



# Ultrasonographic Characteristics of Thyroid Nodules in Predicting the Malignancy and the Role of the Cytopathologist

## Maligniteyi Öngörmede Tiroid Nodüllerinin Ultrasonografik Özellikleri ve Sitopatoloğun Rolü

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### ABSTRACT

**Aim:** It is very important to determine the correct nodules which should be evaluated with thyroid fine-needle aspiration biopsy (FNAB) and avoid unnecessary operations. In this study, we aimed to analyze the relationship and the compatibility between thyroid FNAB results with ultrasonographic features and the role of the pathologist who examines the specimens.

**Material and Methods:** 458 patients with nodules who underwent FNAB were included in the study. The relationships between the ultrasonographic features (echogenicity, shape, margin, vascularization and calcification) and biopsy results and, the factor of the pathologist were evaluated retrospectively. Since there was no Bethesda VI (malignant) result, analyzes were made among 5 subgroups. FNAB procedures were considered as, inadequate or nondiagnostic, benign, atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), follicular neoplasia and suspicion of malignancy.

**Results:** Solid internal structure, irregular margin, hypoechogenicity, presence of microcalcification, nodules with taller than wide (AP> T) were found significantly different between the subgroups of Bethesda classification system ( $p<0.01$ , for all features). All suspicious features were found more common in the malignant suspicious nodules than the benign nodules. 63 of 211 preparations (29.8%), which were evaluated as Bethesda I and Bethesda III, were reported by a single pathologist, and statistically significant differences were found in comparison to the other pathologists ( $p<0.01$ ).

**Conclusion:** Ultrasonography features that include malignancy risk correlate with cytopathology results. The incidence of thyroid nodules is gradually increasing and therefore, it is important to perform biopsies according to ultrasonographic risk stratification. Moreover, working with a thyroid specialist pathologist may minimize the indeterminate results.

**Keywords:** Thyroid nodule, Ultrasound imaging, Aspiration biopsy, Fine-needle

### ÖZ

**Amaç:** Tiroid ince iğne aspirasyon biyopsisi ile değerlendirilmesi gereken doğru nodülleri belirlemek ve gereksiz işlemlerden kaçınmak çok önemlidir. Bu çalışmada tiroid ince iğne aspirasyon biyopsi sonuçları ile ultrasonografik özellikler arasındaki ilişkiyi ve patoloğun rolünü incelemeyi amaçladık.

**Gereç ve Yöntemler:** Çalışmaya tiroid ince iğne aspirasyon biyopsisi yapılan 458 hasta dahil edildi. Ultrasonografik özellikler (ekojenite, şekil, vaskularizasyon, sınır ve kalsifikasyon) ve biyopsi sonuçları ile patoloğ faktörü arasındaki ilişki retrospektif olarak değerlendirildi. Bethesda VI (malign) sonucu olmadığı için 5 alt grup arasında analizler yapıldı. İİAB işlemleri patoloji sonuçları açısından yetersiz, benign, önemi belirsiz atipi/önemi belirsiz folliküler lezyon, foliküler neoplazi ve malignite şüphesi olarak değerlendirilmiştir.



**Bulgular:** Düzensiz sınır, hipoekojenite, mikrokalsifikasyon varlığı, ön-arka çapın transvers çapından büyük olması (AP> T) Bethesda alt grupları arasında istatistiksel olarak farklı bulundu. (ekojenite için  $p<0.05$ , diğerleri için  $p<0.01$ ). Bethesda I ve Bethesda III olarak değerlendirilen 211 preparatın 63'ü (% 29.8) aynı patolog tarafından rapor edilmiş ve diğer patolojlara göre istatistiksel olarak anlamlı farklılık bulunmuştur ( $p<0.01$ ).

**Sonuç:** Ultrasonografik olarak malignite riski içeren özellikler sitopatoloji sonuçları ile korelasyon göstermektedir. Tiroid nodüllerinin görülme sıklığı giderek artmaktadır, bu nedenle ultrasonografik risk sınıflandırmasına göre biyopsi yapılması önemlidir. Ayrıca tiroid spesifik patoloji ile çalışmak indeterminate sonuçları en aza indirebilir.

**Anahtar Sözcükler:** Tiroid nodülü, Ultrasonografi, Aspirasyon biyopsisi, İnce iğne

### INTRODUCTION

Although most thyroid nodules show benign features, thyroid gland carcinomas are the most common malignancies in the endocrine system. According to the United States cancer database system (SEER), the incidence of papillary thyroid carcinoma was 4.8 per 100000 in 1975 and 14.9 per 100000 in 2012. Early diagnosis is important because thyroid malignancies mostly have a slow progression, and survival rates are high with appropriate treatment. For this reason, all nodules should be examined for ultrasonographic features and evaluated histopathologically in the case of suspected ultrasonography (US) findings. None of the ultrasonographic features predicts or excludes malignancy by 100% percent. However, the presence of 2 or more suspicious sonographic findings increases the risk of malignancy (1). The most convenient and reliable method that can reveal the histopathological feature of the nodule is thyroid fine-needle aspiration biopsy (FNAB). Performance of biopsy by an experienced clinician with US guidance and evaluation of samples by an experienced cytopathologist increase the diagnostic value. Retrospective studies have shown that, in comparison to palpation, both the false negativity and the non-diagnostic rates are much lower in FNAB performed with US guidance (2).

In this study, we aimed to retrospectively analyze the relationship and the compatibility between thyroid FNAB results and ultrasonographic features with the recommendation of the guidelines and the relationship between cytological results and role of pathologists in a single center.

### MATERIALS and METHODS

Four hundred and fifty-eight patients who underwent FNAB on their thyroid nodules between June 2015 and October 2017 at the Endocrinology Clinic of Samsun Research and Training Hospital were included in the study. This study was conducted in accordance with the declaration of Helsinki, and the protocol for this retrospective analysis was approved by the Local Ethics Committee (Ondokuz Mayıs University, Clinical Research Ethics Committee, 09.07.2020, 2020/462 decision number). The data of the patients were evaluated retrospectively. In all patients, nodule evaluation

was performed with the high-resolution probe (13-5 MHz, linear) of the same ultrasonography device (Siemens Antares). Based on ultrasonography; solid internal structures, irregular borders, hypoechogenicity, presence of microcalcification and nodules with taller than wide (AP>T) were accepted as suspicious features (3).

Three dimensions were measured, and the largest diameter in millimeters was taken into consideration. The echogenicity of the nodules were compared to the surrounding thyroid parenchyma and classified as isoechoic, hypoechoic, hyperechoic and mixed. Mixed echoic nodules were evaluated based on their internal solid components. The echogenicity of the solid portion was categorized as hyperechoic (for nodules showing hyperechogenicity compared to the normal thyroid), isoechoic (for nodules showing isoechogenicity compared to the normal thyroid), or hypoechoic (for nodules showing hypoechogenicity compared to the normal thyroid). The composition of the nodules was classified as solid, cystic, predominantly solid ( $\leq 50\%$  cystic components), predominantly cystic lesions ( $< 50\%$  solid components), or spongiform. The margin was classified as regular or irregular, while calcification was classified as microcalcification, macrocalcification or no calcification. Vascularization was classified as absent vascularity (type 1), perinodular vascularity greater than intranodular vascularity (type 2) or intranodular vascularity greater than peripheral vascularity (type 3). The feature of AP>T was indicated as present or absent.

Fine-needle aspiration biopsies were performed by applying vacuum with a 22-gauge needle. Air-dried specimens were taken for cytopathological examination. The specimens were randomly examined by 7 different pathologists (1st Pathologist, 2nd Pathologist, 3rd Pathologist, 4th Pathologist, 5th Pathologist, 6th Pathologist, 7th Pathologist) according to their working schedule. The results were reported in six different categories according to the Bethesda classification system (4). Bethesda I: nondiagnostic or inadequate, Bethesda II: benign, Bethesda III: atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), Bethesda IV: follicular neoplasm or suspicious for follicular neoplasm, Bethesda V: suspicious for malignancy, Bethesda VI: malignant.

## Statistical Analysis

The Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the data of the study, descriptive statistical methods (Mean, Standard Deviation, Median, Frequency, Rate, Minimum, Maximum) were used, and the distribution of the data was evaluated with Shapiro-Wilk Test. Kruskal-Wallis Test was used for comparison of three or more groups that did not show normal distribution of the quantitative data, ANOVA was used for comparison of three or more groups with normal distribution, and post hoc tests were performed to determine the difference. The qualitative data were analyzed with Chi-squared test. Qualitative data were analyzed with the Chi-square test. Fisher-Exact analysis was used for two groups and Monte-Carlo analysis for multiple groups. Significance was evaluated at  $p < 0.01$  and  $p < 0.05$  levels.

## RESULTS

A total of 458 people, 396 women (86.5%) and 62 men (13.5%), were included in the study. The mean age of the patients was  $52.07 \pm 12.3$  years. The mean nodule size was  $19.48 \pm 7.46$  mm. 225 (49.1%) nodules were located on the right lobe, 211 (46.1%) were located on the left lobe, and 22 (4.8%) were located on the isthmus. 202 nodules were isoechoic (44.1%), 129 nodules were hypoechoic (28.2%), 4 nodules were hyperechoic (0.9%), and 123 nodules were mixed (26.8%). 321 nodules were solid (70.1%), 13 nodules were cystic (2.8%), 118 nodules were mixed (predominantly cystic and predominantly solid) (25.8%), and 6 nodules were detected in a spongiform (1.3%) structure. 442 nodules had regular margins (96.5%), and 16 nodules had irregular margins (3.5%). There were microcalcifications (5%) in 23 nodules and macrocalcifications (4.4%) in 20 nodules. Calcification was not observed in 415 nodules (90.6%). 164 (35.8%) had Type 1, 290 (63.3%) had Type 2 and 4 (0.9%) had Type 3 vascularization patterns. The results are shown in Table 1.

When the FNAB procedures were evaluated in terms of the pathology results, 80 (17.5%) were inadequate, 189 (41.3%) were benign, 131 (28.6%) were AUS/FLUS, 42 (9.2%) were follicular neoplasia, and 16 (3.5%) were found to be suspected of malignancy.

Since there was no Bethesda VI (malignant) result in the FNAB results, analyses were conducted among 5 subgroups. There was no statistically significant difference between the groups in terms of age and sex ( $p = 0.136$ ,  $p = 0.145$ ). There was no statistically significant difference between the groups in terms of the nodule sizes and localizations ( $p=0.072$ ,  $p= 0.085$ ). While irregular margins were detected by ultrasonography in 10 of 58 nodules with AUS/FLUS and suspected malignancy, none of the benign nod-

ules had irregular margins ( $p < 0.001$ ). 75% of the nodules were hypoechoic in the group with suspected malignancy, and 19% were hypoechoic in the benign group ( $p = 0.001$ ;  $p < 0.01$ ). When the Bethesda subgroups were compared in terms of nodule calcification, a statistically significant difference was found between the groups ( $p < 0.01$ ). Microcalcification was detected by 43.8% in the group with suspected malignancy and by 1.6% in the benign group. While none of the benign nodules had AP> T, 12.5% AP> T was found in the malignancy-suspected group ( $p = 0.001$ ;  $p < 0.01$ ). In terms of vascularization, 75% Type 2 and 12.5% Type 3 vascularization results were found in the nodules with suspicion of malignancy, while none of the benign nodules had Type 3 vascularization. The results are shown in Table 2.

A statistically significant difference was found in terms of the results and the pathologists. Bethesda I and Bethesda III that required repeated biopsy were evaluated in terms of the pathologists who examined the results. 32 (40%) of 80 preparations were reported as Bethesda I by the 1st Pathologist, 13 (16.3%) were reported by the 2nd Pathologist, 11 (13.8%) were reported by the 3rd Pathologist, 13 (16.3%) were reported by the 4th Pathologist, 1 (1.3%) was reported by the 5th Pathologist, 8 (10%) were reported by the 6th

**Table 1.** Characteristics of the patients and thyroid nodules

Characteristics	Results	
<b>Age</b>		
Mean±SD	52.07±12.30	
Min-Max (Median)	17-88 (53.0)	
<b>Nodule Size</b>		
Mean±SD	19.48±7.46	
Min-Max (Median)	1-45 (18.0)	
	<b>Cases [n (%)]</b>	
<b>Gender</b>	Female	396 (86.5)
	Male	62 (13.5)
<b>Echogenicity</b>	Isoechoic	201 (44)
	Hypoechoic	129 (28.1)
	Hyperechoic	4 (0.9)
	Mixed	124 (27)
<b>Margin</b>	Regular	442 (96.5)
	Irregular	16 (3.5)
<b>Calcification</b>	Micro	23 (5)
	Macro	20 (4.4)
	No calcification	415 (90.6)
<b>Vascularization</b>	Type 1	164 (35.8)
	Type 2	290 (63.3)
	Type 3	4 (0.9)
<b>AP&gt;T</b>	Present	4 (0.9)
	Absent	454 (99.1)

Pathologist and, 2 (2.5%) were reported by the 7th Pathologist. Among the 131 preparations reported as Bethesda III, 31 (23.7%) were reported by the 1st Pathologist, 11 (8.4%) were reported by the 2nd Pathologist, 15 (11.5%) were reported by the 3rd Pathologist, 18 (13.7%) were reported by the 4th Pathologist, 15 (11.5%) were reported by the 5th Pathologist, 31 (23.7%) were reported by the 6th Pathologist, and 10 (7.6%) were reported by the 7th Pathologist. The results are shown in Table 3.

**DISCUSSION**

In this study, when the high-risk features of US and cytopathology results were compared, a statistically significant relationship was found between hypoechogenicity, irregular margin, microcalcification, and AP> T and malignancy in accordance with the literature. Additionally, a statistically sig-

nificant relationship was found between the reports which required repeated biopsy (Bethesda I and Bethesda III) and the pathologists who examined the preparations.

Fine-needle aspiration biopsy is the most reliable and inexpensive method to evaluate thyroid nodules. In ultrasonography, hypoechogenicity, intranodular vascularization, irregular margin, microcalcification and AP>T are risk factors for malignancy. The solid component is the most sensitive sonographic finding for malignancy. However, its specificity is 52-56% (5). The risk of malignancy increases with a decrease in echogenicity. The marked hypoechoic nodule has 92% specificity and 26-41% sensitivity for malignancy (6,7). In our study, while 75% of the nodules with suspected malignancy were hypoechoic, this rate was found to be 19% in the benign nodules.

**Table 2:** Comparison of Bethesda system subgroups in terms of nodule ultrasonographic characteristics

		Results n (%)					p
		Bethesda I	Bethesda II	Bethesda III	Bethesda IV	Bethesda V	
Gender	Female	63 (15.9)	164 (41.4)	117 (29.5)	39 (9.8)	13 (3.3)	0.145
	Male	17 (27.4)	25 (40.3)	14 (2.,6)	3 (4.8)	3 (4.8)	
Echogenicity	iso	23 (1.4)	109 (54.2)	49 (24.4)	17 (8.5)	3 (1.5)	0.042*
	Hypo	32 (24.8)	36 (27.9)	37 (28.7)	12 (9.3)	12 (9.3)	
	Hyper	3 (75)	1 (25)	0 (0)	0 (0)	0 (0)	
	Mixed	22 (17.7)	43 (34.7)	45 (36.3)	13 (10.5)	1 (0.8)	
Margin	Regular	78 (17.6)	189 (42.8)	127 (28.7)	39 (8.8)	9 (2)	0.001**
	Irregular	2 (12.5)	0 (0)	4(25)	3 (18.8)	7 (43.8)	
Calcification	Micro	3 (13)	3 (13)	6 (26.1)	4 (17.4)	7 (30.4)	0.001**
	Macro	7 (35)	11 (55)	2 (10)	0 (0)	0 (0)	
Vascularization	Type-1	36 (22)	87 (53)	33 (20.1)	6 (3.7)	2 (1.2)	0.001**
	Type-2	43 (14.8)	102 (35.2)	97 (33.4)	36 (12.4)	12 (4.1)	
	Type-3	1 (25)	0 (0)	1 (25)	0 (0)	2 (50)	
AP>TR	Present	1 (25)	0 (0)	1 (25)	0 (0)	2 (50)	0.001**
	Absent	79 (17.4)	189 (41.6)	130 (28.6)	42 (9.3)	14 (3.1)	

Chi-Square Test \*\*p<0.01, \* p<0.05

**Table 3:** Comparison of cytology results in terms of pathologists who evaluate the specimens

	Results n (%)							p
	1st Pathologist	2nd Pathologist	3rd Pathologist	4th Pathologist	5th Pathologist	6th Pathologist	7th Pathologist	
Bethesda I	32 (40)	13 (16.3)	11 (13.8)	13 (16.3)	1 (1.3)	8 (10)	2 (2.5)	0.001
Bethesda II	3 (1.6)	35 (18.5)	27 (14.3)	2 (1.1)	54 (28.6)	12 (6.3)	56 (29.6)	
Bethesda III	31 (23.7)	11 (8.4)	15 (11.5)	18 (13.7)	15 (11.5)	31 (23.7)	10 (7.6)	
Bethesda IV	0 (0)	2 (4.8)	2 (4,8)	9 (21.4)	5 (11.9)	14 (33.3)	10 (23.8)	
Bethesda V	0 (0)	6 (37.5)	3 (18,8)	0 (0)	2 (12,5)	2 (12.5)	3 (18.8)	



In comparison to a non-calcified solid nodule, the risk of cancer is 3 times higher in the presence of microcalcification. In predicting malignancy, microcalcification's specificity is 93%, its positive predictive value is 70%, but its sensitivity is 36% (8). Macrocalcification is less specific for malignancy and is often observed in benign multiple nodules. However, its presence in a solitary nodule may be a finding in favor of malignancy by 75%. In our study, microcalcification was detected in 43.8% of the nodules with suspected malignancy and 1.6% of the nodules with benign results. While 5.8% of the nodules with benign results had macrocalcification, none of the patients with suspected malignancy had macrocalcification. Although the margin interpretations of nodules show subjective differences, the specificity of an irregular margin in predicting malignancy is 83-91.8%, and its sensitivity is 48.3-55% (6,7). The results in this study were also compatible with the literature. While nodules that have a dominantly peripheral vascularization are benign, nodules showing a chaotic intranodular bleeding pattern have a potential to become malignant. The specificity of internal hypervascularization in predicting malignancy is 81%, and its sensitivity is 74% (1). In our study, 75% Type 2 and 12.5% Type 3 vascularization patterns were found in the nodules with suspicion of malignancy, while none of the benign nodules had Type 3 vascularization.

Although fine-needle aspiration biopsy is an important technique in evaluation of nodules, the adequacy of the specimen, sampling technique, the experience and skill of the pathologist may cause differences in evaluations. In the cytology of thyroid lesions, diagnostic variations and disagreements between pathologists are encountered, especially for the diagnosis of follicular lesions and follicular neoplasms. In a study in which preparations diagnosed as follicular lesions were examined by 4 different pathologists, a moderate to significant evaluation difference was detected among the pathologists (9). Seok et al. found that, with examination of thyroid FNAB by thyroid specialist cytopathologists, more accurate results could be obtained in a shorter time (10). In another study, it was found that, if the preparations that were reported as AUS / FLUS were reevaluated with the joint examination of at least five pathologists, there was an optimal level of inter-observer consistency, less indetermination and excellent cytohistological compatibility (11). In a review, it was shown that, if thyroid FNAB samples were evaluated by a second cytopathologist, the diagnostic accuracy increased, and clinical management was potentially changed (12). It has also been described as an important diagnostic method for precise recognition and improved accuracy for biopsies, which were originally defined as "uncertain". In our study, 63 of 211 preparations (29.8%), which were evaluated as insufficient and indeterminate, were reported by a single pathologist, and statistically significant differences were found in comparison to the other pathologists.

There were some strengths and limitations of our study. All thyroid US and FNAB procedures were performed by the same clinician. It eliminated clinician-related differences. Furthermore, all FNAB procedures were performed with US guidance. Although we aimed to compare the diagnostic accuracy of different pathologists, histopathological diagnosis could not be used as the reference method because of missing data of operation materials. This was because most of the operations were performed out of our hospital. Since it was a retrospective study, the same specimens could not be evaluated by each pathologist. Additionally, the number of the samples evaluated by each pathologist was small.

In conclusion, the incidence of thyroid nodules is gradually increasing, and therefore, it is important to perform biopsies based on ultrasonographic risk stratification. Working with specific cytopathologists who will be able to evaluate thyroid biopsies will be an appropriate strategy to prevent unnecessary repeated biopsies and operations.

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#### Ethical Approval

This study was approved by local ethics committee of Ondokuz Mayıs University Clinical Research Ethics Committee (2020/462).

#### Conflicts of Interest

The author E.K.K. has no conflict of interest.

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Idea, design, data and literature review, resource search, article writing, analysis were done by author.

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