Review article

Thyroid cytology reporting in the local setting based on the Bethesda system: Testing the water and looking beyond!

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Introduction

Thyroid diseases are common in our setting leading to significant morbidity [1]. According to National Cancer incidence data thyroid malignancies were the 3rd commonest malignancy in women in 2010. In men, thyroid malignancies were placed 3rd among the age group 15-34 years and 5th among 35-49 years [2]. Thyroid fine needle aspiration cytology test (FNAC) is a front line investigation to assess the possibility of a neoplasm in patients presenting with thyroid nodules. The original 'Thy' classification of the British thyroid association of the Royal College of pathologists of United Kingdom (RCP UK) formed the basis for thyroid FNAC reporting in Sri Lanka, since 2007[3]. In May 2014, the College of Pathologists of Sri Lanka initiated a dialog with the clinical stakeholders regarding issues related to the 'Thy' based FNAC reporting. Following discussions at a workshop attended by representatives from the Colleges of Pathologists, Radiologists, Surgeons and Endocrinologists, the Bethesda system (TBSRTC) was adopted for thyroid FNAC reporting in our setting [4]. Reporting the risk of subsequent malignancy (ROM) for each category was however not adopted till the availability of local data. The hand book titled 'Thyroid Cytology Reporting. National Guidelines for Sri Lanka' (NGSL) based on TBSRTC was published by the Ministry of Health in 2016 [5]. Currently NGSL forms the frame work for thyroid FNAC reporting in our setting. Some concerns have since been expressed regarding TBSRTC based NGSL guidelines.

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Impact of FNAC method on management guidelines

A specific access method is not mentioned for obtaining FNAC smears in both TBSRTC and NGSL management guides (ultrasound guided: G-FNAC vs palpation guided: P-FNAC) [4,5]. Both management guides are flexible, stating that the actual management may vary based on the clinical and radiological findings. G-FNAC increases the diagnostic accuracy of the procedure [6] targeting specific nodules/areas, and has the advantage of being able to assess impalpable deep lesions and regional lymph nodes. Yet both access methods are practiced in most settings. As per the Royal College of pathologists of Australasia (RCPA) QA

programme, 65% aspirates are performed by radiologists (G-FNAC) while 11% are performed by pathologists (P-FNAC) [7]. RCP UK guidelines mention that *'In most units, the sample taker will be a surgeon, endocrinologist, oncologist or radiologist, rather than a cytopathologist, but this will vary from unit to unit depending on resources and local preference and practice'*. Additionally, it is mentioned that G-FNAC tends to have a higher adequacy rate than P-FNAC [8]. In Sri Lanka both access methods are used, however data on the actual percentages of the two methods are unknown. Both access methods demonstrate certain limitations in our setting.

Limitations of G-FNAC - Most G-FNACs are not performed with pathological collaboration, preventing rapid onsite evaluation of smears (ROSE) for adequacy and smear preparation to ensure quality. Therefore, significant proportions of G-FNAC smears we encounter fall in to the non-diagnostic category due to inadequacy and air drying. As a robust patient recall mechanism is not in place in most settings recall of patients for an interval repeat FNAC may not be feasible.

Limitations of P-FNAC - The radiology appointment for a G-FNAC may take longer, prompting clinicians to refer patients with palpable lesions for P-FNAC. In this instance prior radiological evaluation of the lesion is not available at presentation for P-FNAC. Thus, the pathologist would be unaware of the radiological findings making it unsuitable to render a management recommendation based on P-FNAC. Additionally, when a pathologist is requested to perform a P-FNAC on a multi-nodular goiter without prior radiological evaluation, the pathologist is faced with the dilemma of selecting the nodule/s for sampling. P-FNACs are also performed by pathology registrars and medical officers with a possible impact on the adequacy and the sensitivity of the test. In these situations, rendering a management recommendation is not appropriate.

NGSL [5] is intended to serve as a guide to the pathologist for thyroid FNAC reporting. Therefore, it would be prudent to include management recommendations only when the pathologist is fully aware of the clinical and the radiological context of the patient and the thyroid lesion. Additionally, the pathologist should be aware of other local limitations, prior to rendering management recommendations. Most issues could be discussed and overcome, if regular multidisciplinary meetings are convened with the radiologist and the clinician. Therefore, pathologists are encouraged to discuss management options at multidisciplinary meetings. The problem of non-diagnostic/unsatisfactory smears following G-FNAC could also be avoided if pathological assistance is provided at 'multidisciplinary FNAC clinics' for ROSE for proper smear fixation and adequacy evaluation. Implementation of ROSE at multidisciplinary FNAC clinics with a pathologist, pathology registrar or when their presence cannot be assured even a well trained cytotechnologist is therefore strongly recommended.

Additionally, radiologists should be encouraged to provide an unambiguous radiology report for patients referred for P-FNAC. The 'Thyroid image reporting and data system (TI-RADS), which has been recently introduced includes risk categories for thyroid lesions similar to BI-RADS categories for breast lesions. TI-RADS, which includes a standardized scoring system with guidance on FNAC and follow up was introduced in April 2017, by the American College of Radiology [9] (Table 1). TI-RADS reporting system clearly communicates the radiological impression to the pathologist and the clinician, impacting both the FNAC diagnosis and the management recommendation. It is always preferable for multi-nodular goitres to be sampled by G-FNAC, as nodule/s requiring FNAC cannot be identified by palpation. The clinicians should be made aware of this limitation.

Sample adequacy criteria

It has been questioned whether a sample is adequate, if six groups of ten follicular epithelial cells are present across all the available smears. NGSL and TBSRTC prefer adequacy criterion to be met in a single smear. The revisions recently introduced for TBSRTC in 2017 have retained the original adequacy criterion [10].

Table 1 - Thyroid image reporting and data system (TI-RADS) [9]

TI-RADS category	Interpretation
TI-RADS 1	Normal thyroid gland
TI-RADS 2	Benign lesions
TI-RADS 3	Probably benign lesions
TI-RADS 4	Suspicious lesions (sub classified as 4a,4b,and 4c with increasing risk of malignancy
TI-RADS 5	Probably malignant lesion (more than 80% risk of malignancy)
TI-RADS 6	Biopsy proven malignancy

The thyroid cytology reporting protocol by RCPA endorses TBSRTC adequacy criterion [7]. RCP UK guidelines mentions that samples from solid lesions should have *'at least six groups of thyroid follicular epithelial cells across all the submitted slides, each with at least 10 well-visualized epithelial cells'.* It further mentions that, *'However, this is purely a cytological criterion and does not take into consideration the clinical setting'* and concludes as '*A more pragmatic criterion taking into account the clinical context and findings is advocated'* [8]. Therefore, whenever the adequacy requirement is met only across smears and not on a single smear the pathologist may use his/her discretion to decide on the adequacy in consultation with the clinician by considering the clinical and radiological information.

When should non-diagnostic/unsatisfactory smears be re-aspirated?

NGSL mentions that '*a patient with an initial non-diagnostic(ND)/unsatisfactory(UNS) result should proceed for re-aspiration preferably after about three months to avoid false positive results due to reactive reparative changes. As the risk of malignancy in pure cystic lesions is low, it may not be necessary to re-aspirate those ultrasonically confirmed benign, cystic nodules with an initial ND/UNS result. Cysts with solid areas may need guided re-aspiration from the solid areas'* [5].

Some local pathologists/clinicians prefer to repeat the FNAC without a waiting time to avoid delay and to prevent patients being lost for follow up. The RCPA thyroid cytology protocol [7] states, '*there is no level 1 evidence* [11, 12] *regarding the optimum interval for a repeat aspiration even though a three-month interval has been suggested to prevent false-positive misinterpretations due to reactive/reparative changes*'. RCPA recommends the repeat interval to be decided by the clinician based on the clinical circumstances. The 2017 revisions for TBSRTC recommend a repeat G-FNAC for ND/UNS nodules and endorse the view point of the American Thyroid Association guidelines that a waiting time is not needed before repeating the FNAC [10]. Therefore, we could be guided by the TBSRTC 2017 recommendation, especially when a waiting time of three months is not acceptable.

A new problem in FNAC evaluation of follicular lesions of thyroid

It is well known that thyroid FNAC is unable to accurately identify and separate follicular neoplasms from non-neoplastic follicular lesions. Based on TBSRTC, NGSL attempts to address this by separating follicular pattern smears as Bethesda category 3 (Atypical lesion/follicular lesion of uncertain significance, AUS/FLUS) and category 4 (Follicular neoplasm/Suspicious for follicular neoplasm, FN/SFN) based on the degree of suspicion. More recently the histological entity 'encapsulated, noninvasive, follicular variant of papillary thyroid carcinoma' has been recognized to have a good prognosis and has been renamed as 'Noninvasive follicular thyroid tumours with papillary like nuclear features' (NIFTP) [13]. Diagnosis of NIFTP is based on histological criteria [13, 14] (Tables 2, 3) and its surgical management has been downgraded from total thyroidectomy to hemithyroidectomy, with no additional therapy.

Impact of NIFTP on Thyroid FNAC - As NIFTP diagnosis is based on histological criteria it is not possible to make this diagnosis on FNAC. NIFTP's may therefore be included in the Bethesda categories of 3, 4 and 5 [15, 16]. NIFTP cytology

Table 2 – Diagnostic criteria for NIFTP [13]

Diagnostic criteria for NIFTP
Encapsulation or clear demarcation [a]
Follicular growth pattern [b] with < 1% papillae
No psammoma bodies
< 30% solid/trabecular/insular growth pattern
Nuclear score of 2-3 (as defined in table 3)
No capsular/vascular invasion [c]
No high mitotic activity [d]

[a] Thick, thin or partial capsule or well circumscribed with a clear demarcation from adjacent thyroid tissue

[b] Including microfollicular, normofollicular or macrofollicular architecture with abundant colloid

[c] Requires adequate microscopic examination of tumour capsule interface

[d] High mitotic activity defined as at least 3 mitoses per 10 HPF (400x)

includes follicular groups of cells with nuclear overlap, with some, but not all nuclear features of papillary thyroid carcinoma (PTC). Papillary architectural features such as true papillae, branching cell groups, caps or psammoma bodies are absent [14]. When the cytological features raise the possibility of NIFTP, the case should be assigned to an appropriate Bethesda category based on the nuclear and architectural features observed. Then a comment should be included raising the possibility of NIFTP, as its clinical management may override the management recommendations [14].

Table 3 - Nuclear scoring as a NIFTP diagnostic criterion [13]

Nuclear scoring for NIFTP
Nuclear size and shape (enlargement, overlapping, elongation)*
Nuclear membrane irregularities (irregular contours, grooves, pseudo inclusions)*
Chromatin characteristics (clearing with margination/ glassy nuclei)*

*Each nuclear feature is scored as present (1) or absent (0), if two or three are present, the tumour has nuclear features of NIFTP; if zero or one, it is a follicular neoplasm.

NIFTP has not been considered in the NGSL. Therefore, the local pathologists need to be aware of this new entity, its FNAC appearance and the impact on the management recommendation.

Is molecular testing the way forward?

Immunohistochemical markers (galectin-3, HBME-1, fibronectin-1, cytokeratin 19 and CITED-1) were initially thought to be helpful to differentiate papillary from follicular neoplasms. However, these have been less than satisfactory in clarifying indeterminate categories of TBSRTC (Bethesda categories 3 and 4). Several molecular tests identifying genetic alterations have since become available [17]. These are based on gene mutations and rearrangements that are identified in papillary and follicular neoplasms. Around 70% of PTC have shown point mutations in the *BRAF* and *RAS* genes or *RET/PTC* or *TRK* gene rearrangements [18, 19, 20]. *BRAF* gene mutation is the most common and specific, seen in 40-45% of PTC, with *RET/PTC* gene mutation in 20%. Alteration of the *RAS* gene is seen in 40-50% of follicular neoplasms, with 30-35% showing *PAX8/PPARr* gene rearrangements [21]. NIFTP is reported to have a similar molecular profile as follicular carcinoma [22].

Molecular tests currently available as commercial kits include Afirma Gene Expression Classifier, Afirma Malignancy Classifier, ThyGenX and ThyroSeq tests. The molecular markers tested in these kits impact the diagnostic accuracy and the yield of FNAC results. It is reported that the presence of gene mutations or rearrangements significantly increase the likelihood of malignancy among cases with indeterminate FNAC results. A positive molecular test may therefore alter the medical decision in favour of upfront thyroidectomy as the initial surgical management. ThyroSeq test based on next generation sequencing on gene mutation and fusion is reported to have the best negative and positive predictive values with 100% analytic accuracy and is considered both a 'rule out' and a potential 'rule in' test [17]. However molecular testing based on commercial kits is not feasible in Sri Lanka due to its high cost.

The *BRAF*^{V600E} mutation analysis has emerged as the most promising marker for PTC (commonest thyroid malignancy) on thyroid FNAC. A recent meta analysis by a Chinese group on *BRAF*^{V600E} mutation analysis using residual material obtained from routine FNAC showed that it improves the diagnostic accuracy and reduces the false-negative rate. They demonstrated that *BRAF*^{V600E} mutation analysis has diagnostic value, especially in the 'Suspicious for malignant cells' group (Thy 4 in the British system and category 5 of TBSRTC). However, they concluded that its value in FN/SFN category of TBSRTC was doubtful and recommended expanded panels containing other diagnostic markers for this category [23]. *BRAF*^{V600E} specific mutant protein assessment on cell blocks by IHC appears to be a more feasible method [24], which could be implemented in Sri Lanka as an adjunct for thyroid FNAC to improve the yield of PTC.

The 2017 Bethesda System for reporting Cytopathology

Revisions to the original TBSRTC is made available recently in 2017 [10]. These revisions have been inspired by the availability of new data and other new developments such as NIFTP and adjunct molecular testing. Highlights of the TBSRTC 2017 are as follows. The original six diagnostic categories (Bethesda categories) are retained with the names unchanged. However, it is encouraged for categories to be named (E.g. Malignant) without only stating the numerical designation of the category (E.g. Bethesda 6). For categories with alternate names (E.g. AUS/FLUS, FN/SFN) laboratories have been asked to choose one term for exclusive use and not use both terms interchangeably. The risk of malignancy for the categories are recalculated based on post 2010 data and are presented in two ways; when NIFTP is considered non-malignant and when it is

Table 4 – The 2017 TBSRTC: implied risk of malignancy and recommended clinical management [10]

Bethesda category	Risk of malignancy if NIFTP ≠ CA (%)	Risk of malignancy if NIFTP = CA (%)	Usual management [a]
Category 1 - Non diagnostic/unsatisfactory	5-10	5-10	Repeat FNA with ultrasound guidance
Category 2 – Benign	0-3	0-3	Clinical and sonographic follow up
Category 3 - Atypia of undetermined significance (AUS) or Follicular lesion of undetermined significance (FLUS)	6-18	~ 10-30	Repeat FNA, molecular testing or lobectomy
Category 4 - Follicular neoplasm or Suspicious for a follicular neoplasm	10-40	25-40	Molecular testing, lobectomy
Category 5 - Suspicious for malignancy	45-60	50-75	Near total thyroidectomy or lobectomy [b,c]
Category 6 – Malignant	94-96	97-99	Near total thyroidectomy or lobectomy [c]

[a] Actual management may depend on other factors (eg, clinical, sonographic) besides the FNA interpretation

[b] Some studies have recommended molecular analysis to assess the type of surgical procedure (lobectomy versus total thyroidectomy)

[c] In the case of 'Suspicious for metastatic tumour' or a 'Malignant' interpretation indicating metastatic tumour rather than a primary thyroid malignancy, surgery may not be indicated

considered malignant. The usual management for AUS/FLUS and FN/SFN now includes the option of molecular testing (Table 4). It is recommended to include cases with mild nuclear changes associated with PTC with FN/SFN. Hence the definition and diagnostic criteria for FN/SFN category are revised. It is also suggested to limit the use of category Malignant; PTC, only for cases demonstrating the classical features of PTC. Use of optional educational notes is recommended for FN/SFN with cytomorphological features suggestive of follicular variant of PTC and NIFTP. Incorporating an educational note is also encouraged for 'Malignant; PTC' mentioning that a small proportion of cases may prove to be NIFTP.

In conclusion it is important for the local pathologists to be aware of ways to overcome issues faced locally when reporting thyroid FNAC smears, be aware of the recent developments in the field that impact thyroid FNAC reporting and the revisions that have been made to the original TBSRTC recently in 2017.

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