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Cytologic-histopathologic correlation of bethesda category III diagnosed cases in thyroid cytology and analysis according to the bethesda system for reporting thyroid cytopathology 2023

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Abstract

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has been used by pathologists since 2010, providing a standardized language, updated in 2017 and finally in 2023. According to the latest update, category III has been divided into two subgroups: " Atypia of uncertain significance (AUS) -nuclear atypia" and "AUS-other." We aimed to analyze the risk of malignancy in patients diagnosed with category III (AUS, follicular lesion of uncertain significance (FLUS), or AUS/FLUS) and compare them based on recent changes in cytologic-histopathologic correlation. We retrospectively evaluated thyroid fine needle aspiration materials diagnosed as category III. We categorized the cases as AUS, FLUS, or AUS/FLUS. The cytopathologic and histopathologic diagnoses in the resection materials were then compared. Based on detailed microscopic descriptions in our reports, we reclassified the cases as "AUS-nuclear atypia" and "AUS-other" according to the updated 2023 TBSRTC.Our study included 73 patients, who had undergone thyroidectomy. Of these, 45.21% were reported as FLUS, 47.94% as AUS, and 6.85% as AUS/FLUS in thyroid cytology. Surgical follow-up showed no significant differences in malignancy risk, both between the FLUS and AUS groups (p=.232) and between cases diagnosed as "AUS-nuclear atypia" and "AUS-other" (p=.826). Within the indeterminate group, category III has the lowest risk of malignancy, with a predicted risk between 10-30%. However, since this group is heterogeneous, the reported malignancy risk in the literature ranges from 6-72.9%, depending on the type of atypia leading to the AUS interpretation. According to the literature, it was reported that the subgroup exhibiting the highest nuclear atypia had the highest risk of malignancy.

Keywords: Thyroid, cytology, category III, fine needle aspiration

Introduction

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) is a system used in reporting thyroid cytology, aiming to increase cytopathologist-clinician agreement and to manage the patient correctly in terms of diagnosis and treatment [1]. TBSRTC has been used by pathologists since 2010, providing a standardized language, updated in 2017 and finally in 2023 [2]. When reporting on thyroid fine needle aspiration (FNA) materials, based on architectural and cytologic changes, cytologic materials are evaluated in six categories according to the TBSRTC (1-3). These categories are: I - non-diagnostic, II - benign, III - atypia of uncertain significance or follicular lesion of uncertain significance (AUS/FLUS), IV - follicular lesion or suspected follicular lesion, V - suspected malignancy, and VI - malignant (1). The main architectural features considered are the formation of macrofollicle and/or microfollicle patterns, the presence of papillae or papilla-like groups, discohesion, and the amount of colloid. Nuclear changes such as coarsening, contour irregularity, elongation, clearing or coarse chromatin, presence of grooves, inclusions, equal distances between nuclei, or overlapping are also considered (1). This classification helps clinicians predict the malignancy risk of the thyroid nodule and influences patient management, including repeat FNA, molecular testing, surgery, and the type of possible surgical intervention (1-3). According to the final update, there have been some changes in the Category III group [3]. For category III, reporting as AUS

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or FLUS was advised in previous versions [1]. According to the latest update, category III has been divided into two subgroups: "AUS-nuclear atypia" and "AUS-other." It was reported that the malignancy risk of the "AUS-nuclear atypia" was higher than the "AUS-other" [2,3].

We aimed to analyze the risk of malignancy in patients diagnosed with category III (AUS, FLUS, or AUS/FLUS) and compare them based on recent changes in cytologic-histopathologic correlation.

Material and Methods

We obtained a total of 8035 thyroid FNA materials between January 1, 2016, and June 10, 2020, at Istanbul Medeniyet University Prof. Dr. Süleyman Yalçın City Hospital's Department of Pathology. We conducted a study on 526 samples that were categorized as category III. We categorized the cases as AUS (Figure 1), FLUS (Figure 2), or AUS/FLUS (Figure 3). We then compared the cytopathologic and histopathologic diagnoses in the resection materials. Based on detailed microscopic descriptions in our reports, we reclassified the cases as "AUS-nuclear atypia" (Figure 4) and "AUS-other" (Figure 5) according to the updated 2023 TBSRTC. The cytologic findings were re-correlated with surgical follow-up. Our analysis focused on changes in the updated TBSRTC.

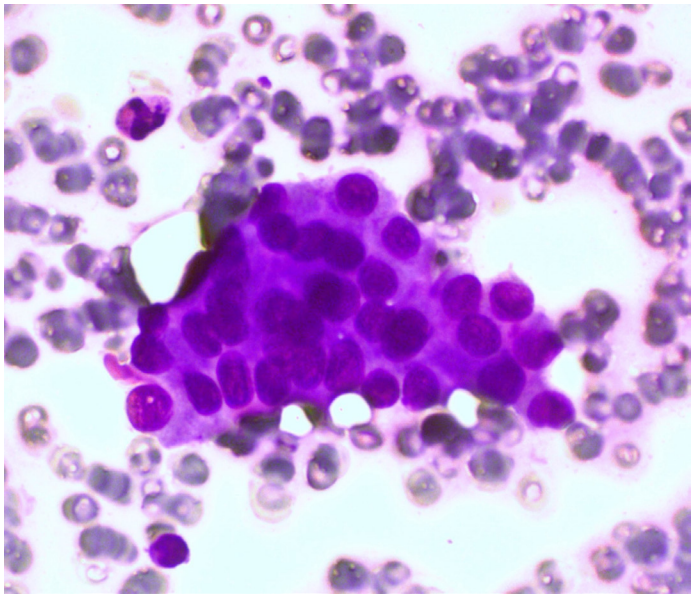

Figure 1. Atypia of uncertain significance (AUS): Follicular cells show mild enlargement of nuclei and mildly irregular nuclear membranes /May Grunwald-Giemsa stain (MGG)x400

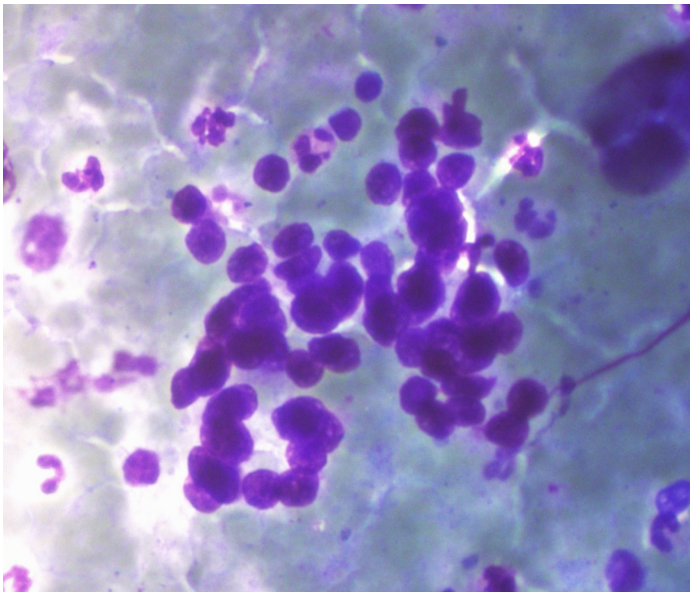

Figure 3. Atypia of uncertain significance/Follicular lesion of uncertain significance (AUS/ FLUS): Presence of both mild cytologic and architectural atypia/ May Grunwald-Giemsa stain (MGG)x400

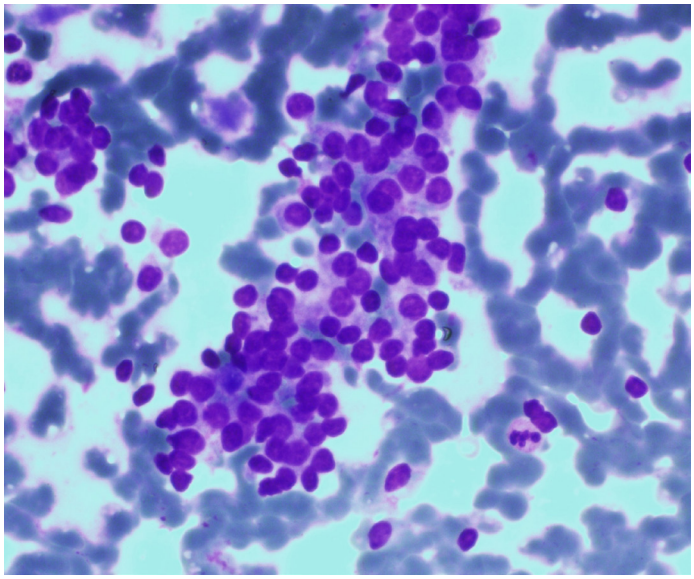

Figure 2. Follicular lesion of uncertain significance (FLUS): The smear shows a sparsely cellular specimen with a predominance of microfollicles without nuclear atypia/May Grunwald-Giemsa stain (MGG)x400

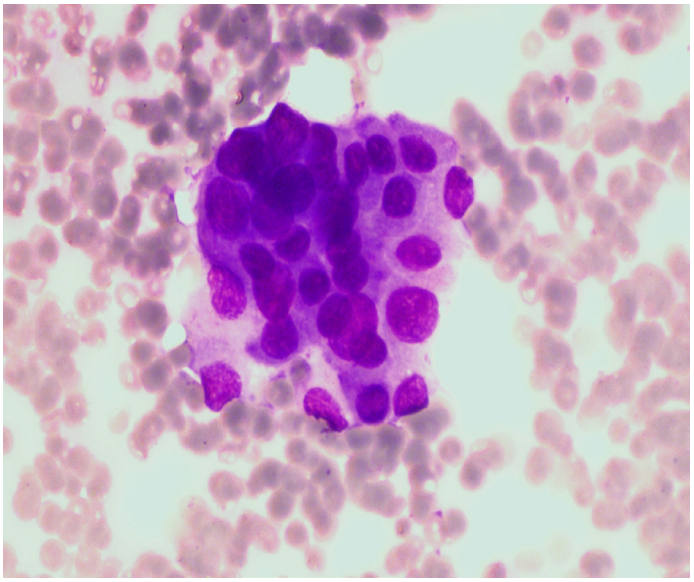

Figure 4. Atypia of uncertain significance (AUS) - other: The smear shows oncocytic cells with mildly nuclear atypia in cohesive flat sheets/May Grunwald-Giemsa stain (MGG)x400

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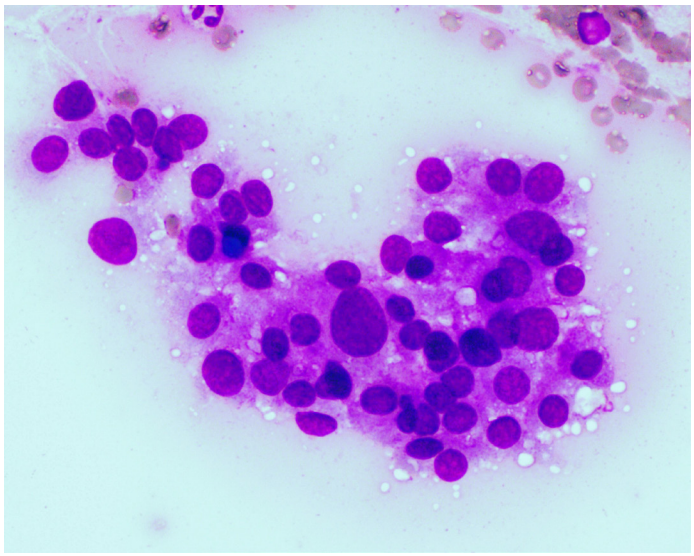

Figure 5. Atypia of uncertain significance (AUS) with nuclear atypia: The smear shows rare cells with mild nuclear enlargement, and mildly irregular nuclear contours/May Grunwald-Giemsa stain (MGG)x400

Statistical analyses

SPSS version 22 software (IBM, New York City, New York) was used for statistical analysis, descriptive statistical methods (mean, median value, group distribution, and percentages) were used to evaluate the study data, and the Pearson Chi-Square test was used to compare qualitative data. Significance was evaluated as $p<0.05$.

Statement of ethics

The Ethics Review Committee of Istanbul Medeniyet University Goztepe Training and Research Hospital reviewed and approved this study protocol, approval number 2023/0545.

Results

We conducted a study on 8035 samples of thyroid FNA cytology in our laboratory. Of these, 526 samples were categorized as category III (AUS, FLUS, or AUS/FLUS), and 184 were categorized as category VI (malignant). This means that 6.54% of all thyroid FNA cytology samples were found to be category III. Moreover, the ratio of category III diagnosis to category VI diagnosis was 2.85.

After excluding recurrent cases, we analyzed the cases of 481 patients with a cytologic diagnosis of category III. Of these, 45.74% were diagnosed as FLUS, 45.53% as AUS, and 8.73% as AUS/FLUS (Table 1).

Table 1. Distribution of FNA cytology diagnosed as Bethesda Category III in the study population

Bethesda category III	n	%
FLUS	220	45.74
AUS	219	45.53
AUS/FLUS	42	8.73
Total	481	100

AUS: Atypia of uncertain significance, FLUS: Follicular lesion of uncertain significance, FNA: Fine needle aspiration

Upon a retrospective review for histopathologic correlation, we found that 73 patients had undergone thyroidectomy. Of these, 45.21% were reported as FLUS, 47.94% as AUS, and 6.85% as AUS/FLUS in thyroid cytology (Table 2).

Table 2. Distribution of FNA cytology diagnosed as Bethesda Category III with surgical follow-up in the study population

Bethesda category III	n (%)	AUS-nuclear atypia (n)	AUS-other (n)
FLUS	33 (45.21)	17	16
AUS	35 (47.94)	12	23
AUS/FLUS	5 (6.85)	1	4
TOTAL	73 (100)	30 (41.10)	43 (58.90)

AUS: Atypia of uncertain significance, FLUS: Follicular lesion of uncertain significance, FNA: Fine needle aspiration

Our study included 73 patients, comprising 49 females and 24 males, who had undergone thyroidectomy. The ages of these patients ranged from 21 to 77 years, with a mean value of 50.08 and a median value of 51.

The distribution of FNA cytology results and surgical follow-up data in the study group is shown in Figure 6.

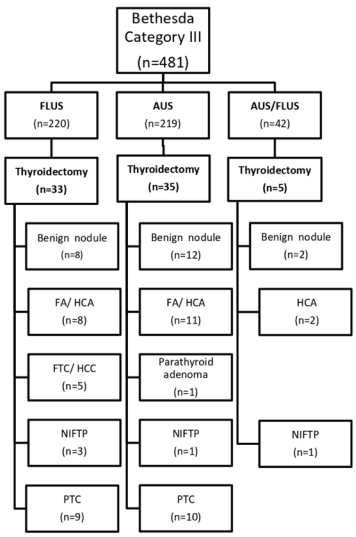

Figure 6. The distribution of FNA cytology results and surgical follow-up data in the study. AUS: Atypia of uncertain significance, FA: Follicular adenoma, FLUS: Follicular lesion of uncertain significance, FNA: Fine needle aspiration, FTC: Follicular thyroid carcinoma, HCA: Hürthle cell adenoma, HCC: Hürthle cell carcinoma, NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear, PTC: Papillary thyroid carcinoma

When noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was not classified as malignant, 49 cases (67.12%) were diagnosed as benign, while 24 cases (32.88%) were diagnosed as malignant after surgical resection. When NIFTP was considered malignant, 44 cases (60.27%) were diagnosed as benign, and 29 cases (39.73%) were diagnosed as malignant after surgical resection. The histopathological diagnoses included papillary thyroid carcinoma in 19 cases, follicular carcinoma in 3 cases, Hürthle cell carcinoma in 2 cases, and NIFTP in 5 cases. However, 10 of the 19 cases diagnosed

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as papillary thyroid carcinoma were actually follicular variant papillary thyroid carcinoma. Since NIFTP is known to behave less aggressively [4], we did not consider it malignant for our study. Therefore, the total risk of malignancy in category III was 32.88% (24/73). Of all the cases diagnosed as FLUS, 42.42% (14/33) were malignant, and 28.57% (10/35) of the AUS cases were malignant. However, no cases diagnosed as AUS/FLUS showed histopathologic malignancy. 75.75% (25/33) of FLUS cases, 65.71% (23/35) of AUS cases, and 60% (3/5) of AUS/FLUS cases were diagnosed as neoplasms.

On surgical follow-up, there was no significant difference in the risk of malignancy between the FLUS and AUS groups (p=.232).

Additionally, there was no significant difference in the risk of neoplasm between the FLUS, AUS, and AUS/FLUS groups (p=.588).

Our reports provided detailed microscopic descriptions that allowed us to reclassify cases as either "AUS- nuclear atypia" or "AUS- other" based on TBSRTC 2023 guidelines.

Figure 7 shows the distribution of FNA cytology in the study population with surgical follow-up. Furthermore, there were no significant differences in the risk of malignancy (p=.826) or neoplasm (p=.619) between the cases diagnosed as "AUS-nuclear atypia" and "AUS-other" on surgical follow-up.

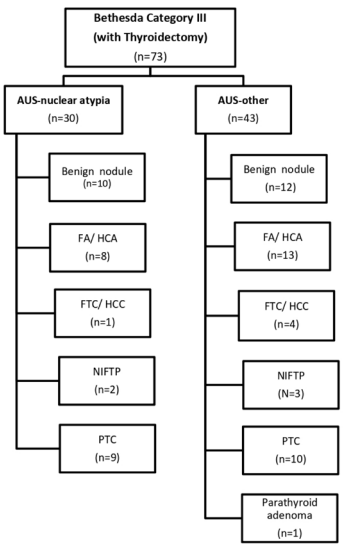

Figure 7. According to reclassification, the distribution of FNA cytology results and surgical follow-up data in the study. AUS: Atypia of uncertain significance, FA: Follicular adenoma, FNA: Fine needle aspiration, FTC: Follicular thyroid carcinoma, HCA: Hürthle cell adenoma, HCC: Hürthle cell carcinoma, NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear, PTC: Papillary thyroid carcinoma

Discussion

The TBSRTC recommends that each thyroid FNA report should begin with a general diagnostic category for clarity of communication. The estimated cancer risk for each group ranges from 1% to 2% overall for the 'Benign' category to over 100% for the 'Malignant' category [3]. Based on cytologic examination, the majority of cases are diagnosed as benign, while a small percentage are malignant, and around 20-30% fall into the "indeterminate" group, which includes categories III, IV, and V [5]. The rate of AUS/FLUS within the indeterminate group can range from 1-20% [6]. It is recommended that laboratories aim for AUS/FLUS diagnoses to make up around 7% of all thyroid cytology, with a maximum of 10% [7]. Additionally, the ratio of AUS-diagnosed cases to malignant cytology cases should not exceed 3, as this is an indicator of laboratory quality [7]. Our AUS/FLUS ratio was 6.5%, and our AUS to malignant ratio was 2.61, which meets the recommended quality criteria.

Within the indeterminate group, category III has the lowest risk of malignancy, with a predicted risk between 10-30% [2,8]. However, since this group is heterogeneous, the reported malignancy risk in the literature ranges from 6-72.9% [9], depending on the type of atypia leading to the AUS interpretation [10-13]. In our study, the risk of malignancy for category III was 32.88%, slightly higher than the upper limit recommended by TBSRTC. This may be due to the small number of AUS/FLUS cases that underwent surgical excision, and the data was based solely on clinical follow-up.

The 2017 update for Category III recommends using consistent nomenclature and selecting either AUS or FLUS [1]. We documented cases as AUS, FLUS, or AUS/FLUS instead of a single designation. However, 50% of cases in this study were documented before the 2017 revision. Furthermore, pathologists tend to classify cases with significant architectural atypia and follicular origin as FLUS, and cases with pronounced cytologic atypia as AUS, as seen in the initial version of the TBSRTC in 2010 [9,14]. In some studies, cases were divided into two groups: "AUS/FLUS with cytologic/nuclear atypia" and "AUS/FLUS with architectural atypia". It was reported that the group with cytologic/nuclear atypia had a higher risk of malignancy than the other group [10-13]. In 2019, Huhtemella et al. divided Category III into 8 subgroups and discovered varying rates of malignancy in each subgroup [15]. In 2021, Zhao et al. conducted a study and classified category III cases into 6 subgroups based on their cytologic features. It was observed that the subgroup with the highest risk of malignancy was the one exhibiting the highest nuclear atypia [16]. The results of this study indicate that there is no significant difference in the risk of cancer between patients classified as FLUS and AUS who underwent surgical follow-up. However, a small number of individuals categorized as AUS/FLUS did not show any signs of cancer, which may be attributed to the limited number of cases and poor quality of certain samples due to hypocellularity.

Category III was divided into two subgroups in the 2023 update: "AUS-nuclear atypia" and "AUS-other". The "AUS-nuclear atypia" group was found to have a higher risk of malignancy than the "AUS-other" group [2]. The ‘’AUS-nuclear atypia’’ subgroup in cytologic samples may display various variations such as "focal prominent nuclear atypia," "diffuse but mild nuclear atypia," "atypical cyst lining cells," and "histiocytoid cells." Moreover, the presence of both nuclear and architectural atypia is also a possibility [2]. It is suggested to report cases with architectural atypia, oncocytic atypia, atypical lymphocytic

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cells, and only psammomatous calcification as ‘’AUS-other’’ [2]. With the latest update, we have reclassified these cases as "AUS-nuclear atypia" and "AUS-other" based on the microscopic descriptions provided in our pathology reports. We did not observe a significant difference in the risk of cancer or neoplasms between the two groups, as was the case in the previous distribution. This could be due to the fact that we categorized the cases based on their microscopic descriptions, and some of the cases were suboptimal due to hypocellularity, even though they were diagnosed as category III. It is important to note that not all nodules diagnosed as AUS are removed through surgery. Instead, some nodules are monitored through repeated fine needle aspiration biopsies or molecular examination results. As a result, it is difficult to determine the actual malignancy rate of these nodules and subgroups through histopathology [17]. The reported rates instead reflect the risks of malignancy or neoplasia in cases that underwent surgical resection. In addition, the risk assessment presumes that cases that do not undergo resection are benign, leading to the belief that the true malignancy risk falls somewhere in between these two rates [1,17].

Out of the patients in category III group, nineteen of them were diagnosed with papillary thyroid carcinoma through surgery. Among these cases, 10 (52.63%) were found to be follicular variants. This indicates that diagnosing the follicular variants of papillary thyroid cancer is especially difficult and they are often classified as indeterminate.

Our study is not devoid of limitations. Firstly, the small sample size and single-center design of the study may affect the generalizability of the results. Another limitation is the reduced predictive value observed in 12 cases due to their hypocellularity. Also, it's essential to consider that not all nodules classified as AUS/FLUS underwent surgical resection, which may impact the overall assessment. Furthermore, the categorization of cases into new subgroups based on their microscopic descriptions may introduce some variability in our analysis.

Conclusion

To summarize, our laboratory's analysis shows that the prevalence of category III diagnoses in thyroid fine needle aspiration samples aligns with current TBSRTC guidelines and standards. Our findings suggest that the risks of malignancy and neoplasm in category III cases are slightly higher than recommended by TBSRTC. Separating Category III into subgroups revealed no significant differences, whether using the new or old classification. This may be a sign of our internal consistency. We hope that this study will help pathologists stay up-to-date on TBSRTC updates and inspire further research in this area.

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

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval

The Ethics Review Committee of Istanbul Medeniyet University Goztepe Training and Research Hospital reviewed and approved this study protocol, approval number 2023/0545.

References

1. Ali SZ, Cibas ES. The bethesda system for reporting thyroid cytopathology. Defnitions, criteria and explanatory notes. New York, NY: Springer; 2018.

2. Ali SZ, VanderLaan PA. The bethesda system for reporting thyroid cytopathology. Defnitions, criteria and explanatory notes. (E-bbok) New York, NY: Springer; 2023.

3. Ali SZ, Baloch ZW, Cochand-Priollet B, et al. The 2023 bethesda system for reporting thyroid cytopathology Thyroid. 2023 Jul 8. doi: 10.1089/thy.2023.0141. [Epub ahead of print].

4. Haaga E, Kalfert D, Ludvikova M, et al. Non-invasive follicular thyroid neoplasm with papillary-like nuclear features is not a cytological diagnosis, but it infuences cytological diagnosis outcomes: a systematic review and meta-analysis. Acta Cytol. 2022;66:85-105.

5. Zhang M, Lin O. Molecular testing of thyroid nodules: a review of current available tests for fine-needle aspiration specimens. Arch Pathol Lab Med. 2016;140:1338-44.

6. Straccia P, Rossi ED, Bizzarro T, et al. A meta-analytic review of the bethesda system for reporting thyroid cytopathology: has the rate of malignancy in indeterminate lesions been underestimated?. Cancer Cytopathol. 2015;123:713-22.

7. Krane JF, Vanderlaan PA, Faquin WC, et al. The atypia of undetermined significance/follicular lesion of undetermined significance:malignant ratio: a proposed performance measure for reporting in The Bethesda System for thyroid cytopathology. Cancer Cytopathol. 2012;120:111-6.

8. Ali SZ, Cibas ES. The bethesda system for reporting thyroid cytopathology. Defnitions, criteria and explanatory notes. New York, NY: Springer; 2010.

9. Hathi K, Rahmeh T, Munro V, et al. Rate of malignancy for thyroid nodules with AUS/FLUS cytopathology in a tertiary care center-a retrospective cohort study. J Otolaryngol Head Neck Surg. 2021;50:58.

10. VanderLaan PA, Marqusee E, Krane JF. Usefulness of diagnostic qualifers for thyroid fneneedle aspirations with atypia of undetermined signifcance. Am J Clin Pathol. 2011;136:572-7.

11. Wu HH, Inman A, Cramer HM. Subclassifcation of “atypia of undetermined signifcance” in thyroid fne-needle aspirates. Diagn Cytopathol. 2014;42:23-9.

12. Crescenzi A, Palermo A, Trimboli P. Cancer prevalence in the subcategories of the indeterminate class III (AUS/FLUS) of the Bethesda system for thyroid cytology: a meta-analysis. J Endocrinol Investig. 2021;44:1343-51.

13. Chen JC, Pace SC, Khiyami A, et al. Should atypia of undetermined signifcance be subclassified to better estimate risk of thyroid cancer? Am J Surg. 2014;207:331-6.

14. Kwon H, Kim WG, Eszlinger M, et al. Molecular diagnosis using residual liquid-based cytology materials for patients with nondiagnostic or indeterminate thyroid nodules. Endocrinol Metab (Seoul). 2016;31:586-91.

15. Huhtamella R, Kholová I. Thyroid bethesda category AUS/FLUS in our microscopes: three-year-experience and cyto-histological correlation. Cancers (Basel). 2019;11:1670.

16. Zhao H, Guo H, Zhao L, et al. Subclassification of the bethesda category III (AUS/FLUS): a study of thyroid fna cytology based on thinprep slides from the national cancer center in China. Cancer Cytopathol. 2021;129:642-8.

17. Iskandar ME, Bonomo G, Avadhani V, et al. Evidence for overestimation of the prevalence of malignancy in indeterminate thyroid nodules classified as Bethesda category III. Surgery. 2015;157:510-7.