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Evaluation of repeat fine-needle aspiration biopsy according to ACR-EU-K TIRADS scores in the management of nodules with Bethesda III (AUS) cytology

Bethesda III (ÖBA) sitolojisi olan nodüllerin yönetiminde ACR-EU-K TIRADS skorlarına göre tekrarlanan ince iğne aspirasyon biyopsisinin değerlendirilmesi

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ABSTRACT

Aim: There is no consensus on the management of nodules with Bethesda III (atypia of undetermined significance [AUS]) cytology. This study aimed to evaluate the diagnostic contribution of repeat fine-needle aspiration biopsy (FNAB) in nodules with AUS and to investigate whether it provides additional value when interpreted together with ultrasound-based risk stratification systems.

Material and Methods: Patients whose initial FNAB results were AUS and who underwent thyroidectomy were included. The nodules were classified into two distinct categories, benign and malignant, based on their histopathological features. Single and repeat FNAB groups were compared, and their distributions according to ACR, EU, and K-TIRADS scores were analyzed. In addition, malignancy rates of repeat FNAB cytology were assessed based on Bethesda categories and TIRADS 4–5 (intermediate-to-high risk) classifications.

Results: While 87 (28.2%) of the nodules that underwent thyroidectomy had undergone a single FNAB before surgery, 222 (71.8%) had undergone repeat FNAB. 35 (40.2%) of the nodules that underwent single FNAB and 106 (47.7%) of the nodules that underwent repeat FNAB were reported as malignant on final histopathology results (p = 0.233). The distributions of ACR, EU, and K-TIRADS scores were generally similar between the groups. The most frequent repeat FNAB result was AUS (50.9%) with a malignancy rate of 40.7%. High malignancy rates were also observed in benign (30.0%) and nondiagnostic (31.2%) repeat FNAB results. A substantial proportion of these subgroups were classified as TIRADS 4–5, where malignancy rates reached 60–70% across the ACR, EU, and K-TIRADS systems.

Conclusion: Repeat FNAB in AUS nodules showed limited diagnostic utility, as malignancy rates remained high regardless of cytological results, particularly in nodules classified as TIRADS 4–5. Our findings suggest that ultrasound-based risk stratification may be more reliable than repeat FNAB alone in guiding clinical decision-making. However, further prospective multicenter studies are needed to validate these results.

Keywords: Atypia of undetermined significance (AUS), repeat FNAB, ACR-TIRADS, EU-TIRADS, K-TIRADS

ÖZ

Amaç: Bethesda III (önemi belirsiz atipi [ÖBA]) sitolojisi olan nodüllerin yönetimi konusunda bir fikir birliği yoktur. Bu çalışmada, ÖBA tanılı nodüllerde tekrarlanan ince iğne aspirasyon biyopsisinin (İİAB) tanısal katkısını değerlendirmek ve ultrasonografi temelli risk sınıflama sistemleri ile birlikte yorumlandığında ek bir değer sağlayıp sağlamadığını araştırmak amaçlanmıştır.

Gereç ve Yöntemler: İlk İİAB sonuçları ÖBA olan ve tiroidektomi uygulanan hastalar çalışmaya dahil edildi. Nodüller, histopatolojik özellikleri temel alınarak benign ve malign olarak iki ayrı sınıfa ayrıldı. Tek ve tekrarlanan İİAB grupları karşılaştırıldı ve ACR, EU ve K-TIRADS skorlarına göre dağılımları incelendi. Ayrıca, tekrarlanan İİAB sitolojisinin malignite oranları Bethesda kategorileri ve TIRADS 4–5 (orta-yüksek risk) sınıfları temelinde analiz edildi.

Bulgular: Tiroidektomi uygulanan nodüllerin 87'sine (%28,2) cerrahi öncesi tek İİAB yapılırken, 222'sine (%71,8) mükerrer İİAB yapılmıştı. Tek İİAB yapılan nodüllerin 35'i (%40,2) ve mükerrer İİAB yapılan nodüllerin 106'sı (%47,7) nihai histopatoloji sonuçlarına göre malign olarak raporlandı (p = 0,233). ACR, EU ve K-TIRADS sınıflamalarına göre tek ve tekrarlanan İİAB grupları arasında dağılımlar genellikle benzerdi. Tekrarlanan İİAB sitolojisi en sık ÖBA idi (%50,9) ve bu nodüllerde malignite oranı %40,7 bulundu. Benign (%30,0) ve nondiagnostik (%31,2) tekrar İİAB sonuçlarında da yüksek malignite oranları saptandı. Bu alt grupların önemli bir kısmı TIRADS 4–5 kategorisinde yer almakta olup, bu nodüllerde malignite oranları ACR, EU ve K-TIRADS sistemlerinde %60–70 düzeyine ulaştı.

Sonuç: ÖBA tanılı nodüllerde tekrarlanan İİAB sınırlı tanısal fayda sağlamaktadır; çünkü sitoloji sonuçlarından bağımsız olarak, özellikle TIRADS 4–5 kategorisinde yer alan nodüllerde malignite oranları yüksek seyretmektedir. Bulgularımız, klinik karar verme sürecinde ultrasonografi temelli risk sınıflamasının, tekrar İİAB'ye kıyasla daha güvenilir olabileceğini göstermektedir. Ancak bu sonuçların doğrulanması için ileriye dönük ve çok merkezli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Önemi belirsiz atipi (ÖBA), İİAB tekrarı, ACR-TIRADS, EU-TIRADS, K-TIRADS

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Highlights

- Repeated FNAB in Bethesda III (AUS) nodules does not alter the malignancy rate, regardless of the ACR-EU-K TIRADS category, indicating limited diagnostic benefit of performing a second biopsy.
- Repeat FNAB in nodules with an initial AUS result may complicate clinical decision-making, as high malignancy rates persist even in benign or nondiagnostic repeat cytology results.
- Ultrasound-based TIRADS scoring appears more reliable than repeat FNAB, and determining treatment based on TIRADS categories may be more useful in guiding management strategies.

INTRODUCTION

Ultrasonography (USG)-guided fine-needle aspiration biopsy (FNAB) is the widely accepted standard diagnostic approach for thyroid nodules worldwide (1). FNAB cytology is reported in six different categories using the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), a standardized reporting system (2). Approximately 20% of thyroid nodules are reported as atypia of undetermined significance (AUS), which is Bethesda category III (Bethesda III), in cytology reports (2 - 3). The risk of malignancy (ROM) of the AUS category varies between 13% and 30% according to the results of previous studies (4). Nevertheless, some studies have reported a markedly higher malignancy rate of 42.5%, even when nonmalignant neoplasms such as non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) are excluded (5). Current guidelines and recent comprehensive studies recommend a variety of clinical approaches for nodules with AUS, including repeat FNAB, molecular testing, follow-up, or diagnostic surgery (1, 4).

USG is a very important tool in the follow-up of thyroid nodules and in determining the next step in management. There are studies in the literature regarding treatment decisions based on suspicious USG features in the management of Bethesda III nodules (6, 7). Suspicious USG features of a thyroid nodule that suggest malignancy are markedly hypoechoic character, anteroposterior (AP) diameter>transverse diameter, irregular margins, and presence of microcalcification (8 - 10). Since none of these USG features alone can reliably predict malignancy, the Thyroid Imaging Reporting And Data System (TIRADS) system was introduced in 2009 by combining various features to increase the diagnostic value of USG (11). In recent years, this system has been improved and European Thyroid Association, American College of Radiology, and Korean-TIRADS (EU-TIRADS, ACR-TIRADS, and K-TIRADS, respectively), which have proven to be reliable in the initial evaluation of thyroid nodules, are widely used (12-14). EU-TIRADS, ACR-TIRADS and K-TIRADS scores are determined according to the size, composition, echotexture, echogenicity, margin regularity, shape and calcification status of the thyroid nodule (12-14).

More recently, advances in artificial intelligence have led to the integration of decision support systems into thyroid nodule management, aiming to improve diagnostic precision and minimize unnecessary procedures (15).

There is no consensus on the approach to Bethesda III nodules. Repeat biopsy is usually the first choice because molecular testing is not available in most institutions. However, there are conflicting results regarding the consequences of repeat FNAB. There are studies suggesting that the incidence of cancer increases, decreases, or is unaffected by repeat FNAB (16-23). Nonetheless, our review of the existing literature indicates that no prior research has examined the implications of selecting repeat FNAB on a nodule-specific basis. Therefore, we aimed to evaluate the performance of each TIRADS scoring system in the decision of repeat FNAB in patients with AUS, and thus to evaluate whether the number of unnecessary FNABs could be reduced. In addition, we aimed to evaluate the performance of ACR-EU-K TIRADS in predicting malignancy.

MATERIAL and METHODS

Patients who were followed up in the Department of Endocrinology and Metabolism of Necmettin Erbakan University (NEU) Faculty of Medicine between January 2018 and January 2024 and whose thyroid USG reports were available, whose initial FNAB result was reported as AUS, and who underwent thyroidectomy were included in the study. The study received approval from the NEU Ethics Committee under approval number 2024/4896, dated 05/04/2024. All patients had previously provided written informed consent for FNAB and surgical procedures as part of routine clinical practice. Exclusion criteria were incomplete information about nodule features in the thyroid USG report, discordance in the localization of the nodule in the preoperative USG and postoperative histopathology reports.

Demographic characteristics of the patients such as age and gender, USG features of nodules, thyroid-stimulating hormone (TSH) and thyroid antibody values were recorded from the patient files. For thyroid autoantibodies, anti-thyroglobulin (anti-TG) and anti-thyroid peroxidase (anti-TPO) values higher than the upper limit of normal (ULN) of the ref-

erence values were evaluated as positive, and those lower than the ULN were evaluated as negative (ULN for anti-TG and anti-TPO antibodies are 115 IU/mL, and 34 IU/L, respectively). According to the USG features of the nodules, ACR-EU-K TIRADS scores were calculated and recorded. The study cohort was categorized into two groups based on whether they underwent thyroidectomy following a single FNAB or a repeat FNAB. Cytology results of those who underwent repeat FNAB were recorded. Based on the final histopathological evaluations, the nodules were classified into benign and malignant categories, with NIFTP assigned to the benign group.

In our department, FNAB is carried out for nodules in line with the indications outlined in the ACR-EU-K TIRADS and American Thyroid Association (ATA) guidelines, and in addition, it is performed for reasons such as the patient being concerned about a malignant tumor or having a family history of thyroid cancer. In our department, USG-guided FNAB procedure is performed by specialized endocrinologists using a 22-gauge needle mounted on a 10-mL single-use plastic syringe that has been pre-rinsed with a methanol–water solution (ThinPrep CytoLyt, Hologic). A minimum of two passes are made for each lesion.

Thyroid USG evaluation before FNAB was performed by experienced endocrinologists using the high-resolution USG device (SIEMENS Healthineers, Acuson Juniper Ultrasound System, linear-array transducer, 12L3, Berlin, Germany) in the frequency range of 3.6-12.9 MHz. Each nodule included in the study was evaluated and reported in terms of size, composition, echogenicity, shape, margin regularity and echogenic focus. Nodules were classified in terms of composition as cystic, spongioform, mixed cystic, solid; in terms of echogenicity as anechoic, isoechoic, hyperechoic, moderately hypoechoic, and markedly hypoechoic relative to the thyroid parenchyma; in terms of shape as wider than tall and taller than wide; and in terms of margins as regular, lobulated, irregular, and with extrathyroidal extension. If the nodule had an echogenic focus, it was classified as comet-tail, macrocalcification, peripheral calcification, and microcalcification. In addition, the presence of cervical lymphadenopathy was evaluated and if present, it was classified as reactive or pathological. Moderate and marked hypoechoic echogenicity, irregular border, taller than wide shape, presence of microcalcifications, extrathyroidal extension and pathological lymphadenopathy were considered as USG features of the nodule suggestive of malignancy. ACR-EU-K TIRADS scores were categorized according to the USG features of the nodules in accordance with their reporting criteria. ACR-TIRADS (13) was categorized from 1 to 5, EU-TIRADS (12) from 2 to 5 and K-TIRADS (14) from 2 to 5.

Statistical Analysis

SPSS version 22.0 (Statistical Package for the Social Sciences) was used to carry out all statistical analyses. Continuous variables were reported as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Chi-square test or Fisher Exact test was used to evaluate the relationship between preoperative clinical and USG variables and postoperative malignancy. The independent samples t-test was used to evaluate the relationship between continuous variables showing normal distribution and postoperative malignancy, and the Mann-Whitney U-test was used to evaluate the relationship between continuous variables not showing normal distribution and postoperative malignancy. Univariate logistic regression analysis was performed to identify predictors of malignancy, and then multivariate regression analysis was performed to eliminate the effect of confounding factors. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy values for malignancy of ACR-EU-K TIRADS categories 4 and 5 were calculated. For differences, $p < 0.05$ value was considered statistically significant.

RESULTS

During the time period in which our study was conducted, FNAB was performed on 6903 nodules in our institution, and the cytology results of 683 (9.8%) of them were reported as AUS. Of the 683 nodules with AUS, 309 met the inclusion criteria and were included in our study. The final histopathology results of the 309 nodules included in the study were reported as benign in 168 (54.3%) and malignant in 141 (45.6%) (Figure 1). Benign and malignant groups determined according to final histopathology results were similar in terms of age and gender distributions ($p = 0.239$ and $p = 0.570$) (Table 1).

In the comparison of preoperative USG features of nodules in benign and malignant groups, the frequency of nodules with a nodule diameter smaller than 10 mm was significantly higher in the malignant group than in the benign group ($p<0.001$). The frequency of nodules with solid composition, hypoechoic echogenicity, a taller-than-wide shape, irregular borders, extrathyroidal extension, microcalcifications, and cervical lymphadenopathy was significantly higher in the malignant group than in the benign group ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.042$, $p < 0.001$, and $p < 0.001$, respectively) (Table 1).

ACR-EU-K TIRADS 5 nodule frequency was higher in the malignant group, ACR-EU-K TIRADS 3 nodule frequency was higher in the benign group ($p<0.001$ and $p<0.001$, respectively). There was a trend for ACR TIRADS 4 nodule frequency to be higher in the malignant group, EU TIRADS

Table 1: Comparison of demographic, clinical and ultrasonographic features of histopathologically confirmed benign and malignant nodules

Variables	Benign n=168 (%54.3)	Malign n=141 (%45.6)	p value
Age (years), mean±SD	46.57±12.74	44.72±14.76	0.239
Gender			0.570
Female	140 (83.3)	114 (80.9)	
Male	28 (16.7)	27 (19.1)	
Repeat FNAB			0.233
No	52 (31.0)	35 (24.8)	
Yes	116 (69.0)	106 (75.2)	
Nodule Size			<0.001*
<10 mm	24 (14.5)	60 (42.6)	
>10 mm	142 (85.5)	81 (57.4)	
Composition			
Completely cystic	1 (0.6)	0 (0.0)	-
Spongioform	2 (1.2)	0 (0.0)	-
Mixed cystic	67 (39.9)	12 (8.5)	-
Solid	98 (58.3)	129 (91.5)	<0.001*
Echogenicity			
Anechoic	2 (1.2)	0 (0.0)	-
Iso-Hyperechoic	127 (75.6)	35 (24.8)	-
Slightly hypoechoic	38 (22.6)	82 (58.2)	-
Markedly hypoechoic	1 (0.6)	24 (17.0)	-
Hypoechoic			
No	129 (76.8)	35 (24.8)	<0.001*
Yes	39 (23.2)	106 (75.2)	
Shape			<0.001*
width > height	168 (99.4,)	116 (82.3)	
width < height	1 (0.6)	25 (17.2)	
Margins			<0.001*
regular	158 (94.0)	73 (51.8)	
irregular	10 (6.0)	68 (48.2)	
Extrathyroidal extension			0.042*
No	168 (100.0)	137 (97.2)	
Yes	0 (0.0)	4 (2.8)	
Echogenic foci			
None or comet-tail	142 (84.5)	62 (44.0)	-
Macrocalcifications	16 (9.5)	9 (6.4)	-
Peripheral calcifications	3 (1.8)	4 (2.8)	-
Microcalcifications	7 (4.2)	66 (46.8)	<0.001*
Cervical lymphadenopathy			
None	160 (95.2)	107 (75.9)	-
Reactive	7 (4.2)	20 (14.2)	-
Pathological	1 (0.6)	14 (9.9)	-
Cervical lymphadenopathy			
No	160 (95.2)	107 (75.9)	<0.001 *
Yes	8 (4.8)	34 (24.1)	
ACR-TIRADS			
1 (Benign)	3 (1.8)	0 (0.0)	-
2 (Not suspicious)	49 (29.2)	3 (2.1)	<0.001*
3 (Mildly suspicious)	64 (38.1)	9 (6.4)	<0.001*
4 (Moderately suspicious)	50 (29.8)	52 (36.9)	0.185
5 (Highly suspicious)	2 (1.2)	77 (54.6)	<0.001*

Table 1 continue

EU-TIRADS			
2 (Benign)	3 (1.8)	0 (0.0)	-
3 (Low-risk)	120 (71.4)	16 (11.3)	<0.001*
4 (Intermediate-risk)	31 (18.5)	17 (12.1)	0.122
5 (High-risk)	14 (8.3)	108 (76.6)	<0.001*
K-TIRADS			
2 (Benign)	4 (2.4)	0 (0.0)	-
3 (Low suspicion)	120 (71.4)	18 (12.8)	<0.001*
4 (Intermediate suspicion)	36 (21.4)	44 (31.2)	0.051
5 (High suspicion)	8 (4.8)	79 (56.0)	<0.001*
TSH (mIU/L), mean±SD	1.98±1.74	2.08±1.38	0.129
anti-TG			0.952
negative	71 (77.2)	70 (78.7)	
positive	21 (22.8)	19 (21.3)	
anti-TPO			0.065
negative	53 (60.9)	63 (74.1)	
positive	34 (39.1)	22 (25.9)	

All values are presented as mean value ± SD or n (%). **ACR-TIRADS:** American College of Radiology Thyroid Imaging Reporting and Data System, anti-TG: anti-thyroglobulin, anti-TPO: anti-thyroid peroxidase, **EU-TIRADS:** European Thyroid Imaging Reporting and Data System, **FNAB:** Fine needle aspiration biopsy, **K-TIRADS:** Korean Thyroid Imaging Reporting and Data System, **TSH:** thyroid-stimulating hormone.

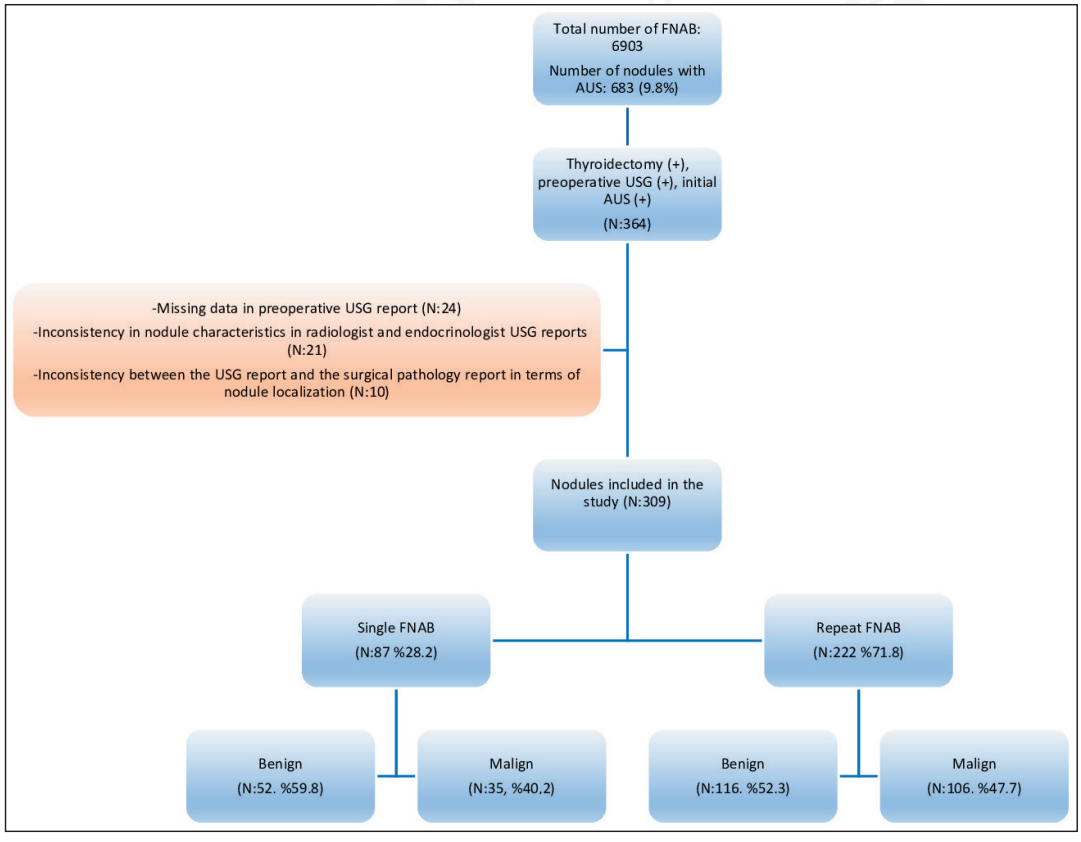

Figure 1: Final histopathology results of single and repeat FNAB groups in nodules with AUS, and summary of the study design.
AUS: atypia of undetermined significance, **FNAB:** fine needle aspiration biopsy, **USG:** ultrasonography

4 nodule frequency in the benign group and K TIRADS 4 nodule frequency in the malignant group, but this was not statistically significant (p = 0.185, p = 0.122 and p = 0.051, respectively). ACR TIRADS 2 nodule frequency was higher in the benign group (p<0.001). ACR TIRADS 1 and EU-K TIRADS 2 nodule numbers were insufficient, so comparison between the groups could not be made (Table 1).

Of the nodules, 87 (28.2%) underwent surgery after a single FNAB and 222 (71.8%) underwent surgery after a repeat FNAB (Figure 1). The final histopathology results of

35 (40.2%) of the nodules that underwent a single FNAB before thyroidectomy and 106 (47.7%) of the nodules that underwent a repeat FNAB were reported as malignant (p = 0.233) (Figure 1) (Table 1).

The distribution of ACR-TIRADS, EU-TIRADS, and K-TIRADS scores between the single and repeat FNAB groups is presented in Table 2. According to the ACR-TIRADS classification, the frequency of category 2 nodules was significantly higher in the single FNAB group compared to the repeat FNAB group (25.3% vs. 13.5%, p = 0.020). The distributions of categories 3, 4, and 5 were similar between the groups (p = 0.987, p = 0.071, and p = 1.000, respectively). In the EU-TIRADS classification, there were no statistically significant differences between the groups for categories 3, 4, and 5 (p = 0.087, p = 0.161, and p = 0.543, respectively). According to the K-TIRADS classification, category 4 nodules were more frequent in the repeat FNAB group compared to the single FNAB group (29.7% vs. 16.1%, p = 0.020). Categories 3 and 5 showed no significant differences (p = 0.069 and p = 0.777, respectively) (Table 2).

Table 2: Comparison of TIRADS Score Distributions Between Single and Repeat FNAB Groups

TIRADS	single FNAB n=87	repeat FNAB n=212	p value
ACR-TIRADS			
1	1 (1.1)	2 (0.9)	-
2	22 (25.3.)	30 (13.5)	0.020*
3	20 (23.0)	53 (23.9)	0.987
4	22 (25.3)	80 (36.0)	0.071
5	22 (25.3)	55/57 (25.7)	1.000
EU-TIRADS			
2	1 (1.1)	2 (0.9)	-
3	45 (51.7)	91 (41.0)	0.087
4	9 (10.3)	39 (17.6)	0.161
5	32 (36.8)	90 (40.5)	0.543
K-TIRADS			
2	1 (1.1)	3 (1.9)	-
3	46 (52.9)	92 (41.4)	0.069
4	14 (16.1)	66 (29.7)	0.020*
5	26 (26.9)	61 (27.5)	0.777

All values are presented as n (%). **ACR-TIRADS:** American College of Radiology Thyroid Imaging Reporting and Data System, **EU-TIRADS:** European Thyroid Imaging Reporting and Data System, **FNAB:** Fine needle aspiration biopsy, **K-TIRADS:** Korean Thyroid Imaging Reporting and Data System.

Cytological results of the nodules that underwent repeat FNAB were nondiagnostic in 32 (14.4%), benign in 30 (13.5%), AUS in 113 (50.9%), follicular neoplasm in 6 (2.7%), suspicious for malignancy in 17 (7.6%), and malignant in 24 (10.8%).

The corresponding malignancy rates in the final histopathology results were 31.2%, 30.0%, 40.7%, 16.7%, 94.1%, and 100.0%, respectively. Among these, a substantial proportion of nodules were classified as intermediate-to-high risk (TIRADS 4–5). In the nondiagnostic group, 46.8% (15/32) were TIRADS 4–5 with malignancy rates of 60.0% (ACR), 64.3% (EU), and 69.2% (K). In the benign group, 46.7% (14/30) were TIRADS 4–5 with malignancy rates of 57.1%, 50.0%, and 50.0%, respectively. In AUS nodules, 54.0% (61/113) were TIRADS 4–5 with malignancy rates of 60.7%, 64.9%, and 64.3%, respectively (Table 3).

In the logistic regression analyses, preoperative USG features of the nodules such as hypoechoic appearance (OR: 3.330, 95% CI: 1.212-9.147, p = 0.020), a taller-than-wide shape (OR: 12.113, 95% CI: 1.122-130.793, p = 0.040), irregular borders (OR: 4.009, 95% CI: 1.193-13.472, p = 0.025), microcalcifications (OR: 16.990, 95% CI: 3.497-82.549, p = 0.000) and the presence of lymphadenopathy (OR: 5.614, 95% CI: 1.487-21.195, p = 0.011) were identified as factors independently associated with malignancy (Table 4).

For malignancy, specificity and PPV of ACR TIRADS 5 were 98.8% and 97.5%, respectively, while sensitivity and NPV of EU TIRADS 5 were 76.6% and 82.4%, respectively. For malignancy, accuracy of EU TIRADS 5 was the highest

Table 3. Malignancy rates of repeat FNAB results by Bethesda category and TIRADS 4–5 (intermediate-to-high risk) classification

Bethesda category	Overall malignancy n/N (%)	ACR-TIRADS 4–5 malignancy n/N (%)	EU-TIRADS 4–5 malignancy n/N (%)	K-TIRADS 4–5 malignancy n/N (%)
Non-diagnostic	10/32 (31.2)	9/15 (60.0)	9/14 (64.3)	9/13 (69.2)
Benign	9/30 (30.0)	8/14 (57.1)	6/12 (50.0)	6/12 (50.0)
AUS	46/113 (40.7)	37/61 (60.7)	37/57 (64.9)	36/56 (64.3)
Follicular neoplasm	1/6 (16.7)	1/3 (33.3)	1/3 (33.3)	1/3 (33.3)
Suspicious for malignancy	16/17 (94.1)	15/15 (100.0)	15/15 (100.0)	15/15 (100.0)
Malignant	24/24 (100.0)	23/23 (100.0)	23/23 (100.0)	23/23 (100.0)

All values are presented as n/N (%), where n = number of malignant nodules and N = total number of nodules in each category.
AUS: atypia of undetermined significance.

Table 4: Univariate and multivariate logistic regression analysis of nodule characteristics for malignancy

	Univariate		Multivariate	
Variables	**OR (%95 CL)**	**p**	**OR (%95 CL)**	**p**
Nodule Size <10 mm	4.44 (2.574-7.673)	<0.001*	2.601 (0.856-7.902)	0.092
Solid	7.679(3.944-14.951)	<0.001*	1.825 (0.555-6.000)	0.322
Hypoechoic	10.018(5.934-16.913)	<0.001*	3.330 (1.212-9.147)	0.020*
Height > width	35.991(4.809-269.360)	<0.001*	12.113(1.122-130.793)	0.040*
Irregular margins	14.718(7.168-30.219)	<0.001*	4.009 (1.193-13.472)	0.025*
Microcalcifications	20.240(8.862-46.226)	<0.001*	16.990(3.497-82.549)	<0.001*
Cervical lymphadenopathy	6.355(2.832-14.259)	<0.001*	5.614 (1.487-21.195)	0.011*
negative anti-TPO	1.837(0.960-3.515)	0.066	1.670 (0.643-4.338)	0.293
Repeat FNAB	1.358(0.821-2.245)	0.233	-	-

anti-TPO: anti-thyroid peroxidase, **CI:** confidence interval, **FNAB:** Fine needle aspiration biopsy, **OR:** odds ratio.

Table 5: Diagnostic performance of ACR-EU-K TIRADS in predicting malignancy

	Sensitivity	Specificity	PPV	NPV	Accuracy
ACR TIRADS 4	36.9	70.2	51.0	57.0	55.0
EU TIRADS 4	12.1	81.5	35.4	52.5	49.8
K TIRADS 4	31.2	78.6	55.0	57.6	56.9
ACR TIRADS 5	54.6	98.8	97.5	72.2	78.6
EU TIRADS 5	76.6	91.7	88.5	82.4	84.7
K TIRADS 5	56.0	95.2	90.8	72.1	83.8
ACR TIRADS 4-5	91.5	69.0	71.3	90.6	79.2
EU TIRADS 4-5	88.7	73.2	73.5	88.5	80.2
K TIRADS 4-5	87.2	73.8	73.7	87.3	79.9

All values are presented as n (%). **ACR-TIRADS:** American College of Radiology Thyroid Imaging Reporting and Data System, **EU-TIRADS:** European Thyroid Imaging Reporting and Data System, **K-TIRADS:** Korean Thyroid Imaging Reporting and Data System, **NPP:** negative predictive value, **PPV:** positive predictive value

(84.7%). For ACR-EU-K TIRADS 4, the highest specificity value was found for EU TIRADS 4 (81.5%), the highest sensitivity value was found for ACR TIRADS 4 (36.9%), and the highest PPV, NPV, and accuracy were found for K TIRADS 4 (55%, 57.6%, and 56.9%, respectively) (Table 5).

DISCUSSION

ATA guidelines and the Bethesda system recommend repeat FNAB for AUS nodules (1,4). However, there are different recommendations regarding repeat FNAB. There are studies suggesting that the malignancy rate does not change with repeat FNAB (20-23). On the other hand, there are also studies reporting that the malignancy rate increases or decreases with repeat FNAB (16-19). In our study, although not statistically significant, there was a trend towards a higher malignancy rate in repeat FNAB than in single FNAB. Unlike previous studies, our analysis incorporated ultrasound-based risk stratification (ACR, EU, and K-TIRADS) to explore whether repeat FNAB provides additional diagnostic value beyond sonographic features. This perspective is particularly important because TIRADS scoring has already demonstrated high predictive performance, and our data suggest that repeat FNAB contributes little when nodules are classified as intermediate-to-high risk.

Of the nodules that underwent repeat FNAB, 18.5% were reported as suspicious for malignancy or malignant, which may appear useful in guiding surgical decisions. However, since only surgically resected nodules were included in our study, this rate is subject to selection bias and is likely lower in the general population. Moreover, almost all nodules with suspicious or malignant repeat FNAB results were already classified as TIRADS 4–5. Given that TIRADS 4–5 nodules are known to have high positive and negative predictive values, repeat FNAB did not provide additional diagnostic value in these high-risk nodules. The most frequent repeat FNAB result was AUS (50.9%), with a malignancy rate of 40.7%, which was similar to the malignancy rate in the single FNAB group (40%). This indicates that a repeat AUS diagnosis did not alter the malignancy risk and, if used as the sole indica-

tion for surgery, may inevitably lead to unnecessary resections of many benign nodules. Importantly, high malignancy rates were also observed in nodules with benign (30.0%) and nondiagnostic (31.2%) repeat FNAB results. However, since only surgically treated nodules were included in our cohort, the outcomes of patients who avoided surgery after receiving a benign repeat FNAB result could not be evaluated; therefore, the true clinical benefit of repeat FNAB in reducing unnecessary surgeries could not be demonstrated in this study. Nevertheless, in the subgroup of nodules classified as TIRADS 4–5, malignancy rates in benign and nondiagnostic repeat FNAB results increased to 60–70%. This finding suggests that benign or nondiagnostic results on repeat FNAB should be interpreted with caution, particularly in nodules with high-risk ultrasonographic features. Previous studies have similarly reported malignancy rates of 18–29% in benign repeat FNAB results, which were not significantly different from those in nodules that underwent direct surgery (21,24,25). Our findings are consistent with these results. In conclusion, the diagnostic reliability of repeat FNAB in AUS nodules appears limited. The observation that even benign and nondiagnostic repeat FNAB results carry a considerable malignancy risk underscores the need to integrate cytology with TIRADS-based ultrasonographic risk stratification when making clinical decisions.

USG features remain the most consistent predictors of malignancy in AUS nodules. In line with previous studies (6,7,26,27), we found hypoechogenicity, taller-than-wide shape, irregular margins, microcalcifications, and lymphadenopathy to be independent risk factors, with microcalcifications showing the strongest association. We also observed that nodules <1 cm tended to have higher malignancy rates. Previous studies have similarly reported an association between smaller nodules and malignancy (6,18,28,29), although others have suggested that larger nodules >2 cm may also be linked to thyroid cancer (30,31). These findings, including ours, should be interpreted cautiously due to the selection of surgically treated, high-risk nodules.

In our study, ACR-EU-K TIRADS 5 showed a good performance in predicting malignancy with high specificity and PPV, and the most successful in this regard was ACR-TIRADS 5. When we evaluate ACR-EU-K TIRADS 4-5 or score ≥ 4, we see that sensitivity and NPV increase. This shows that ACR-EU-K TIRADS score < 4 exhibits a successful performance in terms of ruling out malignancy in nodules. Xing Z et al. conducted a systematic review and meta-analysis on the diagnostic performance of USG risk classification systems in cytologically indeterminate thyroid nodules. And the authors reported that in the evaluation of high-risk categories of USG risk classification, the sensitivity of EU-TIRADS 5 was the highest (59%), and the specificity of USG risk classification by Kwak JY et al (99%) was the highest. However, among ACR-EU-K TIRADS, they reported that ACR TIRADS 5 had the highest specificity (91%) (32, 33). In our study, among these 3 TIRADS classifications, the category with the highest specificity was ACR-TIRADS 5 and the category with the highest sensitivity was EU-TIRADS 5. In this respect, the results of our study are consistent with the aforementioned meta-analysis in terms of comparing the performance of ACR-EU-K TIRADS 5. We believe that our study is important in contributing to the literature in terms of evaluating the performance of all 3 TIRADS in nodules with AUS and that these results may contribute to the determination of the treatment approach to nodules with AUS.

Our study has some limitations. First, although USG is performed by experienced endocrinologists, it is an operator-dependent procedure. In addition, USG data were also examined retrospectively. Second, we only included patients with AUS who underwent surgery. Since we do not know the final histopathology results of patients who did not undergo surgery, the true malignancy rate may differ from that found in our study. Third, for patients who underwent surgery with a single FNAB, the treatment choice may be due to suspicious USG features of the nodules, which may cause selection bias. However, in our study, we see that the rates of ACR-EU-K TIRADS 5 nodules, which are indicators of high risk for malignancy, are similar in the single and repeat FNAB groups, which increases the reliability of the results of our study. Fourth, due to the retrospective nature of the study, no evaluation was made regarding whether the nodules with AUS had nuclear atypia, as most cytopathology results did not include information on nuclear atypia. Finally, our study has a single-center design and small sample size.

Conclusion

repeat FNAB in AUS nodules showed limited diagnostic utility, as malignancy rates remained high regardless of cytological results, particularly in nodules with intermediate-to-high TIRADS scores. These findings suggest that ultrasound-based risk stratification may be more reliable than repeat FNAB alone in guiding management decisions, but further prospective multicenter studies are needed.

Author Contributions

Study conception and design: **Yusuf Öztürk, Muhammet Kocabaş,** data collection: **Yusuf Öztürk,** analysis and interpretation of results: **Yusuf Öztürk, Muhammet Kocabaş,** draft manuscript preparation: **Yusuf Öztürk, Muhammet Kocabaş.** The author(s) reviewed the results and approved the final version of the article.

Conflicts of Interest

The authors have no conflict of interest to declare.

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Ethical Approval

The Ethics Committee of the Necmettin Erbakan University Medical Faculty approved the study (approval no: 2024/4896 and date: 05/04/2024).

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