Coexisting sonographic features of “tumor neovascularization-like pattern” and “echogenic areas” in thyroid nodules: Diagnostic performance in prediction of papillary carcinoma

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Abstract

Purpose To determine the diagnostic performance of the sonographic finding of tumor neovascularization-like patterns and echogenic areas in differentiating papillary carcinomas from benign nodules.

Materials and methods We prospectively analyzed ultrasound-guided fine-needle aspiration (FNA) of thyroid nodules for the two features. In total, 299 nodules (evenly distributed benign vs papillary carcinoma) were evaluated by two blinded readers for intense small penetrating vessels and homogenous mild hyper-echoic areas within the nodules and correlated with FNA-confirmed pathologic diagnosis. A set of nodules with and without the features was selected for further pathologic review and comparison to establish a pathologic basis for the finding.

Results The specificity of the “tumor neovascularization-like pattern” and “echogenic areas” in papillary thyroid cancers (PTCs) was 90.6% and 91.3%, respectively. There was a significant correlation, with $P < 0.0001$, between the “tumor neovascularization-like pattern” and “echogenic areas” in PTCs on both sonographic and pathologic levels. Additionally, the newly identified features as a single variable were more predictive of malignancy than the well-studied features. Inclusion of these new features markedly improved the predictive ability of PTC (AUROC = 0.873 versus 0.801, $P < 0.0001$).

Conclusion Despite their relatively low sensitivity, the “tumor neovascularization-like pattern” and “echogenic areas” are highly specific for PTC and can be a useful sonographic finding in decisions regarding FNA.

Introduction

Although nodules within the thyroid are common due to the advent of high-resolution ultrasound detection, only a small minority of the nodules are proven to be malignant(1–3). Not all detected nodules require fine-needle aspiration (FNA) or surgical excision for definitive treatment, and identification of features that help determine which nodules require further evaluation by FNA is critical(4). Therefore, dedicated thyroid ultrasound, as a reliable and noninvasive method, is recommended by consensus guidelines in determining which nodules warrant FNA on the basis of a reasonable likelihood of malignancy(5, 6). Ultrasound has demonstrated advantages in thyroid evaluation due to the ability to dynamically visualize nodule morphology and characterize internal architecture with high resolution that exceeds other imaging approaches.

Many well-studied sonographic features have been used clinically to evaluate thyroid nodules for malignancies. The American College of Radiology (ACR) Thyroid Imaging, Reporting and Data System (TI-RADS) provided guidelines for risk stratification designed to identify most clinically significant malignancies while reducing the number of biopsies performed on benign nodules. Sonographic grayscale features proposed by TI-RADS include composition, echogenicity, shape, margin and echogenic

foci(4). However, as ultrasound device advances and image resolution have improved, other features, such as vascularity imaging, have gradually been recognized by sonographers.

Sustained angiogenesis is one of the critical hallmarks of tumor pathophysiology(7) and is considered an acquired event during tumor initiation and progression via an angiogenic switch from vascular quiescence. Sustained angiogenesis results in tumor-specific vasculature compared to normal tissues in the context of architecture and biological behavior. Specifically, tumor neovascularization shows abnormal maturation, manifesting irregular sprouting and branching of angiogenic vessels with disordered coverage of pericytes(8, 9). Therefore, vascularity is critical for the evaluation of malignancy in thyroid nodules. Color Doppler flow imaging (CDFI), as the most well-established technology with the capability of visualizing flow patterns, is theoretical to classify malignancy on the basis of the vascular information. Although CDFI has been used to predict suspicious malignancy of thyroid nodules(5, 10–13), existing studies have not reached an agreement regarding its assessment(14, 15). One of the reasons is that the previous studies simply took the abundance and distribution of vascular flow into consideration, regardless of vascular architecture.

In view of the abundance of vascular flow, hypervascularity has traditionally been considered to be suggestive of benignity, and a lack of vascularity was more frequent in papillary thyroid cancers (PTC) given the existence of extensive fibrosis(16–19). Sonographers have rarely observed the vascular architecture using CDFI because nodular hypervascularity could potentially be confused for benignity. On the other hand, although recent studies demonstrated that central vascularization was a characteristic of malignancy in consideration of the distribution of vascular flow(12, 13), the conclusions of different researchers have been controversial(14–16). Therefore, a reliable assessment to identify which nodules warrant FNA on the basis of vascular architecture would be highly desirable. In view of this, we have found that nodules with intense small penetrating vessels on CDFI are often associated with PTC. Moreover, the vascularity observed on sonography often indicates neovascularity in tumors(20, 21), suggesting that a dedicated evaluation of the vascular architecture on CDFI is merited. Thus, we sought to study this previously undescribed vascular architecture, termed tumor neovascularization-like pattern, in more detail.

Anecdotally, we have observed a correlation between the tumor neovascularization-like pattern and echogenic areas on ultrasound. Although recent studies have demonstrated a relationship between echogenic areas and malignancy(22), the coexistence of tumor neovascularization-like patterns and echogenic areas within the malignant nodules has not been previously described but may be a helpful sonographic finding that could increase suspicion for malignancy. Therefore, the purpose of this study was to determine whether tumor neovascularization-like pattern and concomitant echogenic areas contained within thyroid nodules seen on ultrasound can be used to identify potential papillary carcinomas of the thyroid.

Materials And Methods

Patient cohort and collection of ultrasound images

The patient cohort in this study comprised consecutive patients with thyroid nodules who underwent regular ultrasound detection and ultrasound-guided FNA between June 2018 and October 2018 at the Ultrasound Department of the First Affiliated Hospital of Dalian Medical University. All ultrasound studies were prospectively generated by a trained sonographer using 5-18 MHz frequencies of the HITACHI HI VISION Ascendus (HITACHI, Japan). Ultrasound dynamic video records were obtained with patients positioned supine and with slightly hyperextended necks. Transverse and longitudinal videos and images of each nodule, using both grayscale and CDFI, were preserved by the ultrasonic machine for the subsequent analysis. Doppler amplification was set to a level at which normal thyroid tissue did not display any noise and just under the level at which random noise appeared. All studies were checked by expert radiologists at the time of the examination to ensure image quality and adequacy. After acquisition of the images, ultrasound-guided FNAs were prospectively performed on these patients by a trained sonographer with 10 years of experience in interventional ultrasound. From the initial patient cohort, all FNA results were evaluated by the expert pathologist for the clinical diagnosis, and patients with unclear pathological diagnosis (Bethesda I, III and IV) or non-papillary carcinoma were excluded. The final nodule population was 299, including 161 malignant nodules (PTCs) and 138 benign nodules. Figure 1 is the detailed diagram of this study group.

Evaluation of ultrasound images

We reviewed electronic medical records to obtain pertinent patient information including sex, age, FNA-proven pathologic diagnosis, and ultrasound dynamic video records. The videos and images of each nodule were evaluated on the ultrasonic machine with Hitachi color monitor (Hitachi, Ltd., Higashi-Ueno, Taito-ku, Tokyo, Japan) with 2-megapixel 1280 × 1024 resolution. The location, size, adjacency, composition, echogenicity, shape, margin and echogenic foci of each nodule were evaluated by two trained sonographers and documented for the following analyses.

To evaluate the nodular microvascular flow, each nodule was visualized with CDFI by the video mode in both transverse and longitudinal section. The video clips were about 5 seconds. In terms of the merging methods recommended by Shin et al(23), Chen et al(24) and our experience, nodular vascularity on CDFI was classified into four types (Fig. 2): type I, absence of nodule vascularity; type II, predominantly perinodular vascularity with continuous (II a) or discontinuous circumferential vascularity at the margin of a nodule (II b); type III, predominantly intranodular vascularity, homogenous linear (III a) or branching (III b) with or without perinodular vascularity; type IV, heterogeneous short-line, strip-like, or microbubble colorful signals, which penetrated the margin of the nodule (perinodular neovascularization, IV a) or dispersely distributed within the nodule (internal neovascularization, IV b). Specifically, the presence of tumor neovascularization-like pattern in CDFI was identified as vascularity of type IV. The vascularity of type I, II and III were excluded from the tumor neovascularization-like pattern. Nineteen freeze-frame shots of these nodules from 19 video clips are provided in Figure 2.

For the evaluation of the echogenic areas, each nodule was evaluated in a binary manner in both transverse and longitudinal sections. Specifically, the echogenic area features (present or absent) were defined as homogeneous mild hyperechoic areas (excluding microcalcifications) located within the relatively hypoechoic nodule. The morphology of the hyperechoic areas was either conglomerate (Fig. 3A) or flocculent (Fig. 3B).

All of the above evaluations were performed independently by two trained sonographers, and the discrepant cases were reassessment by an expert radiologist to reach a consensus. All evaluators were blinded to the pathologic results.

Tissue samples

Tissue samples from 10 patients with malignant thyroid nodules (presence of the echogenic areas on binary), 10 patients with malignant nodules (presence of the tumor neovascularization-like pattern on CDFI) and 10 patients with benign nodules (absence of the tumor neovascularization-like pattern on CDFI) treated at the First Affiliated Hospital of Dalian Medical University (Dalian, Liaoning) were randomly selected. All tissue slides were reviewed by an expert pathologist for verification of the clinical diagnosis. Each tissue sample had at least 3 independent tissue slides for immunohistochemistry (IHC) staining.

IHC staining

IHC was performed on the 5-μm formalin-fixed, paraffin-embedded tissue slides. The DAB chromogenic reagent kit (Cat. ZLI-9019, ZSGB-BIO, Beijing, China) was used for IHC. Slides were dewaxed, rehydrated, antigen retrieved and endogenous peroxidase blocked following the protocol of the kit. For immunolabeling of CD34, mouse monoclonal CD34 (Cat. Kit-0004, MXB Biotechnologies, Fujian, China) was applied. Thereafter, secondary antibody testing, DAB chromogenic reaction, counterstaining, dehydration and transparency were performed according to the protocol of the kit.

IHC scoring system

Immunoreactivity of the vascular endothelial cells was first evaluated for each tissue slide. For all cases, slides that showed distinct immunostaining in vascular endothelial cells were further evaluated. Thereafter, the intensity of CD34 expression for vascular endothelial cells in each field was classified into five grades: extremely high (score 4), high (score 3), moderate (score 2), low (score 1), and negative (score 0). Final scores for each case were calculated as the mean score of all the individual field scores of each slide.

Measuring microvessel density (MVD)

Microvessels of thyroid nodules were highlighted by anti-CD34 immunostaining in formalin-fixed, paraffin-embedded slides. The MVD quantification for each slide was performed according to the detailed method described previously(25, 26). Briefly, the stained slides were examined at low-power magnification (40 and 100 total magnification) to identify the areas of highest neovascularization of the

tumor. In each slide, the three most vascular areas were chosen. The microvessel counts in a 200 field (Olympus microscope) in each of these 3 areas was counted. The average counts of the 3 fields in each slide were calculated and thereafter were referred to as the density counts. Any brown-staining endothelial cell clearly separated from adjacent microvessels, tumor cells, or other connective tissue elements was considered to be a single, countable microvessel. Large vessels with thick, muscular walls and large vessels with lumina greater than approximately eight red blood cells were excluded from the count. All measurements were performed independently by two observers.

Scoring system for the individual features

For multivariate analyses, covariates for prediction models included the newly identified features, as well as established risk factors. Specifically, the absence and presence of tumor neovascularization-like pattern were scored as 0 and 1 point, respectively, as well as the echogenic areas. The traditional grayscale features were scored according to the ACR TI-RADS guideline(4). In brief, the composition of cystic, spongiform, mixed and solid was scored as 0, 0, 1, and 2 points, respectively; the echogenicity of anechoic, hyperechoic or isoechoic, hypoechoic and very hypoechoic was scored as 0, 1, 2, and 3 points, respectively; the shapes of wider-than-tall and taller-than-wide were scored as 0 and 3 points, respectively; the margins of smooth, ill-defined, lobulated or irregular and extrathyroidal extension were scored as 0, 0, 2, and 3 points, respectively; and the echogenic foci of none or large comet-tail artifacts, macrocalcifications, peripheral calcifications and punctate echogenic foci were scored as 0, 1, 2, and 3 points, respectively.

Statistical analysis

Data included in this study were analyzed using SPSS version 23.0 (IBM Corporation, Armonk, NY). Chi squared test was used to analyze differences of clinical parameters between two groups of patients. FNA-proven diagnosis was used as the reference standard to determine sensitivity and specificity. Fisher's exact test was used to analyze associations between the sonographic features and malignancy. Student’s t test was used to analyze differences in IHC scores and MVD counts. Logistic regression analysis was performed to evaluate the sonographic features as the predictor for malignancy and the associated odds ratio (OR). Additionally, the relative importance of individual covariates in multivariate logistic regression models was estimated by examining the partial Wald Chi-squared statistic. $P < 0.05$ was considered statistically significant.

Results

Tumor neovascularization-like pattern shown by CDFI is a specific characteristic in thyroid malignant nodules

Between June 2018 to October 2018, a total of 215 patients (Table 1) underwent ultrasound-guided FNA of the thyroid and had clear cytological diagnosis, complete clinical information and ultrasonic images. These patients had 299 nodules, with 161 malignant nodules (PTCs) and 138 benign nodules. Of these

161 PTCs, 112 PTCs were confirmed by histopathologic diagnosis. Nodular microvascular flow was preoperatively evaluated under dynamic video mode in the 299 nodules, and the specific neovascularization-like pattern (including perinodular and internal neovascularization, Fig. 2) on CDFI was more commonly found in malignant nodules, which had a sensitivity of 45.3%, a specificity of 90.6%, a positive predictive value (PPV) of 84.9%, and a negative predictive value (NPV) of 58.7% (Table 2). In our study, the presence of the tumor neovascularization-like pattern increased the risk of PTC by an odds ratio of 7.976 (95% CI, 4.164–15.279, P < 0.0001, Table 3). Analysis of the tumor neovascularization-like pattern by Fisher's exact test found it to be a statistically significant predictor of PTC, with a P-value of < 0.0001 (Table 2).

Table 1
Clinical and ultrasound findings of 299 nodules

Parameter	Malignancy (n = 161)	Benign (n = 138)	P-value (Chi squared test)
Age 50	56 (34.8%)	67 (41.3%)	
50	105 (65.2%)	71 (51.4%)	0.016
Sex Female	128 (79.0%)	109 (79.5%)	
Male	33 (20.5%)	29 (21.5%)	1.000
Size 1.5 cm	86 (53.4%)	87 (63.0%)	
1.5 cm	75 (46.6%)	51 (37.0%)	0.093
Lobe L	72 (44.7%)	66 (47.8%)	
R	75 (46.6%)	67 (48.6%)	
I	14 (8.7%)	5 (3.6%)	0.199
Abbreviations: L, left lobe; R, right lobe; I, Isthmus			

Table 2

Diagnostic value of different ultrasound features for the identification of malignancy

Feature	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95%s CI)	P-value (Fisher's exact test)
TNLP	45.3 (37.6, 53.4)	90.6 (84.1, 94.7)	84.9 (75.2, 91.4)	58.7 (51.7, 65.3)	0.0001
EA	59.0 (51.0, 66.6)	91.3 (85.0, 95.2)	88.7 (80.9, 93.8)	65.6 (58.4, 72.2)	0.0001
TNLP & EA	37.3 (29.9, 45.3)	97.1 (92.3, 99.1)	93.8 (84.0, 98.0)	57.0 (50.4, 63.4)	0.0001
Solidness	96.9 (92.5, 98.9)	29.0 (21.7, 31.4)	61.4 (55.1, 67.3)	88.9 (75.1, 95.8)	0.0001
Hypoechogenicity	95.0 (90.1, 97.7)	37.0 (29.0, 45.6)	63.8 (57.3, 69.8)	84.6 (74.5, 93.6)	0.0001
Taller-than-wide	31.7 (24.7, 39.5)	92.8 (86.7, 96.3)	83.6 (71.5, 91.4)	53.8 (47.2, 60.2)	0.0001
Extrathyroidal extension	3.1 (1.1, 7.4)	99.3 (95.4, 99.9)	83.3 (36.5, 99.1)	46.8 (41.0, 52.6)	0.0013
Punctate echogenic foci	10.5 (6.5, 16.6)	98.6 (94.3, 99.7)	89.5 (65.5, 98.2)	48.6 (42.6, 54.6)	0.2224

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; TNLP, tumor neovascularization-like pattern; EA, echogenic areas.

Table 3
Results of logistic regression analysis of different ultrasound features and malignancy

Feature	Coefficient	S.E.	P-value	OR (95% CI)
TNLP	2.076	0.332	0.0001	7.976 (4.164, 15.279)
EA	2.716	0.342	0.0001	15.114 (7.732, 29.543)
TNLP & EA	2.991	0.533	0.0001	19.901 (7.002, 56.561)
Solidness	2.544	0.492	0.0001	12.735 (4.859, 33.733)
Hypoechogenicity	2.417	0.403	0.0001	11.211 (5.086, 24.713)
Taller-than-wide	1.781	0.369	0.0001	5.935 (1.516, 2.158)
Extrathyroidal extension	1.480	1.102	0.179	4.391 (0.507, 38.047)
Punctate echogenic foci	2.083	0.757	0.006	8.028 (1.821, 35.399)

Abbreviations: TNLP, tumor neovascularization-like pattern; EA, echogenic areas. S.E., standard error; OR, odds ratio; CI, confidence interval.

We next investigated the pathological basis of the specific vascular pattern on CDFI by comparing the differences in the angiogenesis of benign (n = 10) and malignant nodules (n = 10), demonstrated by IHC and MVD analyses of CD34 immunostaining. The intensity of CD34 expression was significantly stronger in the microvessels of malignant tissues than in benign nodules (P = 0.036) and paracancerous tissues (P = 0.002). Moreover, in morphology, tumor angiogenic vessels showed abnormal maturation, irregular sprouting and branching with discontinuous and disordered coverage of endothelial cells, whereas the microvessels of benign nodules and paracancerous tissues displayed a constant density of endothelial cells that was identified by the CD34 immunostaining (Fig. 4). Subsequently, the MVD was significantly higher for malignant tissues than for benign nodules (P = 0.037) and paracancerous tissues (P = 0.012). These findings thus suggested that thyroid malignant nodules showed higher endothelial activity and sustained and immature angiogenesis, which was consistent with the tumor neovascularization-like pattern on CDFI.

Echogenic areas are highly specific for malignant thyroid nodules

The feature of echogenic areas on ultrasound was preoperatively evaluated in the 299 nodules. Based on our interpretations (described in Methods), the feature had a sensitivity of 59.0%, a specificity of 91.3%, a PPV of 88.7%, and an NPV of 65.6% (Table 2). Moreover, the presence of the echogenic areas increased the risk of PTC by an odds ratio of 15.114 (95% CI, 7.732–29.543, P < 0.0001) (Table 3). Analysis of the feature by Fisher's exact test found it to be a statistically significant predictor of PTC, with a P-value of < 0.0001 (Table 2). Moreover, we found a significant correlation between tumor neovascularization-like pattern and echogenic areas on ultrasound in the malignant nodules (r = 0.430, P < 0.0001). Specifically, the distribution of the two features within one nodule was not overlapped. Flocculent echogenic areas

often existed in the nodules with internal neovascularization (Fig. 2 Type IV b), manifested as the microvascular signals and flocculent echogenic areas dispersed within the nodules. Conglomerate echogenic areas often existed in the center of the nodules with perinodular neovascularization (Fig. 2. Type IV a).

To investigate the pathological basis of the echogenic areas on binary, a total of 10 malignant nodules found to have echogenic areas on binary were randomly selected for pathological specimen review, demonstrating extensive fibrosis. Moreover, we found the pathologic correlation of the tumor neovascularization and fibrosis in malignant nodules, manifested as a complementary and integrated distribution. Specifically, in the nodules with perinodular neovascularization, few viable cells and microvascular vessels were located in the regions of central echogenic areas, as the tissue had been almost entirely replaced by conglomerate fibrosis (Fig. 3A). In the nodules with internal neovascularization, intranodular flocculent fibrosis was surrounded by tumor cells and microvascular networks (Fig. 3B).

The newly identified features are helpful for better determination of malignant risk

We next tested the ability of the two features to predict the malignancy of nodules. In the present study, the newly identified features as a single variable were more predictive of malignancy than the well-studied features recommended by TI-RADS, which include solidness, hypoechogenicity, taller-than-wide shape, extrathyroidal extended margin and presence of punctate echogenic foci (Table 2 and Table 3). In multivariate logistic regression models that also considered composition (cystic, spongiform, mixed, solid), echogenicity (anechoic, hyperechoic or isoechoic, hypoechoic, very hypoechoic), shape (wider-than-tall, taller-than-wide), margin (smooth, ill-defined, lobulated or irregular, extrathyroidal extension) and echogenic foci (none or large comet-tail artifacts, macrocalcifications, peripheral calcifications, punctate echogenic foci), inclusion of the newly identified features markedly improved predictive ability (Fig. 5A; AUROC = 0.873 versus 0.801, $P < 0.0001$), and the presence of echogenic areas was the most significant covariate as measured by the Wald chi-squared statistic (Fig. 5B). Multivariate models that included either tumor neovascularization-like pattern or fibrotic echogenic areas had comparable predictive value for malignancy of nodules (with tumor neovascularization-like pattern: AUROC = 0.850 versus 0.801, $P < 0.0001$; with fibrotic echogenic areas: AUROC = 0.864 versus 0.801, $P < 0.0001$). These results demonstrate that the newly identified features improve the ability to predict the malignancy of nodules.

Discussion

We have found that the tumor neovascularization-like pattern and fibrotic echogenic area findings on ultrasound were highly predictive of PTC, with a sensitivity and specificity that were comparable to the well-established sonographic features (Table 2). To our knowledge, the newly identified features have never been evaluated in the literature prior to this study and could potentially be extremely useful in determining which nodules should be further evaluated with FNA (Table 3 and Fig. 5).

Tumors are highly dependent on angiogenesis, which presents different features in malignant and benign processes(27–29). Vascularity was usually related to cellular proliferation in a neoplastic condition(30). The examination of vascular flow of thyroid nodules is an age-old issue and has played a role in the evaluation of nodules. However, in the latest edition of the American Thyroid Association guideline(6), vascular flow was removed, indicating that this feature is not sufficiently specific to predict a nodule as malignant, and sensitivity is also lower than other features(14–16). We have reviewed previous studies and found that they may have emphasized the abundance and distribution of vascular flow on CDFI over the details of vascular architecture. One reason that previous studies overlooked important variations in vascular architecture is that many of these studies were performed using lower-frequency probes (7–12 MHz)(13, 15, 16), which may have obscured fine details. Therefore, to provide more detailed descriptions and obtain the full vascular information, we have completed this study using 5–18 MHz probes and critically adjusted amplification to see details that may not have otherwise been evident at lower frequencies. As ultrasound technology and imaging resolution improve, impactful and new features could be found. Consequently, we have evaluated vascular architecture of small vessels on CDFI and attempted to establish that tumor neovascularization-like pattern and its background echogenic areas within the nodule can be useful in the assessment of malignancy. Moreover, recent evidence has demonstrated that neovascularity in tumors can also be depicted with greater detail and clarity with superb microvascular imaging (SMI)(24). However, this technology is limited by the particular type of ultrasound machine and cannot be generalized like CDFI.

The lack of vascularity has traditionally been considered to be suggestive of PTC(31), possibly because extensive fibrosis frequently exists in PTCs. Therefore, sonographers have frequently overlooked the vascular architecture observed with CDFI given that nodular hypervascularity could potentially be confused for benignity. In the present study, we have skipped the ideological restraints and found that a specific vascular architecture was significantly associated with PTCs. Moreover, this tumor neovascularization-like pattern was not excluded from fibrotic echogenic areas. Instead, these features frequently coexisted in PTCs on ultrasound evaluation, leading to elevated specificity in diagnosing PTCs (Table 2 and Table 3). Although the newly identified features had high specificity, the sensitivity was relatively lower. Similar to the other well-established sonographic features, vascularity and echogenic areas alone were insufficient for determination of PTCs because not all PTCs harbor these features, and there is overlap between PTCs and benign nodules. To seek a more effective method to recognize PTCs, we brought suspicious grayscale features proposed by ACR TI-RADS and the newly identified features on CDFI into a multivariate logistic regression analysis. The results showed that inclusion of the newly identified features markedly improved predictive ability, and the most significant covariate was presence of echogenic areas, followed by tumor neovascularization-like pattern. Our results indicated that the newly identified features combined with conventional features may become the choice for differentiating PTCs from benign nodules. Therefore, when we make the decision of whether to conduct FNA with regard to the indeterminate nodules on ultrasound, especially for the solid and hypoechoic nodules without any other suspicious features, the presence of the newly identified features can potentially serve to influence the management decisions of suspicious malignancies.

There were a few limitations in our study. First, malignant nodules, including follicular and medullary carcinoma, were not evaluated; however, some studies have claimed that vascularity plays an important role in these conditions(32, 33). Second, the morphologic characteristic of microvessels evaluated by CDFI was not sufficiently objective, and multimodal approaches combined with SMI and contrast-enhanced ultrasonography (CEUS) should be verified in the future. Third, the presence of tumor neovascularization-like pattern evaluated by CDFI was possibly affected by the nodular size and presence of diffuse thyroid hypervascularity. Nodules of tiny size (< 1 cm) or with diffuse background hypervascularity were difficult to evaluate on CDFI.

In conclusion, we found the tumor neovascularization-like pattern and coexisting fibrotic echogenic areas to be helpful in predicting the risk of PTC in thyroid nodules. This finding helps refine the schema for vascularity evaluation. The newly identified features combined with conventional features may become the choice for differentiating benign from malignant nodules in determining FNA.

Declarations

Ethics approval and consent to participate

Standard institutional board review approval was obtained from our research compliance office prior to implementation of this study. Thyroid cancer and nodular goiter samples were obtained from the archives of the Pathology Department of the First Affiliated Hospital of Dalian Medical University (Dalian, Liaoning) following the approval of the institutional review board. These patients were contacted by telephone to obtain verbal informed consent for the tissue slides used in this study. The patient data were anonymized.

Consent for publication

The material has not been published and was not under active consideration by another journal. If accepted, it will not be published elsewhere. In case of acceptance of the manuscript copyright will be allowed to be transferred to BMC Medical Imaging.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MT, MQ and SL performed experiments, analyzed the data and wrote the paper; XF, LG, WX and JJ carried out additional analyses and supported the study; YC oversaw and directed the study. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

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Figures

Figure 1

Diagram of the study group. Abbreviations: TR, American College of Radiology (ACR) Thyroid Imaging, Reporting and Data System (TI-RADS); FNA, Fine-needle aspiration; PTC, Papillary thyroid carcinoma

Figure 2

Schematic diagram and sonographic images illustrating the CDFI vascularity types of thyroid nodules. Type I: absence of nodule vascularity. Type II: predominantly perinodular vascularity with continuous (II a) or discontinuous circumferential (II b) vascularity at the margin of a nodule. Type III: predominantly intranodular vascularity, linear (III a) or branching (III b) with or without perinodular vascularity. Type IV: penetrating (perinodular neovascularization, IV a) or diffusing (internal neovascularization, IV b) vascularity of a nodule.

Figure 3

Representative histology for fibrotic echogenic areas in the individual cases of PTCs. (A) Conglomerate fibrotic echogenic area in the center of the nodule. (B) Flocculent fibrotic echogenic areas dispersed within the nodule. Sonographic image of binary manner (left), cut surface of the resected nodule (middle) and HE staining (100×, right). Scale bars indicate 200 μm. The arrows indicate fibrosis.

Figure 4

Immunohistochemical features of endothelial cells within microvessels in benign and malignant thyroid nodules. (A) Representative histology for IHC staining of CD34 expression in the individual cases of benign and malignant thyroid nodules. Scale bars indicate 200 μm, 100 μm and 50 μm in the top, middle and bottom columns, respectively. All slides were counterstained with hematoxylin (light blue). (B) Boxplots depicting distribution of CD34 expression (top) and MVD (bottom) in benign and malignant nodules. (C) Representative histology for IHC staining of CD34 expression in the individual cases of malignant thyroid nodules. Scale bars indicate 200 μm, 100 μm and 50 μm in the top, middle and bottom columns, respectively. All slides were counterstained with hematoxylin (light blue). (D) Boxplots depicting distribution of CD34 expression (top) and MVD (bottom) in cancer and paracancerous tissues.

Figure 5

The newly identified features predict malignancy. (A) Receiver operating characteristic (ROC) curves for prediction of malignancy, using logistic regression models that include composition, echogenicity, shape, margin and echogenic foci as covariates, with (green line) or without (blue line) TNLP and echogenic areas. (B) Significance (chi-squared statistic) of each covariate for prediction of malignancy in the multivariate model that includes TNLP and EA. TNLP, tumor neovascularization-like pattern; EA, echogenic areas; df, degrees of freedom.