratio was 0.64. The final frame of the cineloop is shown without additional image processing in (a), while (b) depicts the same frame after the image was notch filtered to remove signal from background noise and large vessels. Only signal from flow in the testicular microvasculature remains, indicated by shades of grey. The curve fit algorithm in (c) demonstrates the raw data as points and the exponential curve fits as lines. The spectral content of the derivative of the reperfusion curves for each testis is displayed in (d). R: right testis; L: left testis.

Table 2. Number of automated studies performed at each nominal occlusion level.

	Power level		
Nominal occlusion level	MI 0.16	MI 0.09	Total
Baseline	35	32	67
Mild	35	32	67
Moderate	31	29	60
Severe	29	27	56
Total	130	120	250

MI: mechanical index.

Data from 250 separate experiments were available for automated analysis. Of the 250 experiments, it was not possible to analyse 7 (3%) because the exponential fit using the Marquardt algorithm did not converge after 1000 iterations. The remaining 243/250 (97%) experiments were processed by the automated program (table 2). The graphical output of the Matlab® program for a sample experiment is illustrated in figure 1.

Table 3. Mean ± SD Perfusion[a] and intervention/control ratio by nominal occlusion level.

Nominal occlusion level	*N*	Perfusion of intervention testis (mL g^{-1} min^{-1})	Perfusion of control testis (mL g^{-1} min^{-1})	Intervention/control ratio
Baseline	35	0.225 ± 0.084	0.284 ± 0.091	0.803 ± 0.205
Mild	35	0.216 ± 0.087	0.306 ± 0.111	0.725 ± 0.222
Moderate	32	0.196 ± 0.078	0.331 ± 0.108	0.607 ± 0.230
Severe	30	0.086 ± 0.072	0.295 ± 0.123	0.287 ± 0.225

N: number of experimental animals.
[a] Perfusion measured with radiolabelled microspheres.

Table 4. Percent correct identification of the lesser-perfused testis by analytical method, stratified according to mechanical index setting and I/C radiolabelled microsphere perfusion ratio.

	All studies			I/C perfusion ratio < 0.6			I/C perfusion ratio < 0.33		
	MI 0.16	MI 0.09	MI 0.16 and 0.09 combined	MI 0.16	MI 0.09	MI 0.16 and 0.09 combined	MI 0.16	MI 0.09	MI 0.16 and 0.09 combined
p_7	67%	68%	68%	80%	85%	82%	90%	83%	87%
p_final	68%	65%	67%	81%	81%	81%	90%	83%	87%
A	70%	59%	65%	81%	67%	75%	85%	83%	84%
S	62%	46%	54%	67%	50%	58%	80%	67%	74%
F_w	69%	72%	70%	85%	88%	87%	85%	89%	87%
Reader A	40%	38%	40%	67%	67%	67%	85%	94%	89%
Reader B	36%	35%	35%	59%	67%	63%	80%	89%	84%

MI: mechanical index; I/C: intervention/control; p_7: value of the US signal intensity at $t = 7$ s, p_final: final value of the US signal intensity; A: final value of the nonlinear curve as $t \to \infty$; S: time constant; F_w: weighted frequency content.

Baseline perfusion as measured by radiolabelled microspheres averaged 0.225 ± 0.084 mL g^{-1} min^{-1} in the intervention testis and 0.284 ± 0.091 mL g^{-1} min^{-1} in the control testis. The average I/C ratio at baseline was 0.803 ± 0.205. These values were very similar whether the intervention side was right or left. Perfusion of the intervention side and the I/C ratio both decreased as the degree of occlusion increased ($p < 0.001$ for both by repeated measures analysis of variance), while perfusion of the control side remained fairly stable ($p = 0.08$, table 3). When perfusion of the control side was combined over all occlusion levels, the mean ± SD testicular perfusion was 0.304 ± 0.108 mL g^{-1} min^{-1}.

We used the A, S, p_7 and p_final values calculated from the nonlinear curve fit algorithm, and the F_w value calculated from the spectral algorithm as the parameters to compare with the qualitative assessment of testicular perfusion by the two readers. When the experiments were analysed together, the nonlinear curve fit methods p_7 and p_final and the spectral method F_w correctly determined the lesser-perfused testis in 68%, 67% and 70% of all cases, respectively. The metrics A and S were correct in 65% and 54% of all cases, respectively. In comparison, reader A and reader B correctly identified the lesser-perfused testis in 40% and 35% of all cases, respectively. The data reveal a general trend of increasing accuracy for all five metrics as the I/C perfusion ratio decreases (table 4).

When we considered correct identification of the lesser-perfused testis as a function of the radiolabelled microsphere I/C perfusion ratio, we noted that the two automated algorithms more closely matched the radiolabelled microsphere ratios than the two readers when the

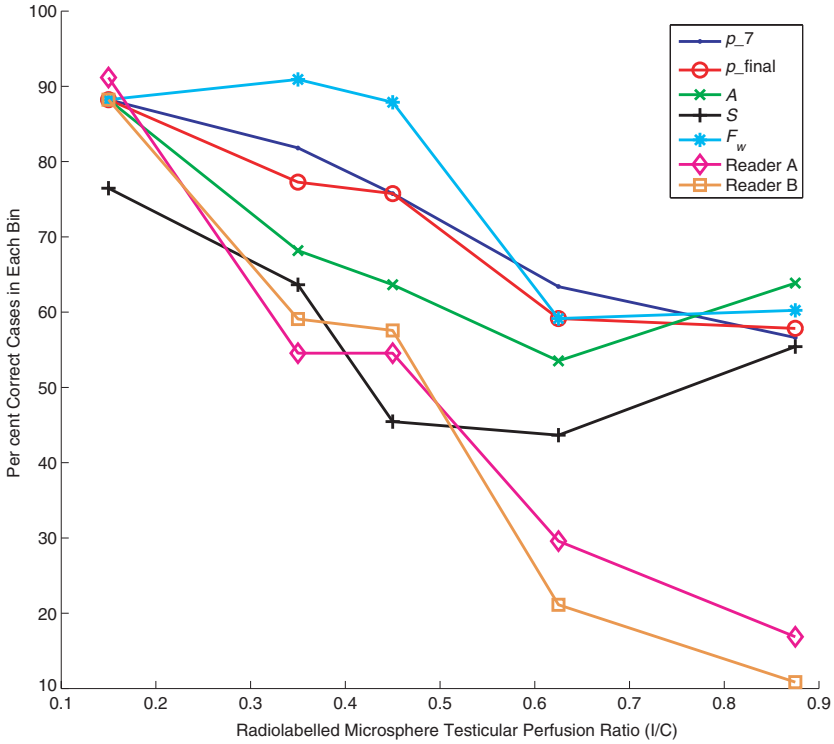

Figure 2. Percentage of experimental cases where the automated algorithms and the two readers correctly determined the lesser-perfused testis using the I/C radiolabelled microsphere perfusion ratio as the reference standard. The data is divided into five bins encompassing I/C ratios of 0 to 1.0 with the data plotted at the centre point of each bin. I/C: intervention/control.

differences in testicular perfusion were small. As the I/C perfusion ratio decreased to 0.2 and below, correct identification of the lesser-perfused testis by both automated algorithms and both readers converged to approximately 90% accuracy. These data are displayed graphically in figure 2.

Nonparametric Spearman correlations between the I/C perfusion ratios determined algorithmically and by the radiolabelled microsphere technique ranged from 0.05 to 0.64. The strongest correlations were found for the US signal intensity measurements p_7 and $p_$final (table 5).

4. Discussion

In the clinical setting of acute testicular pain, it is critical to determine accurately whether or not the symptomatic testis is ischaemic. Large discrepancies in perfusion do not usually pose a diagnostic dilemma whereas more subtle differences may be diagnostically challenging. Since current examination techniques are not always reliable, the potential contribution of an easily performed, accurate quantitative method for analysis of tissue perfusion is considerable.

Contrast-enhanced pulse inversion imaging of testicular perfusion is an experimental technique not currently approved for clinical use in the United States. A recent study by Paltiel *et al* (2006) compared qualitative assessment of conventional colour and power

Table 5. Spearman correlations between algorithmically-based and radiolabelled microsphere-based I/C perfusion ratios.

	Spearman correlation coefficient		
Parameter	MI 0.16 and 0.09 combined	MI 0.16	MI 0.09
Both transducers			
p_7	0.45	0.40	0.53
p_final	0.45	0.41	0.51
A	0.31	0.34	0.29
S	0.15	0.19	0.10
F_w	0.34	0.35	0.33
L12-5 transducer			
p_7	0.50	0.39	0.64
p_final	0.51	0.40	0.64
A	0.29	0.28	0.30
S	0.17	0.19	0.15
F_w	0.34	0.32	0.37
L7-4 transducer			
p_7	0.42	0.42	0.43
p_final	0.41	0.46	0.40
A	0.39	0.47	0.34
S	0.16	0.25	0.05
F_w	0.34	0.40	0.28

I/C: intervention/control; MI: mechanical index; p_7: US signal intensity value at $t = 7$ s; p_final: final value of the US signal intensity; A: value of the nonlinear curve as $t \to \infty$; S: time constant; F_w: weighted frequency content.

Doppler imaging of testicular perfusion in a rabbit model of acute testicular ischaemia to qualitative and quantitative assessment of contrast-enhanced pulse inversion imaging. The qualitative assessment of testicular perfusion by two readers blinded to experimental conditions was assessed and compared to quantitative measures of perfusion based on testicular ROIs subjectively determined by a third independent investigator. This earlier study showed that when all cases were considered, the quantitative measures based on ROI analysis correctly determined the lesser-perfused testis in 75–79% of all cases, whereas the two readers were correct in 40% and 34% of all cases, respectively. When the readers assessed perfusion performed with conventional Doppler techniques, they correctly determined the lesser-perfused testis in 60% and 48%, respectively, of all cases studied with colour Doppler US; and 53% and 39%, respectively, of all cases studied with power Doppler US. One significant limitation of this earlier study was the subjective determination of ROIs for quantitative analysis, with the potential for inclusion of large vessels not representative of parenchymal perfusion, as well as the unvalidated assumption that the selected ROIs accurately reflect whole organ perfusion.

In this study, we investigated the use of two automated algorithms in an attempt to eliminate the subjective determination of ROIs for a more accurate reflection of true tissue perfusion. Both algorithms grey scale transformed the US images using a threshold method to remove background noise and signal from large vessels. This simple technique appears to be a useful method for image preprocessing that eliminates noise without eliminating areas of decreased perfusion. The lower threshold of 1.1 times the mean noise and the upper threshold of 1.3 times the mean noise were chosen by empirical observation, but proved reasonably

effective for the experimental cases considered. More objective methods for choosing these threshold values should prove a fruitful topic for future work.

The results demonstrate that each algorithm in this investigation correctly determined the lesser-perfused testis more often than either reader when all experimental cases were considered. The improvement over the readers in determining the lesser-perfused testis using the automated methods was most striking for the more equivocal cases in which one testis was only slightly less well-perfused than the other. This enhanced accuracy for mildly discrepant perfusion is potentially useful for early diagnosis of a progressive condition or the diagnosis of otherwise subtle perfusion abnormalities. These initially promising results indicate a need for further investigation and development of automated methods of contrast-enhanced US image analysis. The automated system may prove even more sensitive to small intensity changes in future work using uncompressed data so as not to limit the dynamic range. Another potential focus of future investigation would be a comparison of the readers' performance in qualitatively determining the lesser-perfused testis after a review of the post-processed grey scale images versus the original contrast-enhanced images.

The automated methods worked less well when attempting to quantify the degree of perfusion abnormality. Perfusion analysis based on data acquired from a single tissue plane is unlikely to be representative of the entire organ. We believe that volumetric perfusion information should more accurately determine perfusion 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 tissue planes over a short time interval would result in an improved estimate of total organ perfusion. Data from multiple planes would also yield information on perfusion changes in the direction orthogonal to the image planes and might provide an opportunity for more accurate alignment between the left and right testes for comparison.

Clinical US diagnosis of testicular torsion relies on a purely subjective assessment of relative testicular perfusion. There are no data currently available regarding testicular perfusion values or perfusion ratios that will permit a distinction between normal variation in bilateral perfusion, pathological hypoperfusion, or the prediction of longterm testicular viability based on perfusion measurements at the time of initial presentation. We plan to investigate these issues in the future.

5. Conclusion

The results of this preliminary investigation indicate that automated analysis of contrast-enhanced pulse inversion US images may provide useful information regarding differential testicular perfusion in the setting of acute testicular pain. Improved image processing techniques including the use of uncompressed data, more sophisticated signal intensity filtering methods, and acquisition of volumetric data should lead to improved accuracy of this approach. While the focus of this work is the diagnosis of ischaemia, an automated, objective method for assessing differential perfusion may eventually prove more widely applicable in the diagnosis of other vascular disorders of the scrotum such as varicocele, as well as to vascular disorders of other organ systems.

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