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Original Article

Assessment of cytomorphological features and risk of malignancy of Bethesda category III in thyroid cytopathology

Aneesha Asok Kumar[1], Feroze M[1], Ranjith Sukumar[1], Sneha Rajan[1]

[1]*MES Medical College, Perithalmanna, India*

Keywords:

Bethesda system; Risk of malignancy; Thyroid cytology; Thyroid neoplasm;

ABSTRACT

Background: In the Bethesda System for Reporting Thyroid Cytopathology, category III is characterized by architectural and nuclear features that are indeterminate. This category continues to pose diagnostic and therapeutic dilemmas, with a variable risk of malignancy across different populations. Our study aimed to assess the risk of malignancy associated with Bethesda category III and the correlation of malignancy with each cytomorphologic feature within this category.

Materials and Methods: All cases identified as Bethesda category III on fine needle aspiration cytology at The MES Medical College, Perithalmanna, India between January 2022 and September 2024 that had also had subsequent thyroidectomy at the same facility were retrospectively reviewed. The Ethical Committee gave its approval to the study (IEC/MES/F6/2025). In Bethesda Category III cytology, patient demographics, architectural characteristics, and cytological features were evaluated and compared to the final histopathological diagnosis in resected tissues.

Results: Nuclear atypia was observed in 29 (61.7%) of the 47 Bethesda category III cases, with a 51.7% risk of malignancy. Features such as nuclear pseudo-inclusion (Risk of Malignancy-100%), membrane irregularity ($p = 0.013$), microfollicular pattern ($p = 0.001$), and crowded three-dimensional clusters ($p = 0.003$) were associated with an increased risk of malignancy.

Conclusions: Risk of malignancy varies with different cytomorphological features, even within the AUS category. It is important to identify the nuclear and architectural features associated with a higher risk of malignancy and highlight them in the cytopathology report, which will help the clinician in patient management.

Correspondence:
Dr Aneesha Asok Kumar, MD
Department of Pathology,
MES Medical College, Perithalmanna, India
ORCID ID: 0009-0009-4467-1387
Email: aneesha.ea@gmail.com

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INTRODUCTION

With the widespread adoption of sensitive imaging technologies, the incidence of thyroid malignancies is on the rise globally, with notable variation in prevalence across different geographic and demographic cohorts. The incidence of thyroid cancers has increased across India, with a large difference between rural and urban areas.[1] The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) is a 6-tiered system that links each diagnostic category with risk of malignancy (ROM) and possible management recommendations.[2,3] Bethesda Category III - Atypia of Undetermined Significance (AUS) is a

heterogeneous category of thyroid nodules with uncertain malignant potential exhibiting variable degrees of nuclear and architectural atypia. The usual management of AUS may vary from repeat Fine Needle Aspiration Cytology (FNAC), molecular testing, diagnostic lobectomy, or surveillance based on clinical and sonological factors.[2] The ROM of AUS is essential for guiding and determining optimal management and varies significantly across different populations. Population-specific ROM for this indeterminate category is required to tailor the management of AUS.[4] In the initial Bethesda classification, AUS/ Follicular Lesion of Undetermined Significance(FLUS) was a single category, and ROM was not separately calculated for nuclear or non-nuclear atypia. Several studies pointed out the difference in ROM for nuclear and non-nuclear atypia.[5] The 3rd edition TBSRTC subcategorizes the AUS based on ROM as AUS with nuclear atypia and AUS-other.[2] The 5th edition of the World Health Organization (WHO) Endocrine and Neuroendocrine Tumors introduced a new category of low-risk neoplasms to reduce the risk of over-treatment.[6] The 3rd edition of TBSRTC incorporated the changes in the latest WHO. This new category of low-risk neoplasm, for which the incidence of spread is extremely low, will reduce the ROM of Bethesda category III.[2]

The presence and predominance of distinct cytologic characteristics in AUS correlate with benign or malignant potential. The reporting of cytologic characteristics strongly associated with malignancy in AUS enables clinicians to develop targeted management strategies and improve patient outcomes.[3,7] This study aimed to find out the ROM in AUS in a tertiary care center in Kerala, India and to assess the risk of malignancy of each cytologic feature in AUS, which will aid in optimal patient management.

MATERIALS AND METHODS

Approval for the study was given by the Ethical Committee of MES Medical College, Perithalmanna (IEC/MES/F6/2025), India. This was a retrospective study from January 2022 to September 2024 of patients diagnosed with Bethesda category III thyroid nodules on FNAC from the Department of Pathology, MES Medical College, who underwent thyroidectomy (lobectomy/subtotal thyroidectomy/total thyroidectomy) in the same institution. Exclusion from the study was applied to cases whose cytology and histopathology slides were unavailable in the archives. Information on the patient's sex, age at the time of FNAC, Free T4 (fT4), and TSH values was extracted from electronic medical records for a total of 47 cases included in the study. One pathologist, blinded to clinical information and final histopathology reports, analyzed the cytology slides and reclassified them as AUS nuclear and AUS-other based on the criteria of the 3rd edition TBSRTC, which included architectural atypia, oncocyte atypia, and atypia NOS.[2] Cytomorphological features like anisocytosis, nuclear atypia, nuclear overlapping, nuclear grooves, granular chromatin, membrane irregularity, pseudo-inclusions, micro follicles, and crowded three-dimensional clusters were studied. Also the respective histopathology slides of the thyroidectomy specimen were retrieved and reclassified based on the 5th edition WHO Endocrine and Neuroendocrine Tumors[8] as Benign, Low-risk neoplasms, and Malignant. The overall malignancy rate was calculated based on the total number of patients who had undergone thyroidectomy at our institution. Data analysis was done in IBM SPSS version 26. Using appropriate descriptive and inferential statistical tests, the collected data were tabulated, summarized, computerized, and analyzed. Descriptive statistics were used to compute demographic data. The Chi-square test or Fisher's exact test was used to find an association. The level of significance at $p < 0.05$ was used as the cut-off value for statistical significance.

RESULTS

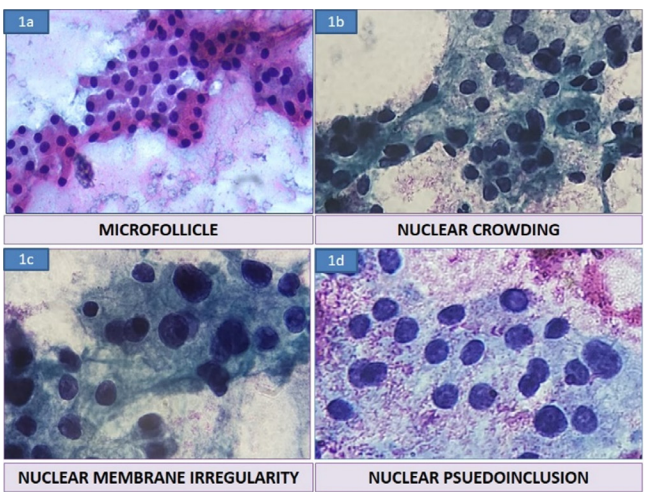

Figure 1: *Cytolomorphological features of thyroid showing (a) Micofollicular pattern, (b) nuclear crowding, (c) nuclear membrane irregularity, (d) nuclear pseudo-inclusion.*

Among 47 Bethesda III cases, 29 (61.7%) were AUS nuclear, and 18 (38.3%) were AUS-other; the majority of the cases of AUS that underwent surgery in our institution were AUS nuclear. Indicating a significant overlap in sonologic characteristics, 51.7% of AUS nuclear and 50% of AUS-other were TIRADS 4 nodules. The mean age of AUS patients was 46±12. In both the AUS nuclear and AUS-other categories, a female preponderance was observed. Mean TSH was higher in AUS nuclear than in AUS-other (Table 1).

Table 1: Demographic details and mean TSH of patients

Bethesda subcategorization	Age in years(Mean)	Male-to-female ratio	Mean TSH (µunits/ml)
AUS nuclear	49±11	1:11	7.49 ±15.02
AUS other	42±11	1:6	2.23±.3.10

Low cellularity and obscuring blood or drying/fixation artifacts may lead to an AUS interpretation in thyroid cytology. 12 cases in our study showed low cellularity and obscuring factors. Scant colloid was found in 58.6% of AUS

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nuclear. (Table 2)

Table 2: Presence of other cells, colloid, cellularity, and obscuring factors in AUS cytology

Cytological features	AUS nuclear (Number of cases and percentage)	AUS-other (Number of cases and percentage)	Total Number of cases
Cyst macrophages	8 (27.5%)	4 (22.2%)	12 (25.5%)
Scant colloid	17 (58.6%)	6 (33.3%)	23 (48.9%)
Low cellularity	8 (27.5%)	1 (5.5%)	9 (19.1%)
Obscuring factors	4 (13.7%)	Nil	4 (8.5%)

Histopathology diagnoses of AUS were classified as benign, low risk, and malignant based on the essential and desirable criteria of the 5th edition WHO classification[8] of endocrine tumors (Table 3). The majority of benign cases were follicular nodular disease (FND). The low-risk neoplasms can influence the ROM of the AUS category. In our study, 6 cases were low-risk neoplasms. Of these, 4 cases were Non-invasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features (NIFTP), with 3 reported as AUS nuclear and one as AUS-other on cytology. There was one case each of Follicular Tumor of Uncertain Malignant Potential (FTUMP) and Well-Differentiated Tumor of Uncertain Malignant Potential (WDTUMP). FTUMP was interpreted as AUS nuclear, and WDTUMP as AUS-other in cytology. Most AUS nuclear cases that proved malignant were papillary carcinoma of the thyroid (PTC). Among the 3 malignant cases of AUS-other, 2 were follicular carcinoma, and one was invasive encapsulated FVPTC. We identified 2 cases of invasive encapsulated FVPTC, now recognized as a separate category in the 5th edition WHO[8] one was classified as AUS nuclear and the other as AUS-other in cytology.

Table 3: Histopathology diagnosis of AUS based on the 5th edition WHO endocrine tumors

Histopathology diagnosis	WHO category	AUS nuclear (n=29)	AUS-other (n=18)	Total number of cases
Folliclar nodular disease	Benign	10	10	20
Hashimoto thyroiditis	Benign	4	2	6
Follicular adenoma	Benign	Nil	1	1
NIFTP	Low-risk neoplasm	3	1	4
FTUMP	Low-risk neoplasm	1	Nil	1
WDTUMP	Low-risk neoplasm	Nil	1	1
Minimally invasive encapsulated FVPTC	Malignant	1	1	2
Minimally invasive follicular carcinoma	Malignant	1	1	2
Encapsulated angioinvasive follicular carcinoma	Malignant	Nil	1	1
Papillary thyroid carcinoma	Malignant	9	Nil	9

NIFTP: Non-invasive follicular thyroid neoplasm with papillary-like nuclear features; FTUMP: Follicular tumor of uncertain malignant potential; WDTUMP: Well differentiated thyroid tumor of uncertain malignant potential; FVPTC: Follicular variant of papillary thyroid carcinoma

ROM of AUS nuclear and AUS other were calculated considering low-risk neoplasm as both benign and malignant (Table 4). ROM of AUS nuclear was found to be higher when compared to AUS-other. Including low-risk neoplasms in the malignant category significantly increased the ROM of AUS nuclear to 51.7%.

Table 4: Risk of malignancy of Atypia of undetermined significance

Bethesda subcategorization	ROM, considering Low-risk neoplasms as benign	ROM considering Low-risk neoplasms as malignant
AUS nuclear	37.9%	51.7%
AUS-other	16.6%	27.7%

ROM: Risk of malignancy

Cytomorphological features

Membrane irregularity: The presence of membrane irregularity was associated with malignancy (ROM-60.8%) with a statistically significant p-value (p-0.013).

Microfollicles: Based on the criteria of the 3rd edition TBSRTC, the AUS category applies to cases in which 50-70% of follicular cells exhibit a microfollicular pattern without a marked predominance. In our study, cases with a 50-70% microfollicular pattern all turned out to be either low-risk neoplasms (4 cases) or malignant (3 cases). Among the 4 cases of low-risk neoplasms, 3 cases were NIFTP and one was a well-differentiated tumor of uncertain malignant potential (WDT-UMP). The presence of a predominant microfollicular pattern showed a statistically significant association with malignancy (p-0.001).

Crowded three-dimensional clusters: Crowded three-dimensional clusters showed a ROM of 88.8% with a significant p-value of 0.003.

Nuclear Pseudo-inclusions: Out of 47 cases, 3 cases showed nuclear pseudo-inclusions; all of them turned out to be malignant with a ROM of 100%.

Crowded three-dimensional clusters and membrane irregularity showed a significant Odds ratio of 17.33 and 4.667, respectively.

Our study found no significant correlation between AUS ROM and cytomorphological features, including granular chromatin, anisocytosis, nuclear atypia, nuclear enlargement, nuclear grooves, and nuclear overlapping.

Table 5: Risk of malignancy, Odds ratio, and p-value of cytomorphological features in Atypia of Undetermined Significance

Number of cases with distinct cytologic features (N=47)		Malignant diagnoses on Histopathology	Risk of malignancy	ODDS ratio Lower	Confidence Interval Upper		p-value
Granular chromatin	23	11	47.8%	1.528	0.478	4.88	0.474
Anisocytosis	14	5	35.7%	0.667	0.183	2.422	0.537
Nuclear atypia	12	7	58.3%	2.369	0.622	9.020	0.200
Nuclear enlargement	36	14	38.8%	0.530	0.136	2.072	0.358
Nuclear grooves	23	12	52.17%	2.182	0.671	7.092	0.192
Nuclear overlapping	20	11	55%	2.44	0.744	8.036	0.137
Membrane irregularity	23	14	60.8%	4.667	1.341	16.239	0.013
Pseudoinclusions	3	3	100%				0.070
Microfollicles	7	7	100%				0.001
Crowded three-dimensional clusters	9	8	88.8%	17.33	1.943	154.644	0.003

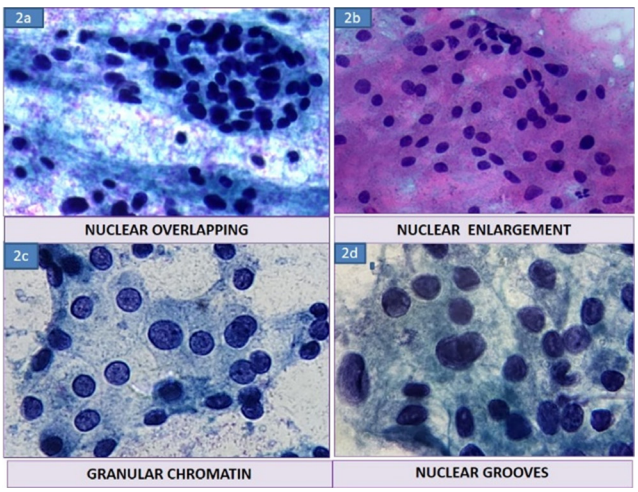

Figure 2: *Cytomorphological features showing (a) nuclear overlapping, (b) nuclear enlargement, (c) granular chromatin, and (d) nuclear grooves.*

DISCUSSION

In our study, 91.5% of cases diagnosed as AUS were females. The male-to-female ratio in the AUS nuclear category was 1:11. A female predominance was found in the cases diagnosed as AUS in studies conducted by Elomami et al. (82.2%),Yoon et al.(85.7%) andGan et al. (76.4%).[4,9,10] Worldwide, there is a gender disparity in the incidence of thyroid disease.Thyroid cancers are 2.9 times more common in women than men. Dietary, environmental, or molecular factors were not found to be accountable for this disparity. Studies suggest reproductive factors as a logical hypothesis to account for this.[11] The median age of patients with AUS diagnosis was 47 years in the study conducted by Elomami et al. which was similar to our study.[4]

The AUS category has low inter-pathologist agreement due to subjective morphology interpretation and limited surgical follow-up, which hinders ROM determination. The ROM of the AUS category, as per the 3rd edition TBSRTC is 13-30% with approximately 2-fold higher ROM for AUS nuclear compared to AUS-other.[2] Our study also showed a difference in ROM between AUS subtypes, with AUS nuclear showing a 2-fold increase. The estimated risk of AUS-associated malignancy exhibits significant heterogeneity in the literature, with reported rates ranging from 17% to 83%. There is a discrepancy between international guidelines and clinical practice in India regarding thyroid nodule management, potentially driven by apprehensions about patient follow-up.[12] This may be the reason for an increased ROM for AUS in the study conducted by Roy et al. in India compared to other studies.[13] The studies conducted by Yoon et al., Gan et al., and Roy et al., were all based on the 2007 TBSRTC criteria.[9,10,13] The more recent studies by Arocho et al and Bagis et al, were based on 3rd edition TBSRTC criteria similar to ours.[14,15] Our study's ROM estimates for AUS nuclear and AUS-other were similar to the study conducted by Bagis et al in Turkey.[15] However, the study conducted by Arocho et al in the US showed a lower ROM.[14] The variability in AUS ROM is likely to be driven by a combination of factors like inter-pathologist variability, ethnic factors, and differences in clinical management.

Table 6: Comparison of Risk of Malignancy (ROM) of Atypia of Undetermined Significance (AUS) in different studies.

Study, Year	ROM of AUS nuclear	ROM of AUS-other
Yoon et al., 2016[9]	45.6%	32.6%
Gan et al., 2017[10]	36.8%	14.7%
Roy et al., 2018[13]	78.3%	75.3%
Arocho et al., 2024[14]	21%	6%
Bagis et al., 2024[15]	48.2%	21%
Our study (low-risk as benign)	37.9%	16.6%
Our study (low-risk as malignant)	57.1%	27.7%

The 5th edition WHO classification[8] of endocrine tumors incorporated several changes, including the introduction of a distinct low-risk neoplasm category and reclassification of invasive encapsulated FVPTC as a separate entity, owing to its association with RAS-like mutation. Histological classification of the AUS category in our study was based on

the 5th edition of WHO. In a study conducted by Gan et al., 17 out of 21 cases of AUS with nuclear atypia were PTC[10]. Elomami et al found that 80% of the cases with nuclear atypia and 19.5% of cases with architectural atypia were PTC.[4] In the study conducted by Roy et al in CMC Vellore, India; 56.5% of cases with nuclear atypia and 40% of cases with architectural atypia were PTC.[13] All three above-mentioned studies were based on the 4th edition classification of WHO Endocrine tumors. As in our study, Bagis et al used the 5th edition WHO classification for histologic categorization of AUS.[15] In their study of 260 cases of AUS, 53.1% cases were benign, 11.2% low-risk neoplasms, and 30.7% malignant. 81.9% of benign neoplasms were FND. Out of 29 cases of low-risk neoplasm, 65.6% were WDT-UMP, 20.6% were NIFTP, and 13.8% were FT-UMP. Among the malignant neoplasms, 76.3% were PTC and 18.8% were Invasive encapsulated FVPTC. In our study, 57.4% of cases were benign, 12.7% of cases were low-risk neoplasms, and 29.7% were malignant. 74% of benign cases were FND. Among the low-risk neoplasms, NIFTP comprised 66.6%, while WDT-UMP and FT-UMP accounted for 16.6% each. Among the malignant neoplasms in our study, 64.2% were PTC and 14.2% were invasive encapsulated FVPTC.

Cytomorphological analysis of AUS in various studies revealed varying predictive values of malignancy for different features. This necessitates the identification and reporting of specific features associated with malignancy, which will aid in enhanced cancer suspicion, repeat FNA with molecular testing, and timely surgical intervention. Nuclear grooves and pseudo inclusions were found to be reliable predictors of malignancy in studies conducted by Chen et al, Kaymaz et al, and Bagis et al.[3,16,15] In our study, the ROM of nuclear grooves and pseudo inclusions was 52.17% and 100% respectively. Similar to the studies conducted by Kaymaz et al. and Bagis et al., we also found membrane irregularity as an independent predictor of malignancy with a ROM of 60.8% with a significant p-value of 0.013.[16,15] In our analysis, three-dimensional clusters showed a significant association with malignancy (p=0.003), similar to Bagis et al's findings.[15] Unlike previous research, our study identified microfollicular pattern (50-70% of follicular cells) as a significant indicator of malignancy (p=0.001).

Table 7: Statistically significant cytomorphological features of AUS in various studies

Study, year	Statistically significant cytomorphological features associated with malignancy
Chen et al.,[3] 2014	Nuclear grooves, Nuclear pseudo-inclusion
Kaymaz et al.,[16] 2020	Nuclear groove, irregular contours, pseudo-inclusion
Bagis et al.,[15] 2024	Nuclear molding, nuclear irregularity, nuclear groove, nuclear overlapping, three-dimensional groups

The limitations of our study include the evaluation of cytology and histopathology specimens by a single pathologist, precluding the inter-observer variability assessment, and the small sample size (n=47).

CONCLUSIONS

AUS is the most heterogeneous category of TBSRTC with varying ROM across studies. The management strategies of AUS vary from repeat FNA to molecular diagnostics and surgery. The incidence of thyroid varies with region, ethnicity, and socio-economic status. The prevalence of malignancy for this indeterminate category varies substantially among centers; hence, it is crucial to know the prevalence of malignancy of AUS in one's institution, which will help the surgeon in tailoring the patient management. Not only the overall ROM, but the ROM associated with different cytomorphological features varies in AUS. So it is important to identify the nuclear and architectural features associated with a higher risk of malignancy and highlight them in the cytopathology report, which will help the clinician in patient management.

Conflict of interest: None

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