Quantitative Analysis of Tumor Vascularity in Benign and Malignant Solid Thyroid Nodules

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Objective. The purpose of our study was to analyze the accuracy of quantitative analysis of tumor vascularity on power Doppler sonograms in differentiating malignant and benign solid thyroid nodules using tumor histologic evaluation as the reference standard. Methods. Eighty-six solid thyroid tumors (46 malignant and 40 benign) in 56 consecutive patients (mean age ± SD, 53.1 ± 11.6 years; 12 male and 44 female) referred for the surgical treatment were included in our study. Visual and qualitative analysis of patterns of nodule vascularity was performed for all tumors. Quantification of the power Doppler sonograms was performed with normalized and weighted vascular indices (VIs). The accuracy of sonographic criteria for thyroid cancer was evaluated with univariate analysis. Results. Among benign thyroid tumors, there was a statistically significant increase in the levels of intranodular vascularity with an increase in tumor size (P < .001). In all tumors, increased intranodular vascularity showed 65.2% sensitivity, 52.5% specificity, and 58.9% overall accuracy in differentiation between benign and malignant thyroid lesions. In tumors smaller than 2 cm, it had 65.5% sensitivity, 85.7% specificity, and 72.1% overall accuracy. Quantitative analysis of tumor vascularity significantly outperformed visual analysis of power Doppler patterns (P < .05). Among thyroid lesions with diameters of less than 2 cm, a normalized VI of greater than 0.14 had 72.4% sensitivity, 100% specificity, and 86.2% overall accuracy. A weighted VI of greater than 0.24 showed compatible results, with 69.0% sensitivity, 100% specificity, and 84.5% overall accuracy. Conclusions. Our study indicates that in small thyroid nodules, quantitative analysis of tumor vascularity has benefits over visual inspection and can be useful in differentiation between benign and malignant thyroid tumors. Key words: power Doppler sonography; thyroid tumors; vascular index.
The use of power Doppler sonography for thyroid cancer diagnosis was widely studied in the past. Power Doppler scans performed with high-frequency transducers allowed identification of low-velocity blood flow in superficial tissues and were shown to be useful in the differential diagnosis of thyroid tumors. Color and power Doppler sonography has been evaluated as a diagnostic tool for predicting thyroid cancer, with the hypothesis that flow that is predominantly at the periphery of a nodule is suggestive of a benign nodule, whereas flow predominantly in the central portion of the nodule is suggestive of malignancy. The results of these studies have been mixed, with some reporting that Doppler sonography was helpful and others reporting that Doppler sonography did not improve diagnostic accuracy. Many experts agree that color Doppler sonography alone cannot be used to diagnose or exclude malignancy with a high degree of confidence; rather, the color Doppler sonographic finding of predominantly internal or central blood flow appears to increase the chance that a nodule is malignant.

This controversy may be explained by the fact that virtually all studies on power Doppler sonography used visual analysis of tumor vascularity, and the diagnosis of thyroid malignancy was based on the presence or absence of increased intranodular vascularization. It is well known that this analysis has a great potential for intraobserver and interobserver variability. Therefore, more accurate, quantitative criteria of tumor vascularity may be useful to improve the diagnostic value of power Doppler sonography in thyroid cancer diagnosis. The purpose of our study was to analyze the accuracy of quantitative analysis of tumor vascularity on power Doppler sonograms in differentiating malignant and benign solid thyroid nodules using tumor histologic evaluation as the reference standard.

Materials and Methods

Patients
The study protocol was approved by the Institutional Review Board. Before enrollment, each patient gave written informed consent. The inclusion criteria were the presence of solid thyroid nodules with the preoperative suspicion of thyroid cancer based on the clinical, imaging, and cytologic findings. Patients who refused to give informed consent or who did not receive surgical treatment were excluded from the study. A total of 86 solid thyroid tumors in 56 consecutive patients were included in our study. All patients underwent surgery, and the final diagnosis was based on histopathologic evaluation of the resected specimens. The mean age of examined patients ± SD was 53.1 ± 11.6 years (range, 25–74 years), and there were 12 male and 44 female patients. All patients had normal serum levels of thyroid and thyroid-stimulating hormones.

Sonographic Examination
Power Doppler sonography was performed for all patients by the same investigator (A.L.) using a Sonoline Elegra scanner (Siemens Medical Solutions, Issaquah, WA) with a 7.5L40 linear array transducer (B-mode frequency range, 5–9 MHz; Doppler frequency range, 5–7 MHz). Settings of the ultrasound scanner remained the same throughout the study. All power Doppler images were acquired with the same standard power Doppler color map, where minimal tissue perfusion was coded as a dark red signal; moderate perfusion was coded as a red-to-orange signal; and markedly increased tissue perfusion was mapped as an orange-to-yellow signal.

During sonographic examinations in the power Doppler mode, the type and intensity of nodular blood flow were evaluated for all lesions. Doppler amplification was controlled so that normal thyroid tissue did not display any random color noise. For each thyroid nodule, 4 or 5 power Doppler images were acquired in transverse and longitudinal planes. Of them, one 2-dimensional frame representing the slice of the tumor with the most prominent vascularization was chosen for future quantitative analysis by consensus of radiologists performing sonographic image analysis (A.L. and T.H.). The intensity of perinodular or intranodular blood flow was assessed subjectively by comparing it with surrounding thyroid tissue. In total, 3 types of nodule vascularization were identified: type 1, absence of flow signals; type 2, increased perinodular vascularization; and type 3, increased perinodular and intranodular vascularization.
Image Processing
Sonograms were digitally stored in the scanner’s memory and exported to an external computer for future analysis. Quantitative analysis of thyroid nodule vascularity was performed independently by 2 observers blinded to the patient’s final diagnosis and clinical or fine-needle aspiration findings. Interobserver variability was assessed by the calculation of the difference in the values of normalized and weighted vascular indices (VIs) between observers. The images used for this investigation were B-mode sonograms with an overlaid color flow image based on power Doppler signal processing (Figure 1). To maintain a consistent analysis among subjects, all Doppler measurements were constrained to the tumor region of interest (ROI). A graphical user interface was designed with commercially available software (MATLAB version 7.0; The MathWorks Inc, Natick, MA), which allowed for manual segmentation of the tumor region and subsequent quantification of the Doppler signal within the ROI. The additional time required for quantitative image processing was no more than 2 to 3 minutes.

Each image was processed to calculate 2 parameters: the ratio of flow area to total tumor area, defined as the normalized VI, and the strength of flow in the tumor, designated the weighted VI. Characterization of the ROI with these parameters provides an understanding of the extent of vascularity with the former and the magnitude of flow with the latter.

Figure 1. Quantitative analysis of tumor vascularity. A, Transverse B-mode sonogram from a 48-year-old female patient shows papillary thyroid carcinoma with prominent perinodular and intranodular vascularization. The ROI for image processing inside the tumor is outlined. Perinodular vascularity was excluded from the ROI. B, The normalized VI represents the ratio of color-coded pixels to the area of the tumor. C, The weighted VI represents the ratio of weighted color-coded pixels to the area of the tumor. Values of normalized and weighted VI in this patient were 0.62 and 1.27, respectively.
The color flow signal consisted of 3 channels of color information, corresponding to red, blue, and green intensities at each pixel location. Within the designated tumor boundary, the number of pixels containing color flow data was summed and divided by the total number of pixels within the ROI to determine the normalized VI. As such, the normalized VI represents a ratio of the area of flow within the tumor to the total tumor area.

The weighted VI was used to extend this characterization by incorporating the strength of the flow at each pixel. To quantify the signal strength, the intensity of the color flow signal was calculated by taking the magnitude of the vector defined by the 3 color channels. This calculation was only performed on pixels containing color flow information; other pixels within the tumor ROI that did not contain a color signal were assigned an intensity value of 0. The result was an image map representing the intensity, or strength, of the color signal for each pixel of interest. The weighting of the intensities was determined by using the Doppler color bar that accompanied each image. With the intensity associated with the color bar, a 3-level grading scheme was used to classify the magnitude of the color signal (each level consisted of a range of equally distributed intensity values). Grades of 1, 5, and 10 were assigned to the low-, medium-, and high-flow categories, respectively. The assigned values of 1, 5, and 10 were arbitrary selections. They were chosen to provide delineation between the 3 strength categories. The numbers were correlated with the color bar in that the lowest number (1) was assigned to the lowest third of the color bar intensities, the middle number (5) to the middle intensities, and the highest (10) to the highest intensities. Any 3 numbers could have been chosen to represent the categories. Different number assignments would have changed the absolute value of the weighted vascular intensity; however, the relationship between the weighted VI for different tumors would still be discernible, although the specific relationship would have to be evaluated.

On completion, the weighted VI was calculated by summing the number of pixels containing a color flow signal as weighted by the 3-level grading system over the tumor region and then divided by the tumor area. As a result, the weighted normalized VI allowed the degree of vascularization between subjects to be compared because it related to the amount of flow. It should be noted that whereas the selection of a 3-level grading scheme was somewhat subjective, it did serve as an initial strategy to stratify Doppler data for the purposes of differential diagnosis of thyroid tumors in a simple manner.

Surgery and Histologic Evaluations

All patients with malignant cytologic findings underwent total thyroidectomy with lateral neck lymph node dissection. Twenty patients with benign or borderline cytologic findings having clinical indications, such as comparatively large or quickly growing nodules, underwent hemithyroidectomy. Histologic evaluations were performed by a pathologist with more than 15 years of experience in thyroid histologic evaluation. The pathologist was blinded to sonographic and cytologic findings.

Statistical Analysis

Comparison of qualitative variables was performed with the \( \chi^2 \) test; quantitative variables were compared with the paired Student \( t \) test. Sonographic characteristics of each thyroid nodule were registered separately and processed blindly for statistical evaluation. The unit of analysis was each nodule rather than each patient. For each diagnostic criterion, the sensitivity, specificity, and overall accuracy of differentiation between benign and malignant lesions were calculated by standard procedures. To determine the size dependency of sonographic criteria for thyroid cancer, different cutoff values of the nodular size were tested to find the one that could divide examined lesions into 2 groups with statistically significant differences in their vascularity. After the cutoff value of the nodular size was established, all sonographic characteristics and their diagnostic accuracy were analyzed for nodules smaller and larger in diameter than selected threshold value. Quantitative data are presented as mean ± SD. Significance was defined as \( P < .05 \).

Results

A total of 86 solid thyroid nodules were included in this study. At histologic examination, papillary thyroid carcinoma was diagnosed in 40 nodules...
(46.5%), a follicular variant of papillary carcinoma in 6 (7.0%), follicular adenoma in 22 (25.6%), and adenomatous goiters in 18 (20.9%).

**Qualitative Visual Analysis of Power Doppler Sonograms**

Results of the qualitative assessment of power Doppler sonograms are summarized in Table 1. Although most of the malignant tumors (65.2%) showed increased intranodular vascularization, the same feature was observed in 47.5% of the benign tumors. Thus, the diagnostic accuracy of increased intranodular vascularity in thyroid cancer diagnosis was comparably low (sensitivity, 65.2%; specificity, 52.5%; and overall accuracy, 58.9%).

The distribution of benign and malignant nodule sizes is shown in Figure 2. Benign thyroid tumors showed a statistically significant increase in the levels of intranodular vascularization with an increase in the tumor size ($R = 0.5; P < .001$; Figure 3). Among the benign tumors with diameters of less than 2 cm, only 14.3% showed intranodular vascularization (Table 1). That was significantly less than that among the benign thyroid tumors with diameters of greater than 2 cm (65.4%; $P < .01$). Conversely, the distribution of the tumor vascularization patterns among malignant lesions did not show significant dependence on tumor size ($R = 0.04; P = .8$). Thus, among small (<2-cm) thyroid nodules, detection of the intranodular blood flow on power Doppler sonography showed specificity of 85.7% and overall accuracy of 72.1% in thyroid cancer detection. There was no statistically significant difference between histologic subclassifications of small and large benign lesions.

**Quantitative Analysis of Power Doppler Sonograms**

There were significant differences in the values of both normalized and weighted VIs between benign and malignant thyroid tumors. The normalized VI for malignant tumors was almost 2-fold higher than that for benign ones (0.31 ± 0.22 versus 0.16 ± 0.12; $P < .001$). A similar trend was observed for the weighted VI, which for malignant lesions was almost twice as high as for benign ones (0.70 ± 0.65 versus 0.40 ± 0.42; $P < .01$).

Table 1. Qualitative Analysis of Thyroid Tumor Vascularity

<table>
<thead>
<tr>
<th>Vascularity</th>
<th>Benign, n (%)</th>
<th>Malignant, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD type 1</td>
<td>10 (25.0)</td>
<td>12 (26.1)</td>
</tr>
<tr>
<td>PD type 2</td>
<td>11 (27.5)</td>
<td>4 (8.7)</td>
</tr>
<tr>
<td>PD type 3</td>
<td>19 (47.5)</td>
<td>30 (65.2)</td>
</tr>
<tr>
<td>Tumor diameter &lt;2 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD type 1</td>
<td>9 (64.3)*</td>
<td>7 (24.1)</td>
</tr>
<tr>
<td>PD type 2</td>
<td>3 (21.4)</td>
<td>3 (10.3)</td>
</tr>
<tr>
<td>PD type 3</td>
<td>2 (14.3)*</td>
<td>19 (65.5)</td>
</tr>
<tr>
<td>Tumor diameter &gt;2 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD type 1</td>
<td>1 (3.8)*</td>
<td>5 (29.4)</td>
</tr>
<tr>
<td>PD type 2</td>
<td>8 (30.8)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>PD type 3</td>
<td>17 (65.4)*</td>
<td>11 (64.7)</td>
</tr>
</tbody>
</table>

PD indicates power Doppler.
*Difference between tumors with diameters of less than and greater than 2 cm ($P < .01$).

Interobserver errors of normalized and weighted VI measurements were 4.7% ± 1.3% and 4.9% ± 1.5%, respectively.

As well as the number of benign thyroid tumors with intranodular vascularization, both normalized and weighted VIs in benign tumors showed a significant increase with an increase in tumor size (both $R = 0.5; P < .01$; Figures 4 and 5). Conversely, values of normalized and weighted VIs in malignant tumors were independent of tumor size ($R = –0.1; P = .5$; and $R = 0.05; P = .8$, respectively). Thus, among all tumors, none of

![Figure 2. Distribution of benign and malignant tumor sizes. The bold line represents distribution of malignant nodules; the dotted line represents distribution of benign nodules. An area of 19,000 pixels corresponds to 2 cm in nodule diameter.](image-url)
the quantitative criteria of tumor vascularization showed adequate accuracy in differentiation between benign and malignant thyroid tumors (Figures 6 and 7).

A normalized VI of greater than 0.278 showed 82.5% sensitivity, but the specificity and overall accuracy of this criterion were low (54.3% and 68.4%, respectively). A weighted VI of greater than 0.176 was not specific (50.0%) and had low overall accuracy (64.2%) but showed somewhat high sensitivity (78.3%).

Taking into account the significant size dependency of benign thyroid tumor vascularization, we tested numerous cutoff values of tumor size to establish the one that could improve the diagnostic accuracy of the examined diagnostic criteria for cancer. Among the numerous tested cutoff values of tumor diameter, we found that a threshold of 2 cm (corresponding value of a tumor area of 19,000 pixels) could divide examined lesions into 2 groups with the most significant differences in echographic characteristics (Table 1). After the
cutoff value of the nodular size was established, all sonographic characteristics and their diagnostic accuracy were analyzed for nodules smaller and larger in diameter than selected threshold value. Unlike in all tumors, among thyroid lesions with diameters of less than 2 cm, both the normalized and weighted VIs could be successfully used for thyroid cancer diagnosis. Among thyroid lesions with diameters of less than 2 cm, a normalized VI of greater than 0.14 had 72.4% sensitivity, 100% specificity, and 86.2% overall accuracy. A weighted VI of greater than 0.24 showed comparable results, with 69.0% sensitivity, 100% specificity, and 84.5% overall accuracy.

**Discussion**

Growth and progression of malignant tumors are substantially dependent on the available blood supply.8 Neoangiogenesis, with increased formation of irregular vessels and arteriovenous shunts, is a characteristic feature shown by many tumors.
tumors. Therefore, assessment of the difference in vascular signatures between malignant and benign tumors may provide valuable diagnostic criteria for cancer. Power mode Doppler sonography has been available for clinical application since the early 1990s. Modern Doppler technology allows blood flow imaging of vessels with very small diameters (<100 µm) and slow flow rates of only a few millimeters per second. Therefore, power mode Doppler sonography proved to be quite superior to color flow Doppler imaging when it came to visualizing the smallest blood flow pattern still detectable.

Power and color Doppler examinations have become established imaging techniques for assessing patients with thyroid nodular disease. Some authors have shown their ability to identify lesions with a higher probability of malignancy with good sensitivity and specificity. Many authors have noted that malignant thyroid nodules predominantly had central vascularization, whereas benign nodules predominantly had peripheral vascularization. In a recent study, Frates et al showed that solid hypervascular thyroid nodules had a high likelihood of malignancy (nearly 42%). Conversely, they concluded that color characteristics of a thyroid nodule could be used to exclude malignancy because 14% of solid nonhypervascular nodules were malignant.

There is some controversy between particular institutions on the appearances of benign thyroid nodules and on the power Doppler criteria for thyroid cancer. This is mainly because the visual criteria of nodule vascularity depend considerably on the Doppler settings, resolution of the ultrasound scanner, and training and experience of the examiner.

A certain improvement in the diagnostic value of Doppler sonography in thyroid cancer diagnosis was achieved by measuring intratumoral vascular resistance. The resistive index (RI) is a popular parameter for characterizing the arterial waveform produced on Doppler sonography and is thought to be a useful duplex parameter for assessing thyroid nodules because it is not dependent on the angle of insonation, that is, the angle at which the ultrasound beam intercepts the axis of the vessel. However, there is still a disagreement in the literature over the adequate cutoff values of the RI for thyroid cancer diagnosis, mainly because of inconsistent results of RI values in benign thyroid tumors.

Our results showed significant size dependency of both quantitative and qualitative power Doppler criteria for thyroid cancer. Among benign thyroid tumors, there was a statistically significant increase in the levels of intranodular vascularization with an increase in the tumor size. Less than 15% of the small benign tumors showed...
intranodular vascularization on power Doppler sonograms. Conversely, more than half of the large benign thyroid tumors showed prominent intranodular vascularization. Thus, both qualitative and quantitative criteria for thyroid cancer had higher specificity among small tumors. Our results are comparable with results of some previous studies, which showed that visual analysis of tumor vascularity in small tumors had reasonable accuracy in thyroid cancer diagnosis. Papini et al\(^6\) reported that in nonpalpable thyroid tumors, increased intranodular vascularization on color Doppler sonography as an individual feature had sensitivity of 74.2% and specificity of 80.8% for thyroid cancer diagnosis, which were close to the results of our study.\(^6\)

We also showed that quantitative analysis of power Doppler sonograms can significantly improve the accuracy of thyroid cancer diagnosis. Of the 2 investigated methods of power Doppler quantification, the normalized VI showed somewhat better sensitivity than the weighted VI; however, this difference was not significant. Our study revealed no statistically significant differences between the power Doppler appearances of papillary and follicular nodules. This, however, may be explained by the small number of follicular tumors included in our study and should be reconfirmed by future studies.

Some limitations need to be addressed. We analyzed only one 2-dimensional sonographic frame per tumor, which may not have accurately represented spatial vascular architecture. Therefore, further, more standardized studies based on 3-dimensional sonographic image analysis using the above-mentioned software with boundary detection upgrades and volume flow analysis will be needed in a larger population to determine the value of quantitative sonographic criteria for thyroid cancer and their size dependence to support confidence in the results within appropriate limits. In addition, in this study, tumor vascularity was assessed in a group of patients referred for surgery. Although this could provide an accurate histologic confirmation of the final diagnosis, this patient selection may have influenced the rate of detection and patterns of flow in the nodules. Therefore, future studies in larger populations of unselected patients will be required to verify the value of quantitative analysis of thyroid nodule vascularity.

In conclusion, our findings showed that the quantitative analysis of tumor vascularity on power Doppler sonograms has benefits over visual inspection in differentiating malignant and benign solid thyroid nodules, especially for smaller lesions. Among these nodules, a normalized VI of greater than 0.14 had 100% specificity and 72.4% sensitivity. Conversely, both qualitative and quantitative criteria for cancer showed very low accuracy in the large thyroid nodules. This was mainly because of the significant increase in the levels of intranodular vascularization in the benign thyroid lesions with an increase in tumor size. Therefore, we conclude that in small thyroid nodules, quantitative analysis of tumor vascularity can be useful in differentiation between benign and malignant nodules.

References
Quantitative Analysis of Thyroid Tumor Vascularity


