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Cytology of thyroid and parathyroid glands in oncology diagnosis – a contemporary review of updates and innovations

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Abstract

Fine needle aspiration (FNA) is widely used in the examination of head and neck lesions and has been considered an important diagnostic tool in the evaluation of thyroid and parathyroid nodules. Thyroid nodules are frequent findings in the general population, although 90-95% of these nodules are benign. FNA plays a crucial role to determine which nodules are at greatest risk of malignancy and which nodules are benign and do not require surgical intervention. In the case of the parathyroid glands, the US-guided parathyroid FNA is an effective method for the identification of intrathyroidal or ectopic parathyroid tissue, and distinguish it from thyroid and other surrounding anatomical structures. In addition, the use of FNA can significantly increase the accuracy of parathyroid gland location in patients with hyperparathyroidism who are candidates for surgical treatment in cases where imaging techniques fail to identify the parathyroid. Widespread US guidance in FNA procedures, constellation of clearly defined, reproducible key diagnostic cytopathological criteria for individual lesions in conjunction with images and clinical data as well as evolutions in FNA techniques and ancillary tests facilitate further diagnostic and clinical management. This paper aims to review the current state of the art in cytological evaluation of thyroid and parathyroid lesions.

Key words: cytology, fine needle aspiration, thyroid, parathyroid, cytological diagnosis

Introduction

Most thyroid and parathyroid nodules represent benign colloid nodules and parathyroid adenomas or hyperplastic parathyroid glands respectively. Malignant tumors represent less than 5% of all thyroid tumors and less than 1% of parathyroid tumors [1–4]. The selection of patients with malignant thyroid tumors and parathyroid tumors who are eligible for surgical treatment is the greatest challenge in the work up of thyroid and parathyroid nodules. Surgical treatment can result in complications such as post-operative thyroid hormone imbalance, hypoparathyroidism, recurrent laryngeal nerve injury, bleeding and infection. Surgical treatment of benign tumors should be limited to cases of hyperthyroidism and hyperparathyroidism, nodular lesions in conjunction with Graves's disease and patients with compressive symptoms. Numerous studies have found fine needle aspiration (FNA) to be a minimally invasive, safe, accurate, and cost-effective diagnostic tool for management of thyroid and parathyroid nodules. As ultrasound- (US) guided thyroid FNA is recommended for initial evaluation of thyroid nodules and to triage patients based on its results [5,6], the cytologic examination of parathyroid has not been recommended in the past [7–9]. Dense fibrotic reaction to the needle, disruption of the lesion and seeding along the needle tract has been reported as complications caused by parathyroid FNA [10–15]. However, FNA is an efficient technique for the identification of parathyroid tissue in patients with intrathyroidal or ectopic parathyroid gland location, and to distinguish it from thyroid and other surrounding anatomical structures. In cases of persistent hypercalcemia after a failed surgery or in a recurrent disease when neck anatomy is distorted, the use of US-guided FNA can significantly increase the accuracy of parathyroid gland localization [16–21].

Clinical perspectives and role of image studies

Thyroid

Thyroid nodules are common with a higher prevalence in woman, and with palpable nodules found in 4–7 % of adults [1, 3, 22–25] and subclinical nodules identified in approximately 70% of adults [26, 27]. Although 90–95% of thyroid nodules are benign, the rate of thyroid cancer has been on the rise

over the last 3 decades [5, 27, 28]. Factors such as an increase in radiation exposure and more frequent diagnostic imaging with higher resolution US as well as overall diagnostic improvements contribute to this trend. Increased detection of microcarcinomas is also attributed to increasing prevalence of thyroid cancer worldwide [28–31].

Thyroid nodules are more frequent in women than in men and the female risk of carcinoma is approximately 3-fold compared to that of men. Pregnancy and the effect of estrogen have been suggested as factors associated with increased risk of malignancy [32]. Chronic iodine deficiency has been known to be a risk factor for goiter and follicular thyroid carcinoma [32, 33]. On the other hand, according to some epidemiologic studies, iodine excess might increase the incidence of papillary thyroid carcinoma [34]. Other risk factors include environmental factors such as ionizing radiation. The risk of radiation-related thyroid carcinoma was shown to be 3-fold higher in iodine deficient areas than elsewhere [35–37].

Tumors of the thyroid gland are the most common endocrine neoplasm. The majority of these derived from follicular epithelial cells, a smaller number from calcitonin-secreting C cells and rarely from both follicular and C cells [38]. Somatic rearrangements of the *RET* proto-oncogene occur in about 35% and *BRAV* V600E mutations in 45% of adult sporadic papillary carcinoma [38–42]. Follicular thyroid carcinoma and thyroid neoplasms with follicular architecture and an expansive but not infiltrative growth pattern are characterized by a high incidence of *RAS* mutations [43]. Medullary thyroid carcinoma, tumor derived from C cells, is characterized by *RET* and *RAS* mutations being detected in 80–90% of cases [44].

US examination provides valuable information about the sonographic characteristics of thyroid nodules. Sonographic characteristics for suspicious malignant nodules include irregular margins, solid structure, markedly hypoechogenicity, microcalcifications, larger vertical than horizontal dimensions and dominant central vascularity. Benign nodules are usually well-defined, isoechoic, with regular borders, without microcalcifications and commonly cystic.

In 2009 a standardized risk-stratification system for thyroid lesions (TI-RADS), assessing the risk of malignancy of thyroid nodules based on ultrasound features, was proposed. TI-RADS scale informs practitioners about the risk of malignancy and further management of the lesion. Since then, there have been various modifications to this scale, and similar systems were established by the European Thyroid Association (EU-TI-RADS), the American College of Radiology (ACR-TI-RADS), the American Thyroid Association (ATA guidelines) and the Korean Society of Thyroid Radiology (K-TI-RADS) [45–48]. In 2022, Polish Scientific Societies and the National Oncological Strategy introduced updated recommendations referring to diagnosis and treatment of thyroid cancer in adult patients. In that guideline, the EU-TI-RADS-PL classification, modified based on EU-TIRADS classifier, was introduced. According to EU-TI-RADS-PL, the malignancy risks of thyroid lesions are evaluated as non-suspicious (TR2), low-risk (TR3), intermediate-risk (TR4) and high-risk (TR5) nodules. The most significant modification, compared to EU-TI-RADS, referred to EU-TIRADS-PL 5 class with indication for FNA in TR5 nodules of dimension ≥5mm (instead of ≥10mm in EU-TI-RADS 5 lesions) [49].

Parathyroid

Typically, parathyroid glands are small, weighing between 30–50 mg, with an oval or kidney-shaped appearance, and their color ranges from yellow to brown. They usually measure 2–7 mm in size. It's common for a person to have four parathyroid glands, but variations exist, and about 15% of individuals may have additional parathyroid glands. The upper parathyroid glans, which emerge from the fourth branchial pouches alongside the thyroid gland lateral anlages, are generally found behind the middle section of the thyroid at the level of isthmus. Less than 2% of upper glands occur in ectopic location. Conversely, the lower parathyroids and the thymus originate from the third branchial pouch, usually located lateral to, or less commonly slightly below the thyroid's lower pole. Due to their shared embryonic origins with the thyroid gland and thymus, ectopic parathyroid glands can be found in locations like the mediastinum or near the carotid sheath in the aortopulmonary

window in 10–20% of individuals. A small subset of intrathyroidal parathyroids account for about 2% of all cases.

Sporadic hyperparathyroidism is a leading cause of hypercalcemia, with parathyroid adenomas being identified in the majority (more than 85%) of primary hyperparathyroidism cases. Hyperactivity in all four glands is the second most common cause of primary hyperparathyroidism (10% to 15%) while parathyroid carcinoma is found in less than 1% of cases [16, 50, 51]. The diagnosis of hyperparathyroidism hinges on elevated levels of parathyroid hormone (PTH) and calcium in the blood. Both PTH and calcium are elevated in the majority of the cases. The standard care of primary hyperparathyroidism is preoperatively identification and subsequent surgical removal of the affected gland or glands. Surgical removal of the affected glands has evolved from extensive, bilateral neck explorations to less invasive techniques such as minimally invasive parathyroidectomy.

A different approach including radical surgery is required in cases of suspicious parathyroid carcinoma The localization of parathyroid abnormalities in patients with primary hyperparathyroidism is essential for surgical planning. Techniques like ultrasound (US) and Technetium 99 m sestamibi scans (99 Tc-MIBI) are commonly employed, with other methods including 4-dimensional computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) serving as alternatives, especially when initial imaging is inconclusive [7, 16]. Both US and sestamibi scintigraphy studies show that the effectiveness of these imaging modalities varies by the specific location of pathologic parathyroid glands. Both methods are more sensitive in detecting lower left adenomas than upper right ones. Overall, the positive predictive value for all parathyroid gland sites is approximately 54% for sestamibi scintigraphy and 59% for US, respectively. High-resolution US, in particular, has demonstrated a broad range of sensitivity and specificity between 51% and 87% and between 90% and 98% respectively. The PET/CT scans with 18F-labeled choline analogues might be considered as the alternative imaging method of parathyroid glands, especially in patients with negative or equivocal first-line imaging tool findings [19, 20, 52, 53]. The size of the parathyroid glands does not necessarily correlate with their function, and common limitations of US are the

identification of small, multiple, or ectopic parathyroid glands and to differentiate parathyroid tissue from thyroid nodules or other neck structures. Although imaging studies are used to localize the lesion and not to diagnose hyperplasia or parathyroid neoplasms, up to 25% of parathyroid adenomas might not be detectable via US or sestamibi scans, highlighting the challenges evident in localizing these glands [16, 52, 53].

The role of cytopathology and sampling techniques

FNA is widely accepted as a safe, cost-effective, and accurate diagnostic modality that may be performed in an outpatient setting.

For FNA of thyroid and parathyroid lesions, 27- to 22-gauge (0.4–0.7 mm) needles are suitable, either using a capillary technique without aspiration or a plastic disposable 10- or 20-ml syringe attached to a plastic or metal syringe holder (FNA with aspiration). Both air-dried and alcohol-fixed smears as well as liquid-based preparations have been used in evaluating thyroid and parathyroid FNAs. Adjunct use of cell block preparations may also be helpful in certain situations. There are two common fixation methods: air drying or wet fixing using either 95% ethanol or ethanol based Cytospray as a fixative. Both fixation methods are largely determined by local practice patterns and provide comparable results for a reliable diagnosis performance. Wet fixation usually better demonstrates such details as nuclear pattern, chromatin structure and nucleoli, and match nuclear and cytoplasmic structures observed in histologic sections. Air-dried specimens give better information on cytoplasmic details and the background material. Air dried smears can be stained with Diff-Quik or May-Grünwald-Giemsa (MGG), and wet fixed smears with hematoxylin and eosin (H&E) or Papanicolaou (Pap) [54].

The liquid-based preparation techniques have been reported as a valid method for cytologic diagnosis of thyroid and parathyroid lesions. Cellular morphology and nuclear details may appear more prominent, and the architectural pattern may show only minor differences as compared to conventional smears. However, smears from thyroid nodules may contain less colloid, the nuclear

hallmarks of papillary carcinomas may be vague, and cell shrinkage and disruption of the cytoplasm may be more pronounced.

In many institutions, aspirations are routinely performed by a clinician or radiologist without the assistance of a cytopathologist or cytotechnologist. A less skilled aspirator may see a higher percentage of unsatisfactory FNA smears, and on-site adequacy evaluations (ROSE) can be helpful in reducing the number of nondiagnostic specimens. ROSE allows the cytopathologists to achieve important clinical information and is a prerequisite for the multimodal approach. The ROSE procedure, however, is costly and time-consuming with divergent results in respect to the FNA adequacy rate. The benefit of on-site evaluations depends on the experience of the operator and the skill and expertise of the interpreting cytopathologist or cytotechnologist [55–57].

FNA of the thyroid has emerged as a minimally invasive, precise and reliable method for managing thyroid nodules. Cytopathology has a low false-negative rate for diagnosis of thyroid malignancy and offers crucial insights into the characteristics of thyroid nodules, aiding in the differentiation between benign and suspicious nodules. US-guided FNA has significantly increased the triage efficiency, decreased the rate of unnecessary surgery for benign thyroid nodules and helped to identify nodules that are most appropriate for surgical management [54]. In cases of benign FNA**,** the recommendation is to monitor the patient periodically with imaging. In cases of malignant FNA or suspicion of malignancy, the recommendation is to undergo surgical treatment such as lobectomy or total thyroidectomy. Controversy still exists regarding the accuracy of FNA, and the clinical management for thyroid nodules smaller than 1 cm or greater than 4 cm [6, 58]. FNA of a palpable thyroid mass may be performed by any clinician or cytopathologist with appropriate experience and in the past, aspirations were often performed only with manual aid. In the last decades, thyroid FNA is increasingly performed using ultrasound US guidance. The US-guided FNA is a safe and effective method that has proven to be superior to palpation-guided FNA to reduce inadequate sampling and the need for repeated FNA with inadequate sample rates of 14–21% *versus* 32–50 %, respectively

[57–60]. Complications due to FNA of the thyroid gland are rare and may include persistent pain, hematoma, infection, and recurrent laryngeal nerve palsy.

In the past, thyroid FNA reporting generated much confusion for both clinicians and pathologists due to multiple different reporting schemes and descriptive reports that did not clearly convey malignancy risk. In 2007, more uniform and evidence-based reporting schemes have been instituted, including the widely implemented six-tiered Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), arising from the National Cancer Institute in Bethesda, Maryland. As a result, the Bethesda system provides clarity of communication, facilitating the exchange of data across institutions, as well as having an implicit cancer risk associated with each category to guide appropriate clinical management [54]. Since its introduction and publishing the first edition of TBSRTC, two additional editions of the Bethesda system have been published. The updated 3rd edition published in 2023 [61, 62] reflects advances in the field of thyroid cytology in the last decade such as the expanded use of ancillary testing in the cytological diagnosis of neoplastic disease. A simplified reporting structure; updated and recalculated risk of malignancy (ROM) for each category and harmonization of nomenclature with the 2022 World Health Organization classification of thyroid tumors [38, 61] (tab. I) are important topics that have been expanded and updated in the 3rd edition of TBSRTC. After the introduction of TBSRTC, the system has been most widely accepted in Poland with some minor modifications. Polish scientific society recommendations regarding the cytological diagnosis of thyroid nodules and treatment of thyroid malignancies were updated and published in 2022 [48]. The terminology, diagnostic categories, risk of malignancy and patient management are consistent with the Bethesda recommendations.

FNA diagnosis of many thyroid lesions is based on cytological patterns and the distinctive cytological features of FNA smears, and can be a precise match to the endocrine pathology diagnosis. In others, the cytological examination can show a particular pattern that can help to place the lesion in a specific diagnostic category of TBSRTC but may not provide a specific histological diagnosis [61, 62].

Normal components of a thyroid FNA are comprised of follicular cells and colloid. Follicular cells may appear as intact macrofollicles or flat sheets of uniformly spaced follicular cells with small round nuclei and condensed chromatin. Microfollicles composed of 15 or fewer follicular cells are absent or only a minor component in aspirates of non-neoplastic thyroid nodules. Follicular cells that underwent oncocytic metaplasia show abundant granular cytoplasm with enlarged, round, eccentrically placed nuclei with prominent nucleoli. Colloid has a variable appearance and may appear as rounded or irregular aggregates with a jagged “cracking” artifact. Smears of common cystic thyroid nodules exhibit hemosiderin-laden macrophages and “cyst-lining” cells with elongated, drawn-out cytoplasm. Multinucleated giant cells may be seen in thyroid aspirates, although these cells are not specific for either benignity or malignancy. Nonthyroidal elements may be obtained through transit of the needle to the target lesion. Of nonthyroidal elements, smears of parathyroid nodules and lymph nodes may mimic thyroid lesions [54].

Adequacy in thyroid aspirates generally requires identification of six or more groups of at least 10 follicular cells per group. Any aspirate with a diagnostic abnormality is counted as adequate regardless of cellularity. Classification of cystic lesions lacking adequate follicular cells as nondiagnostic has been a controversial topic and in the 3rd edition of TBSRTC, FNA smears of colloid nodules that consist of abundant colloid without minimum number of follicular cells are considered satisfactory for evaluation and benign (TBSRTC category II).

Key diagnostic cytopathological features and ancillary tests

Key cytological description and clearly defined and reproducible cytological criteria are established for most benign and malignant diagnoses in thyroid pathology. Benign diagnoses with distinctive cytologic diagnostic criteria include follicular nodular diseases, colloid cysts, and thyroiditis. Papillary carcinoma accounts for 80% of all thyroid cancers and has readily recognizable cytologic features. (fig. 1A–D) Several histologic variants of papillary carcinoma have been described. The cytologic features

of most of these overlap and specific recognition of subtypes is difficult or impossible. However, diagnosis of a specific subtype of papillary carcinoma is not clinically necessary in most instances [54].

In the new 2022 WHO classification scheme, tumors of the thyroid gland are stratified into the following main categories:

- follicular cell-derived neoplasms,
- C-cell derived neoplasms,
- mixed medullary and follicular cell derived neoplasms,
- salivary gland type carcinomas,
- thyroid tumors of uncertain histogenesis,
- thymic tumors within the thyroid, and
- embryonal thyroid neoplasms.

Even though cytologic criteria for most malignant entities exists, specific and accurate diagnosis of some thyroid malignancies requires synergies with immunocytochemistry and molecular tests [63–72]. Thyroglobulin indicates follicular thyroid cells and calcitonin parafollicular cells with high specificity. Thyroglobulin positivity occur in most follicular neoplasms of the thyroid, and for the columnar cell variant of papillary carcinoma. Thyroid neoplasms of follicular origin show immunopositivity for thyroid transcription factor-1 (TTF-1) and the cells of oncocytic tumors for thyroglobulin and for low-molecular-weight keratin. TTF-1 shows nuclear expression by IHC in thyroid follicular and parafollicular cells and lungs. TTF-1 is diffusely expressed in papillary thyroid carcinoma, follicular thyroid carcinoma, high-grade follicular-derived non-anaplastic thyroid carcinoma, and medullary thyroid carcinoma. Immunopositivity for calcitonin, carcinoembryonic antigen (CEA) and neuroendocrine markers, such as chromogranin, synaptophysin, and rarely CD56 is appropriate for the diagnosis of medullary carcinoma. In addition, the second-generation neuroendocrine markers insulinoma-associated protein 1 (INSM1) is a highly sensitive and specific marker in the diagnosis of medullary thyroid carcinoma and C-cell hyperplasia. The anaplastic type of thyroid cancer shows inconsistent positive reactivity for cytokeratin, PAX8, p53 and occasionally TTF-1. An immunopanel

comprising thyroglobulin, TTF-1, GATA-3, PTH and chromogranin is helpful to distinguish cells of thyroid origin from those of parathyroid origin [63-65]. Example of immunoprofiles for primary thyroid neoplasms and parathyroid lesions are presented in Table 2. In addition, the selective panels of antibodies may be applicable to differentiate tumors of thyroid origin from neoplasms metastatic to the thyroid gland. For example, panel of CK7, CK20, TTF-1, CDX2, CEA, MUC1, MUC5AC, SATB2 and MOC31 helps to distinguish secondary esophagus, stomach and colorectal malignancies from primary thyroid neoplasms; panel of CK7, CK20, GATA3, mammaglobin, GCDFP15, ER, PR, TTF-1 and TG, metastasis of mammary carcinoma from primary thyroid neoplasms and panel of SOX10, Melan-A, S100, HMB45, CK7 and CK20, metastatic melanoma from primary thyroid neoplasms.

The TBSRTC may reliably establish a benign or malignant nodule diagnosis in 70% to 80% of all cases. The FNA diagnosis for the remaining 20 to 25% of nodules, falls in indeterminate cytology categories such as follicular lesion of undetermined significance/atypia of uncertain significance (FLUS/AUS, Bethesda category III), follicular neoplasm/suspicious for follicular neoplasm (FN/SFN, Bethesda category IV) [61, 62, 66].

The majority of lesions representative of these categories are benign on surgical pathology, indicating unnecessary surgical interventions [67]. Molecular tests have been increasingly applied to complement cytopathology [68] and improve risk-based stratification of indeterminate thyroid nodules [69, 70]. Molecular tests are based on detection of thyroid tumor specific mutations, sometimes added by gene expression profiling (Mutations in papillary thyroid carcinoma: *BRAF* mutation (29–69%), *RET* rearrangement (13–43%), *NTRK1* rearrangement (5–13%), *RAS* mutation (0–21%), the infiltrative follicular variant of papillary carcinoma (higher rates of *BRAF* than *RAS* mutations), follicular thyroid carcinoma: *RAS* mutation (40–53%), *PPARG* rearrangement (25–63%) [41–43, 71–73]. Although molecular testing has been useful for the diagnosis of indeterminate thyroid FNA, there are no molecular panel confidently discriminate malignancy.

While cytopathology offers valuable insights in the evaluation of head and neck masses, it has been of limited value in the diagnosis of parathyroid disorders as the differentiation between normal, hyperplastic, or neoplastic parathyroid tissue solely on FNA samples can be challenging or impossible [16, 74–76]. However, FNA guided by US can enhance the precision of locating parathyroid glands prior to potential surgical treatment of hyperparathyroidism, especially in complex cases such as recurrent disease or following unsuccessful surgeries. Yet, certain cytomorphologic characteristics can help differentiate parathyroid tissue from thyroid and other anatomical structures in cases of unintentional aspiration of intrathyroidal and ectopic parathyroid glands, although overlaps exist [16–21].

The effectiveness of FNA for cytopathological analysis can be hindered by factors like the small size, divergent location and number of parathyroid glands, as well as coexisting lesions of the thyroid gland or previous neck surgeries. An experience and skill of the aspirator in US-guided FNA and the expertise of the interpreting cytopathologist may significantly affect the adequacy and accuracy of the procedure.

Potential complications of parathyroid FNA include hematoma, parathyroid abscess, disruption of the lesion and seeding along the needle tract (parathyromatosis) or dense fibrotic reaction to the needle [11–15]. The number of needle pass, the size of the needle and the skill of the aspirator may influence these complications. However, these uncommon events may rarely convert a minimally invasive surgery to a standard surgical approach, and in the majority of reported series of parathyroid FNA no severe complication occurred associated with FNA procedure of parathyroid gland [16].

Recent studies have addressed the cytomorphological aspects of parathyroid lesions, revealing a range of diagnostic sensitivities (40.4% to 88.9%) in identifying parathyroid tissue [16–18, 76–78]. The availability of clinical and radiological data at the time of FNA can improve diagnostic accuracy. In two reported series, sensitivity was found to be 50% and 71% in cases without clinical and radiologic data and 86.7% and 88.9%, respectively, in cases with available serum PTH and results

of the US examination [7, 77]. Distinguishing parathyroid and thyroid lesions is not easy because of their adjacent anatomical location and the overlapping of cytological and radiological features. Knowledge of cytomorphologic features of parathyroid is essential in distinguishing parathyroid from thyroid lesions and to avoid misdiagnosis. Many studies have focused on the cytomorphological aspects of parathyroid lesions. Yet, certain cytomorphologic characteristics enhance the accuracy of FNA in identifying parathyroid tissue and distinguishing parathyroid and thyroid lesions [7, 16, 77, 79–84].

Three-dimensional tight or loose fragments with variable architectural patterns are common in parathyroid aspirates, whereas flat sheets are more common in thyroid. A mixture of scattered cells and naked nuclei in the background and nuclei with stippled chromatin are common features of parathyroid aspirates, while microfollicles, papillary and papillary-like features may be present in both thyroid and parathyroid aspirates [16, 77, 80, 85]. According to the studies published to date, FNA smears of the parathyroid show certain reproducible architectural patterns, as well as characteristic features of individual cells, nuclei, and background of the smears. Consistent cytomorphology comprises high to moderate cellular smears consisting of tight or loose three-dimensional clusters with cribriform, trabecular and wedge-shaped architectural patterns. These cohesive or loose cellular clusters of round, uniform, or slightly pleomorphic cells, have an overlapping appearance with numerous naked nuclei and/or isolated single cells in the background (fig. 2A). Less common findings include disorganized or follicular/microfollicular sheets, papillary fragments with fibrovascular cores, and capillary networks with associated epithelial cells [16–18, 77–81, 84]. Parathyroid cells in FNA smears are smaller than cells from thyroid follicular lesions and are usually round to oval in shape. The cells have a moderate to high nuclear to cytoplasmic ratio with pale to finely granular, occasionally oxyphilic cytoplasm and uncommon cytoplasmic vacuoles.

The nuclei are uniform with regular nuclear membrane, absent or inconspicuous nucleoli, coarsely granular, and typically stippled chromatin. Nuclear grooves, nuclear molding, and nuclear inclusions are fewer common features, while anisonucleosis may be seen in relatively many cases [7,

16, 17, 78]. Colloid-like material, macrophages and lymphocytes may be occasionally present in the background of the smears [16, 79–81, 84]. Cytologic pitfalls leading to misdiagnosis include the presence of cells with oxyphilic cytoplasm (oncocytic pattern) [16, 83–85]. Some reported series describes possible diagnostic criteria to classify parathyroid lesions and to distinguish benign parathyroid lesions from parathyroid carcinoma. The preliminary observations suggested that evident nucleoli, mitoses and possibly a papillary-solid pattern may guide the differentiation between parathyroid adenoma and parathyroid carcinoma [17, 18, 20, 77]. Differentiating between benign and malignant parathyroid lesions, however, remains complex due to overlapping cytomorphological features of parathyroid hyperplasia, parathyroid adenoma and parathyroid carcinoma [16]. Most parathyroid lesions can be highly variable in terms of cytomorphologic features and many different patterns may be seen in an aspirated specimen from one lesion. True Parathyroid cysts are rare and most represent non-functional cysts. Parathyroid cysts usually yield thin water-clear fluid as opposed to the thick colloid or bloody or colored cystic fluid with macrophages obtained from cystic thyroid lesions. A pathologically altered parathyroid gland can take on the form of a cystic or partly cystic lesion which in our experience may yield bloody fluid as opposed to water-clear fluid of pure parathyroid cysts. Such cystic parathyroid lesions may be difficult to distinguish from cystic lesions of the thyroid, especially if the FNA material is just a fluid without cells on microscopic examination. In such cases, the high level of PTH in the needle rinsed fluid speak for the parathyroid cyst [16].

Assessing PTH levels in the needle washout fluid (FNA-PTH assay) and applying immunocytochemistry (IC) to the aspirated material are ancillary techniques that enhance the accuracy of FNA in identifying parathyroid lesions. Immunohistochemistry, in particular, helps distinguish parathyroid from thyroid tissue by testing for specific markers. Parathyroid cells are immunoreactive for PTH, GATA-3, and chromogranin (98%) [86–88] and negative for TTF-1, INSM1, and thyroglobulin. A small subset of parathyroid tissue is immunoreactive for synaptophysin. IC can be applied to any of the several types of preparations such as direct smears, cytospin, liquid-based preparations or cell blocks (fig. 2B–D).

The usefulness of detecting PTH in the rinse material obtained from FNAB has been a well established technique with a strong correlation observed between levels of PTH and the cytologic findings [7, 16, 21, 80–83]. The FNA-PTH assay is especially useful in the location of pathological parathyroid glands while immunostainings on aspirated material can improve the discrimination between parathyroid and thyroid nodules. There are studies suggesting that the FNA procedure with PTH assay is more sensitive than parathyroid scanning or US alone and is also superior to FNA alone. [16, 21, 89–91] The PTH washout greater than 436.5 pg/ml has been reported to be 90% sensitive and 89% specific in localizing parathyroid tissue [89, 91].

Conclusions

US-guided FNA is a reliable and sensitive method to diagnose thyroid nodules and to detect parathyroid lesions. A key role of cytopathology in evaluation of thyroid nodules depends in part on evolutions in reporting criteria and the creation of uniform reporting systems such as TIRADS and TBSRTC. Those reporting systems facilitate easier and more reliable interpreting and sharing the results of FNA examination of thyroid with clinicians and improve patient management. In addition, the recent development of thyroid cytopathology and the growing role of parathyroid cytopathology includes widespread US guidance in FNA procedures, improved resolution of US for nodule detection, evolutions in FNA techniques and increasingly growing ancillary tests on cytopathology samples. However, clinical judgment remains of crucial importance in interpreting FNA results.

Article information and declarations

Conflicts of Interest

None declared

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Table I. Diagnostic categories in the 3rd edition of the Bethesda System for Reporting Thyroid Cytopathology*

I. Nondiagnostic
cyst fluid only
virtually acellular specimen
other (obscuring blood, clotting artifact, drying artifact, etc.)
II. Benign
consistent with follicular nodular disease (includes adenomatoid nodule, colloid nodule, etc.)
consistent with chronic lymphocytic (Hashimoto) thyroiditis in the proper clinical context
consistent with granulomatous (subacute) thyroiditis
other
III. Atypia of undetermined significance
specify if AUS-nuclear atypia or AUS-other
IV. Follicular neoplasm
specify if oncocytic (formerly Hürthle cell) type
V. Suspicious for malignancy
suspicious for papillary thyroid carcinoma
suspicious for medullary thyroid carcinoma
suspicious for metastatic carcinoma
suspicious for lymphoma
other
VI. Malignant

papillary thyroid carcinoma
high-grade follicular-derived carcinoma
medullary thyroid carcinoma
undifferentiated (anaplastic) carcinoma
squamous cell carcinoma
carcinoma with mixed features (specify)
metastatic malignancy
non-Hodgkin lymphoma
other

* Adapted from Ali SZ, VanderLaan PA. The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria, and Explanatory Notes, 3rd ed. Springer: New York, NY, USA; 2023

Table II. Examples of immunoprofile of thyroid tumors and parathyroid

Thyroid tumors	**Immunoprofiles**
thyroid tumors of follicular cells origin	CK7+, CK20–, TTF1+, PAX8+, thyroglobulin+, calcitonin–, synaptophysin–, chromogranin–
thyroid tumors of parafollicular c- cells origin	CK7+, TTF-1+, PAX8–, calcitonin+, CEA+, synaptophysin+, chromogranin+, thyroglobulin–
mixed medullary and follicular cell-derived thyroid carcinomas	Calcitonin+, TTF1+, thyroglobulin+
oncocytic carcinoma	TTF1+, PAX8+, CK7+, thyroglobulin+, calcitonin–
high-grade follicular cell-derived non-anaplastic carcinoma	TTF1+, PAX8+, CK7+, thyroglobulin+, calcitonin–
anaplastic thyroid carcinoma	inconsistent positive reactivity; CK+ (75% of cases), PAX8+ (50% of cases with epithelioid morphology), P53+ (50% of cases), squamous cell carcinoma phenotype: P63+, P40+, 34BE12+, CK5/6+
cribriform morular thyroid carcinoma	b-catenin, TTF1+ (mainly in cribriform components), PAX8–, thyroglobulin–
hyalinizing trabecular tumor	TTF1+, PAX8+, thyroglobulin+, MIB1 (membranous)
parathyroid	TTF1–, PAX8–, thyroglobulin–, calcitonin– (rarely +), PTH+, GATA3+, chromogranin+, synaptophysin+

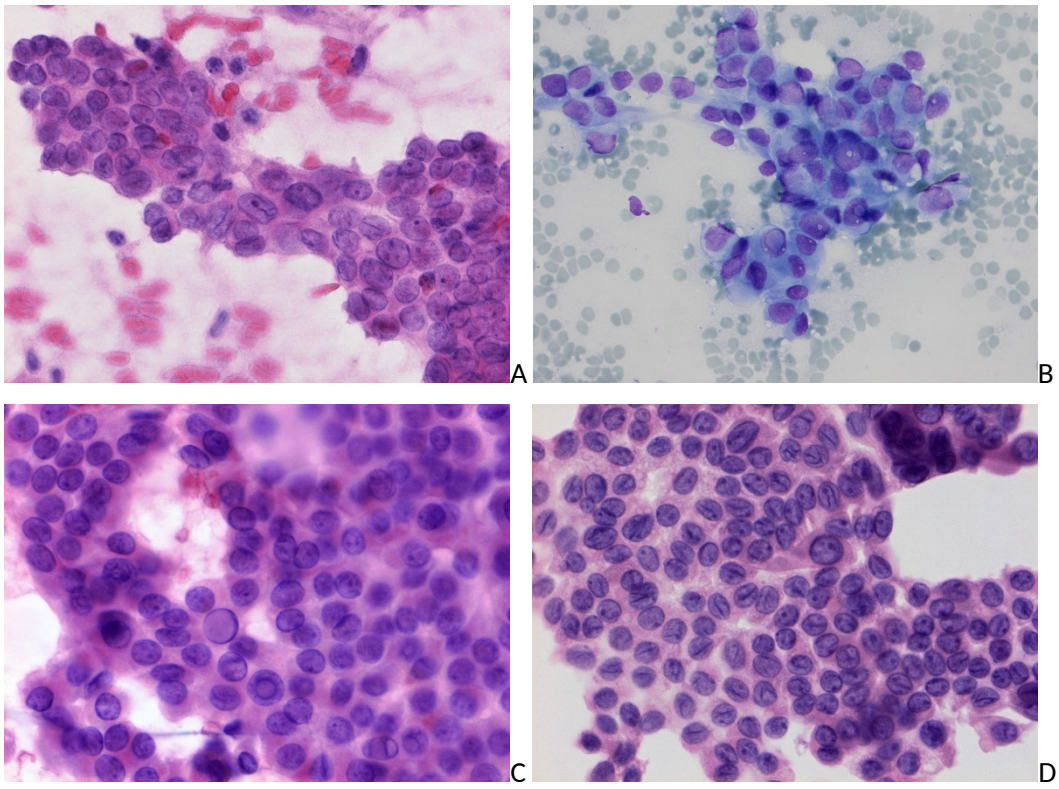
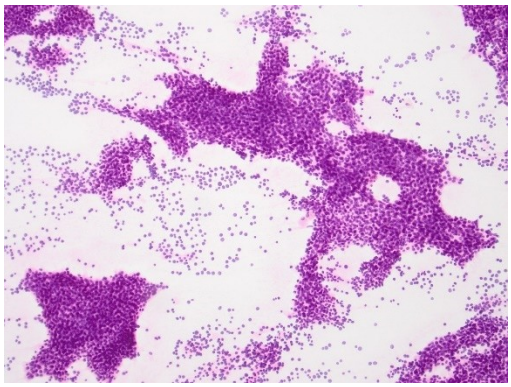
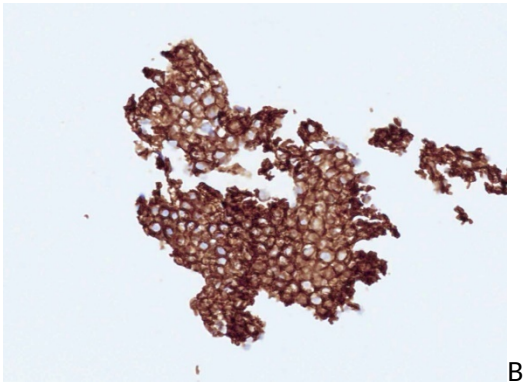


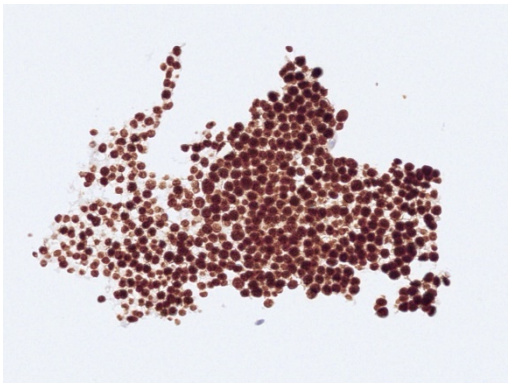
Figure 1. FNA of papillary thyroid carcinoma. (**A**) Tumor sheets with extensive nuclear changes of papillary carcinoma: grooves, nuclear membrane abnormalities, powdery chromatin, crowding, and nuclear enlargement. (hematoxilin and eosin staining). The most specific nuclear finding of papillary carcinoma is that of intranuclear pseudoinclusions. (**B**) This air-dried smear juxtaposes two and (**C**) alcohol fixed smear three true nuclear pseudoinclusions. (MGG and hematoxilin and eosin stains) (**D**) Another case of papillary thyroid carcinoma which also exhibits the common nuclear features of papillary carcinoma: nuclear enlargement, pale and powdery chromatin and irregular nuclear membranes with grooves. (hematoxilin and eosin stain)



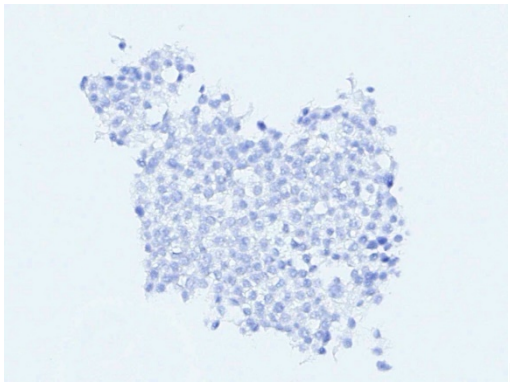
A



B



C



D

Figure 2. FNA of parathyroid. (**A**) Three-dimensional, crowded and loose clusters of uniform cells with small round nuclei without nucleoli and scant granular cytoplasm (hematoxilin and eosin staining). Cell block sections. Positive immunostainings for (**B**) parathyroid hormone (**C**). GATA-3 and and (**D**) negative TTF-1, confirming parathyroid tissue (cell block, PTH+, FATA-3+, TTF-1–)