

CLINICAL STUDY

Thyroid B-flow twinkling sign: a new feature of papillary cancer

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Abstract

Background: Microcalcifications (aggregated with psammoma bodies), detected by ultrasound (US), are the most specific feature of papillary thyroid cancer (PTC). Using B-flow imaging (BFI), we identified a new sign (the twinkling sign; BFI-TS) in 'suspect' PTC nodules, which appeared to be generated by microcalcifications.

Objective: To evaluate whether the BFI-TS was predictive of malignancy, we correlated the BFI-TS with the results of fine needle aspiration cytology and histology.

Design: Cross-sectional cohort study from September 2006 to April 2008.

Setting: Department of Radiology and Endocrinology, University of Naples Federico II, and Department of Endocrinology, Second University of Naples.

Patients: A total of 306 consecutive patients with 539 thyroid nodules > 8 mm in diameter.

Main outcome measure: US and BFI examinations were performed with the Logiq 9 system (General Electric Company, Milan, Italy); all patients underwent cytological examination.

Results: Cytology revealed 455 (84.4%) benign nodules and 84 (15.6%) malignant nodules; the latter were confirmed by postsurgical histological examination (76 cases of PTC, 7 follicular carcinoma, and 1 Hürthle cell carcinoma). All suspect nodules, namely, nodules with potential predictors of thyroid malignancy (e.g., microcalcifications and intra-nodal vascularity), were analyzed by cytology or histology (or both). Of 84, 68 (80.9%) of malignant nodules had ≥ 4 or more BFI-TSs in at least one scan versus only 12 of 455 (2.6%) of benign lesions.

Conclusions: Our results indicate that the BFI-TS could be a reliable diagnostic technique in the management of suspect thyroid nodules.

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Introduction

Thyroid nodular disease is very common. Depending on the method of evaluation, the prevalence of thyroid nodules in the general population is 20–50% (1, 2). The prevalence of malignant nodules is 5–15% (1, 3–7) depending on age, gender, radiation exposure, and family history (1). Papillary thyroid cancer (PTC) is the most frequent thyroid carcinoma (80%). Its annual incidence varies considerably, namely from 1.2 to 2.6 cases per 100 000 individuals in men and 2.0 to 3.8 cases per 100 000 in women (8, 9). The incidence of PTC has increased by more than 5% per year in the last decade (10, 11).

Ultrasound (US) is the morphological imaging modality of choice for the study of thyroid nodules. US-guided fine needle aspiration (FNA) is the gold standard for diagnosis and it is particularly important in the assessment of small or non-palpable nodules (12, 13).

A consensus conference on several US characteristics potentially predictive of thyroid malignancy

(microcalcifications, hypoechogenicity, irregular margins, absence of a halo, predominantly solid composition, intra-nodal vascularity, and regional lymphadenopathy) concluded that calcifications within the nodule increase the likelihood of malignancy (14). In particular, microcalcifications in a predominantly solid nodule are associated with an approximately threefold higher cancer risk whereas coarse calcifications are associated with a twofold increased risk, compared with predominantly solid nodules (14).

B-flow imaging (BFI) is a non-Doppler technique widely used to evaluate carotid artery stenosis and other vascular diseases (15, 16). While evaluating the application of BFI for studies of nodular vascularization, we serendipitously identified a new sign (the 'twinkling sign'; BFI-TS) in 'suspect' PTC nodules, which appeared to be generated by microcalcifications. This sign resembles the color Doppler TS generated by renal stones (17). The BFI-TS is a rapidly flashing white light behind such stationary objects as microcalcifications, which gives the appearance of

movement. When an incidental sonographic beam impinges a rough interface composed of sparse reflectors, the sign is generated by the phase shift, thereby causing a faint variation of the sonographic beam at the interface. The sign is also caused by the increase of pulse duration, which results in multiple reflections in the medium. Thyroid microcalcifications increase in size by aggregating with primary psammoma bodies (PBs); they consist mainly of highly reflecting crystalline aggregates of calcium (18).

The aim of this study was to determine whether the BFI-TS is predictive of malignancy. To this aim, we investigated whether the sign was correlated with microcalcifications of thyroid nodules and the results of US-guided FNA.

Materials and methods

Patients

From a sample of 2686 patients from the Campania Region (Naples metropolitan area), a mild iodine-deficient region (19), referred to the Department of Radiology and Endocrinology, University of Naples Federico II, and to the Department of Endocrinology, Second University of Naples, between September 2006 and April 2008, we enrolled 306 subjects based on the selection criteria of the 2005 US Consensus Conference Statement of Society of Radiologists (14), i.e., these patients were affected by solid thyroid lesions larger than 8 mm and had normal serum levels of thyroid hormone, thyroid-stimulating hormone, and calcitonin. These 306 consecutive patients (age range 19–74 years, mean age 42.2 ± 9.3 years; 98 males and 208 females) had 539 palpable and non-palpable solid thyroid nodules. All 539 nodules had the following US characteristics of potential predictors of thyroid malignancy: hypoechogenicity, irregular margins, absence of a halo, microcalcifications, solid composition, and intranodular vascularity.

The study was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of the University of Molise.

Methods

US and BFI US examinations were performed with the GE Logiq 9 machine (General Electric Company), a commercially available real-time US system, equipped with a 9–14 and 6–8 MHz linear array transducer. All examinations were performed separately by two radiologists, and data analysis was performed by another investigator. The following US characteristics were recorded for each nodule: size, parenchymal composition, echogenicity, presence or absence of halo, margin appearance, and presence or absence of microcalcifications. The size was recorded as three orthogonal

dimensions. The echogenicity of each nodule was determined by comparing the solid portion of the nodule to the surrounding thyroid parenchyma and designated 'hyperechoic' when it was more echogenic, 'isoechoic' when it was similar, and 'hypoechoic' when it was less echogenic than the thyroid tissue (3). The presence of hyperechoic spots (calcifications or colloidal crystals) was evaluated in relation to the surrounding thyroid tissue (20, 21).

BFI was performed at 10 MHz (M12L) and 7 MHz (7L) with the BFI capability at the level of the nodule. The pulse repetition index was set at 3. BFI gain was not fixed and was adjusted to allow a better visualization of the signs.

We examined the BFI images for the presence, appearance, and intensity of TSs. For each nodule, we recorded the maximum number of well-differentiated signals per scan; we excluded scans in which TSs were less than 2 mm apart in a single scan. Each US examination took 15 ± 4.3 min, whereas it took only 5 min to identify the thyroid BFI-TS.

After US features were assessed, a cytological examination was carried out and the findings were recorded by a radiologist. FNA was performed by the endocrinologist under the US guidance of the radiologist. Physicians were highly trained in the technique of US-guided FNA with 27 and 22 gauge needles, according to the procedure described elsewhere (22, 23). Three or four smears were prepared; the first was air dried and immediately stained with Diff-Quik stain, which is a May-Grunwald–Giemsa modified stain in which smears are stained in less than 1 min. The other smears were fixed in absolute alcohol for subsequent AbTPO smear or immunostaining. Diff-Quik smears were immediately evaluated by a cytopathologist who assessed the adequacy of the samples; the FNA was repeated in case of inadequate or scarcely cellulated smears. Cases classified 'benign' from cytology were scheduled for a clinical and US follow-up to monitor the size and US features of the nodules. Patients with suspect or malignant cytological features underwent surgery during which intra-surgical frozen sections were examined to verify diagnosis. If the cytological analysis was negative, the patient was re-examined at 3 and 6 months to monitor changes in nodule size. We used cytospray fixation of frozen intra-operative sections to optimize the reading of frozen intra-operative sections (24) and to compare cytological imprints. Lastly, the χ^2 test and the predictivity test were used for statistical analysis.

Results

Cytology and histology

The cytology specimens of 171 of 539 nodules (31.7%) were not evaluable; thus FNA was repeated 1 month

later. The cytological examination showed 455 benign nodules (84.4%), 11 suspect nodules (2%), and 73 malignant nodules (13.6%). The 84 patients with suspect or malignant nodules underwent surgery, and histology showed PTC in 76 (90.5%) cases, follicular thyroid carcinoma (FTC) in 7 (8.3%) cases, and Hürthle cell carcinoma (HCC) in 1 (1.2%) case. The prevalence of cancer was 39% in nodules measuring between 9 and 15 mm and 61% in nodules larger than 15 mm.

Conventional US and color Doppler flow sonography

Table 1 shows the conventional sonographic features of thyroid cancer as well as the new BFI-TS identified in our patients together with their specificity, sensitivity, and predictive value for malignancy.

BFI evaluation

The BFI-TS was identified in 147 of the 539 nodules (26.3%; inter-observer variation: $4.32 \pm 2.18\%$). As shown in Table 1, 4 PTCs had between 1 and 3 BFI-TSs per scan, and 68 PTCs had 4 or more BFI-TSs per scan separated by more than 2 mm. Of the 67 nodules with less than 3 signs/scan, 4 (6%) were PTC and 63 (94%) were benign. Of the 80 nodules with 4 or more signs/scan, 68 (85%) were PTC and 12 (15%) were benign. The BFI-TS was not found in non-PTC tumors (FTC or HCC).

The specificity and sensitivity of the BFI-TS for all thyroid cancers with ≥ 4 or more signs were 97.4 and 80.9% respectively (Table 1). These values are significantly higher than the values we obtained for conventional US features of thyroid malignancy (microcalcifications: specificity 92.3% and sensitivity 41.7%; hypoechogenicity: specificity 40.9% and sensitivity 72.6%). They are also significantly higher ($X^2 = 337.13$; $P < 0.0001$) than the values previously reported for the

conventional US features (14). The sensitivity of the BFI-TS was even greater when we considered only PTC (89.5 vs 80.9% for non-PTC tumors).

Discussion

The early diagnosis of PTC reduces the incidence of metastatic lesions and increases 5-year survival (96%) (14). It is generally agreed that fine punctuate calcifications on US are the most reliable indicators of malignancy because they correspond to PBs on microscopic examination. PBs are concentric laminated calcified structures most commonly found in neoplasms such as meningiomas, papillary carcinoma of the ovary, and PTC (25, 26). The coalescence of various PBs results in larger calcifications (26, 27). The aim of this study was to evaluate the diagnostic value of the BFI-TS in visualizing microcalcifications generated by PB aggregation in solid thyroid nodules.

In our study, the BFI-TS was unrelated to intranodular vascularization on computational flow dynamics, but was detected only at the site of microcalcifications. It is feasible that the signal resulted from an aggregation of PBs. Our histological data are in line with this concept (see Figs 1D and 2D).

We also examined the sensitivity, specificity, and positive predictive values of conventional US features associated with an increased risk of thyroid cancer (microcalcifications, hypoechogenicity, irregular margins, absence of a halo, and intra-nodular vascularity). Our results are in agreement with a previous study on conventional US features in which microcalcifications had the highest positive predictive value and specificity for malignancy (24.3–70.7% and 85.8–95.0% respectively) (14).

We found that the BFI-TS identified significantly more microcalcifications than B-mode US. In fact, based on the BFI-TS with 4 or more signs/scan, 80 thyroid nodules were positive versus 66 identified by B-mode US (Fig. 1). In addition, of the 80 BFI-TS-positive lesions,

Table 1 Ultrasound features associated with thyroid cancer in the 539 nodules examined.

US features	Papillary cancer	Non-papillary cancer	Benign	Significance	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
Irregular margins or no halo	38/76	5/8	155/455	$X^2 = 8.95$ $P = 0.003$	65.9	51.2	21.7	88
Microcalcifications	31/76	4/8	35/455	$X^2 = 72.43$ $P < 0.0001$	92.3	41.7	50	89.5
Hypoechogenicity	55/76	6/8	269/455	$X^2 = 5.44$ $P = 0.02$	40.9	72.6	18.5	89
CDF intra-nodular	46/76	7/8	93/455	$X^2 = 65.33$ $P < 0.0001$	79.6	63.1	36.3	92.1
No BFI-TS	5/76	8/8	380/455	$X^2 = 166.21$ $P < 0.0001$	16.5	15.5	3.3	51.4
1–3 BFI-TSs	4/76	0/8	63/455	$X^2 = 5.38$ $P = 0.02$	86.1	4.8	6	83
≥ 4 BFI-TSs	68/76	0/8	12/455	$X^2 = 337.13$ $P < 0.0001$	97.4	80.9	85	96.5

US, ultrasound; PPV, positive predictive value; NPV, negative predictive value; CDF, color Doppler flow; BFI, B-flow imaging; TS, twinkling sign.

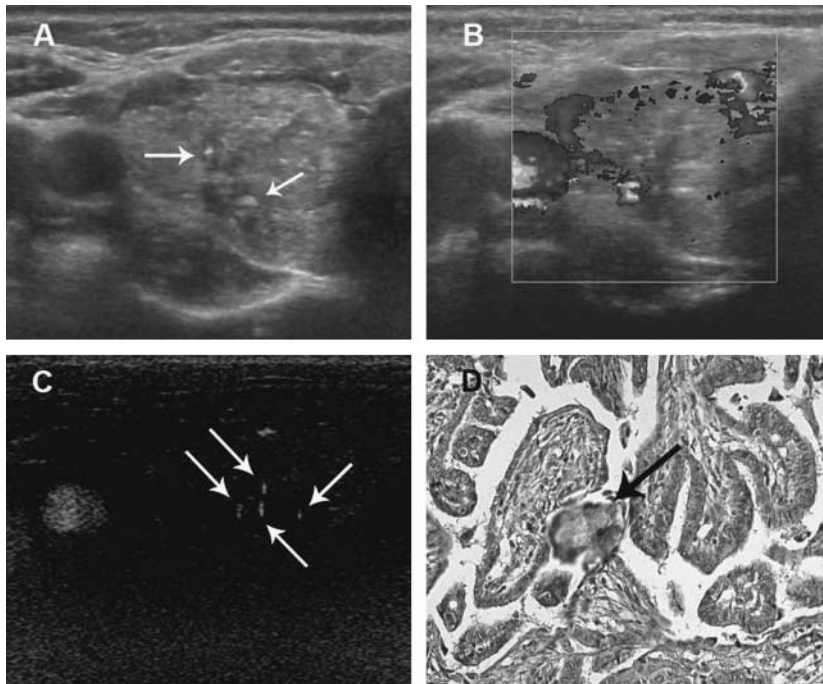


Figure 1 Ultrasound B-mode of a suspect thyroid nodule. (a) Transverse US image of a nodule containing multiple fine echogenicities (arrow) with no comet-tail artifact. (b) Addition of color Doppler mode shows flow within the mural component. (c) Transverse B-flow image of a nodule containing multiple twinkling signs, highly suggestive of malignancy. Fine needle aspiration and surgery confirmed papillary carcinoma. (d) Histological image showing a papillary cancer and psammoma body (arrow).

cytology showed that 68 were malignant (68 of 80, 85%, all PTC) and 12 were benign (12 of 80, 15%). Of the 66 cases of microcalcifications identified with B-mode US, 31 were PTC and 35 were benign lesions. Thirty-seven microcalcification-associated PTCs were detected only by the BFI-TS; B-mode US failed to identify these cases (Fig. 2). Consequently, BFI-TS is more sensitive than B-mode US in detecting PBs (sensitivity: 80.9 vs 41.7%).

The BFI-TS was more frequent in PTC than in other thyroid lesions. Moreover, the specificity, sensitivity, and positive predictive value of ≥ 4 or more BFI-TS/scan for PTC were higher than those of the conventional US features (Table 1). Our study indicates that nodules with at least 4 signs per scan are highly suggestive of malignancy. This assessment, based on statistical criteria, is supported by the histological observation, within PTCs of PBs.

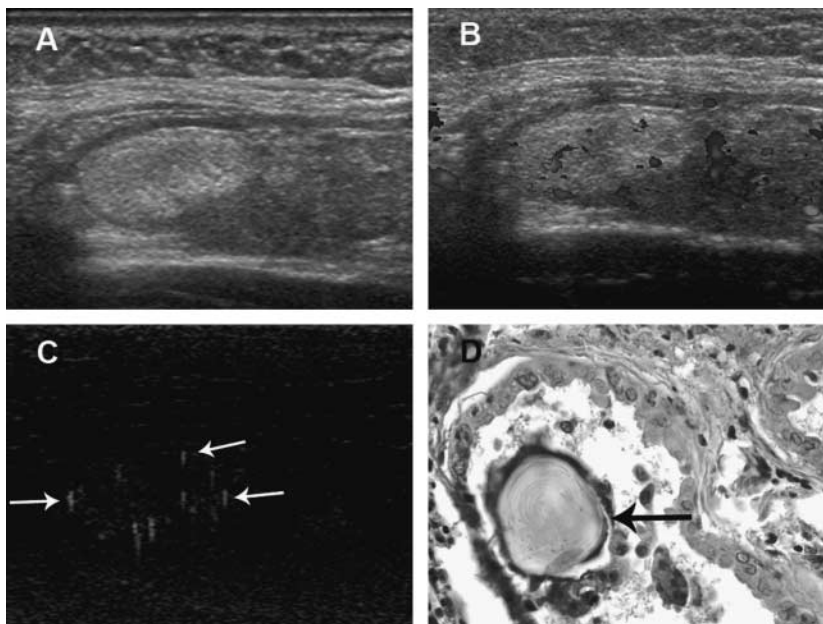


Figure 2 Mode and color Doppler of a non-suspect thyroid nodule. (a) Sagittal image of a solid nodule suggesting a benign lesion. (b) Addition of color Doppler mode did not show marked internal vascularity. (c) Sagittal B-flow image of a nodule containing multiple twinkling signs, suggesting malignancy. Fine needle aspiration and surgery confirmed papillary carcinoma. (d) Histological image showing a papillary cancer and psammoma body (arrow).

PBs are formed from the progressive, lamellar appositions of calcium salts and other minerals (see Fig. 2D). It is likely that the lamellar layer morphology of PBs determines a number of calcium interfaces that produce the twinkling phenomenon. It is conceivable that studies of BFI-TS features in benign and malignant lesions might reveal differences between lesions that produce large amounts of PBs PTC and lesions that produce lower amounts, or that produce microcalcifications with physical characteristics different from PBs.

Thus far, the BFI technique has been used to study the vascular circulation mainly in the carotid artery. Our results indicate that this technique can be applied to the studies of thyroid nodules, and that its sensitivity and specificity is higher than those of traditional US diagnostic techniques. However, longitudinal studies on a large population are required to verify the efficacy of BFI in the diagnosis of thyroid carcinoma.

Lastly, the GE Logiq 9 BFI apparatus was not designed to visualize the TS but to study vascularization. One may envisage the development of software that enhances the TS to a point where it reveals PBs as small as 10–100 μm , thereby increasing the early detection rate of PTCs.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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