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The Impact of the 2022 WHO Classification of Thyroid Neoplasms on Everyday Practice of Cytopathology

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Abstract

This review outlines how the alterations in the 5th edition of the WHO Classification of Endocrine and Neuroendocrine Tumors of the thyroid gland are likely to impact thyroid cytopathology. It is important to note that WHO subclassifies thyroid tumors into several new categories based on increased comprehension of the cell of origin, pathologic features (including cytopathology), molecular classification, and biological behavior. The 3rd edition of the *Bethesda System for Reporting Thyroid Cytopathology* (TBSRTC) will debut in the near future and will include changes in diagnostic category designations. The changes in the 5th edition of the WHO will in some instances subtly, and in other instances significantly, impact the cytological diagnoses. Moreover, these changes will also affect other thyroid FNA classification schemes used internationally for classifying thyroid FNA specimens.

Keywords WHO · Thyroid cancers · Thyroid cytology · Thyroid diagnostic categories · Molecular testing · Personalized medicine

Introduction

Thyroid cancer is the most common endocrine malignancy. For that reason, much of the 5th edition of the WHO Classification of Endocrine and Neuroendocrine Tumors is focused on thyroid tumors [1, 2]. The approach adopted in this edition is different from the prior ones in that it focuses not only on the cell of origin, but also highlights the molecular profile of each entity, defines the pathologic features, and reviews the clinical behavior [3–5]. It stands to reason that any change and revision adopted for the classification of histological specimens will have implications on the cytological interpretation and reporting schemes currently utilized for the diagnosis and management of thyroid nodules [6, 7].

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Thyroid nodules may be non-neoplastic or neoplastic. Some neoplasms occur either as a single or a dominant mass in the background of multiple nodules [8, 9]. The detection of thyroid nodules is on the rise in the general population due to the widespread use of radiologic screening techniques [10]. By ultrasound, up to 60% of the population in the USA are found to harbor one or more thyroid nodules, with a malignancy risk of < 10% [8–11]. The prevalence of thyroid nodules increases with age, with the majority of nodules occurring in individuals older than 40 years of age. The rise in thyroid nodule detection rates is, in part, responsible for the fivefold increase in the incidence of thyroid carcinoma, most of which are papillary thyroid carcinomas measuring 1.0 cm or under [12].

Fine needle aspiration (FNA) cytology has proven to be an indispensable tool for the diagnosis and management of thyroid lesions. It is a rapid and safe method that is most often performed in the outpatient setting with very few to no complications and can discriminate between benign and malignant nodules with high accuracy [8, 13–15]. Nevertheless, as expected from a limited tissue sample of a large lesion, there are inherent limitations to thyroid FNA, mostly due to both benign and malignant follicular-patterned lesions with or without nuclear atypia [6, 16, 17]. Such specimens are often classified as indeterminate for malignancy on cytologic evaluation [6].

Prior to 1996, the thyroid FNA specimens were classified and reported according to the classification schemes developed and used only within institution-specific cytology practices. The Papanicolaou Society of Cytopathology proposed a tiered classification scheme for reporting thyroid FNA specimens in 1996, paving the way for the development of future classification schemes, formulated by various professional societies [18]. Among them, *The Bethesda System for Reporting Thyroid Cytopathology* (TBSRTC), with its two editions published in 2010 and 2018, respectively (Table 1), provided a diagnostic framework well-aligned with the most updated histologic classification and management of thyroid lesions [7, 19].

The diagnostic framework of TBSRTC is tiered; it is comprised of six diagnostic categories which span the spectrum of benign and malignant thyroid lesions including the so called "Gray Zone/Indeterminate" diagnoses, i.e., "Atypia/Follicular Lesion of Undetermined Significance" (AUS/FLUS) and "Follicular Neoplasm/Suspicious for Follicular Neoplasm" (FN/SFN) (Fig. 1). These indeterminate diagnoses clearly represent a spectrum of cytologic diagnoses rendered for various thyroid lesions and reflect the fact that for follicular-patterned lesions, thyroid FNA is a screening test that gives information on how the nodule is best triaged clinically, rather than a diagnostic test, since a diagnosis of malignancy rests upon demonstration of invasive characteristics that cannot be assessed on FNA. Each diagnostic category of TBSRTC is linked to an implied risk of malignancy and possible recommendations for patient management (Table 1) [20].

It is essential to realize "the importance of the timing" of the first edition of TBSRTC. The first edition was published in the setting of an explosion in knowledge regarding the molecular profile of thyroid tumors, and multiple practice recommendations by various clinical, radiologic, and pathology professional organizations to provide optimal clinical management of thyroid nodules. Thus TBSRTC has provided a diagnostic framework which is congruent with the present and the future paradigms of thyroid nodule management [6, 21].

The Achievement of TBSRTC

The tremendous success of TBSRTC as a reporting framework cannot be understated; within a few years of the debut of the 1st edition, it became widely used in pathology practices across the USA and in many European and Asian countries [6, 22–24]. The TBSRTC also served as a model for tiered classification schemes for reporting thyroid FNA specimens developed by international pathology organizations and clinical disciplines [25–27]. Regarding its applicability in everyday practice of cytopathology, numerous studies were published representing experiences of both academic and non-academic institutions regarding its use in reporting of thyroid nodule FNA specimens and clinical management [17, 22, 28–30]. In addition, various practice paradigms were proposed regarding instituting adjunct reflex molecular testing based on diagnostic categories of TBSRTC [31–33]. The 2nd edition of TBSRTC included modified ranges for the risk of malignancy associated with diagnostic categories based on literature published following the 1st edition which took into account changes in the histopathologic classification of thyroid neoplasms, especially the renaming of non-invasive encapsulated follicular variant of papillary thyroid carcinoma as a non-invasive follicular tumor with papillary like nuclei (NIFTP), and updated recommendations for reflex molecular testing and management; additionally, it included explanatory notes [6, 20, 34].

Table 1 The *Bethesda System for reporting Thyroid Cytopathology*, 1st and 2nd Editions [7, 89, 90]

Diagnostic category	ROM (%) 1st ed	ROM (%) 2nd ed	ROM (%) 2nd ed with NIFTP Dx	Usual management
Non-diagnostic or unsatisfactory	1–4	5–10	NSC	Repeat FNA with ultrasound guidance
Benign	0–3	0–3	NSC	Clinical follow-up
Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS)	~5–15	10–30	6–18	Repeat FNA
Follicular neoplasm or suspicious for a follicular neoplasm (specify if Hurthle type or oncocytic)	15–30	25–40	10–40	Surgical lobectomy
Suspicious for malignancy	60–75	50–75	45–60	Near-total thyroidectomy or surgical lobectomy
Malignant	97–99	97–99	94–96	Near-total thyroidectomy

ROM risk of malignancy, *NIFTP* noninvasive follicular tumor follicular thyroid neoplasm with papillary like nuclear features, *Dx* diagnosis, *NSC* no significant change, *FNA* fine-needle aspiration

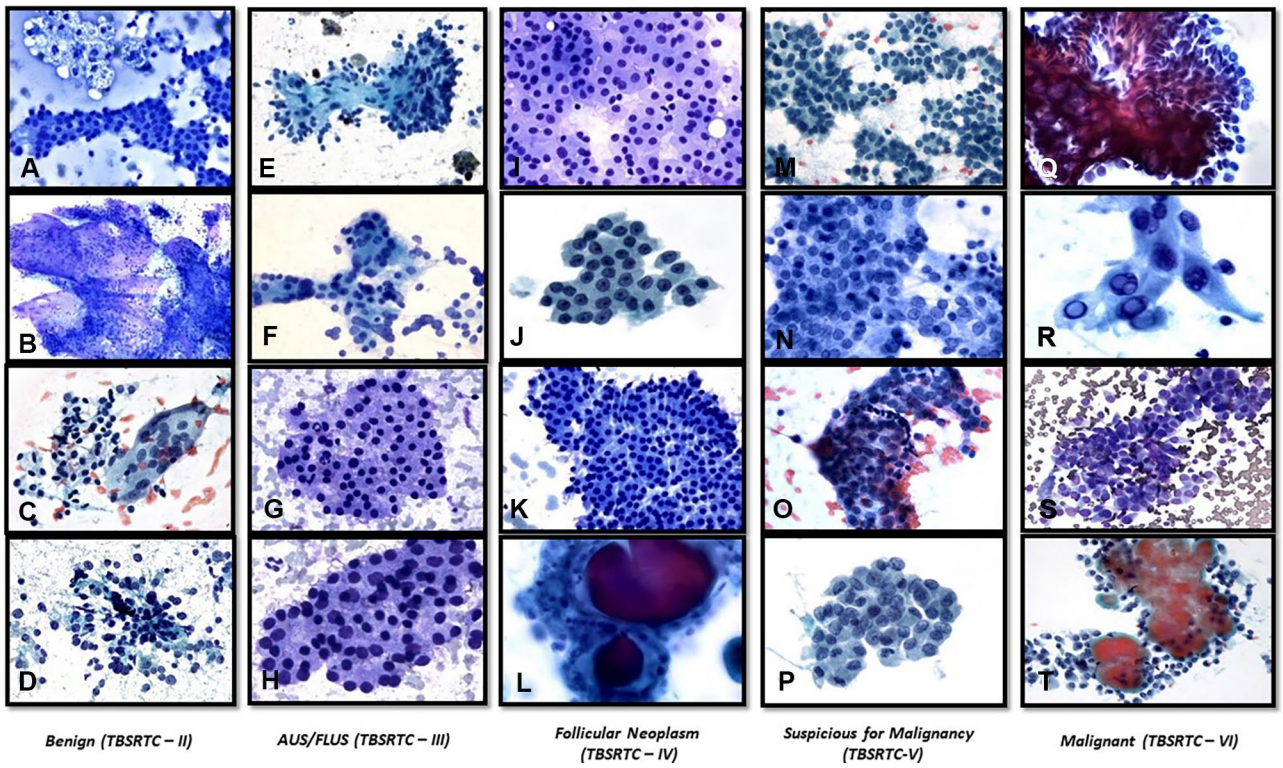

Fig. 1 An illustrative depiction of various diagnostic categories of the Bethesda System for Reporting Thyroid Cytopathology

The Aftereffect of TBSRTC

The ranges for the implied risk of malignancy associated with each diagnostic category of TBSRTC are based on a comprehensive analysis of the literature [6, 20]. However, these numerical values of risk of malignancy represent institutional experiences; thus, it is also understood that these values may not reflect all real world practices due to diverse patient populations, variabilities in the use of clinical and radiologic selection criteria for thyroid nodules for FNA, and variable assessment of criteria for the cytologic diagnoses of thyroid lesions/neoplasms [6] (Table 2). This is evident in follow-up studies using TBSRTC, which have shown that the risk of malignancy in cases diagnosed as follicular neoplasm/suspicious for follicular neoplasm was similar or even in some cases lower than cases classified as AUS/FLUS [17, 22, 26, 28, 35] (Table 2). Hence, some authors have recommended to reduce the TBSRTC categories to 4 instead of 6 or stratify the AUS/FLUS based on nuclear or architectural features [30, 36–39].

Table 2 Meta-analyses comparing risk of malignancy in 1st and 2nd editions of TBSRTC to published literature [7, 17, 19, 20, 91–93]

Diagnostic category	ROM (%) 1st ed	ROM (%) 2nd ed	ROM (%) Bongiovanni et al.	ROM (%) Krauss et al.	ROM (%) Vuong et al.	ROM (%) Haaga et al.
Non-diagnostic or unsatisfactory	1–4	5–10	16.8	12.0	2.0–19.1	28.9
Benign	0–3	0–3	3.7	5.0	0.7–8.0	12.7
Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS)	~5–15	10–30	15.9	17.0	9.2–30.5	36.6
Follicular neoplasm or suspicious for a follicular neoplasm (specify if hurthle type or oncocytic)	15–30	25–40	26.1	25.0	28.9	35.1
Suspicious for malignancy	60–75	50–75	75.2	72.0	79.6	82.8
Malignant	97–99	97–99	98.6	98.0	99.1	97.7

ROM risk of malignancy

However, before raising questions regarding the validity of TBSRTC in real world practice of thyroid nodule cytology, the following need to be considered: (1) majority of the literature represents patients who are treated at large academic or tertiary referral centers with malignancy risks dissimilar to the general population, (2) wide variation in interpreting thyroid FNA specimens and subsequent surgical resections specimens, and (3) the ranges and average risk of malignancy associated with each diagnostic category of TBSRTC are extracted from the retrospective review of the literature. It is also prudent to note that the risk of malignancy value for each diagnostic category is most likely an overestimation because it is based predominantly on cases undergoing surgical excision; moreover, a sizeable number of studies do not provide acorrelation between thyroid lesion/nodule seen on radiologic studies and biopsied, and surgical pathology follow-up [40, 41].

Regardless of these "so-called" shortcomings, both clinicians and pathologists agree to the fact that a tiered classification scheme such as TBSRTC is essential for the diagnosis, reporting, and management of thyroid nodules.

The new WHO thyroid tumor classification scheme employs a morpho-molecular approach to classify thyroid tumors which is aligned with clinical risk-based management strategies. The following account discusses how the new WHO classification scheme will impact the current and future trends in cytologic interpretation, reflex molecular testing, and management of thyroid nodules [1, 2].

The New Categorization of Benign Follicular Cell-Derived Thyroid Lesions

In the benign category of follicular cell-derived lesions, the 4th edition of the WHO classification of endocrine tumors included only a single benign entity of follicular adenoma (FA) [5]. The 5th edition has expanded this category to include thyroid follicular nodular disease, follicular adenoma with papillary architecture and oncocytic adenoma [1, 2].

It is well known that cytomorphologic features alone cannot definitively differentiate between non-neoplastic and neoplastic follicular-patterned thyroid lesions. The FNA samples of an adenomatous nodule, follicular adenoma, and even follicular carcinoma can be cellular and show a monotonous population of follicular cells arranged in cohesive groups with nuclear overlapping and crowding, microfollicles, and thick colloid. Therefore, some cases of adenomatous nodules are classified into indeterminate TBSRTC categories, Atypia/Follicular lesion of Undetermined Significance (AUS/FLUS) andFollicular Neoplasm/Suspicious for Follicular Neoplasm (FNA/SFN) [6, 7, 16, 42–44].

It is interesting that a sizeable proportion of adenomatous nodules that occur in the context of "multinodular goiter" are clonal proliferations and are indeed neoplasms [1, 45]. The question arises considering these molecular findings, should the indeterminate diagnoses AUS/FLUS and FN/SFN rendered for FNA specimens of cellular adenomatous nodules not be considered as "false positive"? However, in the current era of adjunct molecular testing of thyroid FNA specimens most adenomatous nodules are classified as benign or having equal to or less than 3–5% risk of malignancy [34, 46, 47].

Among the list of benign neoplasms, a clinically important benign neoplasm classified as "follicular adenoma with papillary architecture" has been included [2]. The key histopathologic features of this tumor include encapsulation, papillary architecture with tips of the papillae directed towards the center of the lesion, and the core of papillae with edematous stroma containing follicles [2]. On ultrasound, these tumors appear as hypoechoic solid nodules with increased vascularity. The FNA specimens from these nodules are usually cellular, demonstrate papillary architecture, and most lack nuclear features of papillary carcinoma [48] (Fig. 2). However, some authors have reported atypical cytologic features such as elongated nuclei with intranuclear groves and chromatin clearing. Therefore, it is not surprising that FNA specimens from these nodules may be classified as AUS/FLUS, FN/SFN, and even, in rare cases, as suspicious for papillary thyroid carcinoma [48–50]. A majority (up to 70%) of cases of follicular adenoma with papillary architecture harbor TSH-receptor (*TSHR*) mutations and small minority have *GNAS* and/or *EZH1* mutations. These mutations can be detected in molecular analysis of FNA specimens preventing unnecessary or aggressive management strategies [1, 2, 46, 51].

The diagnostic category of low-risk follicular neoplasms which was introduced in the 4th edition WHO classification of endocrine tumor refers to borderline tumors that are morphologically and clinically intermediate between benign and malignant tumors, with an extremely low risk of locoregional and/or distant recurrence. The list of low-risk follicular–derived neoplasms in the 5th edition of WHO classification of endocrine tumors includes the following: (1) non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), (2) thyroid tumors of uncertain malignant potential (UMP), and (3) hyalinizing trabecular tumor (HTT) [1, 2]. A majority of FNA specimens from either UMP or NIFTP will be classified as AUS/FLUS or FN/SFN. Some cases of NIFTP will be classified as suspicious for PTC due to atypical nuclei, and rare cases are diagnosed as consistent with PTC [52, 53]. It has been shown that the histologic diagnosis of NIFTP will significantly decrease the implied ROM of the indeterminate diagnostic categories of TBSRTC, especially for AUS/FLUS, FN/SFN, and SFM [7, 54]. At present, adjunct molecular analysis of thyroid FNA specimens by various home-brewed or commercially available tests can confirm that these low-risk follicular patterned lesions are usually RAS driven (commonly harbor

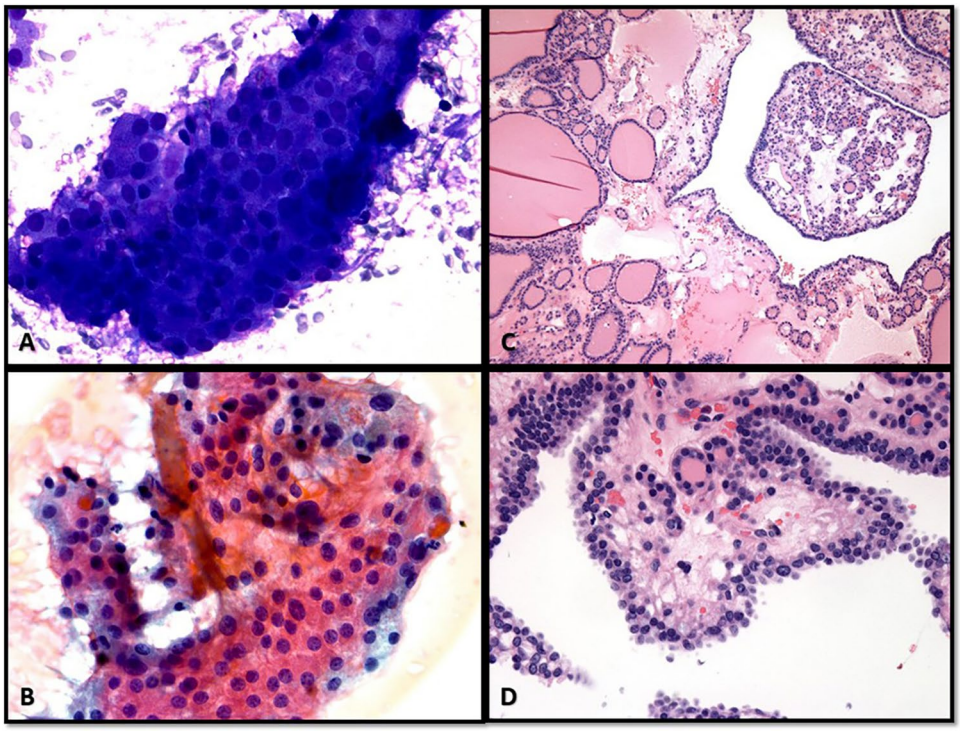

Fig. 2 Follicular adenoma with papillary architecture. Fine-needle aspiration cytology demonstrating lesional cells arranged in papillary configuration (**A** air-dried smear preparation—Diff-Quik®) stain and the nuclei are round with evenly distributed nuclear chromatin lacking nuclear features of papillary thyroid carcinoma (**B** alcohol-fixed smear preparation—Papanicolaou stain). The histologic follow-up is compatible with follicular adenoma with papillary architecture (**C** and **D** hematoxylin and eosin stain)

NRAS mutations), though some may have other alterations including *PAX8::PPARγ* fusions [55, 56].

The FNA specimen from HTT can be misdiagnosed as suspicious or consistent with PTC, due to the fact that the polygonal or spindle-shaped cells of HTT frequently have nuclear pseudo-inclusions and grooves [57–61]. HTT can also mimic medullary thyroid carcinoma in FNA specimens due to hyaline material appearing similar to amyloid. Membranous MIB-1 performed on cell block specimen with adequate tumor cellularity can help to arrive at a diagnosis of HTT [59]. For FNA specimens that undergo molecular analysis, the finding of *GLIS3* fusions (or less frequently a *GLIS1* fusion) is diagnostic of HTT [57, 62].

Papillary Thyroid Carcinoma, the Subtypes Including Follicular Variant

The 2nd edition of TBSRTC II describes all the morphological criteria for the various subtypes of PTC, including classic (Fig. 3) and infiltrative follicular variant of PTC [7]. Nonetheless, it is well understood both among experts in cytology and treating clinicians that recognition of PTC subtypes and documenting subtype in the cytology report is not necessary [7] while infiltrative FVPTC is included as a subtype of PTC. Invasive encapsulated FVPTC (Fig. 4) is in a separate section of the WHO based on clinical behavior and molecular alterations. Whereas infiltrative FVPTC spreads to lymph nodes and may be associated with a *BRAF* p. V600E mutation, invasive encapsulated FVPTC spreads to distant sites and are RAS-driven tumors. Hence, the main difference between invasive encapsulated FVPTC and FTC is basedon the presence or absence or nuclear features of PTC. Nonetheless, on cytological samples, it is impossible to render a diagnosis of either invasive encapsulated FVPTC or FTC; as both are diagnosed based on criteria of capsular and/or vascular invasion [6]. These entities, in the presence of adequate cytomorphological features, are likely to be diagnosed as FN or SFM [7].

"High Grade" Non-Anaplastic Follicular Cell-Derived Thyroid Carcinoma

One of the remarkable changes in the 5th edition of WHO is the introduction of the diagnostic category "high-grade non-anaplastic follicular cell-derived thyroid carcinoma" [1, 2]. It defines a group of thyroid carcinomas with a prognosis intermediate between the favorable outcome of differentiated follicular cell-derived thyroid carcinomas (papillary and follicular carcinoma) and the very poor outcome of anaplastic carcinoma [1, 2].

This group includes poorly differentiated thyroid carcinoma (PDTC) and differentiated high-grade follicular cell-derived thyroid carcinoma (DHGTC). DHGTC do not meet morphologic criteria for poorly differentiated thyroid carcinoma (either because the tumor lacks solid/trabecular/insular growth or has maintained nuclear features of papillary

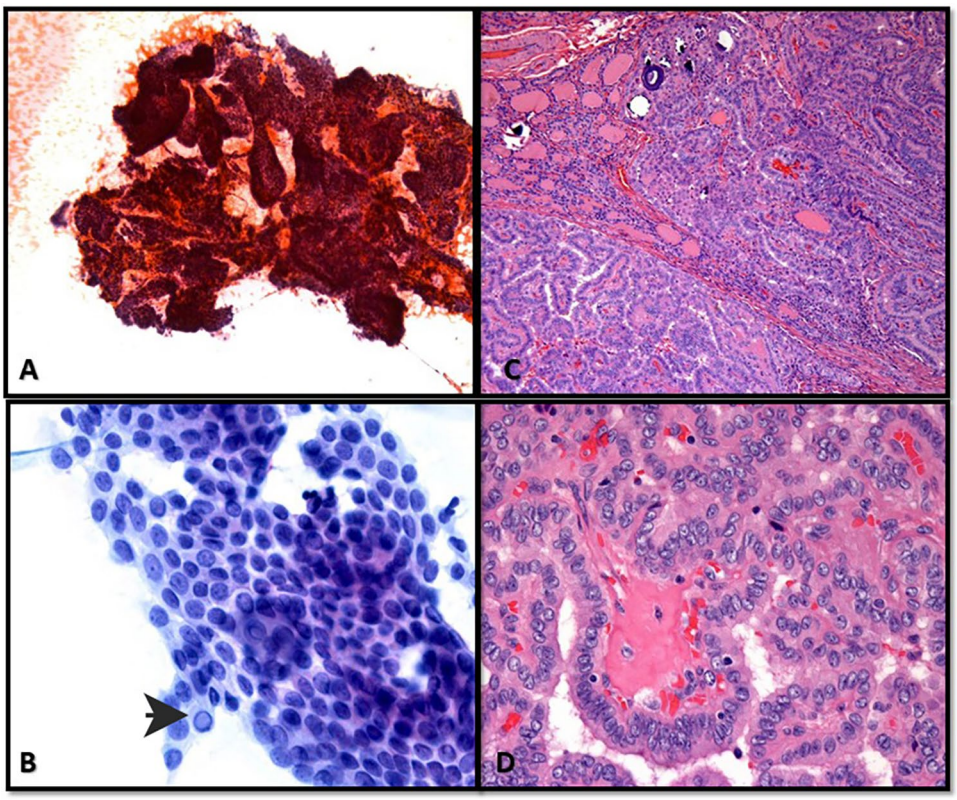

Fig. 3 Papillary thyroid carcinoma, classic subtype. Fine-needle aspiration cytology showing tumor with complex papillary architecture and the tumor cells demonstrating diagnostic nuclear features of papillary thyroid carcinoma including intranuclear inclusion – arrowhead (**A** and **B** alcohol fixed smear preparation—Papanicolaou stain)

thyroid carcinoma) but are high grade on the basis of an increased mitotic count (5 or more mitoses per 2 mm^2) or tumor necrosis [1, 2].

A "clear cut" diagnosis of poorly differentiated thyroid carcinoma in FN specimens can be difficult; most are diagnosed as FN/SFN (TBSRTC IV) because their cytomorphologic features overlap with both benign and malignant well-differentiated follicular cell-derived neoplasms, especially those with follicular growth pattern [63, 64]. Rare cases of PDTC are reported as suspicious for malignancy or malignant due to the presence of marked nuclear pleomorphism, foci of tumor necrosis and/or mitoses [63]. Because most DHGTC are high-grade PTC, a majority will be diagnosed as either suspicious for malignancy or malignant due to clinical presentation, suspicious ultrasound features, nuclear cytomorphology, or necrotic debris and mitoses [6, 20].

Immunohistochemical studies can be performed on cell blocks containing an adequate number of lesional cells. Both PDTC and DHGTC will be positive for TTF1, PAX8, cytokeratins (usually cytokeratin 7), and thyroglobulin [1].

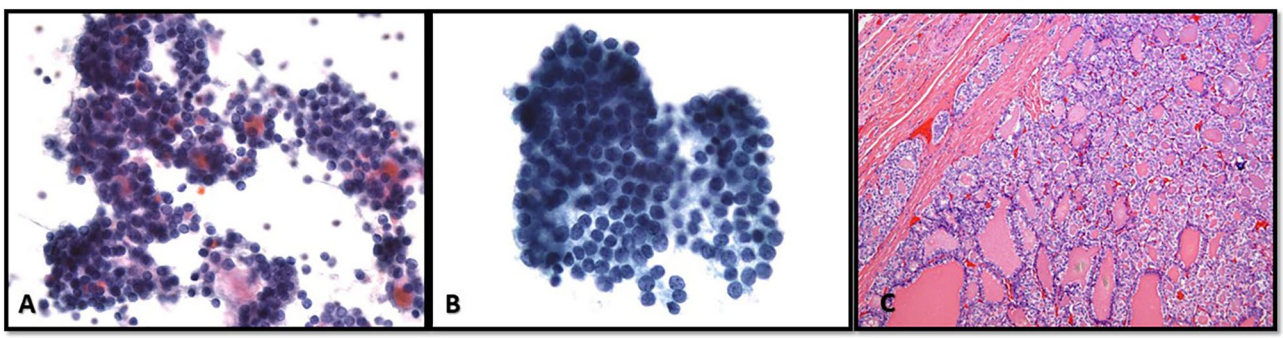

Fig. 4 Invasive encapsulated follicular variant of papillary thyroid carcinoma. The tumor cells are monotonous in appearance and forming microfollicles (**A** alcohol fixed smear preparation—Papanicolaou stain) and show focal nuclear elongation and nuclear chromatin clearing (**B** ThinPrep® preparation). The histologic follow-up shows an encapsulated follicular patterned tumor with capsular and angioinvasion (**C** hematoxylin and eosin stain). The high power showed nuclear cytology of papillary thyroid carcinoma

The Ki67 proliferation index is high, usually in the range of 10 to 30% and helps to confirm the diagnosis when performed on cytology cell block specimens. Molecular profiling can also be performed on cell block specimen as well as on the needle rinse. Whereas PDTC is generally a RAS-driven tumor, because most DHGTC are high-grade PTC, most harbor driver mutations in *BRAF* (*BRAF* p. V600E), or much less frequently, gene fusions such as *RET* or *NTRK3*. Both PDTC and DHGTC often carry aggressive secondary mutations, most frequently of the *TERT* promoter and in some cases, *PIK3CA* and *TP53* mutations [1, 2].

Squamous Cell Carcinoma: A Type of Anaplastic Carcinoma

In the 4th edition of WHO classification of thyroid tumors, squamous cell carcinoma of the thyroid was defined as a primary thyroid carcinoma composed almost entirely of squamous cells without a differentiated carcinoma component; it was considered a separate entity from anaplastic thyroid carcinoma [5]. Since then, studies have shown that it is a morphologic subtype of anaplastic thyroid carcinoma [1, 2, 65, 66]. In TBSRTC II Edition, ATC and SCC are described as separate entities; however, both are described in the same chapter [7]. The upcoming III edition of TBSRTC will include SCC among the ATC subtypes. Thyroid SCC is morphologically and immunochemically similar to squamous cell carcinomas originating in other organs, though p16 immunoreactivity favors non-thyroid origin, whereas PAX8 supports squamoid ATC. Correlation with clinical and imaging findings is essential for excluding a metastasis. The behavior of the squamous subtype is similar to ATC, as is the clinical management [1, 2, 65, 66].

A further implication on clinical management of ATC is testing of all anaplastic carcinoma cases for the presence of *BRAF* p.V600E mutation, which can be easily performed on cytological samples (preferably cell blocks), or as next-generation sequencing (NGS) [1, 2]. PDL1 testing is also frequently performed in most clinical practices. However, it is essential that cytology specimens should meet the adequacy requirement for PDL1 staining and interpretation [67].

Oncocytic Neoplasms and Neoplasms with Oncocytic Features

The differential diagnosis of a thyroid neoplasm with oncocytic cells includes the following: oncocytic adenoma, oncocytic carcinoma, oncocytic poorly differentiated carcinoma, oncocytic PTC and oncocytic medullary thyroid carcinoma. The term "oncocytic carcinoma of the thyroid" in the 5th edition of WHO refers to invasive malignant follicular cell neoplasms containing at least 75% oncocytic cells in which the nuclear features of PTC and high-grade features are absent [1, 2]. This term replaces the misnomer "Hürthle cell carcinoma," because Karl Hürthle described C cells located in a parafollicular location in canine thyroid glands [68].

TBSRTC uses the term "follicular neoplasm, oncocytic cell type" when a FNA specimen of a thyroid nodule is cellular and consists exclusively (or almost exclusively) of oncocytic cells; however, the oncocytic cells with nuclear features of PTC are classified as malignant, likely representing a subtype of PTC [20].

Similar to FTC, the diagnosis of oncocytic carcinoma is not possible in thyroid FNA specimens and will be classified as a follicular neoplasm with oncocytic features (Bethesda Category IV).

Molecular Profiling of Thyroid FNA Specimens, Beyond Diagnostic Confirmation

The last decade has witnessed how the molecular profiling of the FNA samples has revolutionized the clinical management of thyroid nodules [11, 34, 55, 69–73]. A large body of literature has shown that adjunct molecular testing of thyroid FNA specimens can serve in risk stratification and the identification of potentially actionable genetic events, such as *BRAF* p. V600E, *TERT* promoter mutations, *RET*, *NTRK1-3*, *BRAF*, and *ALK*, in cases of therapy-resistant disease progression [31, 55].

Immunostains for specific molecular targets can also be applied to cytology specimens with an adequate number of lesional cells as an adjunct to cytologic diagnosis [74]. This may also be useful for cytology laboratories around the world that are not able to offer next-generation sequencing due to monetary concerns [75]. The list of available and validated molecular immunostains that can be successfully employed in routine thyroid surgical pathology preparations is growing at a steady pace [76–78]. However, from this list, only VE1-BRAF immunostain has shown high sensitivity and specificity in confirming the diagnoses of PTC in thyroid FNA specimens [79–81].

Other Thyroid Neoplasms

The 5th WHO edition has suggested histopathologic grading of the medullary thyroid carcinoma based on recommendations of an international panel utilizing a multi-institutional case cohort. This is a two-tiered grading system consisting of low and high-grade tumors; the high-grade tumors are defined as having at least one of the three following features: tumor necrosis, mitotic count ≥ 5 per 2 mm^2, and/or a Ki67 proliferation index $\geq 5\%$ [1, 2, 82].

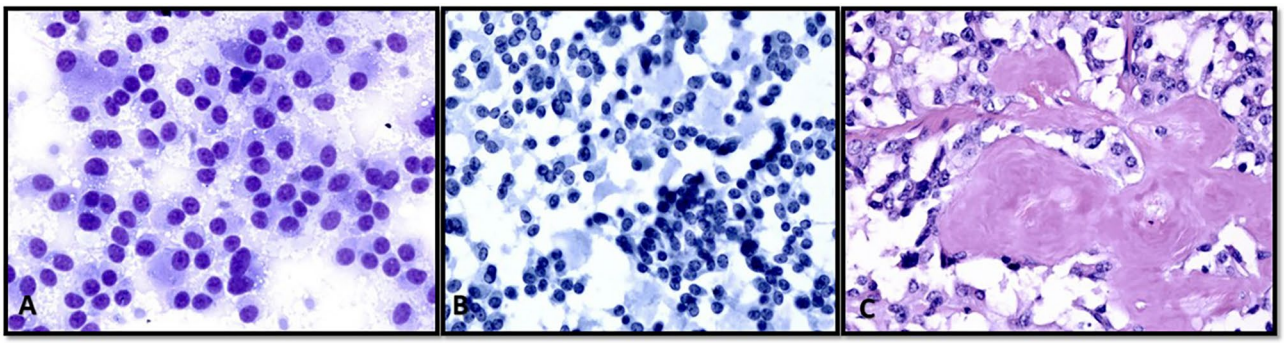

Fig. 5 Medullary thyroid carcinoma. The specimen shows plasmacytoid tumor cells with granular cytoplasm (**A** air-dried smear preparation—Diff-Quik®) and neuroendocrine nuclear chromatin "salt and pepper appearance" and background shows acellular material compatible with amyloid (**B** alcohol fixed smear preparation—Papanicolaou stain). The histologic follow-up shows medullary thyroid carcinoma with amyloid (**C** hematoxylin and eosin stain)

Cytologically, most cases of MTC show specific morphological features, a distinctive immunophenotype and a variable amount of amyloid (Fig. 5). The cytologic diagnosis of MTC needs to be confirmed by employing immunostains for calcitonin, CEA, TTF1, thyroglobulin, and neuroendocrine markers [2, 83].

It is well known that MTC show a wide range of cellular and architectural features in FNA samples. Despite the morphologic heterogeneity of MTC and various morphologic subtypes; TBSRTC states that the identification of a specific subtype MTC is not required in FNA samples [20]. The use of a grading system is discouraged on FNAC from MTC.

The diagnostic features of rare tumors of the thyroid categorized in the 5th edition of WHO classification of thyroid neoplasms including, salivary gland type neoplasms, thymic tumors, and tumors of uncertain histogenesis—including cribriform carcinoma of the thyroid (formerly classified as cribriform morular variant of PTC) and sclerosing mucoepidermoid carcinoma with eosinophilia [1, 2], are well described in cytologic literature and have been included in the TBSRTC [20, 84–88].

Conclusions

As highlighted in the current review, the changes in the 5th WHO edition will subtly or significantly impact the cytological diagnoses of some but not all entities. However, these changes will also affect the other thyroid FNA classification schemes used internationally for reporting thyroid FNA specimens.

Author Contribution Conception and design: ZB, ER; data collection and analysis: all authors; manuscript preparation and editing: all authors; approval of final manuscript: all authors.

Availability of Data and Material Not applicable.

Declarations

Ethics Approval Not applicable.

Consent for Publication All authors consent to the publication.

Conflict of Interest The authors declare no competing interests.

References

1. Christofer Juhlin C, Mete O, Baloch ZW The 2022 WHO classification of thyroid tumors: novel concepts in nomenclature and grading. Endocr Relat Cancer 30, 2023.
2. Baloch ZW, Asa SL, Barletta JAet al. Overview of the 2022 WHO Classification of Thyroid Neoplasms. Endocr Pathol 33: 27-63, 2022.
3. Hedinger C WE, Sobin LH: WHO. Histologic Typing of Thyroid Tumors, New York: Springer-Verlag, 1988.
4. DeLellis RA, Lloyd RD, Heitz PU, Eng C, eds.: WHO: Pathology and Genetics. Tumours of Endocrine Organs., Lyon, France: IARC Press, 2004, Pages.
5. WHO Classification of Tumours of Endocrine Organs, Lyon, France: WHO/IARC Press, 2017.
6. Baloch Z, LiVolsi VA The Bethesda System for Reporting Thyroid Cytology (TBSRTC): From look-backs to look-ahead. Diagn Cytopathol, 2020.
7. The Bethesda System for Reporting Thyroid Cytopathology. Definitions, Criteria, and Explanatory Notes., Switzerland, 2018.
8. Rosario PW, de Castro Nicolau T The value of ultrasonography for the indication of fine-needle aspiration in autonomous thyroid nodules. Diagn Cytopathol 49: 363-366, 2021.
9. Mistry R, Hillyar C, Nibber A, Sooriyamoorthy T, Kumar N Ultrasound Classification of Thyroid Nodules: A Systematic Review. Cureus 12: e7239, 2020.
10. Garber JR, Papini E, Frasoldati A et al. American Association of Clinical Endocrinology And Associazione Medici Endocrinologi Thyroid Nodule Algorithmic Tool. Endocr Metab Immune Disord Drug Targets 21: 2104-2115, 2021.

11. Grani G, Sponziello M, Pecce V, Ramundo V, Durante C Contemporary Thyroid Nodule Evaluation and Management. J Clin Endocrinol Metab 105, 2020.
12. Kim PH, Suh CH, Baek JH, Chung SR, Choi YJ, Lee JH Unnecessary thyroid nodule biopsy rates under four ultrasound risk stratification systems: a systematic review and meta-analysis. Eur Radiol 31: 2877-2885, 2021.
13. Maxwell C, Sipos J Clinical diagnostic evaluation of thyroid nodules. Endocrinol Metab Clin North Am 48: 61-84, 2019.
14. Wang Z, Vyas CM, Van Benschoten Oet al. Quantitative Analysis of the Benefits and Risk of Thyroid Nodule Evaluation in Patients >/=70 Years Old. Thyroid 28: 465-471, 2018.
15. Cordes M, Schmidkonz C, Horstrup K, Weppler M, Kuwert T Fine-needle aspiration biopsies of thyroid nodules. Nuklearmedizin 57: 211-215, 2018.
16. Anand B, Ramdas A, Ambroise MM, Kumar NP The Bethesda System for Reporting Thyroid Cytopathology: A Cytohistological Study. J Thyroid Res 2020: 8095378, 2020.
17. Vuong HG, Ngo HTT, Bychkov Aet al. Differences in surgical resection rate and risk of malignancy in thyroid cytopathology practice between Western and Asian countries: A systematic review and meta-analysis. Cancer Cytopathol 128: 238-249, 2020.
18. Guidelines of the Papanicolaou Society of Cytopathology for the examination of fine-needle aspiration specimens from thyroid nodules. The Papanicolaou Society of Cytopathology Task Force on Standards of Practice. Diagn Cytopathol 15: 84–89, 1996.
19. Cibas ES, Ali SZ The bethesda system for reporting thyroid cytopathology. THYROID 19: 1159-1165, 2009.
20. Ali SZ, Cibas ES The bethesda system for reporting thyroid cytopathology : definitions, criteria, and explanatory notes, 2018.
21. Durante C, Grani G, Lamartina L, Filetti S, Mandel SJ, Cooper DS The Diagnosis and Management of Thyroid Nodules: A Review. JAMA 319: 914-924, 2018.
22. Nguyen TPX, Truong VT, Kakudo K, Vuong HG The diversities in thyroid cytopathology practices among Asian countries using the Bethesda system for reporting thyroid cytopathology. Gland Surg 9: 1735-1746, 2020.
23. Park VY, Lee HS, Kim EK, Kwak JY, Yoon JH, Moon HJ Frequencies and malignancy rates of 6-tiered Bethesda categories of thyroid nodules according to ultrasound assessment and nodule size. Head Neck 40: 1947-1954, 2018.
24. Chakravarthy NS, Chandramohan A, Prabhu AJet al. Ultrasound-guided Fine-needle Aspiration Cytology along with Clinical and Radiological Features in Predicting Thyroid Malignancy in Nodules >/=1 cm. Indian J Endocrinol Metab 22: 597-604, 2018.
25. Hirokawa M, Suzuki A, Higuchi Met al. The Japanese reporting system for thyroid aspiration cytology 2019 (JRSTAC2019). Gland Surg 9: 1653-1662, 2020.
26. Trimboli P, Crescenzi A, Castellana M, Giorgino F, Giovanella L, Bongiovanni M 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 63: 430-438, 2019.
27. Cross PA, Poller D The Bethesda thyroid terminology and progress towards international agreement on thyroid FNA cytology reporting. Cytopathology 21: 71-74, 2010.
28. Poller DN, 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 Cytopathol 128: 36-42, 2020.
29. Pusztaszeri M, Rossi ED, Auger Met al. The Bethesda System for Reporting Thyroid Cytopathology: Proposed Modifications and Updates for the Second Edition from an International Panel. Acta Cytol 60: 399–405, 2016.
30. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW The Bethesda System for Reporting Thyroid Cytopathology: A Meta-Analysis. Acta Cytol 56: 333-339, 2012.
31. Nylen C, Mechera R, Marechal-Ross Iet al. Molecular Markers Guiding Thyroid Cancer Management. Cancers (Basel) 12, 2020.
32. Nishino M, Nikiforova M Update on Molecular Testing for Cytologically Indeterminate Thyroid Nodules. Arch Pathol Lab Med 142: 446-457, 2018.
33. Bizzarro T, Martini M, Capodimonti Set al. Young investigator challenge: The morphologic analysis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features on liquid-based cytology: Some insights into their identification. Cancer 124: 699-710, 2016.
34. Carty SE, Ohori NP, Hilko DAet al. The Clinical Utility of Molecular Testing in the Management of Thyroid Follicular Neoplasms (Bethesda IV Nodules). Ann Surg 272: 621-627, 2020.
35. Sheffield BS, Masoudi H, Walker B, Wiseman SM Preoperative diagnosis of thyroid nodules using the Bethesda System for Reporting Thyroid Cytopathology: a comprehensive review and meta-analysis. Expert Rev Endocrinol Metab 9: 97-110, 2014.
36. Brown C, Mangano W, Thompson S, Richmond B Factors Predicting Thyroid Malignancy in Fine Needle Aspiration Biopsy Specimens Classified as Atypia of Uncertain Significance/Follicular Lesion of Uncertain Significance. Am Surg 84: 1207-1213, 2018.
37. Mosca L, Silva L, Carneiro PCet al. Malignancy rates for Bethesda III subcategories in thyroid fine needle aspiration biopsy (FNAB). Clinics (Sao Paulo) 73: e370, 2018.
38. Valderrabano P, Khazai L, Thompson ZJet al. Cancer risk stratification of indeterminate thyroid nodules: A cytological approach. THYROID, 2017.
39. Renshaw AA Accuracy of thyroid fine-needle aspiration using receiver operator characteristic curves. Am J Clin Pathol 116: 477–482., 2001.
40. Faquin WC The thyroid gland: recurring problems in histologic and cytologic evaluation. Arch Pathol Lab Med 132: 622-632, 2008.
41. VandenBussche CJ, Baloch ZW The cytologic diagnosis of "atypical": Criteria and controversies. Diagn Cytopathol 50: 143-145, 2022.
42. Poller DN, Megadmi H, Ward MJA, Trimboli P Hurthle Cells on Fine-Needle Aspiration Cytology Are Important for Risk Assessment of Focally PET/CT FDG Avid Thyroid Nodules. Cancers (Basel) 12, 2020.
43. Sengul D, Sengul I, Van Slycke S Risk stratification of the thyroid nodule with Bethesda indeterminate cytology, category III, IV, V on the one surgeon-performed US-guided fine-needle aspiration with 27-gauge needle, verified by histopathology of thyroidectomy: the additional value of one surgeon-performed elastography. Acta Chir Belg: 1–9, 2019.
44. Granel-Villach L, Fortea-Sanchis C, Laguna-Sastre JM, Escrig-Sos J Performance of the Bethesda system in the citopathological diagnosis of the thyroid nodule. Cirugia espanola 96: 599-600, 2018.
45. Jovanovic L, Delahunt B, McIver B, Eberhardt NL, Grebe SK Thyroid gland clonality revisited: the embryonal patch size of the normal human thyroid gland is very large, suggesting X-chromosome inactivation tumor clonality studies of thyroid tumors have to be interpreted with caution. J Clin Endocrinol Metab 88: 3284-3291, 2003.
46. Landau MS, Nikiforov YE, Ohori NP, Chiosea SI Impact of molecular testing on detecting mimics of oncocytic neoplasms in thyroid fine-needle aspirates diagnosed as follicular neoplasm of Hurthle cell (oncocytic) type. Cancer Cytopathol, 2021.
47. Ohori NP Molecular testing and thyroid nodule management in North America. Gland Surg 9: 1628–1638, 2020.
48. Khayyata S, Barroeta JE, LiVolsi VA, Baloch ZW Papillary hyperplastic nodule: pitfall in the cytopathologic diagnosis of papillary thyroid carcinoma. Endocr Pract 14: 863-868, 2008.

49. Albores-Saavedra J, Wu J The many faces and mimics of papillary thyroid carcinoma. Endocr Pathol 17: 1-18, 2006.
50. Khurana KK, Baloch ZW, LiVolsi VA Aspiration cytology of pediatric solitary papillary hyperplastic thyroid nodule. Arch Pathol Lab Med 125: 1575-1578., 2001.
51. Guan H, Matonis D, Toraldo G, Lee SL Clinical Significance of Thyroid-Stimulating Hormone Receptor Gene Mutations and/or Sodium-Iodine Symporter Gene Overexpression in Indeterminate Thyroid Fine Needle Biopsies. Frontiers in endocrinology 9: 566, 2018.
52. LiVolsi VA, Baloch Z Noninvasive Follicular Tumor With Papillary-like Nuclear Features: A Practice Changer in Thyroid Pathology. Arch Pathol Lab Med, 2020.
53. Rossi ED, Faquin WC, Baloch Zet al. Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features (NIFTP): Update and Diagnostic Considerations-a Review. Endocr Pathol 30: 155-162, 2019.
54. Zhou H, Baloch ZW, Nayar Ret al. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP): Implications for the risk of malignancy (ROM) in the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). Cancer Cytopathol 126: 20-26, 2018.
55. Macerola E, Poma AM, Vignali Pet al. Molecular Genetics of Follicular-Derived Thyroid Cancer. Cancers (Basel) 13, 2021.
56. Basolo F, Macerola E, Ugolini C, Poller DN, Baloch Z The Molecular Landscape of Noninvasive Follicular Thyroid Neoplasm With Papillary-like Nuclear Features (NIFTP): A Literature Review. Adv Anat Pathol 24: 252-258, 2017.
57. Nikiforova MN, Nikiforov YE, Ohori NP GLIS rearrangements in thyroid nodules: A key to preoperative diagnosis of hyalinizing trabecular tumor. Cancer Cytopathol 127: 560-566, 2019.
58. Bishop JA, Ali SZ Hyalinizing trabecular adenoma of the thyroid gland. Diagn Cytopathol 39: 306-310, 2011.
59. Agarwal S, Mathur SR, Ray R, Sharma SC, Sinha P Cytopathological diagnosis of hyalinizing trabecular tumour, a rare thyroid neoplasm. Cytopathology 21: 133-134, 2010.
60. Gupta S, Modi S, Gupta V, Marwah N Hyalinizing trabecular tumor of the thyroid gland. J Cytol 27: 63-65, 2010.
61. Baloch ZW, LiVolsi VA Cytologic and architectural mimics of papillary thyroid carcinoma. Diagnostic challenges in fine-needle aspiration and surgical pathology specimens. Am J Clin Pathol 125 Suppl: S135–144, 2006.
62. Nikiforova MN, Nikitski AV, Panebianco Fet al. GLIS Rearrangement is a Genomic Hallmark of Hyalinizing Trabecular Tumor of the Thyroid Gland. Thyroid 29: 161-173, 2019.
63. Bongiovanni M, Sadow PM, Faquin WC Poorly differentiated thyroid carcinoma: a cytologic-histologic review. Adv Anat Pathol 16: 283-289, 2009.
64. Bongiovanni M, Bloom L, Krane JFet al. Cytomorphologic features of poorly differentiated thyroid carcinoma: a multi-institutional analysis of 40 cases. Cancer Cytopathol 117: 185-194, 2009.
65. Xu B, Fuchs T, Dogan Set al. Dissecting Anaplastic Thyroid Carcinoma: A Comprehensive Clinical, Histologic, Immunophenotypic, and Molecular Study of 360 Cases. THYROID 30: 1505-1517, 2020.
66. Deeken-Draisey A, Yang GY, Gao J, Alexiev BA Anaplastic thyroid carcinoma: an epidemiologic, histologic, immunohistochemical, and molecular single-institution study. Hum Pathol 82: 140-148, 2018.
67. Pusztaszeri MP, Bongiovanni M, Brimo F Do we need PD-L1 as a biomarker for thyroid cytologic and histologic specimens? Cancer Cytopathol 128: 160-165, 2020.
68. Hurthle K Beitrage zur kenntnis des sekretionsvorganges in der schilddruse. Arch FD Ges Physiol 56: 1, 1894.
69. Nikiforova MN, Lepe M, Tolino LAet al. Thyroid cytology smear slides: An untapped resource for ThyroSeq testing. Cancer Cytopathol, 2020.
70. Kumar N, Gupta R, Gupta S Molecular testing in diagnosis of indeterminate thyroid cytology: Trends and drivers. Diagn Cytopathol 48: 1144-1151, 2020.
71. Ngo HTT, Nguyen TPX, Vu THet al. Impact of Molecular Testing on the Management of Indeterminate Thyroid Nodules Among Western and Asian Countries: a Systematic Review and Meta-analysis. Endocr Pathol, 2020.
72. Ohori NP, Landau MS, Manroa P, Schoedel KE, Seethala RR Molecular-derived estimation of risk of malignancy for indeterminate thyroid cytology diagnoses. J Am Soc Cytopathol 9: 213-220, 2020.
73. Spaulding SL, Ho R, Everest S, Chai RL The role of molecular testing in the diagnosis of medullary thyroid cancer: A case report of oncocytic medullary thyroid carcinoma and review of the literature. Am J Otolaryngol 41: 102312, 2020.
74. Rossi ED, Raffaelli M, Minimo Cet al. Immunocytochemical evaluation of thyroid neoplasms on thin-layer smears from fine-needle aspiration biopsies. Cancer 105: 87-95, 2005.
75. Rossi ED, Raffaelli M, Mule Aet al. Relevance of Immunocytochemistry on Thin-layer Cytology in Thyroid Lesions Suspicious for Medullary Carcinoma: A Case-control Study. Appl Immunohistochem Mol Morphol, 2008.
76. Papathomas TG, Nose V New and Emerging Biomarkers in Endocrine Pathology. Adv Anat Pathol 26: 198-209, 2019.
77. Dvorak K, Higgins A, Palting J, Cohen M, Brunhoeber P Immunohistochemistry with Anti-BRAF V600E (VE1) Mouse Monoclonal Antibody is a Sensitive Method for Detection of the BRAF V600E Mutation in Colon Cancer: Evaluation of 120 Cases with and without KRAS Mutation and Literature Review. Pathol Oncol Res 25: 349-359, 2019.
78. Baloch Z, Mete O, Asa SL Immunohistochemical Biomarkers in Thyroid Pathology. Endocr Pathol 29: 91-112, 2018.
79. Straccia P, Brunelli C, Rossi EDet al. The immunocytochemical expression of VE-1 (BRAF V600E-related) antibody identifies the aggressive variants of papillary thyroid carcinoma on liquid-based cytology. Cytopathology 30: 460-467, 2019.
80. Smith AL, Williams MD, Stewart Jet al. Utility of the BRAF p.V600E immunoperoxidase stain in FNA direct smears and cell block preparations from patients with thyroid carcinoma. Cancer Cytopathol 126: 406–413, 2018.
81. Lee SR, Yim H, Han JHet al. VE1 antibody is not highly specific for the BRAF V600E mutation in thyroid cytology categories with the exception of malignant cases. Am J Clin Pathol 143: 437-444, 2015.
82. Alzumaili B, Xu B, Spanheimer PMet al. Grading of medullary thyroid carcinoma on the basis of tumor necrosis and high mitotic rate is an independent predictor of poor outcome. Mod Pathol 33: 1690-1701, 2020.
83. Liu CY, Bychkov A, Agarwal Set al. Cytologic diagnosis of medullary thyroid carcinoma in the Asia-Pacific region. Diagn Cytopathol 49: 60-69, 2021.
84. Baloch ZW, LiVolsi VA Special types of thyroid carcinoma. Histopathology 72: 40-52, 2018.
85. Solomon AC, Baloch ZW, Salhany KE, Mandel S, Weber RS, LiVolsi VA Thyroid Sclerosing Mucoepidermoid Carcinoma With Eosinophilia: Mimic of Hodgkin Disease in Nodal Metastases. Arch Pathol Lab Med 124: 446-449, 2000.
86. Baloch ZW, Solomon AC, LiVolsi VA Primary mucoepidermoid carcinoma and sclerosing mucoepidermoid carcinoma with eosinophilia of the thyroid gland: a report of nine cases. Mod Pathol 13: 802-807., 2000.
87. Erickson LA Unique Clinical Significance of the Cribriform-Morular Variant of Papillary Thyroid Carcinoma. Mayo Clin Proc 95: 831–833, 2020.
88. Sakurai K, Onouchi T, Yamada Set al. Cytohistology of morule in cribriform-morular variant of papillary thyroid carcinoma. Malays J Pathol 41: 339-343, 2019.
89. Ali SZ, Cibas ES: The Bethesda System for Reporting Thyroid Cytopathology Definitions, Criteria and Explanatory Notes, Boston, MA: Springer US, 2010.

90. Ali SZ Thyroid cytopathology: Bethesda and beyond. Acta Cytol 55: 4-12, 2011.
91. Bongiovanni M, Faquin WC, Giovanella L, Durante C, Kopp P, Trimboli P Impact of non-invasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP) on risk of malignancy in patients undergoing lobectomy/thyroidectomy for suspected malignancy or malignant fine-needle aspiration cytology findings: a systematic review and meta-analysis. Eur J Endocrinol 181: 389-396, 2019.
92. Haaga E, Kalfert D, Ludvikova M, Kholova I Non-Invasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features Is Not a Cytological Diagnosis, but It Influences Cytological Diagnosis Outcomes: A Systematic Review and Meta-Analysis. Acta Cytol 66: 85-105, 2022.
93. Krauss EA, Mahon M, Fede JM, Zhang L Application of the Bethesda Classification for Thyroid Fine-Needle Aspiration: Institutional Experience and Meta-analysis. Arch Pathol Lab Med 140: 1121-1131, 2016.