

The Cytological Grey Zone: Mapping Concealed Morphological Signatures in Indeterminate Thyroid Fine-Needle Aspiration Cytology

Dr. Ajit Pal Sing

Associate Professor, Department of Medical Lab Technology, School of Allied Sciences, Galgotias University, Greater Noida, India, 203201

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ABSTRACT

Background

Thyroid nodules rank as one of the most prevalent endocrine discoveries, with fine-needle aspiration cytology (FNAC) serving as the primary minimally invasive method for their assessment. Despite the recent 2023 revision of the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), which has established standardized terminology and malignancy-risk assessments, a significant number of aspirates continue to be classified as indeterminate specifically atypia of undetermined significance (Bethesda III), follicular neoplasm (Bethesda IV), and suspicious for malignancy (Bethesda V). These classifications possess intersecting nuclear, architectural, cytoplasmic, and contextual characteristics that constrain the distinguishing capability of any individual morphological standard.

Aim

The goal is to propose a structured methodology for detecting and categorizing nuanced, composite ("hidden") morphological indicators in indeterminate thyroid FNAC, as well as to explain how these indicators may be linked to histopathology, supplementary/molecular assessments, and malignancy risk, thereby improving diagnostic differentiation within the cytological grey area.

Methods

A systematic narrative synthesis of the 2023 TBSRTC and current literature regarding nuclear, architectural, cytoplasmic, and background characteristics in Bethesda III–V thyroid FNAC was conducted, enhanced by data on cytohistological correlation, supplementary immunocytochemistry, molecular classifiers, and AI-assisted digital cytology. A five-domain conceptual framework known as the Cytological Grey-Zone Morphological Signature (CGZ-MS) was developed from this synthesis, categorising characteristics into Nuclear, Architectural, Cytoplasmic, and Background domains that culminate in an Integrated Risk domain.

Results

Composite cytomorphological patterns, as opposed to singular cytological anomalies, seem to be more reliably linked with malignant or borderline histology in Bethesda III–V thyroid tumours. The simultaneous occurrence of nuclear atypia, structural disarray, modified cytoplasmic differentiation, and a conducive cytological environment like psammomatous calcification and little colloid might offer enhanced diagnostic significance compared to depending solely on isolated characteristics. Incorporating immunocytochemical indicators such as thyroglobulin, TTF-1, PAX8, and calcitonin alongside genetic mutations and gene-expression classifiers such as BRAF, RAS, RET/PTC, and TERT, as well as Afirma and ThyroSeq platforms, could enhance risk categorization. The advent of AI-enhanced digital cytology may offer an extra dimension of objective analysis;

yet, these methods should be viewed as supplementary to, rather than substitutes for, professional cytomorphological examination.

Conclusion

Systematic, multi-domain mapping of concealed morphological signatures may convert descriptive atypia into reproducible morphological phenotypes that complement, rather than replace, the 2023 Bethesda System. The proposed CGZ-MS framework is a conceptual, hypothesis-generating model that requires prospective, multicentre validation against histopathological and molecular outcomes before any diagnostic application.

Keywords: Thyroid FNAC; Bethesda System 2023; cytological grey zone; atypia of undetermined significance; follicular neoplasm; morphological signature; molecular cytopathology; digital pathology; artificial intelligence.

INTRODUCTION

Thyroid nodules are among the most commonly observed irregularities in endocrine medicine, and their identified occurrence is increasing due to the extensive application of high-resolution ultrasonography and cross-sectional imaging. Over 95% of thyroid nodules are non-cancerous; nevertheless, differentiating between benign and malignant lesions is difficult due to similarities in their physical and cytological characteristics and significant biological variability. FNAC serves as the fundamental assessment for thyroid nodules due to its minimally invasive nature, cost efficiency, speed, and direct influence on clinical decision-making, thereby minimizing unnecessary surgical interventions [1-2]. The primary constraint occurs when cytological characteristics lack adequate differentiation for a conclusive diagnosis, leading to a cytological "grey zone" that includes lesions exhibiting a mix of benign, borderline, and malignant traits, along with significant inter-observer heterogeneity. The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) was established in 2010 and updated in 2017 to create a standardized six-tier reporting framework. The 2023 third edition enhanced diagnostic terminology and malignancy-risk estimates by aligning with the 2022 WHO classification of thyroid neoplasms, reclassifying atypia of undetermined significance (AUS), and broadening recommendations for ancillary and molecular testing. Morphology continues to serve as the foundation for cytological analysis, and nuanced nuclear anomalies, chromatin modifications, cytoplasmic characteristics, and contextual indicators may provide diagnostic insights that are overlooked when evaluating features independently. This critique suggests a systematic methodology, the Cytological Grey-Zone Morphological Signature (CGZ-MS) framework, for methodically identifying these "hidden" morphological markers and correlating them with histopathological, molecular, and clinical results [3-6].

DEFINING THE CYTOLOGICAL GREY ZONE: A CONCEPTUAL FRAMEWORK

The phrase "cytological grey zone" denotes the diagnostically intricate spectrum within thyroid FNAC, where cellular, nuclear, and architectural anomalies exist between unequivocal benignity and unmistakable malignancy. In the 2023 TBSRTC, the ambiguous classifications include Bethesda III (AUS), Bethesda IV (follicular neoplasm), and Bethesda V (suspicious for malignancy). The 2023 update enhanced terminological accuracy by incorporating "follicular neoplasm" and preserving "AUS" and "suspicious for malignancy" as specified classifications associated with certain malignancy-risk assessments [7]. The biological foundation of this diagnostic ambiguity encompasses intra-tumoral heterogeneity, sporadic or fluctuating expression of diagnostic characteristics, overlap between benign and malignant morphologies, and the intrinsic sampling constraints of FNAC. A solitary cytological characteristic often possesses restricted specificity; nevertheless, its intensity and distribution, when evaluated alongside accompanying nuclear, architectural, cytoplasmic, and background traits, can uncover clinically significant patterns. This approach endorses the perspective of thyroid cytology as a continuum — morphological phenotype → cytological phenotype → molecular change → histological phenotype → clinical risk, which may, in theory, be systematically mapped instead of being confined to a single category designation [8].

Table 1. Approximate malignancy-risk estimates by 2023 Bethesda category

Bethesda category	Descriptor	Approx. risk of malignancy (adults)	Typical management
I	Non-diagnostic	~13% (5–20%)	Repeat US-guided FNAC
II	Benign	~4% (2–7%)	Clinical / sonographic follow-up
III	Atypia of undetermined significance (AUS)	~13–30%	Repeat FNAC ± molecular testing
IV	Follicular neoplasm	~30% (23–34%)	Molecular testing / diagnostic lobectomy
V	Suspicious for malignancy	~74% (67–83%)	Lobectomy / near-total thyroidectomy
VI	Malignant	~97–100%	Definitive surgery per histotype

Risk estimates are approximate, adapted from the 2023 Bethesda System and associated literature; institution-specific rates may vary with population, technique and sampling.

MORPHOLOGICAL SUBSTRATE: NUCLEAR, ARCHITECTURAL, CYTOPLASMIC AND BACKGROUND FEATURES

Evaluation of diagnostically uncertain thyroid FNAC requires assessment of nuclear, architectural, cytoplasmic, colloidal, background and stromal attributes in combination, so that an isolated atypical feature is not over-interpreted [9].

Nuclear features include nuclear enlargement, elongation, overlap and crowding, chromatin clearing or margination, irregular nuclear outlines, membrane abnormalities, grooves and intranuclear cytoplasmic pseudo-inclusions. Their predictive value increases when several nuclear abnormalities are present concurrently and are relatively homogeneous across the cell population [10].

Architectural features include microfollicular and macrofollicular patterns, trabecular structures, three-dimensional cell nests, papillary-like formations, streaming, and disruption of normal follicular architecture; these patterns, combined with specific nuclear features, help separate follicular-patterned from papillary-type lesions [11].

Cytoplasmic features — volume, density, granularity, oncocytic (Hürthle-cell) transformation and vacuolisation — provide further information on differentiation. Colloid characteristics (quantity, density, texture and dispersion) help distinguish benign hyperplastic processes from follicular-patterned lesions, while background elements (macrophages, lymphocytes, necrotic material, blood, calcification, inflammatory cells and psammoma bodies) and stromal or extracellular-matrix change add contextual information. These findings should be interpreted as correlated, composite features rather than independent diagnostic markers [12].

3.1 Bethesda III: Atypia of Undetermined Significance — the Essential Grey Area

Bethesda III specimens show atypia exceeding that expected for a clearly benign lesion but insufficient for a higher-risk category. The 2023 TBSRTC subdivides AUS into nuclear-atypia and "AUS–other" subgroups, reflecting their differing morphological and molecular associations. Nuclear atypia in AUS typically comprises localised or minimal nuclear enlargement, pale or clear chromatin, grooves, irregular nuclear shapes and cell overlap; AUS–other includes atypical architectural or cytoplasmic change without sufficient criteria for a definitive category, such as focal micro follicular change, oncocytic change, or features associated with inflammation or cystic degeneration [13].

A central challenge in Bethesda III is distinguishing true lesional atypia from reactive, degenerative, cystic or technical change, since follicular/hyperplastic nodules, Hashimoto thyroiditis and cystic degeneration can each mimic neoplastic atypia. Diagnostic suspicion is heightened when nuclear enlargement, chromatin clearing, irregular nuclear shapes, grooves, crowding and architectural disarray occur concurrently and are prominent or

multifocal, particularly alongside papillary-type structures or psammoma bodies — but these findings must always be read within the whole cytological and clinical context rather than as independent markers of malignancy [14].

3.2 Bethesda IV: Follicular Neoplasm

The 2023 TBSRTC replaced "suspicious for a follicular neoplasm" with the definitive term "follicular neoplasm," carrying an estimated malignancy risk of ~30% (23–34%) in adults. Cytologically, these aspirates are moderately to highly cellular, with cells arranged in microfollicular, trabecular, three-dimensional or dispersed patterns and typically scant colloid. A conspicuous microfollicular architecture is most meaningful when accompanied by increased cellularity and a relatively monomorphous population; isolated or rare microfollicles alone are not diagnostic.

Nuclear atypia is generally milder than in papillary thyroid carcinoma (PTC), though mild enlargement, overlap, crowding and chromatin clearing may occur. Oncocytic (Hürthle-cell) variants require careful distinction from hyperplastic or thyroiditis-related Hürthle-cell change. Because FNAC cannot reliably assess capsular or vascular invasion, a definitive distinction between follicular adenoma and follicular carcinoma generally requires histopathological examination of the resected specimen; molecular testing may add value for selected Bethesda IV nodules[15].

3.3 Bethesda V: Suspicious for Malignancy

Bethesda V comprises aspirates highly suggestive of, but not conclusive for, malignancy, with an estimated malignancy risk of ~74% (67–83%) in adults. This heterogeneous category includes suspected PTC, medullary thyroid carcinoma (MTC), lymphoma and metastatic carcinoma. Suspected PTC typically shows nuclear enlargement, crowding and overlap, membrane irregularity, grooves, chromatin clearing and intranuclear cytoplasmic pseudo-inclusions, with or without papillary architecture; findings that fall short of the threshold for definitive (Bethesda VI) diagnosis define the boundary between "suspicious" and "malignant." Suspected MTC may show dispersed plasmacytoid, spindled or polygonal cells with eccentric nuclei and granular cytoplasm, for which calcitonin immunocytochemistry is informative. Lymphoma and metastatic disease should be considered when the cytological pattern is discordant with a primary thyroid epithelial neoplasm, prompting flow cytometry, immunophenotyping or immunocytochemical confirmation as appropriate.

CHARTING CONCEALED MORPHOLOGICAL SIGNATURES

Concealed morphological signatures are nuanced, targeted or composite cytomorphological patterns that individually lack diagnostic specificity but gain discriminatory value when evaluated alongside other cellular and structural features. In inconclusive thyroid FNAC, diagnostic information may reside not in an isolated feature but in the pattern, intensity, distribution and co-occurrence of multiple findings. A systematic, signature-based approach can convert subjective impressions of "atypia" into reproducible morphological profiles suitable for correlation with histopathology, molecular findings and clinical outcome.

4.1 Nuclear Signature Mapping

Nuclear morphology is treated as a set of interrelated features rather than a single abnormality: nuclear enlargement, shape change (elongation, irregularity, distortion), membrane contour abnormalities, chromatin pattern (pallor, clearing, margination, coarseness), grooves, and intranuclear cytoplasmic pseudo-inclusions, each considered for severity, distribution and reproducibility across the follicular cell population. Spatial distribution matters: focal change may carry different significance from widespread, consistently expressed alteration, and the confluence of several nuclear attributes is generally more informative than any single finding.

4.2 Architectural Signature Mapping

Architectural signatures, micro follicular aggregation, dense follicular clustering, trabecular configuration, three-dimensional grouping, papillary-like structures, streaming, and disruption of normal follicular

architecture — provide a complementary axis of assessment. Predominantly micro follicular arrangement with scant colloid suggests a follicular-neoplastic phenotype; papillary architecture with characteristic nuclear irregularity raises suspicion for PTC; and preserved macro follicular architecture with abundant colloid favours a benign hyperplastic process. Architecture is best interpreted jointly with nuclear phenotype rather than as a stand-alone criterion.

4.3 Cross-Domain Interactions

Nuclear and architectural features interact: a microfollicular pattern with largely uniform nuclei suggests a follicular-patterned lesion, whereas papillary-like architecture combined with nuclear enlargement, grooves, chromatin clearing and membrane irregularity raises suspicion for PTC. Cytoplasmic traits should likewise be read against nuclear findings — abundant granular (oncocyctic) cytoplasm with regular nuclei favours a Hürthle-cell process, whereas oncocyctic change accompanying marked nuclear atypia or cellular disorganisation raises greater concern. Background/contextual elements modulate interpretation further: lymphocytes may explain reactive nuclear change in chronic thyroiditis, macrophages suggest cystic degeneration, and psammomatous calcification or an atypical inflammatory context in association with epithelial irregularity raises suspicion of malignancy. Figure 3 illustrates, in schematic and qualitative form, how these four domains are conceptually interdependent rather than independent [16].

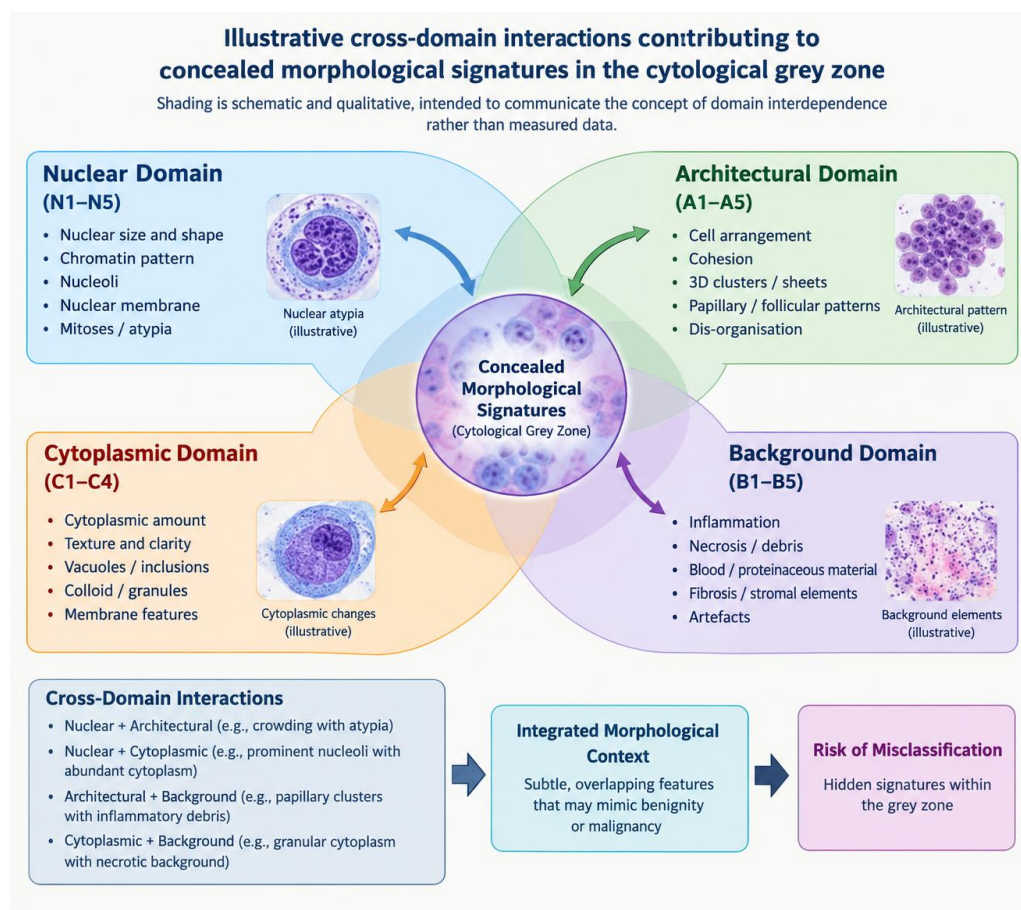


Figure 1. Illustrative cross-domain interactions contributing to concealed morphological signatures in the cytological grey zone. Shading is schematic and qualitative, intended to communicate the concept of domain interdependence rather than measured data.

4.4 Signature Clustering

Signature clustering groups thyroid FNAC specimens by the co-occurrence pattern, rather than isolated presence, of nuclear, architectural, cytoplasmic and background features. Illustrative clusters include a nuclear-dominant cluster (enlargement, chromatin clearing, membrane irregularity, grooves, overlap), an architectural cluster (microfollicular, trabecular or three-dimensional predominance), a papillary-associated cluster (papillary architecture with characteristic nuclear change and calcific material), and an oncocyctic cluster (abundant granular cytoplasm in a reactive or neoplastic context). Additional groupings may reflect colloid

abundance, macrophage burden, lymphocytic infiltration or degenerative change. Clustering does not itself establish malignancy; rather, it identifies reproducible morphological patterns whose clinical significance must be tested against Bethesda category, ultrasound features, histopathology and molecular results, ideally with quantitative scoring of feature frequency, intensity and distribution to improve inter-observer reproducibility.

5. THE PROPOSED CYTOLOGICAL GREY-ZONE MORPHOLOGICAL SIGNATURE (CGZ-MS) FRAMEWORK

Rather than treating atypia as a purely qualitative, single-observation judgement, the CGZ-MS framework organises cytomorphological features into five domains (Figure 2):

Domain I — Nuclear Signature: N1 nuclear enlargement; N2 nuclear shape variation; N3 membrane irregularity; N4 chromatin alteration; N5 nuclear grooves / intranuclear cytoplasmic pseudo-inclusions.

Domain II — Architectural Signature: A1 microfollicular; A2 trabecular; A3 papillary; A4 three-dimensional; A5 streaming / crowding patterns.

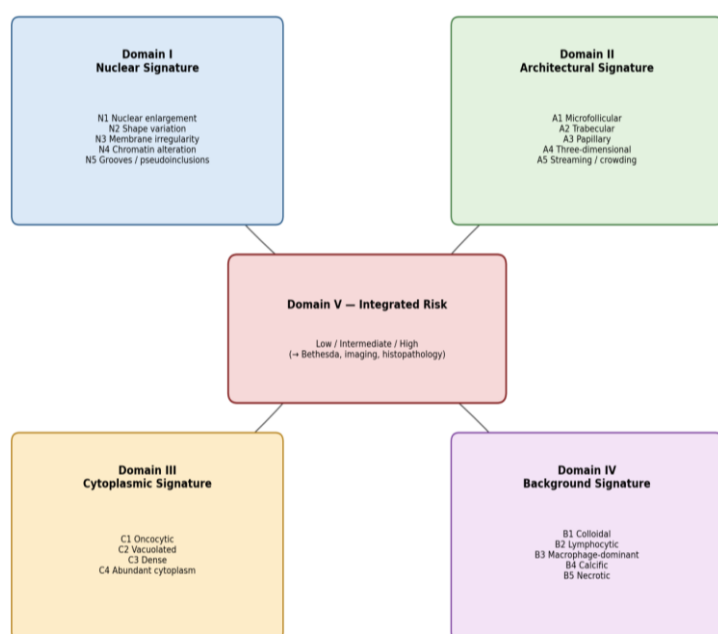
Domain III — Cytoplasmic Signature: C1 oncocytic; C2 vacuolated; C3 dense; C4 abundant cytoplasm.

Domain IV — Background Signature: B1 colloidal; B2 lymphocytic; B3 macrophage-dominant; B4 calcific; B5 necrotic components.

Domain V — Integrated Risk: a synthesis of Domains I–IV into a low-, intermediate- or high-risk tier, subsequently correlated with Bethesda category, ultrasound findings, histopathology and molecular results.

The domains are intended to be assessed holistically, with emphasis on the incidence, variation and connectivity of findings rather than on individual anomalies. The CGZ-MS is proposed as an adjunct to, not a replacement for, the Bethesda System, which remains the accepted reference framework for reporting thyroid cytopathology. It is intended to provide a structure for systematic scoring, inter-observer assessment, digital image analysis and computerised feature extraction, moving from category-driven diagnosis toward signature-based morphological risk mapping. Validation in well-characterised clinical cohorts is required before any of these associations can be regarded as established.

Figure 2. Cytological Grey-Zone Morphological Signature (CGZ-MS) five-domain conceptual framework



Domains I–IV are assessed holistically for incidence, intensity and co-occurrence rather than as isolated features, and converge on Domain V for risk-tier assignment, which is then correlated with Bethesda category, imaging and histopathology.

Figure 2. Cytological Grey-Zone Morphological Signature (CGZ-MS) five-domain conceptual framework. Domains I–IV converge on Domain V for integrated risk-tier assignment.

5.1 Illustrative Differential-Diagnosis Matrix

Table 2 summarises, in illustrative and non-exhaustive form, how dominant feature patterns across the four morphological domains might map onto differential diagnoses relevant to Bethesda III–V. This matrix is intended as a conceptual aid for hypothesis generation and structured reporting, not as a validated diagnostic algorithm.

Dominant pattern	Nuclear features	Architecture	Favoured differential
Reactive / hyperplastic	Minimal, scattered atypia	Macrofollicular, abundant colloid	Benign hyperplastic / adenomatoid nodule
Follicular-neoplastic	Mild enlargement, overlap	Microfollicular, scant colloid, uniform cells	Follicular neoplasm (Bethesda IV)
Oncocytic	Regular nuclei, granular cytoplasm	Sheets / clusters of Hürthle cells	Hürthle-cell change vs. oncocytic neoplasm
Papillary-type	Enlargement, grooves, chromatin clearing, pseudo-inclusions	Papillary fragments ± psammoma bodies	Suspicious for / consistent with PTC
Inflammatory / degenerative	Focal reactive atypia	Disrupted, admixed with lymphocytes/macrophages	Hashimoto thyroiditis, cystic degeneration

CORRELATION BETWEEN CYTOMORPHOLOGY AND HISTOPATHOLOGY

Correlating FNAC with subsequent histopathology is central to validating any morphological signature, since cytology provides a limited sample-based extrapolation while histopathology allows comprehensive assessment of architecture, encapsulation, and vascular or capsular invasion. This correlation is particularly informative for Bethesda III–V lesions. While PTC typically shows characteristic nuclear irregularities on cytology, follicular adenoma and follicular carcinoma can appear cytologically similar, with malignancy determined only by invasive growth on histology. Non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) may show overlapping follicular-patterned cytology with variable papillary-type nuclear features, illustrating a specific pitfall of cytology-only interpretation [11,12]. Oncocytic neoplasms show abundant granular Hürthle-cell cytoplasm, with invasion again determining clinical classification, and MTC may be under-recognised without ancillary immunohistochemical or molecular confirmation.

Discordance between cytology and histology — false positives from reactive, degenerative or thyroiditis-related atypia, and false negatives from sampling error, low cellularity or subtle malignant features — highlights lesions in which systematic correlation is most needed. Identifying which nuclear, architectural, cytoplasmic and background features most reliably associate with histopathological outcome is the central empirical task for validating the CGZ-MS framework [17].

TOWARD QUANTITATIVE MORPHOLOGICAL SCORING

Converting subjective impressions of atypia into an objective, reproducible and observer-independent assessment requires quantitative morphological scoring. Individual features such as nuclear enlargement, membrane irregularity and chromatin clearing may have limited specificity in isolation, but scoring them jointly against a structured rubric can better separate benign, indeterminate and malignant lesions. A practical approach scores nuclear features (enlargement, shape, membrane, chromatin, grooves/inclusions) and architectural features (microfollicular predominance, trabecular pattern, papillary-like structures,

cellularity/architectural disruption) independently, normalising for severity, extent and distribution, before integrating them into a combined score that yields low-, intermediate- and high-risk tiers.

This approach should explicitly distinguish the presence of a feature from its diagnostic weight: isolated nuclear enlargement, grooves or pseudo-inclusions may have low specificity, whereas concurrent abnormality across at least three morphological domains may provide stronger evidence for neoplasia. Inter-observer reproducibility should be measured with appropriate agreement statistics (e.g., weighted kappa), and diagnostic performance reported using sensitivity, specificity, positive and negative predictive value, likelihood ratios and ROC-based calibration (area under the curve). Quantitative morphology is proposed as one component of a broader diagnostic toolkit that also includes histopathology, ultrasonography and molecular testing.

INCORPORATION OF ANCILLARY AND MOLECULAR DIAGNOSTICS

The 2023 TBSRTC gives increased weight to ancillary and molecular studies for diagnostically challenging Bethesda III and IV samples [4–7]. Immunocytochemistry can clarify cell lineage: thyroglobulin and TTF-1 support thyroid follicular-cell origin, PAX8 supports thyroid lineage more broadly, and calcitonin is informative when MTC is suspected; results should always be interpreted alongside cytomorphological, clinical and radiological data.

Molecular testing detects alterations in genes such as BRAF, the RAS family, RET, NTRK and TERT. Point-mutation panels have high specificity and positive predictive value as "rule-in" tests but comparatively low sensitivity, whereas RNA-based gene-expression or genomic-sequencing classifiers (e.g., Afirma-type platforms) have been validated as "rule-out" tests with high negative predictive value, and next-generation-sequencing-based classifiers (e.g., ThyroSeq-type platforms) combine mutation and expression data to refine risk stratification further [8–10]. Since a mutated gene is not synonymous with malignancy, and RAS-family alterations occur in some histologically benign nodules, molecular results are best interpreted as complementary to, rather than a substitute for, careful cytomorphological evaluation. A logical diagnostic sequence is morphological evaluation → molecular profiling (where indicated and available) → risk stratification → clinical correlation, with or without histopathological confirmation[18].

ARTIFICIAL INTELLIGENCE AND DIGITAL CYTOPATHOLOGY

Digital cytopathology and whole-slide imaging (WSI) are increasingly used to support objective, reproducible feature extraction in thyroid FNAC, although WSI adoption in cytology has lagged behind histopathology because of image-quality, scanning-speed and cost considerations [19]. Machine-learning and deep-learning approaches — including convolutional neural networks — have been applied to segment follicular cells, lymphocytes, colloid and atypical aggregates, and to classify thyroid cytology as benign, malignant or indeterminate, but external validation across institutions and populations remains limited. A recent systematic review of AI and WSI applications specifically in AUS/FLUS cytology identified a small number of eligible studies using varied classifiers (convolutional and feedforward neural networks, gradient-boosted trees and others) with promising but heterogeneous performance, underscoring the early stage of this evidence base[19–20].

AI-based feature extraction can, in principle, translate qualitative morphological impressions (nuclear, architectural, cytoplasmic and background features) into quantitative, multidimensional metrics compatible with the CGZ-MS domains. Explainable AI (XAI) approaches are particularly relevant here, since diagnostic tools that cannot relate their output to recognisable cytomorphological features are unlikely to gain clinical trust; the most realistic near-term model combines automated feature extraction with pathologist-led contextual interpretation rather than autonomous algorithmic diagnosis. Persistent barriers include dataset bias, inconsistent staining/preparation protocols, lack of annotation consensus, limited external validation, interoperability constraints, and regulatory and generalisability challenges across institutions [21–22].

DIAGNOSTIC PITFALLS AND SOURCES OF VARIABILITY

Diagnostic accuracy in thyroid FNAC is shaped by pre-analytical, analytical, morphological and interpretive factors, with false positives and false negatives concentrated in the equivocal categories. Sampling error is a major limitation, since malignant foci nested within otherwise benign tissue may not be adequately targeted; suboptimal cellularity, haemorrhage, coagulation artefact, and preparation- or staining-related artefact can further distort nuclear, chromatin and architectural appearances. Blood contamination may obscure atypical cells with subtle features, while cystic degeneration can generate macrophages, necrotic debris and reactive lining-cell atypia that mimics carcinoma. Hashimoto thyroiditis frequently produces Hürthle-cell transformation, nuclear enlargement and irregularity that can mimic neoplastic atypia.

Observer variability is particularly important in Bethesda III–V lesions, where subtle nuclear and architectural differences determine categorisation; standardised terminology, quality assurance, second-opinion review and collaborative assessment can help reduce this variability. "Category inflation" — overuse of indeterminate categories — can trigger unnecessary repeat procedures or surgery, whereas over-weighting isolated features such as focal grooves or minimal enlargement risks false-positive diagnosis in benign, inflammatory or degenerative states, and under-recognition of focal atypia limited to a small portion of the aspirate risks false-negative diagnosis. These considerations reinforce the case for composite, multi-domain morphological assessment supported by adequate sampling and appropriate ancillary testing.

STRENGTHS, LIMITATIONS AND RESEARCH GAPS

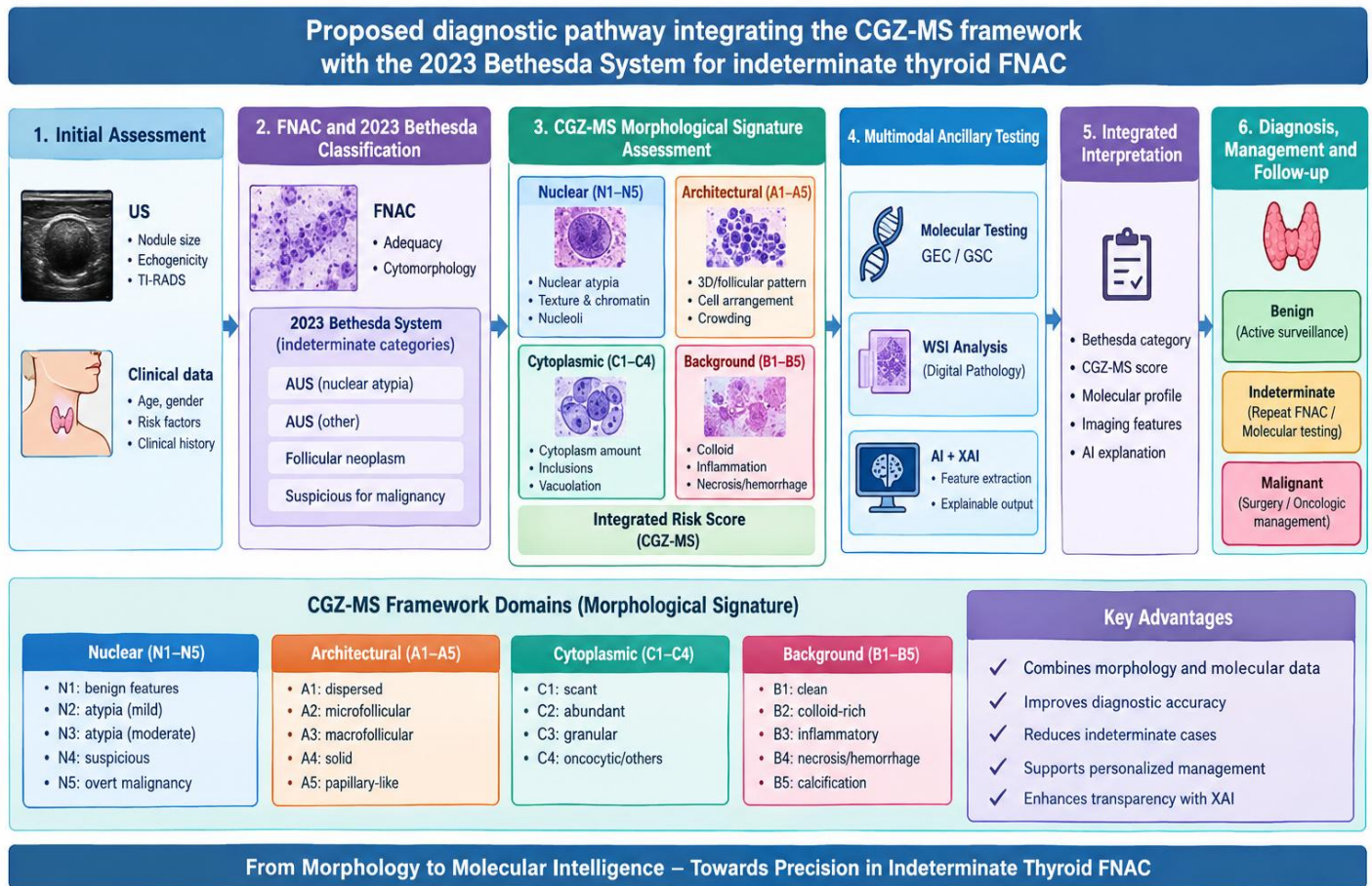
The proposed framework's principal strength is its morphology-focused, multi-domain methodology, which moves beyond categorical reporting by systematically incorporating nuclear, architectural, cytoplasmic, colloidal, background and stromal attributes into potentially reproducible signatures. Its alignment with the 2023 TBSRTC provides a contemporary reference structure, and its explicit accommodation of histopathological correlation, molecular diagnostics and AI-assisted digital cytology positions it for future multimodal integration.

Important limitations must be acknowledged. Cytomorphological analysis remains fundamentally subjective, particularly for minor or focal abnormalities, and sampling variability may under-represent heterogeneous lesions. Inter-observer variability in molecularly relevant indeterminate categories can be considerable, as shown in multicentre studies. Molecular testing is not universally available because of cost, infrastructure and technical-expertise constraints, which may limit implementation in many healthcare settings. Key research gaps include the absence of a globally accepted morphological grading system, limited prospective data linking individual or composite signatures to long-term outcomes, and variable definitions of "significant" minor atypical change. The integration of morphology with AI-derived quantitative features and molecular data also remains at an early stage. Future work should prioritise multicentre prospective studies, standardised image-based datasets, formal reproducibility assessment, morphology–molecular correlation studies, and independent validation of the CGZ-MS framework to determine whether concealed morphological signatures add diagnostic or prognostic value beyond the Bethesda classification alone[23–25].

FUTURE DIRECTIONS

Indeterminate thyroid FNAC should progressively move beyond category-based diagnosis toward systematic, quantitative, multimodal and prospectively validated morphological risk assessment. This requires developing an objective morphological scoring system with clearly defined parameters, weighting and reproducibility thresholds; testing this scoring across multiple institutions, populations, staining protocols and imaging platforms; and building large, well-curated, multi-institutional digital cytology datasets linking Bethesda category, ultrasound findings, molecular results, histopathology and clinical outcome. Explainable AI-enhanced morphological mapping may support automated cell identification, nuclear quantification, architectural pattern recognition and multidimensional signature extraction, provided algorithmic predictions remain traceable to recognisable cytomorphological features. Fusion of cytomorphology with ultrasound risk stratification and, prospectively, with genomic data, may enable more individualised malignancy-risk

estimation. Development of standardised reference atlases depicting representative low-, intermediate- and high-risk morphological profiles could support training and improve global inter-observer agreement, ultimately helping individualise management — active surveillance, repeat FNAC, molecular testing, or diagnostic surgery — according to comprehensive biological risk rather than cytological category alone.



US = ultrasonography; GEC/GSC = gene-expression / genomic-sequencing classifier; WSI = whole-slide imaging; XAI = explainable artificial intelligence.

Figure 3. Proposed diagnostic pathway integrating the CGZ-MS framework with the 2023 Bethesda System for indeterminate thyroid FNAC. US = ultrasonography; GEC/GSC = gene-expression / genomic-sequencing classifier; WSI = whole-slide imaging; XAI = explainable artificial intelligence.

CONCLUSION

The cytological grey zone of indeterminate thyroid FNAC should not be viewed only as a realm of diagnostic ambiguity but as a biologically heterogeneous morphological spectrum in which clinically relevant information may be hidden within nuanced, localised and interrelated cytological features. Systematic mapping of nuclear, architectural, cytoplasmic, background and stromal signals offers a conceptual route for converting descriptive atypia into organised morphological phenotypes. Combining these signatures with the standardised lexicon and risk framework of the 2023 Bethesda System, ultrasound findings, clinical data, histopathological correlation and judiciously selected ancillary or molecular testing could improve diagnostic clarity in Bethesda III–V lesions. The proposed CGZ-MS framework offers a potential foundation for quantitative assessment, inter-observer comparison, digital cytology and AI-assisted morphological analysis; its clinical relevance, however, depends entirely on prospective, multicentre validation. The framework is intended to complement, not replace, expert cyto-pathological assessment and the established Bethesda classification, by systematically identifying composite morphological markers and their biological associations — ultimately working toward a more personalised, evidence-based approach to malignancy-risk assessment in the thyroid cytological grey zone.

DECLARATIONS

Ethics approval and consent to participate

Not applicable — this is a conceptual/narrative review proposing a framework and does not report new patient-level data.

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Conflicts of interest

The author declare no conflicts of interest relevant to this manuscript (to be confirmed/completed by the author team).

Author contributions

To be completed using a standard taxonomy (e.g., CRediT) prior to submission.

Data availability

Not applicable; no primary dataset was generated. The conceptual framework and figures are provided within this manuscript.

REFERENCES

1. Juhlin, C. C., & Baloch, Z. W. (2024). Pitfalls In Thyroid Fine-Needle Aspiration Cytopathology: An Approach To Atypical Findings. *Acta Cytologica*, 68(3), 179-193.
2. Canberk, S., & Baloch, Z. W. (2026). The Current Status Of Thyroid Cytology In The Era Of New Classifications, Molecular Profiling, And Clinical Risk Stratification. *Acta Cytologica*.
3. Saini, T., Kundu, R., Rohilla, M., Gupta, P., Gupta, N., Srinivasan, R., ... & Dey, P. (2024). Gray Zone Bethesda Category Iii–Atypia Of Undetermined Significance Lesions Of The Thyroid: Potential Diagnostic Issues And Image Morphometry As A Useful Adjunct To Cytomorphology. *Cytojournal*, 21, 38.
4. Piga, I., L'imperio, V., Capitoli, G., Denti, V., Smith, A., Magni, F., & Pagni, F. (2023). Paving The Path Toward Multi-Omics Approaches In The Diagnostic Challenges Faced In Thyroid Pathology. *Expert Review Of Proteomics*, 20(12), 419-437.
5. Guo, Y., Zhao, T., Zhang, L., Liu, Y., Liu, K., Han, B., ... & Zhao, L. (2026). Artificial Intelligence In Thyroid Ultrasound: Clinical Applications And Perspectives. *Frontiers In Endocrinology*, 17, 1877780.
6. Bilookyi, O. V., Hovornyan, S. L., & Bilookyi, V. V. (2026). Possibilities And Prospects Of Application Of Modern Methods In The Diagnostics Of Thyroid Diseases. *International Journal Of Endocrinology (Ukraine)*, 22(5), 537-546.
7. Rossi, E. D., Piermattei, A., Cianfrini, F., Cappoli, N., Mulè, A., & Pantanowitz, L. (2026). Thyroid Cytology: Practical Tricks And Pitfalls. *Virchows Archiv*, 488(1), 129-146.
8. Tralongo, P., Ballato, M., Fiorentino, V., Zuccalà, V., Pizzimenti, C., Pepe, L. R., ... & Fadda, G. (2026). The Role Of Artificial Intelligence In Thyroid Cytology Of Indeterminate Nodules: From Digital Cytology To Multimodal Precision Triage. *Frontiers In Endocrinology*, 17, 1800918.
9. Ribeiro, M., Canberk, S., & Bongiovanni, M. (2026). The Role Of Cytology, Histology And Molecular Pathology In The Diagnostic Process Of Thyroid Nodules. *Cancers*, 18(11), 1814.
10. Bordoni, M., Aboud, N., Silvetti, F., Tacaliti, A., Balercia, G., & Salvio, G. (2025). Towards An Integrated Multi-Omic Approach To Improve The Diagnostic Accuracy Of Fine-Needle Aspiration In Thyroid Nodules With Indeterminate Cytology. *Diagnostics*, 15(12), 1506.
11. Capitoli, G., Piga, I., L'imperio, V., Clerici, F., Leni, D., Garancini, M., ... & Pagni, F. (2022). Cytomolecular Classification Of Thyroid Nodules Using Fine-Needle Washes Aspiration Biopsies. *International Journal Of Molecular Sciences*, 23(8), 4156.

12. Monia, B., Nairus, A., Francesca, S., Augusto, T., Giancarlo, B., & Gianmaria, S. (2025). Towards An Integrated Multi-Omic Approach To Improve The Diagnostic Accuracy Of Fine-Needle Aspiration In Thyroid Nodules With Indeterminate Cytology. *Diagnostics*, 15(12), 1506.
13. Suo, W., Guo, C., Zhang, X., Li, H., Lai, W., Zhang, J., ... & Zhang, J. (2026). Radiomics In Thyroid Nodule Assessment. *Bmc Medical Imaging*.
14. Braga, E. R., Braga Júnior, R. M., Braga, M. S., Cerutti, J. M., & Salcedo, W. J. (2026). Portable Multispectral Optoelectronic System For Thyroid Cancer Detection. *Sensors*, 26(14), 4448.
15. Kumar, S., Dutta, A., Singh, B. P., Vallur, S., & Singh, S. (2026). Ai-Driven Early Diagnosis And Management Of Head-And-Neck Malignancies: Current Insights And Future Directions. *Indian Journal Of Otolaryngology And Head & Neck Surgery*, 78(6), 3543-3556.
16. Li, M., Yang, D., Liu, T., Liu, S., Sun, W., Cai, P., ... & Li, J. (2026). Label-Free Molecular Profiling Of Cancer Using Raman Spectroscopy: From Fundamentals To Clinical Applications. *Frontiers In Oncology*, 16, 1822181.
17. Radhachandran, A. (2026). Deep Learning For Ultrasound Imaging: From Task-Specific Architectures To Foundation Models (Doctoral Dissertation, University Of California, Los Angeles).
18. Singh, A. P., Siddiqui, J., & Singh, V. (2026). Advanced Tissue Processing: Automation And Emerging Technologies. *Indian Journal Of Pathology: Research And Practice*, 15(1).
19. Verma, K., Kumar, N., Singh, R., & Singh, A. P. Path Lab-Ai: An Autonomous Framework For Error-Free Histopathology Slide Interpretation. *Research & Reviews: A Journal Of Bioinformatics*. 2026; 13 (1): 19–30p. *Stm Journals* 2026. All Rights Reserved, 20, 2.
20. Singh, A. P., Siddiqui, J., & Singh, V. (2026). Safety And Ethics In The Histopathology Laboratory. *Indian Journal Of Pathology: Research And Practice*, 15(1), 31-45.
21. Semwal, S., Mishra, P., Kumar, A., & Singh, A. P. (2026). Integrated Assessment Of Haematological, Inflammatory And Hormonal Biomarkers In Women With Polycystic Ovary Syndrome Aged 15–40 Years. *Biochemical & Cellular Archives*, 26(1), 951.
22. Singh, R., Verma, K., Kumar, N., & Singh, A. P. (2026). Beyond The H&E Slide: Uncovering Hidden Histopathological Signatures With Spatial Omics And Artificial Intelligence. *Journal Of Basic And Applied Research International*, 32(5), 75-91.
23. Singh, A. P., Saxena, R., & Saxena, S. (2023). Cytopathology: An Important Aspect Of Medical Diagnosis. *Research & Reviews: Journal Of Oncology And Hematology*. 2023; 12 (3): 13–18p. Cytopathology: An Important Aspect Of Medical Diagnosis Singh Et Al. *Stm Journals*, 2.
24. Rahman, H. A., & Singh, A. P. (2024). Section Cutting In Histopathology: An Update. *Research & Reviews: A Journal Of Health Professions*. 2024; 14 (1): 29–34p. Section Cutting In Histopathology: An Update Rahman And Singh *Stm Journals*, 2.
25. Cruz, S., & Murugan, J. (2026). Comprehensive Review Of Fuzzy Systems In Cancer Research. *Archives Of Computational Methods In Engineering*, 1-45.