Radiology

Pulse-Inversion US Imaging of Testicular Ischemia: Quantitative and Qualitative Analyses in a Rabbit Model[1]

Harriet J. Paltiel, MD
Leslie A. Kalish, ScD
Ricardo A. Susaeta, MD
Ferdinand Frauscher, MD
Patrick L. O'Kane, MD
Luiz G. Freitas-Filho, MD

Purpose: To quantitatively and qualitatively assess perfusion with pulse-inversion (PI) ultrasonography (US) in rabbit model of acute testicular ischemia.

Materials and Methods: Institutional animal care committee approval was obtained. After 35 rabbits underwent unilateral spermatic cord occlusion, testicular Doppler US and contrast material–enhanced PI imaging were performed. Enhancement data yielded perfusion measurements including mean value during the first 10 seconds, mean value over entire recorded replenishment curve, and curve slope during the first 5 seconds. Calculated perfusion ratios were compared with radiolabeled microsphere–derived perfusion ratios. Two readers assessed testicular perfusion as none, possible, or definite and relative perfusion as greater to the right testis than to the left, greater to the left testis than to the right, or as equal to both testes. With κ statistics, interobserver agreement for all imaging methods was determined. Association between qualitative perfusion categories and radiolabeled microsphere–based perfusion measurements was assessed. Quantitative and qualitative determinations of relative perfusion were compared with radiolabeled microsphere–based measurements.

Results: Correlations between calculated and radiolabeled microsphere–based perfusion ratios were determined (r = 0.49–0.64). Interobserver agreement for presence of perfusion was excellent (κ = 0.76), and that for relative perfusion assessment was good (κ = 0.55). Neither κ value varied significantly with imaging method. The percentage of times a testis classified as having definite perfusion had greater perfusion as measured with radiolabeled microspheres than a testis classified as having no perfusion or possible perfusion was higher with PI imaging than with Doppler US (85%–98% vs 72%–89%). Identification of the testis with less perfusion was better with quantitative methods than with qualitative assessment of images by the readers (75%–79% vs 34%–60%, $P < .004$).

Conclusion: PI imaging, compared with conventional Doppler US methods, provides superior assessment of perfusion in the setting of acute testicular ischemia.

[1] From the Departments of Radiology (H.J.P.) and Urology (R.A.S., L.G.F.) and the Clinical Research Program (L.A.K.), Children's Hospital Boston, Harvard Medical School, 300 Longwood Ave, Boston, MA 02115; Department of Radiology, University Hospital, Innsbruck, Austria (F.F.); and Department of Radiology, Thomas Jefferson University, Philadelphia, Pa (P.L.O.). Received February 7, 2005; revision requested April 6; revision received July 20; accepted August 25; final version accepted September 12. Supported in part by National Institutes of Health grant 1 R03 EB00543-01 and by Bristol-Myers Squibb Medical Imaging grant CG#21129. **Address correspondence to** H.J.P. (e-mail: *harriet.paltiel@childrens.harvard.edu*).

Acute scrotal pain is common, with an estimated risk of development in one in 160 males by the age of 25 years. The overall incidence of testicular torsion, the most serious cause of acute scrotal symptoms, is approximately one in 4000 (1). Because of the risk of infarction, testicular torsion must be immediately excluded in all patients who present with acute scrotal symptoms. Torsion can occur at any age but is most frequent in the pediatric population, with peaks of incidence in the neonatal period and adolescence (2). Clinical evaluation of acute scrotal symptoms in children often is unreliable because of small testicular size, presence of a reactive hydrocele, and lack of patient cooperation. The need to exclude the diagnosis of testicular torsion to prevent unnecessary surgery must be balanced by the requirement to rapidly and accurately diagnose the condition in those patients who require urgent surgical intervention (3).

Color Doppler ultrasonography (US) currently is the imaging test of choice in the evaluation of acute scrotal symptoms because of its capability for direct visualization of the testicular blood supply and for documentation of alterations in blood flow without the need for ionizing radiation (4–7). Its use in children, however, often is limited by the difficulty in detection of flow in testes of small volume (8–12). Contrast material–enhanced US techniques that have been developed have resulted in greater sensitivity to flow in small blood vessels (13–20). Harmonic imaging is a US method in which transducers transmit ultrasound at one frequency (the fundamental) and receive signals at twice that frequency (the second harmonic). When harmonic imaging is used in conjunction with microbubble-based US contrast agents, there is greater enhancement of the vascular signal compared with that obtained with conventional contrast-enhanced color Doppler US methods (21,22). With pulse-inversion (PI) imaging, a two-pulse sequence is used with a 180° phase difference to cancel the effect of transmitted second harmonics from nonvascular background tissues on the received signal. This technique results in improved distinction between parenchymal vessels and background tissues (14–18).

The purpose of our study was to quantify relative testicular perfusion with PI imaging in a rabbit model of acute testicular ischemia and to compare PI and Doppler US images in the qualitative assessment of testicular perfusion.

Advances in Knowledge

- The technique of contrast-enhanced PI imaging was successfully applied to an animal model of acute testicular ischemia.
- Quantitative and qualitative contrast-enhanced PI imaging, compared with conventional Doppler US methods, provided a superior assessment of perfusion in the setting of acute testicular ischemia.

Materials and Methods

The contrast agent used in the study (Definity; Bristol-Myers Squibb Medical Imaging, Billerica, Mass) was provided by the manufacturer. The authors had control of the data and the information submitted for publication.

Selection of Animals

The study was performed according to a protocol approved by the Animal Care and Use Committee of Children's Hospital Boston, Boston, Mass, 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 ischemia is well established (23–25). Thirty-five adult male New Zealand white rabbits (Millbrook Breeding Labs, Amherst, Mass) with a mean weight of 4.0 kg were examined.

Animal Preparation

General anesthesia was induced by intramuscularly administering atropine sulfate (Baxter, Deerfield, Ill), 0.04 mg per kilogram of body weight, and intravenously administering ketamine (Ketaset; Fort Dodge, Fort Dodge, Ind), 10 mg/kg, and acepromazine maleate (Phoenix Pharmaceutical, St Joseph, Mo), 0.5 mg/kg (Appendix).

Surgical Procedure

A unilateral inguinal incision with exposure of the spermatic cord was performed by one of two urologists (R.A.S., L.G.F.) with both clinical (8 and 19 years, respectively) and large-animal surgical experience (1 year and 13 years, respectively). A right-sided inguinal incision and then a left-sided inguinal incision were performed for consecutive experimental animals. An inflatable vascular occluder with a 6- or 8-mm luminal diameter (OC6, OC8; Kent Scientific, Torrington, Conn) was placed around the spermatic cord, followed by cuff inflation with normal saline to produce, in succession, mild, moderate, and severe degrees of occlusion of the testicular artery and vein (Appendix).

Contrast Agent Administration

The US contrast agent was intermittently infused intravenously by the first author (H.J.P.) at a rate of 1.3 mL/min, with a maximal dose of 20 mL per experimental animal (Appendix). The duration of each infusion was less than 4 minutes.

Image Acquisition

Serial color Doppler, power Doppler, and PI US imaging was performed by the first author (H.J.P., a pediatric radiologist with 16 years of experience) at baseline and af-

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Abbreviations:
M_{10} = mean value during first 10 seconds
MI = mechanical index
M_{max} = mean value over entire recorded replenishment curve
PI = pulse inversion
ROI = region of interest
S_{05} = curve slope during first 5 seconds

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Guarantor of integrity of entire study, H.J.P.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, H.J.P.; experimental studies, H.J.P., R.A.S., F.F., P.L.O., L.G.F.; statistical analysis, L.A.K.; and manuscript editing, H.J.P., L.A.K.

See Materials and Methods for pertinent disclosures.

ter mild, moderate, and severe degrees of spermatic cord occlusion. All studies were performed by using a US unit (model HDI 5000; Philips Medical Systems, Bothell, Wash) and a linear transducer (Philips Medical Systems). Of the 35 experimental animals in the study, 22 were investigated by using a 12–5-MHz linear transducer, and 13 were investigated with a 7–4-MHz linear transducer. Color and power Doppler US images were acquired prior to contrast material administration. PI images were then obtained by using the so-called negative bolus technique, which is based on the destruction-reperfusion principle (Appendix) (26,29,37).

Regional Perfusion Measurements

Reference testicular perfusion measurements were obtained by the first author by using a radiolabeled-microsphere technique (38). At the conclusion of the experiment, each rabbit was sacrificed with an intravenous overdose of pentobarbital (1 mL/4.5 kg). The testes were then removed by one of two individuals (R.A.S. or L.G.F.) and immediately sectioned for regional perfusion determination (Appendix).

Quantitative Image Analysis

Gray-scale testicular parenchymal enhancement during the PI imaging sequences was measured with manufacturer's software (QLAB, version 1.0; Philips Medical Systems), which allows quantification of pixel intensity before logarithmic compression for video display. For each imaging sequence, identical regions of interest (ROIs) were manually placed by the first author over each testis, with four on each side; care was taken to avoid the large vessels located at the testicular periphery. ROIs varied in size from 1.52 to 21.46 mm^2 (mean, 7.2 mm^2). An effort was made to place corresponding ROIs in the right and left testes at similar depths from the transducer face to avoid differences in signal intensity values caused by differential beam attenuation. Replenishment curves were estimated on the basis of temporal changes in image signal intensity within each ROI.

Qualitative Image Analysis

Image processing.—The digital cine loops were converted to a multimedia file format (Audio Video Interleave; Microsoft, Redmond, Wash) by the first author (H.J.P.) and an administrative assistant with manufacturer's software (QLAB, version 1.0; Philips Medical Systems) and stripped of all identifying information, including date of the study, animal number, and experimental side. Each image file was assigned a randomly generated index number and exported to a password-protected Web site for review. Identifying links to each animal were available only to the first author and to the first author's administrative assistant and were stored in password-protected computer files.

Image review.—Qualitative assessment of all images was performed independently by two readers (F.F., P.L.O.),

Table 1

Number of Animals Tested at Each Occlusion Level

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

Table 2

Radiolabeled Microsphere–based Perfusion Measurements and Intervention-Control Testicular Perfusion Ratios according to Occlusion Level

Occlusion Level	No. of Animals	Perfusion of Intervention Testis ([mL · g^{-1}]/min)	Perfusion of Control Testis ([mL · g^{-1}]/min)	Intervention-Control Testis 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

Note.—Data are the mean ± standard deviation.

Table 3

Number of Studies Performed with PI Imaging and Intervention-Control Testicular Perfusion Ratio according to Occlusion Level

A: No. of Studies with PI Imaging

Occlusion Level	MI of 0.16	MI of 0.09	All
Baseline	35	32	67
Mild	33	31	64
Moderate	31	30	61
Severe	29	27	56
All	128	120	248

B: Intervention-Control Testicular Ratio

Occlusion Level	M10*	Mmax*	S05*
Baseline	0.955 ± 0.439	0.961 ± 0.416	1.020 ± 0.695
Mild	0.745 ± 0.335	0.763 ± 0.336	0.724 ± 0.359
Moderate	0.623 ± 0.350	0.638 ± 0.345	0.653 ± 0.427
Severe	0.509 ± 0.614	0.506 ± 0.618	0.564 ± 0.693

Note.—MI = mechanical index.

* Data are the mean ± standard deviation.

both radiologists with particular expertise in US (10 and 8 years, respectively), who were blinded as to the side on which the intervention testis and the control testis were located. They classified the perfusion to each testis on every image as either none, possible, or definite. The criterion used to categorize perfusion as possible or definite consisted of a qualitative judgment comparable to that which would ordinarily be employed in clinical practice. They also categorized relative testicular perfusion as right greater than left, right equal to left, or left greater than right (Appendix).

Statistical Analyses

All statistical analyses were performed by one of the authors (L.A.K.). All reported *P* values were from two-sided tests and were considered to indicate a statistically significant difference when the *P* value was less than .05.

Quantitative analysis.—Each replenishment curve plotted with data derived from ROI measurements was analyzed separately for the estimation of three measurements of perfusion: *(a)* The mean value during the first 10 seconds (M_{10}) was used to measure the average perfusion during the first 10 seconds. (The mean total imaging time differed for sequences performed with the lower- and higher-frequency transducers. Use of a common maximum time interval helped to standardize this difference.) *(b)* The mean value over the entire recorded replenishment curve (M_{max}) was used to measure the average perfusion level over the whole curve. Relative to M_{10}, the first few seconds are downweighted in M_{max} because they generally represent a smaller percentage of the entire imaging sequence duration. *(c)* The curve slope during the first 5 seconds (S_{05}) was used to measure how quickly the testis was reperfused. S_{05} was calculated by fitting a straight line through the data obtained during the first 5 seconds, with the restriction that the line start at (0,0) (Appendix).

Qualitative analysis.—Interobserver agreement about the presence of perfusion and relative perfusion was assessed with the weighted κ statistic (35). In the analysis of agreement on the presence of perfusion, the control testis and the intervention testis at the baseline occlusion level were classified as having definite perfusion 97% of the time (1251 of 1296 assessments). Therefore, most of this analysis focused on the intervention testis at mild, moderate, and severe levels of occlusion. For the purpose of analysis, relative perfusion of the right versus the left testis was restated as intervention versus control testis (viz, perfusion in the intervention testis less than that in the control testis, equal perfusion in both intervention and control testes, perfusion in the intervention testis greater than that in the control testis). The readers were blinded to which side was the intervention side (Appendix).

Comparison of Quantitative and Qualitative Analyses

For each of the calculated and qualitative methods, we tabulated the percentage of times each calculated method was correct in the identification of the lesser perfused testis by using the radiolabeled microsphere–based measurements as the reference standard (Appendix).

Figure 1

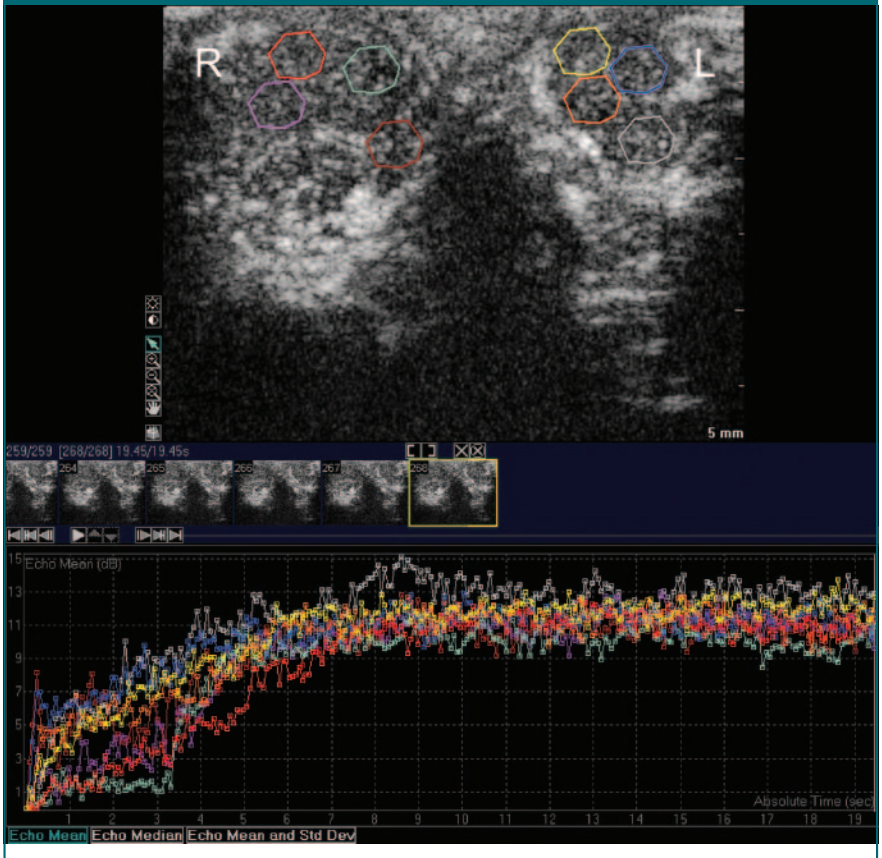

Figure 1: Baseline study. Top: Final frame from contrast-enhanced PI imaging sequence shows four ROIs over right *(R)* and left *(L)* testes. Middle: Individual image frames are shown, with final frame outlined with yellow box. Bottom: Corresponding testicular replenishment curves. The intervention-control (right-left) radiolabeled microsphere–based testicular perfusion ratio was 1.04. A spread in reperfusion values was observed not only between the right and left testes but also within each testis: Intratesticular perfusion was not uniform.

Results

Thirty-five animals were examined. Three rabbits 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 decreases in numbers of animals tested at the moderate and severe occlusion levels (Table 1).

The mean baseline perfusion value, as measured with radiolabeled microspheres, was 0.225 (mL · g^{-1})/min ± 0.084 (standard deviation) in the intervention testis and 0.284 (mL · g^{-1})/min ± 0.091 in the control testis. The mean intervention-control testicular perfusion ratio at baseline was 0.803 ± 0.205. These values were similar whether the intervention side was the right or the left. Perfusion values on the intervention side and the intervention-control testicular perfusion ratio both decreased as the degree of occlusion increased ($P < .001$ for both with repeated-measures analysis of variance), whereas perfusion values on the control side remained fairly stable ($P = .08$) (Table 2). When perfusion values on the control side were combined for all occlusion levels, the mean testicular perfusion was 0.304 (mL · g^{-1})/min ± 0.108.

Quantitative Analysis

Data for analysis were available for 248 (89%) of a possible set of 280 US studies (Table 3, Figs 1–3). The PI data provided calculated measurements of perfusion expressed as the mean intervention-control testicular perfusion ratios ± standard deviation, according to occlusion level (Table 3).

Nonparametric Spearman correlations between the calculated and the radiolabeled microsphere-based intervention-control testicular perfusion ratios were moderate and ranged from 0.49 to 0.68 and were significantly higher for the M_{10} and M_{max} measurements, compared with the S_{05} measurement ($P < .02$) (Table 4). On the right side of Table 4, analysis is restricted to the subgroup of animals that were imaged with the lower-frequency 7–4-MHz probe alone. However, the ratios did not vary significantly according to probe frequency or MI. The mean differences indicate that the calculated perfusion ratios caused overestimation of the radiolabeled microsphere–based perfusion ratios by 0.08 to 0.19. These estimates were all significantly nonzero ($P < .05$). Results obtained from a repeated-measures analysis of variance model indicated that the mean differences did not vary significantly according to calcu-

Figure 2

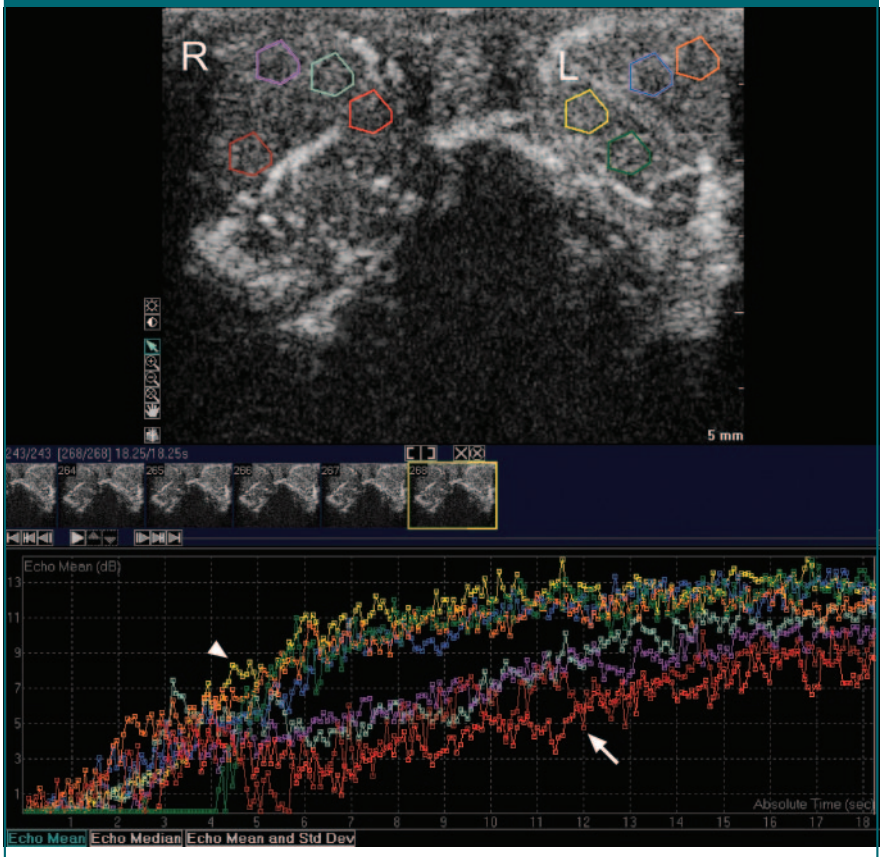

Figure 2: Moderate right spermatic cord occlusion. Top: Final frame from contrast-enhanced PI imaging sequence shows four ROIs over right *(R)* and left *(L)* testes. Middle: Individual image frames are shown, with final frame outlined with yellow box. Bottom: Corresponding testicular replenishment curves. The intervention-control (right-left) radiolabeled microsphere–based testicular perfusion ratio was 0.44. A delay in the rate of rise of the right testicular replenishment curves (arrow), compared with the left testicular curves (arrowhead), was observed; a decrease in the amplitude of the plateau levels of the right testis, compared with the left testis, was seen.

Table 4

Spearman Correlations and Mean Differences between Calculated and Radiolabeled Microsphere–based Intervention-Control Testis Perfusion Ratios

Calculated Measurement	PI with 12–5- and 7–4-MHz Transducers Combined		PI with 7–4-MHz Transducer Alone	
	MI of 0.16	MI of 0.09	MI of 0.16	MI of 0.09
	Spearman Correlations			
M_{10}	0.56	0.63	0.68	0.62
M_{max}	0.56	0.64	0.67	0.67
S_{05}	0.49	0.53	0.54	0.52
	Mean Differences*			
M_{10}	0.12 ± 0.49	0.08 ± 0.31	0.10 ± 0.39	0.11 ± 0.27
M_{max}	0.12 ± 0.48	0.10 ± 0.29	0.10 ± 0.35	0.15 ± 0.23
S_{05}	0.17 ± 0.65	0.09 ± 0.38	0.19 ± 0.63	0.09 ± 0.31

* Values for mean differences indicate calculated ratio larger than radiolabeled microsphere–based ratio.

Figure 3

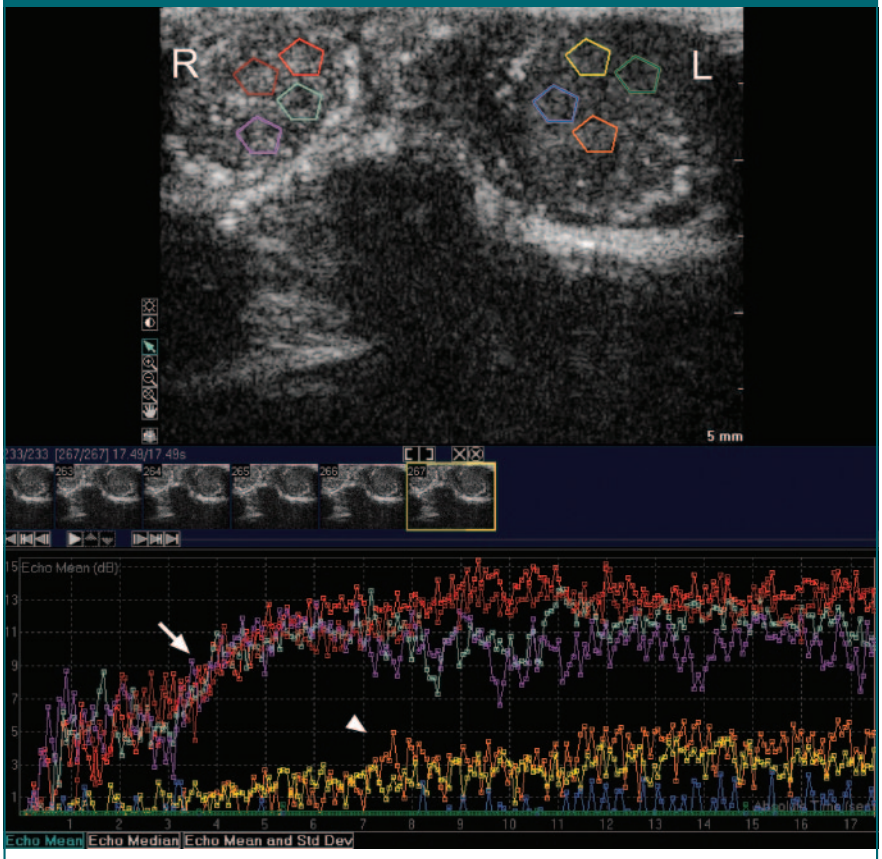

Figure 3: Severe left spermatic cord occlusion. Top: Final frame from contrast-enhanced PI imaging sequence shows four ROIs over right *(R)* and left *(L)* testes. The left testis appears swollen and hypoechoic, compared with the right testis, and is surrounded by a small hydrocele. Middle: Individual image frames are shown, with the final frame outlined with yellow box. Bottom: Corresponding testicular replenishment curves. The intervention-control (left-right) radiolabeled microsphere–based testicular perfusion ratio was 0.05. A pronounced delay in the rate of rise of the left testicular replenishment curves (arrowhead), compared with the right testicular curves (arrow), was observed; a decrease in the amplitude of the plateau levels of the left testis, compared with the right testis, was seen.

Table 5

Interobserver Agreement about Presence of Perfusion to Intervention Testis with all Imaging Methods Combined for Mild to Severe Occlusion Levels

	Reader B			
Reader A	None	Possible	Definite	All
None	45	3	0	48 (13)
Possible	14	8	3	25 (7)
Definite	2	30	269	301 (80)
All	61 (16)	41 (11)	272 (73)	374 (100)

Note.—The κ value was 0.76 (95% confidence interval: 0.70, 0.83). Data indicate number of imaging sequences. Numbers in parentheses are percentages.

lated measurement, MI, or probe frequency (Table 4).

Qualitative Analysis

Assessment of intraobserver reliability revealed agreement about the presence of perfusion (ie, none, possible, or definite) of the intervention testis in 38 (95%) of 40 cases and of the control testis in 40 (100%) of 40 cases for each reader. With respect to relative perfusion assessment (ie, perfusion of the right testis greater than that of the left, equal perfusion of both testes, perfusion of the left testis greater than that of the right), reader A agreed with himself 29 (72%) of 40 times, and reader B agreed with himself 37 (92%) of 40 times.

The readers agreed with each other about the presence of perfusion on the control side in 487 (95%) of 511 imaging sequences, with 97% of the 511 sequences classified as having definite flow. There was excellent interobserver agreement about the presence of perfusion on the intervention side at mild, moderate, and severe occlusion levels (86% agreement; $\kappa = 0.76$; 95% confidence interval: 0.70, 0.83) (Table 5). The PI method with an MI of 0.09 demonstrated the highest interobserver agreement ($\kappa = 0.86$ vs 0.73–0.74 for the other three methods), although a test of variation across the methods was not significant ($P = .39$). When κ statistics were calculated separately according to occlusion level, the highest level of agreement beyond chance occurred with the greatest occlusion level (Table 6).

PI at both MI settings more accurately helped to distinguish different degrees of testicular perfusion than color or power Doppler US (Table 7).

The two readers agreed about the degree of relative perfusion between the testes (ie, perfusion of the intervention testis less than that of the control testis, equal perfusion of both testes, or perfusion of the intervention testis greater than that of the control testis) 76% of the time ($\kappa = 0.55$; 95% confidence interval: 0.49, 0.62) (Table 8). The level of agreement was fairly uniform across the imaging methods ($\kappa = 0.50–0.59$, $P = .79$).

There were no statistically signifi-

cant differences in the results obtained with the two US probes for the qualitative portions of the study.

Comparison of Quantitative and Qualitative Analyses

As the ratio of testicular perfusion measured with radiolabeled microspheres decreased (ie, as the difference between the perfusion values of each testis increased), the percentage of correct identification of the less perfused testis increased for all methods. There was generally a large improvement from the perfusion ratio category of 0.60 or more to less than 0.80 to the perfusion ratio category of 0.40 or more to less than 0.60. Therefore, results are presented for all ratios combined and separately for ratios less than 0.60 (Table 9).

Overall, the performance of the six calculated measurements was better than that of the eight qualitative measurements (Table 9). For all perfusion ratios combined, comparison of each calculated measurement with each qualitative measurement was statistically significant ($P < .004$ for all comparisons), and this result indicated that, with the quantitative methods, identification of the side with lower perfusion was significantly more likely than it was with the readers.

When the analysis was restricted to cases with the greatest difference in perfusion (low-high perfusion ratios of <0.60), it was easier to identify the less perfused testis, which resulted in a higher percentage of correct assessments. Again, performance of the calculated measurements exceeded that of the qualitative assessments, although not all of the comparisons were significant. The performance of calculated measurements was especially promising, with an MI of 0.09. The best performance of the qualitative assessments was for color Doppler US, with rates of 74% and 84% for the two readers.

Table 6

Intervention Testis: κ Statistics for Perfusion according to Nominal and Measured Occlusion Categories

Category	No. of Imaging Sequences	κ Value
Nominal occlusion		
Baseline	137	0.25
Mild	134	0.29
Moderate	124	0.64
Severe	116	0.75
Measured perfusion ([mL · g^{-1}]/min)*		
≥0.30	59	NE†
≥0.20 to <0.30	168	0.49
≥0.10 to <0.20	195	0.53
<0.10	89	0.76

* Measured with radiolabeled microspheres.

† NE = could not be evaluated. The κ statistic could not be calculated because one reader classified all cases in one category.

Discussion

Our data demonstrated that *(a)* calculated intervention-control perfusion ratios derived from testicular replenishment curves compared favorably with ratios derived from radiolabeled microsphere–based perfusion measurements, *(b)* different levels of testicular perfusion were qualitatively distinguished better with PI imaging than with color or power Doppler US, and *(c)* identification of the less perfused testis was significantly more likely with quantitative methods than with readers when a direct comparison between quantitative and qualitative analyses was performed.

Limitations of the study included restriction of imaging to a single testicular tissue plane and inclusion of testicular parenchymal vessels of varying size within each ROI.

When testicular perfusion measurements are based on imaging limited to a single tissue plane, one presumes that these measurements are representative of the entire organ. There may, in fact, be substantial in-plane testicular flow variability. There is, therefore, a need for volumetric flow information to more accurately determine perfusion within the testis as a whole. Current scrotal imaging devices do not permit simultaneous acquisition of reperfusion data from multiple tissue planes. It is possible, however, that averaging of data obtained from two or more tissue planes during a short time 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.

Table 7

Comparison of Classifications of Testicular Perfusion according to Imaging Method and Reader

Imaging Method	Reader A	Reader B
Color Doppler	87 (78, 95)	77 (68, 85)
Power Doppler	89 (81, 96)	72 (60, 83)
PI		
MI of 0.16	98 (95, 100)	85 (72, 96)
MI of 0.09	96 (92, 99)	88 (71, 98)
All	89 (83, 93)	76 (70, 82)

Note.—Numbers are the percentages of times a testis classified as having definite perfusion had greater perfusion according to radiolabeled microsphere–based measurements than did a testis classified as having none or possible perfusion, according to imaging method. Numbers in parentheses are 95% confidence intervals.

Quantitative analysis in this study relied on a totally subjective determination of parenchymal ROIs, with the potential for erroneous inclusion of large feeding vessels and structures adjacent to the testes. Physiologic differences in flow velocity within the macro- and microcirculation, as well as size and position of the ROIs, have been shown to influence tissue replenishment curves (39–41). The exponential function $y = A[1 - e^{-\beta t}]$ has been applied to replenishment curves obtained in vivo to assess perfusion of kidney, myo-

cardium, and brain (40,42–45). As noted by Lucidarme et al (39), however, this exponential model is valid only if the concentration of microbubbles that enter the ROI immediately after the destruction pulse is constant. For this to occur, the contrast medium infusion rate must be constant and blood vessels entering the ROI must not have been previously subjected to the US beam. These authors showed that the plateau of tissue enhancement depends on the fractional blood volume in the ROI, the length of the feeding vessels subjected to the US beam before they reach this region, and the flow rate. Potdevin et al (41) demonstrated in phantom experiments that the shape of the replenishment curve is substantially affected by the range of velocities within an ROI. Testicular replenishment curves have not been previously investigated. In many cases, because the assumed parametric model was not well suited to the data, we abandoned it in favor of three estimates of perfusion detailed before, namely M_{10}, M_{max}, and S_{05}. In the future, we intend to perform studies that will incorporate replenishment data derived from multiple tissue planes and will investigate a variety of curve-fitting algorithms.

A possible limitation of the study was the use of an occlusive ischemic model as a surrogate for testicular torsion. The degree of ischemia induced by a defined degree of torsion can be variable. We therefore employed a more straightforward method of producing ischemia through direct occlusion of the spermatic cord to investigate the capability of PI imaging to depict perfusion differences between intervention and control testes.

In conclusion, quantitative and qualitative PI imaging, compared with conventional Doppler US methods, provides a superior assessment of perfusion in acute testicular ischemia. The results of our investigation hold promise in regard to the utility of PI imaging for the diagnosis of testicular ischemia in patients with acute scrotal pain and warrant further methodological investigation and refinement.

Practical application: PI imaging is of potential utility in the diagnosis of testicular ischemia in patients with acute scrotal pain, especially in children with small testes in whom conventional Doppler imaging methods provide a suboptimal assessment of both the presence of perfusion and relative perfusion to the symptomatic and contralateral testes.

Table 8

Interobserver Agreement about Relative Perfusion in Intervention and Control Testes

	Reader B			
Reader A	Intervention Less than Control	Equal in Both	Intervention Greater than Control	All
Intervention less than control	178	76	1	255 (50)
Equal in both	31	208	4	243 (48)
Intervention greater than control	0	10	3	13 (3)
All	209 (41)	294 (58)	8 (2)	511 (100)

Note.—Data are numbers of imaging sequences. Numbers in parentheses are percentages. The κ value was 0.55 (95% confidence interval: 0.49, 0.62).

Table 9

Correct Identification of Testis with Lower Perfusion Determined with Radiolabeled Microspheres

Measurement and Imaging Method	All Ratios*	Ratios <0.60†
Quantitative		
M_{10}		
PI with MI of 0.16	77	91
PI with MI of 0.09	78	98
M_{max}		
PI with MI of 0.16	77	91
PI with MI of 0.09	79	100
S_{05}		
PI with MI of 0.16	78	91
PI with MI of 0.09	75	92
Qualitative		
Reader A		
PI		
MI of 0.16	40	67
MI of 0.09	38	67
Color Doppler	60	84
Power Doppler	53	75
Reader B		
PI		
MI of 0.16	36	62
MI of 0.09	34	67
Color Doppler	48	74
Power Doppler	39	58

Note.—Numbers are percentages.

* Sample size range was 120–138.

† Sample size range was 52–57.

Appendix

Animal Preparation

The first rabbit in this series received a higher dose of intravenous ketamine (25 mg/kg) and was given intramuscular xylazine (Xylazine HCl Injection; IVS Animal Health, St Joseph, Mo)(5 mg/kg) instead

of acepromazine. All subsequent rabbits received ketamine and acepromazine because of an across-the-board change in anesthetic medication instituted by the veterinary department of our animal facility. Anesthesia was maintained with 1%–3% isoflurane (Baxter, Deerfield, Ill) mixed with oxygen and delivered intratracheally. An endotracheal tube was placed to protect the airway, and a catheter was inserted into an ear vein for venous access. A central venous catheter (Intramedic PE 90; Becton Dickinson, Sparks, Md) was placed into a jugular vein for contrast agent administration. Straight polyethylene catheters were inserted into the left ventricle (4-F; Cook, Bloomington, Ind) for radiolabeled-microsphere injection and into one femoral artery (Intramedic PE 50; Becton Dickinson) for blood pressure monitoring and for obtaining reference blood samples. Heparin flushes were administered through the venous and arterial catheters. Animal temperature was maintained with a warming blanket, and the ambient temperature was held constant. Heart rate, respiratory rate, and oxygen saturation were continuously monitored.

Surgical Procedure

The volume of saline required to produce mild to severe levels of occlusion varied from animal to animal, with a range of approximately 0.05–1.50 mL. The degree of occlusion was visually determined by the first author in every instance by monitoring flow within the ipsilateral testis with color Doppler US while increasing pressure within the cuff and comparing the resultant flow within this testis to flow within the contralateral control testis. The criteria used to categorize perfusion as mildly, moderately, or severely diminished relative to that in the control testis consisted of a qualitative judgment comparable to that which would ordinarily be employed in clinical practice.

Contrast Agent Administration

The contrast agent used in this study is a perflutren lipid–coated microsphere composed of octafluoropropane encapsulated in an outer lipid shell. The mean range in diameter of the microsphere particles was 1.1–3.3 μm. One milliliter of this contrast agent suspension contains a maximum of 1.2×10^{10} perflutren lipid microspheres. For each experimental animal, 1.3 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 jugular venous catheter by means of an infusion pump (model 55-1111; Harvard Apparatus, South Natick, Mass). Between infusions, the syringe was gently rotated and inverted to resuspend the contrast agent, which had a tendency to settle out of solution over time.

Image Acquisition

A steady infusion of the contrast agent was established, and a US probe was placed over an ROI. Delivery of a burst of high-energy US pulses into the tissues caused microbubble destruction within the imaging plane (26–28). Low-energy US scanning performed immediately after delivery of the high-energy US pulses permitted imaging of a progressive increase in tissue US signal intensity due to replenishment of the microbubbles through arterial perfusion.

We investigated the 12–5-MHz and 7–4-MHz probes since, in our practice, both are employed in the evaluation of the pediatric scrotum. We were interested in comparing the results from data acquired with these two probes because the image resolution and sensitivity to low flow provided by the higher-frequency probe are generally considered superior to those obtainable with the lower-frequency probe, whereas the resonance frequency of the microbubbles used in the contrast agent interacts better with the 7–4-MHz frequencies than with the 12–5-MHz frequencies. This observation theoretically would result in more robust PI imaging data. Imaging was performed with the US probe fixed by means of a mechanical arm over a midtransverse plane of the scrotum to include both testes within the field of view. The same machine settings were used for acquisition of all of the color and power Doppler images, as well as for all of the PI images. One or two focal zones were positioned adjacent to the mid-to-lower portions of the testes. Gray-scale and color gain were adjusted during the course of the color and power Doppler sequences for image optimization. PI imaging was performed by using a 170-dB dynamic range with persistence turned off. Gray-scale gain was adjusted prior to contrast agent administration and was not altered after it.

At baseline and at each level of spermatic cord occlusion, an intravenous infusion of the contrast agent was established, followed by intermittent PI imaging at a low MI setting 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 seconds. Continuous PI imaging at a low MI was then performed for several seconds, followed first by delivery of seven destructive image pulses into the scrotum at maximal power (0.6–0.9 MI) and then by a reversion to low-MI imaging. Images were acquired uniformly over time for an average of 14 seconds (range, 7–20 seconds) during which contrast agent replenishment to a steady-state level within the imaging plane was achieved. The sampling interval was 0.075 seconds in 90% of the imaging sequences, with a range of 0.0375–0.115 seconds. A scanning sequence performed with a low MI setting of 0.16 was immediately followed by a second scanning sequence performed with a low MI setting of 0.09.

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 Perfusion Measurements

A different radioisotope was used for each perfusion determination: cerium 141 (baseline), ruthenium 103 (mild occlusion), niobium 95 (moderate occlusion), and scandium 36 (severe occlusion) (PerkinElmer Life and Analytical Sciences, Billerica, Mass). Each of these

radioisotopes has a unique photon energy and therefore can be measured independently. For every perfusion measurement, a 0.2-mL solution containing 10 μCi (0.74 MBq) of 15-μm-diameter radiolabeled microspheres was injected into the left ventricular catheter. A reference blood sample was simultaneously removed from the femoral artery with a 5-mL syringe and withdrawal pump (model 55-1143; Harvard Apparatus) at a rate of 2.0 mL/min for 1.25 minutes. The withdrawal interval was measured with an electronic timer.

After sacrifice, each testis was used in its entirety for perfusion measurements. In the first three rabbits, the testis was divided longitudinally into two strips; in the remaining rabbits, the testis was divided 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 reference blood samples was measured in a deep-well gamma counter (Packard, Downers Grove, Ill). Regional perfusion measurements (expressed as $[mL \cdot g^{-1}]$/min of testicular tissue) for each tissue sample were determined. The measurements for the individual tissue strips were averaged to obtain a mean perfusion measurement for each testis. By averaging the perfusion measurements from four tissue samples as compared with two tissue samples, we were able to increase the stability of the data.

Image Review

Readers A and B accessed the password-protected Web site and sequentially downloaded the randomly ordered image files. Each image file could be reviewed repeatedly without penalty and was classified by means of a pop-up menu. Once an image file was electronically classified and submitted, it was no longer available for review and its classification could not be altered.

Intraobserver reliability was evaluated by having each reader assess 40 (8%) of 511 imaging sequences twice. The percentage of times each reader agreed with himself in regard to the classification of the presence of perfusion in each testis (ie, none, possible, or definite) and in regard to the classification of relative perfusion (ie, perfusion of the right testis greater than left, equal perfusion of both testes, or perfusion of the left testis greater than right) were tabulated.

Statistical Analyses

Quantitative analysis.—In the preliminary analysis, a nonlinear curve that has been used in the literature was fit to each ROI, with $y = A[1 - e^{-\beta t}]$, where β represents mean microbubble velocity, t is time in seconds, A represents fractional blood volume, and the product of β and A represents tissue perfusion (26,29,30). The assumed parametric model was not, in many cases, well suited to the data and did not permit an estimation of model parameters. This approach was not pursued further.

For each PI imaging sequence, perfusion estimates from the four left and four right testicular ROIs were averaged to produce single left and right summary statistics, and ratios of the perfusion estimate for the intervention testis to that for the control testis were calculated. Thus, the ROI data for each PI imaging sequence were reduced to intervention-control testicular perfusion ratios for M_{10}, M_{max}, and S_{05}. These calculated intervention-control perfusion ratios derived from the testicular replenishment curves were then compared with the ratios derived from the radiolabeled microsphere–based regional perfusion measurements.

The performance of the calculated measurements of relative testicular perfusion was assessed by determining *(a)* the nonparametric Spearman correlation between calculated perfusion ratios and radiolabeled microsphere–based perfusion ratios and *(b)* the mean difference between calculated perfusion ratios and radiolabeled microsphere–based perfusion ratios. Visual examination of the replenishment curves suggested that there was less noise in the data derived from the lower-frequency (7–4-MHz) US transducer. When we tested whether correlations varied significantly according to calculated measurement (M_{10}, M_{max}, S_{05}) or MI (0.16 or 0.09), we accounted for the nonindependence induced by estimating different ratios in the same animal (31). This was not necessary when we compared transducer frequencies (12–5-MHz transducer vs 7–4-MHz transducer); results of a test for independent data were sufficient (31). A repeated-measures analysis model with a robust variance estimate was used to assess whether the mean differences were significantly different from zero and whether they varied according to calculated measurement, MI, or transducer frequency (32).

Qualitative analysis.—The κ value was calculated separately for each imaging method—namely color Doppler imaging, power Doppler imaging, and PI imaging at MI levels of 0.16 and 0.09—transducer frequency, and all imaging methods combined. To investigate whether there might be a graded association with the degree of spermatic cord occlusion, κ for the intervention testis was calculated separately according to occlusion level. In addition to the baseline, mild, moderate, and severe categorizations, occlusion categories were created on the basis of radiolabeled microsphere–based perfusion measurements of 0.30 or greater, of 0.20 or greater to less than 0.30, of 0.10 or greater to less than 0.20, and less than 0.10 $(mL \cdot g^{-1})$/min to analyze the results in terms of more homogeneous perfusion categories.

Qualitative measurements of perfusion were compared with radiolabeled microsphere–based perfusion measurements by calculating the percentage of times a testis classified as having definite flow had greater flow as measured with radiolabeled microspheres than a testis rated as having none or possible flow. This is equivalent to calculating the area under a receiver operating characteristic curve for comparison of radiolabeled-microsphere perfusion measurements in testes classified as having definite perfusion with those in which perfusion was classified as possible or none (33). In contrast to the usual receiver operating characteristic curve setting, the roles of the reference standard and the diagnostic test are reversed here. Confidence intervals for

these quantities were constructed by using the bootstrap method (34).

Comparison of Quantitative and Qualitative Analyses

For quantitative assessments, if the side for which a lower calculated measurement of testicular perfusion (ie, M_{10}, M_{max}, S_{05}) was the same as the side for which the radiolabeled microsphere–based perfusion measurement was lower, then the calculated measurement was considered correct. For qualitative assessments, if a reader classified perfusion of the left testis as less than that of the right and the perfusion measurement of the left testis was lower or, similarly, if a reader classified perfusion of the right testis as less than that of the left and the perfusion measurement of the right testis was lower, then the reader was considered correct. If a reader classified perfusion as equal to both testes, the reader was considered incorrect regardless of the perfusion measurement. (In fact, for the right and left testes, radiolabeled microsphere–based perfusion measurements never were exactly the same.)

The percentage of correct assessments was determined separately for the six calculated measurements (M_{10}, M_{max}, and S_{05} applied to PI imaging with an MI of 0.16 and to PI imaging with an MI of 0.09) and for the eight qualitative assessments (the four imaging methods of color Doppler US, power Doppler US, PI imaging with an MI of 0.16, and PI imaging with an MI of 0.09, each assessed by two readers). For each combination, the results were further stratified according to the true relative perfusion measurements obtained with radiolabeled microspheres by using four categories that were based on the ratio of lower perfusion to higher perfusion measurements: perfusion ratio category of 0.80 or more to less than 1.00, perfusion ratio category of 0.60 or more to less than 0.80, perfusion ratio category of 0.40 or more to less than 0.60, and perfusion ratio category of zero or more to less than 0.40. Comparison of each calculated measurement with each qualitative measurement was performed by using the McNemar test (35). The Bonferroni-Holm method was used to adjust for multiple comparisons (36).

References

1. Williamson RC. Torsion of the testis and allied conditions. Br J Surg 1976;63:465–476.
2. Scorer CG, Farrington GH. Torsion of the testis. In: Congenital deformities of the testis and epididymis. London, England: Butterworths, 1971; 118–135.
3. Cass AS, Cass BP, Veeraraghavan K. Immediate exploration of the unilateral acute scrotum in young male subjects. J Urol 1980;124:829–832.
4. Lerner RM, Mevorach RA, Hulbert WC, Rabinowitz R. Color Doppler US in the evaluation of acute scrotal disease. Radiology 1990;176:355–358.
5. Burks DD, Markey BJ, Burkhard TK, Balsara ZN, Haluszka MM, Canning DA. Suspected testicular torsion and ischemia: evaluation with color Doppler sonography. Radiology 1990;175:815–821.
6. Dogra VS, Gottlieb RH, Oka M, Rubens DJ. Sonography of the scrotum. Radiology 2003;227:18–36.
7. Middleton WD, Siegel BA, Melson GL, Yates CK, Andriole GL. Acute scrotal disorders: prospective comparison of color Doppler US and testicular scintigraphy. Radiology 1990;177:177–181.
8. Paltiel HJ, Rupich RC, Babcock DS. Maturational changes in arterial impedance of the normal testis in boys: Doppler sonographic study. AJR Am J Roentgenol 1994;163:1189–1193.
9. Paltiel HJ, Connolly LP, Atala A, Paltiel AD, Zurakowski D, Treves ST. Acute scrotal symptoms in boys with an indeterminate clinical presentation: comparison of color Doppler sonography and scintigraphy. Radiology 1998;207:223–231.
10. Kalfa N, Veyrac C, Baud C, Couture A, Averous M, Galifer RB. Ultrasonography of the spermatic cord in children with testicular torsion: impact on the surgical strategy. J Urol 2004;172:1692–1695.
11. Hormann M, Balassy C, Philipp MO, Pumberger W. Imaging of the scrotum in children. Eur Radiol 2004;14:974–983.
12. Nussbaum Blask AR, Bulas D, Shalaby-Rana E, Rushton G, Shao C, Majd M. Color Doppler sonography and scintigraphy of the testis: a prospective, comparative analysis in children with acute scrotal pain. Pediatr Emerg Care 2002;18:67–71.
13. Grant EG. Sonographic contrast agents in vascular imaging. Semin Ultrasound CT MR 2001;22:25–41.
14. Hope Simpson D, Chin CT, Burns PN. Pulse inversion Doppler: a new method for detecting nonlinear echoes from microbubble contrast agents. IEEE Trans Ultrason Ferroelectr Freq Control 1999;46:372–382.
15. Bauer A, Hauff P, Lazenby J, et al. Wideband harmonic imaging: a novel contrast ultrasound imaging technique. Eur Radiol 1999;9(suppl 3):S364–S367.
16. Harvey CJ, Blomley MJ, Eckersley RJ, et al. Hepatic malignancies: improved detection with pulse-inversion US in late phase of enhancement with SHU 508A—early experience. Radiology 2000;216:903–908.
17. Halpern EJ, Rosenberg M, Gomella LG. Prostate cancer: contrast-enhanced US for detection. Radiology 2001;219:219–225.
18. Burns PN, Wilson SR, Hope Simpson D. Pulse inversion imaging of liver blood flow: improved method for characterizing focal masses with microbubble contrast. Invest Radiol 2000;35:58–71.
19. Postert T, Federlein J, Rose J, Przuntek H, Weber S, Buttner T. Ultrasonic assessment of physiological echo-contrast agent distribution in brain parenchyma with transient response second harmonic imaging. J Neuroimaging 2001;11:18–24.
20. Federlein J, Postert T, Meves S, Weber S, Przuntek H, Buttner T. Ultrasonic evaluation of pathological brain perfusion in acute stroke using second harmonic imaging. J Neurol Neurosurg Psychiatry 2000;69:616–622.
21. Goldberg BB, Liu JB, Forsberg F. Ultrasound contrast agents: a review. Ultrasound Med Biol 1994;20:319–333.
22. Burns PN. Harmonic imaging with ultrasound contrast agents. Clin Radiol 1996;51(suppl 1):50–55.
23. Frush DP, Babcock DS, Lewis AG, et al. Comparison of color Doppler sonography and radionuclide imaging in different degrees of torsion in rabbit testes. Acad Radiol 1995;2:945–951.
24. O'Hara SM, Frush DP, Babcock DS, et al. Doppler contrast sonography for detecting reduced perfusion in experimental ischemia of prepubertal rabbit testes. Acad Radiol 1996;3:319–324.
25. Coley BD, Frush DP, Babcock DS, et al. Acute testicular torsion: comparison of unenhanced and contrast-enhanced power Doppler US, color Doppler US, and radionuclide imaging. Radiology 1996;199:441–446.
26. Wei K, Skyba DM, Firschke C, Jayaweera

AR, Lindner JR, Kaul S. Interactions between microbubbles and ultrasound: in vitro and in vivo observations. J Am Coll Cardiol 1997;29:1081–1088.

27. Chomas JE, Dayton P, Allen J, Morgan K, Ferrara K. Mechanisms of contrast agent destruction. IEEE Trans Ultrason Ferroelectr Freq Control 2001;48:232–248.

28. Sirlin CB, Girard MS, Baker KG, Steinbach GC, Deiranieh LH, Mattrey RF. Effect of acquisition rate on liver and portal vein enhancement with microbubble contrast. Ultrasound Med Biol 1999;25:331–338.

29. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S. Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. Circulation 1998; 97:473–483.

30. Cosgrove D, Eckersley R, Blomley M, Harvey C. Quantification of blood flow. Eur Radiol 2001;11:1338–1344.

31. Kleinbaum DG, Kupper LL, Muller KE, Nizam A. Applied regression analysis and other multivariable methods. 3rd ed. Pacific Grove, Calif: Duxbury, 1998; 99–100.

32. Diggle PJ, Heagerty PJ, Liang KY, Zeger SL. Analysis of longitudinal data. 2nd ed. Oxford, England: Oxford University Press, 2002; 70–80.

33. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143: 29–36.

34. Efron B, Tibshirani RJ. An introduction to the bootstrap. London, England: Chapman & Hall, 1993.

35. Fleiss JL, Levin B, Paik MC. Statistical methods for rates and proportions. 3rd ed. New York, NY: Wiley, 2003.

36. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat 1979;6:65–70.

37. Becher H, Burns PN. Methods for quantitative analysis, chap 4. In: Handbook of contrast echocardiography: LV function and myocardial perfusion. Berlin, Germany: Springer, 2000; 153–171.

38. Heymann MA, Payne BD, Hoffman JI, Rudolph AM. Blood flow measurements with radionuclide-labeled particles. Prog Cardiovasc Dis 1977;20:55–79.

39. Lucidarme O, Franchi-Abella S, Correas JM, Bridal SL, Kurtisovski E, Berger G. Blood flow quantification with contrast-enhanced US: "entrance in the section" phenomenon—phantom and rabbit study. Radiology 2003; 228:473–479.

40. Schlosser T, Pohl C, Veltmann C, et al. Feasibility of the flash-replenishment concept in renal tissue: which parameters affect the assessment of the contrast replenishment? Ultrasound Med Biol 2001;27:937–944.

41. Potdevin TC, Fowlkes JB, Moskalik AP, Carson PL. Analysis of curve refill shape in ultrasound contrast agent studies. Med Phys 2004;31:623–632.

42. Villanueva FS, Abraham JA, Schreiner GF, et al. Myocardial contrast echocardiography can be used to assess the microvascular response to vascular endothelial growth factor-121. Circulation 2002;105:759–765.

43. Linka AZ, Sklenar J, Wei K, Jayaweera AR, Skyba DM, Kaul S. Assessment of transmural distribution of myocardial perfusion with contrast echocardiography. Circulation 1998;98:1912–1920.

44. Rim SJ, Leong-Poi H, Lindner JR, et al. Quantification of cerebral perfusion with "real-time" contrast-enhanced ultrasound. Circulation 2001;104:2582–2587.

45. Seidel G, Meyer K. Harmonic imaging: a new method for the sonographic assessment of cerebral perfusion. Eur J Ultrasound 2001; 14:103–113.