This is a peer-reviewed, accepted author manuscript of the following research article, van der Horst, C., Wright, S., Young, D., Tailor, H., & Clark, L. (2020). What is Thy3a? a study of 336 Thy3a (AUS/FLUS) thyroid FNAs with histology compares UK RCPath with other reporting systems and shows how Thy3a subclassification can improve risk stratification and help address overuse of this category. *Cytopathology*, *32*(1), 29-36. https://doi.org/10.1111/cyt.12910

Title page

Title:

What is Thy3a? A study of 336 Thy3a (AUS/FLUS) thyroid FNAs with histology compares UK RCPath with other reporting systems and shows how Thy3a subclassification can improve risk stratification and help address overuse of this category.

Running title:

Thy3a subclassification improves risk stratification and helps address overuse

Manuscript word count: 2992
Figure count: 8
Table count: 5

Author details:

Cynthia van der Horst[1], Sylvia Wright[1], David Young[2], Hitesh Tailor[3], Louise Clark[3]

1. Department of Pathology, Queen Elizabeth University Hospital Glasgow

2. Department of Mathematics and Statistics, University of Strathclyde
NHS, Greater Glasgow and Clyde

3. ENT Surgery, Queen Elizabeth University Hospital Glasgow

Corresponding author: Cynthia van der Horst
Corresponding author address:
Department of Pathology,
Queen Elizabeth University Hospital
1345 Govan Road
Glasgow G51 4TF

Corresponding author telephone number: 0141 3549480

Corresponding author email: cafeeley@doctors.org.uk

Abstract:

Introduction:

Thy3a (AUS/FLUS) is an indeterminate and heterogenous category in thyroid cytology. Thy3a reporting rates vary widely, with many laboratories documenting overuse. Subclassification of Thy3a helps with risk stratification. We aimed to investigate whether subclassification can also help address Thy3a overuse. We compare the UK reporting system with other terminologies.

Methods:

An audit of Thyroid FNAs reported at our institution between 2012 and 2017 was performed. Thy3a FNAs followed by histology were reviewed and subcategorised into four subgroups: Scanty Atypia (SA), Scanty Microfollicular (SMF), Mixed (MX) and Thyroiditis versus Neoplasm (TVN). Review and subclassification were blinded to histology outcomes. The FNAs were then correlated with the histology results and statistical analysis was performed.

Results:

Our Thy3a rate was high (24% of all thyroid FNAs). 336 Thy3a FNAs with histology were included. Following subcategorization, the malignancy rates of the four subgroups were: SA 68%, SMF 20%, MX 4%, TVN 31%. There was a significant association between subgroup and malignancy risk. On histology, SA had more malignancies than expected and MX had fewer. There was also a significant association between subgroup and tumour risk. On histology, SA and SMF had more tumours than expected and MX had fewer. There was no significant difference in the tumour and malignancy outcomes for SMF and Thy3f FNAs. We propose that some MX and SMF cases could safely diverted to Thy2 and Thy3f respectively.

Conclusions:

Subclassification of Thy3a FNAs into four described subgroups is recommended. This can improve risk stratification and help address overuse of Thy3a.

Keywords:

1. Thy3a
2. Subclassification
3. Malignancy risk
4. Thyroid FNA

INTRODUCTION

Fine needle aspiration (FNA) is an accurate, minimally invasive and cost−effective procedure, widely used to triage patients with thyroid nodules into surgical or conservative management.

The Royal College of Pathologist's guidance for reporting thyroid cytology (RCPG) [1] and the Bethesda system for reporting thyroid cytology (TBSRTC) [2] promote standardized reporting of thyroid cytology, to facilitate patient management. Each system has equivalent diagnostic categories and TBSRTC assigns a malignancy risk to each category.

Thy3a corresponds to AUS/FLUS in TBSRTC and may be used if there is uncertainty if a lesion is benign, a follicular neoplasm, or suspicious of malignancy. It can also be applied to specimens of compromised quality that are difficult to interpret. Thy3a is therefore a heterogenous group and not surprisingly, the most controversial category. Its use varies widely [3,4] reflecting differences in reporting practices and in part, FNA quality. Many laboratories find it difficult to keep their indeterminate rates low [2] and studies focusing on limiting overuse are lacking.

The second edition of TBSRTC proposes a malignancy risk of 10-30% for this category, or 6-18% to take NIFTP diagnoses into account [2]. A recent meta-analysis of pre-NIFTP (Non-Invasive Follicular Thyroid neoplasm with Papillary–like Nuclear Features) literature using the UK Thy terminology estimated the risk of malignancy of Thy3a to be 25% in operated patients [5]. This figure may not have changed greatly since the introduction of NIFTP, as the prevalence of this diagnosis appears relatively low in the UK [6].

However, many other studies found that AUS/FLUS had a higher malignancy risk ranging from 6 – 48% [4]. As it is a heterogenous group, it can be difficult to accurately estimate the risk for an individual patient and clinicians are often unsure whether surgery should be performed. While molecular tests such as BRAF/V600E, RAS and TERT promotor mutations and RET/PTC gene rearrangements may help in some cases, they are not widely available, costs are high, and their clinical utility is still being developed [7,8]. Consequently, management of patients with Thy3a cytology still largely relies on a combination of cytology, clinical and ultrasound findings.

Subcategorization of indeterminate FNAs has been proposed to help estimate malignancy risk, for example cases with nuclear atypia are of higher risk [9,10]. However, there is no clear consensus in the literature as to how indeterminate FNAs should be subclassified or how many subgroups should be used.

As a British centre using RCPG, we wished to take a closer look at Thy3a and compare different reporting terminologies. We investigate whether subclassification can improve risk stratification and help address overuse. Our series of 336 Thy3a FNAs with histologic follow-up is to our knowledge, one of the largest published studies of this kind to date.

METHODS

A search of our archive at Queen Elizabeth University Hospital, Glasgow was done for all Thy3a FNAs reported from 2012 to 2017 for audit purposes.

FNAs were mostly performed under ultrasound guidance. Direct smears from each pass and were submitted air−dried for Diff−Quick staining and/or alcohol-fixed for Papanicolaou staining. On average, two passes were performed for each nodule. In most cases, a needle wash was also submitted. Rapid on-site evaluation (ROSE) of adequacy was not performed. All FNAs were taken locally with no external referrals included. All were reported according to RCPG.

We serve over 9 sites across the greater Glasgow and Clyde region and receive approximately 400 thyroid FNAs annually from a large number of operators. In our department, seven consultants report thyroid cytology. We routinely confer with one another over difficult or equivocal cases to reach a consensus opinion. Thy3a FNAs are not routinely discussed at the thyroid multidisciplinary team (MDT) meeting unless specifically requested for clinical reasons.

Thy3a FNAs with histological follow up were reviewed by an experienced cytopathologist, who was blinded to the histology to avoid bias. Four main morphological Thy3a patterns were observed: Scanty Atypia (SA); Scanty Microfolliclular (SMF); Mixed (MX) and Thyroiditis vs neoplasm (TVN). Each reviewed FNA was assigned to one of these four subgroups. For repeated cases with a second Thy3a result pre-surgery, the morphologic subgroup of the first Thy3a was taken as the index case.

SA encompasses situations described in RCPG as scanty atypical cells, such that malignancy cannot be excluded, but insufficient to warrant Thy4; for example scanty groups or nuclei showing features suggestive of papillary carcinoma in an otherwise predominantly benign−appearing sample, or scanty atypical cells with assessment compromised by artefact, or atypia related to cystic change, repair or therapy (figure 1). Cases showing papillary structures with frequent nuclear grooves and inclusions are excluded.

SMF refers to cases described in RCPG as hypocellular, with most of the cells arranged in microfollicles [1]. These samples are too scanty for a Thy3f assessment (figure 2). Cases

with cellular atypia such as nuclear grooves and inclusions are excluded.

MX encompasses scenarios described in RCPG as: “comprising 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” (figure 3) [1].
Cellular cases with a predominance of microfollicles and scanty colloid are excluded (Thy3f pattern), as are FNAs with mostly flat, loose sheets of follicular epithelial cells and plentiful thin colloid (Thy2 pattern). Cases showing nuclear atypia are also excluded.

TVN cases comprise oncocytic cells with a lymphocytic component. Colloid is usually scant. They are often cellular, making it difficult to distinguish a possible Hurthle cell neoplasm from Hashimoto’s thyroiditis. Alternatively, a prominent lymphocytic population may make it difficult to exclude a lymphoma, particularly if clonality studies are not available (figure 4).

For histology correlation, only cases with a definitive histopathological diagnosis were included (for example cases with core biopsies showing a follicular lesion but not otherwise diagnostic, were excluded). Only the histology of the nodule targeted by the FNA was considered; incidental findings, including microcarcinomas found elsewhere in the resection specimen were excluded. Non-Invasive Follicular Tumour with Papillary–like nuclear features (NIFTP)s were not considered malignant. The results of all repeat FNAs were recorded.

Associations between subgroups and malignancy risks and between subgroups and tumour risks were investigated using chi-squared tests. A Z-test for 2 proportions was done to compare the outcomes of SMF with Thy3f and to compare outcomes of one Thy3a with two Thy3a FNAs. All analyses were done using Minitab (version 18) at a 5% significance level. Colloid and cellularity for MX cases were recorded against outcomes.

RESULTS

A total of 2583 thyroid FNAs were reported (table 1). Of 569 patients with a Thy3a FNA, 173 had FNAs repeated. This yielded 59 additional Thy3a results, giving a total of 628 Thy3a FNAs.

148 patients had clinical follow up only. 247 (39%) had surgery following a single Thy3a. The decision to undergo surgery directly was based on various factors including

ultrasound features, family history, patient preference and in some cases, MDT discussion. Of the 173 patients that had FNAs repeated, 89 subsequently had surgery.

In total, diagnostic histology was available following 336 Thy3a FNAs from 331 patients comprising 58 men and 273 women, mean age 52 years (standard deviation 14.0, range 14 - 90). The mean nodule size was 3.0cm (standard deviation 1.7, range 0.2cm – 13cm).

The malignancy rate for Thy3a FNAs with histology was 16% and for all Thy3a FNAs was at least 8% (table 1). The outcomes of the four Thy3a subgroups are shown in table 2.

There was a significant association between subgroups and malignancy risk (chi-squared $p<0.001$). On histology, the SA subgroup had more malignancies than expected (assuming no difference in malignancy between the four subgroups) and MX had fewer.

There was also a significant association between subgroups and tumour risk (chi-squared $p<0.001$). The SA and SMF subgroups had more tumours than expected and the MX subgroup had fewer.

There was no significant difference in the tumour rate ($p=0.208$) or malignancy rate ($p=0.353$) between the SMF subgroup and Thy3f FNAs.

Review of MX cases identified 8 that could be re-assigned to Thy2. When statistical analysis without these 8 cases was repeated, SA, SMF and TVN had more malignancies than expected and MX had fewer ($p<0.001$). SA and SMF had more tumours than expected and MX had fewer ($p<0.001$).

Of 173 patients undergoing repeat FNA (table 3), 56 (32%) had a second Thy3a. Thy2/2c, Thy3f, Thy4 and Thy5 accounted for 49% of the repeat results. The remaining 19% were Thy1/1c. 30 patients had surgery following two Thy3a results. 7 of these (23%) were of a different Thy3a subgroup to the first Thy3a FNA.

DISCUSSION AND CONCLUSIONS

TBSRTC and RCPG describe a wide variety of situations that can be included in the indeterminate category, which paradoxically should be used sparingly [1,2]. The initial publication of the TBSRTC proposed a rate of use of 7% or less, but the most recent edition acknowledged that 10% may be more realistic [2]. Reproducibility of AUS/FLUS varies substantially, with one study reporting frequency of use across institutions to range from 3.3 to 14.9% and amongst pathologists from 2.5 to 28.6% [3].

Some studies have reported rising indeterminate rates following the introduction of RCPG and TBSRTC [11-13], with one UK centre documenting a rise from 11.0% over

2008/10, to 20.8% in 2014 [11]. The numerous and varied scenarios proposed in the guidelines could potentially impact upon the numbers and types of cases being classed as indeterminate. Sample quality is also relevant, because it can be difficult to provide a definitive result on scanty or compromised material. However, there are many other contributing factors, including local thyroid disease, ultrasound use and case selection, aspirator skill, experience of the cytopathologist, cytologic diagnostic thresholds and local histology reporting. Clearly, for numerous potential reasons, many laboratories have difficulty in limiting the indeterminate rate. Our Thy3a rate of 24% was far in excess of what is recommended.

Subclassification of indeterminate FNAs enables identification of high-risk cases with nuclear atypia [9,10], and is encouraged by TBSRTC [2]. However, there is no clear consensus in the literature on how best to subclassify. Atypia and Microfollicular subgroups are used by most authors, with some studies using only these [10,14], while some propose an additional "Other" subgroup to encompass all remaining scenarios [15]. Others consider "Hurthle cell proliferations" as a fourth subgroup [9,16] while Ho added a fifth "AUS-favour benign" subcategory [17].

All of our Thy3a FNAs could be easily designated to one of four described morphologic subgroups, which on histology have clearly different malignancy risks.

Our SA had a malignancy rate of 68%, with rates of 65.8%, 54% and 48% cited elsewhere [18,16,15]. Some studies suggest that this higher risk may relate to under-diagnosis of papillary carcinoma [11,18]. Although most of our malignancies in this subgroup were papillary carcinoma (table 4), all FNAs showed atypia that was too scant or subtle for a Thy4 assessment. Furthermore, this subgroup accounted for only 7% of our Thy3a cases. We think it therefore unlikely that undercalling potential Thy4 cases was the main reason for our high Thy3a rate.

Cases with atypia are clearly high risk. This raises a debate around how they are currently classified, because RCPG and TBSRTC include them in the lower risk Thy3a and AUS/FLUS categories respectively. Importantly, this contrasts with the Italian consensus for the classification and reporting of thyroid cytology (ICCRTC) which includes indeterminate FNAs with cytological atypia in the higher risk TIR3B category rather than the lower risk TIR3A group [19]. Potential advantages of the latter approach may include better stratification of malignancy risk across categories and a reduction in the heterogeneity of Thy3a (AUS/FLUS). Further studies on this subject are recommended.

Atypia related to degenerative changes and cyst lining cells accounted for the majority of our SA cases with a benign outcome. Although the changes were not considered sufficient for Thy4 or Thy5 in our series, atypia in cyst lining cells is a well-documented reason for false positive FNAs [20].

The literature varies on the malignancy risk of the microfollicular subgroup, ranging from 7 – 56.4% [10,17]. Our SMF malignancy and tumour rates of 20% and 77% respectively

were not significantly different to those of our Thy3f FNAs (24% and 71% respectively) over the same period. Olson also documented similar malignancy risks for AUS-microfollicular (27%) and Suspicious for Follicular Neoplasm (28%) [15]. Some SMF cases could therefore potentially be diverted to Thy3f, which could help reduce the Thy3a rate. Follicular carcinomas accounted for most SMF malignant outcomes in our series (table 6).

MX accounted for the majority (52%) of our Thy3a FNAs. 96% had a benign outcome, suggesting overuse of Thy3a for benign cases, also documented elsewhere [4,21]. Ho's AUS 'favour benign' had a similar low malignancy risk of 7.7% [17]. The nature of our practice as a large tertiary centre receiving FNAs from several operators is significant. However, reporting thresholds within departments are an important factor. A cautious approach can become established following the experience of false negatives, for example during this period we had four carcinomas assessed as Thy2 (table 1). TBSRTC proposes a malignancy rate of 0-3% for the Benign category, acknowledging that malignancy can rarely masquerade as a benign nodule. This risk is particularly associated with follicular-patterned tumours, including follicular carcinoma and follicular variant of papillary carcinoma [22].

There are few studies focusing on the problem of high indeterminate rates. Two recommend removing "AUS favour benign" cases from AUS [23,24]. Similarly, we propose that some MX cases could be safely re-assigned to Thy2.

Review of the MX cases identified only 8 that could be easily diverted to Thy2. These had low cellularity comprising macrofollicles and plentiful thin colloid and all had a non-tumour outcome. We observed that moderate cellularity, even in the presence of plentiful thin colloid, tended to prompt a Thy3a report. However, TBSRTC reminds us that rather than considering cellularity alone, microfollicle morphology and the relative proportions of microfollicles to macrofollicle fragments must be evaluated, to avoid overcalling benign cases [2.] Our analysis of cellularity and colloid (table 5), found plentiful thin colloid to be frequently associated with a non-tumour result, but our numbers are too small to be conclusive on the significance of cellularity and this would require further investigation.

TVN accounted for only 5% of our Thy3a cases. Although relatively uncommon, these cases form a distinct subgroup posing a specific diagnostic dilemma. Histology (table 2) showed a relatively high tumour rate (44%) and malignancy rate (31%). All malignancies were either Hurthle cell carcinomas or lymphomas (table 3), with Hurthle cell adenomas and Hashimoto's thyroiditis accounting for the remaining cases. Other subclassification studies utilising an oncocytic subgroup recorded malignancy rates ranging from 19-33% [21,23,13]. Clinical history, thyroid autoantibodies and clonality can aid interpretation. Clonality was unknown in all of our cases because a needle wash was either not submitted or was too hypocellular or degenerate for analysis. 8 of our 16 cases had documented raised thyroid autoantibodies at the time of FNA and a clinical history of Hashimoto's thyroiditis was provided for four of these. However, two were malignant;

one was a marginal zone lymphoma and the other a Hurthle cell carcinoma, each with background Hashimoto's thyroiditis. Therefore a known history of Hashimoto's thyroiditis cannot always exclude neoplasia. However, Thy3a results may have been avoided on the lymphoma cases had clonality been available.

In our series, scanty cellularity was a significant problem, with SA and SMF together accounting for 42% of our Thy3a cases, with many of our MX cases also of low cellularity. Our Thy1 rate is also high (25%). Although this suggests a need to review regional aspiration practice, a recent review of literature using the RCPath terminology reported Thy1 rates to range from 7.9% - 43.3% [25]. ROSE appears to lower Thy1 rates [25,26]. While the introduction of ROSE would therefore be desirable in our setting, implementation has not been practical due to the number of sites that we serve, with other mitigating factors including lack of space in radiology departments and time constraints with ultrasound lists.

Repeating the FNA (table 4) yielded a more definitive Thy result category in 49%. For those with a second Thy3a, 23% had a different subgroup pattern compared to the initial Thy3a. These differing outcomes may reflect a change in the quality of the repeat FNA or sampling of a different focus within the same nodule. We found no significant difference in the incidence of malignancy after one Thy3a (13%), compared with after two Thy3a results (17%), (p=0.603). This mirrors findings by other authors [9,27]. 20 of our cases had a Thy2 repeat result and only one had surgery, which was a benign hyperplastic nodule. However, some authors question whether a second benign result carries more weight than the initial indeterminate result and can justify avoiding surgery [9,27]. These patients need careful ultrasound correlation and may need follow-up. Larger studies are required to further determine appropriate management.

This study has a number of weaknesses. It documents the experience of one laboratory, and considering the known variability in reporting trends across institutions, may not necessarily be applicable elsewhere. Ours was a retrospective analysis and we haven't attempted to prospectively re-assign MX cases to Thy2 or SMF cases to Thy3f, to check for a reduction in Thy3a use whilst maintaining sensitivity. We did not investigate for differences in the ultrasound findings amongst the nodules in each of the four subgroups.

In conclusion, Thy3a is a heterogenous category and we recommend subclassification into four subgroups; SA, SMF, MX and TVN. Subclassification helps with risk stratification and management planning. Indeterminate lesions with nuclear atypia (SA) have a high risk of malignancy and should be distinguished from other subgroups. We question how these cases should be classified and highlight important differences between various reporting terminologies. Subclassification can help identify causes and potential solutions for overuse of Thy3a. The Thy3a category should not be a 'waste basket' for difficult cases and efforts should be made to avoid overuse. Collaboration with FNA takers to optimise sample quality is mandatory. Along with subclassification of

Thy3a FNAs, we recommend rigorous audit with cytological-histological correlation and cytology review.

References:

1. Cross P, Chandra A, Giles T et al Guidance on the reporting of thyroid cytology specimens. Second version 2016. https://www.rcpath.org/resourceLibrary/g089-guidancereportingthyroidcytology-jan16.html

2. Ali S, Cibas E, eds The Bethesda System for Reporting Thyroid Cytopathology. Definitions, Criteria and Explanatory Notes. *Second edition* Springer, 2018

3. Layfield L, Morton J, Cramer J, Hirschowitz, S. Implications of the proposed thyroid fine needle aspiration category of "follicular lesion of undetermined significance": A five-year multi-institutional analysis. Diagnostic cytopathology 2009;37(10):710-714

4. Kholová I, Ludvíková M. Thyroid atypia of undetermined significance or follicular lesion of undetermined significance: an indispensable Bethesda 2010 diagnostic category or waste garbage? Acta Cytol. 2014;58(4):319-29.

5. Poller D, Bongiovanni M, Trimboli P. Risk of Malignancy in the Various Categories of the UK Royal College of Pathologists Thy Terminology for Thyroid FNA Cytology: A Systematic Review and Meta-Analysis. Cancer Cytopathology 2020; Jan;128(1):36-42.

6. An audit of reporting thyroid cytology specimens and their correlation with thyroid histology. www.rcpath.org.

7. Al-Abaddi M, Shareef S, Yousef M et al. A follow-up study on thyroid aspirates reported as atypia of undetermined significance/follicular lesion of undetermined significance and follicular neoplasm/suspicious for follicular neoplasm: A multicenter study from the Arabian Gulf region. Diagn Cytopathol. 2017 Nov;45(11):983-988.

8. Decaussin-Petrucci M, Descotes F, Depaepe L et al. Molecular testing of BRAF, RAS and TERT on thyroid FNAs with indeterminate cytology improves diagnostic accuracy. Cytopathology 2017 Dec;28(6):482-487

9. Shrestha R, Hennessey J. Cytologic subclassification of atypia of undetermined significance may predict thyroid nodules more likely to be malignant at surgery. Diagn Cytopathol 2016 Jun;44(6):492-8

10. Horne M, Chhieng D, Theoharis C et al
Thyroid follicular lesion of undetermined significance: Evaluation of the risk of malignancy using the two-tier sub-classification. Diagn Cytopathol 2012 May;40(5):410-5

11. Parkinson D, Aziz S, Bentley R, Johnson S. Thyroid cytology-histology correlation using the RCPath terminology for thyroid cytology reporting.J Clin Pathol. 2017 Aug;70(8):648-655.

12. Sullivan P, Hirschowitz S, Fung P, Apple S. The impact of atypia/follicular lesion of undetermined significance and repeat fine-needle aspiration: 5 years before and after implementation of he Bethesda System. Cancer Cytopathol 2014 Dec;122(12):866-72.

13. Broome J, Solorzano C. The impact of atypia/follicular lesion of undetermined significance on the rate of malignancy in thyroid fine-needle aspiration: evaluation of the Bethesda System for Reporting Thyroid Cytopathology. Surgery. 2011 Dec;150(6):1234-41.

14. Hyeon J, Ahn S, Shin J, Oh Y. The prediction of malignant risk in the category "atypia of undetermined significance/follicular lesion of undetermined significance" of the Bethesda System for Reporting Thyroid Cytopathology using subcategorization and BRAF mutation results. Cancer Cytopathol. 2014 May;122(5):368-76

15. Olson M, Clark T, Erozan M, Ali S. Spectrum of Risk of Malignancy in Subcategories of `Atypia of Undetermined Significance'. Acta Cytologica 2011;55: 6(6):518-525

16. Mathur A, Najafian A, Schneider E et al. Malignancy risk and reproducibility associated with atypia of undetermined significance on thyroid cytology. Surgery 2014 Dec;156(6):1471-6

17. Ho A, Sarti E, Jain K et al. Malignancy rate in thyroid nodules classified as Bethesda category III (AUS/FLUS) Thyroid 2014 May;24(5):832-9.

18. Kim S, Roh J, Baek J et al. Risk of malignancy according to sub-classification of the atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS) category in the Bethesda system for reporting thyroid cytopathology. Cytopathology. 2017 Feb;28(1):65-73.

19. Trimboli P, Crescenzi A, Castellana M et al. Italian consensus for the classification and reporting of thyroid cytology: the risk of malignancy between indeterminate lesions at low or high risk. A systematic review and meta-analysis. Endocrine 2019;63:430–438

20. Faquin W, Cibas E, Renshaw A. "Atypical" cells in fine-needle aspiration biopsy specimens of benign thyroid cysts. Cancer Cytopathol. 2005;105(2);71-9.

21. Vanderlaan P, Krane J, Cibas E. The frequency of 'atypia of undetermined significance' interpretations for thyroid fine-needle aspirations is negatively correlated with histologically proven malignant outcomes. Acta Cytol 2011;55(6):512-7

22. Giles W, Maclellan R, Gawande A et al. False negative cytology in large thyroid nodules. Annals of Surgical Oncology. 2015;22(1):152-7.

23. Renshaw A, Krane J. Can changing the terminology for benign aspirates reduce the atypia of undetermined significance/follicular lesion of undetermined significance rate in thyroid fine-needle aspirates? Cancer Cytopathol. 2013 Apr;121(4):175-8.

24. Renshaw A, Gould E Reducing indeterminate thyroid FNAs. Cancer Cytopathol. 2015 Apr;123(4):237-43.

25. Poller D, Doyle V, Trimboli P et al. Rates of Thy 1-non-diagnostic thyroid fine needle aspiration using the UK Royal College of Pathologists Thy Terminology. A systematic review of the literature comparing patients who undergo rapid on-site evaluation and those who do not. Cytopathology 2020;00:1-7.

26. Shield, P, Cosier, J, Ellerby, G et al. Rapid on-site evaluation of fine needle aspiration specimens by cytology scientists: a review of 3032 specimens. Cytopathology 2014, Vol.25(5), p.322(8)

27. VanderLaan P, Marqusee E, Krane J. Clinical outcome for atypia of undetermined significance in thyroid fine-needle aspirations: should repeated FNA be the preferred initial approach? Am J Clin Pathol. 2011 May;135(5):770-5.

Table 1: Thyroid FNA results 2012 - 2017. Note: As only a proportion of FNAs had follow-up histology, the true malignancy rate for each category cannot be calculated, but is likely to be between the values for operated and total cases.

RCPath Diagnostic Category	Total number	% of total	Number operated	Number malignant	% malignant operated cases	% malignant total cases
Thy1	664	25%	135	33	24%	5%
Thy1c	198	8%	26	7	27%	4%
Thy2	309	12%	24	4	17%	1%
Thy2c	102	4%	6	1	17%	1%
Thy3a	628	24%	336	53	16%	8%
Thy3f	534	21%	363	86	24%	16%
Thy4	73	3%	67	66	99%	90%
Thy5	75	3%	65	65	100%	87%
Total	2583					

Table 2: Histologic outcomes of all 336 patients that had surgery (after a single Thy3a or a Thy3a followed by a repeat FNA) and the frequency of use of each of the 4 subgroups and the outcomes and malignancy rates for each.

	Scant Atypia	Scant Microfollicles	Mixed Pattern	Thyroiditis vs Neoplasm	Total
Non-tumour histology	3	28	117	9	157
Follicular Adenoma	3	67	48	2	120
NIFTP	2	3	1	-	6
Malignant	17	24	7	5	53
Total	25 (7%)	122 (36%)	173 (52%)	16 (5%)	336
Malignant rate in cases with histology	68%	20%	4%	31%	16%

Table 3: Outcomes of patients who underwent a repeat FNA

Repeat FNA result	Total	No histology	Histology	Histology: non-tumour	Histology: Follicular adenoma	Histology: NIFTP	Histology: Malignant
Thy1	30	20	10	4	2	-	4
Thy1c	3	3	-	-	-	-	-
Thy2	17	16	1	1	-	-	-
Thy2c	6	5	1	-	1	-	-
Thy3a	56	26	30	14	9	2	5
Thy3f	55	12	43	14	21	2	6
Thy4	1	-	1	-	-	-	1
Thy5	5	2	3	-	-	-	3
Total	173	84	89	33	33	4	19

Table 4: Malignancy types in each subgroup

Malignancy type	Scanty Atypia	Scanty Microfollicular	Mixed	Thyroiditis versus neoplasm	Total
Papillary ca	12	3	4		19
Follicular variant papillary carcinoma	1	4	1		6
Follicular carcinoma	1	14	1		16
Hurtle cell carcinoma		2		2	4
Medullary carcinoma		1			1
Lymphoma				3	3
Metastatic carcinoma (Primaries: Adenoid cystic carcinoma, Squamous carcinoma, Renal cell carcinoma)	3				3

Anaplastic carcinoma			1		1
Total	17	24	7	5	53

Table 5: Analysis of cellularity and colloid for Thy3a MX FNAs

Colloid	Cellularity	Histology: Colloid nodule	Histology: Follicular adenoma	Histology: NIFTP	Histology: Malignant	Total
plenty thin	Low	13	4	-	-	17
plenty thin	Moderate	27	6	-	1 (FVPC)	34
Moderate thick	Low	1	-	-	1 (FCC)	2
Moderate thick	Moderate	6	2	1	-	9
Scant thick	Low	4	5	-	-	9
Scant thick	Moderate	2	3	-	2	7
Scant thin/none	Low	41	12	-	2	55
Scant thin/none	Moderate	23	16	-	1	40
Total		117	48	1	7	173

FVPC: Follicular variant of papillary carcinoma, FCC: Follicular carcinoma