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Evaluating the Effect of Multiple Passes to the Same Thyroid Nodule in the Fine-Needle Aspiration Biopsy Session on Obtaining Adequate or the Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Cytological Result

Fatma Dilek Dellal Kahramanca[1], Fatma Neslihan Cuhaci Seyrek[2], Afra Alkan[3], Oya Topaloglu[2], Reyhan Ersoy[2], Bekir Cakir[2]

[1]Division of Endocrinology and Metabolism, Ankara City Hospital, Ankara, Türkiye
[2]Division of Endocrinology and Metabolism, Ankara Yıldırım Beyazıt University Faculty of Medicine, Ankara, Türkiye
[3]Division of Biostatistics, Ankara Yıldırım Beyazıt University Faculty of Medicine, Ankara, Türkiye

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ABSTRACT

Background: To determine whether multiple fine-needle passes to the same thyroid nodule in the fine-needle aspiration (FNA) biopsy session affect sufficient and/or atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) cytological result.

Methods: Ultrasonography(US) and cyto-histopathology results of the nodules of patients were retrospectively analyzed. The nodules were divided into 2 groups according to the number of needle passes performed in the same FNA session as those with 1 pass (1-pass group) and those with 2 or 3 passes (multiple-pass group).

Results: Totally, 1409 (93.9%) nodules were performed 1 pass, and 91 (6.1%) were performed multiple pass(n = 91).The rates of cystic/mixed nodule, macrocalcification, and Thyroid Imaging Reporting and Data System 3 score were higher in the multiple-pass group ($P = .001$, $P = .039$, $P = .006$, respectively). Adequacy and AUS/FLUS rates were similar between the 2 groups. When nodules with macrocalcifications and cystic/mixed structures were evaluated as 2 separate subgroups, the rates of the adequacy and AUS/FLUS were similar between 1-pass and multiple-pass groups within each subgroup.The number of passes showed no univariate or multivariate significant effect on sufficient and/or AUS/FLUS cytology results ($P > .05$).

Conclusion: Although more passes are performed in cystic/mixed and macrocalcified nodules estimating that the material would be insufficient with macroscopic on-site evaluation, needle insertion of 2 or 3 times does not affect obtaining sufficient and/or AUS/FLUS cytology compared to 1 pass.

Keywords: Thyroid nodule, fine-needle biopsy, number of needle pass, adequacy, AUS/FLUS

Introduction

Nodular thyroid disease is a commonly encountered endocrinological disorder with a prevalence of 3%-7% by palpation,[1] and 19%-70% by high-resolution ultrasonography (US).[2,3] Lifetime expectancy of thyroid nodule development is reported to be 10%.[4] Although the majority of thyroid nodules are benign, 5% of palpable nodules are malignant.[5]

Fine-needle aspiration (FNA) biopsy is a safe, accurate, and cost-effective diagnostic modality used for the evaluation of thyroid nodules.[6,7] Major limitations of the technique are higher rates of nondiagnostic (in other words inadequate/insuffici ent) or indeterminate results, particularly atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) cytology. Nondiagnostic and indeterminate results lead to repetitive FNAs, increased patient anxiety,

Corresponding author: Fatma Dilek Dellal Kahramanca, e-mail: drdellal@yahoo.com
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treatment delay, unnecessary surgery, financial burden, and waste of time.

Nondiagnostic cytology rates are reported as 5.0%-53.8%.[8] These nodules harbor 5%-10% malignancy risk.[9] The rates of inadequate FNA can be affected by the experience of operator, criteria of adequacy, and radiological features of the nodule.[10] Technical factors such as use of rapid on-site evaluation/adequacy assessment (ROSE), the number of needle pass, needle size, use of US- or palpation-guided technique, and use of aspiration or capillary technique are other factors related with inadequate results.[6,10] The American National Cancer Institute recommends 2-5 needle passes to decrease the risk of nondiagnostic result if ROSE is not available.[11]

Rapid on-site evaluation/adequacy assessment refers to the evaluation of cytological adequacy by a cytopathologist in the biopsy room after FNA. Number of insertions to the nodule is decided according to the cytopathologist's judgement. The contribution of this method to the adequacy rates and cost-effectiveness are still controversial.[12] In addition, it is associated with higher cost and need for a longer time. Besides, a cytopathologist and equipment for ROSE are not always available.[13] Therefore, ROSE is not routinely performed in all institutions, as in ours.

The term AUS/FLUS is used when the FNA fluid contains cells with architectural and/or nuclear atypia and the aspirated material is adequate but not cytologically enough to diagnose as benign, follicular neoplasm or malignant.[14] This is an indeterminate cytology result that requires follow-up and biopsy repetition. Studies have reported AUS/FLUS cytology with a wide range of 1%-28%.[15] Malignancy rate in this lesion varies between 6% and 30% depending on whether noninvasive follicular thyroid neoplasm with papillary-like nuclear features is considered a malignant lesion or not.[9] After FNA, reactive changes in the nodule, such as papillary endothelial hyperplasia, fibrosis, cystic change, hemorrhage, and vascular proliferation can be seen.[16] If a second biopsy is performed before these changes resolve, the nodule cytology might be misjudged as AUS/FLUS. However, whether an increased number of insertions in the same FNA session causes AUS/FLUS misdiagnosis is unknown.

In this retrospective observational study, our aim was to determine whether the number of needle passes affects the rates of nondiagnostic or AUS/FLUS cytology results.

Main Points

- We hypothesized that the possible hemorrhage that would occur with too many insertions and the resulting fibrin might cause an incorrect diagnosis of atypia of undetermined signi ficance/follicular lesion of undetermined significance (AUS/FLUS), especially if the multiple biopsy was performed on the same thyroid nodule in the fine-needle aspiration biopsy session.
- According to our knowledge, this is the first study to evaluate the relationship between the number of passes and other US features and Thyroid Imaging Reporting and Data System scores.
- As we know, this is the first study to investigate the effect of the number of biopsy needle insertions in the same fine-needle aspiration session on the AUS/FLUS diagnosis rate.
- This study includes the highest number of nodules among studies evaluating the effect of the number of the passes on cytological results.
- Although more passes are performed in cystic/mixed and macrocalcified nodules, estimating that the material would be insufficient with macroscopic on-site evaluation, needle insertion 2 or 3 times does not affect obtaining sufficient and/or AUS/FLUS cytology compared to 1 pass.

Methods

The data of patients diagnosed with thyroid nodule/nodules in our outpatient clinic and underwent FNA between May and August 2021 was retrospectively analyzed. Lesions with non-thyroidal cytology result (lymph node, parathyroid adenoma) were excluded from the study. Demographical features (age, gender), clinical diagnosis, thyroid status, thyroid autoantibody (anti-thyroglobulin, anti-thyroid peroxidase, and thyroid stimulating hormone (TSH) receptor antibodies) positivity of patients, US and cyto-histopathological features of the nodules, and the number of needle passes into the same nodule in the same FNA session were recorded.

Electrochemiluminescence immunoassay methods were used for measurements of serum TSH, free T3 (FT3), free T4 (FT4), anti-thyroid peroxidase, anti-thyroglobulin, and TSH receptor antibody levels. They were measured by Siemens Advia Centaur XPT immunoassay (Tarrytown, NY, USA). Reference ranges were 0.55-4.78 µLU/mL for TSH, 2.3-4.2 ng/L for FT3, 0.89-1.76 ng/dL for FT4, <60 U/ML for anti-thyroid peroxidase antibody, and <1.5 IU/mL for anti-thyroglobulin antibody. Thyroid stimulating hormone receptor antibody level was determined by radioimmunoassay method using a gamma counter (≤1 IU/L negative, 1-1.5 borderline, >1.5 IU/L positive). When any of the thyroid autoantibody was higher than the upper level of normal, it was considered positive. Thyroid status was classified as euthyroid if TSH was within the reference range. When free thyroid hormones were within normal limits, subclinical hypothyroidism was diagnosed if TSH was between 4.78 and 10 µIU/mL, and subclinical hyperthyroidism was diagnosed if TSH was below the lower limit of the reference value.

Toshiba Aplio XV (Japan) US device and linear transducer (PLT-805AT) were used for thyroid US. Nodule diameters and volume, localization (left or right thyroid lobe, isthmus), structure (solid, cystic, mixed), echogenity (isoechoic, hypoechoic, hyperechoic, heterogenous), border regularity, presence of cystic degeneration, calcification (microcalcification, macrocalcification), and peripheral halo were recorded. Thyroid Imaging Reporting and Data System (TIRADS) scores for US features of the nodules were determined. Suspicious US features for malignancy were solid texture, hypoechoic, irregular or microlobular borders, microcalcifications, and taller than-wide. Presence of each feature was scored as 1. If the total score of the nodule was 0, 1, 2, 3, and ≥4, TIRADS groups were determined as category 3 (probably benign), 4a (low suspicion), 4b (intermediate suspicion), 4c (moderate suspicion), and 5 (high suspicion), respectively.[17]

Fine-needle aspiration was performed under US (General Electric Logiq pro 200 US, Model number 2270968, GE Healthcare Korea, Seongnam-SI, Gyean GGI-DO, Korea) guidance with a 5.5-7.5 MHz probe using 20- or 21-gauge needle without attaching syringe. The needle was fixed to the 20 mL syringe and obtained material was gently sprayed on glass slides for cytological assessment. Written informed consent was obtained from all patients before the FNA procedure. All nodules with 1 cm or larger size were evaluated by FNA. Smaller than 1 cm nodules were evaluated by FNA if the patient had clinical risk factors (family history of thyroid cancer, radiotherapy history to the neck region) or nodule had suspicious US features such as hypoechoic texture, microcalcification, irregular borders and taller than wide shape.[14] Same endocrinologist with 11 years of experience in this field executed FNA.

Cytological findings were classified according to Bethesda System by the experienced cytopathologist and grouped as non-diagnostic, benign, AUS/FLUS, follicular neoplasm/suspicious for follicular neoplasm, suspicious for malignancy, and malignant. The cytologic material with obscuring blood, poor cell preservation, and an insufficient sample of follicular cells (absence of at least 6 groups of benign follicular cells with each group composed of at least 10 cells) was considered nondiagnostic or insufficient.[9] Nodules were divided into 2 categories as insufficient or sufficient (all cytological results other than nondiagnostic cytology). When the cellular changes were not sufficient to diagnose suspicious for malignancy or follicular neoplasm cytologically but there were follicular cells with architectural and/or nuclear atypia, AUS/FLUS was reported.[15] Nodules were also grouped into AUS/FLUS group and non-AUS/FLUS group (all cytological results other than AUS/FLUS cytology). Thyroidectomy was decided depending on the size, US features, and cytopathological result of the nodule. Histopathological diagnosis was made in accordance with the 2004 World Health Organization criteria[18] and nodules were divided into benign and malignant ones.

Nodules were also divided into groups according to the number of needle passes in the same FNA session. Those with 1 pass were named as the 1-pass group, and 2 or 3 passes were named as the multiple-pass group. The 1-pass and multiple-pass groups were compared with regards to US and cyto-histopathology features.

The institutional ethical committee of Ankara City Hospital approved the study (approval number: E1-21-2095, October 20, 2021). It was performed in accordance with the Declaration of Helsinki. Informed consent was waived because of the retrospective nature of the study.

The distributions of age, nodule sizes, and volume were examined by Shapiro–Wilk's test, normality plots, and skewness/kurtosis statistics. Mean ± standard deviation (mean ± SD) and median (interquartile range, IQR: first quartile-third quartile) were provided for age and nodule measurements, respectively. All categorical variables were reported by frequency (%).

Nodule characteristics were compared between 1-pass and multiple-passes groups by Mann–Whitney *U*-test and chi-square tests, considering the type of variables and the minimum expected frequency in the 2-way tables. Besides, nodule characteristics were compared based on the cytology sufficiency and AUS/FLUS cytology results by Mann–Whitney *U*--test and chi-square tests. The effects of the number of passes on obtaining sufficient cytology and AUS/FLUS cytology were investigated by univariate and multivariate logistic regression models. Other nodule characteristics were analyzed by univariate logistic regression analysis to determine the possible predictors. The predictors with a *P*-value < .250 were considered as candidates for multivariate model. Then, the multivariate model was constructed with the main effects of the candidate predictors and the number of passes, and the interaction effect of the number of passes with nodule structure and macrocalcification by using the enter method. The regularity of the border, TIRADS score, and histopathology result were excluded from the univariate and multivariate analysis due to the missing data and correlation with other predictors. The odds ratio (OR) and its 95% confidence interval (CI) were provided for the predictors in the multivariate model. The statistical significance level was considered as $P < .05$. All statistical analyses were performed via IBM SPSS® Statistics 22.0 (IBM SPSS Corp.; Armonk, NY, USA).

Results

In total, 1529 thyroid lesions of 727 patients were retrospectively reviewed. Lesions diagnosed as lymph node (n = 5) or parathyroid adenoma (n = 4) with clinical and cytological evaluation, and lesions with largely incomplete ultrasonographical and cytological information (n = 20) were excluded from the study. Finally, 1500 nodules of 708 patients were included in the study. There were 575 females (81.2%) and 133 males (18.8%), and the mean age was 51.57 ± 12.51 years (minimum–maximum: 19-92). Clinical features of the patients are given in Table 1.

There were 1409 (93.9%) nodules in 1-pass group, and 91 (6.1%) nodules in multiple-pass (85 two passes and 6 three passes) group. Considering the nodule structure; the rate of solid nodule was significantly higher in the 1-pass group and the rate of mixed/cystic nodule was significantly higher in the multiple-pass group. While the macrocalcification rate was higher in the multiple-pass group, the presence of peripheral halo was higher in the 1-pass group ($P = .039$, $P = .040$; respectively). On the other hand, the rate of the multiple passes was 10.5% in macrocalcified nodules and 5.6% in other nodules; 5.5% in solid nodules and 13.9% in cystic/mixed nodules (data not shown in the table). Other US features were similar between the 2 groups ($P > .05$). There was a significant difference in terms of TIRADS scores between the 2 groups ($P = .006$). TIRADS 3 rate was significantly higher in multiple-pass group although other scores were similar (Table 2).

The nondiagnostic rate was 26.7% (n = 376) in the 1-pass group and 28.6% (n = 26) in the multiple-passes group ($P = .694$). The AUS/FLUS rate was 17.0% (n = 240) and 9.9% (n = 9), respectively, and there was no difference between them ($P = .103$). Surgery was performed in 65 nodules, and the 2 groups were similar in terms of histopathological results ($P = .430$) (Table 2). Ultrasonographical and cyto-histopathological features of the nodules in terms of needle pass groups are shown in Table 2.

Table 1. Clinical Features of the Patients

Features (n = 708)	n (%)
Clinical diagnosis	
Euthyroid/hypothyroid (multi) nodular goiter	552 (78.0)
Toxic (multi) nodular goiter	37 (5.2)
Toxic diffuse (multi) nodular goiter	119 (16.8)
History of thyroidectomy	48 (6.8)
History of radioactive iodine treatment	2 (0.3)
History of radiotherapy to the head and neck region	2 (0.3)
Anti-thyroid peroxidase positivity (n = 666)	157 (23.6)
Anti-thyroglobulin positivity (n = 657)	172 (26.2)
TSH receptor antibody positivity (n = 124)	
Positive	19 (15.3)
Borderline	27 (21.8)
Negative	78 (62.9)
Drug use	168 (23.7)
Levothyroxine	112 (15.8)
Anti-thyroid drugs	56 (7.9)
Thyroid functional status (n = 689)	
Euthyroid	561 (81.4)
Subclinical hyperthyroid	107 (15.5)
Subclinical hypothyroid	21 (3.0)

TSH, thyroid-stimulating hormone.

In nodules with macrocalcification, the rates of adequacy or AUS/FLUS did not show a significant difference between the 1-pass and multiple-pass groups (P = .526 and P = .519, respectively). Similar results were found within cystic/mixed nodules as well (P = .116 and P > .999) (Table 3).

The multivariate model examining the effect of multiple passes on obtaining sufficient cytology is given in Table 4. Accordingly, isoechoic nodules compared to heterogeneous nodules, and nodules with a peripheral halo compared to nodules without a peripheral halo were found to have a higher likelihood of resulting in sufficient cytology, considering the other nodule features in the model were fixed (P < .05). The multiple passes had an odds ratio of 2.616 (95% CI: 0.274-24.948), indicating a higher likelihood of obtaining sufficient cytology in cystic/mixed nodules or nodules without macrocalcification. However, this was not significant (P = .403). The interaction effect of multiple passes with nodule structure and macrocalcification was not significant (P > .05).

Considering the AUS/FLUS cytology, hypoechoic nodules were found to have a higher likelihood to result AUS/FLUS cytology compared to heterogeneous nodules, considering that other features in the model were similar between nodules (P = .031, Table 5). The macrocalcification showed increased likelihood of obtaining AUS/FLUS cytology result in the nodules that 1 pass was performed (P = .049). The multiple passes were found to have insignificant increasing effect on obtaining AUS/FLUS cytology in the nodules cystic/mixed nodules or nodules without macrocalcification (OR: 1.773, 95% CI: 0.269-11.710, P = .552). Further, the interaction effect of multiple passes with nodule structure or macrocalcification was not significant on obtaining AUS/FLUS cytology (P > .05).

Discussion

In our study, adequacy and rate of AUS/FLUS cytology were similar between thyroid nodules evaluated with 1-pass and multiple passes during FNA. The number of passes did not show a significant effect on obtaining sufficient cytology and/or AUS/FLUS cytology. Multiple passes were used with a higher frequency in nodules with cystic/mixed structure or macrocalcification.

In the majority of studies investigating the effect of the number of biopsy needle insertions on cytological adequacy, it is recommended to make a minimum of 2-3 passes to the nodule;[3,5,19-21] although other studies have determined this number to be 4-6.[22-25] Naim et al[20] prospectively evaluated 252 nodules performing 1-3 times needle insertions and reported that the rate of unsatisfactory results decreased by 13.7% with 3 passes using a 25-gauge needle. In another prospective study, in which 1-4 number of passes were done to 121 nodules, it was found that a minimum of 2 and a maximum of 3 insertions should be made in order to obtain adequate cytological results.[5] In their retrospective study, Kavanagh et al[24] evaluated a higher number of nodules (n = 724) with the capillary technique and grouped the nodules as 1, 2, 3, and 4-5 needle passes. They showed that the rate of cytological insufficiency in a single needle pass was 6 times higher than 4-5 passes.

Rapid on-site evaluation/adequacy assessment was introduced as a complementary technique that might be used to increase the adequacy of FNA results. While some studies reported lower insufficient cytology results with ROSE, some others showed no contribution of this technique on increasing the adequacy of FNA.[26] Rapid on-site evaluation/adequacy assessment is not routinely performed in our center due to the low number of cytopathologists. In the absence of an on-site cytologist, the biopsy material is evaluated grossly (macroscopic on-site evaluation) by the biopsy operator, and if it is considered insufficient, the biopsy is repeated as long as allowance of the clinical situation of patients (anxiety, complications) and time. In particular, the need for biopsy repetition may arise when cystic fluid is obtained (usually occurs in cystic or mixed cystic nodules), or there is difficulty in needle entry to the nodule and small volume of material can be achieved (usually occurs in nodules with macrocalcification), or the material appears to be contaminated with peripheral blood. In the present study, we made a maximum of 3 needle insertions to the nodules and did not detect any difference in cytological adequacy between the 1-pass and multiple-passes groups.

Nodules having cystic component or macrocalcification were reported to be significantly related with low cellularity and insufficient results.[23,27] Cengic et al[22] determined that the number of needle insertions required for cytological adequacy was higher in nodules with more cystic content than in solid ones. They stated that in the absence of an on-site cytologist,

Table 2. Ultrasonographical and Cyto-Histopathological Features of the Nodules in Terms of Needle Pass Groups

Features	1-Pass (n = 1409)	Multiple-Passes (n = 91)	*P*
Diameters (mm)			
Anteroposterior	8.80 (6.80-12.50)	8.50 (6.10-15.80)	.574
Transverse	12.00 (9.80-16.70)	11.50 (9.00-17.50)	.344
Longitudinal	13.30 (10.90-18.90)	12.60 (10.60-21.30)	.552
Volume* (cm^3)	2.55 (1.43-7.49)	2.06 (1.12-11.13)	.246
Localization			.623
Right	674 (47.9)	47 (51.6)	
Left	641 (45.5)	40 (44.0)	
Isthmus	93 (6.6)	4 (4.4)	
Nodule structure			.001
Solid	1322 (93.8)	77 (84.6)	
Cystic/mixed	87 (6.2)	14 (15.4)	
Echogenicity			.089
Isoechoic	1087 (81.7)	61 (79.2)	
Hypoechoic	58 (4.4)	8 (10.4)	
Hyperechoic	4 (0.3)	0 (0.0)	
Heterogeneous	182 (13.7)	8 (10.4)	
Presence of microcalcification	75 (5.3)	3 (3.3)	.623
Presence of macrocalcification	119 (8.4)	14 (15.4)	.039
Regular border	43 (7.2)	5 (12.5)	.214
Cystic degeneration	1041 (73.9)	60 (65.9)	.096
Presence of peripheral halo	250 (17.7)	8 (8.8)	.040
TIRADS category			.006
3	73 (5.2)**	12 (13.1)**	
4a	702 (49.8)	40 (44.0)	
4b	579 (41.1)	32 (35.2)	
4c	55 (3.9)	7 (7.7)	
5	0 (0.0)	0 (0.0)	
Cytological sufficiency			.694
Insufficient	376 (26.7)	26 (28.6)	
Sufficient	1033 (73.3)	65 (71.4)	
Benign	765 (54.3)	52 (57.1)	
US/FLUS	240 (17.0)	9 (9.9)	
FN/SFN	2 (0.1)	1 (1.1)	
Suspicious for malignancy	9 (0.6)	3 (3.3)	
Malignant	17 (1.2)	0 (0.0)	
AUS/FLUS cytology result			.103
AUF/FLUS	240 (17.0)	9 (9.9)	
Non-AUS/FLUS	1169 (83.0)	82 (90.1)	
Histopathology			.430
Benign	38 (67.9)	4 (50.0)	
Malignant	19 (32.1)	4 (50.0)	

AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; TIRADS, Thyroid Imaging Reporting and Data System.
*Median (IQR) was reported. Other features were assessed by frequency and column proportion.
**Two proportion significantly differ (*P* = .001).

Table 3. Cytological Sufficiency and Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Rates of the 1-Pass and Multiple-Passes Groups Within Macrocalcified Nodules and Cystic/Mixed Nodules

	Cytological Sufficiency		AUS/FLUS Cytology Result	
	Insufficient	Sufficient	AUS/FLUS	Non-AUS/FLUS
	n (%)	n (%)	n (%)	n (%)
Macrocalcified nodules				
1 pass (n = 119)	31 (26.1)	88 (73.9)	29 (24.4)	90 (75.6)
Multiple passes (n = 14)	5 (35.7)	9 (64.3)	2 (14.3)	12 (85.7)
P	.526		.519	
Cystic/mixed nodules				
1 pass (n = 87)	35 (40.2)	52 (59.8)	15 (17.2)	72 (82.8)
Multiple passes (n = 14)	2 (14.3)	12 (85.7)	2 (14.3)	12 (85.7)
P	.116		>.999	

4 entries to solid nodules and 5 entries to cystic nodules were required to obtain an adequate result. Redman et al[23] achieved insufficient results in 15% of cystic nodules and 1% of solid nodules and concluded that the cystic structure of the nodule is one of the factors affecting cytological adequacy. Lee et al[28] reported that nodules with predominantly cystic composition had higher rates of inadequacy in liquid-based cytology. Jiang et al[12] stated that in mixed cystic or macrocalcified nodules

Table 4. Effect of the Number of Passes on Obtaining a Sufficient Cytology Result in the Multivariate Setting

	Univariate Analysis			Multivariate Analysis		
Predictors	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Diameter						
Anteroposterior	1.008	0.989-1.028	.388			
Transverse	1.011	0.997-1.024	.116	0.996	0.950-1.045	.884
Longitudinal	1.009	0.998-1.020	.118	0.999	0.959-1.040	.952
Volume	1.002	0.999-1.006	.203	1.001	0.995-1.008	.676
Localization			.315			
Right	Ref.					
Left	1.068	0.844-1.351	.585			
Isthmus	1.488	0.886-2.498	.133			
Solid structure	1.638	1.074-2.498	.022	1.338	0.674-2.656	.406
Echogenicity*			.023			.034
Isoechoic	1.582	1.138-2.200	.006	1.556	1.113-2.175	.010
Hypoechoic	1.354	0.729-2.517	.337	1.316	0.685-2.529	.410
Heterogeneous	Ref.			Ref.		
Presence of microcalcification	1.233	0.719-2.115	.447			
Presence of macrocalcification	0.985	0.660-1.471	.942	1.291	0.776-2.148	.325
Cystic degeneration	1.168	0.905-1.507	.232	0.900	0.654-1.236	.514
Presence of peripheral halo	1.694	1.212-2.367	.002	1.585	1.123-2.238	.009
Multiple passes	0.910	0.569-1.456	.694	2.616	0.274-24.948	.403
Multiple passes x Solid structure				0.323	0.032-3.294	.340
Multiple passes x Macrocalcification				1.147	0.195-6.746	.879

OR, odds ratio.
*4 hyperechoic nodules were excluded.

Table 5. The Effect of the Number of Passes on Obtaining Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Cytology Result in the Multivariate Setting

	Univariate Analysis			Multivariate Analysis		
Predictors	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Diameters						
Anteroposterior	1.001	0.980-1.023	.910			
Transverse	1.001	0.986-1.016	.901			
Longitudinal	1.003	0.991-1.015	.670			
Volume	1.002	0.999-1.005	.245	1.002	0.999-1.005	.215
Localization			.452			
Right	Ref.					
Left	0.861	0.503-1.473	.585			
Isthmus	0.748	0.435-1.288	.295			
Solid structure	0.982	0.572-1.686	.948	0.704	0.318-1.559	.387
Echogenicity*			.132			.043
Isoechoic	1.038	0.682-1.580	.863	1.008	0.658-1.543	.971
Hypoechoic	1.850	0.942-3.636	.074	2.140	1.073-4.266	.031
Heterogeneous	Ref.			Ref.		
Presence of microcalcification	1.429	0.820-2.492	.208			
Presence of macrocalcification	1.602	1.045-2.456	.031	1.716	1.001-2.941	.049
Cystic degeneration	0.850	0.629-1.148	.288			
Presence of peripheral halo	1.688	1.217-2.343	.002	1.842	1.296-2.618	.001
Multiple passes	0.535	0.265-1.078	.080	1.773	0.269-11.710	.552
Multiple passes x Solid structure				0.245	0.030-2.000	.189
Multiple passes x Macrocalcification				0.899	0.085-9.565	.930
Peripheral halo x Macrocalcification				0.568	0.155-2.081	.393

OR, odds ratio.
*4 hyperechoic nodules were excluded.

the insufficiency rate was diminished in ROSE group in comparison to non-ROSE group in which mean number of passes was 1.7 and 2.8, respectively. In our study, multiple passes were used with a higher frequency in nodules with cystic/mixed structure or macrocalcification. However, we did not detect any difference in the rates of cytological adequacy between 1-pass and multiple-passes groups in these subgroups of nodules. The possible reasons for this indifference might be the presence of an experienced biopsy operator who could precisely refrain from needle insertions into the cystic and/or calcified areas of the nodule.[29] Similarly, Redman et al[23] found that the number of minimally sufficient entries to reach an adequate cytological diagnosis was significantly lower in the presence of an experienced operator. Inadequate results were higher when less-experienced clinicians performed the biopsy according to another study.[30] However, de Koster et al[3] stated that the proficiency rate did not depend on the operator experience. Another possible reason for comparable results between 1-pass and multiple pass in terms of adequacy in our study might be that the number of passes was below the required threshold for cytological adequacy. For example, Zhu et al[25] demonstrated that an insufficient result was significantly diminished by 11% with 4 needle passes, and they suggested optimally 4-6 needle passes for obtaining sufficient results, as others did.[22-25] For the exclusion of this possibility, a prospective study with more insertions may be required.

Zargham et al[29] used ROSE and demonstrated the need for an additional needle pass for obtaining adequate material in nodules larger than 3 cm. They speculated additional passes might be necessary as larger nodules likely had more hemorrhage and fibrosis. In contrast, the volume of the nodule did not influence the number of aspirations necessary for achieving specimen adequacy in another study.[22] We found comparable results in terms of US features including size and volume, except for structure and calcification as mentioned above. Not surprisingly, the proportion of TIRADS 3 score was significantly higher in the multiple needle insertions group compared to the 1 needle insertion group,

possibly because all TIRADS 3 nodules had a cystic or mixed texture, which was an US feature related to a higher rate of multiple passes.

Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance is an indefinite diagnosis and harbors malignancy risk. It requires follow-up, repetition of FNA, and if possible, the mutation analysis of the aspirate. So, it is related to patient anxiety and discomfort as well as financial burden and waste of time. For these reasons, the factors affecting the result of AUS/FLUS have been the subject of interest. Some reactive changes occur in the nodule after biopsy.[16] These changes can be accidentally interpreted as AUS/FLUS in the second biopsy, especially if it is performed in the early post-biopsy period. We hypothesized that the possible hemorrhage that would occur with too many insertions[7] and the resulting fibrin might cause an incorrect diagnosis of AUS/FLUS. While the material is thinned and spread between the slides, fibrins on the slide may cause the cells to be crushed and pulled. This process may result in an elongated appearance of the nucleus. In addition, fibrin filaments can mimic groove structures in cytopathological evaluation. However, the rate of AUS/FLUS result was similar between 1-pass and multiple-passes groups in our study. This indifference may be due to the milder hemorrhage because of the experienced operator performing the biopsy and thereby the relatively shorter biopsy time.

The strengths of the current study are that, to our knowledge, this is the first study to evaluate the relationship between the number of passes and other US features and TIRADS scores. Additionally, as far as we know, there is no study in the literature investigating the effect of the number of biopsy needle insertions in the same FNA session on the AUS/FLUS diagnosis rate. This study includes the highest number of nodules among studies evaluating the effect of the number of the passes on cytological results. On the other hand, our study had some limitations. It was designed in a retrospective style. The needle size was not recorded. Third, thyroid nodules were usually not inserted more than 3 times in the same FNA session in our clinic. The main reasons for that were the high number of patients admitting to our tertiary center, a significant number of patients with multiple nodules requiring biopsy, and the lack of time and experienced staff. Last, possible different causes of nondiagnostic cytological interpretation (such as hemorrhage or hypocellularity) were not compared in the 1-pass and multiple-passes groups.

In conclusion, although there is a tendency to make more insertions into the cystic/mixed nodules or nodules with macrocalcification in clinical practice considering that the macroscopic image of the obtained material would be insufficient, the needle pass of 2 or 3 times does not contribute to cytological sufficiency compared to a single time. Similarly, AUS/FLUS rate did not increase with multiple passes. Our study can be expanded by prospective studies evaluating whether biopsies performed with an on-site cytopathologist and more entrances increase the adequate cytology results.

Ethics Committee Approval: This study was approved by the Ethics Committee of Ankara City Hospital (approval no: E1-21-2095; date: October 20, 2021).

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