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ДИАГНОСТИЧЕСКАЯ ЗНАЧИМОСТЬ МУЛЬТИМАРКЕРНОЙ ИММУНОЦИТОХИМИЧЕСКОЙ ПАНЕЛИ GALECTIN-3, CK19 И HBME-1 В ВЕРИФИКАЦИИ НЕОПРЕДЕЛЕННЫХ КАТЕГОРИЙ BETHESDA ПРИ ТАБ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

Аннотация: Неопределенные результаты тонкоигольной аспирационной биопсии щитовидной железы сохраняют высокую диагностическую значимость вследствие недостаточности цитоморфологических признаков для уверенного разграничения доброкачественных и злокачественных фолликулярно-клеточных поражений. Наибольшие затруднения связаны с категориями Bethesda III и IV. Цель исследования заключается в оценке диагностической значимости сочетанного применения Galectin-3, CK19 и HBME-1 при неопределенных результатах ТАБ и разработке последовательности интерпретации мультимаркерного иммунопрофиля. Использованы анализ научных публикаций, сопоставление диагностических характеристик маркеров и клинико-морфологический анализ наблюдения. Установлено, что совместная оценка трех маркеров повышает информативность дополнительного исследования. Клиническое наблюдение демонстрирует значение согласованной тройной позитивности при фолликулярно-паттернированном поражении с последующей гистологической верификацией папиллярного рака щитовидной железы.

Ключевые слова: щитовидная железа, Bethesda, тонкоигольная аспирационная биопсия, иммуноцитохимия, Galectin-3, CK19, HBME-1, папиллярный рак.

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DIAGNOSTIC SIGNIFICANCE OF THE GALECTIN-3, CK19 AND HBME-1 MULTIMARKER IMMUNOCYTOCHEMICAL PANEL IN THE VERIFICATION OF INDETERMINATE BETHESDA CATEGORIES IN THYROID FINE-NEEDLE ASPIRATION

Abstract: Indeterminate thyroid fine-needle aspiration results remain diagnostically significant because cytomorphological findings may be insufficient for reliable differentiation between benign and malignant follicular-cell lesions. The greatest difficulties are associated with Bethesda III and IV categories. The study aims to evaluate the diagnostic significance of combined Galectin-3, CK19, and HBME-1 testing and to develop a sequence for interpreting multimarker immunoprofiles. Scientific literature analysis, comparison of marker diagnostic characteristics, and clinicopathological assessment of an illustrative case were used. Combined evaluation of the three markers increased the informative value of ancillary testing. The clinical case demonstrates the significance of concordant triple positivity in a follicular-patterned lesion followed by histological verification of papillary thyroid carcinoma.

Keywords: thyroid, Bethesda, fine-needle aspiration, immunocytochemistry, Galectin-3, CK19, HBME-1, papillary thyroid carcinoma.

Fine-needle aspiration (FNA) is the primary method for the initial morphological assessment of thyroid nodules. The Bethesda System standardizes cytological reporting and provides a basis for determining subsequent diagnostic management. In the 2023 edition, category III is

designated as atypia of undetermined significance (AUS), while category IV is designated as follicular neoplasm (FN). Both categories are characterized by cytological indeterminacy, in which the observed features are insufficient to reliably determine the nature of the lesion [1].

In Bethesda III, cytological specimens may show moderate nuclear changes, microfollicular structures, cellular crowding, and alterations in the relative proportions of cellular and colloid components. However, these features are insufficiently pronounced to classify the specimen into a more definitive category. Bethesda IV is characterized by increased cellularity and a predominantly follicular or microfollicular pattern. A major limitation of FNA in such lesions is the inability to assess capsular and vascular invasion in cytological specimens. Therefore, definitive characterization of certain follicular-patterned neoplasms requires examination of surgically excised tissue [2].

Additional diagnostic information can be obtained through the use of immunocytochemical markers. Galectin-3, CK19, and HBME-1 are of particular interest. Galectin-3 is a β-galactoside-binding protein involved in cell adhesion, apoptosis, and tumor progression. CK19 is a low-molecular-weight cytokeratin, strong expression of which is characteristic of papillary differentiation. HBME-1 is used as a membranous marker in the evaluation of follicular-cell tumors. The diagnostic performance of these antibodies depends on the type of specimen examined, positivity criteria, techniques used, and the morphological variant of the tumor [3] (Table 1):

Table 1. Comparative Characteristics of the Immunocytochemical Markers in the Galectin-3, CK19, and HBME-1 Panel

Marker	**Biological role**	**Staining localization**	**Sensitivity / specificity for PTC**	**Diagnostic limitations**
Galectin-3	Lectin-family protein involved in cell adhesion,	Cytoplasm and nucleus	Sensitivity: 85–92%; Specificity: 80–	Weak positivity may occur in Hürthle cell adenomas and

	apoptosis, and metastasis		88%	severe autoimmune thyroiditis
CK19	Low-molecular-weight cytokeratin; component of intermediate filaments	Cytoplasm; membranous and perinuclear staining patterns	Sensitivity: 90–95%; Specificity: 65–75%	High sensitivity is accompanied by limited specificity; positivity may occur in follicular adenomas and thyroiditis
HBME-1	Membranous antigen used in the evaluation of follicular-cell lesions	Membrane; apical/luminal region	Sensitivity: 80–87%; Specificity: 85–90%	False-positive results may occur in nodular goiter
Combination	Combined assessment of the tumor immunoprofile	Integrated assessment of all three markers	Sensitivity: >95%; Specificity: >92%	Requires correlation with cytomorphological, ultrasound, and clinical findings

The diagnostic yield of immunocytochemical analysis is largely determined by the quality of the cytological material. The use of cell blocks in Bethesda III specimens allows the expression of multiple markers to be compared within the same cell groups. Insufficient sample cellularity remains a limitation of the method and may complicate interpretation or result in a non-diagnostic finding [4].

The diagnostic significance of the panel is determined by the overall immunoprofile. Isolated CK19 positivity, weak focal Galectin-3 staining, or isolated membranous HBME-1 staining has limited diagnostic value when considered independently. A more significant finding is concordant strong expression of two or three markers in morphologically suspicious cells. Incorporating CK19, Galectin-3, and HBME-1 (Fig. 1) into the diagnostic assessment alongside cytological findings increases the diagnostic yield in indeterminate thyroid lesions [5].

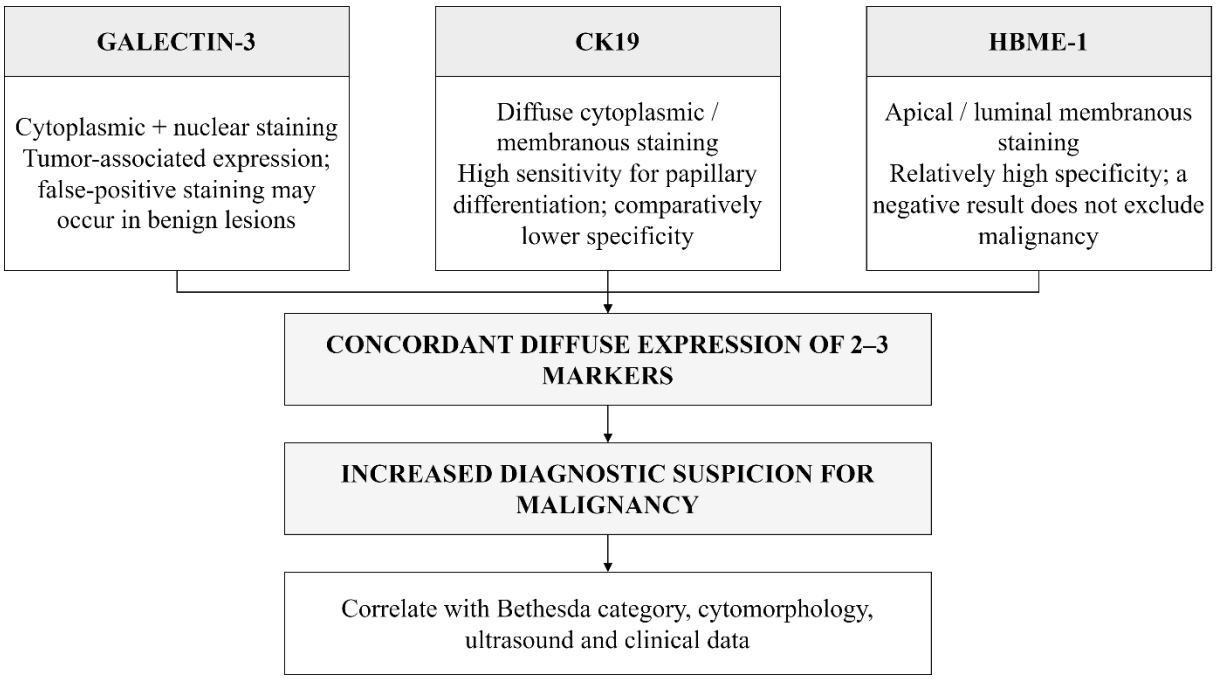

Fig. 1. Comparative interpretation of the Galectin-3, CK19, and HBME-1 multimarker panel

Combined assessment of Galectin-3, CK19, and HBME-1 reduces the impact of the diagnostic limitations of individual antibodies. For example, a systematic review and diagnostic meta-analysis demonstrated high specificity for the combined assessment of these markers in differentiating thyroid lesions. The numerical estimates vary depending on the type of specimen and staining methodology. Therefore, triple positivity should be interpreted in the context of the underlying morphological findings [6].

The proposed diagnostic approach (see Figs. 2 and 3) involves sequential assessment of FNA and immunocytochemical findings. Once a Bethesda III or IV diagnosis has been established, specimen adequacy for ancillary testing is assessed. Expression of Galectin-3, CK19, and HBME-1 is then evaluated with regard to staining intensity, extent, and localization. A predominantly negative or focal immunoprofile warrants repeat morphological assessment and determination of subsequent diagnostic management. Concordant diffuse positivity increases the likelihood of malignancy and should be interpreted in conjunction with ultrasound findings and clinical data.

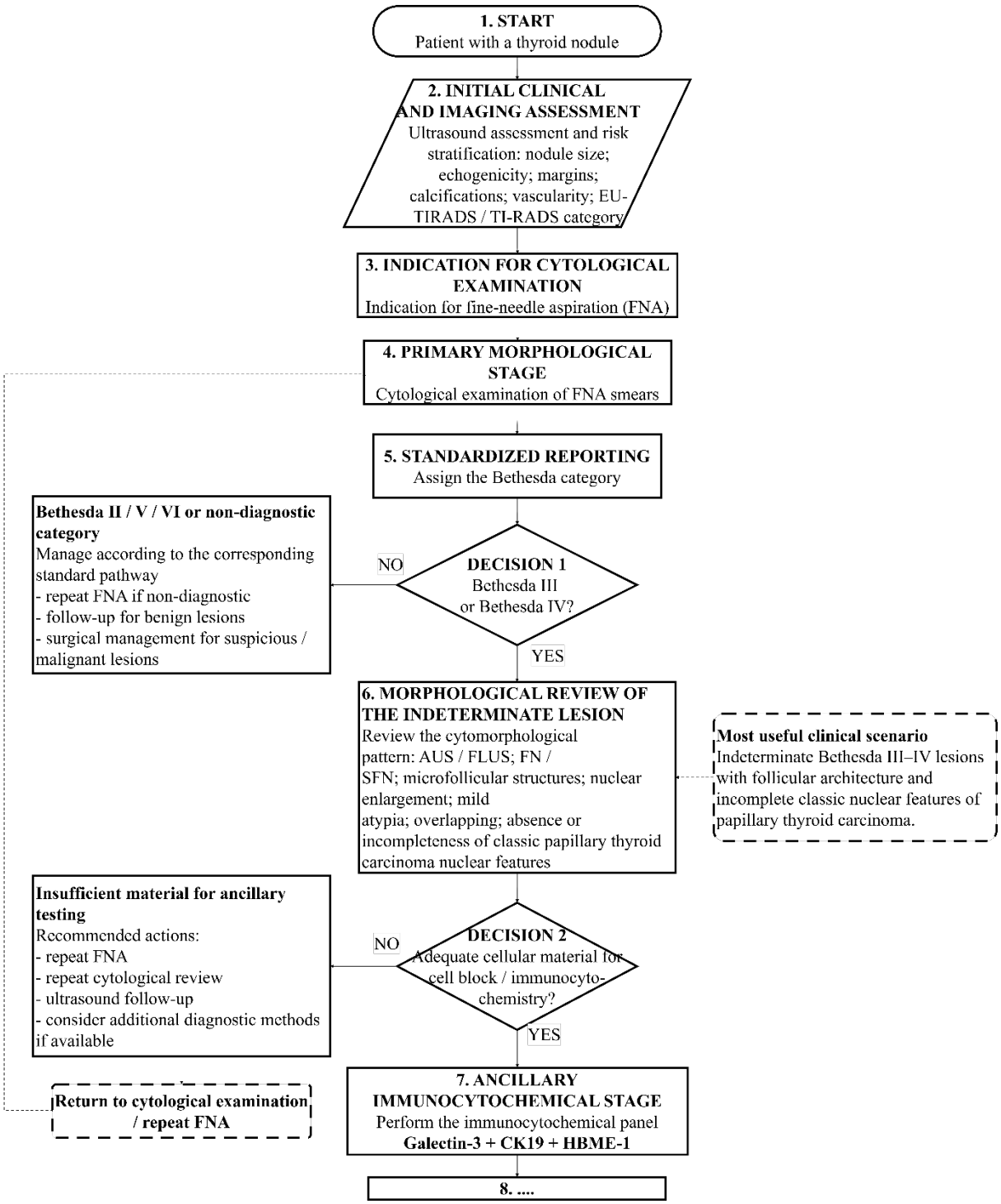

Fig. 2. Diagnostic algorithm for an indeterminate thyroid nodule (1)

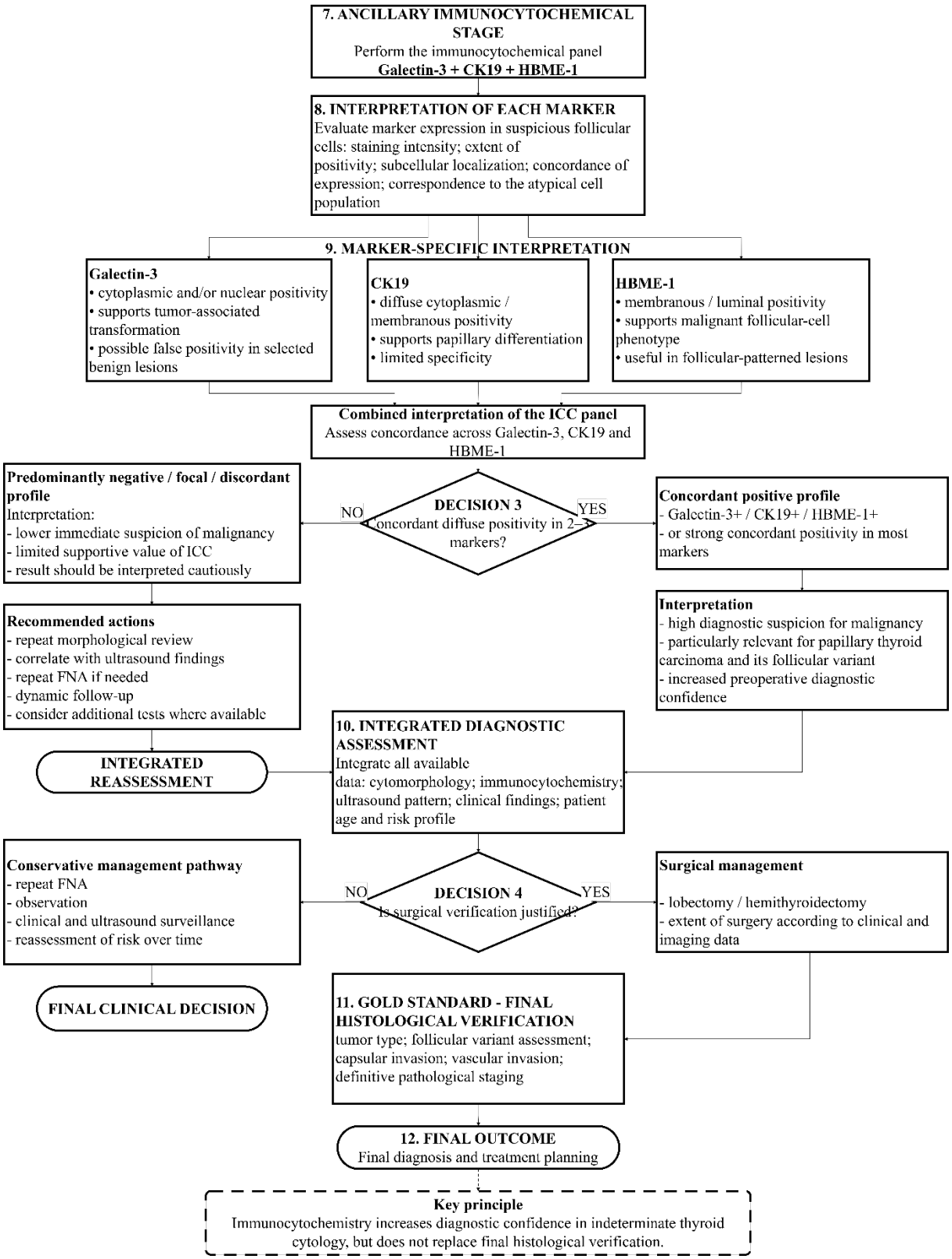

Fig. 3. Diagnostic algorithm for an indeterminate thyroid nodule (2)

The practical applicability of the proposed approach is illustrated by the clinical case of a 34-year-old female patient, J. A nodule in the right thyroid lobe

was incidentally detected during a routine ultrasound examination. Ultrasound revealed a hypoechoic nodule with ill-defined margins measuring 18 × 12 × 14 mm in the right lobe. No microcalcifications were identified. Moderate intranodular vascularity was observed. The nodule was classified as EU-TIRADS 4.

FNA yielded a moderately cellular specimen. The smears showed monomorphic cell groups and microfollicular structures composed of follicular epithelial cells. Moderate nuclear enlargement, mild anisokaryosis, and cellular crowding were observed. Orphan Annie eye nuclei and true intranuclear cytoplasmic inclusions were absent. The smear background contained scant eosinophilic colloid and blood elements. Taken together, these findings were consistent with an indeterminate Bethesda III (AUS) cytological diagnosis.

Immunocytochemical analysis was performed to further assess the malignant potential of the nodule. Galectin-3 showed strong positivity, with diffuse cytoplasmic and nuclear staining in more than 60% of atypical follicular cells. CK19 showed strong positivity, with intense cytoplasmic staining exhibiting membranous and perinuclear patterns in all follicular structures. HBME-1 demonstrated distinct luminal and membranous staining of atypical cells.

The resulting Galectin-3+/CK19+/HBME-1+ profile was characterized by concordant strong expression of all three markers. This finding substantially increased diagnostic suspicion for papillary thyroid carcinoma and provided additional justification for surgical verification.

Based on the clinical findings, the patient underwent right hemithyroidectomy. Subsequent histological examination confirmed papillary thyroid carcinoma with a follicular pattern. The tumor measured 16 mm and was staged as pT1bN0M0. No evidence of invasion of the thyroid capsule or blood vessels was identified. It should be noted that morphological verification of the tumor and assessment of its characteristics are critical for determining subsequent management of patients with differentiated thyroid carcinoma [7].

Thus, the presented case illustrates a major diagnostic challenge associated with follicular-patterned variants of papillary thyroid carcinoma. The absence of prominent classic nuclear features may result in a Bethesda III or IV diagnosis. In this cytological setting, assessment of Galectin-3, CK19, and HBME-1 can provide additional evidence of neoplastic transformation. Concordant strong expression of multiple markers is the most informative finding.

However, immunocytochemical analysis has several limitations, including insufficient sample cellularity, the quality of cell block preparation, fixation conditions, and differences among the antibodies used. Laboratory-specific criteria for positivity also play an important role. False-positive reactions may occur in benign lesions, while false-negative results are possible in certain carcinoma variants. Therefore, the immunoprofile should be interpreted in the context of cytomorphological, ultrasound, and clinical findings.

The analysis demonstrates the diagnostic significance of the Galectin-3, CK19, and HBME-1 multimarker panel in indeterminate Bethesda categories. Each of the markers evaluated has inherent limitations in sensitivity and specificity. Concordant assessment of their expression patterns increases the diagnostic yield of ancillary testing. The greatest diagnostic significance is associated with strong diffuse positivity for multiple markers in morphologically suspicious follicular cells.

Thus, the clinical case supports the utility of the panel in follicular-patterned lesions lacking well-developed nuclear features of papillary thyroid carcinoma. The Galectin-3+/CK19+/HBME-1+ profile increased diagnostic suspicion and provided additional support for surgical verification. The definitive diagnosis of such neoplasms is established by histological examination.

References:

1. Ali S.Z., Baloch Z.W., Cochand-Priollet B., Schmitt F.C., Vielh P., VanderLaan P.A. The 2023 Bethesda System for Reporting Thyroid Cytopathology // Thyroid. – 2023. – Vol. 33. – No. 9. – P. 1039–1044. – DOI 10.1089/thy.2023.0141.

2. Crescenzi A., Baloch Z. Immunohistochemistry in the pathologic diagnosis and management of thyroid neoplasms // Frontiers in Endocrinology. – 2023. – Vol. 14. – Art. 1198099. – DOI 10.3389/fendo.2023.1198099.

3. Xin Y., Guan D., Meng K. et al. Diagnostic accuracy of CK-19, Galectin-3 and HBME-1 on papillary thyroid carcinoma: a meta-analysis // International Journal of Clinical and Experimental Pathology. – 2017. – Vol. 10. – No. 8. – P. 8130–8140.

4. Abram M., Huhtamella R., Kalfert D. et al. The Role of Cell Blocks and Immunohistochemistry in Thyroid Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Bethesda Category // Acta Cytologica. – 2021. – Vol. 65. – No. 3. – P. 257–263. – DOI 10.1159/000514906.

5. Margari N., Giovannopoulos I., Pouliakis A. et al. Application of Immunocytochemistry on Cell Block Sections for the Investigation of Thyroid Lesions // Acta Cytologica. – 2018. – Vol. 62. – No. 2. – P. 137–144. – DOI 10.1159/000485824.

6. de Matos L.L., Del Giglio A.B., Matsubayashi C.O. et al. Expression of CK-19, Galectin-3 and HBME-1 in the differentiation of thyroid lesions: systematic review and diagnostic meta-analysis // Diagnostic Pathology. – 2012. – Vol. 7. – Art. 97. – DOI 10.1186/1746-1596-7-97.

7. Ringel M.D., Sosa J.A., Baloch Z. et al. 2025 American Thyroid Association Management Guidelines for Adult Patients with Differentiated Thyroid Cancer // Thyroid. – 2025. – Vol. 35. – No. 8. – P. 841–985. – DOI: 10.1177/10507256251363120.