

Ultrasonographic Risk Factors for Non-Diagnostic Cytology and Malignant Histopathology in Thyroid Nodules: Results of 607 Biopsies, 138 Thyroidectomies Performed by a Single Surgeon

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Abstract

Aim: Ultrasound-guided fine-needle aspiration biopsy (US-FNAB) is the cornerstone of thyroid nodule evaluation. Although traditionally performed by radiologists, the increasing involvement of endocrine surgeons in this procedure necessitates assessment of its effectiveness. This study aims to evaluate the diagnostic adequacy of US-FNAB performed by a single endocrine surgeon and to identify ultrasonographic risk factors associated with non-diagnostic cytology and malignancy.

Methods: Data from 607 US-FNAB procedures performed by a single surgeon and histopathology of 161 nodules from 138 thyroidectomies were retrospectively reviewed. Nodule characteristics (composition, location, echogenicity, shape, margins, echogenic foci) were correlated with Bethesda cytology categories and final histopathology. Statistical analyses were performed to identify factors associated with non-diagnostic results and malignancy.

Results: The non-diagnostic cytology rate was 8.73%. Anterior location was associated with reduced risk of non-diagnostic cytology ($p=0.019$), while peripheral calcification significantly increased this risk ($p=0.0039$). Malignancy rates were consistent with Bethesda categories, ranging from 13.69% (Bethesda 2) to 100% (Bethesda 6). Marked hypoechogenicity (43.08%, $p<0.001$), taller-than-wide shape (35.29%, $p<0.001$), irregular margins (33.75%, $p<0.001$), and punctate calcifications (25.26%, $p<0.001$) were strong predictors of malignancy. Inferior location was associated with lower malignancy risk ($p=0.029$).

Conclusions: US-FNAB performed by an experienced endocrine surgeon demonstrates high diagnostic adequacy. Anterior location reduces the risk of non-diagnostic cytology, while inferior location lowers malignancy risk. Peripheral calcification increases non-diagnostic risk, whereas marked hypoechogenicity, taller-than-wide shape, irregular margins, and punctate calcifications elevate malignancy risk. These findings support the important and complementary role of endocrine surgeons in thyroid nodule management.

Keywords: Thyroid nodule; US-FNAB; cytology; malignancy; ultrasonography

1. Introduction

Thyroid fine needle aspiration biopsy (US-FNAB) has long been utilized as a fundamental diagnostic tool in the evaluation of thyroid nodules. Traditionally, this procedure has been performed by radiologists under ultrasound guidance in a hospital setting.¹ However, in recent years, with technological advancements and the widespread availability of portable and cost-effective ultrasound devices, clinical endocrinologists have also begun performing this procedure.¹ Today, with an increasing number of surgeons specifically interested in endocrine surgery, this procedure has also started to be performed by surgeons.^{2,3}

The adoption of US-FNAB by endocrine surgeons has redefined the role of surgeons in thyroid nodule management and paved the

way for a multidisciplinary approach to US-FNAB. However, the increasing prevalence of US-FNAB performed by surgeons has also brought about some controversies. The literature indicates that the success of this procedure varies depending on operator experience, nodule characteristics, and the technique used.^{1,4} Intrinsic factors such as the cystic nature of nodules or challenging anatomical locations are known to negatively affect diagnostic yield.^{1,4} Furthermore, reports suggest that inexperienced surgeons may initially encounter higher non-diagnostic rates.⁵ On the other hand, reports also indicate that US-FNAB performed by surgeons reduces patient waiting times, provides cost-effectiveness, and facilitates the diagnostic process due to surgeons' familiarity with neck

anatomy.⁶⁻⁸ Moreover, the ability of surgeons to directly observe nodule characteristics and perform lymph node mapping is cited as significant advantages that improve patient care.¹ Consequently, the literature contains both supportive and opposing reports on the efficacy of US-FNAB performed by surgeons, and discussions on its effectiveness continue.

This study aims to demonstrate the efficacy of US-FNAB performed by a single surgeon and the relationship between nodule characteristics, cytological adequacy, and malignancy, by comparing the results of 607 US-FNABs and the cytology and histopathology outcomes of 138 operated patients with the existing literature.

2. Materials and Methods

A database consisting of prospectively collected data from 500 patients and 607 thyroid nodules that underwent US-FNAB performed by a single surgeon at Antalya Kepez State Hospital between January 2022 and April 2025 was retrospectively reviewed. In addition to demographic data such as age and gender, intrinsic characteristics of the nodules, including size, vascularity, composition, echogenicity, shape, margin, and echogenic foci, were noted. Ultrasound reports were prepared according to ACR-TIRADS, which was also used to determine biopsy indications.⁹ Cytology results were recorded based on the Bethesda classification.² Bethesda category 1 was defined as non-diagnostic, Bethesda category 2 as benign, Bethesda category 3 as atypia of undetermined significance, Bethesda category 4 as follicular neoplasm, Bethesda category 5 as suspicious for malignancy, and Bethesda category 6 as malignant cytology. Surgical indications included the presence of symptomatic nodules, indeterminate cytology, malignancy, toxic adenoma, toxic multinodular goiter (MNG), and Graves' disease. US-FNAB was also performed for the exclusion of possible malignancy in patients with symptomatic nodular goiter not meeting ACR-TIRADS biopsy criteria, those with hyperparathyroidism requiring surgery and a concomitant ipsilateral nodule, or those with toxic MNG or toxic adenoma with a hypoactive nodule on scintigraphy. The study received ethical approval from the Health Sciences University Antalya Training and Research Hospital Ethics Committee (Approval no: 2025-363).

Statistical Analysis

All statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences Version 22.0; SPSS Inc., Chicago, IL, USA) software. The normality assumption of continuous

variables was tested using the Kolmogorov-Smirnov test. Categorical data were presented as frequency (n) and percentage (%), and continuous variables with normal distribution were presented as mean $\pm$ standard deviation. The relationship between categorical variables was examined using Chi-square analysis, Fisher's Exact Test, and Fisher-Freeman-Halton Test, depending on the suitability of the data. The relationship between continuous and categorical variables was examined using the Independent Samples T-test. In the analyses performed, results with a p-value of less than 0.05 were considered statistically significant.

3. Results

A total of 736 US-FNABs were performed on 607 thyroid nodules in 500 patients included in the study. A total of 129 thyroid nodules underwent a second biopsy due to indeterminate or insufficient cytology results. For patients who had repeat biopsies, the highest Bethesda category was considered. A total of 138 patients underwent thyroidectomy, and 161 thyroid nodules from these operated patients were histopathologically evaluated. Demographic data are presented in Table 1.

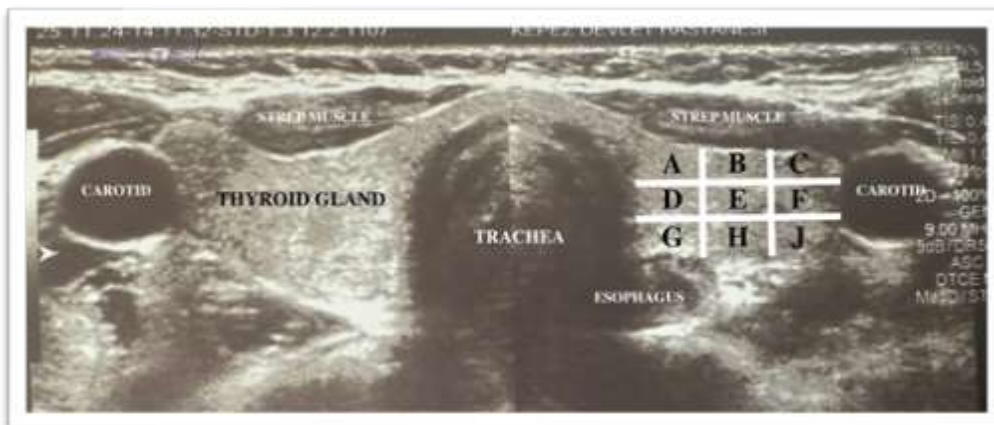
Table 1

Demographic data

Patients (n)		500
Number of biopsied nodules (n)		607
Total number of biopsies		736
Mean age (years)		52.1
Gender(n, %)	Female	493 (81.22%)
	Male	114 (18.78%)
ACR-TIRADS (n, %)	1	4 (0.66%)
	2	75 (12.36%)
	3	170 (28.01%)
	4	176 (29.00%)
	5	182 (29.98%)
	6	53 (8.73%)
	7	345 (56.84%)
Bethesda category (n, %)	3	138 (22.73%)
	4	11 (1.81%)
	5	23 (3.79%)
	6	37 (6.10%)

Figure 1

Transverse view of thyroid gland



In the anterior-posterior axis, A, B, and C represent the anterior location; D, E, and F represent the middle location; and G, H, and J represent the posterior location. In the medial-lateral axis, A, D and G represent the medial location; B, E and H represent the middle location; and C, F and J represent the lateral location.

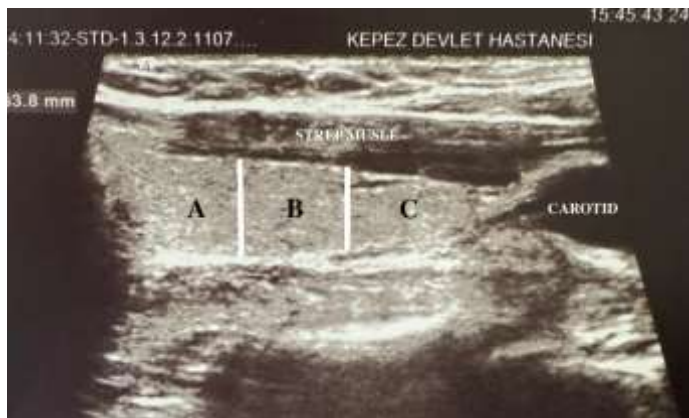
Table 2

Relationship between nodule characteristics and non-diagnostic cytology

Nodule characteristic		Non-diagnostic	Diagnostic	Total	P-value
Composition	Mixed	11 (8.80%)	114	125	0.866
	Solid	42 (8.77%)	437	479	
	Spongiform	0 (0.00%)	3	3	
	Anterior	15 (5.3%)	268	283	
Localization	Middle	16 (12.4%)	113	129	0.019
	Posterior	22 (11.3%)	173	195	
	Inferior	25 (9.03%)	252	277	
	Middle	12 (9.09%)	120	132	
	Superior	16 (8.08%)	182	198	0.912
	Medial	17 (6.97%)	227	244	
	Middle	14 (9.03%)	140	155	
	Lateral	22 (10.58%)	187	208	
Peripheral calcification	Present	7 (24.14%)	22 (75.86%)	29 (100%)	0.0039
	Absent	46 (7.96%)	532 (92.04%)	578 (100%)	
	Total	53 (8.73%)	554 (91.27%)	607 (100%)	

Figure 2

Longitudinal view of thyroid gland



A; Superior, B; Middle, C; Inferior

To investigate factors affecting cytological adequacy, the relationship between nodule composition, localization, and the presence of peripheral calcification with non-diagnostic results was examined, and the results are presented in Table 2. Non-diagnostic cytology was reported in 8.73% (n: 53) of patients. Nodules were grouped as solid, mixed, and spongiform based on their composition; no significant relationship was observed between composition and non-diagnostic results ($p=0.866$). Based on localization, nodules were classified in the transverse plane as anterior, middle, and posterior along the anterior-posterior axis; and as medial, middle, and lateral along the medial-lateral axis in ultrasound images (Figure 1). In sagittal planes, nodules were classified as superior, middle, and inferior along the superior-inferior axis (Figure 2). The rate of non-diagnostic cytology was statistically significantly lower in anteriorly located nodules compared to non-anteriorly located ones along the anterior-posterior axis ($p=0.019$). No significant relationship was observed between nodule localization and non-diagnostic cytology in evaluations along the medial-lateral axis and superior-inferior axis ($p=0.312$, $p=0.912$). Independently of nodule composition and localization, nodules containing peripheral calcification had a statistically significantly higher rate of non-diagnostic cytology

compared to those without peripheral calcification ($p=0.0039$).

Table 3 presents the malignancy rates of Bethesda categories based on preoperative cytology reports for 161 thyroid nodules examined in 138 patients who underwent thyroidectomy.

Table 4 shows the relationship between intrinsic thyroid nodule characteristics and malignancy.

In the localization-based analysis, no significant relationship was observed between nodule localization and malignancy along the anterior-posterior and medial-lateral axes. In the superior-inferior axis evaluation, significantly lower malignancy rates were observed in inferiorly located nodules compared to non-inferiorly located nodules ($p=0.029$). In the composition-based analysis, no significant relationship was observed between nodule composition and malignancy ($p=0.100$). In the evaluation based on nodule echogenicity, markedly hypoechoic nodules had a statistically significantly higher malignancy rate compared to nodules with other echogenicities (43.08% vs 8.85%, $p<0.001$); while isoechoic nodules had significantly lower malignancy rates (6.01% vs 20.43%, $p<0.001$).

Table 3

Malignancy rates according to cytology results

Bethesda category (n)	Number of malignant cases	Total number of nodules	Malignancy rate
1	2	5	40%
2	10	73	13.69%
3	14	49	28.57%
4	1	2	50%
5	12	16	75%
6	16	16	100%
Total	55	161	34.16%

In the evaluation based on nodule shape, "taller than wider" nodules had a statistically significantly higher malignancy rate compared to "wider than taller" nodules (35.29% vs 8.81%, $p<0.001$). Based on margin characteristics, nodules were classified as regular, lobulated, and irregular, with malignancy rates observed as 8.84%, 22.22%, and 33.75%, respectively.

Table 4

Relationship between nodule characteristics and malignancy

Nodule characteristic		Malignant	Non-Malignant	Total	P-value
Localization	Anterior	42 (8.66%)	241	283	0.186
	Middle	11 (8.53%)	118	129	
	Posterior	23 (11.79%)	172	195	
	Superior	30 (15.15%)	168	198	0.029
	Middle	22 (16.67%)	110	132	
	Inferior	24 (8.66%)	253	277	
	Medial	34 (13.93%)	210	244	0.058
Composition	Middle	11 (7.10%)	144	155	
	Lateral	31 (14.90%)	177	208	
	Mixed	9 (7.20%)	116	125	0.100
	Solid	67 (13.99%)	412	479	
	Spongiform	0 (0.00%)	3	3	
	Isoechoic	20 (6.01%)	313	333	< 0.001
	Hyperechoic	0 (0.00%)	10	10	
Echogenicity	Hypoechoic	28 (14.07%)	171	199	
	Markedly hypoechoic	28 (43.08%)	37	65	< 0.001
	Weider than taller	46 (8.81%)	476	522	
Shape	Taller than weider	30 (35.29%)	55	85	< 0.001
	Regular	45 (8.84%)	464	509	
Margin	Lobulated	4 (22.22%)	14	18	< 0.001
	Irregular	27 (33.75%)	53	80	
Extrathyroidal extension	Present	14 (16.47%)	71	85	0.237
	Absent	62 (11.88%)	460	522	
	Absent	36 (9.16%)	357	393	
Echogenic focus	Punctate	24 (25.26%)	71	95	< 0.001
	Coarse	11 (18.03%)	50	61	
	Peripheral	2 (6.90%)	27	29	
	Comet-tail art.	3 (10.34%)	26	29	

Significantly higher malignancy was observed in nodules with irregular margins ($p < 0.001$). Based on echogenic focus content, nodules were grouped as no echogenic focus, comet-tail artifact, peripheral, coarse, and punctate, with malignancy rates observed as 9.16%, 10.34%, 6.9%, 18.03%, and 25.26%, respectively. The malignancy rate was significantly higher in nodules containing punctate calcifications ($p < 0.001$).

4. Discussion

The results of this study indicate that US-FNAB performed by a surgeon yields satisfactory results in terms of cytological adequacy. Furthermore, nodule localization and intrinsic characteristics are independent risk factors for cytological adequacy and malignant histopathology. Anteriorly located nodules reduce the risk of non-diagnostic cytology, while the presence of peripheral calcification increases this risk. The risk of malignancy decreases in inferiorly located nodules; however, marked hypoechoogenicity, "taller than wider" shape, irregular margins, and the presence of punctate calcifications significantly increase the risk of malignancy.

Cytological adequacy in thyroid FNAB varies depending on the patient's neck anatomy, intrinsic characteristics of the nodule such as composition, localization, and calcification content, biopsy technique, and the experience of the operator and cytopathologist.^{4, 10-13} Although the impact levels of these factors on cytological adequacy vary, the largest share undoubtedly belongs to operator experience. Studies in the literature investigating the effect of operator experience on cytological adequacy primarily compare periods before and after the learning curve. In this context, McIvor et al.'s study reported 31.4% non-diagnostic cytology rates during

the learning curve, compared to 6.1% non-diagnostic cytology rates after the learning curve, clearly demonstrating the impact of operator experience.¹⁴ Nevertheless, comprehensive studies in the literature generally show non-diagnostic rates for US-FNAB ranging from 5% to 15%.^{15, 16} The 8.73% non-diagnostic cytology rate observed in our current cohort indicates that a highly satisfactory level of diagnostic efficiency can be achieved even with the experience of a single surgeon. This successful outcome in our study can be attributed to the surgeon's in-depth knowledge of neck anatomy, expertise in trans-cervical approach to palpable and non-palpable nodules, and skill in ultrasound-guided needle tracking. These findings strongly support the trends in the literature suggesting that with appropriate training and continuous practice, endocrine surgeons can provide thyroid nodule biopsy services with high diagnostic adequacy.

The effect of nodule characteristics on cytological adequacy is critically important for optimizing the diagnostic performance of US-FNAB. In our study, anteriorly located nodules were found to significantly reduce the risk of non-diagnostic cytology ($p = 0.019$), while the presence of peripheral calcification statistically significantly increased this risk ($p = 0.0039$). In the literature, some studies indicate that nodule localization (e.g., deeply located, isthmic, or paratracheal nodules) can complicate biopsy and increase the risk of non-diagnostic results¹⁷, while other studies have not supported this relationship.¹⁸ The lower non-diagnostic rate obtained in biopsies of anteriorly located nodules in our study can be explained by the ease of needle penetration, clearer visualization of the needle tip on the ultrasound monitor, easier accessibility of nodules in this region, and the ability to achieve stable positioning. The effect of peripheral calcifications in increasing non-diagnostic cytology has been evaluated to a limited

extent in the literature¹⁷ and may be related to peripheral calcification acting as a physical barrier to the needle, impeding needle penetration, and thus hindering cellular aspiration and the acquisition of adequate cellular material. Some previous studies have shown that thyroid nodules with cystic composition are associated with a higher rate of inadequate sampling; for instance, one study found that nodules with more than 50% cystic component increased the likelihood of non-diagnostic results.^{17,19} However, on-site cytological evaluation and the universal use of ultrasound guidance to ensure sampling of solid components can reduce the frequency of inadequate sampling in cystic nodules.²⁰ The lack of a significant relationship between nodule composition (solid, mixed, spongiform) and non-diagnostic results in our study suggests that a competent operator can collect sufficient cellular material even from cystic or mixed lesions. These results indicate that careful evaluation of the ultrasound characteristics of the nodule before US-FNAB can directly affect the success of the procedure and guide strategies to minimize non-diagnostic rates.

The correlation between ultrasonographic morphological features of thyroid nodules and malignancy risk plays a central role in clinical decision-making processes and in identifying nodules suspicious for malignancy. In our cohort, a significantly lower malignancy risk was found in inferiorly located nodules ($p=0.029$), while marked hypoechogenicity, "taller than wider" shape, irregular margins, and the presence of punctate calcifications statistically significantly increased the risk of malignancy ($p<0.001$). These findings strongly support the general consensus in the literature and the high-risk ultrasonographic features specified in the American Thyroid Association (ATA) and ACR-TIRADS guidelines.^{9,21} Markedly hypoechogenicity, in particular, is one of the most prominent ultrasonographic signs of malignant thyroid nodules, and low echogenicity is thought to result from the denser packing of malignant cells and the acoustic impedance difference between them and surrounding tissues. The "taller than wider" shape reflects the malignant invasion and growth pattern of the nodule, while irregular margins indicate the invasive growth of the tumor towards surrounding tissues. Punctate calcifications, especially microcalcifications with high specificity for papillary thyroid carcinoma, represent psammoma bodies and are a strong indicator of malignancy.⁹ The lower malignancy rate we observed in inferiorly located nodules is similar to some studies in the literature,^{22,23} but may differ from others;²⁴ this could be explained by sample heterogeneity or regional genetic differences, as well as by differences in embryological origin or functional characteristics of this region. The absence of a significant relationship in terms of composition ($p=0.100$) partially contradicts findings in some studies that solid nodules carry a higher risk of malignancy.²⁵ This difference might be explained by mixed nodules in our study having a solid component and targeting solid areas during sampling. The low malignancy risk in isoechoic nodules (6.01% vs 20.43%, $p<0.001$) is an expected finding and suggests that nodules with echogenicity similar to normal thyroid tissue are generally benign. Overall, the results of our study support that ultrasound performed by a surgeon is a reliable method for distinguishing malignant nodules.

Our study has some limitations. The retrospective nature of the study may introduce potential information bias. Being a single-center study may limit the generalizability of the findings, and further research may be needed to determine whether similar results can be obtained in different populations or in centers with different surgical practices. Furthermore, the lack of data on long-term follow-up results for nodules reported as cytologically benign and not undergoing surgery prevents us from fully evaluating the true malignancy risk of these nodules. Future studies should focus

on multi-center, prospective designs with long-term follow-up to address these limitations and more comprehensively elucidate the role of US-FNAB performed by surgeons in thyroid nodule management. Nevertheless, the prospective collection of data from a large US-FNAB cohort performed by a single surgeon and the comparison of these data with both cytological adequacy and histopathological malignancy results can be listed as advantages of the study. The single-operator approach minimizes operator-dependent variability, increasing the consistency of the results. Additionally, correlating histopathology results obtained from operated patients with cytology results provides valuable information about the diagnostic accuracy of US-FNAB.

5. Conclusion

This study supports that ultrasound-guided fine needle aspiration biopsy (US-FNAB) performed by a single surgeon offers high diagnostic adequacy in the evaluation of thyroid nodules and that an experienced endocrine surgeon can safely perform this procedure. The risk of non-diagnostic cytology was found to be reduced in anteriorly located nodules, while the presence of peripheral calcification increased this risk. In inferiorly located nodules, the risk of malignancy decreased; whereas marked hypoechogenicity, "taller than wider" shape, irregular margins, and the presence of punctate calcifications were confirmed as strong predictive factors for malignancy. These findings emphasize that the active involvement of endocrine surgeons in thyroid nodule management has the potential to improve diagnostic accuracy and patient care. In the future, larger-scale prospective studies on the clinical outcomes and cost-effectiveness of US-FNAB performed by surgeons will further solidify the place of this approach in the thyroid nodule management algorithm.

Statement of ethics

The study received ethical approval from the Health Sciences University Antalya Training and Research Hospital Ethics Committee (Approval no: 2025-363).

genAI

No artificial intelligence-based tools or generative AI technologies were used in this study. The entire content of the manuscript was originally prepared, reviewed, and approved by both authors.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

Mehmet Ilker Turan: Acquisition of data (lead); Analysis and interpretation of data (lead); Drafting of manuscript (lead); Critical revision (lead); Statistical expertise (equal).

Nedim Akgul: Analysis and interpretation of data (supporting); Drafting of manuscript (supporting); Critical revision (supporting); Statistical expertise (equal).

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