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MedEst|2026 | Vol.6: e495| ISSN: 2789-7567 |RNPS: 2524| NARRATIVE REVIEW ARTICLE

Pathological anatomy methods in the diagnosis of thyroid cancer. A narrative review

Métodos de anatomía patológica en el diagnóstico del cáncer de tiroides. Revisión narrativa

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RESUMEN

Introducción: El cáncer de tiroides es la neoplasia endocrina más frecuente, con variantes histológicas de comportamiento y pronóstico diferentes. Identificar el tipo tumoral es fundamental para el manejo quirúrgico y la selección de terapias dirigidas. **Objetivo:** Revisar los métodos de anatomía patológica en el diagnóstico del cáncer de tiroides: citología aspirativa con aguja fina (CAAF), histología, inmunohistoquímica, estudios moleculares y su integración con patología digital e inteligencia artificial. **Métodos:** Se realizó una revisión narrativa con búsqueda estructurada de la literatura publicada entre enero de 2019 y diciembre de 2025 en PubMed, Scopus y Web of Science. Se incluyeron estudios originales, revisiones, guías y metaanálisis sobre diagnóstico citológico, histológico, inmunohistoquímico o molecular del cáncer de tiroides; se excluyeron reportes de caso aislados y publicaciones sin revisión por pares. Se identificaron 214 registros, 34 duplicados; se seleccionaron 63 artículos y se incluyeron 52 publicaciones. **Resultados:** La CAAF con estratificación Bethesda es la primera línea diagnóstica. La histología identifica variantes tumorales y evalúa factores pronósticos. La inmunohistoquímica diferencia tumores foliculares de medulares y confirma variantes agresivas. Los estudios moleculares detectan mutaciones y fusiones genéticas que orientan pronóstico y terapias dirigidas. La integración multimodal permite medicina de precisión. La patología digital e inteligencia artificial mejoran la interpretación morfológica y predicen mutaciones, aunque su validación clínica es limitada. **Conclusiones:** El diagnóstico del cáncer de tiroides requiere un enfoque multimodal que combine citología, histología, inmunohistoquímica y análisis molecular, integrados con patología digital e inteligencia artificial para optimizar la estratificación de riesgo y seleccionar terapias dirigidas.

ABSTRACT

Introduction: Thyroid cancer is the most common endocrine neoplasm, comprising histological variants with distinct behaviors and prognoses. Identifying the tumor type is crucial for surgical management and the selection of targeted therapies. **Objective:** To review pathological methods for diagnosing thyroid cancer: fine-needle aspiration cytology (FNAC), histology, immunohistochemistry, and molecular studies, as well as their integration with digital pathology and artificial intelligence. **Methods:** A narrative review was conducted involving a structured search of literature published between January 2019 and December 2025 in PubMed, Scopus, and Web of Science. Original studies, reviews, guidelines, and meta-analyses regarding the cytological, histological, immunohistochemical, or molecular diagnosis of thyroid cancer were included; isolated case reports and non-peer-reviewed publications were excluded. A total of 214 records were identified (with 34 duplicates); 63 articles were selected, and 52 publications were included. **Results:** FNAC with Bethesda stratification serves as the first-line diagnostic approach. Histology identifies tumor variants and assesses prognostic factors. Immunohistochemistry distinguishes follicular from medullary tumors and confirms aggressive variants. Molecular studies detect mutations and gene fusions that guide prognosis and targeted therapies. Multimodal integration enables precision medicine. Digital pathology and artificial intelligence improve morphological interpretation and predict mutations, although clinical validation remains limited. **Conclusions:** Diagnosing thyroid cancer requires a multimodal approach combining cytology, histology, immunohistochemistry, and molecular analysis, integrated with digital pathology and artificial intelligence to optimize risk stratification and the selection of targeted therapies

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INTRODUCTION

Thyroid cancer constitutes the most frequent malignant neoplasm of the endocrine system, representing approximately 3 to 4 % of all tumors diagnosed worldwide [1]. In recent decades, its incidence has shown a sustained increase, a phenomenon attributed in part to the greater use of high-resolution imaging techniques, such as ultrasound and tomography, which allow the early detection of small thyroid nodules [2,3]. However, recent studies suggest that there is also a real increase in the incidence of clinically relevant tumors, indicating the need for a more precise, evidence-based diagnostic approach [4,5].

Thyroid tumors are classified primarily according to their cellular origin and differentiation pattern. Carcinomas derived from follicular cells include papillary, follicular, and poorly differentiated carcinoma, as well as anaplastic carcinoma, while those derived from C cells (parafollicular) correspond to medullary carcinoma [1,4]. This classification has clinical relevance, since each type presents significant differences in biological behavior, risk of recurrence, and response to treatment. Papillary thyroid carcinoma (PTC) is the most frequent, representing between 70 and 80 % of all cases [6].

It generally presents in young adult patients and shows a predilection for regional lymphatic dissemination. Its histological variants include classic, tall cell, diffuse sclerosing, solid, and follicular, each with distinct prognostic characteristics. For example, the tall cell variant is associated with a higher risk of recurrence, while the diffuse sclerosing variant is more frequent in young patients and may present significant lymph node involvement [6,7,8].

Follicular carcinoma (FC) constitutes approximately 10–15 % of thyroid cancers. It is characterized by capsular and/or vascular invasion, with differentiation from a benign follicular adenoma being a frequent diagnostic challenge. Its dissemination pattern is usually hematogenous, which increases the probability of metastasis to bone and lung. The follicular variant of papillary carcinoma combines morphological characteristics of PTC with follicular architecture, which can hinder its identification in cytology and requires careful histological correlation [9,10].

Medullary thyroid carcinoma (MTC) represents around 3–5 % of thyroid tumors and originates in parafollicular C cells. It can be sporadic or familial, associated with Multiple Endocrine Neoplasia syndromes 2A and 2B (MEN2A and MEN2B). The presence of germline mutations in the RET gene has direct implications for clinical management and family approach. MTC can produce calcitonin and other hormones,

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allowing biochemical monitoring and early identification of recurrence [11].

Poorly differentiated carcinoma and anaplastic carcinoma represent less than 5 % of cases, but are characterized by aggressive biology and poor prognosis. The former constitutes an intermediate entity between differentiated and anaplastic tumors, while anaplastic carcinoma presents rapid progression, extensive local invasion, and high mortality. Early identification of these forms requires exhaustive histopathological evaluation and, in many cases, molecular support [12,13].

Risk factors for the development of thyroid cancer include exposure to ionizing radiation, family history of thyroid neoplasia, specific genetic syndromes such as MEN2, and hereditary genetic predisposition. Radiation exposure in childhood, even at low doses, is associated with a higher risk of papillary carcinoma, while germline RET mutations predispose to familial medullary carcinoma [5,14]. The prevalence of histological subtypes has changed in recent decades, with a relative increase in papillary carcinoma and a decrease in follicular carcinoma, a phenomenon attributed to environmental factors and improvements in detection [38,41].

In this context, pathology methods have consolidated as the cornerstone of definitive diagnosis. Fine needle aspiration cytology allows the initial evaluation of thyroid nodules, classifying risk through the Bethesda System. Conventional histology provides diagnostic confirmation, allows the identification of tumor variants and the evaluation of prognostic factors such as capsular and vascular invasion. Immunohistochemistry and molecular studies complement the characterization of aggressive tumors, providing valuable information for risk stratification and the selection of targeted therapies [15,16,17]. International clinical guidelines, such as those of the American Thyroid Association and the European Society for Medical Oncology, recommend a multidisciplinary approach that integrates these methods to optimize patient management [32,34,35,37].

Despite the abundant literature on individual techniques, no integrative synthesis has been published that critically addresses the complementarity of cytological, histological, immunohistochemical, and molecular methods in the context of current clinical practice, particularly regarding morpho-molecular integration and the emerging role of digital pathology and artificial intelligence. Likewise, controversies persist regarding the standardization of molecular panels, accessibility to these technologies in centers with limited resources, and the clinical validation of artificial intelligence algorithms.

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These gaps justify the need for a narrative review that synthesizes the available evidence and offers an integrative perspective.

The present article aims to comprehensively review the pathology methods used in the diagnosis of thyroid cancer, with emphasis on the description of the different types of cancer, their specific diagnostic characteristics in cytology, histology, immunohistochemistry, and molecular analysis, as well as on the evaluation of future perspectives based on digital pathology and artificial intelligence.

A narrative rather than systematic review was chosen due to the breadth and heterogeneity of the topic, which encompasses multiple diagnostic techniques with diverse methodologies (diagnostic accuracy studies, cohorts, case series, expert consensus), making a quantitative synthesis through meta-analysis difficult.

METHODS

To conduct this review on pathology methods in the diagnosis of thyroid cancer, a narrative review design with structured literature search was implemented, aimed at integrating recent evidence published between January 2019 and December 2025 in the main biomedical databases: PubMed, Scopus, and Web of Science. This period was selected because it coincides with the publication of the fifth edition of the World Health Organization classification of endocrine tumors (2022), the update of the Bethesda System (2023), and the most relevant advances in molecular and digital pathology, guaranteeing the scientific currency of the synthesis [3,4,19].

Search strategy

Controlled MeSH vocabulary terms and keywords combined using Boolean operators were used. The main terms employed were: "Thyroid neoplasms," "Fine needle aspiration biopsy," "Bethesda system," "Histopathology," "Immunohistochemistry," "Molecular pathology," and "Thyroid carcinoma variants." Tumor-type terms were combined with diagnostic techniques and histological variants, allowing the identification of articles addressing both methodology and the clinical and prognostic correlation of pathological findings. Publications in English and Spanish were considered, without restriction by study type, to guarantee broad literature coverage.

Inclusion and exclusion criteria

Original studies with adult and pediatric patients diagnosed with any type of thyroid cancer, systematic reviews and meta-analyses evaluating cytological, histological, immunohistochemical, or molecular

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diagnostic techniques, clinical guidelines, and international consensus, such as those of the American Thyroid Association and the WHO Classification of Endocrine Tumours, were included [3,16,32,34,35].

Isolated case reports or small series (<20 patients) were excluded, unless they provided relevant morphological or molecular findings; studies focused exclusively on surgical treatment, radiotherapy, or chemotherapy without diagnostic correlation; and publications with insufficient information on methodology or without peer review.

Selection process

Initially, 214 records were identified, of which 34 were duplicates. After evaluating titles and abstracts, 63 articles were selected for full review. Of these, 52 publications were finally included for meeting the criteria of relevance, methodological quality, and scientific currency. Each article was independently reviewed by two reviewers (LCG and AMMM) to guarantee precision and avoid selection biases. Disagreements were resolved by consensus; if they persisted, a third reviewer (DRG) with experience in oncological pathology was consulted. Clarity in the description of the sample, validity of cytological, histological, immunohistochemical, and molecular techniques, and the degree of evidence provided were considered. Publications with large cohorts, documented clinical follow-up, and morpho-molecular correlation analysis were prioritized.

Data extraction and synthesis

Information was organized into thematic blocks: Fine needle aspiration cytology and Bethesda System; Conventional histological study and tumor variants; Immunohistochemical panels and their correlation with cancer type and variant; Molecular studies, recurrent mutations, and genetic fusions; and Emerging tools: digital pathology and artificial intelligence. From each study, the following variables were extracted: author, year, country, study design, sample size, population, intervention/comparator, main results, and level of evidence. The synthesis had a descriptive and critical approach, integrating recent findings, the specific diagnostic characteristics of each type of cancer, and the limitations of each technique.

Ethical considerations

Since this is a bibliographic review based on published literature, approval by an ethics committee was not required. Nevertheless, strict selection criteria were respected to guarantee the inclusion of studies with informed consent and peer review, ensuring the validity of the cited data.

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RESULTS

Fine needle aspiration cytology (FNAC) and Bethesda System

Fine needle aspiration cytology (FNAC) constitutes the first diagnostic line in the evaluation of thyroid nodules. Its advantage lies in being a minimally invasive, safe, repeatable procedure with high sensitivity for detecting malignancy, although its specificity may be limited in follicular lesions [5,20]. The technique consists of aspirating thyroid cells using fine-gauge needles (23–27G), generally under ultrasound guidance, followed by the preparation of conventional smears, cell blocks in liquid medium, or fixation for immunohistochemical and molecular analysis [15,21,22].

Cytology interpretation has been standardized through the Bethesda System for Reporting Thyroid Cytopathology, second edition, which classifies samples into six categories, each with an estimated risk of malignancy and clinical recommendations [4,19]. The Bethesda System has demonstrated adequate reproducibility and correlation with the risk of malignancy in multiple studies, although variability persists in the indeterminate categories [35,48].

Table 1. Cytology interpretation using the Bethesda System

Bethesda Category	Risk of Malignancy (%)	Clinical Recommendation
I. Non-diagnostic	5–10	Repeat FNAC
II. Benign	<3	Ultrasound follow-up
III. AUS/FLUS	10–30	Repeat FNAC, molecular studies, or surgery
IV. Follicular neoplasm	25–40	Diagnostic lobectomy
V. Suspicious for malignancy	50–75	Surgical resection
VI. Malignant	97–99	Definitive surgery

Source: Adapted from the Bethesda System 2023 [4,19]. Risks of malignancy come from expert consensus (level of evidence 5) supported by multicenter cohorts (level 2b).

Specific cytological findings by type of cancer (mainly level of evidence 2b–4, based on cohort studies and case series):

1. Papillary thyroid carcinoma (PTC): enlarged nuclei with pale chromatin ("ground glass"), nuclear grooves, and nuclear pseudoinclusions. Aggressive variants, such as tall cell or diffuse sclerosing, may show moderate pleomorphism and more intense

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cytoplasmic staining, requiring experience to differentiate from benign lesions [7,20,39].

2. Follicular variant of papillary carcinoma: combines follicular architecture with nuclear alterations typical of papillary, such as discrete nuclear grooves and mild pseudoinclusions. Its cytological identification is more challenging and carries a higher risk of classification as Bethesda III or IV. For this reason, correlation with histological findings is essential [10].

3. Follicular carcinoma (FC): presents in cytology as uniform microfollicles, scarce colloid, and absence of nuclear characteristics of PTC. The distinction between follicular adenoma and follicular carcinoma depends on evidence of capsular or vascular invasion, which cannot be safely determined in FNAC. Therefore, the Bethesda IV category (follicular neoplasm or suspicion) is usually used and diagnostic resection is recommended [9].

4. Medullary thyroid carcinoma (MTC): shows in cytology plasmacytoid or spindle cells with "salt and pepper" chromatin, granular cytoplasm, and frequently the presence of amyloid material in the background. Detection of calcitonin by immunocytochemistry on the smear significantly increases diagnostic sensitivity and allows differentiation from other round cell tumors. Early identification is crucial, especially in patients with MEN2 syndromes and germline RET mutations [10,11,23].

5. Poorly differentiated carcinoma: cytology reveals moderate to marked pleomorphism, increased mitoses, and focal necrosis, differentiating it from well-differentiated follicular and papillary tumors. Cytological diagnosis may suggest high-grade malignancy, but histological confirmation is indispensable [12].

6. Anaplastic carcinoma: highly aggressive and lethal, it is cytologically characterized by pleomorphic giant cells, numerous mitotic figures, and extensive necrosis. The sample may be heterogeneous, and correlation with histology and immunohistochemistry (loss of thyroglobulin and TTF-1) is necessary to confirm the diagnosis [13].

Limitations and prognostic utility of FNAC

FNAC allows: stratifying the initial risk of malignancy according to the Bethesda System categories; selecting patients for clinical follow-up or surgery; and obtaining additional material for immunohistochemical and molecular studies [17,24]. However, its limitations include: indeterminacy in categories III and IV; difficulty distinguishing follicular adenomas from follicular carcinomas; dependence on the cytopathologist's experience; and possible underdiagnosis of

aggressive variants if the sample is not representative [20,25]. The integration of cytological findings with histology, immunohistochemistry, and molecular studies significantly increases diagnostic accuracy and allows more appropriate therapeutic planning [15,16].

Histology and variants of thyroid cancer

Conventional histology remains the gold standard for the definitive diagnosis of thyroid cancer. It allows evaluation of tumor architecture, nuclear characteristics, capsular or vascular invasion, and other essential prognostic factors (6,7,9). Correct interpretation requires adequate processing: 10% formalin fixation, paraffin embedding, serial sections, and hematoxylin-eosin staining. Furthermore, obtaining cell blocks allows complementary immunohistochemical and molecular analysis studies, crucial in cases with atypical morphology or poorly differentiated tumors (15,17).

Classic PTC represents the most common form of thyroid carcinoma. Histologically, it is characterized by: papillary architecture (fibrovascular structures lined by cuboidal or cylindrical epithelial cells); nuclear alterations (enlarged nuclei, pale chromatin, pseudoinclusions, nuclear grooves); and psammoma bodies (concentric calcifications frequent in classic variants) (6,39).

Table 2. Histological findings by tumor type

Tumor Type	Key Histological Characteristics	Prognosis	Notes
Classic PTC	Fibrovascular papillae, pseudoinclusions, psammoma bodies	Low risk (6)	—
Tall cell variant	Tall cells (height:width ratio ≥3), elongated nuclei	Higher risk of recurrence (7)	—
Diffuse sclerosing variant	Diffuse infiltration with fibrosis, lymphocytes	Young patients, lymphatic metastases (8)	—
Solid/trabecular variant	Solid architecture, minimal papillary formation	Associated with radiation (8)	—
Follicular variant	Microfollicles with subtle nuclear alterations	Difficult differentiation (10)	—
Follicular carcinoma	Capsular and/or vascular invasion, uniform follicular	Hematogenous dissemination (9)	—

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	pattern		
Hürthle cell carcinoma	Oncocytic cells, abundant eosinophilic granular cytoplasm	Variable, depends on invasion (10)	—
Medullary carcinoma	Solid/trabecular nests, amyloid, plasmacytoid cells	Calcitonin (+) (11,23)	—
Poorly differentiated	Solid/trabecular/insular pattern, necrosis, increased mitoses	Intermediate (12)	—
Anaplastic	Extreme pleomorphism, extensive necrosis, loss of differentiation	Very aggressive (13,17)	—

Level of evidence of findings: mainly 2b (cohorts) and 4 (descriptive series of rare variants).

Integration with cytological and molecular findings

Correlation between cytology, histology, immunohistochemistry, and molecular analysis allows: confirming the histological type when FNAC is indeterminate; classifying aggressive variants and guiding surgical strategy and follow-up; and identifying mutations targeted for molecular therapy, especially in PTC, MTC, and undifferentiated tumors [15,16]. Surgical management guidelines, such as those of the American Association of Endocrine Surgeons, emphasize the importance of this integration for therapeutic planning [31].

Immunohistochemistry and molecular studies

Immunohistochemistry (IHC) complements morphology and is especially useful in indeterminate cases (Bethesda III/IV categories) and in poorly differentiated tumors (level of evidence 2b–3) [17,24]. Genetic markers are grouped into:

General markers of thyroid differentiation: TTF-1 (Thyroid Transcription Factor-1), positive in tumors derived from follicular cells; PAX8, a nuclear marker useful in papillary, follicular, and poorly differentiated carcinomas; and thyroglobulin, a specific marker of thyroid differentiation, useful for differentiating tumors of thyroid origin from metastatic ones [17].

Table 3. Specific markers by tumor type

Tumor Type	Positive IHC Panel	Negative IHC Panel
PTC	CK19, HBME-1, Galectin-3, TTF-1, PAX8	Calcitonin
FC	TTF-1, PAX8,	CK19, HBME-1

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	thyroglobulin	(usually)
MTC	Calcitonin, CEA	Thyroglobulin, TTF-1
Poorly differentiated	Variable (partial loss of thyroglobulin, TTF-1)	Loss of markers
Anaplastic	Variable (total loss of differentiation)	Thyroglobulin, TTF-1 (generally negative)

Source: Systematic reviews and guidelines (level 1a–2a) [17,24]. Negative panels may present exceptions; joint interpretation with morphology is recommended. The combination of CK19, HBME-1, and Galectin-3 has demonstrated high sensitivity and specificity in meta-analyses [43,44,45].

Clinical applications of IHC (level of evidence 2b): confirmation of thyroid origin in metastases, identification of aggressive variants, and diagnostic support in Bethesda III/IV [17].

Molecular analysis has acquired a fundamental role in the diagnostic and prognostic characterization of thyroid carcinomas. The main methods include polymerase chain reaction (PCR), next-generation sequencing (NGS), fluorescence in situ hybridization (FISH), and analysis of specific genetic panels [15,16,24]. Recurrent mutations by tumor type are:

1. Papillary carcinoma: BRAF V600E (associated with higher risk of recurrence and extrathyroidal extension), RET/PTC, and NTRK1/3. BRAF mutations are frequent in aggressive variants (tall cell and diffuse sclerosing) [15,16,37].

2. Follicular carcinoma: RAS (NRAS, HRAS, KRAS) and PAX8/PPARγ; their presence correlates with follicular behavior and moderate risk of recurrence [16].

3. Medullary carcinoma: germline RET mutations in familial forms; somatic RET mutations in sporadic cases. Genetic identification allows preventive management of family members and surgical planning [11].

4. Poorly differentiated and anaplastic: TP53 and TERT mutations, often combined with BRAF or RAS, indicate aggressive behavior and resistance to conventional therapies [16,13].

Genetic fusions and therapeutic applications: Fusions such as RET/PTC, NTRK, and ALK can be detected by NGS and FISH, with direct implications for targeted therapies. For example, RET inhibitors (selpercatinib, pralsetinib) and NTRK inhibitors (larotrectinib) show efficacy in tumors with these genetic alterations [16,24]. Genomic classifiers, such as ThyroSeq v3 and Afirma, have demonstrated utility in indeterminate nodules, reducing unnecessary surgeries [29,30,36,46,47].

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Integration of IHC and molecular studies: The combination of morphological, immunohistochemical, and molecular analysis allows: confirming the histogenesis of tumors with ambiguous morphology; differentiating follicular adenomas from follicular carcinomas and PTC variants; stratifying poorly differentiated and anaplastic tumors for selection of targeted therapies; and complementing cytological interpretation in indeterminate Bethesda System categories (III and IV) [15,20].

Limitations of immunohistochemistry and molecular studies: Although IHC and genomics offer high diagnostic accuracy, they present limitations that must be considered in clinical practice: limited accessibility in centers with restricted resources; high costs of NGS panels and complementary analyses; and the need for standardization of techniques and multicenter validation to ensure reproducibility [16,24]. Future perspectives include the integration of digital pathology and artificial intelligence, allowing more efficient morpho-molecular correlation and reduction of interobserver variability. This opens the possibility of rapid and automated diagnosis, with prediction of mutations from digitized images [20,19].

Digital pathology, artificial intelligence, and future perspectives

Digital pathology and artificial intelligence (AI) represent significant advances in the diagnosis of thyroid cancer, with the potential to transform clinical practice and improve diagnostic accuracy. These technologies allow the digitization of histological and cytological slides, the creation of image banks, and the computational analysis of complex morphological characteristics, which can be correlated with immunohistochemical markers and molecular profiles [26,27].

Slide digitization allows: storage and remote review of histological and cytological samples, facilitating multicenter consultations; quantitative analysis of nuclear and architectural characteristics, such as cell size, shape, and distribution, which can improve diagnostic objectivity; and traceability and documentation of cases, useful for follow-up and quality audit. Recent studies have shown that digital pathology can reduce interobserver variability, especially in the identification of aggressive variants of papillary carcinoma and in the distinction between adenomas and follicular carcinomas [26,27].

Artificial intelligence and deep learning

Deep learning algorithms applied to digitized images can: classify thyroid nodules according to risk of malignancy, based on cytological and architectural patterns; predict genetic mutations (such as BRAF V600E or RET/PTC) from morphological characteristics, integrating

information from cytology, histology, and immunohistochemistry; and optimize diagnostic workflows, suggesting more precise Bethesda categories and assisting in surgical decision-making. For example, recent studies have reported that AI algorithms can identify typical nuclear characteristics of PTC with sensitivity above 90 % and differentiate follicular and aggressive variants with high concordance with expert diagnosis [20,27]. A recent meta-analysis confirmed that AI systems achieve a sensitivity of 85–92 % in the classification of thyroid nodules, with a specificity of 80–88 % [49].

Morpho-molecular integration

Digital pathology combined with molecular analysis allows: direct correlation between histological patterns and genetic mutations; early identification of poorly differentiated and anaplastic tumors before clinical progression; and selection of patients candidates for targeted therapies based on specific mutations (BRAF, RET, NTRK, TP53) [24,27]. This integration opens new opportunities for precision medicine, allowing therapeutic strategies to be adjusted to the individual characteristics of the tumor.

Current limitations

Despite the potential, important challenges exist: standardization of algorithms and digital platforms (the lack of uniform protocols hinders multicenter comparison of results); technological and economic requirements (digitization equipment, servers, and specialized software generate access barriers in many centers); and clinical validation (evidence is still required that AI improves clinical and long-term prognostic outcomes) [26,28].

Future perspectives

Future research focuses on: complete automation of the diagnostic workflow, from FNAC to integration with IHC and molecular studies; predictive models of recurrence and therapeutic response based on deep learning and morpho-molecular analysis of large patient cohorts; and global implementation of digital pathology, with remote access for centers with limited resources, optimizing multidisciplinary care. The combination of digital pathology, AI, and molecular analysis will allow progress toward a comprehensive diagnostic model, in which morpho-molecular correlation is the basis for risk stratification, surgical planning, and selection of targeted therapies, especially in poorly differentiated tumors, aggressive PTC variants, and anaplastic carcinomas [15,16,20,26,27].

DISCUSSION

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The results of this review confirm that the diagnosis of thyroid cancer has evolved toward a multimodal approach in which cytology, histology, immunohistochemistry, and molecular studies complement each other to maximize diagnostic accuracy and guide therapeutic management and clinical follow-up [6,15,16].

FNAC continues to be the initial tool of choice for the evaluation of thyroid nodules. Its ability to differentiate benign from malignant lesions and classify samples according to the Bethesda System allows informed clinical decisions [20,19]. However, indeterminate categories (Bethesda III and IV) represent a diagnostic challenge, especially in follicular tumors or follicular variants of papillary carcinoma. In these cases, cytology alone cannot determine capsular or vascular invasion, limiting its predictive value.

These findings agree with those reported by Hsiao et al. [5] in their meta-analysis, which found an overall sensitivity of 78% for the detection of malignancy, with greater variability in indeterminate categories. In contrast, studies such as that of Stillman et al. [13] suggest that the routine incorporation of molecular tests in Bethesda III reduces the rate of unnecessary diagnostic surgeries, although with a significant increase in costs. Likewise, Bongiovanni et al. [48] documented an adequate correlation between Bethesda categories and the risk of malignancy, although with heterogeneity in categories III and IV.

Histological study confirms the diagnosis and allows the identification of tumor variants that may have important prognostic implications [6,7,8]. For example, the tall cell variant of PTC or the diffuse sclerosing variant show a higher risk of recurrence and extrathyroidal extension, information that cannot always be inferred from cytology. Histology is also essential for differentiating follicular adenomas from follicular carcinomas, as well as for evaluating poorly differentiated and anaplastic carcinomas, whose aggressive morphology is reflected in necrosis, pleomorphism, and elevated mitoses [12,13]. These findings are consistent with those described by Mete and Asa [8], who emphasize that histological evaluation of vascular invasion is an independent prognostic factor in differentiated carcinomas. Cameselle-Teijeiro et al. [40] have pointed out that diagnostic difficulties exist in thyroid tumors that require correlation with complementary techniques.

IHC panels allow confirmation of tumor histogenesis and differentiation of tumors with ambiguous morphology. In particular, the identification of calcitonin in medullary carcinoma or the loss of differentiation markers in anaplastic tumors provides crucial information for clinical management. The combination of markers such as CK19, HBME-1,

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Galectin-3, TTF-1, and PAX8 allows increasing diagnostic sensitivity and specificity, especially in aggressive PTC variants [17,24].

However, the reviewed evidence shows heterogeneity in the definition of optimal panels and cut-off points, which hinders comparison between studies. This limitation has been previously pointed out by Crescenzi and Baloch [24], who advocate for international standardization of IHC panels in thyroid pathology. The meta-analyses by Papale et al. [43], Matos et al. [44], and Dunderovic et al. [45] have confirmed the diagnostic value of the combination of CK19, HBME-1, and Galectin-3.

Molecular analysis provides an additional level of diagnostic and prognostic precision. The detection of recurrent mutations (BRAF, RAS, RET/PTC, TP53, TERT) and genetic fusions (NTRK, ALK) allows not only confirming the tumor type but also identifying patients candidates for targeted therapies [15,16,24]. In indeterminate tumors of Bethesda III and IV categories, molecular panels can reduce diagnostic uncertainty and guide the surgical decision, minimizing unnecessary resections. However, evidence on the cost-effectiveness of these tests is still limited and heterogeneous, and their accessibility varies considerably between centers and countries. Raghunathan et al. [11] and Hannoush et al. [27] agree that the implementation of molecular panels must be accompanied by an economic evaluation and local validation of the platforms used. The studies by Nikiforov et al. [29,46], Steward et al. [30], and Alexander et al. [47] have demonstrated the utility of genomic classifiers in indeterminate nodules.

Limitations of the reviewed evidence

The quality of the reviewed evidence is heterogeneous. Retrospective cohort studies (level 2b) and case series (level 4) predominate, especially regarding rare tumor variants and the application of emerging techniques such as AI. Meta-analyses and systematic reviews (level 1a–2a) focus on the diagnostic accuracy of FNAC and the utility of molecular panels, but there are few randomized clinical trials evaluating the impact of these technologies on long-term clinical outcomes, such as overall survival or recurrence. Likewise, there is heterogeneity in the definition of outcomes and in patient selection criteria between studies, which limits the comparability of results. Most studies come from high-volume centers and high-income countries, which reduces the generalizability of findings to resource-limited settings.

Limitations of the narrative review itself

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This review presents several limitations inherent to its design. First, bibliographic selection was subject to the authors' criteria and the availability of the consulted databases (PubMed, Scopus, and Web of Science), which could have introduced a selection bias. Second, although a structured search strategy with MeSH terms and keywords was used, a systematic methodology with a registered protocol (e.g., PROSPERO) was not followed, nor was a formal risk of bias assessment of the included studies performed, which limits the reproducibility and comprehensiveness of the search. Third, the restriction to publications in English and Spanish could have excluded relevant literature in other languages. Fourth, dependence on the authors' criteria for the inclusion of sources and narrative synthesis may have introduced interpretation biases. Finally, the absence of a PRISMA flow diagram (although not mandatory in narrative reviews) limits the transparency of the selection process, although the selection flow was described in the Methods section.

Implications for clinical practice, health policy, and future research

The findings of this review have concrete implications for clinical practice. It is recommended to maintain FNAC with Bethesda categorization as the first line of thyroid nodule evaluation, reserving molecular tests for indeterminate cases (Bethesda III and IV) and tumors with characteristics of aggressiveness. IHC panels should be used rationally, prioritizing markers with greater diagnostic and prognostic value. Molecular evaluation should be integrated in centers with available resources to select targeted therapies and avoid unnecessary surgeries. Digital pathology and AI should be considered complements, requiring multicenter validation before routine implementation. It is necessary to promote equity in access to these technologies through investment in infrastructure and training of human resources. Finally, prospective and multicenter studies are required to evaluate the clinical impact of morpho-molecular integration and AI on patient-centered outcomes.

Identification of knowledge gaps

Important gaps persist in the literature. There is no consensus on the optimal molecular panel for each tumor type, nor on the cut-off points for IHC interpretation in aggressive variants. Evidence on the clinical utility of AI in thyroid pathology is still preliminary and based on retrospective single-center studies. Randomized clinical trials comparing AI-guided versus conventional diagnostic strategies are lacking. Furthermore, most cost-effectiveness studies come from high-income countries, so the applicability of these results in resource-limited contexts is unknown. Finally, the molecular characterization of

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poorly differentiated and anaplastic tumors is incomplete, and studies integrating genomic, transcriptomic, and epigenetic data are required to identify new therapeutic targets.

CONCLUSIONS

The diagnosis of thyroid cancer requires a multimodal approach that integrates cytology (Bethesda System), histology, immunohistochemistry, and molecular studies, allowing the classification of tumor variants, stratification of prognostic risk, and selection of targeted therapies. Future perspectives include digital pathology and artificial intelligence for more efficient morpho-molecular analysis. However, the level of certainty varies according to the technique: FNAC and histology have solid evidence (level 2b–1a), molecular panels have moderate evidence (level 2b–3), and AI has preliminary evidence (level 4). The implementation of these technologies faces challenges of accessibility, standardization, and clinical validation, which must be addressed through prospective research and equitable health policies. Multidisciplinary collaboration and updating of protocols guarantee better outcomes for patients.

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