Evaluation of Fine-Needle Aspiration of Thyroid Nodules in a Series of 1,100 Patients: Correlation Between Cytology and Histopathology Original Article.

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Received: 5 February 2014 / Accepted: 8 May 2014 / Published online: 24 May 2014
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Abstract In this study, our aim was to test the usefulness of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) in indeterminate lesions in which cytological and histological diagnosis discordance was high and to investigate the contribution of TBSRTC in this indeterminate diagnostic group. The medical records of the patients who presented to the Haydarpaşa Numune Training and Research Hospital in Istanbul, Turkey, between October of 1999 and September of 2010, for the cytological and histological diagnosis of thyroid nodules, were reviewed retrospectively. We reclassified these patients according to the Bethesda method, which features well-defined cytological criteria for each category within a six-tiered system. For each patient, the diagnosis and reasons for discordance were discussed and a final decision was made for each discordant case. The overall distribution of the cytological diagnoses using an in-house system was as follows: 811 benign (73.7 %), 87 suspicious follicular cell/follicular neoplasia (7.9 %), 52 suspicious for malignancy (4.7 %), and 45 malignant (4.1 %). We reclassified the diagnoses using the

Bethesda system and the results are as follows: 797 benign (73.7 %), 48 follicular lesion of undetermined significance (4.3 %), 68 follicular neoplasia (7.9 %), 40 suspicious for malignancy (3.6 %), and 44 malignant (4 %). Our results showed that using TBSRTC for the lesions in the indeterminate category decreased the ratios of false-positive and false-negative diagnoses.

Keywords Thyroid · Cytology · Fine-needle aspiration · Bethesda classification · Neoplasia

Introduction

Thyroid nodules occur in 4–7 % of the general population. The incidence of malignancy in these nodules is around 5 %, so it is necessary to identify the possible candidates for surgery using standardized and effective screening methods [1]. Although various screening methods exist, such as ultrasonography and scintigraphic methods, fine-needle aspiration cytology (FNAC) has proven to be the method with the highest positive and negative predictive values in determining whether a lesion is malignant or benign. The current published literature shows that the ranges of sensitivity and specificity for FNACs are 57–99 and 45–99 %, respectively [1–4]. To date, there are many terminologies for the reporting of findings [3]; in particular, the use of different diagnostic categories can be misleading and may lead to unacceptable discrepancies in the diagnoses. This is especially problematic in the diagnoses of “indeterminate” nodules. There are no national terminologies for reporting thyroid FNACs in different European countries; the USA (Bethesda) and British (BTA/RCPATH) terminologies are not universally applied; and personal, local, and descriptive terminologies are currently being used by cytopathologists [1]. However, there is agreement about the need for standardization in thyroid FNAC reporting. In 2007,

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the National Cancer Institute (NCI) hosted the Thyroid FNA State of the Science Conference to address these concerns [4]. At the conclusion of the conference, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), a standardized system with clear categorical nomenclature including malignancy risks, was proposed [2].

In this study, our aim was to evaluate the usefulness of TBSRTC in indeterminate lesions in which cytological and histological diagnosis discordance was higher and to investigate the contribution of TBSRTC to this indeterminate diagnostic group.

Materials and Methods

The medical records of the patients who presented to the Haydarpasa Numune Training and Research Hospital in Istanbul, Turkey, between October of 1999 and September of 2010, for cytological and histological diagnoses of thyroid nodules, were reviewed retrospectively. The reports of the FNA analyses and reports from the surgical histological sections were obtained, and the slides of the patients whose histological and cytological diagnoses were discordant were reevaluated using the May-Grunwald-Giemsa (MGG), Papanicolaou (PAP) and hematoxylin, and eosin stains.

The cytology results were categorized (in-house system) according to the original cytology reports into five groups: (1) nondiagnostic specimen (inadequate), (2) benign, (3) suspicious for follicular cell/follicular neoplasm (SFC-FN), (4) suspicious for malignancy (SFM), and (5) malignant. Our detailed criteria were as follows:

1. Nondiagnostic: The specimens that were categorized as unsatisfactory consisted predominantly of blood, lacked both colloid and follicular cells, and/or had six groups of well-preserved cells on at least two slides [3, 5].
2. Benign cytology: Samples that were negative for malignancy included benign follicular nodules (BFN), lymphocytic thyroiditis (LT), or thyroid cysts. This category had abundant colloid, mixed follicular, and Hurthle cells that mainly formed a macrofollicular pattern or flat sheets without crowding or nuclear atypia [6].
3. Suspicious for follicular cells (SFC)/follicular neoplasia (FN): When some features of FN raise strong suspicions of neoplasm, but the findings are not sufficient for a conclusive diagnosis, they were considered to be suspicious for follicular cell (SFC) [6, 7]. Follicular neoplasia refers to a significant alteration in the follicular cell architecture, characterized by cell crowding, microfollicles, and dispersed isolated cells, with scant or moderate amounts of cytoplasm. The nuclei are round and slightly hyperchromatic, with inconspicuous nucleoli. Colloid is scant or absent.
4. Suspicious for malignancy (SFM): The cells were considered to be SFM when some features of malignancy were present, mainly those of papillary thyroid carcinoma (PTC) or medullary thyroid carcinoma (MTC), raising a strong suspicion of malignancy, but the findings were not sufficient for a conclusive diagnosis [6].
5. Malignant specimens: These specimens had cytological features that were consistent with a malignant neoplasm, including papillary thyroid carcinoma (PTC), anaplastic thyroid carcinoma (ATC), and medullary thyroid carcinoma (MTC), which were classified as being positive for malignancy.

For each case, the final histopathological diagnosis after surgical resection was recorded and classified as either benign or malignant. The diagnosis of follicular nodular disease [6], thyroiditis, adenomatoid nodules (AN), follicular adenoma (FA), or Hurthle cell adenoma (HA) was considered to be benign pathologies. Alternatively, the diagnosis of PTC and its subtypes, follicular carcinoma (FC), ATT and MTC, lymphoma, and metastatic cancer were classified as malignant.

In cases of discrepancies between the FNAC and histopathological diagnosis of the same lesion, both the cytology and histology were reevaluated in order to determine possible causes of error. In total, 182 cases had such discrepancies; the cytology of 160 of these patients was obtained, and the fine needle aspirations of 22 patients were sent to other centers for consultation and could, therefore, not be assessed. These 160 cytological preparations were reassessed separately by two different pathologists who were not aware of the original diagnosis (PG/PD). These lesions were reclassified in these patients according to TBSRTC, which features well-defined cytological criteria for each category within a six-tiered system. For each patient, the diagnosis and reasons for discordance were discussed, and a final decision was made for each discordant case. The Bethesda categories are as follows: nondiagnostic, benign, atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), suspicious for follicular neoplasm/follicular neoplasm (SFN/FN), suspicious for malignancy (SFM), and malignant. The criteria for each category were subsequently published in an atlas [2]. The causes of discordance were categorized as follows: diagnosis made with suboptimal or nondiagnostic material, overlapping cytological features, misinterpretation by the assessor, and presumed sampling errors.

The sensitivity and specificity of the cytological diagnoses were evaluated with two different reporting systems using the histological diagnoses as the gold standard. The cytological diagnoses which were referred for surgery according to the in-house reporting system (SFC, FN, SFM, and malignant) and Bethesda reporting system (FN, SFM, and malignant) were considered to be positively diagnostic.

Statistical Analysis

Statistical analyses were performed using SPSS statistical software. The descriptive statistics were calculated as the mean±standard deviation and frequency. The statistical outcomes included sensitivity (TP/[TP+FN]), specificity (TN/[TN+FP]), positive predictive value (TP/[TP+FP]), and negative predictive value (TN/[TN+FN]).

Results

Demographic Data

The pathology department of Haydarpasa Numune Training and Research Hospital selected 1,100 patients out of a total of 8,300 thyroid FNA procedures that took place during the study period, as this cohort also had histopathological verification of disease. The age of the patients varied between 8 and 86 years (43.23 ± 13.25). The male-to-female ratio was 1:5.5, and the size of the thyroid nodules varied from 10 to 130 mm.

The overall distribution of the cytological diagnoses using the in-house system was as follows: 105 (9.5 %) inadequate, 811 benign (73.7 %), 87 SFN/FN (7.9 %), 52 SFM (4.7 %), and 45 malignant (4.1 %). We reclassified the incompatible diagnoses using the Bethesda system and the results are as follows: 103 inadequate (9.3 %), 797 benign (73.7 %), 48 FLUS (4.3 %), 68 SFC/FN (7.9 %), 40 SFM (3.6 %), and 44 malignant (4 %). The results of 1,100 cases, according to TBRSTC and the in-house system (cytological diagnosis and resection diagnosis), are shown in Tables 1 and 2.

We have also reevaluated the diagnosis of the cases with the mismatch of cytology and histology using an in-house system and the Bethesda system. The results according to the in-house system were as follows: 58 benign (36 %), 60 SFN/FN (37 %) (53 SFN, 7 FN), 42 SM (26 %), and 1 (0.09 %) was malignant. According to the Bethesda system, the findings were as

follows: 41 benign (25.4 %), 50 FLUS/AUS (31 %), 38 SFN/FN (23.6 %), 31 SM (19.2 %), and 1 (0.6 %) was malignant.

According to the in-house system, there were 58 patients with benign lesions. These patients were reevaluated according to Bethesda, and reclassified as: 1 SM (1.7 %), 16 FLUS/AUS (27.5 %), and 41 benign (70.6 %). According to the in-house system, 60 patients had SFC/FN, and these patients were reevaluated according to Bethesda and reclassified: 22 FLUS/AUS (36.6 %) and 38 SFL/FN (63.3 %). According to the in-house system, 42 with an SM diagnosis were reevaluated according to Bethesda: 30 were SM (71.4 %) and 12 were FLUS/AUS (28.5 %) (Table 3).

Histological Diagnoses and Review

According to the histopathological classification, 88.1 % ($n=970$) of the cases were classified as benign, 10.1 % ($n=110$) were malignant, and 1.82 % ($n=20$) were micro-papillary thyroid carcinoma.

Of the 969 benign lesions, 321 (29.7 %) were follicular adenoma/adenomatoid nodules, 636 (57.7 %) were follicular nodular disorder, and 12 (1.36 %) were chronic lymphocytic thyroiditis (CLT). Of the 111 malignant tumors, 97 (8.3 %) were PTC, 9 (0.82 %) were FC, and 5 (0.45 %) were MC.

The cytohistological analyses of 1,100 patients are shown in Table 1 (TBRSTC system) and Table 2 (in-house system), respectively. For 79.8 % of the nodules, the diagnosis was benign, and 8.3 % were FA, 10.1 % were malignant, and 1.8 % were micro-papillary thyroid carcinoma. (Figs. 1 and 2)

Table 3 shows the comparison of the mismatched/discordant lesions under the two schemes. In the in-house system, in 20 of 58 patients (34.4 %) with a diagnosis of benign lesions, malignancies were confirmed; whereas, according to the Bethesda system, 14 of the 41 patients (34.1 %) were diagnosed as malignant. In patients with a diagnosis of malignancy under either system, there was no discordance.

Table 1 Classification of the patients for whom histological follow-up data were available, based on the Bethesda reporting system

Cytological category	Histological	Diagnosis			Total (%)
	Nonneoplastic (%)	Follicular adenoma (%)	Malign	Micropapillary carcinoma (%)	
Inadequate	89 (86.4)	10 (9.7)	3 (2.9)	1 (1)	103 (100)
Benign	728 (91.3)	32 (4)	19 (2.4)	18 (2.3)	797 (100)
AUS/FLUS	23 (47.9)	15 (31.3)	10 (20.8)	0 (0)	48 (100)
SFN/FN	28 (41.2)	29 (42.6)	10 (14.7)	1 (1.5)	68 (100)
SFM	9 (22.5)	4 (10)	27 (67.5)	0 (0)	40 (100)
Malign	1 (2.3)	1 (2.3)	42 (95.4)	0 (0)	44 (100)
Total	878 (79.8)	91 (8.3)	111 (10.9)	20 (01.8)	1,100 (100)

AUS/FLUS atypia/follicular lesion of undetermined significance, SFN/FN suspicious for follicular neoplasm, SFM suspicious for malignancy

Table 2 Classification of the patients for whom histological follow-up data were available, based on the in-house reporting system

Cytological category	Histological	Diagnosis			Total (%)
	Nonneoplastic (%)	Follicular adenoma (%)	Malign (%)	Micropapillary carcinoma (%)	
Inadequate	91 (86.7)	10 (9.5)	3 (2.9)	1 (1)	105 (100)
Benign	728 (89.8)	42 (5.2)	23 (2.8)	18 (2.2)	811 (100)
SFN/FN	43 (49.4)	32 (36.8)	11 (12.6)	1 (1.1)	87 (100)
SFM	15 (28.8)	6 (11.5)	31 (59.6)	0 (0)	52 (100)
Malign	1 (2.2)	1 (2.2)	43 (95.6)	0 (0)	45 (100)
Total	878 (79.8)	91 (8.3)	111 (10.9)	20 (01.8)	1,100 (100)

SFN/FN suspicious for follicular neoplasm, *SFM* suspicious for malignancy

In a further analysis of the 58 benign diagnoses of the in-house group, 16 (27.5 %) were reclassified as the AUS/FLUS category of the Bethesda classification. All of these cases went to surgery, and 5 were found to be malignant.

In a group of 102 patients who had diagnoses in the indeterminate category (SFN/FN/SFM) after surgery according to the in-house system, 67 % were benign and 33 % were

diagnosed as malignant. In comparisons under the Bethesda system, 60 % of 70 patients were found to be benign, and the remainder were malignant. The overall detection rate for malignancy in the discordant cases (benign and indeterminate) was 20 %, but only 9.09 % in the indeterminate AUS/FLUS category. The histology of the 160 cases was reevaluated, and the causes of the discordant diagnoses were listed as: 43 with failure in interpretation (26.7 %), 57 with overlapping cytology (35.4 %), 6 sampling errors (3.7 %), and 32 with inadequate material for diagnoses (19.8 %). A group of 22 patients (14.2 %) were considered highly suspicious for malignancy, but were not labeled as such.

The estimates of sensitivity and specificity for the FN cytology and FA histology for both the benign and malignant lesions were as follows: specificity of 93 % in-house and 95 % in the Bethesda system and sensitivity of 60 and 65 %, respectively.

Table 3 Distribution of discordant cases for whom histological follow-up data were available ($n=160$) based on cytologic diagnoses using the in-house and Bethesda reporting systems

Cytologic diagnosis		Histologic diagnosis		
In-house report system	Bethesda report system	Total	Benign	Malignant
Benign ($n=58$)	Benign	41	27	14
	AUS/FLUS	16	11	5
	SFN/FN	0	0	0
	SFM	1	1	0
	Malign	0	0	0
SFN ($n=53$)	Benign	0	0	0
	AUS/FLUS	20	18	2
	SFN/FN	33	30	3
	SFM	0	0	0
	Malign	0	0	0
FN ($n=7$)	Benign	0	0	0
	AUS/FLUS	2	2	0
	SFN/FN	5	3	2
	SFM	0	0	0
	Malign	0	0	0
SFM ($n=42$)	Benign	0	0	0
	AUS/FLUS	12	9	3
	SFN/FN	0	0	0
	SFM	30	8	22
	Malign	0	0	0

Total ($n=160$)

AUS/FLUS atypia/follicular lesion of undetermined significance, *SFN* suspicious for follicular neoplasm, *FN* follicular neoplasm, *SFM* suspicious for malignancy

Discussion

Malignancy rates of FNAC in the literature vary between 1.6 and 18 %, and our results showed it to be just over 4 %. Özlük et al. reported the highest rate (18 %). In their analysis, they believed that this may have been attributable to the use of a highly selective population which included only those patients for whom cytological or histological follow-up data were available and/or to the geographic uniqueness of their population [8]. A rate of 0.8 to 9 % suspicious malignancy, including a follicular neoplasia rate of up to 11.6 %, has been documented in the literature. Our results match these values [6, 9, 10].

The reported rates of false-negative results vary between 1 and 11 %. In our setting, there is a high rate of surgical treatment in patients with a diagnosis of benign lesions (false negatives), therefore, providing a better evaluation. The commonest cause of false negativity was erroneous interpretation, followed by attempted diagnosis in suboptimal

Fig. 1 **a** Case diagnosed as inadequate material on initial FNA (Papanicolaou stain $\times 100$). **b**, **c** Surgical pathology follow-up showed encapsulated cystic lesion exhibiting diagnostic nuclear cytology of

papillary carcinoma (intracystic papillary thyroid carcinoma). **b** H&E stain $\times 20$. **c** H&E stain $\times 400$

material. In the literature, insufficient material remains central to false-negative diagnoses, and experience in the field minimizes incorrect interpretations.

In order to reduce the false-negativity rates in suboptimal material, a benign diagnosis is advisable in cases with sufficient cellularity. In cases of insufficient cellularity, a repeat FNAC is advisable.

The suboptimal material was an important factor towards false-negative results in our study, and this supports the findings of Raab et al. [11], Nayar et al. [12], and Yang et al. [9].

The use of standardized criteria, as in the Bethesda system, and evaluation by cytopathologists is expected to decrease the false-negativity rate [4]. The reevaluations of the discordant cases revealed a high (25 %) rate of misinterpretation, particularly in groups defined as grey zone FA (65.9 %), follicular variant of PTC (22 %), and cystic PTC (4.9 %). Yang et al. found cytological and histological diagnosis mismatch to be 15.3 % and listed reasons such as sampling technique, cystic PTC, and misinterpretation [9].

For the diagnosis of mismatch, Özlük et al. [8] considered histopathological subtyping to be an important element when confronted with follicular variants of papillary thyroid carcinoma, and to a lesser extent, the uncertain potential of differentiated groups. They also emphasized that changes, such as hyalinization, calcification, and intracystic papillary hyperplasia, may reflect atypical nuclear changes [8].

Yang et al. proposed cytological criteria for the difficult-to-differentiate cytologically hyperplastic nodules and the FA. According to their proposal, there are cytological features that may help in distinguishing hyperplastic nodules versus FNs. In a hyperplastic nodule, follicular cells may form large single-

layered sheets that fold over on themselves. Within these sheets of follicular cells, there are well-formed macrofollicles that often demonstrate perifollicular fibrosis or are surrounded by basement membrane-like material. Follicular cells within follicles are cohesive and often “held” together by basement membranes and/or matrix material. Conversely, FNs often have syncytial clusters of follicular cells, 3-dimensional groups, and loosely cohesive follicles (especially microfollicles), with isolated, intact follicular cells [9].

The Bethesda subcategory, AUS/FLUS, constitutes an interesting entity, and its ratio is not expected to exceed 7 %; although, in the literature, it may be as high as 27 % [5, 10, 12]. Our analysis found 4.3 % AUS/FLUS lesions. The rate of detection for malignancy varies from 6 to 48 % and in TBRSTC, from 5 to 15 % [4].

There is an indeterminate diagnostic category located between benign and SFN. Significant inter- and intra-observer variability has been reported in this group. It is not a homogeneous category for predicting the risk of malignancy, since the cellular patterns vary; cytological atypia suggestive of papillary carcinoma appears to have a higher risk of malignancy [4]. The risk of malignancy in this category may be as high as 20 % [5, 9], contrary to the general knowledge of its potential, and may be disturbing for both the clinician and the patient.

Conclusion

Our results showed that the Bethesda reporting system for thyroid FNA has high sensitivity and specificity. The two

Fig. 2 **a**, **b** Case diagnosed as suspicious follicular lesion. **a** Diff-Quik stain $\times 100$. **b** Diff-Quik stain $\times 200$. **c** Surgical pathology follow-up showed an encapsulated follicular variant papillary carcinoma. The tumor

was composed of variously sized follicles which hypereosinophilic colloid; many of which was angulated and irregular in shape. The nuclei have characteristic features of papillary carcinoma (H&E stain $\times 400$)

reporting systems had few statistical differences. Compared to the Bethesda system, our in-house system used very similar categories. However, the AUS/FLUS category is the most problematic category. In order to reduce cytological and histological diagnosis discordance, the use of standardized criteria (as in the Bethesda system), evaluation by cytopathologists, sufficient cellularity, and in cases of insufficient cellularity, repeat FNACs are advisable.

Acknowledgments I would like to thank Dr. Fatih Göktaş and Dr. Nil Çomunoğlu for their technical support.

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