A new power Doppler ultrasound guiding technique for improved testicular sperm extraction

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Objective: To develop a noninvasive procedure that employs image processing of power Doppler ultrasound (PDUS) images of several orthogonal cross-sections of the testis to construct a three-dimensional (3D) mapping of preferential testicular regions in which spermatozoa are most likely to be found in nonobstructive azoospermic testes.

Design: Clinical study.

Setting: Ultrasound and andrology units in a large university-affiliated municipal hospital.

Patient(s): Twenty-four nonobstructive azoospermic men.

Intervention(s): Before testicular sperm extraction was performed, PDUS images were acquired at seven cross-sections to reconstruct a 3D testicular vascularity index (TVI) matrix for spatial mapping of testicular regions in which spermatozoa are most likely to be found. The predictions based on TVI values of 107 regions were compared with the biopsy results.

Main Outcome Measure(s): Prediction of presence or absence of spermatozoa by TVI values.

Result(s): The prediction rate of the TVI matrix for the presence or absence of spermatozoa was 74.8%. The positive predicted value was 72%, negative predicted value was 75.6%, and specificity was 89.8%, but sensitivity was 47.3%.

Conclusion(s): Our technique may obviate the need for arbitrary multiple biopsies that inflict some degree of damage upon testicular tissue and may increase the success rate of identifying viable spermatozoa in testicular tissue. (Fertil Steril 2004;81:430–4. ©2004 by American Society for Reproductive Medicine.)
Multiple biopsies would need to be performed to increase the success rate per patient to 62% (2). It has been suggested that foci of spermatogenesis in nonobstructive azoospermic men may be found in regions with improved blood perfusion. Accordingly, blood vessels were sought and identified at various planes of the testis in azoospermic testes by using power Doppler ultrasound (PDUS) techniques (3). A fine needle aspiration was then performed in those planes with well-defined testicular vascularization, and spermatogenetic cells, including mature spermatozoa, were found in 75% of the trials. The drawbacks to this procedure, however, are that it is done visually, based on information from a single scanned plane, and that the information derived from other scanned planes cannot be exploited to contribute to pinpointing the best site of spermatogenesis in the testis.

In this study, we used a new noninvasive screening procedure that analyzes the testicular vascularity index (TVI) obtained via image processing of PDUS images of the testis at several orthogonal cross-sections to provide a three-dimensional (3D) mapping of the testicular regions at which spermatozoa residuals are most likely to be found in nonobstructive azoospermic testes (11).

**MATERIALS AND METHODS**

This noninvasive procedure for predicting sites with spermatogenesis foci or spermatozoa residuals in nonobstructive azoospermic testes is described in detail elsewhere (11). Its essential components are described below. The procedure was approved by the ethical committee of the Tel Aviv Sourasky Medical Center.

**Ultrasound Imaging of the Testis**

Within 1 month before the biopsy procedure, PDUS images of azoospermic testes were acquired with an advanced ultrasound system (Sonoline Elegra, 7.5-MHz transducer, Siemens Medical Systems Inc., Issaquah, WA) while the subject was lying in a supine position. The head and tail of the epididymis were identified, and the testis ellipsoid axes were aligned to coincide with the body orthogonal axes. The PDUS images acquired from each testis were one frontal, three sagittal, and three transverse cross-sections. All the cross-sections were scanned at the highest frequency and maximal gain that provided the best images of blood flow (Fig. 1A). The images were recorded on a Super Video Home System (VHS) videotape and later digitized into 24-bit RGB files on a personal computer equipped with a video board (DC Miro 30+, Pinnacle Systems, Braunschweig, Germany) for data processing.

**Detection of Blood Vessels**

The PDUS images were processed by means of a computerized search of the relative intensity of the red and yellow colors in each pixel to identify the ones that represent blood vessels in each of the testicular cross-sections generated by the PDUS image (Fig. 1). The threshold values of

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**FIGURE 1**

(A) Power Doppler ultrasound image of an azoospermic patient and (B) the processed image.

**FIGURE 2**

(A) Schematic reconstruction of the testis from seven cross-sections of PDUS images (3 transverse, 3 sagittal, and 1 frontal scanned planes). (B) Thirty-two elements are formed. A and B denote three- and five-face elements, respectively.
each image were then calculated, and those found to represent blood vessels were colored in a bright red color.

**Testicular Vascularity Index**

The seven planes of the PDUS images taken from each testis divided the testis into 32 volume elements (Fig. 2A). Each volume element has three to five orthogonal sides, where each side contains information about the blood vessels from each of the PDUS images (Fig. 2B). The TVI for each element is defined as the sum of the relative area of blood vessels on the orthogonal sides of the volume element. The value TVI of the element at the \(i,j,k\) position is calculated by counting the red pixels on each of its \(N\) orthogonal surfaces as follows:

\[
TVI_{i,j,k} = \frac{1}{N} \sum_{m=1}^{N} \left( \frac{S_{i,j,k}^m}{A_{i,j,k}^m} \right)
\]

where \(N\) is the number of the orthogonal sides, \(S_{i,j,k}^m\) is the number of red pixels found on the surface \(m\) of the element, and \(A_{i,j,k}^m\) is its corresponding area (in pixels). Accordingly, the TVIs for all the volume elements of the testis compose a 3D matrix whose components correspond to the volumetric elements of the testes that are generated by the PDUS images. The value of each TVI\(_{i,j,k}\) ranges from 0 (no blood vessels on the surfaces) to 1 (all the surfaces are part of the blood vessel). Because some of the blood vessels are not perpendicular to the planes of the PDUS images, an oval projection is generated on these surfaces, thereby yielding a larger TVI\(_{i,j,k}\).

**Testicular Biopsy**

All TESE procedures were performed on men with non-obstructive azoospermia who were lying in a supine position and under conditions of general anesthesia. Tissue biopsies were taken from three different locations along the midline of the testis, that is, in the center and near both poles. The presence of spermatozoa was then examined in each biopsy, using mincing techniques and searching under an inverted microscope. It should be noted that the biopsies were not directed by any information regarding the PDUS images or the TVI analysis.

**Analysis of TVI as a Predictor for the Presence of Spermatozoa**

The TVI matrix exhibits the number and the size of blood vessels that may exist in each testicular volume element. Because the biopsies are performed at three different locations along the testis midline (center and two poles), we focused the prediction analysis on the TVIs from the surrounding elements in the frontal layers below and above the site of the biopsy. Accordingly, we defined a region of testicular elements by the 16 volume elements that surround the biopsy, 8 elements in each frontal plane (Fig. 3). The analysis of the 16 TVIs of a region should characterize whether or not spermatozoa were present. A region was considered “positive” when the TVI\(_{i,j,2}\)−TVI\(_{i,j,1}\) was greater than 0 in at least five of eight pairs of elements, and “negative” when the TVI\(_{i,j,2}\)−TVI\(_{i,j,1}\) was smaller than 0 in at least five of eight pairs of elements. When four pairs yielded TVI\(_{i,j,2}\)−TVI\(_{i,j,1}\)>0 and four pairs yielded TVI\(_{i,j,2}\)−TVI\(_{i,j,1}\)<0, the region could not be characterized.

**RESULTS**

Forty-three testes were screened from 24 azoospermic men. One hundred eighteen biopsies were performed; thus, there were fewer than three biopsies in some testes. The TVI matrix was calculated for each testis from the seven PDUS images, which produced 32 volume elements (each about 0.45 cm\(^3\)) of the testis. One hundred twenty-nine regions were calculated, but only 118 biopsied regions were considered because the other 11 regions could not be characterized and were excluded from analysis. The results are summarized in Table 1. Thirty-five (32.7%) regions of a total of 107 were positive, and 72 (67.3%) were negative. The TVI values ranged from 0 to 0.2. We divided the regions into two groups: group A included the positive regions, in which more than 14 elements were greater than 0.001 (high values), and group B included the negative regions and the positive regions with at least two elements smaller than 0.001 (low values). Group A included 25 regions within which spermatozoa were found in 18 (72%) and were missing in 7 (28%). Group B included 82 regions within which spermatozoa were absent in 62 (75.6%) and were present in 20 (24.4%).

**DISCUSSION**

The locations for TESE biopsies are currently chosen arbitrarily and, as a consequence, 50% of these biopsies yield negative results (2). To prevent redundant biopsies, we have developed a noninvasive PDUS-based technique for predicting intratesticular locations of spermatozoa residuals in azoospermic testes. Most of the testicular regions in the azoospermic men were found to be negative: spermatozoa were absent in 75% of the group B regions. Thus, a negative or low-positive region indicates that spermatozoa will not be found. Spermatozoa were found in the majority (72%) of the group A regions. Therefore, high-positive regions indicate
that spermatozoa are present in them. Although the sensitivity of our technique is fairly low (47.3%), the specificity of 89.8% is high, suggesting that our technique may better predict the absence of spermatozoa than their presence.

Foresta et al. (3) also used imaging methodology to predict the presence of viable spermatozoa in testes, but their technique was limited to planar visualization, whereas ours is based on the analysis of testicular volume elements. The approach of examining small testicular volumes is a logical one because spermatogenesis may still survive because of perfusion by blood vessels in the surrounding tissue. Thus, considering the vessels’ contribution to the viability of spermatozoa from all directions would be more valid than such consideration from one plane alone. Foresta et al. (3) reported a 75% success rate in finding spermatozoa per patient with a well-defined testicular vascularization. This implies that spermatozoa are present in 75% of this group and that the problem is a matter of finding them. Hauser et al. (2) reported that spermatozoa were found in 62.1% of the patients, and our results indicated that spermatozoa were present (at least in one of the subject’s biopsies) in 14 patients (58.3%). The real value of our technique, however, is in its ability to correctly predict whether spermatozoa are present or absent in one biopsy. The detection rate was 74.8%.

The current investigation was carried out in two alternative conditions. High-positive regions were proposed as being indicators for the presence of spermatozoa, whereas negative and low-positive regions would indicate the unlikelihood that any sperm would be found. Positive regions were considered to exist when the TVI values in the anterior layer were lower than those in the posterior layer. In azoospermic men, the latter implied that the region was well perfused by the capillary tree within the lobules. When the TVI values were very low, that is, smaller than 0.001, the region was not normally perfused.

The TVI technique has several limitations. The TESE biopsies were performed 5 mm beneath the skin along the midline of the testis’ length, where the size of a biopsy is about 0.125 cm³. The result of a biopsy is correlated with a region surrounding the biopsy site, that is, 2.5–3 cm. If the number of volume elements generated from the PDUS images is increased, the volume of a region will be reduced, and this may refine the biopsy site to a more precise location. The number of the acquired PDUS planes limits the size of the TVI matrix. The TVI values are entirely dependent upon the blood vessel projection on the PDUS images. Thus, the same TVI could be obtained for two different vessels that had the same projection on the scanned planes. Blood vessels that are not detected by at least one of the PDUS images cannot contribute to the TVI values. The TVI value may increase either when the blood vessel does not vertically intersect the PDUS scanned plane, so that its cross-section appears larger than it actually is, or when the PDUS system might overwrite color onto small blood vessels. This shortcoming may be negligible, however, because the prediction of spermatogenesis depends on the relations between the anterior and posterior TVI elements, and the same effects may be introduced in all scanned images.

**FIGURE 3**

Division of regions of a reconstructed testis. Each region contains 16 elements: 8 elements each in the anterior and posterior layers. Shaded area, the region surrounding biopsy A. Hashed area, the region surrounding biopsy B. Dotted area, the region surrounding biopsy C. $i$, $j$, and $k$ = the axes perpendicular to the transverse, sagittal, and frontal cross-sections.

In conclusion, a new method for testicular screening based on the TVI matrix calculated from PDUS images appears to be a highly promising noninvasive guiding tool for testicular biopsies. By identifying testicular locations that are likely not to contain viable spermatozoa in the testes of nonobstructive azoospermic men, the number of biopsies for TESE procedures may be reduced, the outcome improved, and the potential for testicular damage reduced.

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References