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Research Article

DIAGNOSTIC EFFICACY OF THE BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLGY: A TWO-YEAR PROSPECTIVE STUDY AT A TERTIARY CARE CENTRE IN RURAL REGION OF HARYANA (INDIA)

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ABSTRACT

Background and Objective: The objective of current study aimed (i) to analyse the thyroid cytology smears by the Bethesda system, (ii) to determine the distribution of diagnostic categories, (iii) to stratify the malignancy risk, (iv) to correlate cytopathology with histopatholgy, whenever surgery was done.

Material and method: This is a prospective study of 600 Fine needle aspiration cytology (FNAC) of thyroid nodules which were classified according to the Bethesda system and 119 histological evaluation obtained from this group. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy rates were evaluated.

Result: The distribution of various categories were: Nondiagnostic/Unsatisfactory (ND/UNS) 11 (1.8%), Benign 495 (82.5%), Atypia of undetermined significance/Follicular lesion of undetermined significance (AUS/FLUS) 26 (4.3%), Suspicious for follicular neoplasm (SFN) 18 (3%), Follicular neoplasm of Hurthle cell type (FNHCT) 3 (0.5%), Suspicious for malignancy (SM) 8 (1.3%), and malignant 39 (6.5%). In 119 cases, there was follow up histology. The implied malignancy risk in these categories were: 16.6%, 3.5%, 18.18%, 14.28%, 60% and 100% respectively. The sensitivity, specificity, accuracy, PPV and NPV were 75.4%, 60.3, 96.4%, 94.11% and 72% respectively.

Conclusion: We recommended routine ues of Bethesda system for reporting Thyroid cytopathology (BSRTC) for initial workup of patients with thyroid nodule. However risk of malignancy was found to be significantly high in Bethesda III category to warrant further workup including ultrasonography/ thyroid scan in addition to repeat FNAC. It also provides clear management guidelines to the clinicians to go for follow up FNA or surgery and also extent of surgery.

INTRODUCTION

Thyroid nodules are seen in about 8.5% of the population [1]. However, thyroid cancer is quite rare, and the incidence is 8.7 per 10000 people per year though seems to be increasing over the years [2]. In india, the prevalence of a palpable thyroid nodule in the community is about 12.2%. It has been estimated that 42 million people in India suffer from thyroid diseases [3]. Fine needle aspiration cytology (FNAC) of the thyroid nodule occupies the first line diagnostic test, is widely accepted and a cost effective method. It is a simple, safe, rapid, accurate, reliable method for triaging patients with thyoid nodules and reducing the surgery for benign lesions and necessitates surgical inetrvention when there is significant risk of malignancy [4]. Despites of its widespread use, thyroid FNA suffers from a reporting confusion: multiplicity of category names, descriptive reports without categories and variable surgical pathology terminology [5].

To overcome this confusion in diagnostic terminolgy and clinical perception, multiple organizations have proposed diagnostic guidelines for reporting thyroid FNA cytology results, including the Papanicolau society of cytopathology Task forces and American Thyroid Association although none have been universally accepted [6].

In 2007, the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference introduced the Bethesda system for unifying the terminology, morphological criteria along with the corresponding risk of malignancy [7]. It bridges the communication gap between clinicians and the

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pathologists and thus helps the surgeon to take appropriate therapeutic interventions [8].

Objective: To evaluate the disease spectrum and to elucidate the usefulness of the Bethesda system in reporting thyroid FNAs at our institution and report the malignancy risk.

MATERIAL AND METHODS

This is a prospective study conducted in the Department of Patholgy, at our institute the from Jan 2015 to Dec 2016. It comprised of 600 patients who presented with thyroid swelling and underwent FNAC. Relevant clinical history was taken and local physical examination (multinodular, solitary nodules, diffuse goitre etc) done. Patients of all age group and both the sexes were included in the study. FNAs were conducted using standard procedure, after written informed consent. Maximum 2 to 3 passes were made. The smears were prepared using conventional method and stained with Giemsa and Hematoxyline and Eosin. Repeat aspiration was done for inadequate smears. The cytological evaluation and reporting was done according to the Bethesda system by two expierenced cytopathologist. Out of 600 cases 119 patients subsequently underwent surgical intervention by either excision of nodules / lobectomy or subtotal / near total thyroidectomy. The cytological results were correlated with histopathological examintion. Sensitivity, Specificity, positive predictive value (PPV), negative predictive value (NPV) were calculated using histopatholgy as the gold standard. Incidental papillary carcinomas on resection were not considered malignant when contained within the thyroid, expect when prior cytological interpretation was suspicious for malignancy or malignant.

RESULTS

The present study comprised of thyroid FNACs of 600 cases of various thyroid lesions. 119 cases underwent surgical intervention and were subjected for histopathological examination.

The maximum incidence of thyroid swelling was between the age 21-30 years (n=155 cases, 25%). The age at presentation varied from 10 to 82 years with the mean age being 34 years. There was a preponderance of females in our study. Out of 600 cases 479 were females (79.8.%) and 121 (20.1%) were males. Male and female ratio is 3.9:1.

In present study, 61 (10.1%) cases turned out to be non-diagnostic (ND)/ Unsatisfactory (UNS), 447 (74.5%) benign, 25 (4.1%) Atypia of undetermined significance (AUS)/ Follicular lesion of undetermined significance (FLUS), 18 (3%) Follicular neoplasm (FN)/ Suspicious for Follicular Neoplasm (SFN), 3 (0.5%) Follicular neoplasm of Hurthle cell type (FNHCT), 8 (1.3%) Suspicious for malignancy (SM) and 38 (8.3%) malignant.

Of the FNA samples 61 were ND/UNS, these were reaspirated again under USG guidance. On reaspiration 1.8% cases (n=11) remained ND/UNS, categorized as subcategory cyst fluid only. The distribution of cases according to BSRTC in different categories following reaspiration were Benign 495 (82.5%), AUS/FLUS 26 (4.3%), SFN 18 (3%), FNHCT 3 (0.5%), SM 8 (1.3%) and malignant 39 (6.5%) (Table/Figure. 1) and compared it with other published studies (Table/figure 2)

Table 1 Distribution of cases in Bethesda System as per our study (n=600)

	Diagnostic Category	No. of cases in each category after reaspiration
I.	Non diagnostic/Unsatisfactory	11(1.8%)
	Cyst fluid only	8
	Virtually acellular	3
II.	Benign	498(83%)
	• Colloid/Adenomatoid goitre	351
	• Hashimoto's Thyroiditis	141
	• Granulomatous Thyroiditis	6
III.	Atypia of undetermined significance/ Follicular lesion of undetermined significance	26(4x.3%)
IV.	Suspicious for	
	• Follicular neoplasm	18(3%)
	• Follicular neoplasm of hurthle cell type	3(0.5%)
V.	Suspicious for malignancy	8(1.3%)
	• Papillary carcinoma	6
	• Medullary carcinoma	2
	• Metastatic carcinoma	0
	• Lymphoma	0
	Others	0
VI.	Malignant	39(6.5%)
	• Papillary carcinoma thyroid (PTC)	29
	• Follicular variant of PCT	5
	• Medullary carcinoma	2
	• Poorly differentiated carcinoma	1
	• Anaplastic carcinoma	1
	• Lymphoma	1
TOTAL		**600(100%)**

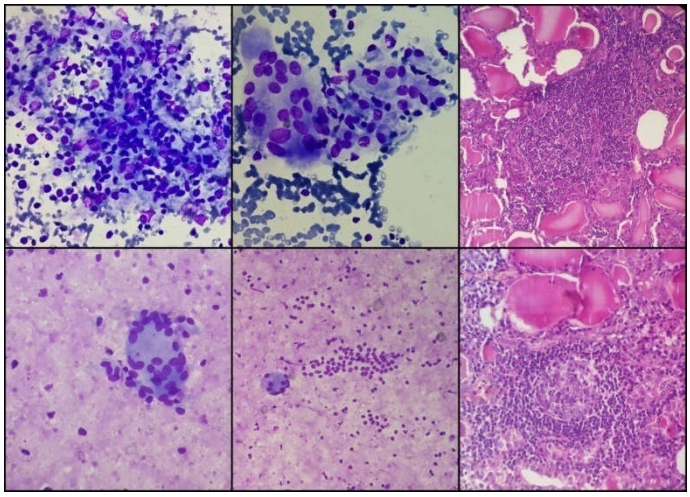

Figure 1 Photomicrographs of a&b) Lymphocytic thyroiditis showing polymorphous lymphoid population and lymphohistiocytic aggregates (MGG, 400X), c) lymphoid follicles alongwith benign thyroid follicles containig colloid, (H&E, 100X) d&e) Granulomatous thyroiditis showing multinucleated giant cell and clusters of epithelioid histiocytes mixed with benign follicular cells in a necrotic background (MGG, 100X), f) lymphoid follicle containing necrosis and giant cell in the centre (H&E, 100X).

Out of 600 cases, 119 underwent surgical intervention and histopathological correlation was done (partial/total thyroidectomy). We compare the original FNA diagnoses of these 119 cases with the diagnoses obtained on HPE and calculated the malignancy risk for each category (Table/Figure. 3) and compared it with other studies (Table/figure 4)

Reasons for surgical intervention included cosmetic consideration and neoplastic pathology. The neoplastic lesions were more common in females (n=30; 76.9%) than males (n=9; 23%)

Table 2 Correlation of cytological diagnosis with final histology, with incidence of malignancy in each Bethesda category.

Cytological diagnosis	No. of cases	No. of cases who underwent surgery	Histological diagnosis	Frequency (%)	Incidence of malignancy (%)
(I) ND/UNS	11 (1.8%)	6 (54.5%)	Nodular goitre Hashimoto thyroiditis PCT	3 (50%) 2 (33.3%) 1 (16.6%)	16.6
(II) Benign	498 (83%)	56 (11.2%)	Nodular goitre Hashimoto thyroiditid Follicular adenoma Follicular carcinoma PCT	35 (62.5%) 16 (28.5%) 3 (5.3%) 1 (1.77%) 1 (1.77%)	3.5
(III) AUS/FLUS	26 (4.3%)	11 (42.3%)	Adenomatoid goitre Follicular adenoma HCA PTC Follicular carcinoma	6 (54.5%) 2 (18.1%) 1 (9%) 1 (9%) 1 (9%)	18.18
(IV) FN/SFN	21 (3.5%)	14 (66.6%)	Adenomatoid goitre Hashimoto thyroiditis FA HCA PTC Follicular carcinoma	6 (42.8%) 1 (7.17%) 4 (28.5%) 1 (7.1%) 1 (7.1%) 1 (7.1%)	14.28
(V) SM	8 (1.3%)	5 (62.5%)	Nodular goitre Lymphocytic thyroiditis PTC MTC PTC	1 (20%) 1 (20%) 2 (40%) 1 (20%)	60
(VI) Malignant	36 (6%)	27 (75%)	Follicular variant PTC Medullary carcinoma Poorly differentiated carcinoma Anaplastic carcinoma Lymphoma	21 (77.7%) 2 (7.4%) 1 (3.7%) 1 (3.7%) 1 (3.7%) 1 (3.7%)	100

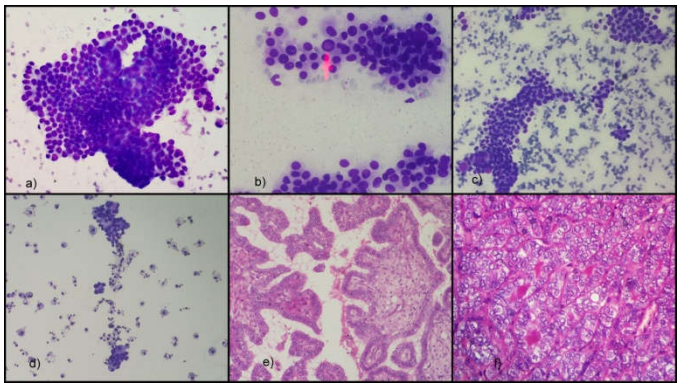

Figure 2 Photomicrographs of Papillary Carcinoma Thyriod showing a) a typical papillae with anatomical borders (MGG, 100X), b) a papillae containing an intranuclear cytoplasmic inclusion (MGG, 100X), c) a papillae containing many hyaline globules (MGG, 100X), d) cystic variant, e) histopatholgy section showing papillary architecture of tumor cells (H&E, 400X), f) follicular variant of PTC (H&E, 400X).

There were 39 cases of malignancy on resection, giving an overall surgery yield of malignancy of 32.7%.

Of the 11 ND cases which remained ND/UNS on repeat FNAC under USG guidance, definitive diagnosis could be arrived in six cases: Nodular goitre, 3; Hashimoto's thyroiditis, 2; Papillary thyroid carcinoma (PTC),1 yielding the malignancy rate of 16.6%.

Of the 600 thyroid samples, 495 were categorized as benign. Follow up histology was available in 56 cases among which 2 cases were found to be malignant leading to overall malignancy risk of 3.5%.

Table 3 Comparasion of Percentage of Distribution of FNAC diagnoses among Published studies

Category	Yassa *et al*	Nayar & Evanoic *et al*	Jo *et al*	Mufti *et al*	Mondal *et al*	Naz *et al*	Garg *et al*	Present study
I) ND/UNS	181 (7%)	260 (5%)	573 (18.6%)	29 (11.6%)	12 (1.18%)	25 (5.7%)	06 (06%)	11 (1.8%)
II) Benign	1707 (66%)	324 (64%)	1817 (59%)	194 (77.6%)	893 (87.5%)	403 (76.3%)	78 (78%)	498 (83%)
III) AUS/FLUS	104 (04%)	935 (18%)	105 (34%)	02 (0.8%)	10 (0.98%)	67 (12.7%)	04 (04%)	26 (4.3%)
IV) SFN/FN	233 (09%)	311 (06%)	299 (2.7%)	10 (4%)	36 (3.58%)	11 (2.1%)	04 (04%)	21 (3.5%)
V) SM	233 (09%)	104 (02%)	71 (2.3%)	06 (2.4%)	14 (1.37%)	18 (3.4%)	14 (14%)	8 (1.3%)
VI) Malignant	129 (5%)	260 (05%)	215 (07%)	09 (3.6%)	48 (4.7%)	04 (0.8%)	04 (04%)	39 (6.5%)
	2587	5194	3080	250	1020	528	100	600

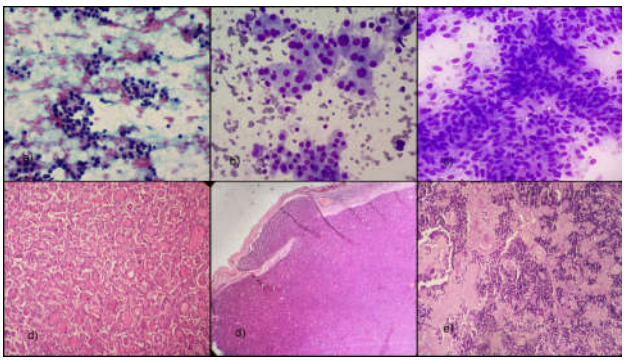

Figure 3 Photomicrograph showing a) Follicular neoplasm (MGG, 100X), b&c) Plasmacytoid and spindle variant of Medullary Carcinoma, d,e&f) Follicular adenoma, follicular carcinoma and medullary carcinoma (H&E, 100X)

Table 4 Comparasion of the Percentage of follow up malignancy of present study with other studies

Category	Yassa *et al*	Nayar & Evanoic *et al*	Jo *et al*	Mufti *et al*	Mondal *et al*	Naz *et al*	Garg *et al*	Present study
ND/UNS	10	09	8.9	20	0		20	16.6
Benign	03	02	1.1	3.1	4.5	11.1	0	3.5
AUS/FLUS	24	06	17	50	20	33.3	25	18.1
SFN/FN	28	14	25.4	20	30.6	25	20	14.2
SM	60	53	70	80	75	100	66	60
Malignant	97	97	98.1	100	97.8	100	100	100

The histological diagnosis were as follows: Nodular goitre, 35; Hashimoto's thyroiditis, 16; Follicular adenoma, 3; Follicular carcinoma,1; PTC, 1.

Out of 26 cases categorized as AUS/FLUS, histopathology was available in 11 cases yielding the following histological diagnoses: Nodular goitre, 6; Follicular adenoma, 2; Hurthle cell adenoma, 1; Follicular carcinoma,1; Follicular variant of PTC,1. Two cases were found to be malignant, giving a malignancy risk of 18.18%.

14 out of 21 cases of SFN category were available for histopatholgical examination yielding the following diagnosis: Nodular Goitre, 6; Hashimoto's thyroiditis, 1; Follicular adenoma, 4; Hurthle cell adenoma, 1; Follicular carcinoma, 1; PTC, 1. Two cases were found to be malignant with the implied risk of malignancy of 14.28%.

Out of 8 cases which were categorized as SM, specimen of 5 cases were available for histology. Of theses 5 cases, 3 were malignant with the following diagnoses: PTC, 2; Medullary carcinoma, 1. The implied risk of malignancy is 60%. 2 cases were benign with the following diagnoses; Nodular Goitre, 1; Lymphocytic thyroiditis, 1.

Of the 39 malignant cases, 27 were followed by thyroidectomy specimen and all 27 were confirmed as malignant. The diagnoses of malignancy on resection were as follows; Papillary carcinoma, 21; Follicular variant of PTC, 2; Medullary carcinoma, 1; Poorly differentiated carcinoma, 1; Anaplastic carcinoma, 1; Lymphoma, 1. The risk of malignancy was 100%.

The sensitivity, specificity, accuracy, positive predictive value and negative predictive value of were 75.4%, 60.3% and 96.4%.to be 94.11% and 72% respectively and compared it with other studies (Table/figure. 5)

Table 5 Comparasion of diagnostic value for malignant lesions

Studies	Sensitivity	Specificity	PPV	NPV	Accuracy
Jo *et al*	88.4	90.9	56.4	98.3	69.4
Gupta *et al*	80	86.6	80	86.6	84
Mufti *et al*	65	96.9	68.4	96.4	82
Mondal *et al*	86.6	86.6	66.3	95.5	65.6
Naz *et al*	66.3	85.1	56.3	88.9	80.3
Muratli *et al*	87.1	64.6	79.5	76.1	77.3
Garg *et al*	88.8	84.3	50	97.7	85
Present study	94.11	72	60.3	96.4	75.4

Cancer of the thyroid gland accounts for 1% of all cancers and is responsible for 0.5% of cancer related deaths. Early dagnosis of thyroid cancer provides higher life expectancy due to slow progression of thyroid cancers [9]. First cytological diagnosis of thyroid cancers with FNAC was made by Martin and Ellis in 1930 [10].

FNAC has excellent patients compliance and minimal morbidity. It has a high diagnostic value and is cost effective. FNAC of thyroid nodules resulted in the decrease in the number of patients who underwent surgical treatment by 25-50% while increasing in the percentage of malignancy in thyroid specimens [5].

Currently FNAC is the preferred diagnostic method for the initial stage of evaluation of thyroid nodules [11].

We compare the results obtained in our study with the studies of Yassa *et al* [5], Jo *et al* [6], Muratli *et al* [9], Mondal *et al* [12], Garg *et al* [13], Nayar & Evanoic et a [14], Naz *et al* [15], and Mufti *et al* [16].

In our study, the maximum number of cases were benign (n=495, 82.5%) which can be attributed to the fact of our institue being the largest tertiary centre in this region, with a large influx of patients from both urban and rural areas. Therefore, the proportion of benign cases that is a lot higher in general population, is reflected proportionately in our study which is comparable to the studies done by Mondal *et al* [12] and Garg *et al* [13] in Indian settings.

The reported non diagnostic test rates range between 1.6% and 20% in the literature [9][11][17][18]. The present study had 61 cases (10.1%) and after reaspiration, this number decreased to 11 (1.8%) which correlates with study done by Mondal *et al* (n=12, 1.18%) [12], Nayar and Ivanoic *et al* (n=260, 5%) [14] and Naz *et al* (n=25, 5.7%) [15]. Jo *et al* (n=573, 18.6%) [6] and Mufti *et al* (n=29, 11.6%) [16] showed higher pecentage of cases in this catgory.

Ali *et al* suggested that the rate of of ND test should be below 10% [18].

The reason for the lower percentage in the ND category can be attributed to the fact that USG guided FNAC is preferred for small nodules or nodules that appear heterogenous on palpation and cytopathologist himself performs the procedure, so that the aspirate can be done from the representative site [12].

Our study showed less number of cases diagnosed as AUS/FLUS (n=26, 4.3%) which is comparable to the studies done by Yassa *et al* [5] (4%). Jo *et al* [6] (3.4%) and The low percentage of cases could be explained by the strict adherence to diagnostic criteria to avoid ambiguity and keep the use of AUS to a minimum.

Nayar and Ivanoic [14] (18%) show higher results and attribute their higher AFLUS rate to a tendency to include adequacy related cases in this diagnostic category which may otherwise been classified as inadequate by other groups. They report that the percentage of AFLUS cases would decrease to 14% if they remove adequacy related cases.

TBSRTC suggests that the frequency of AUS interpretation should be in the range of approximately 7% of all thyroid FNA interpretation. AUS is a category of last resort and should not be used indiscriminately [16].

Our study showed good correlation of FN/SFN including FNHCT (3.5%) with Mondal *et al* [12] (3.58%) and Mufti *et al*

[16] (4%) while it was at variance with that of Yassa *et al* [5] (9%) and Jo *et al* [6] (9.7%) . The higher percentage reported can be attributed to the large sample size and may be geographical distribution.

Our sutudy had 8 cases (1.3%) in SFM category and was comparable with the studies done by Mondal *et al* [12] (1.37%), Nayar and Ivanoic [14] (2%), and Mufti *et al* [16] (2.4%).

Most of the studies conducted to date have revealed a good accuracy of FNAC concordant with the results of our study. In previous studies, the sensitivity of thyroid FNAC ranges from 74% to 92% and specificity ranges from 74% to 100% [9][11][17][19]. In present study we found the sensitivity and specificty to be 94.11% and 72% respectively. The reason for the wide variation of sensitivity and specificity is the difference in categorization of lesions by different pathologists.

The accuracy, positive predictive value and negative predictive value of the present study were 75.4%, 60.3% and 96.4%. The results of our study were concordant with the study done by Jo *et al* [6], Mondal *et al* [12] and Naz *et al* [15].

False negative results may occur because of sampling errors or misinterpretation. The factors that reduce the efficiency include inadequate sampling, inexpierence of the cytopathologist and subjective variation in differentiation of benign and malignant follicular lesions [9]. However it is difficult to calculate the true frequency of false negative result because only a small percentage of patients undergo surgery with benign cytological findings. Most authorities are of the opinion that the true false negative reports are less than 5% even if all patients with thyroid FNAC get an excision done followed by histopathological examination [20]. In the present study two false negative cases were found (5.8%). This in keeping with the literature where false negative rate of 2-7% has been reported [11][17]. In our study, the most common lesions that contribute to false negative results were nodular hyperplasia with dense micropapillary structure. It may be difficult to establish a cytological differentaition between follicular hyperplastic nodules which were diagnosed as SFM or some of the follicular adenomas and well differentiated follicular carcinoma.

In our study the false positive rate (FPR) was 28%. In the studies in the literature the false positive rate has been reported between 1-11% [11][17]. This high false positivity can be attributed to the fact that a number of diagnoses fall in the category of SFN/FN out of which majority turn out to be follicular adenomas or hyperplastic/adenomatous nodules. We included all the cases reported as malignant, but even those reported as SFN or suspicious of PTC.

The rate of malignancy in the present study was comparable in most of the categories to that mentioned in TBSRT and other published studies except in the ND/UNS and AFLUS category. The ND/UNS category in our study yielded a malignancy rate of 16.6% with one case turning out to be PTC, among 6 follow up resections. This was attributed to the underdiagnosis of PTC due to cystic degeneration. Bakhos *et al* [21] have reported that suboptimal material and under diagnosis of PTC due to cystic degeneration are the most common pitfalls for false negative diagnosis in thyroid FNA. Raab *et al* [22] have also emphasized that suboptimal specimens can be a significant source of false negatives in thyroid cytology.

The associated risk of malignancy in AFLUS category was 18.18% as compared to 6% in the study of Nayar and Ivanoic [14]. This can be explained by the fact that the total population studied by us, comprising is a much smaller number compared to that in the study by Nayar and Ivanoic.

CONCLUSIONS

This study validated the accuracy of Bethesda system of reporting thyroid cytopatholgy. Universal application of the new standarized diagnostic categories for reporting thyroid FNA results can improve interlaboratory agreement in the diagnosis of thyroid lesions and may lead to more consistent management approaches. The associated risks found for AFLUS, SFN, SM confirms the importance of these categories in a 6-tiered diagnostic category.

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