Is Subdivision of Atypia of Undetermined Significance AUS/ Follicular Lesion of Undetermined Significance Cases According to Detailed Nuclear Features Vital for Assessing the Risk of Malignancy?

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Abstract

Background: It has been known that the “atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS)” category is the most problematic category in Bethesda system due to its highly heterogeneous morphological features. Recently, it has been reported that aspirates including nuclear atypia in the AUS/FLUS category have a higher risk of malignancy. Aims: This study aimed to assess each nuclear property in aspirates with cytological atypia and also to determine the relationship with the risk of malignancy. Material and Methods: We reviewed 980 AUS/FLUS fine-needle aspirations (FNAs) performed between ‘2012 and 2019’ at a single institution. We classified these aspirates into four groups: AUS-N (nuclear atypia), AUS-A (architectural atypia), AUS-H (Hurthle cell change), and AUS-O (other). Nuclear features were detailed sub-classified; size and shape (enlargement, elongation, and overlapping), membrane irregularities (irregular contours, grooves, pseudo-inclusion), and chromatin characteristics (pale chromatin). The estimated risk of malignancy (ROM) was calculated for each subgroup. Results: Of 980 AUS/FLUS cases, follow-up histological outcome data were available for 209 cases. Among these cases, the estimated ROM was 27.8%. The ROM were 26.4%, 15.4%, and 22.5% for AUS-N, A, and H, respectively. The most common nuclear findings associated with ROM were nuclear groove (67.9%); irregular contours (76.9%) suspected pseudo-inclusion (100%) and overlapping (56%) (P < 0.001). But nuclear findings such as nuclear enlargement, mild pleomorphism, or pale chromatin have a similar ROM as architectural atypia. Conclusion: Although it is known that the presence of cytological atypia in an AUS/FLUS nodule increases the estimated risk of malignancy, all nuclear properties are not equally effective in predicting malignancy risk. Emphasizing nuclear atypia details in reports of AUS case may be a more sensitive way to identify nodules with a high risk of malignancy.

Keywords: Atypia of undetermined significance, nuclear atypia, risk of malignancy

INTRODUCTION

Bethesda system, a diagnostic classification, which has been used for thyroid cytopathology since 2007, has six diagnostic categories based on morphological criteria and includes estimated malignancy risks for each category. This system, revised in 2017, standardizes cytopathological reports of thyroid nodules and also the clinical approach of them.[1] The most problematic category in the Bethesda system is the atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS) due to its heterogeneous morphological characteristics. Malignancy rates of the AUS/FLUS category range from 6 to 48% based on current data.[2] Because of these variable rates, the clinical approach to a nodule diagnosed as AUS/FLUS is not clear. Some authors suggest a more aggressive approach will be needed due to the high risk of malignancy while others believe that it is correct to avoid unnecessary surgery because of its various
complications. So, it has recently been proposed to subdivide this category by using molecular or morphological methods for determining appropriate clinical approach. Considering the heterogeneity of AUS/FLUS cases, it is expected that different cytological features in this category have different risks of malignancy. For this purpose, the researchers subdivided this category into two subgroups: aspirates containing nuclear atypia and aspirates with a predominance of micro follicular pattern in a background of poor colloid. And the recent studies demonstrated that the relationship between nuclear atypia and the risk of malignancy is more specific. However, any case with nuclear atypia does not have the same risk of malignancy. So, based on this, can we suggest that various nuclear features are effective on different malignancy risk rates? Is it possible to get more objective approach by subgrouping of nuclear features?

In our study, we aimed to investigate the AUS/FLUS cases with sub-classification of nuclear features based on more objective criteria as well as subgroups of nuclear and architectural atypia and also to determine the relationship between each subgroup and ROM.

**Materials and Methods**

Thyroid nodules diagnosed as AUS/FLUS in FNA which had been surgically resected were reviewed. Cytological features were basically evaluated in four subgroups based on the Bethesda Classification and according to some meta-analysis studies. Both subgroups compared together for their relationship with the risk of malignancy. And for the essence of the study, nuclear features sub-classified based on nuclear criteria of the noninvasive follicular tumors with papillary like nuclear features (NIFT-P) because of its objectiveness. And all subgroups evaluated their relationship with ROM.

For this purpose, all of the cytological diagnoses of thyroid nodules with fine needle aspirations (FNA) in Zonguldak Bulent Ecevit University Faculty of Medicine between 2012 and 2019 were reviewed. The incidence of each diagnostic category according to Bethesda Classification was determined and compared with expected diagnostic rates. All over the AUS/FLUS cases were included to study. Of these, cases undergoing surgical procedures were selected and reevaluated again. For pathological diagnosis recorded reports on the pathology data system have been used. Some of the slides removed from the pathology archive were reviewed and morphological features were examined by two experienced pathologists. In patients with multiple nodules, each nodule was evaluated separately. To confirm the accuracy of the correlation, nodule size, and localization on ultrasonography (USG) was compared with macroscopic nodule size and localization. Histopathological diagnosis of AUS/FLUS cases with surgical resection (lobectomy/subtotal thyroidectomy/total thyroidectomy) reviewed and according to these reports, thyroid nodules were subdivided into two categories; benign and malignant. Papillary thyroid carcinoma, papillary thyroid microcarcinoma, follicular carcinoma, and medullar carcinoma were included in the malignant category, while nodular hyperplasia, lymphocytic thyroiditis, Hashimoto’s thyroiditis, follicular adenoma, and Hurthle cell adenoma were included in the benign category. Nodules diagnosed as “encapsulated papillary thyroid carcinoma follicular variant” were re-diagnosed as “noninvasive follicular tumor with papillary like nuclear features (NIFT-P)” according to the WHO 2017 classification and these nodules considered to be benign based on this current classification. Nodules without macroscopic and ultrasonographic correlation were excluded from the study. Also, suboptimal evaluation of cytological preparation (broken slides, slides with artifact) has not been included for study.

Sub categorization of cytological features of AUS/FLUS nodules based on classification and nomenclature of meta-analysis studies of Kim et al. and Mathur et al.; cellular atypia (AUS-N), architectural atypia (AUS-A), presence of Hurthle cells (AUS-H), and others (AUS-O).

AUS-A: This group represented the low cellular cytological aspirations with sparse or no colloid which is predominant of micro follicular structure and also with crowded irregular groups but no real papillary structure [Figure 1a-d].

AUS-N: This category was included focal nuclear features that were suspicious for papillary thyroid carcinoma but not sufficient for this diagnosis. These nuclear findings were nuclear pleomorphism, contour irregularity, changes in chromatin structure, nuclear elongation, groove, suspicious pseudo-inclusion, and overlapping [Figure 2a-d].

AUS-H: In this group, aspirates had focal Hurthle cell change but without lymphocytes [Figure 3a, b].

**Figure 1:** Architectural atypia. a, b. Low cellular aspirates containing microfollicular structures with sparse/no colloid (H&E × 40, H and E × 100) c. Architectural atypia as crowded irregular groups (H&E × 200) d. Focal irregular groups resembling papillary structures, but without real fibrovascular cores (H&E × 200)
AUS-O: Widespread bleeding, drying artifact, inadequate specimen, or atypical lymphocytic infiltration was categorized as AUS-O.

Each subgroup was compared with histopathological diagnoses and the estimated malignancy risk ratio was found for each group. Also, the risk of malignancy (ROM) for each group was compared with each other. For further sub-classification of nuclear findings; NIFT-P nuclear criteria have been taken into consideration [Table 1].

In the study, the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation.

Statistical analyses were performed with SPSS 19.0 software. Variables were expressed as frequency and percent. Categorical variables were compared using the Chi-Square test. A P value of less than 0.05 was considered statistically significant for all tests.

**Results**

The diagnoses of the 10743 thyroid nodules in the retrospective analysis have been summarized in Table 2 with the number of cases and the percentages.

The diagnoses of non-diagnostic cytology, benign cytology, and uncertain atypia/indeterminate follicular lesion, was found to be in the range of incidence determined by Bethesda classification. However, the diagnoses of categories 4, 5, and 6 were significantly lower from the intervals determined by Bethesda.

Of 980 nodules diagnosed as AUS/FLUS, 209 cases underwent surgical procedures such as lobectomy, subtotal/total thyroidectomy. Of these, 28 were diagnosed as papillary thyroid carcinoma (13.4%) and 30 were diagnosed as papillary thyroid microcarcinoma (14.4%). As a result, the malignancy rate was 27.8%. The diagnoses of the other 151 resection materials were as follows; follicular adenoma (7.6%), nodular hyperplasia (42.1%), well-differentiated tumor (1.9%), follicular tumor with unknown malignancy potential (2.8%), Hurthle cell adenoma (4.7%), Hashimoto thyroiditis (12.9%).

The subgroups of these 209 thyroid nodules and their relationship with malignancy were summarized in Table 3. Atypical nuclear features such as nuclear size and shape change, chromatin characteristics, and membrane irregularity were detected in 177 samples. Forty-seven cases with nuclear atypia were malignant and the estimated malignancy risk was found to be 26.6%. Also, this ratio was statistically significant (P = 0.04). Architectural atypia, which was detected in 65 of the cases, was associated with malignancy at a rate of 15.4%. Relationship with ROM and architectural atypia of both microfollicular pattern and three-dimension papiloid pattern were not statistically significant (P = 0.12).

In conclusion, in our study nuclear atypia increased the risk of...
malignancy approximately 2-fold compared to architectural atypia. In 111 cases, Hurthle cell change was detected. The estimated malignancy risk for cases with Hurthle cell change is 22.5% ($P = 0.717$).

THE estimated ROM for each nuclear finding was discussed in Table 4. 44 of 172 nodules with changes in nuclear size and shape were diagnosed as malignant (25.6%). The subgroups of nuclear size and shape, such as elongation, overlapping, and enlargement were evaluated with regards to the relationship with malignancy one by one. Resection diagnoses of 42 of 162 cases with nuclear enlargement, 16 of 34 cases with nuclear elongation, and of 14 cases with nuclear overlapping were malignant. As a result, nuclear elongation and overlapping were statistically significant in the prediction of malignancy-risk ($P < 0.001$). Membrane irregularity, another nuclear feature, was detected in 47 cases. The relationship between malignancy and nuclear contour irregularity, suspicious nuclear groove, and pseudoinclusion were; 76.9%, 67.6%, 100% respectively. All of these parameters were found to be statistically related to the risk of malignancy ($P < 0.001$). Five of the nineteen samples were malignant which have pale chromatin patterns and this was not statistically significant (26.3%) ($P = 0.052$).

**Discussion**

AUS/FLUS category is recommended as a last resort by Bethesda classification and not to include more than 7% of total thyroid FNAs.[1] But in practice, many laboratories fail to achieve this figure and different studies have reported variable rates of AUS/FLUS. Horne et al. determined the rate of 2.8%[6] for the diagnosis of AUS/FLUS, while it is reported as 6.5% by Kim et al.,[6] 16.7% by Mathur et al.,[7] 6.7% by Onder et al.,[10] 7.7% by Renshaw et al.[11] A meta-analysis of Koholova and Ludvikula has reported variable rates of this heterogeneous category ranging from 0.09% to 20.5%.[12] Based on these variable rates it is thought that an upper limit of >10% may be more realistic according to the last edition of Bethesda.[1] The incidence of AUS/FLUS as 9.1% from our clinic data was below the limit recommended by the last Bethesda classification. A new and different approach is suggesting a subgroup for the benign category as “Probably benign, absolute clinical follow-up.” So, it is believed that this may limit the diagnosis of the AUS/FLUS category to 2–8%.[13]

Although estimated ROM for the AUS/FLUS category was determined as 5–15% in the 2008 Bethesda classification, studies have observed higher malignancy rates ranging from 25 to 50%. And in the last edition of Bethesda estimated ROM has been determined to be 15–30%. With the introduction of NIFTP terminology in 2016, it is suggested that these figures may change further and ROM can be reduced by 45% in the early data.[14] According to the meta-analysis of Koholova et al., the rates of ROM vary between 6 and 48%.[12] Nishino et al. reported ROM as 39% in a meta-analysis of seven studies.[15] Similar to the other researchers, we reported an estimated ROM as 27.8% for AUS/FLUS.

Due to these wide and variable ranges, many authors emphasized on subcategorizing the AUS/FLUS. Thus, reducing the heterogeneity of this category and also a more

### Table 3: Subclassification of AUS/FLUS category and comparison with ROM

| Cytologic properties | Number of cases | Number of malignancies | Percentage of malignancies (%) | $P$-value |
|----------------------|-----------------|------------------------|-------------------------------|-----------|
| Nuclear Atypia       | 177             | 47                     | 26.6                          | 0.04      |
| Architectural Atypia | 65              | 10                     | 15.4                          | 0.12      |
| Three-dimensional    | 18              | 3                      | 16.7                          | 0.282     |
| Microfollicule       | 47              | 7                      | 14.3                          | 0.135     |
| Hurthle cells        | 111             | 25                     | 22.5                          | 0.717     |
| Others               | 10              | 0                      | 0                             | 1.0       |

### Table 4: Comparison of nuclear findings with estimated ROM rates

| Cytologic abnormalities | Number of cases | Number of malignancies | Percentage of malignancies (%) | $P$-value |
|-------------------------|-----------------|------------------------|-------------------------------|-----------|
| Nuclear findings        | 177             | 47                     | 26.6                          | 0.04      |
| Size and shape          | 172             | 44                     | 25.6                          | 0.128     |
| Enlargement             | 162             | 42                     | 25.9                          | 0.147     |
| Elongation              | 34              | 16                     | 47.1                          | <0.001    |
| Overlapping             | 25              | 14                     | 56                            | <0.001    |
| Membrane                | 47              | 32                     | 68.1                          | <0.001    |
| Irregularities          |                 |                        |                               |           |
| Irregular contours      | 26              | 20                     | 76.9                          | <0.001    |
| Nuclear Groove          | 37              | 25                     | 67.6                          | <0.001    |
| Pseudoinclusion         | 7               | 7                      | 100                           | <0.001    |
| Abnormal chromatin      | 19              | 5                      | 26.3                          | 0.052     |
appropriate prediction of malignancy would be possible. Two subgroups have been observed by several authors: AUS with cytological atypia and AUS with architectural atypia. Some authors defined more subgroups for the same reason; focal nuclear atypia, architectural atypia, focal Hurthle cell changes, and others (predominantly bloody samples, artifacts from air-dried specimens). Ahn et al. reviewed 15 studies reporting the necessity of subgroups in AUS/FLUS. In this recent meta-analysis, the ROM has been reported 44.5% for aspirates with cytological atypia while it was 19.5% for aspirates with a microfollicular pattern and 15.1% for aspirates with Hurthle cell pattern. Valderrebano et al. analyzed 20 studies and found an overall ROM of 47.6% in Bethesda III smears with nuclear atypia and 18.0% in those without nuclear atypia. The results were different in the study of Čuhaci et al. which have determined ROM as 24.3% and 19.8% for subgroups of AUS and FLUS, respectively. Shrestha and Hernesey, in contrast to other studies, found that nuclear atypia and Hurthle cell smears had similar ROM. The researchers also observed that the presence of suspicious radiological features (e.g., nodule diameter), as well as cytological features, would increase the risk of malignancy.

Although the nomenclature is different in several studies, it is observed that nuclear atypia has a higher risk of malignancy rate than architectural atypia with the exceptions. Similarly, in our study, we found that the estimated ROM in AUS-N cases (26.6%) was two times higher than those with AUS-A cases (15.4%). We also found that the relationship with AUS-H cases and ROM was not statistically significant ($P = 0.717$).

Chen et al. assessed the AUS category in more subgroups similar to our research, containing detailed nuclear findings such as nuclear overlapping, nuclear inclinations, nuclear grooves, nuclear enlargement, and hyperchromasia. Among these groups, it has been reported that nuclear grooves or nuclear inclusions are associated with a higher rate of cancer.

According to our research, all nuclear properties are not equally effective in predicting malignancy risk. Although nuclear enlargement or abnormal chromatin pattern is included in the criteria of nuclear atypia, its association with malignancy is not as clear as nuclear membrane irregularity. Also, we reported nuclear elongation was associated with a higher rate of malignancy compared to nuclear enlargement. But these two criteria are very difficult to distinguish from each other. It is possible to say that the most common nuclear features related to the estimated ROM were nuclear groove (67.9%), nuclear contour irregularity (76.9%), rare pseudo-inclusion (100%), and overlapping (56%), supporting Chen et al.

While it is still unclear how the clinical approach to the subcategories of the AUS/FLUS cases should be, Nishino et al. believe that such reorganization will reduce unnecessary surgery and at the same time limit the unnecessary use of molecular testing. Johnson et al. recommended the same clinical approach with benign nodules for AUS/FLUS cases with architectural atypia because of the similar malignancy rates. Also, they suggested that aspiration repeat or lobectomy should be performed in patients with nuclear atypia.

Our study had some limitations. First, our study included cases with surgical procedures. Another limitation was that the study was retrospective. Not all the AUS/PLUS nodules underwent surgery and therefore the estimated ROM rate may differ from that found in our study. In addition, some criteria were observed at the same time in some aspirations and, therefore, intersecting groups have been formed. We disregarded this situation and evaluated the relationship between each criterion and the ROM.

In conclusion, although there are several limitations, we can say that the relationship between nuclear atypia and the estimated risk of malignancy is twice as high as that of architectural atypia. Considering a stronger connection between nuclear groove, membrane irregularity, overlapping, and malignancy compared to other nuclear features, it is possible to conclude that the categorization of nuclear properties may be much more sensitive to assess the risk of malignancy. We recommend a clinical approach, such as repeat FNA or thyroidectomy for the AUS cases with nuclear membrane irregularity and also overlapping. However, clinical follow-up like benign nodules would be more appropriate management for patients with other nuclear findings. We, therefore, believe that the details of nuclear features as indicated in the AUS report are a guide to the clinical approach.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest
There are no conflicts of interest.

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