Thyroid FNA: New Classifications and New Interpretations

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INTRODUCTION

On September 22, 2015, a symposium entitled "Thyroid Fine-Needle Aspiration: New Classifications and New Interpretations" was held at the European Congress of Cytology in Milan, Italy. The main goals of this symposium were to present and discuss the thyroid fine-needle aspiration (FNA) terminologies most commonly used in Europe and the United States and to provide an analysis of their respective advantages and limitations. A comparison of the various international thyroid FNA classification schemes is necessary to understand and establish whether these classification schemes are aligned and can lend themselves to being used interchangeably by cytopathologists around the world. As expected, the discussion also revolved around highlighting discrepancies and putting forth recommendations regarding the modification of existing thyroid FNA terminologies, especially to reduce the number of cases classified as indeterminate.

Nodular lesions of the thyroid represent a common problem for clinicians as well as a diagnostic challenge for pathologists. Up to 50% of the general population may have sonographically detectable thyroid nodules, although only up to 5% of these harbor a malignancy.[1,2] The challenge facing general physicians, endocrinologists, surgeons, and pathologists is to achieve an accurate preoperative diagnosis of malignancy to ensure the appropriate treatment of patients. FNA is the modality most commonly used to help to establish a preoperative diagnosis of malignancy. However, core biopsy has proven to be of value in thyroid nodules with prior nondiagnostic FNA. Regardless of the terminology used, approximately 25% of the thyroid nodules are classified as indeterminate; that is, it is not possible to specify whether a nodule is benign, malignant, or suspicious for malignancy with a high risk of cancer. Indeterminate nodules are challenging for clinicians and may often lead to either unnecessary surgery or additional ancillary tests, which can be expensive and cost-prohibitive in many clinical settings. It is well known that thyroid FNA cases may be diagnosed as indeterminate not only because of the terminology and/or morphologic criteria used but also because some nodules are quite difficult to classify cytologically as benign or malignant as the invasive characteristics (capsular and/or vascular invasion) can be established only after a thorough histopathologic evaluation.

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TABLE 1. The Bethesda System for Reporting Thyroid Cytopathology: Implied Risk of Malignancy and Recommended Clinical Management

Diagnostic Category	Risk of Cancer, %	Clinical Management
Nondiagnostic or unsatisfactory	?	Solid nodule: R-FNA. Cystic nodules correlated with US: re-aspiration of suspicious areas under US guidance at least 3 mo after initial FNA
Benign	0-3	Clinical follow-up at 6- to 18-mo intervals for 3 to 5 y
AUS/FLUS	5-15	R-FNA in 3 to 6 mo with US guidance
FN/HC	15-30	Surgical lobectomy
SUS	60-75	Near total thyroidectomy or surgical lobectomy
Malignant	97-99	Surgical consultation

Abbreviations: AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; FN, follicular neoplasm; FNA, fine-needle aspiration; HC, Hürthle cell neoplasm; R-FNA, repeat fine-needle aspiration; SUS, suspicious for malignancy; US, ultrasonography.

THE BETHESDA SYSTEM FOR REPORTING THYROID CYTOLOGY (TBSRTC)

TBSRTC was proposed in 2007 to provide a framework and address the inconsistencies and limitations of the available diagnostic terminology at that time for thyroid FNA cytology specimens.[3] TBSRTC consists of 6 diagnostic categories that span the spectrum of benign and malignant thyroid lesions and include so-called gray-zone/indeterminate diagnoses. The latter were termed *atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS)* and *follicular neoplasm (FN)/suspicious for follicular neoplasm (SFN)* according to the following rationale.[4] Thyroid FNA is a screening test, not a diagnostic test, for follicular-patterned lesions because the distinction between benign and malignant lesions is based on a demonstration of invasive characteristics, that is, tumor capsule and/or vascular invasion and the presence or absence of nuclear features of papillary thyroid carcinoma (PTC), which are subject to much observer variability in surgical pathology specimens.[5] TBSRTC also includes an implied risk of malignancy for each diagnostic category based on an analysis of the available literature combined with thoughtful recommendations about how to manage patients (Table 1).[4]

Until now, TBSRTC has been used in the majority of pathology practices in North America and has been widely accepted in many European and Asian countries. It has served to generate a body of cytopathology and clinical literature based on institutional experiences focusing on diagnosis, clinical management, and follow-up.[6] It has also served as a springboard for the development of similar tiered classification schemes for reporting thyroid FNA specimens by pathology organizations and clinical disciplines across the globe.[7] Furthermore, the recent paradigms for ancillary testing of thyroid FNA specimens have been based on the diagnostic categories of TBSRTC. Nonetheless, despite its wide applicability and similarity in usage to other reporting schemes for cytopathology specimens, controversies have arisen over the use of the diagnostic designations of AUS/FLUS and FN/SFN, the recommended follow-up (ie, repeat FNA vs surgery), and the implied risk of malignancy. It has been shown that the risk of malignancy of cases diagnosed as AUS/FLUS is approximately the same as or even higher than that in some studies for cases diagnosed as FN/SFN. Thus, it has been proposed by some to condense the 6-tier TBSRTC categories into 4 tiers or subdivide the AUS/FLUS category into subcategories based on the nuclear and architectural features of a particular specimen.[8]

However, one has to consider the following key factors before instituting a change in the current terminology:

1. Most reported studies on the diagnosis and follow-up of thyroid nodules represent patient populations at tertiary referral centers with malignancy risks different than the risk for the general population.
2. There still exists wide variation in the experiences and application of diagnostic criteria among pathologists diagnosing thyroid FNA specimens.
3. The traditional method of calculating the risk of malignancy for a diagnostic category only on the basis of cases that have undergone thyroid surgery outcomes could be considered to lead to overestimations and to be biased.

The last issue is further complicated by some studies that do not provide follow-up by performing a correlation between the biopsied nodule and the nodule by which the surgical pathology diagnosis was rendered or that include incidental microcarcinomas as malignant on the surgical follow-up of thyroid FNA specimens diagnosed as either benign or AUS/FLUS.

In summary, TBSRTC has paved the way to a standardized thyroid FNA specimen terminology. Many studies have shown its positive impact on cytologic diagnosis, clinical management, and the utilization of ancillary testing.[9]

TABLE 2. First Italian Reporting System for Thyroid Cytology

Code	Diagnostic Category	Histologic Correspondence	Suggested Action
TIR 1	Non-diagnostic	Not defined	Repeat US-guided FNA after at least 1 mo
TIR 2	Non-malignant/benign	Goiter, granulomatous, and lymphocytic thyroiditis	Follow-up
TIR 3	Indeterminate/inconclusive (follicular proliferation)	Follicular adenoma, follicular carcinoma, and follicular variant of papillary carcinoma	Surgery
TIR 4	Suspicious for malignancy	Mostly follicular variant of papillary carcinoma	Surgery (consider frozen section)
TIR 5	Malignant	Papillary, medullary, and anaplastic carcinoma, lymphoma, and metastasis	Surgery (only for papillary and medullary carcinoma)

Abbreviations: FNA, fine-needle aspiration; US, ultrasonography.

2014 ITALIAN REPORTING SYSTEM FOR THYROID CYTOLOGY

The new Italian 6-tier reporting system for thyroid cytology was originally intended to update the previous system devised by the Italian Society for Anatomic Pathology and Cytology (SIAPEC) and the Italian Division of International Academy of Pathology (IAP) in 2007.[7,10] The committee that undertook this task, sponsored by SIAPEC-IAP (Italian Division) in agreement with endocrinological societies (Italian Society of Endocrinology, Association of Medical Endocrinologists, and Italian Thyroid Association), comprised 10 specialists in thyroid disease: 5 pathologists and 5 endocrinologists. The previous SIAPEC-IAP reporting system (Table 2) was a 5-tier classification that included the following diagnostic categories: TIR 1 (nondiagnostic), TIR 2 (negative for neoplasia), TIR 3 (indeterminate/follicular proliferation), TIR 4 (suspicious for malignant neoplasm), and TIR 5 (positive for malignancy). The latest Italian reporting system (Table 3) introduces the additional subgroup of TIR 1C (cystic) to the nondiagnostic group and the subdivision of the indeterminate category (TIR 3) into TIR 3A (low-risk indeterminate lesion) and TIR 3B (high-risk indeterminate lesion).

In 2011, an Italian committee composed of 5 pathologists and 5 endocrinologists selected by the national societies of pathology and endocrinology (mentioned previously) was established with the aim of updating the previous reporting system. On the basis of the previously published experiences of other national reporting systems (British and American), the committee also decided to revise the morphologic criteria for inclusion in each category. The categories were further modified with an update regarding clinical management based on information received from novel diagnostic ancillary techniques. Thus, the new reporting system project shows some differences in comparison with the other currently used national systems. The first difference lies in the emphasis of the morphologic criteria of cases classified as atypical.[8,11] Although architectural atypia is the most important morphologic feature for distinguishing low- and high-risk lesions (ie, microfollicular-predominant architecture and high cellularity [TIR 3A from TIR 3B]), a significant degree of nuclear atypia also warrants the inclusion of a lesion in one of the high-risk categories (TIR 3B or TIR 4), which may require the surgical removal of the nodule. Because of this new categorization of follicular cell atypia, the Italian committee expects that the low-risk category (TIR 3A) might result in a 5% to 10% risk of malignancy at histology versus the expected range of 5% to 15% for the similar diagnostic categories of the British and American systems.[6,12] The new Italian reporting system also includes suggested follow-up actions for the nondiagnostic category (TIR 1): the use of the core-needle biopsy technique for repeated nondiagnostic cases. The purpose of core-needle biopsy is to sample a thyroid nodule with a 20- to 22-gauge spring-activated needle, which takes a thin tissue sample to be processed as a histological specimen. This technique has been extensively studied by several groups.[13,14] For the TIR 3 and TIR 4 cytology categories, immunocytochemical stains may be applied to the material processed by liquid-based cytology; however, this is recommended only for institutions with specific experience.[15,16] Immunohistochemical stains may also be used on tissue core biopsies.[13,14]

As a follow-up of the Italian project, the pathologist members of the committee have planned a study for the revision of the morphologic criteria of the reporting

TABLE 3. New Italian Reporting System for Thyroid Cytology

Code	Diagnostic Category	Risk of Malignancy, %	Suggested Action
TIR 1	Non-diagnostic	Not defined	Repeat US-guided FNA after at least 1 mo
TIR 1C	Non-diagnostic–cystic	Low (variable on the basis of clinical findings)	Evaluation in clinical setting and/or R-FNA
TIR 2	Non-malignant/benign	<3	Follow-up
TIR 3A	LRIL	<10[a]	R-FNA/clinical follow-up
TIR 3B	HRIL	15-30[a]	Surgery
TIR 4	Suspicious for malignancy	60-80	Surgery (consider frozen section)
TIR 5	Malignant	>95	Surgery

Abbreviations: FNA, fine-needle aspiration; HRIL, high-risk indeterminate lesion; LRIL, low-risk indeterminate lesion; R-FNA, repeat fine-needle aspiration; US, ultrasonography.
[a] The expected rate of malignancy for the TIR 3 subcategories is mainly based on clinical experience and is only partially supported by the evidence of the published data.

categories based on both individual evaluations and collective discussion of 120 select cytological cases. The committee has also proposed a multicenter, national study to validate this new classification system. The first part (revision of the morphologic criteria) is currently in progress; the multicenter study will begin as soon as the criteria are published.

GUIDANCE ON THE REPORTING OF THYROID CYTOLOGY SPECIMENS FROM THE UK ROYAL COLLEGE OF PATHOLOGISTS (RCPATH)

RCPath's *Guidance on the Reporting of Thyroid Cytology Specimens* was first published in November 2009 and underwent a revision in 2015.[17] It builds on the Thy numerical classification in the British Thyroid Association guidelines for the management of thyroid cancer first published in 2002 (the second and third editions were published in 2007 and 2014, respectively).[18] Numerical reporting categories have long been established in the United Kingdom (eg, the national breast screening program), and some UK cytology departments were using numerical categories for thyroid cytology for decades before the British Thyroid Association document.

Numerical cytology reporting categories do not replace free text reports for the interpretation of the specimen, which remain crucial for providing a specific diagnosis or differential diagnosis for the clinician. Numerical categories do, however, facilitate auditing of outcomes, national standardization, and international comparisons with other systems. To this end, the Thy1 to Thy5 categories equate with the categories of the US Bethesda, Italian, Australian, and Japanese systems, as shown in Table 4. Nevertheless, differences exist. The differences mostly reflect the different health care setups, the application of pathological criteria and resource settings, and differences in some specific categories, which are discussed further in the Discussion section.

Specific comments on the use of the UK Thy categories follow:

- Thy1 reflects specimens that are unsatisfactory because of aspirator or technical reasons (eg, too few follicular epithelial cells or poor cell preservation). For solid lesions, it is recommended that the sample contain at least 6 groups of well-preserved follicular epithelial cells with at least 10 cells per group.
- Thy1c reflects inadequacy due to the cystic nature of the lesion, and separation is needed for auditing purposes.
- Thy2 indicates sufficient epithelial cellularity for a solid lesion (as discussed previously), and a specific diagnosis can usually be given (eg, colloid nodule or Hashimoto's thyroiditis).
- Thy2c indicates a lack of sufficient epithelial cells but abundant colloid with cyst macrophages; it suggests a cystic colloid nodule in the appropriate clinical setting.
- Thy3 means that a neoplasm is possible, and this category is divided into Thy3a and Thy3f:
  a. Thy3a indicates cytological/nuclear or architectural atypia or other features raising the possibility of neoplasia but insufficiently to allow placement into any other category. Some of these may reflect poor-quality samples, and repetition often helps.
  b. Thy3f suggests FNs (including those of oncocytic cells).
- Thy4 indicates suspicion of malignancy. The suspected tumor type should be stated and is often PTC.
- Thy5 indicates that a definite diagnosis of malignancy can be made. The type of malignancy should be stated (eg, PTC, medullary thyroid carcinoma, anaplastic thyroid carcinoma, lymphoma, or metastasis).

TABLE 4. Comparison of the RCPath Thy Categories With Other Internationally Used Systems

RCPath	Bethesda	Italian	Australian	Japanese
Thy1. Non-diagnostic for cytological diagnosis **Thy1c**. Non-diagnostic for cytological diagnosis–cystic lesion	**I**. Non-diagnostic or unsatisfactory	**TIR 1**. Non-diagnostic **TIR 1c**. Non-diagnostic–cystic	**1**. Non-diagnostic	**1**. Inadequate
Thy2. Non-neoplastic **Thy2c**. Non-neoplastic–cystic lesion	**II**. Benign	**TIR 2**. Non-malignant	**2**. Benign	**2**. Normal or benign
Thy3a. Neoplasm possible–atypia/non-diagnostic	**III**. AUS/FLUS	**TIR 3A**. LRIL	**3**. Indeterminate or follicular lesion of undetermined significance	**3**. Indeterminate **B**. Others
Thy3f. Neoplasm possible, suggesting follicular neoplasm	**IV**. Follicular neoplasm or suspicious for a follicular neoplasm	**TIR 3B**. HRIL	**4**. Suggestive of follicular neoplasm	**3**. Indeterminate **A**. Follicular neoplasms **A-1**. Favor benign **A-2**. Borderline **A-3**. Favor malignant
Thy4. Suspicious for malignancy	**V**. Suspicious for malignancy	**TIR 4**. Suspicious for malignancy	**5**. Suspicious for malignancy	**4**. Malignancy suspected
Thy5. Malignant	**VI**. Malignant	**TIR 5**. Malignant	**6**. Malignant	**5**. Malignancy

Abbreviations: AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; HRIL, high-risk indeterminate lesion; LRIL, low-risk indeterminate lesion; RCPath, Royal College of Pathologists.

An assessment of interobserver variation for the UK categories showed good correlation for Thy1 and Thy5, moderate correlation for Thy2 and Thy3f, and poor agreement on Thy3a and Thy4 as expected for the 2 most subjective groups. However, grouping cases into categories suggesting medical management (Thy1, Thy2, and Thy3a) versus surgical management (Thy3f, Thy4, and Thy5) gave a good correlation.[19]

Cases in categories Thy4 and Thy5, Thy3 cases at local discretion, and definitely Thy3a cases yielding a second Thy3a diagnosis on repeat-FNA should be reviewed and discussed in multidisciplinary team meetings, at which there should also be input from radiology, endocrinology, surgery, and oncology to determine the subsequent action. Thy3a is often followed by ultrasound and further cytology. Thy3f and Thy4 lesions usually undergo diagnostic hemithyroidectomy. Thy5 enables therapeutic action, such as total thyroidectomy with appropriate lymph node dissection for PTC or medullary thyroid carcinoma, surgery or oncology treatment for anaplastic thyroid carcinoma, and oncology treatment for lymphoma (although lymphomas usually require core biopsy for precise histological classification).

The UK document strongly recommends that departments undertake regular audits (eg, the frequency of the use of the different categories or the local positive predictive value of each category for malignancy on histology). The only suggested standard is that Thy5 should have a $>$ 99% positive predictive value for malignancy on histology. It is also important that quality assurance be required to maintain accuracy.

Table 5 shows some published ranges of outcomes to date for the frequency of use and prediction of malignancy on histology.[20,21] When one is assessing such predictions, it is important to understand the calculations. For example, if only a histological (not clinical) diagnosis of malignancy is considered to be a positive outcome, then using as the denominator only those cases with histology will give an artificially inflated prediction of malignancy for the lower Thy numbers, for which only a few select cases involve surgery, but a more accurate outcome for the higher Thy numbers, for which most cases do involve surgery. Conversely, if the denominator is all cases in that cytology category, whether there is surgery or not, the reflection is more accurate for the lower Thy numbers but is less useful for the higher Thy numbers because some patients with Thy5 cytology may not undergo surgery (eg, metastatic disease); this means that malignancy is not always histologically confirmed. To facilitate communication between clinicians and patients concerning the need for surgery, it can also be useful to calculate the prediction of neoplasia as well as malignancy.

CLASSIFICATIONS AND MOLECULAR BIOLOGY

Our knowledge of the molecular pathology of thyroid disease has vastly increased in the last few years. A key

TABLE 5. Frequency of Use and Prediction of Malignancy for the Different UK RCPath Thy Categories

RCPath Thy Category	Frequency of Use, %	Positive Predictive Value for Neoplasia on Histology, %[a]	Positive Predictive Value for Malignancy on Histology, %[a]	Risk of Malignancy for All Cytology Cases, %[b]
Thy1/1c	18-27	26-32	16-20	**4-5**
Thy2/2c	42-52	18-26	8-10	**1–2**
Thy3a	5-10	20-58	10-33	**6-17**
Thy3f	7-14	**60-66**	**28-35**	28-30
Thy4	2	**50-85**	**54-68**	44-64
Thy5	2-7	**100**	**100**	67-71

Abbreviation: RCPath, Royal College of Pathologists.
The most useful predictive values are highlighted in bold.
[a] Only for the cases with histology.
[b] Only a histology outcome of malignancy was considered positive.

milestone was the publication in 2014 of the Thyroid Cancer Genome Atlas study of papillary carcinoma of the thyroid.[22] It is now clear that papillary thyroid cancer, which accounts for more than 80% of all newly diagnosed thyroid tumors, is either a BRAF V600E– or a RAS-driven tumor. BRAF V600E–driven papillary cancers tend to be papillary carcinomas of the classic type or variants of classic papillary carcinoma. RAS-driven thyroid tumors are follicular-patterned lesions including follicular variants of papillary carcinoma, follicular carcinomas, and a subset of follicular adenomas. Medullary carcinomas of the thyroid are associated with *RET* gene mutations. Germline *RET* mutations are present in familial tumors, and somatic mutations are present in sporadic medullary carcinomas. HRAS and KRAS mutations are seen in *RET* wild-type tumors, which are predominantly sporadic medullary tumors. Anaplastic or poorly differentiated carcinomas of the thyroid also show a different profile of mutations, including mutations of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α, AKT1, tumor protein 53, catenin β1, telomerase reverse transcriptase, and others.[9]

Although the molecular pathology of thyroid cancer is now better understood, applying this in clinical practice is more complex. TBSRTC, published in 2008, states very little about molecular testing for thyroid FNA.[23] The American Thyroid Association recently published guidelines for the molecular testing of thyroid FNA that detail the Afirma gene expression classifier (GEC) test, the 7-gene mutational panel marketed until recently by Asuragen, and ThyroSeq2, a system developed by Nikiforov and colleagues with targeted next-generation sequencing.[24]

A perusal of the latest international published guidance for thyroid FNA cytology reveals that to some extent published guidance still lags behind recent advances in knowledge. The Italian consensus classification states that molecular testing may be of value for indeterminate FNA, although it makes no specific recommendations about how this should be performed.[10] Similarly, the latest British classification system refers to the value of molecular testing for thyroid FNA but does not give specific recommendations for indications from molecular testing.[17] The Australian structured reporting protocol states that the detection of a BRAF V600E mutation in aspirates should virtually confirm the diagnosis of PTC and, therefore, can be used to confirm a diagnosis in suspicious cases.[25] It also states that RET/PTC and paired box 8/peroxisome proliferator-activated receptor γ translocation use in thyroid cytology is not established and suggests an immunohistochemical panel for HBME1, cytokeratin 19, galectin 3, and BRAF for aspirates suspicious for malignancy, although the protocol states that is not universally accepted. It comments that commercial molecular diagnostic kits for inconclusive categories, including Afirma (Veracyte) and miRInform (Asuragen), are available. It further states that the advantages, cost-effectiveness, and use of these tests in routine practice need more evaluation.[25]

The fundamental point of all molecular tests is that the diagnostic positive and negative predictive values and the clinical decision depend on the risk of malignancy in any particular patient cohort.[24] The Afirma GEC test, for example, is most effective for benign and low-risk thyroid FNA cases as a rule-out test, whereas other systems such as the 7-gene panel are more useful for high-risk FNA cases: a BRAF V600E mutation suggests a virtually certain diagnosis of malignancy, and *RET* PTC or paired box 8/peroxisome proliferator-activated receptor γ rearrangements

suggest a higher probability of malignancy in higher risk FNA cases.[9,24]

For a molecular testing system to have maximum clinical utility, it should have a high positive predictive value for malignancy in both lower risk and higher risk FNA cases across the whole spectrum of FNA malignancy risk (from benign to indeterminate to suspicious for malignancy and malignant).[24] A clinically effective molecular diagnostic system should also be able to detect non–follicular-derived tumors such as medullary carcinomas and parathyroid lesions. Whether FNA is reported with TBSRTC, the Italian consensus terminology, the British terminology, or the Australian protocol is to some extent immaterial because in all these systems, the higher the numerical category is, the higher the risk of malignancy is, and it is the absolute risk of malignancy in any given clinical or practice setting of FNA that in combination with the molecular test determines the positive and negative predictive values of the molecular test being used.[24]

Advances in the molecular classification of thyroid tumors are also leading to changes in how these tumors are classified on the basis of histopathologic examination. If there is no evidence of capsular or vascular invasion, the encapsulated or noninvasive follicular variant of papillary carcinoma is known to behave in a very indolent fashion with virtually no risk of recurrence or metastatic disease during long-term follow-up. From a molecular standpoint, these tumors show a molecular phenotype more similar to that of follicular adenoma or follicular carcinoma than that of classic papillary carcinoma. The recognition that these lesions do not behave in a malignant fashion is leading to a reclassification of these lesions as noninvasive follicular tumors with papillary-like nuclei. This change in the pathology diagnostic gold standard would have implications for cytology because a significant proportion of cancers currently diagnosed on FNA as indeterminate are encapsulated follicular variants of papillary carcinoma without capsular or vascular invasion on follow-up. To illustrate the effect of this change, both Strickland et al[26] and Faquin et al[27] showed that the overall percentage of cancers in Bethesda categories 2, 3, 4, and 5 would be markedly reduced if all lesions that were previously diagnosed at their institutions as follicular variant of papillary carcinoma without capsular and vascular invasion were to be reclassified as a newly proposed non-cancer diagnostic entity (non-invasive follicular tumor with papillary-like nuclei).[26,27]

The other area in which thyroid cytology is undoubtedly going to play a major role in the future is the prediction of responses of thyroid tumors to therapy. Although we are still in the very early days, there is clear anecdotal evidence showing that thyroid tissue or cell samples can be used to detect and predict responses to targeted therapies (eg, the detection of anaplastic lymphoma kinase [ALK] fusions to predict the responses of differentiated, poorly differentiated, or anaplastic thyroid tumors to crizotinib in radioiodine-resistant progressive tumors). Although only a small percentage of tumors show ALK fusions, if an ALK fusion is present, then a patient may respond to crizotinib. A number of other mutations, if present, also predict more aggressive tumor behavior (eg, protein 53, phosphatase and tensin homolog, ALK and telomerase reverse transcriptase) can also be detected in FNA samples.[28,29] There is now some evidence showing that even a very small thyroid papillary carcinoma with multiple gene mutations may behave more aggressively and that these mutations are also amenable to detection on FNA cytology.[30] The implementation of molecular testing in thyroid FNA cytology requires representative cells from the lesion for either DNA or RNA analysis. This usually requires additional needle passes to be performed at the time of FNA for DNA and RNA analysis for next-generation sequencing or polymerase chain reaction methods.

The second point is that so far the most sophisticated technologies are available only as black-box or proprietary systems (eg, Afirma GEC and ThyroSeq 2). At the current time, samples for Afirma GEC or ThyroSeq 2 are analyzed only in North America with logistical implications for specimen transportation. Afirma GEC is most useful for lower risk FNA cases as a rule-out malignancy test.[24] ThyroSeq 2, which has a high positive predictive value for malignancy in both low-risk and higher risk FNA cases, is also a proprietary technology.[24,31,32] Although the Ion Torrent PGM machine platform is commercially available, the DNA and RNA sequences used in the ThyroSeq 2 chips are proprietary, so it is currently impossible for other laboratories to replicate this system and to independently confirm the clinical utility of ThyroSeq2.

One major obstacle holding back implementation is the cost of these new technologies. The cost of standard ultrasound-guided thyroid FNA is comparatively low in developed health care systems, whereas the cost of molecular tests is relatively high. The UK National Health Service tariff cost of a thyroidectomy is just over £2000

(approximately US $3000), and it is possible to show with statistical modeling techniques based on the risk of malignancy for any given FNA category that the implementation of a complex molecular testing algorithm such as ThyroSeq 2 would reduce the number of thyroid surgeries. Some benign thyroid nodules also show gene mutations such RAS mutations, which are also present in follicular carcinoma. Because of the presence of RAS mutations in benign lesions using ThyroSeq 2, it is not possible in the absence of a BRAF V600E gene mutation to accurately predict malignancy with a 97% to 99% positive predictive value for lower risk FNA cases such as AUS/FLUS cases.[32] Other interesting avenues include the use of microRNAs as markers of thyroid cancer.[33]

DISCUSSION

In 2014, a commentary on a symposium held during the 38th European Congress of Cytology in Geneva ("Thyroid FNA: International Perspectives From the European Congress of Cytopathology: Can We Cross the Bridge of Classifications?") was published in this journal.[34] The conclusion of this group of cytopathologists involved in thyroid FNA was that the use of a clearly defined terminology is mandatory and that an international one is called for. One year later, 2 of the 3 already existing terminologies have been updated by the United Kingdom and Italy, and this indicates their wish to retain their own national terminologies. In addition, new terminologies have also been published in Japan and Australia. As discussed by all faculty members of the symposium, there are many similarities among the 3 terminologies highlighted at this symposium; all include benign, indeterminate, malignant, suspicious for malignancy, and nondiagnostic categories. However, there are some obvious differences in the criteria applied for classifying thyroid FNA cases as indeterminate in each terminology scheme; this highlights the need for further evaluation and possible modifications. The RCPath categories Thy3a and Thy3f could overlap with the SIAPEC categories TIR 3A and TIR 3B, respectively. However, Thy3a includes cytological/nuclear or architectural atypia or other features raising the possibility of neoplasia but insufficient to allow placement into any other category, and this better recalls the AUS/FLUS category of the Bethesda system. It differs partially from TIR 3A, which includes only architectural changes; atypias suggestive for papillary carcinoma convert TIR 3A to TIR 3B. In contrast, predominantly oncocytic aspirates would be categorized as Thy3f in the United Kingdom and as TIR 3B in Italy. Thy2c of the RCPath terminology does not obviously appear in TBSRTC. Therefore, comparisons between some categories of the different terminologies should be undertaken cautiously, and one should also keep in mind that direct transposition cannot be performed easily. Some studies using a national terminology propose a transposition into TBSRTC for publication; this is likely to be controversial because the analysis of the risk of malignancy might be biased in the case of transposition. Finally, the management of patients is not always the same. Although TBSRTC and the Italian terminology propose quite the same approach, the distinctive characteristic of the British system is that many of the cases categorized as indeterminate and all of those that are suspicious or malignant should be referred to the multidisciplinary team to establish correct management.

Nevertheless, these various terminologies do now exist and, when properly used, assist in the better understanding of lesions, allow the assessment of the risk of malignancy, offer improved reproducibility between pathologists, give pathologists the opportunity for self-assessment of their own results, and enable more consistent management of patients by clinicians familiar with the respective national terminology being used. Of course, an internationally well-accepted and unique terminology for classifying thyroid FNA cases would be optimal, but it is also not recommended to change existing practices. In Italy and in the United Kingdom, pathologists have worked together and have organized multicenter consensus and/or reproducibility exercises. Italy and the United Kingdom have now adopted their own national terminologies, and asking them to change would be counterproductive, whereas in countries where a national approach has not yet been undertaken, deciding to use an internationally recognized terminology is relevant. In many countries, TBSRTC has been adopted in its entirety. Whatever the terminology used, 20% to 25% of the nodules are going to be classified as indeterminate for malignancy. Liquid-based cytology was proposed several years ago to improve cytomorphology and hence the interpretation; however, the gain was marginal.[17,35] Usually, liquid-based cytology reduces some AUS/FLUS by reducing cellular atypias caused by artifacts; this allows a slight increase in the percentage of benign results due to better cell preservation. Currently, the best results have been obtained by the

application of either immunocytochemical techniques or molecular tests.

Several studies have shown that immunocytochemistry is useful in detecting malignant cases in the indeterminate categories, but its main advantage is its negative predictive value. In all published series using a panel of 2 or 3 antibodies (mostly HBME1, galectin 3, cytokeratin 19, and thyroid peroxidase),[36–40] a benign pattern correlated with a benign histological control in approximately 96% of cases. However, immunocytochemistry results help to achieve a definitive result in only approximately 50% of indeterminate cases.

As for molecular testing as described previously, the most important point is to understand the diagnostic benefits of the available diagnostic tests; some tests are useful for predicting that a biopsied thyroid nodule is benign (rule-out tests), whereas others carry a higher positive predictive value for predicting malignancy (rule-in tests). Furthermore, for the same positive or negative result, the therapeutic implications in terms of the risk of cancer are not the same; they depend on the initial morphological FNA category as explained previously.

Nevertheless, these molecular results seem promising, as stated by the scientific committee of the American Association of Clinical Endocrinologists: "Molecular testing is meant to complement and not to replace the clinical judgment, sonographic assessment and visual cytological interpretation."[24] We believe that molecular testing continues to evolve and will require the input of pathologists in refining and developing various diagnostic and testing strategies for the management of thyroid lesions.

In conclusion, the symposium entitled "Thyroid Fine-Needle Aspiration: New Classifications and New Interpretations" provided an open forum for discussing and debating the terminology modifications required to keep up with the ever-changing field of thyroid FNA cytology. The following topics were highlighted:

1. Tiered classification schemes for thyroid FNA serve the important purpose of establishing streamlined communication among clinicians involved in the care of patients with thyroid nodules.
2. A universally established international terminology most likely represents an ideal option.
3. The 5 current terminologies have already been published and are widely used in clinical practice; however, training, validation, or both among cytopathologists before the chosen terminology is applied are necessary to prevent diagnostic variability.
4. For publication, the careful translation of results into TBSRTC should be considered to facilitate comparisons between different published studies.
5. Ancillary techniques appear to be the only solution to reduce the number of morphologically indeterminate cases.

Techniques for immunocytochemistry are already well established; the current molecular tests have shown greater promise and are in the process of being further modified to provide higher negative and positive predictive values. This all shows how the practice of thyroid FNA continues to evolve along with our knowledge of the molecular pathogenesis of thyroid disease. We believe that this will lead to better preoperative diagnoses and clinical triage of thyroid nodules with dramatic reductions in the rates of unnecessary surgery and to the future prospect of improving drug therapies for radioiodine-resistant thyroid cancer.

A similarly themed symposium for discussing the existing literature and future modifications of TBSRTC in light of ancillary tests will be held at the 2016 International Congress of Cytology in Yokohama, Japan.

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