Spectral Doppler Sonography

A Noninvasive Method for Predicting Dyspermia

Joel H. Hillelsohn, MD, Kai-Wen Chuang, MD, Etai Goldenberg, MD, Bruce R. Gilbert, MD, PhD

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Objectives—Sonography is a noninvasive, office-based diagnostic tool often used for evaluation of subfertile men. Previous studies have suggested that a resistive index (RI) greater than 0.6 is associated with impaired spermatogenesis. We sought to validate this threshold in a urologic patient population presenting for infertility evaluation.

Methods—We retrospectively reviewed 99 consecutive patients seen for nonobstructive male infertility at our institution. Patient demographics, semen analysis parameters, hormone profiles, lipid profiles, and penile and scrotal sonographic measurements were recorded. The RI was calculated from measurements of the peak systolic velocity and end-diastolic velocity. Ninety-one patients fit the inclusion criteria and were subsequently divided into 2 groups based on RI: group 1 with RI values of 0.6 or less (n = 49) and group 2 with RI values greater than 0.6 (n = 42).

Results—Variables that were significantly different between the groups included age, total sperm count, percent motile sperm, total motile sperm, follicle-stimulating hormone, high-density lipoprotein, and testis volume. On the other hand, body mass index, forward progression, World Health Organization score, total testosterone, free testosterone, estradiol, total cholesterol, low-density lipoprotein, and triglycerides were not significantly different between the groups. A receiver operating characteristic curve revealed an area under the curve of 0.64 (confidence interval, 0.52–0.75; $P = .025$). At the threshold of greater than 0.6, the RI had specificity of 63.27% and a 1.56 likelihood ratio to predict total motile sperm less than 20×10^6 at spermatogenesis.

Conclusions—An intratesticular RI greater than 0.6 is associated with impaired spermatogenesis. This finding supports the use of testicular spectral Doppler sonography as a noninvasive tool for evaluation of testicular function.

Key Words—Doppler sonography; infertility; resistive index; semen analysis

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Address correspondence to Bruce R. Gilbert, MD, PhD, Arthur Smith Institute for Urology, Hofstra North Shore–LIJ School of Medicine, 450 Lakeville Rd, Suite M-41, New Hyde Park, NY 11042 USA.

E-mail: bgilbert@gmail.com

Abbreviations

BMI, body mass index; CI, confidence interval; EDV, end-diastolic velocity; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PSV, peak systolic velocity; RI, resistive index; WHO, World Health Organization

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Male factor infertility, either alone or in combination with female factors, is believed to be responsible in as many as 50% of infertile couples.[1] Sonography of the testes is an important diagnostic tool in the evaluation of male subfertility or infertility.[2–4] Recent studies with color Doppler sonography have indicated that the resistive index (RI), measured from the peak systolic and diastolic flow of an intratesticular artery, correlates with testicular function.[5–7]

There have been several studies that demonstrated the RI as a good predictor of testicular function and as a test for distinguishing different causes of dyspermia.[5,7] A higher RI and a higher peak systolic velocity (PSV) were found to be correlated with obstructive

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azoospermia and inversely correlated with the sperm production rate score.[5] Subsequent studies by Pinggera et al[6] revealed that an RI greater than 0.6 is associated with a pathologic sperm count. However, to our knowledge, this threshold has not been previously validated in a urologic population presenting for infertility evaluation. Therefore, our objective was to retrospectively and externally assess whether an RI threshold of greater than 0.6 was associated with impaired spermatogenesis parameters, such as decreased total motile sperm, which studies have shown to be associated with a decreased probability of successful fertility.[8,9] Furthermore, we investigated the lowest RI threshold associated with impaired spermatogenesis.

Materials and Methods

After obtaining Institutional Board Review approval, we performed a retrospective review of a consecutive cohort of 99 patients seen for nonobstructive male infertility between March 2010 and November 2011. Patients with 2 testes, who received testicular sonography and a semen analysis over this period, were included in the study. All patients with azoospermia had a testicular biopsy. Patients with germ cell aplasia or maturation arrest were included, and patients with normal spermatogenesis on biopsy were excluded. The biopsies were graded according to the Johnsen classification system.[10] In this system, all tubular sections in each section of the testicular biopsy are evaluated systematically, and each is given a score from 1 to 10. Complete spermatogenesis with many spermatozoa present is given a score of 10. Other exclusion criteria included an RI that was not measured and obstructive azoospermia diagnosed by testis biospy. Patient demographics, including age, body mass index (BMI), and fertility history, were recorded. In addition, semen analysis parameters, hormone profiles (total testosterone, free testosterone, estradiol, and follicle-stimulating hormone [FSH]), lipid profiles (total cholesterol, high-density lipoprotein [HDL], low-density lipoprotein [LDL], and triglycerides), and scrotal sonographic measurements (testis volume, varicocele status, PSV, end-diastolic velocity [EDV], and RI) were analyzed.

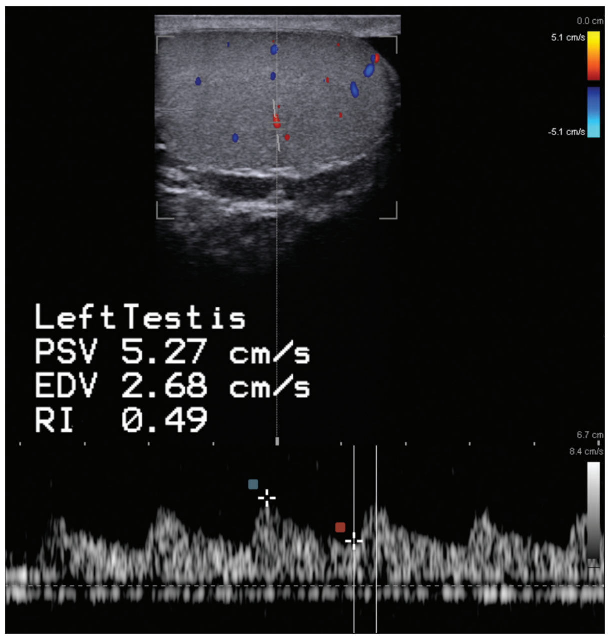
Imaging was performed with a Flex Focus ultrasound system (BK Medical, Peabody, MA) and a multifrequency (9–18 MHz) linear array transducer (8870). Grayscale imaging was performed at 18 MHz. Color and spectral Doppler imaging was performed at 7.5 MHz. The RI of an intratesticular artery from the central portion of each testicle was measured. The RI was calculated by the formula (PSV – EDV)/EDV. At least 3 consecutive waveforms were required for spectral Doppler analysis. The RI used for analysis was the average of the RI values from the right and left testes for each patient (Figures 1 and 2).

Four separate analyses were performed. First, the patients were divided into 2 groups: group 1 with RI values of 0.6 or less (n = 49) and group 2 with RI values greater than 0.6 (n = 42), and the collected parameters from each group were then compared by a standard Student *t* test. Second, the variables collected were compared to total motile sperm and to the average RI in all patients by a Pearson correlation test. Third, the data were analyzed to find the lowest RI threshold associated with impaired spermatogenesis. Last, a receiver operating characteristic curve was generated to see whether the RI could predict total motile sperm less than 20×10^6. All statistical analyses were performed with GraphPad Prism version 5.04 software for Windows (GraphPad Software, La Jolla, CA).

Results

Of the 99 patients reviewed for this study, 8 were excluded: 6 due to azoospermia with normal spermatogenesis found on biopsy, 1 due to congenital absence of the vas deferens bilaterally, and 1 due to a large hydrocele. Three patients had azoospermia but had biopsy-proven atrophy with Johnsen scores less than 2. The demographics and comparison of groups 1 and 2 are summarized in Table 1. The

Figure 1. Testicular spectral Doppler sonogram illustrating an RI less than 0.6.



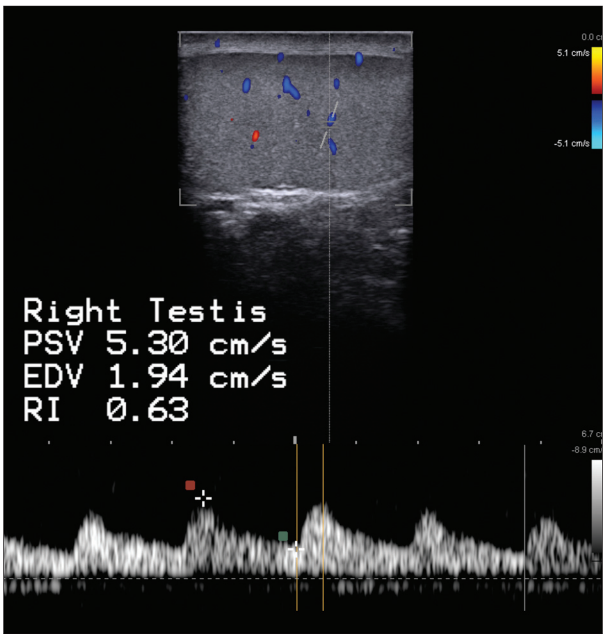
groups differed significantly in age, with the patients with RI values greater than 0.6 being older (37 versus 33 years; $P = .03$). Regarding sonographic parameters, the group with RI values greater than 0.6 had significantly smaller testes bilaterally (right, $P = .04$; left, $P = .01$). The average varicocele size was not significantly different between the groups. The average RI ± SD was 0.52 ± 0.06 in the group with RI values of 0.6 or less, and it was 0.68 ± 0.07 in the group with RI values greater than 0.6.

The total testosterone, free testosterone, and estradiol levels did not significantly differ between the groups. We also did not find a significant difference in total cholesterol, triglyceride, and LDL levels. However, the group with RI values greater than 0.6 had significantly higher FSH levels ($P = .03$) and higher HDL levels ($P = .02$).

For semen analysis parameters, the volume of sperm, forward progression, and World Health Organization (WHO) normal morphology percentage did not significantly differ between the groups. On the other hand, the total sperm count ($P = .02$), percentage of motile sperm ($P = .01$), and total motile sperm ($P < .01$) were significantly lower in the group with RI values greater than 0.6 compared to the group with RI values of 0.6 or less.

A Pearson correlation test was performed between the variables collected and total motile sperm (Table 2). Variables found to be positively associated with total motile sperm included right testis volume ($r = 0.44$; $P < .01$) and left testis volume ($r = 0.36$; $P < .01$). Variables found to be inversely correlated with total motile sperm included FSH ($r = –0.31$; $P < .01$) and average RI ($r = –0.24$; $P = .02$). A correlation test was also performed between the average RI and the variables collected (Table 3). The average RI was found to positively correlate with age ($r = 0.27$; $P = .01$), free testosterone ($r = 0.3$; $P = .03$), and HDL ($r = 0.37$; $P < .01$). The average RI was found to significantly inversely correlate with left testis volume ($r = –0.23$; $P = .03$), percentage of motile sperm ($r = –0.3$; $P < .01$), and total motile sperm ($r = –0.24$; $P = .02$). Results of the correlation test comparing the RI to the other variables were not statistically significant.

Figure 2. Testicular spectral Doppler sonogram illustrating an RI greater than 0.6.



An RI of 0.56 or greater was determined to be the lowest RI associated with dyspermia. The patients were divided into group 1 with RI values less than 0.56 (n = 39) and group 2 with RI values of 0.56 or greater (n = 52). Age, BMI, testes volume, left varicocele size, total and free testosterone, estradiol, total cholesterol, LDL, triglycerides, semen volume, sperm count, forward progression, and WHO score were not significantly different between the groups. The percentage of motile sperm ($P = .01$), total

Table 1. Comparison of Groups With RI Values of 0.6 or Less and Greater Than 0.6

Variable	RI ≤0.6	RI >0.6	*P*
Patients, n	49	42	
Demographics			
Age, y	33 ± 7.5	37 ± 8.7	.03
BMI, kg/m^2	28 ± 5.4	28 ± 3.9	.98
Sonography			
Right testis volume, mL	12.7 ± 3.4	11.1 ± 3.8	.04
Left testis volume, mL	12 ± 2.8	10.2 ± 3.6	.01
Right varicocele, mm	1.9 ± 2.4	2.8 ± 2.4	.09
Left varicocele, mm	2.9 ± 2.8	3.9 ± 2.5	.10
RI	0.52 ± 0.06	0.68 ± 0.07	<.01
Blood			
Total testosterone, ng/dL	359 ± 133	377 ± 171	.59
Free testosterone, ng/dL	12 ± 4.4	14 ± 17.5	.48
Cholesterol, mg/dL	188 ± 36	188 ± 33	.96
HDL, mg/dL	46 ± 9	53 ± 16.8	.02
LDL, mg/dL	115 ± 32	109 ± 28	.41
Triglycerides, mg/dL	147 ± 105	128 ± 99	.4
Estradiol, pg/mL	24 ± 8	25 ± 15.6	.73
FSH, IU/L	4.7 ± 4	7.5 ± 6.9	.03
Semen			
Volume, mL	2.9 ± 1.8	2.7 ± 1.7	.7
Total sperm count, × 10^6	149 ± 192	72 ± 100.4	.02
Motility, %	53 ± 20	39 ± 25	.01
Forward progression	1.7 ± 0.53	1.5 ± 0.7	.2
WHO normal morphology, %	12 ± 14	8 ± 8	.1
Total motile sperm, × 10^6	83 ± 98	37 ± 48	<.01

Data are presented as average ± SD.

Table 2. Correlations Between Total Motile Sperm and the Variables Collected

Variable	*r*	CI	r^2	*P*
Right testis volume	0.44	0.25–0.59	0.19	<.01
Left testis volume	0.36	0.17–0.53	0.13	<.01
Average RI	–0.24	–(0.43–0.04)	0.06	.02
FSH	–0.31	–(0.48–0.10)	0.09	<.01

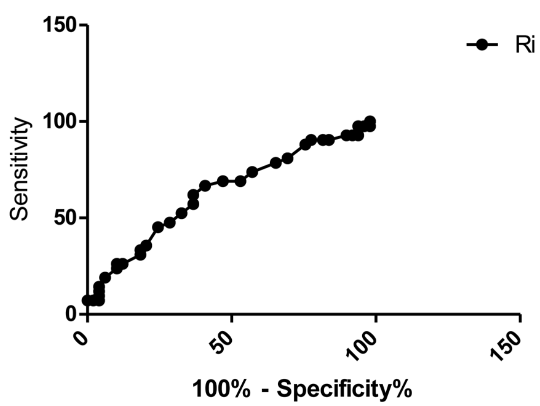
Table 3. Correlations Between Average RI and the Variables Collected

Variable	*r*	CI	r^2	*P*
Age	0.27	0.06–0.45	0.07	.01
Left testis volume	–0.23	–(0.42–0.03)	0.05	.03
Free testosterone	0.30	0.03–0.53	0.09	.03
HDL	0.37	0.17–0.54	0.14	<.01
Motility	–0.3	–(0.47–0.09)	0.09	<.01
Total motile sperm	–0.24	–(0.43–0.04)	0.06	.02

motile sperm (P = .04), and FSH (P = .03) were statistically significantly different between the groups. Right varicocele size (P = .04) and HDL (P = .02) also reached statistical significance.

A receiver operating characteristic curve was generated to see whether the RI could predict total motile sperm less than 20×10^6 (Figure 3). The area under the curve was 0.64 (confidence interval [CI], 0.52–0.75; P = .025). At an RI threshold of greater than 0.6, the sensitivity of the test was 57.14% (CI, 40.96%–72.28%), specificity was 63.27% (CI, 48.29%–76.58%), and the likelihood ratio was 1.56. At a threshold of 0.56, the sensitivity was 69.05% (CI, 52.9%–82.38%), specificity was 46.94% (CI, 32.53%–61.73%), and the likelihood ratio was 1.3.

Figure 3. Receiver operating characteristic curve for the average RI as a predictor of total motile sperm less than 20×10^6.



Discussion

The RI is calculated from measurements of the PSV and EDV. However, the PSV and EDV are dependent on the angle of incidence given by the Doppler formula. The Doppler angle is the angle of incidence between the ultrasound beam and the estimated flow direction. Doppler sonography accurately measures velocity (speed and direction of the movement) only at Doppler angles of 0° and 180°. Angles greater than 60° produce too large of an error in velocity and should not be used. Therefore, variation in the angle of incidence substantially influences the PSV and EDV. The RI, in contrast, is an angle- and operator-independent ratio, making it a reliable indicator. We used the average RI from both testes as an indicator, assuming that both testes contribute equally to semen parameters in spermatogenesis.

The standard RI of the testes was categorized in several animal studies. Carrillo et al[11] examined 5 dogs over a 6-month period to determine that PSV, EDV, and RI measurements remained stable. Tarhan et al[12] showed that the RI did not change in the contralateral artery after unilateral testicular torsion in a study of 24 canines, suggesting that unilateral testicular torsion does not alter contralateral testicular blood flow. Pozor and McDonnell[13] evaluated 52 stallions to determine reference values for the PSV, EDV, and RI in nonpathogenic testes and found that obtaining the RI was feasible and that RI measurements for left and right testes were similar.

The testicular arterial RI has also has been studied for its predictive values in testicular disease. Jee et al[14] studied the RI in scrotal inflammatory disease. They found that the RI could provide a diagnostic criterion for scrotal inflammatory disease if the values for the intratesticular and epididymal arteries were less than 0.5 and less than 0.7, respectively. Lefort et al[15] studied the RI in 5 patients with testicular infarction caused by epididymo-orchitis. They found that an elevated RI can be suggestive of ischemia.

Further studies have examined the role of the RI in testicular microcirculation. Unsal et al[16] examined RI values of 49 healthy patients. Fifteen were classified by sonography as having left-sided varicoceles and were compared to the other group of 34. The RI values were found to be significantly higher in the varicocele group compared to the control group (0.68 versus 0.64, respectively; $P < .05$).

The potential alteration of the RI in dyspermia has been investigated in 2 studies.[5,6] Biagiotti et al[5] assessed whether sonographic values such as the PSV, EDV, and RI may be useful in distinguishing the various causes of dyspermia compared to FSH and testicular volume.

They recruited 161 patients: 9 with obstructive azoospermia, 20 with nonobstructive azoospermia, 17 with oligoasthenospermia, 38 with undetermined oligoasthenospermia, 19 with male accessory gland inflammation, 11 with clinical varicoceles, 32 with normal sperm analysis results plus recent paternity, and 15 with normal sperm analysis results plus recent paternity and varicoceles. They found that only the RI and PSV were correlated with the sperm production rate score, whereas FSH, testicular volume, and the EDV were not. In our study, we found that FSH does correlate with total motile sperm, whereas the PSV does not. The PSV is limited by its angle-dependent characteristic. Follicle-stimulating hormone has been shown to correlate with sperm production in previous studies.[17,18]

Pinggera et al[6] also examined whether the RI can be used to predict dyspermia. They recruited 160 patients and divided them into 2 groups of 80. One group had mild oligoasthenozoospermia on semen analysis, whereas the control group had normal semen analysis results as well as paternity within 14 months of recruitment. The control group had a mean RI of 0.54 ± 0.05, whereas the cohort had a mean RI of 0.68 ± 0.06. The upper RI limit for a patient with normal semen analysis results was 0.6. They concluded that an RI greater than 0.6 may be indicative of a pathologic sperm count in urologic patients. Therefore, the objective in our study was to validate whether this conclusion was true in a urologic population presenting for a subfertility or infertility workup. We also sought to determine whether a lower RI threshold can be associated with impaired spermatogenesis.

When comparing the groups with an RI greater than 0.6 and an RI of 0.6 or less, we found a significant difference in total motile sperm ($P < .01$). This finding confirms the hypothesis by Pinggera et al[6] that an RI greater than 0.6 is associated with dyspermia. Furthermore, we found that at an RI greater than 0.6, the sensitivity for total motile sperm less than 20×10^6 was 57.14%, and specificity was 63.27%, with a 1.56 likelihood ratio. An RI of 0.56 or greater was also significantly associated with lower total motile sperm ($P = .04$). At that level, the sensitivity for total motile sperm less than 20×10^6 was 69.05%, and specificity was 46.94%, with a likelihood ratio of 1.3.

Patients with obstructive azoospermia were excluded from the study, since we do not know the effect of tubal obstruction on the RI, and we wanted to examine the relationship between the RI and semen analysis. However, it was noted that in patients with nonobstructive azoospermia, the average Johnsen score was 2.5, and the average RI was 0.63. This RI was higher than the RI recorded for the excluded patients from our study who had obstructive azoospermia. Their collective average RI was 0.5, and their average Johnsen score was 10. Additional study is needed to determine whether this observation of a higher RI in nonobstructive azoospermia is valid, whether the RI varies in testes with nonobstructive azoospermia, and whether the RI can be used to identify localized pockets of spermatogenesis.

The explanation behind the association of testicular blood flow and spermatogenesis has yet to be fully elucidated. Testicular arteries are targets for androgens,[19] and a study by Jezek et al[20] showed that the testicular blood vessels in hyalinized human testes had an enlarged endothelial layer. More research is needed to clarify whether the impaired testicular microcirculation as reflected by an elevated RI is secondary to systemically impaired vascular functioning or a consequence of decreased testicular function.

A correlation of the RI with testicular biopsy is needed to state that an altered RI identifies spermatogenic dysfunction. Nonetheless, spectral Doppler analysis of the subfertile man has several present and potential clinical applications. Presently, available studies suggest that the RI should be used together with semen analysis and hormonal studies as part of the clinical evaluation of the subfertile man. It is a direct method of evaluating intratesticular blood flow and, as suggested by this and prior articles, yields reproducible data. Spectral Doppler sonography is a noninvasive technique that adds unique information about the intratesticular vasculature that can guide the physician in counseling the subfertile couple. Future studies will define the association of spectral Doppler findings and spermatogenesis as well as determine the spectral Doppler changes occurring with medical and/or surgical therapy and, by extension, spermatogenesis.

In conclusion, an intratesticular RI greater than 0.6 is associated with decreased total motile sperm, decreased testicular size, and increased FSH, supporting its use as an independent indicator of testicular function. Although further correlation with testis biopsy is needed, our data support the use of testicular sonography, and in particular spectral Doppler imaging, as a noninvasive tool for evaluation of testicular function in the subfertile man.

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