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Atypia of Undetermined Significance in Thyroid Fine Needle Aspirates: a 4-Year Audit of Thy3a Reporting

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Abstract
Objective: Thyroid nodules are common within the general population. Cytological analysis of fine needle aspirates (FNAs) of these lesions allows for identification of those that require further surgery. A numerical classification system is in place to streamline reporting. The 3a category is used for lesions that are neither benign nor malignant but show atypia of undetermined significance. We reviewed our use and clinical outcomes of Thy3a over a 4-year period. ***Methods:*** All thyroid FNAs performed at this institute from January 2012 to December 2015 were identified from our laboratory information system using SNOMED codes. Cytology was correlated with histology. ***Results:*** Of the 1,259 FNAs reported at this institute, Thy3a constituted only 1.2% (n = 16) of all cases, with a malignancy rate of 7%. Five Thy3a cases had a repeat FNA that was reported as Thy2 (benign), 1 as Thy1c (cyst), 1 as Thy3f (follicular lesion), and 1 as Thy5 (malignant). Six cases without repeat FNA were follicular adenomas at resection. Two cases were lost to follow-up. Within all thyroid cytology categories in this 4-year period, we had a false-positive rate of 1.9% and a false-negative rate of 0.3%. ***Conclusions:*** The Thy3a subclassification has varied diagnostic criteria and lacks reproducibility. Despite the rare use of the Thy3a category at our centre, our diagnostic accuracy remained high. At this time, further Thy3a cohort studies are required to assess the real benefits of this category.

Introduction

Thyroid nodules are a common finding in the general population with estimates of up to 50% of the population having a sonographically detectable thyroid lesion [1]. Only 5% of these are malignant, and the use of fine needle aspirate (FNA) cytology has allowed for a minimally invasive yet diagnostically reliable means of triaging such lesions. Multiple cytology classification systems exist in-

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ternationally, helping to streamline the reporting of these specimens, allowing for guidance of clinical management.

In November 2009, the Royal College of Pathologists published a document entitled "Guidance on the Reporting of Thyroid Cytology Specimens," which was revised in January 2016 [2]. This utilises a numerical system for categorical reporting and ranges from Thy1 to Thy5. Thy4 to Thy5 lesions are malignant or suspicious for malignancy, and the management of these lesions is usually taken to the multidisciplinary team meeting, with surgical intervention as a recommended outcome. The Thy3 category, which indicates that a neoplasm is possible, is divided into 2 subgroups. Thy3f suggests a follicular neoplasm. It is not possible to use cytology to distinguish benign from malignant follicular neoplasms in Thy3f aspirates and ultimately, surgery for histological examination, to exclude capsular or vascular invasion, is usually recommended.

Thy3a includes atypical nuclear, cytoplasmic and/or architectural features but is insufficient to be placed in any of the other diagnostic categories, and so it is not possible to classify with confidence whether a lesion is benign, suspicious for malignancy, or malignant. The clinical management of these lesions is very difficult due to this undetermined risk of malignancy.

The introduction of this Thy3a category by the Royal College of Pathologists parallels category III atypia of undetermined significance (AUS) used in the American Bethesda system of thyroid cytology reporting [3].

The Thy3a category encompasses:

1. Samples in which there is architectural "atypia", in the form of a mixed micro- and macrofollicular pattern (approximately equal proportions of each) and/or little colloid, where a definite distinction between a follicular neoplasm and hyperplastic nodule is difficult; useful phrasing might be that "the appearances may represent a cellular colloid nodule but a follicular neoplasm is not excluded."
2. Sparsely cellular samples containing predominantly microfollicles.
3. Focal nuclear atypia or other cytological changes, which are most probably benign but where a papillary carcinoma cannot be confidently excluded.
4. A compromised specimen (e.g., obscured by blood, or a poorly spread smear), where some cells appear to be mildly abnormal but are not obviously from a follicular neoplasm or suspicious of, or indicative of, malignancy.
5. Atypical "cyst lining cells."
6. Predominance of lymphoid cells with very scanty epithelium provided a lymphocytic thyroiditis has been excluded [2].

The rate of use of the "atypia of undetermined significance" category will vary amongst pathologists. Studies in the US quote ranges varying between 3 and 6% [1, 4] in all thyroid FNAs. The American Bethesda system recommends a 7% upper limit in AUS reporting, with a 5–15% implied malignancy risk [3].

There is little published data on Thy3a outcomes. In this study, we aim to look at the use of the Royal College of Pathologists' Thy3a category in the 4 years since its introduction in our department. We want to assess its clinical applicability and rates of malignancy, and to address the influence it may have on the sensitivity and specificity of all thyroid cytology reporting at our institution.

Methods

Thy3a reporting commenced in our department in January 2012. We retrospectively reviewed all thyroid FNA cytology reports issued between January 2012 and December 2015. They were identified from our laboratory information system using SNOMED (Systematized Nomenclature of Medicine – clinical terms) codes.

All of the aspirates were taken under ultrasound guidance with 25-G needles by radiologists. There were no rapid on-site assessment specimens. No specimens were drawn by cytopathologists.

Aspirates were directly smeared and air dried. They were then stained with a May-Grünwald-Giemsa stain. A ThinPrep® Papanicolaou-stained slide was also prepared on each case from a specimen collected in CytoLyt® during the same procedure. Cytology was reported by 4 in-house cytopathologists with a particular interest in thyroid FNA cytology, using the Royal College of Pathologists thyroid cytology classification. Radiological findings were included in the clinical history section of the pathology request form by the requesting radiologist.

Within our institution, all thyroid cytology cases reported as Thy3, Thy4, and Thy5 are individually discussed at the thyroid multidisciplinary team meeting. In addition, all aspirates that are reported as Thy2 benign but are clinically or radiologically suspicious are also discussed. Disciplines in attendance include endocrinology (both medical and surgical), radiology, oncology, radiation oncology, and pathology. The need for repeat FNA or surgery is discussed on a case by case basis through integration of clinical, pathological, and radiological findings.

All repeat FNA sampling and surgical procedures with histological outcomes were documented. Cytology was correlated with the final histology. Incidental occult papillary carcinomas arising outside the biopsied lesion were not included as false negatives. Thy2 aspirates were used to represent a true negative rate. Thy5 aspirates and their histological results were used to represent true positive rates.

Statistical analysis was performed on Excel and MedCalcs.

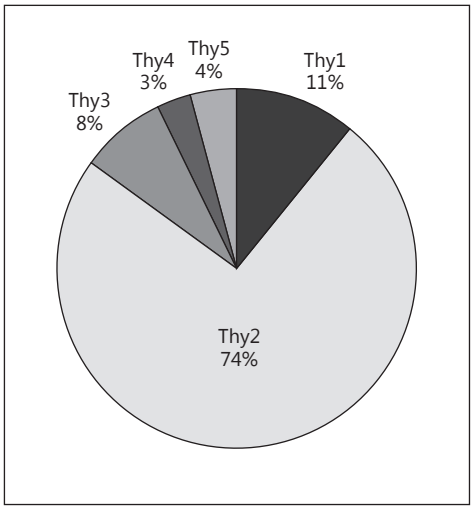

Fig. 1. Frequency of thyroid FNA category reporting over a 4-year period. In the 4-year period, only 8% of cases were reported as Thy3. The majority of our thyroid FNAs were benign, with 74% reported as Thy2.

Table 1. Percentage use of thyroid FNA category reporting over a 4-year period

	2012	2013	2014	2015
Thy1	13.7	7.1	13.0	14.8
Thy2	73.6	78.9	69.6	68.7
Thy3	6.2	5.4	9.5	10.4
Thy4	2.4	2.9	3.3	3.7
Thy5	3.4	5.7	4.6	2.4

Results

In the 4-year period, 1,259 thyroid FNAs were performed on 1,096 patients. Allowing for repeat FNAs, this amounted to a total of 1,195 final diagnoses. The majority of the lesions sampled (74%) were benign, reported as Thy2 or Thy2c (Fig. 1), and the use of the reporting categories over the 4 years was consistent (Table 1).

Overall, 882 aspirates were reported as Thy2, and 80 of these cases underwent surgery. Three of these cases were reported as papillary carcinoma, giving us a false-negative rate of 0.3%. One of the 52 Thy5 cases was a follicular adenoma on resection, giving a false-positive rate of 1.9%. The overall sensitivity was 94.4%, and specificity was 99.9%. The overall positive predictive value, reflecting the quality of cytological examination, was 98%.

A statistical breakdown of the individual malignancy-containing categories is shown in Table 2. Resections were not performed on cases that were anaplastic, lymphoma related, metastatic from another site, or in patients with multiple co-morbidities which rendered them unfit for surgery.

Since 2012, 95 cases have been reported as Thy3, with 16 cases reported as Thy3a (1.2%) and 79 cases as Thy 3f (6.2%). Seven cases from 2012 that were reported as Thy3 but not further subclassified were excluded.

Eight of the Thy3a cases had a repeat FNA; 1 case was reported as Thy1c, 5 cases were Thy2, 1 was Thy3f, and 1 was Thy5 (Table 3). The case reported as Thy3f was a follicular adenoma on resection. Six cases proceeded to surgery without a further FNA and were follicular adenomas on histology. One of these cases had an occult papillary carcinoma. The patient who had a Thy5 on repeat FNA underwent a total thyroidectomy for papillary carcinoma. Two cases were lost to follow-up.

Of the 79 cases reported as Thy3f, 4 had repeat FNAs; 2 remained as Thy3f and 2 were downgraded to Thy2. Following a multidisciplinary team meeting discussion, there were 52 surgeries, the outcomes of which are summarised in Table 4. The incidence of malignancy was 7% (1/14) in Thy3a cases and 21% in Thy3f (11/52).

Discussion

The use of an indeterminate thyroid cytology category is still very much in its infancy, and the purpose of this audit was to analyse our use of Thy3a and its clinical outcomes. Its introduction to our reporting classification has not impacted on diagnostic accuracy, with a sensitivity of 94% and specificity of 99.9% continuing to fall within the Royal College of Pathologists' guidelines of 65–98% and 76–100%, respectively.

We had a low rate of Thy3a usage, equating to only 1.2% of all thyroid cytological assessments and 9.4% of all final Thy3 diagnoses. The rate of malignancy within this group was 7%: 1 case. In the January 2016 updated Guidelines for Thyroid Cytology from the Royal College of Pathologists, the suggested rate of Thy3a usage is 5–10%, with a positive predictive value for malignancy of 17% [5]. Our usage, at 1.2%, is lower than the expected 5–10% quoted in the Royal College of Pathologists' guidelines. Our 7% malignancy rate is also below their 17% standard. At another similar institution, their Thy3a study [6] reported a higher incidence of Thy3a usage. This retrospective study of 748 thyroid nodules saw 109 being reported

Table 2. Sensitivity, specificity, and positive predictive values (PPV) for disease subcategories

	Patients, *n*	Surgeries, *n*	Malignancies, *n*	Sensitivity calculation[a]	Sensitivity, %	Specificity, %	PPV, %
Thy3a	16	7	1	1/4	25	99.3	14.3
Thy3f	79	54	11	11/14	78.6	95.3	20.4
Thy4	38	21	11	11/14	78.6	98.9	52.4
Thy5	52	36	35	35/38	92.1	99.9	97.2

[a] Sensitivity = true malignancies/true malignancies + Thy2 false negatives (n = 3).

Table 3. Outcomes of Thy3a cases from January 2012 to December 2015

Repeat FNA results	Histological diagnosis
Thy3a → Thy1c	No surgery
Thy3a → Thy1 → Thy2	No surgery
Thy3a → Thy2	No surgery
Thy3a → Thy2	No surgery
Thy3a → Thy2	No surgery
Thy3a → Thy2	No surgery
Thy3a → Thy3f	Follicular adenoma
Thy3a → Thy5	Papillary carcinoma (2 foci: 9 mm, 4 mm)
Not repeated	Follicular adenoma
Not repeated	Follicular adenoma
Not repeated	Follicular adenoma
Not repeated	Follicular adenoma
Not repeated	Follicular adenoma
Not repeated	Follicular adenoma (with a 6-mm micropapillary carcinoma)
Not repeated	Lost to follow-up
Not repeated	Lost to follow-up

Table 4. Outcomes of Thy3f surgical cases from January 2012 to December 2015

Cases, *n*	Histological diagnosis
30[a]	Follicular adenoma
7	Follicular carcinoma
4	Papillary carcinoma, follicular variant
11[a]	Benign (multinodular goitre, Hashimoto thyroiditis, nodular hyperplasia)

[a] One case had an occult micropapillary carcinoma.

as Thy3a with a malignancy rate of 13.4%. Their Thy3a usage rate of 14.5% is above the suggested rate, but their malignancy rate is within standards. Although these figures may reflect series size, it may also demonstrate the numerous diagnostic differences with lack of reproducibility within this category. Inter-observer agreement is generally poor for Thy3a. A 2011 study by Kocjan et al. [7] reported kappa values of 0.11, among expert cytopathologists, for the Royal College of Pathologists' subclassification. A similar lack of standardisation is seen with AUS in the US. A review article published by Bongiovanni et al. [8] discussed the various cytomorphological features seen in AUS/FLUS (follicular lesion of undetermined significance) reporting and concluded that there is marked heterogeneity within the atypia category with resultant questionable reproducibility.

Repeat FNA resulted in the reclassification of 50% of our Thy3a cases, with 31% of the 8 cases being reclassified to benign (Thy2). Repeat sampling is usually performed 3–6 months after the initial FNA [9]. Within the Thy3f category, our malignancy rate of 21% is within the expected Royal College of Pathologists' guideline of 15–30% [4] and similar to the rates seen with the Bethesda system. Our malignancy rate in Thy3a lesions is lower than that of Thy3f, and this observation has been documented in the literature [10]. Although falling under the indeterminate Thy3 category, and unlike Thy3a, Thy3f diagnoses are generally more straightforward both cytologically and in terms of management. The study by Kocjan et al. [7] reported improved agreement within Thy3f over Thy3a, with a kappa value of 0.59 amongst expert cytopathologists. In addition, there are clear guidelines for Thy3f management, usually resulting in a diagnostic thyroid lobectomy [11].

In our practice, we have a very low rate of Thy3a category use. This has not impacted negatively on our sensitivity or specificity and has not influenced our diagnostic accuracy. How useful this category is remains to be seen. At this time, lack of reproducible criteria with variation in rates in various practices raise doubts about its use. The purpose of thyroid cytological classification is to stream-

line cytological reporting and patient management. In our experience, most cases possibly classifiable as Thy3a can be adequately assigned to Thy2, Thy3f, or Thy4 without impacting on diagnostic accuracy. Yassa et al. [1] described indeterminate groups as a "disservice" to patients, requiring further FNA sampling before a decision on surgical intervention can be confidently ascertained. All our Thy3a cases that had had a repeated FNA resulted in a different, more useful categorisation on the repeat. Additional studies which involve large Thy3a cohorts are required in order to distinguish the clinical relevance of this subclassification. We suggest that the Thy3a category be critically analysed to establish the appropriate frequency and real benefit of this diagnostic category.

The use of molecular panels [12] may greatly assist in the identification of malignant thyroid cancers, allowing for more definitive preoperative diagnoses for indeterminate nodules in the near future. Studies on BRAF and RET/PTC suggest that those patients with Thy3 nodules who are mutation positive would be strong candidates for total thyroidectomies and that lobectomies are a more appropriate surgical management in those who are mutation negative [13]. These advances may make debates about cytological subclassification less relevant in future.

In conclusion, the Thy3a diagnosis is rarely used in our practice without impacting on accuracy. This raises the question of the clinical usefulness of this diagnosis which we feel should be further investigated in larger series.

Disclosure Statement

All authors have no conflicts of interest to declare.

References

1 Yassa L, Cibas ES, Benson CB, Frates MC, Doubilet PM, Gawande AA, Moore FD, Kim BW, Nosé V, Marqusee E, Larsen PR, Alexander EK: Long-term assessment of a multidisciplinary approach to thyroid nodule diagnostic evaluation. Cancer 2007;111:508–516.

2 Cross P, Chandra A, Giles T, Johnson S, Kocjan G, Poller D, Stephenson T: Guidance on the Reporting of Thyroid Cytology Specimens. London, Royal College of Pathologists, 2016. https://www.rcpath.org/resourceLibrary/g089-guidancereportingthyroidcytology-jan16.html.

3 Cibas ES, Ali SZ: The Bethesda system for reporting thyroid cytopathology. Am J Clin Pathol 2009;132:658–665.

4 Yang J, Schnadig V, Logrono R, et al: Fine-needle aspiration of thyroid nodules: a study of 4,703 patients with histological and clinical correlations. Cancer 2007;111:306–315.

5 Lobo C, McQueen A, Beale T, Kocjan G: The UK Royal College of Pathologist's thyroid fine-needle aspiration diagnostic classification is a robust tool for the clinical management of abnormal thyroid nodules. Acta Cytol 2011;55:499–506.

6 Brophy C, Mehanna R, McCarthy J, Tuthill A, Murphy M.S, Sheahan P: Outcome of subclassification of indeterminate (Thy-3) thyroid cytology into Thy-3a and Thy-3f. Eur Thyroid J 2015;4:246–251.

7 Kocjan G, Chandra A, Cross A, Giles T, Johnson S, Stephenson T, Roughton M, Poller D: The interobserver reproducibility of thyroid fine-needle aspiration using the UK Royal College of Pathologists' classification system. Am J Clin Pathol 2011;135:852–859.

8 Bongiovanni M, Krane JF, Cibas ES, Faquin WC: The atypical thyroid fine-needle aspiration: past, present, and future. Cancer Cytopathol 2012;120:73–86.

9 Layfield LJ, Cibas ES, Gharib H, Mandel SJ: Thyroid aspiration cytology: current status. CA Cancer J Clin 2009;59:99–110.

10 British Thyroid Association, Royal College of Physicians: Guidelines for the Management of Thyroid Cancer, ed 3. Report of the Thyroid Cancer Guidelines Update Group. London, Royal College of Physicians, 2014.

11 Ohori NP, Singhal R, Nikiforova MN, Yip L, Schoedel KE, Coyne C, McCoy KL, LeBeau SO, Hodak SP, Carty SE, Nikiforov YE: BRAF mutation detection in indeterminate thyroid cytology specimens. Cancer Cytopathol 2013;121:197–205.

12 Nikiforov Y, Steward D, Robinson-Smith T, Haugen B, Klopper J, Zhu Z, Fagin J, Falciglia M, Weber K, Nikiforova M: Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab 2009;94:2092–2098.

13 Noureldine SI, Najafian A, Aragon Han P, Olson MT, Genther DJ, Schneider EB, Prescott JD, Agrawal N, Mathur A, Zeiger MA, Tufano RP: Evaluation of the effect of diagnostic molecular testing on the surgical decision-making process for patients with thyroid nodules. JAMA Otolaryngol Head Neck Surg 2016;142:676–682.