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Original Article

Initial AUS cytology is associated with favorable prognostic features in differentiated thyroid carcinoma

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Abstract

Aim: To evaluate whether differentiated thyroid carcinoma (DTC) cases that follow an initial fine-needle aspiration biopsy (FNAB) result of atypia of undetermined significance (AUS) differ in their clinicopathological characteristics and prognostic features compared with DTC cases without prior AUS cytology.
Materials and Methods: Medical records of 936 patients with an initial FNAB result of AUS between January 2019 and December 2023 were retrospectively reviewed. Among them, 307 underwent surgery, and 139 (45.3%) were found to have malignant lesions on final histopathology. Of these, 131 were diagnosed with DTC and comprised the "AUS-preceded DTC" group, which was compared with 376 patients in the "non-AUS-preceded DTC" cohort. Data on histopathological type, invasion patterns, metastasis, Tumor-Node-Metastasis (TNM) stage, and American Thyroid Association (ATA) risk classification were analyzed.
Results: Microcarcinoma (≤10 mm) was significantly more frequent in the AUS-preceded DTC group (45.8% vs. 29.0%, p<0.001). The frequencies of extrathyroidal extension (6.1% vs. 20.2%, p<0.001), capsular invasion (6.1% vs. 16.8%, p=0.040), and lymphatic invasion (12.2% vs. 24.7%, p=0.030) were significantly lower in the AUS-preceded group. Lymph node metastasis (16.8% vs. 32.2%, p=0.001) and distant metastasis (0.0% vs. 3.7%, p=0.026) were also less common. Only one patient (0.8%) in the AUS-preceded DTC group was classified as ATA high risk compared with 39 (10.4%) in the non-AUS-preceded DTC group (p=0.001).
Conclusion: DTC preceded by AUS cytology exhibits more favorable pathological and prognostic features than the non-AUS-preceded DTC population. These tumors are typically smaller, less invasive, and associated with lower ATA risk categories. AUS cytology may therefore provide prognostic insight beyond its diagnostic role and support more individualized, conservative management strategies in low-risk patients. Prospective multicenter studies incorporating molecular data are warranted to validate these findings and elucidate the biological mechanisms underlying the favorable behavior of AUS-related malignancies.

Keywords: Atypia of undetermined significance, fine-needle aspiration biopsy, differentiated thyroid carcinoma, bethesda system, ata risk classification

INTRODUCTION

Since its first edition in 2010, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has been a widely accepted reporting system for fine-needle aspiration biopsy (FNAB) cytology by clinicians and researchers caring for thyroid nodules (1). Developed to standardize thyroid nodule cytology reporting, TBSRTC consists of 6 different categories, and a specific risk of malignancy (ROM) has been proposed for each of these categories. In the recently published third edition of TBSRTC, these six categories are expressed as: (I) nondiagnostic; (II) benign; (III) atypia of undetermined significance (AUS); (IV) follicular neoplasm; (V) suspicious for malignancy; and (VI) malignant (2). In the third edition, the AUS category was simplified by removing the term "follicular lesion of undetermined significance (FLUS)" and subdivided into "AUS-nuclear" and "AUS-other" (2).

When the Bethesda system was first introduced, a ROM of 5-15% was recommended for category 3 (called AUS/FLUS at the time) (1). However, a wide range of malignancy rates have been reported since that time, ranging from 6% to 72.9% (3-8). For nodules in Bethesda category 3 based on the initial FNAB cytology result, the 2015 American Thyroid Association

(ATA) guidelines recommend repeat FNAB, diagnostic surgical excision, or surveillance, depending on clinical risk factors, ultrasonographic features, and individual patient preference (9). Because the ROMs reported for nodules in this category are highly heterogeneous, numerous clinical studies have investigated features (especially ultrasonographic patterns) that help predict the ROMs of thyroid nodules with AUS as the initial FNAB cytology result (10-12).

There is ample evidence reporting the ROMs of nodules categorized as AUS, as well as additional cytological and clinical features that assist in predicting malignant potential. However, evidence remains limited regarding whether differentiated thyroid cancer (DTC) patients with AUS cytology preceding surgery exhibit different clinicopathological prognostic features compared with DTC patients without prior AUS cytology. Therefore, in this study, we aimed to investigate whether the clinicopathological prognostic factors of DTC patients whose initial FNAB results were reported as AUS differ from those of patients with DTC in the non-AUS-preceded cohort, ensuring a comparison between two distinct and independent patient groups.

MATERIALS AND METHODS

All patients who were followed up with AUS as a result of initial FNAB at the Department of Endocrinology and Metabolism at Necmettin Erbakan University Medical School between January 2019 and December 2023, and all patients with DTC as a result of histopathology after surgical treatment at the same institution in the same period, were retrospectively reviewed. Necmettin Erbakan University Ethics Committee approved the study with the approval no: 2025/6104 and the date: 14.11.2025. In patients with DTC, histopathological prognostic features such as tumor histopathological type, tumor diameter, angioinvasion, lymphatic invasion, perineural invasion, extrathyroidal invasion and surgical margin involvement were recorded from the patient files. In addition, prognostic parameters such as lymph node involvement status, distant metastasis status, Tumor-Node-Metastasis (TNM) stage and ATA risk category were recorded for all patients included in the study.

This retrospective, observational and cross-sectional study was conducted in the Department of Endocrinology and Metabolism of a single, large institution that serves as a tertiary referral center for patients with thyroid nodules or patients referred upon detection of thyroid cancer as a result of histopathology following surgical resection. Patients whose FNAB results or surgical histopathology results were from a different institution, whose AUS diagnosis could not be determined whether it was the initial FNAB result or a repeat FNAB result, or whose data were missing were excluded from the study. In addition, parameters that may be the subject of future studies, such as whether repeat FNAB is performed in the decision-making process before surgical treatment in patients with AUS, repeat FNAB results and other factors that may affect the decision-making process for surgical treatment, and the detailed preoperative cytological distribution of the non-AUS-preceded DTC cohort (e.g., Bethesda V–VI categories), were not within the scope of the current study.

For comparative analyses, patients were categorized into two mutually exclusive groups based on their preoperative cytological findings: the AUS-preceded DTC group, consisting of patients whose FNAB cytology prior to surgery was reported as AUS, and the non-AUS-preceded DTC group, consisting of DTC patients without a prior AUS cytological diagnosis. Patients with AUS-preceded DTC were excluded from the comparator cohort, ensuring statistical independence between groups. Clinicopathological features and oncological outcomes were compared between the two groups.

Statistical Analysis

Statistical analysis was performed using the SPSS 21.0 (Statistical Package for Social Sciences) program. Categorical variables were expressed as numbers and percentages, and continuous variable (age) was presented as mean ± standard deviation, as it followed a normal distribution. Comparisons between independent groups were performed using the chi-square test or Fisher's exact test for categorical variables, depending on expected cell counts. Categorical variables with more than two levels were analyzed using appropriate exact tests. The continuous variable was compared using Student's t-test. For differences, $p<0.05$ value was considered statistically significant.

RESULTS

When the medical records of a total of 936 patients diagnosed with Bethesda category III AUS cytology by initial FNAB during a 5-year period were retrospectively reviewed, it was determined that 307 (32.8%) of them underwent thyroidectomy or thyroid lobectomy. Of the 307 patients with AUS who underwent surgery, 139 (45.3%) had malignancy and 168 (54.7%) had benign lesions. In terms of the distribution of thyroid malignancy types, 131 (94.2%) of 139 patients with thyroid malignancy had DTC and 8 (5.8%) had malignancies other than DTC. The final histopathology results of patients with AUS are shown in Figure 1.

During the study period, 376 patients without prior AUS cytology were diagnosed with DTC according to histopathology results after surgical treatment, and 342 (91.0%) of them were diagnosed with papillary thyroid cancer (PTC) (Figure 2).

When the "DTC preceded by AUS" group (n=131) and the "non-AUS-preceded DTC" group (n=376) were compared, there was no difference in age and sex distribution (p=0.168 and p=0.401, respectively). The tumor diameter in 60 (45.8%) of 131 patients in the DTC preceded by AUS group and in 109 (29%) of 376 patients in the non-AUS-preceded DTC group was smaller than 10 mm, and the frequency of microcarcinoma was significantly higher in the DTC preceded by AUS group than

in the non-AUS-preceded DTC group (p<0.001). In addition, T classification differed significantly between the two groups, with earlier T stages being more frequent in the DTC preceded by AUS group compared with the non-AUS-preceded DTC group (p=0.012). Similarly, N classification also showed a significant difference between groups, with a lower frequency of lymph node metastasis observed in the DTC preceded by AUS group (p=0.009). The frequency of extrathyroidal invasion, capsular invasion and lymphatic invasion was significantly lower in the DTC preceded by AUS group than in the non-AUS-preceded DTC group (p<0.001, p=0.040 and p=0.030, respectively). The frequency of surgical margin involvement, angioinvasion and perineural invasion was similar between the groups (p=0.133, p=0.115 and p=0.327, respectively). Lymph node metastasis was detected in 22 (16.8%) patients in the DTC preceded by AUS group and 121 (32.2%) patients in the non-AUS-preceded DTC group, and the frequency of lymph node metastasis was significantly lower in the DTC preceded by AUS group (p=0.001). While distant metastasis was not detected in any patient in the DTC preceded by AUS group, it was detected in 14 (3.7%) patients in the non-AUS-preceded DTC group (p=0.026). While only 1 (0.8%) patient in the DTC preceded by AUS group was in the ATA high risk category, 39 (10.4%) of the patients in the non-AUS-preceded DTC group were in the ATA high risk category, and the ATA high risk was significantly lower in the DTC preceded by AUS group (p=0.001) (Table 1).

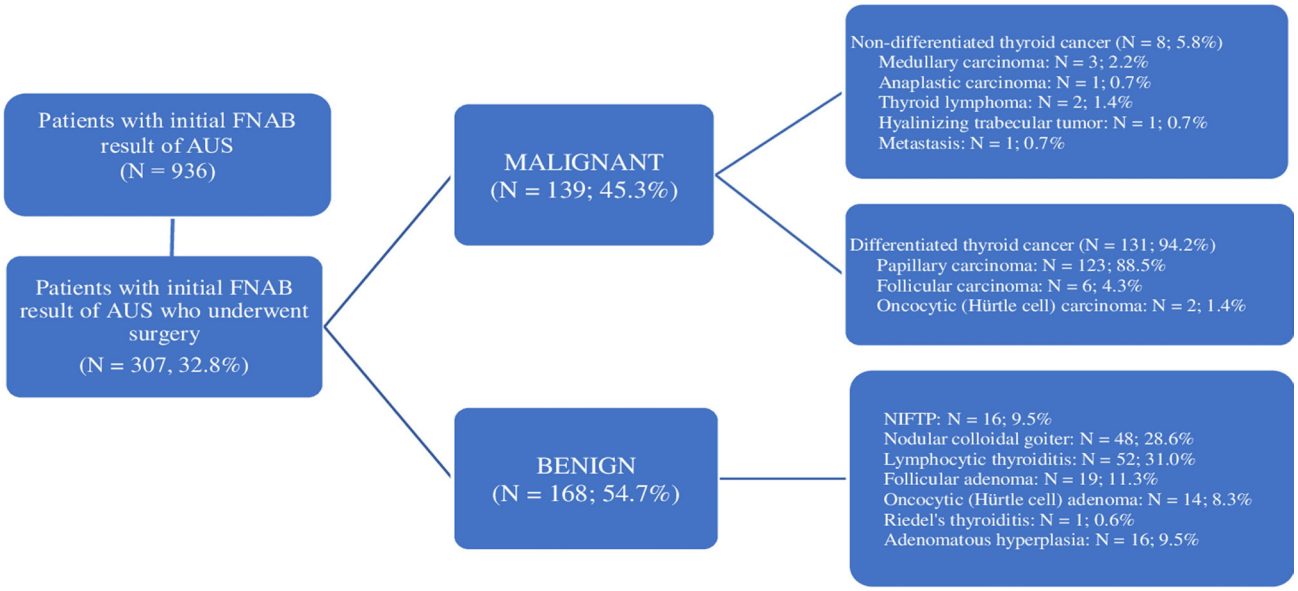

Figure 1. Distribution of final histopathological diagnoses in patients who underwent surgery with an initial FNAB result of AUS over a 5-year period Abbreviations: AUS: Atypia of undetermined significance, FNAB: Fine-needle aspiration biopsy, NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features

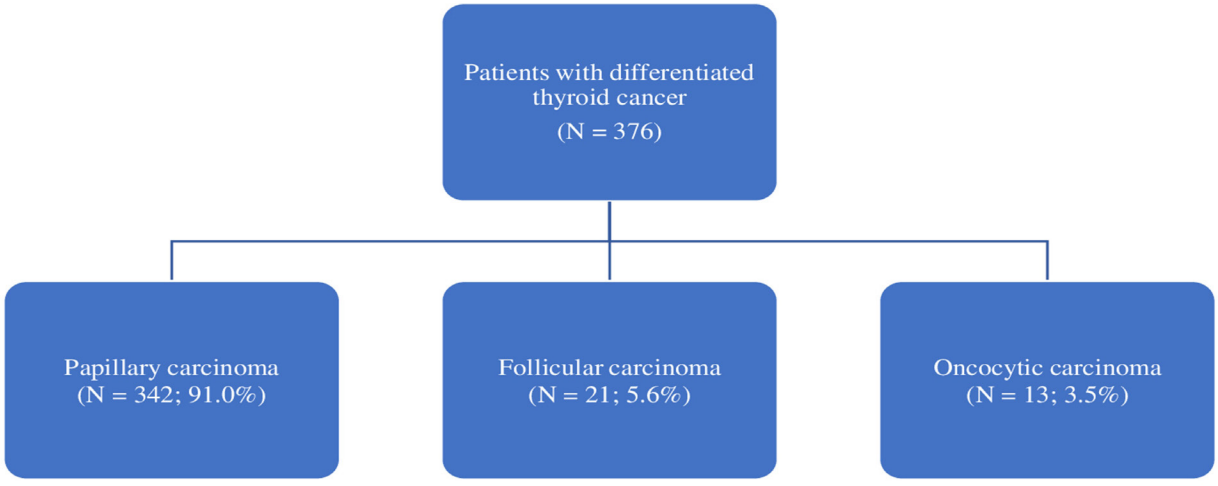

Figure 2. Distribution of surgical histopathological findings in patients with non-AUS-preceded differentiated thyroid cancer over a 5-year period

Table 1. Clinicopathological features of AUS-preceded DTC patients compared with the non-AUS-preceded DTC cohort

Variables	DTC patients preceded by AUS (N=131)	Overall DTC (N=376)	p
Gender			
Female, n (%)	105 (80.2%)	288 (76.6%)	0.401
Male, n (%)	26 (19.8%)	88 (23.4%)	
Age (years), mean±SD	43.95±12.74	45.91±14.76	0.168
Tumor type			
Papillary carcinoma (PTC)	123 (93.9%)	342 (91.0%)	0.260
Follicular carcinoma (FTC)	6 (4.6%)	21 (5.5%)	
Oncocytic (Hürthle cell) carcinoma (HCC)	2 (1.5%)	13 (3.5%)	
Tumor size			
≤10 mm (Microcarcinoma)	60 (45.8%)	109 (29.0%)	<0.001
>10 mm	71(54.2%)	267 (71.0%)	
Surgical margin			
Positive	8 (6.1%)	42 (11.2%)	0.133
Negative	123 (93.9%)	334 (88.8%)	
Vascular invasion			
Present	8 (6.1%)	43 (11.4%)	0.115
Absent	123 (93.9%)	333 (88.6%)	
Extrathyroidal extension (ETE)			
Present	8 (6.1%)	76 (20.2%)	<0.001
Absent	123 (93.9%)	300 (79.8%)	
Capsular invasion			
Present	8 (6.1%)	63 (16.8%)	0.040
Absent	123 (93.9%)	313 (83.2%)	
Lymphatic invasion			
Present	16 (12.2%)	93 (24.7%)	0.030
Absent	115 (87.8%)	283 (75.3%)	
Perineural invasion (PNI)			
Present	3 (2.29%)	18 (4.8%)	0.327
Absent	128 (87.8%)	358 (95.2%)	
Lymph node metastasis			
Present	22 (16.8%)	121 (32.2%)	0.001
Absent	109 (83.2%)	255 (67.8%)	
T classification			
T1a	59 (45.0%)	109 (29.0%)	
T1b	54 (41.2%)	160 (42.6%)	
T2	10 (7.6%)	61 (16.2%)	0.012
T3	7 (5.3%)	37 (9.8%)	
T4a	1 (0.8%)	9 (2.4%)	
T4b	0 (0.0%)	0 (0.0%)	

Data are presented as n (%) for categorical variables and mean ± standard deviation (SD) for continuous variables. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. The continuous variable was compared using Student's t-test. Abbreviations: AUS, Atypia of Undetermined Significance; DTC, Differentiated Thyroid Cancer; FNAB, Fine-Needle Aspiration Biopsy; ETE, Extrathyroidal Extension; PNI, Perineural Invasion; LNM, Lymph Node Metastasis; ATA, American Thyroid Association; AJCC, American Joint Committee on Cancer. $p<0.05$ was considered statistically significant

Table 1. Clinicopathological features of AUS-preceded DTC patients compared with the non-AUS-preceded DTC cohort

Variables	DTC patients preceded by AUS (N=131)	Overall DTC (N=376)	p
N classification			
N0	109 (83.2%)	255 (67.8%)	0.009
N1a	13 (9.9%)	50 (13.3%)	
N1b	9 (6.9%)	71 (18.9%)	
M classification			
M0	131 (100.0%)	362 (96.3%)	0.026
M1	0 (0.0%)	14 (3.7%)	
Stage (AJCC 8th edition)			
Stage I	121 (92.4%)	324 (86.2%)	
Stage II	7 (5.3%)	37 (9.8%)	
Stage III	1 (0.8%)	2 (0.5%)	
Stage IVA	2 (1.2%)	0 (0.0%)	0.583
Stage IVB	0 (0.0%)	13 (3.5%)	
Grouped stages:			
Low stage (I-II)	128 (97.7%)	361 (96.0%)	
High stage (III-IVA-IVB)	3 (2.3%)	15 (4.0%)	
ATA risk classification			
Low risk	109 (83.2%)	192 (51.1%)	
Intermediate risk	21 (16.0%)	145 (38.6%)	
High risk	1 (0.8%)	39 (10.4%)	
Grouped risk categories:			
Low-Intermediate risk	130 (99.2%)	337 (89.6%)	0.001
High risk	1 (0.8%)	39 (10.4%)	
Grouped risk categories:			
Low risk	109 (83.2%)	192 (51.1%)	<0.001
Intermediate-high risk	22 (16.8%)	184 (48.9%)	

Data are presented as n (%) for categorical variables and mean ± standard deviation (SD) for continuous variables. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. The continuous variable was compared using Student's t-test. Abbreviations: AUS, Atypia of Undetermined Significance; DTC, Differentiated Thyroid Cancer; FNAB, Fine-Needle Aspiration Biopsy; ETE, Extrathyroidal Extension; PNI, Perineural Invasion; LNM, Lymph Node Metastasis; ATA, American Thyroid Association; AJCC, American Joint Committee on Cancer. $p<0.05$ was considered statistically significant

DISCUSSION

In our study, patients with DTC preceded by AUS on initial FNAB were more likely to have microcarcinoma compared to the non-AUS-preceded DTC cohort. Consistent with this finding, tumors in the AUS-preceded DTC group were more frequently classified as earlier T stages and were associated with a lower rate of lymph node involvement, reflecting significant differences in both T and N classifications between the two groups. Moreover, adverse prognostic factors such as extrathyroidal extension, capsular invasion, and lymphatic invasion were significantly less frequent in the AUS-preceded group. Additionally, lymph node metastasis, distant metastasis, and ATA high-risk disease were all observed at lower rates in patients with DTC preceded by AUS.

These findings suggest that DTC preceded by AUS may represent a biologically less aggressive and more indolent subtype of thyroid carcinoma. Nodules categorized as AUS in the Bethesda System often display a follicular growth pattern and mild nuclear and/or architectural atypia that are qualitatively or quantitatively insufficient for a more definitive classification (13,14). Therefore, it is biologically plausible that carcinomas arising from these nodules exhibit less aggressive histopathological behavior. Consistent with our results, previous studies have also reported lower rates of extrathyroidal extension, lymph node metastasis, and aggressive histological variants in patients with thyroid carcinoma preceded by AUS cytology (15-17).

Another possible explanation is that patients diagnosed with AUS are often referred for surgery at an earlier stage. Due to both physician and patient concern regarding the indeterminate nature of AUS, early surgical intervention may be preferred. This tendency could lead to the detection of tumors with smaller diameters, as reflected by the significantly higher frequency of microcarcinoma in the AUS group in our study. In contrast, patients whose preoperative cytology is categorized as "suspicious for malignancy" or "malignant" are usually referred for surgery after more apparent clinical or radiological findings have developed. Consequently, these patients are more likely to present with advanced-stage disease and aggressive histopathological features.

Our observations align with previous literature evaluating the clinicopathologic characteristics of AUS-related thyroid carcinomas. Malignant outcomes following AUS cytology are most frequently PTCs, often including the follicular variant and a measurable proportion of papillary thyroid microcarcinomas (PTMCs). For instance, in a tertiary-center cohort of AUS nodules, all malignant cases were variants of PTC, with follicular-variant PTC being the most common subtype and several cases identified as PTMC (18). In parallel, studies correlating Bethesda categories with pathologic behavior have demonstrated that higher-risk cytology (Bethesda V/VI: "suspicious for malignancy" or "malignant") is associated with more adverse features, specifically higher rates of extrathyroidal extension and lymph-node metastasis, whereas AUS cytology tends to exhibit these features less frequently. These findings are consistent with our results showing lower rates of extrathyroidal extension, lymph-node metastasis, and ATA high-risk classification in the AUS-preceded DTC group (15). Collectively, these data support the hypothesis that thyroid carcinomas preceded by AUS cytology generally display a less aggressive biological behavior and may represent a distinct, more indolent subset within the spectrum of DTCs.

In our study, malignant cases following AUS cytology demonstrated more favorable prognostic features compared with the non-AUS-preceded DTC population. This finding suggests that malignancy diagnosed after an AUS result does not necessarily reflect an aggressive disease course. Most AUS-related malignancies in our cohort were microcarcinomas with limited invasion and classified within the low ATA risk category. Therefore, a more conservative postoperative management approach may be appropriate for this subset of patients.

These low-risk malignancies arising after AUS cytology highlight the biological heterogeneity of thyroid carcinoma and suggest that the AUS category may represent a cytological precursor pattern of well-differentiated, indolent malignancies. Accordingly, our findings indicate that AUS cytology provides not only diagnostic but also prognostic insights into the biological behavior of thyroid carcinoma.

This study has several limitations. First, its retrospective and single-center design limits the generalizability of the findings. In addition, only patients who underwent surgery were included; therefore, cases with AUS cytology that were managed non-surgically were not evaluated. This may have introduced a potential selection bias. Furthermore, although patients were appropriately categorized into AUS-preceded and non-AUS-preceded DTC cohorts, the detailed preoperative cytological distribution (e.g., Bethesda V/VI categories) of the non-AUS-preceded group was not available. Therefore, we could not fully adjust for potential baseline differences in cytologic risk between the comparison groups. As a result, the more favorable clinicopathological outcomes observed in the AUS-preceded DTC group may, in part, reflect differences in underlying cytological risk rather than a true prognostic effect. Moreover, the AUS category could not be subdivided into "AUS-nuclear atypia" and "AUS-other" subtypes as defined in the third edition of TBSRTC (2), precluding analysis of potential prognostic differences between these subgroups. Additionally, complementary molecular data were not available, which limited the ability to explore the biological mechanisms underlying the observed prognostic variations.

Despite these limitations, the study's strengths include its relatively large sample size, the uniform evaluation of all cases within a single tertiary center, and pathological examinations conducted by the same laboratory. These factors enhance the internal validity of the results and reinforce the reliability of the observed favorable prognostic features in patients with malignancy following AUS cytology.

CONCLUSION

This study showed that patients with DTC preceded by AUS cytology generally have more favorable clinicopathological and prognostic features than the non-AUS-preceded DTC population. These malignancies often represent small, indolent tumors with lower rates of invasion and metastasis, and fewer cases classified as ATA high risk. The relatively favorable outcomes of AUS-related DTCs may guide clinicians toward more individualized management and follow-up strategies, potentially helping to avoid overtreatment in low-risk cases. Nevertheless, further multicenter prospective studies incorporating molecular data are warranted to validate these findings and to better elucidate the biological basis of the prognostic differences observed in AUS-related malignancies.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that there is no financial support.

Ethical Approval
The study protocol received approval from the Necmettin Erbakan University (NEU) Medical Faculty approved the study with the approval no: 2025/6104 and the date: 14.11.2025.

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