Endocrine (2016) 54:156–168
DOI 10.1007/s12020-016-0982-x

ORIGINAL ARTICLE

Malignancy is associated with microcalcification and higher AP/T ratio in ultrasonography, but not with Hashimoto's thyroiditis in histopathology in patients with thyroid nodules evaluated as Bethesda Category III (AUS/FLUS) in cytology

Oya Topaloglu[1] · Husniye Baser[2] · Fatma Neslihan Cuhaci[1] · Nuran Sungu[3] · Abdussamed Yalcin[4] · Reyhan Ersoy[1] · Bekir Cakir[1]

Received: 14 December 2015 / Accepted: 3 May 2016 / Published online: 12 May 2016

Abstract The predictors of malignancy are important for the decision of appropriate management in nodules with atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS). Our aim was to determine the ultrasonographical, clinical, and biochemical predictors of malignancy in these patients. A total of 427 patients with cytologically Bethesda Category III (AUS/FLUS) thyroid nodules were included in this retrospective study. We divided the nodules into two subgroups according to the histopathology as benign and malignant, and compared the preoperative ultrasonographical, clinical, and biochemical findings. In overall, 427 patients with 449 AUS/FLUS nodules who had undergone surgery, the rate of malignancy was 23.4 % (105/449). When evaluated separately, the rate of malignancy was 25.8 % in nodules with AUS (82/318) and 17.6 % in nodules with FLUS (23/131) ($p = 0.061$). The vast majority of malignant specimens in histopathology consisted of papillary thyroid carcinoma (PTC) ($n = 91$, 86.7 %). Preoperative ultrasonographic features of 105 malignant nodules in histopathology were compared with the 344 benign nodules in histopathology. Anteroposterior/Transverse (AP/T) ratio was significantly higher in malignant group compared to benign group ($p = 0.013$). In multiple logistic analysis, we found that higher AP/T ratio and microcalcification were independently associated with malignancy ($p < 0.05$). The malignancy-associated cut-off value of AP/T ratio at maximum sensitivity and specificity was $\geq$0.81. We did not find any correlation between malignancy and Hashimoto's thyroiditis in histopathology in multivariate analysis ($p > 0.05$). In Bethesda Category III nodules with higher AP/T ratio and microcalcification, surgery might be considered as a first therapeutic option instead of repeat fine-needle aspiration biopsy or observation.

Keywords Atypia of undetermined significance (AUS) · Follicular lesion of undetermined significance (FLUS) · Malignancy · Ultrasonography · Hashimoto's thyroiditis

Introduction

Fine-needle aspiration biopsy (FNAB) is the most accurate, widely accepted, sensitive, and specific diagnostic method for identification of malignancy in thyroid nodules [1, 2]. The Bethesda System for Reporting Thyroid Cytopathology (BSRTC) has been used widely in cytopathological assessment of the thyroid nodules since 2009 [3]. It describes 6 categories based on FNAB cytological diagnosis, and also it gives risk of malignancy and recommendations for management of each group [3]. According to the Bethesda system, atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) termed as Category III is a heterogenous subgroup of thyroid cytopathology. It means that this is a category including mainly sparse and compromised samples with suspicious atypical cytological or architectural features, but not enough to be

✉ Oya Topaloglu
oyasude@gmail.com

1 Department of Endocrinology and Metabolism, Ankara Yildirim Beyazit University School of Medicine, Ankara, Turkey

2 Department of Endocrinology and Metabolism, Ataturk Education and Research Hospital, Ankara, Turkey

3 Department of Pathology, Ankara Yildirim Beyazit University School of Medicine, Ankara, Turkey

4 Department of General Surgery, Ankara Yildirim Beyazit University School of Medicine, Ankara, Turkey

diagnosed as follicular neoplasm (FN) or suspicious for malignancy (SM) [4, 5]. The estimated risk of malignancy for this group of nodules was reported to be between 5 and 15 % [6]. The BSRTC recommends performing a repeat FNAB for decision of management. Recent studies have showed that malignancy rates in nodules with AUS/FLUS cytology are higher than previously estimated, such as 22.6–48 % [7–12]. So, assistant methods for estimation of malignancy in these patients such as thyroid ultrasound (US), molecular tests, fluorodeoxy glucose-positron emission tomography (FDG-PET), and US-elastography seem to be important for the management decision-making [13–16]. However, molecular tests and FDG-PET are not available at most of centers and not cost-effective.

Hashimoto's thyroiditis (HT) is an autoimmune inflammatory disease of the thyroid gland. Thyroid nodules have been frequently detected in patients with HT [17, 18]. Firstly, Dailey et al. reported the association between HT and thyroid malignancy [19]. Since that time, many studies have been published regarding the association between malignancy and HT. However, some studies have reported an increased risk of malignancy in thyroid nodules associated with HT, others not [20–25].

High-resolution US is an excellent imaging method for assessment of thyroid pathologies such as nodule morphology, detection of suspicious lymph nodes, guidance for FNAB procedure, FNAB decision-making, and it is also valuable in nodule and thyroid malignancy follow-up. The American Thyroid Association (ATA) guideline has described the US features highly suspicious for malignancy as follows: solid hypoechoic nodule or solid hypoechoic component of a partially cystic nodule with one or more of these features such as irregular margins, microcalcifications, taller than wide shape, rim calcifications with small extrusive soft tissue component, and evidence of extrathyroidal extension [26]. Ultrasonography of cytologically indeterminate thyroid nodules could provide supportive information for the prediction of malignancy and help in thyroidectomy decision [9, 27]. The Thyroid Imaging Reporting and Data System (TI-RADS) has been developed for the risk stratification of thyroid nodules using suspicious US features [28], and it provides category assessment of nodule according to the predicted malignancy risk [29]. In the literature, there have been few studies which evaluated the value of ultrasonography for prediction of malignancy in the nodules with AUS/FLUS cytology [8, 30–33]. Therefore, the aim of present study was to evaluate the preoperative ultrasonographic features of patients with cytologically Bethesda Category III thyroid nodules, and also to determine the ultrasonographical, clinical, and biochemical predictors of malignancy in these patients who underwent operation.

Materials and methods

We reviewed the medical records of 1041 patients with Bethesda Category III (AUS/FLUS) thyroid nodules. Of these, 427 patients who underwent thyroidectomy were included to the study. The exclusion criteria were as follows: 1. Patients with FNAB results demonstrating Bethesda Category other than Category III. 2. Patients with a history of neck irradiation or radioactive iodine therapy. 3. Patients without a histopathological diagnosis after FNAB.

Demographic characteristics, preoperative thyroid functions, and thyroid autoantibodies of patients were recorded. Preoperative US findings of nodules such as localization, diameter [anteroposterior (AP), transverse (T), longitudinal], nodule AP/T ratio, volume, echogenicity, texture, marginal regularity, micro- and/or macro-calcification, presence of a peripheral halo, and vascularization pattern were evaluated. Moreover, postoperative histopathology (benign/malignant) and presence of HT in histopathology were determined. We divided the nodules into two groups based on histopathology as benign and malignant, and compared the preoperative US findings of two groups. HT was defined as the presence of diffuse lymphoplasmacytic infiltration, germinal centers, and enlarged epithelial cells with large nuclei and eosinophilic cytoplasm (Hurthle cells) in histopathology.

Local ethical committee approval was obtained in accordance with the ethical standards of Helsinki declaration.

Laboratory

Serum thyrotropin (TSH), free triiodothyronine (fT3), and free thyroxine (fT4) levels were measured within at least 3 months prior to surgery in all patients. Chemiluminescence methods (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA, USA and UniCel DXI 800, Beckman Coulter, Brea, CA) were used for measurement of serum TSH, fT3, fT4, anti-thyroid peroxidase antibody (anti-TPOAb), and anti-thyroglobulin antibody (anti-TgAb) levels. The normal ranges for TSH, fT3, fT4, anti-TPOAb, and anti-TgAb were 0.4–4 μIU/mL, 1.57–4.71 pg/mL, 0.61–1.12 ng/dl, <10 U/mL, and <30 U/mL, respectively. The thyroid antibody level over the upper range of normal was accepted as positive.

Ultrasonography and US-guided FNAB procedure

Esaote color Doppler US (Model 796FDII; MAG Technology Co. Ltd., Yung-Ho City, Taipei, Taiwan) and a superficial probe (Model LA523 13–4, 5.5–12.5 MHz)

were used for US evaluation. We evaluated morphological features of thyroid nodules on US as follows: size was the AP, transverse and longitudinal diameters as measured; shape was AP/T ratio; margin was irregular; echogenicity was isoechoic, hypoechoic, or iso-hypoechoic; calcification was microcalcification and/or macrocalcification; and presence of hypoechoic halo; vascularisation pattern was peripheral and/or intranodular vascularization. The suspicious US findings for malignancy in a thyroid nodule were determined as irregular margins, hypoechogenicity, taller than wide (higher AP/T ratio) appearance, solid texture, and presence of microcalcifications, or coexistence of micro- and macro-calcifications.

US-guided FNAB was performed with a 27-gauge needle attached to a 20 mL syringe for following nodules: (1) All solid hypoechoic nodules $\geq$1 cm in diameter. (2) Solid hypoechoic component of a partially cystic nodule $\geq$1 cm in diameter with one or more suspicious US findings. (3) Isoechoic solid nodules $\geq$1.5 cm in diameter. (4) Nodules $\leq$1 cm with highly suspicious US findings, or patients with a family history of thyroid malignancy. All patients gave informed consent prior to the procedure. Aspirated materials were placed onto appropriately labeled glass slides, smeared, air-dried, and stained with Giemsa stain. Cytological diagnosis was classified into six categories according to the BSRTC [3]. AUS determined in thyroid nodules in which cellular and/or nuclear features were more dominantly present than abnormal architecture, but criteria for the diagnosis of SM or malignancy were not met. FLUS determined in thyroid nodules in which low cellularity was present with a predominant microfollicular pattern with no or scant colloid.

Data and statistical analyses

All statistical analyses were performed with the SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA). Descriptive statistics for the constant variables were expressed as mean $\pm$ standard deviation or median (range), and categorical variables were noted as numbers and percent. The χ^2 test was used to investigate the difference between the groups regarding the categorical variables. Significant differences between the means of different groups were evaluated by Student's *t*-tests (Independent-Samples *t* test), and Mann–Whitney *U* test was used to evaluate the significant differences in median values. Multivariate analysis was performed with Forward stepwise (Likelihood ratio) method to assess the predictive US factors for malignancy in thyroid nodules with AUS/FLUS in cytology.

A Thyroid Imaging Reporting and Data System (TI-RADS) category was assigned to each nodule based on a number of suscipious US features present as follows; irregular margins, hypoechogenicity, taller than wide (higher AP/T ratio) shape, solid texture, and presence of microcalcifications, or coexistence of micro- and macro-calcifications. We applied TI-RADS category 3 to nodules with no suspicious US feature, category 4a to nodules with one suspicious US feature, category 4b to nodules with two suspicious US features, category 4c to nodules with three or four suspicious US features, and category 5 to nodules with five suspicious US features [29].

Results

A total of 427 patients (86 men, 341 women) with preoperative cytology results as Bethesda Category III were included. Among the 307 patients who had AUS (71.9 %) included in our study, 69 were male and 238 were female. Among the 120 patients who had FLUS (28.1 %), 17 were male and 103 were female.

Of the 427 patients with the Bethesda category III thyroid nodules, 190 patients (44.5 %) with malignant histopathology were classified as malignant group whereas 237 patients (55.5 %) with benign histopathology were classified as benign group.

The mean age of malignant group was 49.10 $\pm$ 12.23 years, and mean age of benign group was 47.78 $\pm$ 12.39 years ($p = 0.275$). Gender distribution was not significantly different between the two groups ($p = 0.365$). Presence of HT, positivity of anti-TPO and anti-Tg antibodies were significantly higher in malignant group compared to benign group ($p = 0.033$, $p = 0.043$, and $p = 0.007$; respectively). fT3 levels were significantly lower in malignant group ($p = 0.021$) (Table 1). There was no difference between malignant and benign groups regarding functional status of thyroid both in overall patients and in patients with HT. The operation indications of these patients were as follows; 338 (79.2 %) had coexistence of AUS/FLUS cytology and suspicious US findings, 59 (13.8 %) had $\geq$4 cm nodule size, 2 (0.5 %) had hyperthyroidism, 3 (0.7 %) had coexistence of cytologically AUS/FLUS thyroid nodule and parathyroid adenoma, 15 (3.5 %) had multiple thyroid nodules with other cytologies (6 with malignant cytology, 7 with SM, 2 with suspicious for follicular neoplasm). We did not obtain the operation indication data in 10 (2.3 %) patients (Table 1).

AUS/FLUS cytology was detected in a total of 449 nodules (318 AUS, 131 FLUS) from 427 patients. There were 200 nodules (149 AUS, 51 FLUS) in malignant group and 249 nodules (169 AUS, 80 FLUS) in benign group ($p = 0.125$). In malignant group; however, 105 nodules (82 AUS, 23 FLUS) had malignancy in histopathology, 95 nodules had benign histopathology. In last group,

Table 1 Demographic and baseline characteristics of patients in malignant and benign groups

	Malignant group ($n = 190$)	Benign group ($n = 237$)	p
Age (years)	49.10 ± 12.23	47.78 ± 12.39	0.275
Gender			0.365
Male	42 (22.1 %)	44 (18.6 %)	
Female	148 (77.9 %)	193 (81.4 %)	
	N = 171	*N = 128*	
Anti-TPOAb positivity	51 (29.8 %)	25 (19.5 %)	0.043*
Anti-TgAb positivity	54 (31.6 %)	23 (17.8 %)	0.007*
Presence of HT	73 (38.4 %)	70 (29.5 %)	0.033*
Functional status of all patients	$N = 187$	$N = 235$	0.278
Euthyroid	145 (77.6 %)	191 (81.3 %)	
Hypothyroid	22 (11.8 %)	19 (10.1 %)	
Hyperthyroid	20 (10.6 %)	25 (10.6 %)	
Functional status of patients with HT	$N = 73$	$N = 70$	0.641
Euthyroid	56 (76.7 %)	55 (78.6 %)	
Hypothyroid	11 (15.1 %)	11 (15.7 %)	
Hyperthyroid	6 (8.2 %)	4 (5.7 %)	
TSH (μIU/mL)	1.72 ± 1.63	1.80 ± 1.09	0.731
fT3 (pg/mL)	3.19 ± 0.47	3.33 ± 0.67	0.021*
fT4 (ng/dL)	1.17 ± 0.30	1.22 ± 0.69	0.424
Operation indications			<0.001*
AUS/FLUS+suspicious US findings	150 (78.9 %)	188 (79.3 %)	
Nodule size ≥4 cm	17 (8.9 %)	42 (17.7 %)	
Presence of hyperthyroidism	1 (0.5 %)	1 (0.4 %)	
Coexistence of parathyroid adenoma	1 (0.5 %)	2 (0.8 %)	
Another cytology in the same patient			
Malignant	6 (3.2 %)	0	
SM	5 (2.6 %)	2 (0.8 %)	
SFN	1 (0.5 %)	1 (0.4 %)	
Unknown	9 (4.7 %)	1 (0.4 %)	

Histopathological HT was detected in 143 patients

Anti-TPOAb positivity Anti-Thyroid peroxidase antibody positivity, *Anti-TgAb positivity* Anti-Thyroglobulin antibody positivity, *Presence of HT* Presence of Hashimoto thyroiditis, *TSH* Thyrotropin, *fT3* free triiodothyroinine, *fT4* free thyroxine, *AUS/FLUS* atypia of undetermined significance/follicular lesion of undetermined significance, *US* Ultrasound, *SM* suspicious for malignancy, *SFN* suspicious for follicular neoplasm

* $p < 0.05$

malignant nodule was distinct from the target nodule evaluated as AUS/FLUS in cytology.

In overall, of the 449 nodules with AUS/FLUS cytology that went on surgery, the rate of malignancy was 23.4 % (105/449). When evaluated separately, the rate of malignancy was 25.8 % in nodules with AUS cytology (82/318) and 17.6 % in nodules with FLUS cytology (23/131) ($p = 0.061$).

The vast majority of malignant specimens in histopathology consisted of papillary thyroid carcinoma (PTC) ($n = 91$, 86.7 %) followed by the follicular cancer (FC) ($n = 6$, 5.7 %), thyroid tumor of uncertain malignant potential (TT-UMP) ($n = 7$, 6.6 %), and hurthle cell cancer (HCC) ($n = 1$, 1.0 %) (Table 2). However, PTC incidence was higher in nodules with AUS cytology, FC and TT-UMP incidences were higher in nodules with FLUS cytology ($p < 0.001$). Patients with PTC had different variants as follows; 48 (52.7 %) classical variant, 23 (25.3 %) nonencapsulated follicular variant, 5 (5.5 %) oncocytic variant, 5 (5.5 %) encapsulated follicular variant, 1(1.1 %) tall cell variant, 1 (1.1 %) solid variant, and 8 (8.8 %) other variants (Table 2).

Among 449 nodules with AUS/FLUS that went on surgery, preoperative ultrasonographic features of 105 malignant nodules in histopathology were compared with the 344 benign nodules in histopathology. There were no statistically

Table 2 Histopathological distribution of nodules with AUS/FLUS in cytology

	AUS/FLUS ($n = 105$)		
	AUS ($n = 82$)	FLUS ($n = 23$)	p
PTC	78 (95.1 %)	13 (56.5 %)	<0.001*
Classical	40 (51.3 %)	8 (61.5 %)	
Nonencapsulated follicular	20 (25.6 %)	3 (23.1 %)	
Oncocytic	4 (5.1 %)	1 (7.7 %)	
Tall cell	1 (1.3 %)	0	
Solid	1 (1.3 %)	0	
Encapsulated follicular	4 (5.1 %)	1 (7.7 %)	
Other	8 (10.3)	0	
FC	2 (2.4 %)	4 (17.4 %)	
TT-UMP	2 (2.4 %)	5 (21.7 %)	
HCC	–	1 (4.3 %)	

AUS/FLUS Atypia of undetermined significance/Follicular lesion of undetermined significance, *PTC* papillary thyroid carcinoma, *FC* follicular cancer, *TT-UMP* thyroid tumor of uncertain malignant potential, *HCC* hurthle cell cancer

* $p < 0.05$

significant differences between two groups according to AP, transverse, longitudinal diameters of nodules, and nodule volumes ($p > 0.05$, for all). AP/T ratio was found as significantly higher in malignant group compared to benign group ($p = 0.013$) (Table 3). There were no statistically significant differences with regard to nodule location, texture, presence of irregular margin, and presence of halo between the two groups ($p > 0.05$, for all).

Ultrasound features of the 449 AUS/FLUS nodules were categorized according to TI-RADS as follows; 5 (1.1 %) with category 3, 89 (19.8 %) with category 4a, 186 (41.4 %) with category 4b, 168 (37.4 %) with category 4c, and only 1 (0.2 %) nodule with category 5. When applying TI-RADS, significant difference was seen in TI-RADS category between benign and malignant nodules ($p = 0.010$). All of the nodules in category 3 were evaluated as benign in histopathology. The malignancy rates in TI-RADS categories 4a and 4b were 21(23.6 %) and 32 (17.2 %), respectively. Fifty-one (30.4 %) nodules in category 4c and 1 (100 %) nodule in category 5 were found as malignant in final pathology (Table 3).

Microcalcification was more prevalent in malignant group compared to benign group (38.1 vs 24.4 %, $p = 0.006$). Benign group had lower rate of hypoechogenicity than malignant group (5.2 vs 14.3 %, $p = 0.007$) (Table 3). However, 22 (20.9 %) nodules had both micro- and macro-calcifications in malignant group, coexistence of micro- and macro-calcifications was found in 39 (11.3 %) nodules in benign group ($p = 0.006$). There was no difference between malignant and benign groups with regard to macrocalcification (9.5 vs 6.1 %, $p = 0.116$) (Table 3).

The malignancy-associated cut-off value of AP/T ratio at maximum sensitivity and specificity was calculated as ≥0.81. For the AP/T ratio; the calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 50.5, 61.6, 21.9, and 79.9 %, respectively. US findings relating to hypoechogenicity had a sensitivity of 56.2 % and specificity of 50.6 %, and findings related to microcalcification had a sensitivity and specificity of 38.1 and 75.6 %, respectively. Combined analysis of AP/T ratio ≥0.81 and microcalcification improved the specificity to 91.1 %. Sensitivity, specificity, PPV, and NPVs of significant US findings and combination of "microcalcification and AP/T ratio ≥0.81" are summarized in Table 4.

Ultrasound features of the nodules with AUS/FLUS cytology were also evaluated according to nodule size. Of the 449 AUS/FLUS nodules, 46 (10.2 %) had longitudinal nodule size <1 cm and 403 (89.8 %) ≥1 cm. For the nodules <1 cm; AP/T ratio was found as higher in malignant group compared to benign group ($p = 0.004$), and coexistence of micro- and macro-calcification was more frequent in malignant group ($p = 0.033$). Other US findings were found as similar between the benign and malignant groups in nodules <1 cm ($p > 0.05$, for all). For the nodules with longitudinal size ≥1 cm, microcalcification and coexistence of micro- and macro-calcification were more frequent in malignant group ($p = 0.009$ and $p = 0.022$, respectively) (Table 5). The nodules <1 cm in size with AP/T ratio ≥0.81 had a sensitivity of 78.9 % and specificity of 69.2 %, and microcalcification in nodules with ≥1 cm had sensitivity and specificity values of 39 and 74.9 %, respectively.

Table 3 Comparison of the preoperative ultrasonographic features of AUS/FLUS nodules with malignant and benign final histopathology

	Malignant ($n = 105$)	Benign ($n = 344$)	p
Nodule AP diameter (mm)	14.15 ± 7.75	13.82 ± 7.67	0.700
Nodule transverse diameter (mm)	18.39 ± 12.02	18.90 ± 16.66	0.770
Nodule longitudinal diameter (mm)	22.77 ± 16.52	23.14 ± 16.52	0.840
Nodule volume (mL)	1.26 (range 0.06–88.28)	1.58 (range 0.06–157.36)	0.493
Nodule AP/T ratio	0.83 ± 0.21	0.77 ± 0.20	0.013*
Nodule location			0.659
Right lobe	58 (55.2 %)	194 (56.4 %)	
Left lobe	43 (41.0 %)	130 (37.8 %)	
Isthmus	4 (3.8 %)	20 (5.8 %)	
Texture			0.525
Solid	103 (98.1 %)	331 (96.2 %)	
Cystic	1 (1.0 %)	10 (2.9 %)	
Mixed	1 (1.0 %)	3 (0.9 %)	
Echogenicity			0.007*
Isoechoic	46 (43.8 %)	174 (50.6 %)	
Hypoechoic	15 (14.3 %)	18 (5.2 %)	
Isoechoic+hypoechoic	44 (41.9 %)	152 (44.2 %)	
Microcalcification	40 (38.1 %)	84 (24.4 %)	0.006*
Coexistence of macrocalcification and microcalcification	22 (20.9 %)	39 (11.3 %)	0.006*
Macrocalcification	10 (9.5 %)	21 (6.1 %)	0.116
Hypoechoic halo	43 (40.9 %)	128 (37.2 %)	0.447
Irregular margins	56 (53.3 %)	185 (53.8 %)	0.853
TI-RADS			0.010*
Category 3	0 (0 %)	5 (1.4 %)	
Category 4a	21 (20.0 %)	68 (19.8 %)	
Category 4b	32 (30.5 %)	154 (44.8 %)	
Category 4c	51 (48.6 %)	117 (34.0 %)	
Category 5	1 (0.9 %)	0 (0 %)	

Volume is calculated by multiplying AP(cm) × transverse(cm) × longitudinal diameter(cm) × π/6

AP/T ratio Anteroposterior/Tranverse ratio, *TI-RADS* Thyroid Imaging Reporting and Data System

* $p < 0.05$

Table 4 Diagnostic accuracy of significant ultrasonographic features in AUS/FLUS nodules

	AUC (Std. Err)	p	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Nodule AP/T Ratio	0.579 (0.033)	0.015*	≥0.81	50.5	61.6	29.1	79.9
Echogenicity (Hypoechoic)	–		–	56.2	50.6	25.8	79.1
Microcalcification	–	–	–	38.1	75.6	32.3	80.0
Microcalcification and AP/T ≥0.81	–	–	–	23.8	91.1	45.5	79.3

AUS/FLUS Atypia of undetermined significance/Follicular lesion of undetermined significance, *AUC* area under the curve, *AP/T ratio* Anteroposterior/Transverse ratio, *PPV* positive predictive value, *NPV* negative predictive value

* $p < 0.05$

Preoperative US features were compared in nodules discriminating the AUS and FLUS subgroups with benign and malignant final histopathology. In AUS subgroup, AP/T ratio was significantly higher in malignant group compared to benign group (0.85 ± 0.21 vs 0.77 ± 0.20, $p = 0.005$). Microcalcification and coexistence of micro- and macro-calcification were more frequently seen in malignant group ($p = 0.015$ and $p = 0.035$, respectively),

Table 5 Comparison of preoperative ultrasonographic features of nodules according to nodule size (<1 cm and ≥1 cm) with malignant and benign final histopathology

	<1 cm ($n = 46$)		p	≥1 cm ($n = 403$)		p
	Malignant ($n = 19$)	Benign ($n = 27$)		Malignant ($n = 86$)	Benign ($n = 317$)	
Nodule AP/T ratio	0.96 ± 0.26	0.77 ± 0.16	0.004*	0.81 ± 0.20	0.78 ± 0.20	0.225
Texture						0.595
Solid	19 (100 %)	27 (100 %)	–	84 (97.6 %)	304 (95.9 %)	
Cystic	0	0		1 (1.2 %)	10 (3.2 %)	
Mixed	0	0		1 (1.2 %)	3 (0.9 %)	
Echogenicity			0.19			0.487
Isoechoic	4 (21.1 %)	11 (40.7 %)	7	41 (47.7 %)	155 (48.9 %)	
Hypoechoic	4 (21.1 %)	3 (11.2 %)		9 (10.5 %)	16 (5.0 %)	
Isoechoic+hypoechoic	11 (57.8 %)	13 (48.1 %)		36 (41.8 %)	146 (46.1 %)	
Microcalcification	6 (31.6 %)	4 (14.8 %)	0.175	34 (39.5 %)	80 (25.2 %)	0.009*
Coexistence of macro- and microcalcification	3 (15.8 %)	0	0.033*	19 (22.1 %)	39 (12.3 %)	0.022*
Irregular margins	14 (73.7 %)	19 (70.4 %)	0.806	42 (48.8 %)	166 (52.4 %)	0.725

AP/T ratio Anteroposterior/Tranverse ratio

* $p < 0.05$

and other parameters were not significantly different ($p > 0.05$, for all) (Table 6). In FLUS subgroup, all parameters including AP/T ratio, microcalcification, coexistence of micro- and macro-calcification were similar between benign and malignant groups ($p > 0.05$, for all) (Table 6; Figs. 1, 2).

Multiple logistic regression analyses were performed to determine the association between malignancy and age, presence of HT in histopathology, presence of halo, AP/T ratio, and microcalcification. Higher AP/T ratio ($p = 0.030$; odds ratio 0.307; confidence interval 0.105–0.892) and microcalcification ($p = 0.003$; odds ratio 2.087; confidence interval 1.291–3.375) were found as independently associated with malignancy. In multiple logistic regression analyses, after discriminating the nodules according to nodule size, we found that there were significant independent associations between malignancy and AP/T ≥0.81 in nodules <1 cm in size ($p = 0.002$; odds ratio 8.437; confidence interval 2.118–33.607), and malignancy and microcalcification in nodules ≥1 cm in size ($p = 0.008$; odds ratio 1.979; confidence interval 1.196–3.273) (Table 7).

Discussion

In present study, we found that the rate of malignancy was 23.4 % in 449 nodules evaluated as AUS/FLUS in cytology. The malignancy rates between AUS and FLUS subgroups were not significantly different, 25.8–17.6 %, respectively. Although, the presence of thyroid autoantibodies was more frequent in patients with malignancy significantly, we did not find any correlation between malignancy and histopathological HT in multivariate analysis. Furthermore, we demonstrated that some US findings could be predictive factors to detect malignancy in thyroid nodules with AUS/FLUS cytology such as higher AP/T ratio, hypoechogenicity, microcalcification, and coexistence of micro- and macro-calcification. The malignancy-associated cut-off value of AP/T ratio was found as ≥0.81. According to multiple logistic analyses, higher AP/T ratio and microcalcification were detected as independent predictive factors of malignancy. Microcalcification, higher AP/T ratio, and coexistence of micro- and macro-calcification were found as significantly associated with malignancy in AUS group after discrimination of AUS and FLUS subgroups. Thus, these ultrasonographic features can be used as supportive findings for the decision of surgery in nodules with Bethesda Category III in cytology.

According to the BSRTC, AUS/FLUS has been determined as a heterogenous category of cytology regarding thyroid nodules that are not obviously benign, suspicious, or malignant [34]. The management options that are recommended by current guidelines and recent studies for this category are as follows; follow-up, repeat fine-needle aspiration, thyroidectomy, or perform core needle biopsy [26, 35–38]. Thus, determination of malignancy predictors is necessary for management decision in nodules with FLUS/AUS cytology. Previous studies showed that the estimated risk of malignancy for AUS/FLUS was 5–15 %

Table 6 Comparison of the preoperative ultrasonographic features of nodules discriminating the AUS and FLUS subgroups with malignant and benign final histopathology

	AUS ($n = 318$)		p	FLUS ($n = 131$)		p
	Malignant ($n = 82$)	Benign ($n = 236$)		Malignant ($n = 23$)	Benign ($n = 108$)	
Nodule AP diameter (mm)	13.70 ± 7.25	13.68 ± 7.84	0.981	15.77 ± 9.30	14.14 ± 7.30	0.357
Nodule transverse diameter (mm)	17.30 ± 10.54	19.05 ± 10.05	0.431	22.27 ± 15.93	18.58 ± 9.68	0.148
Nodule longitudinal diameter (mm)	21.58 ± 14.87	23.32 ± 18.13	0.435	27.00 ± 21.26	22.75 ± 12.39	0.198
Nodule volume (mL)	1.31 (0.06–88.28)	1.5 (0.06–157.36)	0.467	0.96 (0.21–54.85)	2.13 (0.09–46.41)	0.791
Nodule AP/T ratio	0.85 ± 0.21	0.77 ± 0.20	0.005*	0.79 ± 0.22	0.78 ± 0.21	0.961
Nodule location			0.644			0.307
Right lobe	43 (52.4 %)	137 (58.1 %)		15 (65.2 %)	57 (52.8 %)	
Left lobe	35 (42.7 %)	87 (36.9 %)		8 (34.8 %)	43 (39.8 %)	
Isthmus	4 (4.9 %)	12 (5.1 %)		0	8 (7.4 %)	
Texture			0.749			0.575
Solid	80 (97.6 %)	228 (96.6 %)		23 (100 %)	103 (95.4 %)	
Cystic	1 (1.2 %)	6 (2.5 %)		0	4 (3.7 %)	
Mixed	1 (1.2 %)	2 (0.8 %)		0	1 (0.9 %)	
Echogenicity			0.052			0.877
Isoechoic	33 (40.2 %)	124 (52.5 %)		14 (60.8 %)	58 (53.7 %)	
Hypoechoic	12 (14.6 %)	11 (4.7 %)		1 (4.3 %)	6 (5.6 %)	
Isoechoic+hypoechoic	37 (45.1 %)	101 (42.8 %)		8 (34.8 %)	44 (40.7 %)	
Microcalcification	32 (39.0 %)	59 (25 %)	0.015*	8 (34.8 %)	25 (23.1 %)	0.243
Macrocalcification	9 (10.9 %)	15 (6.4 %)	0.098	2 (8.7 %)	6 (5.6 %)	0.470
Coexistence of Macrocalcification and microcalcification	18 (21.9 %)	31 (13.1)	0.035*	4 (14.4 %)	8 (7.4 %)	0.115
Hypoechoic halo	34 (41.5 %)	91 (38.6 %)	0.643	9 (39.1 %)	37 (34.3 %)	0.552
Irregular margins	42 (51.2 %)	124 (52.5 %)	0.836	14 (60.9 %)	61 (56.5 %)	0.848

Volume is calculated by multiplying AP(cm) × transverse(cm) × longitudinal diameter(cm) × π/6

AUS atypia of undetermined significance, *FLUS* follicular lesion of undetermined significance, *AP/T ratio* Anteroposterior/Tranverse ratio

* $p < 0.05$

by the Bethesda System [3]. In a meta-analysis of 8 studies, Bongiovanni et al. reported that actual risk of malignancy in surgically excised nodules was 6–48 % [39]. In this study, we evaluated the malignancy rate in the AUS/FLUS category. The malignancy incidence for Bethesda Category III was 23.4 % in our institution. In the Bethesda III subcategory, AUS represents AUS with nuclear atypia and FLUS represents AUS with microfollicular architecture. It has been reported that AUS has higher malignancy rate than FLUS (48 and 27 %, respectively) [40]. In present study, malignancy rates were not significantly different between Bethesda III subgroups (25.8 % for AUS and 17.6 % for FLUS). In another study, malignant rates of AUS and FLUS were reported as 42.1 % (64 of 152 patients) and 9.4 %, respectively [9]. In the same study, the authors also reported that all the malignant thyroid nodules with AUS were PTC and mostly classical type of PTC (55 of 64 patients). Rosario et al. demonstrated that the rate of malignancy was lower in nodules initially classified as FLUS (10.8 % with FLUS vs 41.3 % with AUS) [8]. Choi et al. reported that malignancy risk of group AUS/FLUS was 32.4 %, and malignancy in group AUS (65 %) was significantly higher than that of group FLUS (14.3 %) [37]. In the same study, authors demonstrated that among the patients with malignancy, occurrence of PTC was significantly higher in Group AUS, whereas follicular carcinoma was higher in Group FLUS, and all PTCs were classical types. Similarly, in our study, PTC constituted the 86.7 % of all malignant thyroid nodules. In addition, we found that PTC, mostly classical type, was higher in nodules with AUS and follicular carcinoma was more frequent in nodules with FLUS.

High-resolution US is a useful method for the evaluation of thyroid nodule and nodule follow-up. Recent ATA guideline has described sonographic patterns of thyroid nodules according to US features as high, intermediate,

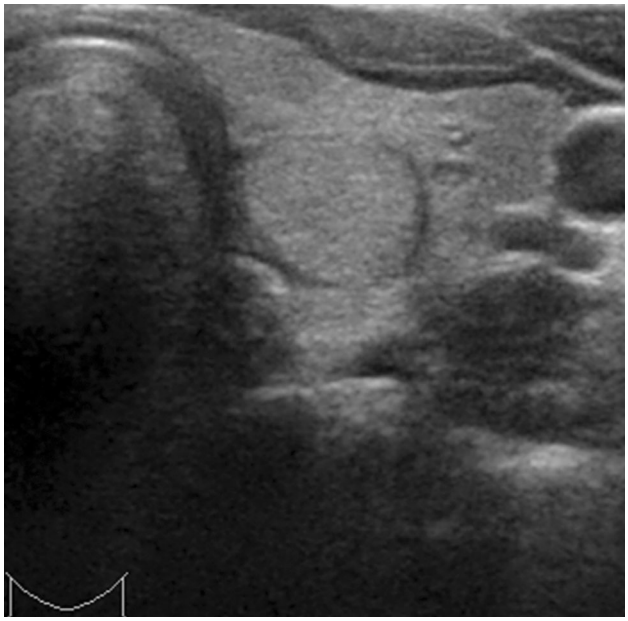

Fig. 1 A 45-year-old woman with a nodule on *left* thyroid lobe evaluated as follicular lesion of undetermined significance (FLUS) in cytology. *Gray scale* ultrasound appearance of nodule in transverse view; solid isoechoic nodule with *oval shape*, smooth margin, and no microcalcification. Final histopathology was evaluated as benign

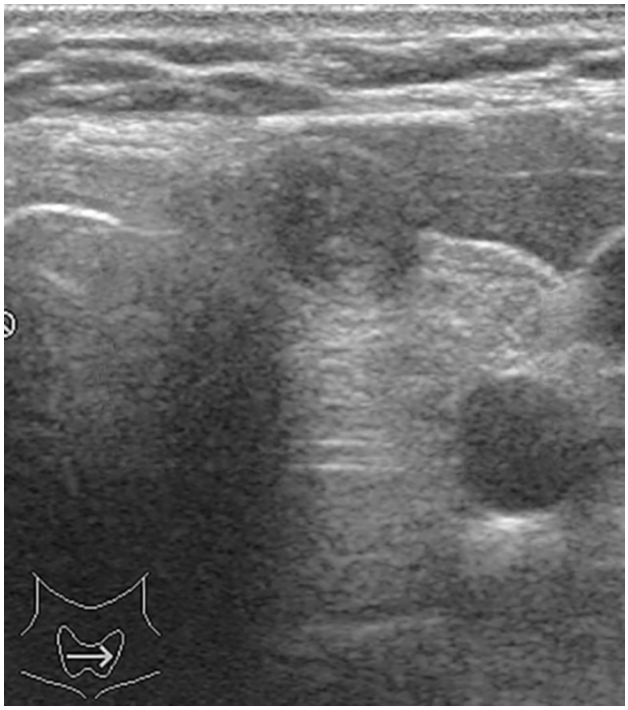

Fig. 2 A 55-year-old woman with a nodule on *left* thyroid lobe evaluated as atypia of undetermined significance (AUS) in cytology. *Gray scale* ultrasound appearance of nodule in transverse view; solid hypoechoic nodule with higher anteroposterior/transverse (AP/T) ratio, irregular margin, and microcalcifications. Final histopathology was evaluated as papillary thyroid carcinoma

low, very low suspicion for malignancy, and benign [26]. Malignancy rates for these groups have been reported as >70–90 %, 10–20 %, 5–10 %, <3 %, and <1 %, respectively. According to recent ATA guideline, high suspicion US features have been described as follows; solid hypoechoic nodule, presence of irregular margins, microcalcifications, taller than wide shape, evidence of extrathyroidal extension [26]. However, a previous study reported that preoperative US findings might help in decision-making for nodules with indeterminate results [41]. Some investigators demonstrated that US features did not influence the decision of performing surgery in patients with indeterminate cytological results [42]. Rosario reported that US was also useful for predicting malignancy, with sensitivity, specificity, and positive and negative predictive values of 79.4, 90.5, 71, and 93.75 %, respectively [8]. Lee et al. demonstrated that hypoechogenicity, marked hypoechogenicity, and malignant US diagnosis were predictive factors of malignancy [9]. In another study, predictive features of malignancy were reported as hypoechogenicity in the AUS group and peripheral vascularization in the FLUS group [10]. In contrary, Rago et al. reported that the malignancy rate in nodules with indeterminate cytology was 21.8 %, and they also demonstrated that gray-scale US alone was insufficient for distinguishing between malignant and benign lesions [43].

Microcalcification is the most specific diagnostic indicator of thyroid malignancy especially in PTC [44]. However, coexistence of macro- and micro-calcification has been found as associated with the same malignancy risk as microcalcifications alone, presence of intranodular macrocalcificaion alone has not been found as consistently associated with thyroid cancer [45, 46]. In the literature regarding AUS/FLUS cytology, however presence of microcalcification was found more frequent in histopathologically malignant nodules compared to benign ones, macrocalcification was reported in similar rates [9, 30]. In our study, we found that higher AP/T ratio, hypoechogenicity, microcalcification, coexistence of micro- and macro-calcification were significantly more prevalent in malignant group. After discrimination of the AUS and FLUS groups, we found similar results in AUS group but not in FLUS group. Finally, in multivariate analysis AP/T ratio ≥0.81 and microcalcification was found as significantly associated with malignancy. In present study, we found that most specific US finding for prediction of malignancy was microcalcification. Specificity of AP/T ratio ≥0.81 was 61.6 %. Furthermore, nodules with microcalcification and AP/T ratio ≥0.81 showed 91.1 % specificity for malignancy. As in present study, combination of several US findings can be used in order to increase diagnostic accuracy of US. Ozel et al. evaluated the

Table 7 Multiple logistic analysis of predictive factors for malignancy in thyroid nodules with AUS/FLUS on preoperative FNAB

Characteristic	*p*	Odds ratio	95.0 % CI	
			Lower	Upper
Age	0.614	1.005	0.986	1.024
Presence of HT in histopathology	0.194	1.379	0.849	2.239
Presence of halo	0.398	1.226	0.765	1.966
AP/T ratio	0.030*	0.307	0.105	0.892
Microcalcification	0.003*	2.087	1.291	3.375
Nodule size <1 cm and AP/T ratio ≥0.81	0.002*	8.437	2.118	33.607
Nodule size ≥1 cm and microcalcification	0.008*	1.979	1.196	3.273

AUS/FLUS atypia of undetermined significance/follicular lesion of undetermined significance, *HT* Hashimoto's thyroiditis, *AP/T ratio* Anteroposterior/Transverse ratio, *FNAB* fine-needle aspiration biopsy, *CI* Confidence interval

* $p < 0.05$

diagnostic accuracy of US in differentiating benign and malignant nodules, and they determined cut-off values of US score (different numbers of the sonographic features) for nodules larger and smaller than 1 cm as two and three, respectively. In the last study, calculated specificities were 94.9 % for nodules ≤1 cm and 91.5 % for nodules >1 cm in size [47]. We evaluated the association between US features and malignancy according to nodule size such as <1 cm and ≥1 cm in size. We demonstrated that in Bethesda Category III nodules <1 cm in size, malignancy was independently associated with AP/T ratio ≥0.81. The calculated sensitivity and specificity were 78.9 and 69.2 %, respectively. Furthermore, we found that malignancy was significantly associated with microcalcification in nodules ≥1 cm in size. Microcalcification in nodules with ≥1 cm had a sensitivity and specificity of 39 and 74.9 %, respectively. TI-RADS can be used for the risk stratification of thyroid nodules using one or more suspicious US features [28]. Yoon et al. evaluated the clinical significance of TI-RADS in AUS and FLUS subcategories, and found that malignancy rates in TI-RADS category 3, 4a, 4b, 4c, and 5 were 15.4, 22.2, 33.3, 57.1, and 80 % ($p < 0.001$) in AUS nodules and 40, 50, 23.5, 22.2, and 0.0 % ($p = 0.414$) in FLUS nodules, respectively [48]. We evaluated the overall study population according to the TI-RADS category 3, 4a, 4b, 4c, and 5, and found malignancy rates as 0.0, 23.6, 17.2, 30.4, and 100 % ($p = 0.010$), respectively.

In the literature, there has been conflicting data concerning association between nodule size and cancer risk assessment. Some studies have reported that nodule size is not associated with malignancy rate [31–33]. Whereas, Mehta et al. reported the absence of carcinoma in mutation-negative nodules <1.85 cm, and demonstrated a linear increase in risk for larger nodules with AUS/FLUS [13]. In another study with a large series of indeterminate or suspicious nodules, a threshold effect was detected at a nodule diameter of approximately 2.5 cm, and it was reported that every 1 cm increase in nodule size causes an increase in the malignancy risk up to 39 % [49]. Lee et al. reported that there was no difference in nodule size between benign and malignant thyroid nodules [9]. Similarly, Rosario demonstrated that nodule size was not a predictor of malignancy [8]. In our study, we also found no difference in nodule size and nodule volume between malignant and benign nodules.

Gender or age does not seem to be a predictor of carcinoma in AUS/FLUS nodules [8, 9, 13, 31–33]. It has been suggested that TSH levels of larger serum concentrations are associated with an increased risk of malignancy in patients with thyroid nodules. However, no difference in TSH levels was detected between patients with benign and malignant disease in a previous study or in another study specifically evaluating patients with AUS/FLUS cytology [8, 31]. Similarly in our study, no difference was found between malignant and benign groups concerning age, gender, and TSH levels.

The linkage between lower thyroid hormones and thyroid malignancy is a novel finding. In the literature, there has been a few controversial data about this topic. Jonklaas et al. reported a relationship between low total T3 level and thyroid cancer [50]. Gul et al. demonstrated that even in normal ranges, higher serum TSH, additionally lower serum fT3 and fT4, are risk factors for the prediction of thyroid malignancy [51]. However, Fiore et al. did not find a linkage between serum thyroid hormones and thyroid cancer [52]. T3 is a strong regulator of gene expression via its action on nuclear receptors [53]. It is likely that lower T3 levels might be a determinant of cellular dedifferentiation [54]. In this study, we also found decreased fT3 levels in patients with malignant histopathology, but we did not find any difference in fT4 levels between benign and malignant groups.

There has been also conflicting data concerning association between thyroid malignancy and HT. Anil et al. have reported that HT is not associated with increased risk of thyroid cancer in patients with thyroid nodules on the

basis of the cytopathological criteria [24]. In contrast, Gul et al. have suggested that there is an association between HT and thyroid cancer [25]. They found that the malignancy rates were 45.7 % in patients with HT and 29 % in patients without HT ($p = 0.001$). The same authors also reported that the prevalence of HT in the patients with thyroid cancer was 21.8 % and in patients without thyroid cancer was 11.9 % ($p = 0.001$). In a recent study, it has been reported that HT does not affect ultrasonographical, cytological, and histopathological features in patients with PTC when they evaluated the 919 PTC patients with and without HT [55]. A recent meta-analysis has evaluated the 10648 PTC patients and showed that PTC is significantly associated with pathologically confirmed HT [56]. However, we found that anti-TPO and anti-Tg antibody positivity were significantly higher in malignant group compared to benign group in patients with AUS/FLUS cytology. We did not find any association between histopathological HT and increased malignancy risk in multivariate analysis.

This study has several limitations. First, the study design was retrospective and we selected patients who had undergone thyroid surgery. Thus, the rate in this study may not give better information about malignancy because of the patient selection bias. Second limitation was the lack of determination of molecular markers. As the Bethesda Category III is accepted as most heterogenous group for thyroid nodules in cytology, complementary methods can be used in the management decision. Although molecular tests can be insufficient to define appropriate management, they can be useful in defining the nature of nodule. In our institution, the molecular tests have not been used for the evaluation of indeterminate nodules. According to Bethesda classification, most of the patients with category III cytology have been advised to follow-up without operation. In present study, we only reviewed the ultrasonographic findings of AUS/FLUS patients who underwent operation. We did not evaluate the US features of patients who were followed-up. So, our study population cannot reflect the overall patients with AUS/FLUS cytology. We could not obtain the color Doppler US data of most patients. In present study, vascularisation pattern could not be evaluated for AUS/FLUS nodules.

In conclusion, the risk of malignancy in AUS/FLUS thyroid nodules is higher than that defined by the BSRTC. Thus, biochemical and ultrasonographic evaluation can be the most important complementary methods in the management of thyroid nodules with AUS/FLUS cytology. In clinical practice, coexistence of microcalcification and higher AP/T ratio can be accepted as highly suspicious US findings for malignancy in cases with AUS/FLUS nodules. It seems that particularly in AUS subgroup of Bethesda Category III nodules with higher AP/T ratio and microcalcification, surgery might be the first therapeutic option instead of repeat FNAB or observation. Presence of HT does not seem as a risk factor for malignancy in AUS/FLUS nodules but this must be investigated in further studies.

Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest.

References

1. H. Gharib, J.R. Goellner, D.A. Johnson, Fine-needle aspiration cytology of the thyroid: a 12 year experience with 11,000 biopsies. Clin. Lab. Med. **13**, 699–709 (1993)
2. H. Gharib, J.R. Goellner, Fine-needle aspiration biopsy of the thyroid: an appraisal. Ann. Intern. Med. **118**, 282–289 (1993)
3. E.S. Cibas, S.Z. Ali, The Bethesda system for reporting thyroid cytopathology. Am. J. Clin. Pathol. **132**, 658–665 (2009)
4. S.Z. Ali, E.S. Cibas, *The Bethesda system for reporting thyroid cytopathology* (Springer, New York, 2010)
5. M. Bongiovanni, J.F. Krane, E.S. Cibas, W.C. Faquin, The atypical thyroid fine-needle aspiration: past, present, and future. Cancer. Cytopathol. **120**, 73–86 (2012)
6. E.S. Cibas, S.Z. Ali, The Bethesda system for reporting thyroid cytopathology. Thyroid. **19**, 1159–1165 (2009)
7. A.S. Ho, E.E. Sarti, K.S. Jain, H. Wang, I.J. Nixon, A.R. Shaha, J.P. Shah, D.H. Kraus, R. Ghossein, S.A. Fish, R.J. Wong, O. Lin, L.G. Morris, Malignancy rate in thyroid nodules classified as Bethesda category III (AUS/FLUS). Thyroid. **24**, 832–839 (2014)
8. P.W. Rosario, Thyroid nodules with atypia or follicular lesions of undetermined significance (Bethesda Category III): importance of ultrasonography and cytological subcategory. Thyroid. **24**, 1115–1120 (2014)
9. K.H. Lee, J.H. Shin, Y.L. Oh, S.Y. Hahn, Aytpia of undetermined significance in thyroid fine-needle aspiration cytology: prediction of malignancy by US and comparison of methods for further management. Ann. Surg. Oncol. **21**, 2326–2331 (2014)
10. N. Çuhaci, D. Arpaci, R. Üçler, A.K. Yazgan, G. Kıyak, S. Yalçin, P.E. Ersoy, G. Güler, R. Ersoy, B. Çakir, Malignancy rate of thyroid nodules defined as follicular lesion of undetermined significance and atypia of undetermined significance in thyroid pathology and its relation with ultrasonographic features. Endocr. Pathol. **25**, 248–256 (2014)
11. N.P. Ohori, K.E. Schoedel, Variability in the atypia of undetermined significance/follicular lesion of undetermined significance diagnosis in the Bethesda system for reporting thyroid cytopathology: sources and recommendations. Acta. Cytol. **55**, 492–498 (2011)
12. P.A. VanderLaan, E. Marqusee, J.F. Krane, Clinical outcome for atypia of undetermined significance in thyroid fine-needle aspirations: should repeated fna be the preferred initial approach? Am. J. Clin. Pathol. **135**, 770–775 (2011)
13. R.S. Mehta, S.E. Carty, N.P. Ohori, S.P. Hodak, C. Coyne, S.O. LeBeau, M.E. Tublin, M.T. Stang, J.T. Johnson, K.L. McCoy, M.N. Nikiforova, Y.E. Nikiforov, L. Yip, Nodule size is an independent predictor of malignancy in mutation-negative nodules with follicular lesion of undetermined significance cytology. Surgery. **154**, 730–736 (2013)
14. E.K. Alexander, G.C. Kennedy, Z.W. Baloch, E.S. Cibas, D. Chudova, J. Diggans, L. Friedman, R.T. Kloos, V.A. LiVolsi, S.J. Mandel, S.S. Raab, J. Rosai, D.L. Steward, P.S. Walsh, J.I.

Wilde, M.A. Zeiger, R.B. Lanman, B.R. Haugen, Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N. Engl. J. Med. **367**, 705–715 (2012)
15. D. Vriens, J.H. de Wilt, G.J. van der Wilt, R.T. Netea-Maier, W.J. Oyen, L.F. de Geus-Oei, The role of [18F]-2-fluoro-2-deoxy-D-glucose-positron emission tomography in thyroid nodules with indeterminate fine-needle aspiration biopsy: systematic review and meta-analysis of the literature. Cancer. **117**, 4582–4594 (2011)
16. V. Cantisani, P. Maceroni, V. D'Andrea, G. Patrizi, M. Di Segni, C. De Vito, H. Grazhdani, A.M. Isidori, E. Giannetta, A. Redler, F. Frattaroli, L. Giacomelli, G. Di Rocco, C. Catalano, F. D'Ambrosio, Strain ratio ultrasound elastography increases the accuracy of colour-Doppler ultrasound in the evaluation of Thy-3 nodules. A bi-centre university experience. Eur. Radiol. (2015). doi:10.1007/s00330-015-3956-0
17. N. Hayashi, N. Tamaki, J. Konishi, Y. Yonekura, M. Senda, K. Kasagi, K. Yamamoto, Y. Iida, T. Misaki, K. Endo, K. Torizuka, T. Mori, Sonography of Hashimoto thyroiditis. J. Clin. Ultrasound **14**, 123–126 (1986)
18. R. Gutekunst, W. Hafermann, T. Mansky, P.C. Scriba, Ultrasonography related to clinical and laboratory findings in lymphocytic thyroiditis. Acta. Endocrinol. (Copenh). **121**, 129–135 (1989)
19. M.E. Dailey, S. Lindsay, R. Skahen, Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland. AMA. Arch. Surg. **70**, 291–297 (1955)
20. R.N. Hirabayashi, S. Lindsay, The relation of thyroid carcinoma and chronic thyroiditis. Surg. Gynecol. Obstet. **121**, 243–252 (1965)
21. D.R. Maceri, M.J. Sullivan, K.D. McClatchney, Autoimmune thyroiditis: pathophysiology and relationship to thyroid cancer. Laryngoscope. **96**, 82–86 (1986)
22. C. Cipolla, L. Sandonato, G. Graceffa, S. Fricano, A. Torcivia, S. Vieni, S. Latteri, M.A. Latteri, Hashimoto thyroiditis coexistent with papillary thyroid carcinoma. Am. Surg. **71**, 874–878 (2005)
23. M.L. Shih, J.A. Lee, C.B. Hsieh, J.C. Yu, H.D. Liu, E. Kebebew, O.H. Clark, Q.Y. Duh, Thyroidectomy for Hashimoto's thyroiditis: complications and associated cancers. Thyroid. **18**, 729–734 (2008)
24. C. Anil, S. Goksel, A. Gursoy, Hashimoto's thyroiditis is not associated with increased risk of thyroid cancer in patients with thyroid nodules: a single-center prospective study. Thyroid. **20**, 601–606 (2010)
25. K. Gul, A. Dirikoc, G. Kiyak, P.E. Ersoy, N.S. Ugras, R. Ersoy, B. Cakir, The association between thyroid carcinoma and Hashimoto's thyroiditis: the ultrasonographic and histopathologic characteristics of malignant nodules. Thyroid. **20**, 873–878 (2009)
26. B.R. Haugen, E.K. Alexander, K.C. Bible, G.M. Doherty, S.J. Mandel, Y.E. Nikiforov, F. Pacini, G.W. Randolph, A.M. Sawka, M. Schlumberger, K. Schuff, S.I. Sherman, J.A. Sosa, D.L. Steward, R.M. Tuttle, L. Wartofsky, American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. (2015). doi:10.1089/thy.2015.0020
27. I. Kholova, M. Ludvıkova, Thyroid atypia of undetermined significance or follicular lesion of undetermined significance: an indispensable Bethesda 2010 diagnostic category or waste carbage? Acta. Cytol. **58**, 319–329 (2014)
28. J.Y. Park, H.J. Lee, H.W. Jang, H.K. Kim, J.H. Yi, W. Lee, S.H. Kim, A proposal for a thyroid imaging reporting and data system for ultrasound features of thyroid carcinoma. Thyroid. **19**, 1257–1264 (2009)
29. J.Y. Kwak, K.H. Han, J.H. Yoon, H.J. Moon, E.J. Son, S.H. Park, H.K. Jung, J.S. Choi, B.M. Kim, E.K. Kim, Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. Radiology. **260**, 892–899 (2011)
30. H.M. Gweon, E.J. Son, J.H. Youk, J.A. Kim, Thyroid nodules with Bethesda system III cytology: Can ultrasonography guide the next step? Ann. Surg. Oncol. **20**, 3083–3088 (2013)
31. W.S. Yoo, H.S. Choi, S.W. Cho, J.H. Moon, K.W. Kim, H.J. Park, S.Y. Park, S.I. Choi, S.H. Choi, S. Lim, K.H. Yi, J. do Park, H.C. Jang, Y.J. Park, The role of ultrasound findings in the management of thyroid nodules with atypia or follicular lesions of undetermined significance. Clin. Endocrinol. (Oxf). **80**, 735–742 (2014)
32. R. Carr, B. Ustun, D. Chhieng, K. Schofield, C. Theoharis, L. Hammers, A.J. Adeniran, Radiologic and clinical predictors of malignancy in the follicular lesion of undetermined significance of the thyroid. Endocr. Pathol. **24**, 62–68 (2013)
33. S.W. Lee, H.J. Lee, H.J. Kim, J. Lee, J.Y. Park, S.H. Kim, J. Kim, Combined categorical reporting systems of US and cytology findings for thyroid nodules: guidance on repeat fine-needle aspiration cytology. Radiology. **266**, 956–963 (2013)
34. Z.W. Baloch, V.A. LiVolsi, S.L. Asa, J. Rosai, M.J. Merino, G. Randolph, P. Vielh, R.M. DeMay, M.K. Sidawy, W.J. Frable, Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. Diagn. Cytopathol. **36**, 425–437 (2008)
35. National Comprehensive Cancer Network (NCCN). NCCN Clinical practice Guidelines in Oncology: Thyroid Carcinoma version2. NCCN, Fort Washington, PA. Available at: www.nccn.org (2013)
36. P.W. Rosario, L.S. Ward, G.A. Carvalho, H. Graf, R.M. Maciel, L.M. Maciel, A.L. Maia, M. Vaisman, Thyroid nodules and differentiated thyroid cancer: Update on the Brazilian consensus. Arq. Bras. Endocrinol. Metabol. **57**, 240–264 (2013)
37. Y.J. Choi, J.H. Baek, E.J. Ha, H.K. Lim, J.H. Lee, J.K. Kim, D.E. Song, Y.K. Shong, S.J. Hong, Different risk of malignancy and management recommendations in subcategories of thyroid nodules with atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS): The role of US-guided core-needle biopsy (CNB). Thyroid. **24**, 494–501 (2014)
38. D.G. Na, J.H. Kim, J.Y. Sung, J.H. Baek, K.C. Jung, H. Lee, H. Yoo, Core- needle biopsy is more useful than repeat fine-needle aspiration in thyroid nodules read as nondiagnostic or atypia of undetermined significance by the Bethesda System for reporting thyroid cytopathology. Thyroid. **22**, 468–475 (2012)
39. M. Bongiovanni, A. Spitale, W.C. Faquin, L. Mazzucchelli, Z.W. Baloch, The Bethesda system for reporting thyroid cytopathology: a meta-analysis. Acta. Cytol. **56**, 333–339 (2012)
40. M.T. Olson, D.P. Clark, Y.S. Erozan, S.Z. Ali, Spectrum of risk of malignancy in subcategories of atypia of undetermined significance. Acta. Cytol. **55**, 518–525 (2011)
41. K.T. Park, S.H. Ahn, J.H. Mo, Y.J. Park, J. do Park, S.I. Choi, S.Y. Park, Role of core needle biopsy and ultrasonographic finding in the management of indeterminate thyroid nodules. Head Neck **33**, 160–165 (2011)
42. S.S. Nagarkatti, W.C. Faquin, C.C. Lubitz, D.M. Garcia, G. Barbesino, D.S. Ross, R.A. Hodin, G.H. Daniels, S. Parangi, Management of thyroid nodules with atypical cytology on fine-needle aspiration biopsy. Ann. Surg. Oncol. **20**, 60–65 (2013)
43. T. Rago, M. Scutari, F. Santini, V. Loiacono, P. Piaggi, G. Di Coscio, F. Basolo, P. Berti, A. Pinchera, P. Vitti, Real-time elastosonography: useful tool for refining the presurgical diagnosis in thyroid nodules with indeterminate or nondiagnostic cytology. J. Clin. Endocrinol. Metab. **95**, 5274–5280 (2010)
44. P. Trimboli, N. Nasrollah, S. Amendola, F. Rossi, G. Ramacciato, F. Romanelli, P. Aurella, A. Crescenzi, O. Laurenti, E.

Condorelli, C. Ventura, S. Valabrega, Should we use ultrasound features associated with papillary thyroid cancer in diagnosing medullary thyroid cancer? Endocr. J. **59**, 503–508 (2012)
45. J.Y. Kwak, K.H. Han, J.H. Yoon, H.J. Moon, E.J. Son, S.H. Park, H.K. Jung, J.S. Choi, B.M. Kim, E.K. Kim, Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. Radiology. **260**, 892–899 (2011)
46. H.J. Moon, J.M. Sung, E.K. Kim, J.H. Yoon, J.H. Youk, J.Y. Kwak, Diagnostic performance of gray-scale US and elastography in solid thyroid nodules. Radiology. **262**, 1002–1013 (2012)
47. A. Ozel, S.M. Erturk, A. Ercan, B. Yılmaz, T. Basak, V. Cantisani, M. Basak, Z. Karpat, The diagnostic efficiency of ultrasound in characterization for thyroid nodules: how many criteria are required to predict malignancy? Med. Ultrason. **14**, 24–28 (2012)
48. J.H. Yoon, H.J. Kwon, E.K. Kim, H.J. Moon, J.Y. Kwak, Subcategorization of atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS): a study applying Thyroid Imaging Reporting and Data System (TIRADS). Clin. Endocrinol. (2016). doi:10.1111/cen.12987
49. N.D. Banks, J. Kowalski, H.L. Tsai, H. Somervell, R. Tufano, A.P. Dackiw, M.R. Marohn, D.P. Clark, C.B. Umbricht, M.A. Zeiger, A diagnostic predictive model for indeterminate or suspicious thyroid FNA samples. Thyroid. **18**, 933–941 (2008)
50. J. Jonklaas, H. Nsouli-Maktabi, S.J. Soldin, Endogenous thyrotropin and triiodothyronine concentrations in individuals with thyroid cancer. Thyroid. **18**, 943–952 (2008)
51. K. Gul, D. Ozdemir, A. Dirikoc, A. Oguz, D. Tuzun, H. Baser, R. Ersoy, B. Cakir, Are endogenously lower serum thyroid hormones new predictors for thyroid malignancy in addition to higher serum thyrotropin? Endocrine. **37**(2), 253–260 (2010)
52. E. Fiore, T. Rago, M.A. Provenzale, M. Scutari, C. Ugolini, F. Basolo, G. Di Coscio, P. Berti, L. Grasso, R. Elisei, A. Pinchera, P. Vitti, Lower levels of TSH are associated with a lower risk of papillary thyroid cancer in patients with thyroid nodular disease: thyroid autonomy may play a protective role. Endocr. Relat. Cancer **16**, 1251–1260 (2009)
53. J. Zhang, M.A. Lazar, The mechanism of action of thyroid hormones. Annu. Rev. Physiol. **62**, 439–466 (2000)
54. E.L. de Souza Meyer, J.M. Dora, M.S. Wagner, A.L. Maia, Decreased type 1 iodothyronine deiodinase expression might be an early and discrete event in thyroid cell dedifferentation towards papillary carcinoma. Clin. Endocrinol. **62**, 672–678 (2005)
55. H. Baser, D. Ozdemir, N. Cuhaci, C. Aydin, R. Ersoy, A. Kilicarslan, B. Cakir, Hashimoto's thyroiditis does not affect ultrasonographical, cytological, and histopathological features in patients with papillary thyroid carcinoma. Endocr. Pathol. **26**, 356–364 (2015)
56. J.H. Lee, Y. Kim, J.W. Choi, Y.S. Kim, The association between papillary thyroid carcinoma and histologically proven Hashimoto's thyroiditis: a meta-analysis. Eur. J. Endocrinol. **168**, 343–349 (2013)