



Cytological and histopathological correlations of the Bethesda 3 categories in thyroid cytopathology: A 4-year single-center experience

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■ MAIN POINTS

- The malignancy rate of repeat cytologies was similar to those directly operated after the first FNA (86.17% vs 77.2%). This suggests that the contribution of the second FNA to clinical decision making is limited.
- The postoperative malignancy rate was 83.45%, of which 36.8% were papillary microcarcinomas, most of which (<5 mm) may have been incidentally detected in the resection material. This may lead to an overestimation of the ROM.
- Malignancy rates were higher in single nodular goiters (45.5%) and significantly lower in multinodular goiters (14.1%) and cases with concomitant thyroiditis (5%). This suggests that multinodularity and chronic lymphocytic thyroiditis are associated with low ROM ($p=0.0033$).

■ ABSTRACT

Aim: This study aimed to investigate the correlation between thyroid fine needle aspiration biopsy (FNAB) and thyroidectomy materials diagnosed as atypia of undetermined significance (AUS) with histopathological diagnoses over the last four years at our hospital.

Materials and Methods: This retrospective study included 251 thyroid FNAB cases with an AUS diagnosis referred to our laboratory between January 1, 2020, and December 31, 2023, and 133 of these patients who subsequently underwent surgery at our hospital.

Results: A histopathological diagnosis was made in 133 cases. Ninety-four patients were diagnosed after the first AUS diagnosis, and 39 after at least one control aspiration. Of the 39 patients who underwent a second cytological control, 56.4% ($n=22$) were diagnosed again with AUS. Of the surgical patients, 36.8% ($n=49$) were diagnosed with papillary microcarcinoma, 33.83% ($n=45$) with papillary carcinomas, 9.02% ($n=12$) with adenomatous hyperplasia and nodular hyperplasia, 6.7% ($n=9$) with invasive encapsulated follicular variant of papillary thyroid carcinoma, 3.75% ($n=5$) with follicular carcinoma, 3% ($n=4$) with chronic lymphocytic thyroiditis, 2% ($n=3$) with follicular adenoma, 1.5% ($n=2$) with well differentiated thyroid tumor of uncertain malignant potential, and 0.75% ($n=1$) each with medullary carcinoma, oncocytic carcinoma, anaplastic carcinoma-not otherwise specified, and paraganglioma. Because the majority (91.83%) of the cases diagnosed with papillary microcarcinoma were ≤ 5 mm in size, and there were benign nodular structures in non-tumor areas, aspiration was performed from benign nodules, and postoperative papillary microcarcinoma was detected incidentally.

Conclusion: In our study, most of the patients diagnosed with AUS had a diagnostic operation by the clinician, and the patients who underwent repeat cytology were mostly persistently diagnosed with AUS. It was remarkable that the majority (36.8%) of the patients diagnosed with AUS from an FNAB were diagnosed with incidental papillary microcarcinoma after resection. Since papillary microcarcinoma tumors are smaller than 1 cm, these cells are not sufficiently included in the cytology material from an FNAB.

Cite this article as: Demirtas R, Tamer HD. Cytological and histopathological correlations of the Bethesda 3 categories in thyroid cytopathology: A 4-year single-center experience. *Ann Med Res*. 2025;32(5):192–198. doi: [10.5455/annalsmedres.2024.12.277](https://doi.org/10.5455/annalsmedres.2024.12.277).

Keywords: Histopathology, FNAB, Atypia of uncertain significance, Thyroid

Received: Dec 25, 2024 **Accepted:** Apr 18, 2025 **Available Online:** May 26, 2025



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■ INTRODUCTION

Thyroid function is crucial for maintaining growth, development, and metabolic homeostasis [1]. A range of diseases are commonly associated with the thyroid gland, such as goiter, hyperthyroidism, hypothyroidism, thyroiditis, and neoplasms [1]. Most neoplasms are considered benign; however, the rate of malignant tumors in solitary nodules is 10% [1]. Although thyroid cancers are relatively rare, they account for 90% of all endocrine tumors [1]. A fine needle aspiration biopsy

(FNAB) is an efficient and low-cost diagnostic technique that can be applied to palpable or non-palpable thyroid nodules suspected by radiologists [2,3]. FNAB is performed under ultrasound guidance in non-palpable lesions [2,3]. FNAB's sensitivity and specificity are 55%–98% and 73%–100%, respectively; however, it has limitations including diagnostic problems in inadequate samples, demanding sampling technique, and an overlap of benign and malignant cytologic features [1]. Two systems are used worldwide for the cytologic clas-

sification of thyroid lesions: the British Thyroid Association and the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) [2]. The TBSRTC classifies thyroid cytology specimens into six categories for more efficient reporting (Table 1) [2,4]. Not only the numerical classification but also the name of the category and numerical notation should be reported to avoid confusion; for example, “Atypia of uncertain significance (AUS) (Bethesda 3)” [4]. This has become the accepted mode of communication among the various specialties that deal with thyroid nodule diseases [5].

The AUS category defined by the TRBSRTC is used for cases without sufficient atypia for follicular neoplasia or malignant tumor and for which a definite diagnosis of benign or malignant is not made [4]. The term “follicular lesion of uncertain significance” (FLUS) was included in the “AUS” group to avoid confusion in the TBSRTC of 2023 [4].

Each category has a cancer risk, and the widespread adoption of the TBSRTC provides an approach to determine the risk of malignancy (ROM) probability for each category [4]. As a result of a large-scale survey conducted in 2017, information on the ROM for each category was updated [4], as presented in Table 1.

The number of patients with cancer after subsequent surgery was considered when calculating the ROM [1]. However, the ROM is overestimated because the number of nodules resected glands in nondiagnostic, benign, or AUS-diagnosed cytologies is lower [4,5].

The effect of “Non-invasive follicular variant papillary thyroid carcinoma” (NIFTP) on ROM is another issue [4]. NIFTP is a surgical diagnosis that cannot be known for sure using an FNAB [4]. The examination of NIFTP cytological features indicates that these nodules tend to be classified as AUS (Bethesda 3), follicular neoplasia (Bethesda 4), or suspected malignancy (Bethesda 5) from an FNAB, which affects the ROM results [4].

Compared with the histopathologic resection results of patients diagnosed with AUS, the ROM was 22% (range 20–32%) (Table 1) [4]. ROM is also affected by whether the diagnosis of AUS is based on nuclear or structural atypia [4,6]. According to the most recently reported data, the ROM in AUS cases diagnosed based on nuclear atypia was 59%, while it was 6.5% in those diagnosed based on structural or oncocytic atypia, which is close to the ROM rate of cases with benign cytology (1–3%) [4,6]. TBSRTC 2023 classified AUS into two subcategories, “nuclear” and “other,” and emphasized the importance of cases diagnosed with nuclear atypia-based AUS to improve patient management for clinicians and cytopathologists [4]. In cases diagnosed with AUS, the absence of nuclear atypia should be considered to avoid unnecessary surgery [6]. The incidence of malignancy is similar between patients diagnosed with AUS from initial cytology and those diagnosed with malignancy after consecutive cytology; therefore, consecutive FNABs may not have a clear benefit

in terms of clinical decision-making [5]. The present study aimed to examine thyroid FNABs diagnosed with AUS in the last 4 years at the hospital where the study was conducted and to determine the correlation of these cases with postoperative histopathologic diagnoses.

MATERIALS AND METHODS

A retrospective study was conducted between 01.01.2020 and 31.12.2023, and cases with histopathological diagnosis data among those diagnosed with AUS using an FNAB in our laboratory were included in the study. Cases with histopathologic right/left lobectomy or total thyroidectomy were included. Core biopsies, recurrent malignancies, and completion thyroidectomy were excluded.

FNAB was performed by head and neck specialists, endocrinologists, and radiologists using ultrasound guidance or palpation with a 25-or 27-gauge needle. Nodules diagnosed as AUS for the first time underwent repeat FNAB or resection, depending on the consent of the patient and the clinician following the patient. Patients who were diagnosed with AUS repeatedly were resected for diagnosis and treatment, again with the consent of the patient. Increased nodule size, large multinodular appearance, clinical suspicion, and patient preference were among the operative criteria.

The evaluations were performed by two pathologists. The diagnosis of AUS was evaluated according to the criteria defined in the TRBSRTC guidelines, taking into evaluation nuclear atypia (nuclear enlargement and contour irregularity), architectural atypia (prominent microfollicles and oncocytic atypia (presence of predominant oncocytic features) [7]. Cases diagnosed with FLUS were reclassified according to the 2023 Bethesda system [4].

Pathology reports of each case were obtained digitally from the hospital's information record system. Approval was obtained from the authorized units for files analysis in the digital environment.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, Version 26.0 (Armonk, NY). Power analysis was conducted using F-tests and by calculating effect sizes to determine if the statistical tests had sufficient power. The normality of continuous variables was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Continuous measurements were summarized as mean and standard deviation. Categorical variables were analyzed using Chi-square tests (including Fisher-Freeman-Halton where appropriate), Kruskal-Wallis, and ANOVA tests. The statistical significance level was set at 0.05 for all tests.

Within this context, 251 FNAB cases with an initial diagnosis of AUS and 133 patients who underwent surgery were included in the study. The rate of control cytology samples was analyzed in cases with an initial AUS diagnosis. Fi-

nally, the correlation between the cytologic and corresponding histopathologic diagnoses was evaluated.

■ RESULTS

There were 251 patients diagnosed with AUS in our department, and 133 of these cases had resection material available in our unit. Of these cases, 73% (n=97) were female, 27% (n=36) were male, and the female-to-male ratio was 2.69:1. These cases were aged between 17 and 79 years, with a mean age of 48.6 ± 12.3 years. According to the results of the analysis, the mean age of the malignant group was 48.92 ± 12.46 years, that of the benign group was 49.15 ± 10.50 years, and that of the group with uncertain malignancy potential was 33.00 ± 21.21 years ($p = 0.193$). The age range with the highest number of cases was 41–50 (30.7%), followed by 54–60 (24.06%). The patient sex and age distributions are shown in Figure 1.

Power analysis using the F-test revealed a statistical power of 99.99%, confirming that the analyses were adequately powered to detect statistically significant differences, should they be present.

Following an initial diagnosis of AUS, a histopathologic diagnosis was reached in 70.6% of cases (n=94), while an additional 29.3% (n=39) received a histopathologic diagnosis after at least one control aspiration. Among the resected thyroid tissues with a single AUS diagnosis, 86.17% (n=81) were malignant, 12.7% (n=12) were benign, and 1.06% (n=1) were lesions of uncertain malignant potential (Figure 2). Of the 39 patients undergoing a second cytology control that had resection material, 10.2% (n=4) were diagnosed with non-diagnostic cytology, 15.3% (n=6) with benign cytology, 56.4% (n=22) with recurrent AUS, 2.5% (n=1) with follicular neoplasia, 12.8% (n=5) with suspected malignancy, and 2.5% (n=1) with malignant cytology. Compared with using the resection material, 76.9% (n=30) of the patients undergoing a second cytology were malignant, 20.5% (n=8) benign, and 2.5% (n=1) neoplasia of uncertain malignancy potential (Figure 3). The resection of 22 cases diagnosed with AUS after the second cytology resulted in 77.2% (n=17) malignant and 22.8% (n=5) benign thyroid lesions.

Among the 133 patients who underwent surgery, 83.45% (n=111) were histopathologically reported as malignant, 15.03% (n=20) as benign thyroid lesions, and 1.5% (n=2) as uncertain malignant potential. The individual analysis of the diagnoses revealed that 36.8% (n=49) of the patients had papillary microcarcinoma, 33.83% (n=45) papillary carcinoma, 9.02% (n=12) follicular nodular disease, 6.7% (n=9) invasive encapsulated follicular variant papillary thyroid carcinoma (IEFV-PTC), 3.75% (n=5) follicular carcinoma, 3% (n=4) chronic lymphocytic thyroiditis (CLT), 2.2% (n=3) follicular adenoma, and 1.5% (n=2) well-differentiated thyroid carcinoma of uncertain malignant potential (WDT-UMP). In addition, 0.75% (n=1) of the patients was diagnosed with medullary carcinoma, oncocytic carcinoma, anaplastic carcinoma-NOS, and paraganglioma.

Table 1. 2023 Bethesda system for reporting thyroid cytopathology.

Diagnostic category		ROM** mean % (range)
Category 1	Non-diagnostic	13 (5–20)
Category 2	Benign	4 (2–7)
Category 3	AUS*	22 (13–30)
Category 4	Follicular neoplasia	30 (23–34)
Category 5	Suspicion of malignancy	74 (67–83)
Category 6	Malignant	97 (97–100)

*AUS: Atypia of Undetermined Significance **ROM: Risk of malignancy.

The analyses of the cases diagnosed with papillary microcarcinoma indicated that 45 of 49 were 5 mm or less in size, and structures such as follicular nodular disease of the thyroid (n=25), chronic lymphocytic thyroiditis (n=16), follicular adenoma (n=5), oncocytic adenoma (n=1), and neoplasia of uncertain malignancy potential (n=2) were observed in non-microcarcinoma areas. Most of the cases (91.83%) diagnosed with papillary microcarcinoma were ≤ 5 mm in size, and there were lesions observed in non-tumor areas; therefore, it was concluded that aspiration was performed from other accompanying nodules and that papillary microcarcinoma was detected incidentally postoperatively. When the incidental papillary microcarcinoma diagnoses were excluded, a malignant tumor rate of 49.62% was obtained. The histopathologic classifications of the nodules resulting in the diagnosis of AUS are presented in Table 2.

Twenty-two cases had nodular formation, 71 cases had multinodular formation, 20 cases had chronic lymphocytic thyroiditis, and 20 cases had no additional features in non-tumor areas. In order to evaluate the malignancy rates according to the criteria of nodular goiter (group 1), multinodular goiter (group 2), CLT and no feature in non-tumor areas, and CLT and no feature (group 3) were included in a single group, and statistical analysis revealed a significant difference ($p=0.0033$). In the first group, the malignant tumor rate was 45.5%. In the second and third groups, this rate gradually decreased. The malignant tumor rates were 14.1% and 5% in the second and third groups, respectively. When malignancy rates were analyzed, it was seen that malignant tumor rates were significantly higher in nodular goiter patients compared to other groups. There was a significant decrease in malignant tumor rates with an increase in the presence of multinodular formation, chronic lymphocytic thyroiditis, and cases with no features.

When the median, minimum and maximum values were calculated in the statistical Kruskal-Wallis test ($p = 0.559$) in terms of TSH hormone level according to malignancy status, the median TSH hormone values in the malignant group were 1.491 mU/L (0.005 - 103.72) in the malignant group, 1.789 mU/L (0.398 - 8.470) in the benign group and 2.318 mU/L (1.817 - 2.819) in the group with uncertain malignancy potential. The mean T4 hormone levels calculated using one-way ANOVA test was 0.977 ± 0.284 ng/dL in the malignant

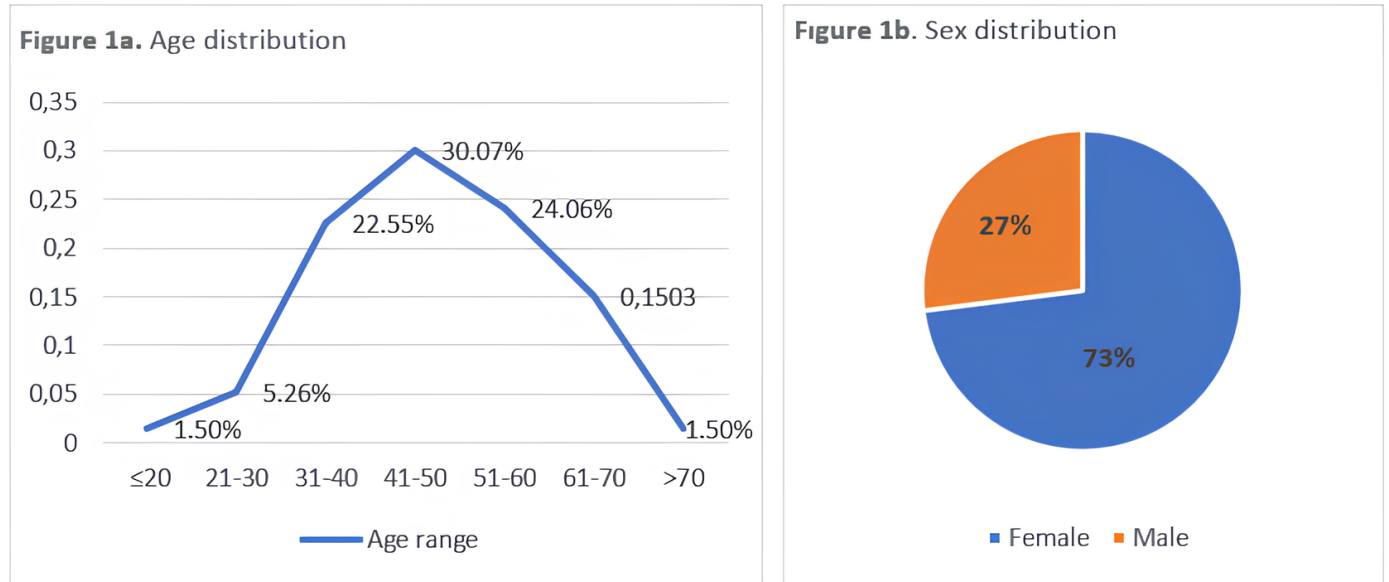


Figure 1. a,b. The distribution of the age (a) and sex of the study population (b).

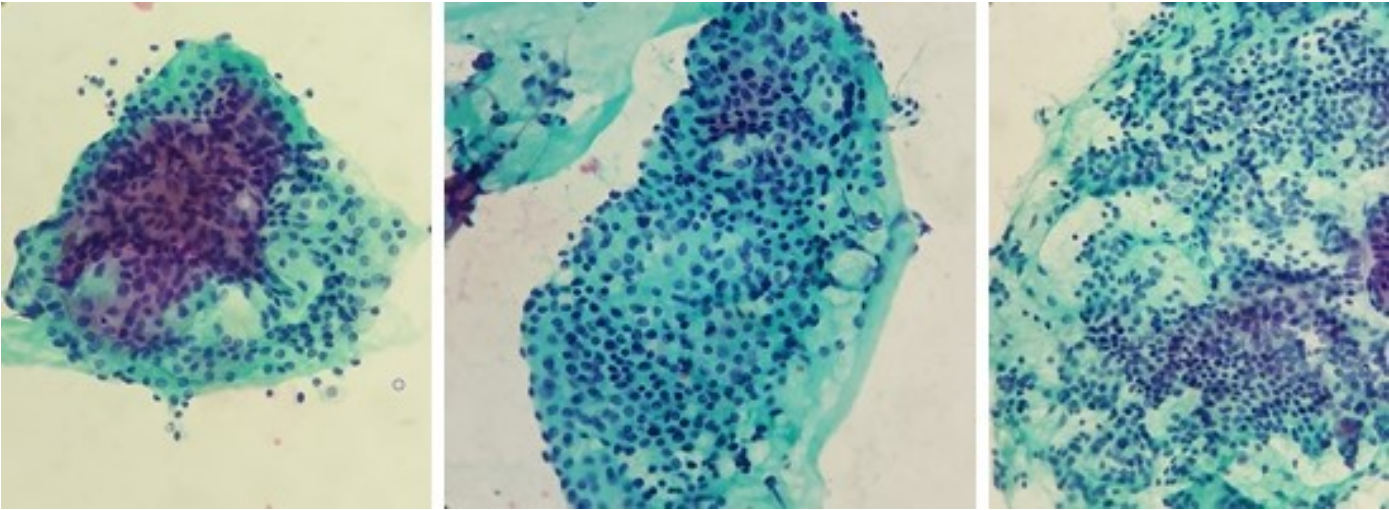


Figure 2. Bethesda 3 category in cytology; 40x, pap.

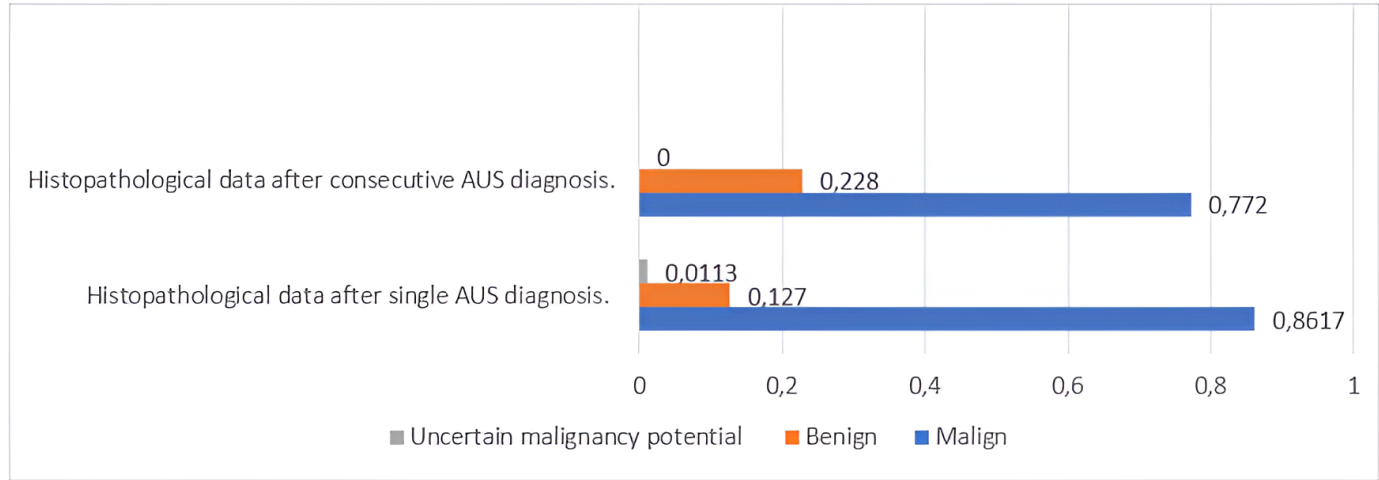


Figure 3. Comparison of the histopathologic results of patients diagnosed with AUS after a single diagnosis of Atypia of Undetermined Significance and cases diagnosed with AUS after repeated fine needle aspiration biopsy.

Table 2. Histopathologic distribution of cases diagnosed with atypia of undetermined significance.

Histopathologic diagnosis	Papillary carcinoma*	Papillary microcarcinoma	Follicular carcinoma	Medullary carcinoma	Anaplastic carcinoma	Oncocytic carcinoma	Other **	Benign ***
Number of cases	54	49	5	1	1	1	3	19
Distribution (%)	40.6%	36.8%	3.8%	0.75%	0.75%	0.75%	2.25%	14.3%

*IEFV-PTC: included in papillary carcinoma ** WDT-UMP and paraganglioma were included in this group *** Follicular nodular disease of the thyroid, chronic lymphocytic thyroiditis, and follicular adenoma were included in this group

group, 0.960 ± 0.207 ng/dL in the benign group and 0.920 ± 0.113 ng/dL in the group with uncertain malignancy potential ($p = 0.927$). According to these results, there was no statistically significant difference between the malignant, benign, and certain malignant tumor potential groups in terms of TSH hormone($p=0.887$) and T4 hormone levels ($p=0.927$).

■ DISCUSSION

Thyroid nodules are detected in 4–8% of adults using physical examination, 41% using radiological methods, and 50% during autopsy [8]. The American Thyroid Association and the National Comprehensive Cancer Network recommend the use of FNAB as the first diagnostic test because of its diagnostic reliability and cost-effectiveness [8]. FNAB avoids unnecessary surgery for benign nodules [8]. Cytopathologic data obtained after FNAB are classified into six categories based on the Bethesda system: non-diagnostic, benign, AUS, follicular neoplasm, suspicion of malignancy, and malignant [4]. Our department included cases diagnosed with FLUS classified according to the pre-2023 Bethesda system, and these cases were reclassified and evaluated according to the current Bethesda classification.

The ages of the patients in this study ranged from 17 to 79 years, and the mean age was 48.6 years. Of these patients, 73% ($n=97$) were female and 27% ($n=36$) were male, with a female-to-male ratio of 2.69:1. The incidence of thyroid lesions was higher in women than in men, a rate similar to the incidence of higher thyroid lesion rates in women (77.34%) in the study conducted by Machata et al. examining all Bethesda categories [9]. In addition, the age distribution of lesions in the study by Machata et al was most frequently between 41 and 50 (25–59%), followed by those between 51 and 60 (22.35%). In the present study, the age distribution was most frequent in the fifth decade, followed by the sixth decade, which was a similar range [9]. Furthermore, Machata et al. found that the post-resection malignancy rate of cases in the Bethesda 3 category was 17.1% [9].

The study conducted by Ho et al. included 350 patients diagnosed with AUS who had immediate surgery, and the ROM was 38.6%, whereas 31 patients underwent repeated FNAB, and the ROM was 37.8% [5]. The majority of cases resulting in malignant cytology were diagnosed as papillary thyroid carcinoma [5]. The cases with benign surgical results were diagnosed with nodular hyperplasia [5]. The rate of cases with benign lesions in the resection material was 14.3% ($n=19$) in

the present study. Follicular nodular disease of the thyroid, chronic lymphocytic thyroiditis, and follicular adenoma were among them. Ho et al. also found incidental thyroid cancer in 85 patients, of whom 76 (89.4%) had a diagnosis of papillary microcarcinoma and the ROM rate was 57.2% with the inclusion of incidental cases. In the present study, papillary carcinoma and papillary microcarcinoma were the most common malignancies, followed by follicular carcinoma. Moreover, the ROM in the present study was 49.62%; with the inclusion of incidental carcinoma cases (papillary microcarcinoma), the ROM was 83.4%, which was higher than that in the literature. This shows that incidental malignant lesions increase the ROM rate in cases diagnosed with AUS and reveals that this risk ratio is not clear in cases diagnosed with AUS.

VanderLaan et al. conducted a study with 331 cases and found that the malignancy rate was 41% in cases diagnosed with AUS after the first FNAB who underwent immediate surgery and 43% in those cases after a repeated FNAB. They determined the rthat the of malignant tumor after a single AUS or recurrent AUS diagnosis was similar and higher than 40% [10]. However, in the present study, the postoperative malignant tumor risk of cases with a first diagnosis of AUS was 86.17% and that of cases with consecutive FNABs was 77.2%, which were close rates. These data suggest that consecutive FNABs do not provide a clear benefit in terms of clinical resection decisions. In addition, VanderLaan et al reported the malignant tumor rate of 45.7% in all surgical cases, a rate higher than that reported in the literature [10]. In the present study, the malignant tumor risk obtained by excluding incidental papillary microcarcinomas was 49.62%, which was similar to that of that study. In the study conducted by VanderLaan et al., 89% ($n=81$) of the cases diagnosed with malignancy were papillary carcinoma, followed by follicular carcinoma at a rate of 9% ($n=8$) [10]. The most common malignant tumor was papillary carcinoma in the present study.

Başçeken et al evaluated the correlation between the diagnosis of AUS and the histopathological correlation and found the rate of malignant tumor was higher than the literature, with a rate of 43.5% [11]. In addition, another study conducted in Turkey found that ROM was 49.1% in AUS cases [12]. These results are similar to the ROM rates calculated in the present study by excluding incidental cases.

In another study, although the risk of malignant tumor was approximately 35% in cases diagnosed with AUS, it was 51.9% in those with a second diagnosis of AUS. The study also re-

ported that the ROM decreased to 5.3% if radiologic data did not suggest malignant tumor when the results were evaluated together with ultrasonographic data. In addition, the same study emphasized that clinicopathological and ultrasound results should also be considered when evaluating the malignant tumor rate in cases diagnosed with AUS [13].

In an analytical review of 47 studies including 4,475 cases of AUS, Huhtamella et al reported the malignant tumor risk rate of 27% (23–31%). Histopathologically confirmed ROM after the first FNAB was 38.2%; however, the ROM after the second FNAB was 21.7%. The study also found a significant correlation between nuclear atypia, which we mentioned earlier, and a higher ROM rate in cytology materials diagnosed with AUS [14]. In addition, the authors reported ROM of 16.7% with focal features suggestive of papillary carcinoma, 12.2% with cytologic atypia, and 12% with architectural atypia [14]. These data show that cases with nuclear atypia have a higher ROM rate and partially explain these variations in ROM rates [14].

Of the 33 cases diagnosed with papillary microcarcinoma, 31% (n=10) had a cytologic diagnosis of AUS in the study conducted by Şeker et al. The study also emphasized that false-negative results were obtained, especially in nodules smaller than 5 mm, because of inadequate sampling [15]. In another similar study, the resection result of papillary microcarcinoma in cases with a false-negative diagnosis may have resulted from the lack of optimal sampling of these areas or the presence of other accompanying nodules and also attributed the reason for resection in these cases to the result of evaluation with detailed clinical, radiological, and pathological data [16]. In the present study, 49 of the resected thyroidectomy materials had a diagnosis of papillary microcarcinoma, and in 45 of these cases, the carcinoma size was 5 mm or smaller. In addition, benign lesions, such as follicular nodular disease and chronic lymphocytic thyroiditis, were observed in the investigation of thyroid tissue outside these carcinoma areas.

Some studies have excluded papillary microcarcinomas [3] or have not included them in the ROM because they are detected incidentally [5]. Some patients are not detected by clinical examination or are diagnosed incidentally. In the present study, it is thought that these tumors, which are incidental and smaller than 1 cm in size, may not contribute cells to the slides from an FNAB and should not be included because they increase the ROM rate after resection. Accordingly, more research is needed on this subject. In addition, clinical, radiological, and pathological data should be considered by clinicians in cases diagnosed with AUS because many factors affect ROM.

■ CONCLUSION

This study determined that the patients diagnosed with AUS in our unit were mostly taken to surgery directly, and in cases with repeated FNABs, the cytopathologic result was mostly AUS. In addition, the malignant tumor rates were similar in

AUS cases that directly underwent surgery or surgery after consecutive FNABs. In this study, most cases diagnosed as AUS (84.9%) were malignant.

However, some papillary microcarcinomas are not detected during clinical examination but are detected during the pathologic examination of thyroid specimens after surgery. Compared with the resection specimens in our unit, it is noteworthy that papillary microcarcinoma (n=45), which was detected in the resection specimens of patients diagnosed with AUS, constituted a large proportion of the diagnoses (33.83%). This suggests that because papillary microcarcinomas are tumors smaller than 1 cm, these cells may not be included in the cytology material from an FNAB, and cytological sampling is performed from other accompanying nodules. The high ROM rate in the cases with AUS is attributed to papillary microcarcinoma features. The rate of ROM was 49.62%, excluding incidental cases of papillary microcarcinoma.

Tumoral cells may not be sampled on the slide from tumors smaller than 1 cm in size and may be incidentally detected in the resection material during FNAB. FNAB is a valuable method that deserves the importance given to it in the diagnosis and follow-up of thyroid nodules; however, the most appropriate decision should be reached by evaluating the patient's clinical, radiological, and laboratory results as a whole. In addition, there are many factors affecting ROM, and incidental malignancies should not be included in the calculation of ROM.

Ethics Committee Approval: Ethical approval was obtained from Atatürk University Non-Interventional Research Ethics Committee with a decision dated March 29, 2024, meeting number: 2 and, decision number: 19.

Informed Consent: Since the current study was a retrospective analysis, ethics committee approval was obtained and informed consent form was not necessary for this manuscript.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept: RD, Design: HDT, Supervision, Material support: RD, Data collection and/or processing: HDT, Data analysis and interpretation: RD, Literature Review: HDT, Writing: HDT, Critical revision of manuscript: RD, HDT.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: The authors declare no financial support.

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