Section: Pathology

Original Research Article

IMMUNOHISTOCHEMICAL EVALUATION OF GALECTIN-3 AS A DIAGNOSTIC MARKER IN THYROID NODULES

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Received : *31/12/2025*
Received in revised form : *20/02/2026*
Accepted : *06/03/2026*

Keywords:
Thyroid nodules, Galectin-3, Immunohistochemistry, Papillary carcinoma, Follicular carcinoma.

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DOI: 10.47009/jamp.2026.8.2.50

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026; 8 (2); 264-270

Abstract

Background: Thyroid nodules are common endocrine lesions, with a small but significant proportion being malignant. On regular histology, distinguishing benign from malignant nodules—especially those with follicular architecture—remains a diagnostic problem. An emerging immunohistochemical marker in thyroid disease is Galectin-3, a β-galactoside-binding lectin involved in tumor growth and apoptosis regulation. The purpose of this study was to determine whether Galectin-3 is expressed in thyroid nodules and whether it may be used to differentiate between benign and malignant nodules. **Materials and Methods:** This prospective and retrospective observational study included 50 histopathologically confirmed nodular thyroid lesions over five years (June 2014–June 2019). Cases comprised 28 non-neoplastic lesions, 8 benign neoplasms (follicular adenoma), and 14 malignant tumors (12 papillary carcinoma, 2 follicular carcinoma). Hematoxylin and eosin staining was performed for diagnosis, followed by immunohistochemistry using Galectin-3 antibody (clone b2c10). Cytoplasmic ± nuclear staining in ≥5% of cells was considered positive and graded based on intensity and proportion. **Results:** Non-neoplastic lesions constituted 56% of cases, benign neoplasms 16%, and malignant tumors 28%. A strong female predominance (90%) was observed, with the majority of cases occurring in the 31–40-year age group (44%). Most non-neoplastic and benign lesions were negative or weakly (1+) positive for Galectin-3. In contrast, malignant tumors demonstrated higher expression, with 2+ and 3+ staining predominantly seen in papillary carcinoma. Strong (3+) positivity was observed exclusively in malignant lesions. Using a cut-off of ≥2+, Galectin-3 showed high sensitivity and specificity in differentiating malignant from benign lesions. **Conclusion:** Galectin-3 is a sensitive and specific adjunct immunohistochemical marker for distinguishing benign from malignant thyroid nodules. Higher staining intensity (≥2+) strongly correlates with malignancy, supporting its routine use in diagnostically challenging thyroid lesions.

INTRODUCTION

Thyroid nodules are among the most frequently encountered endocrine abnormalities in clinical practice. Over the past several decades, the detection rate of thyroid nodules has significantly increased due to the growing use of high-resolution ultrasonography and other imaging modalities.[1] According to epidemiological research, thyroid nodules are more prevalent among women, the elderly, those living in iodine-deficient areas, and people with a history of head and neck radiation exposure. Accurate diagnosis is crucial for proper patient care since, despite the fact that most thyroid nodules are benign, a clinically significant percentage of them are malignant.[2]

When patients come with thyroid nodules, the most significant worry is thyroid cancer. Geographical and demographic factors influence the reported prevalence of thyroid nodule cancer, which ranges from 1.5% to 17%. Because treatment approaches for benign and malignant nodules differ significantly, it is essential to distinguish between the two.[3,4] While malignant tumors may require complete thyroidectomy, lymph node dissection, and adjuvant treatment, benign lesions frequently require conservative care or minimal surgical excision. Establishing a trustworthy and precise diagnosis method is therefore essential to preventing both the overtreatment of benign illnesses and the undertreatment of malignant lesions.

The most reliable method for identifying thyroid lesions is still histopathological analysis. But certain thyroid nodules are quite difficult to diagnose, especially if they have follicular architecture. Because their physical characteristics overlap, it can be challenging to distinguish between follicular adenomas, follicular carcinomas, and hyperplastic nodules. In addition to displaying degenerative alterations such calcification, bleeding, and fibrosis, hyperplastic nodules may have microfollicular or macrofollicular patterns. Follicular carcinomas are characterized by capsular and/or vascular invasion, whereas follicular adenomas are benign encapsulated neoplasms. To detect invasion, a thorough inspection of the entire capsule is necessary, and this may not always be simple.[5]

Additionally, the follicular form of papillary thyroid cancer complicates the diagnosis. The follicular form of papillary carcinoma may not have any visible papillary architecture and closely resemble benign follicular lesions, whereas classical papillary carcinoma is distinguished by unique nuclear characteristics such nuclear clearance, grooves, and pseudoinclusions. Routine hematoxylin and eosin staining alone might not be enough in some situations to provide a conclusive diagnosis. Despite being a popular preoperative diagnostic technique, fine needle aspiration cytology (FNAC) has drawbacks, especially when it comes to differentiating between follicular adenoma and follicular cancer since cytology is unable to measure capsular or vascular invasion.[6]

In thyroid pathology, immunohistochemistry has become a useful adjuvant due to these diagnostic difficulties. The capacity of many immunohistochemical markers to distinguish between benign and malignant thyroid lesions has been investigated. Although they are helpful in verifying thyroid origin, markers such thyroid transcription factor-1 (TTF-1), thyroglobulin, cytokeratins, and PAX8 are not able to accurately differentiate between benign and malignant follicular-derived lesions.[7] Finding markers that are selectively expressed in malignant thyroid cancers has so been a focus.

Galectin-3, a lectin that binds β-galactosides, is a member of the lectin family and has a molecular weight of about 30 kDa. Numerous biological functions, such as cell adhesion, proliferation, apoptosis control, inflammation, and tumor growth, are significantly impacted by it. In many organs, galectin-3 is ordinarily expressed at modest levels; nevertheless, in a number of cancers, including thyroid carcinoma, its expression is markedly elevated.[8] Through its modulation of apoptosis pathways, including interactions with p53 and Bcl-2 family proteins, Galectin-3 has been shown in experimental experiments to be involved in neoplastic transformation and tumor cell survival.

Galectin-3 has been shown to be highly expressed in papillary thyroid cancer and, to varying degrees, follicular carcinoma in thyroid pathology. On the other hand, benign diseases like follicular adenoma and multinodular goiter usually have little to no expression. Galectin-3 immunohistochemical staining often shows cytoplasmic positivity, and semiquantitative grading of the intensity and percentage of stained cells is possible. Although there have been reports of sporadic positive in benign lesions, several investigations have documented the excellent sensitivity and specificity of Galectin-3 in differentiating between benign and malignant thyroid nodules.[10]

Galectin-3's diagnostic utility is further supported by its biological significance in thyroid carcinogenesis. It has been linked to the promotion of angiogenesis, tumor cell proliferation, resistance to apoptosis, and the potential for metastasis. Galectin-3 overexpression in cancerous thyroid cells raises the possibility that it plays a role in the aggressiveness and advancement of tumors.[5,8] Furthermore, it is a good prospective marker for cancer due to its comparatively low expression in benign and normal thyroid tissue.

Variability in technique, staining interpretation, and case selection has resulted in conflicting results despite the large number of studies assessing Galectin-3. To understand its function in standard histopathological practice, it is crucial to evaluate its diagnostic efficacy in a well-defined cohort of thyroid nodules. Galectin-3's immunohistochemistry expression in distinct thyroid nodules will be assessed, and its usefulness as a diagnostic marker for distinguishing benign from malignant thyroid lesions will be ascertained.

Aims and Objectives

Aim

To evaluate the immunohistochemical expression of **Galectin-3** in nodular thyroid lesions and to assess its utility as a diagnostic marker in differentiating non-neoplastic, benign, and malignant thyroid nodules.

Objectives

1. To study the histopathological spectrum of nodular thyroid lesions using Hematoxylin and Eosin staining.
2. To analyze the immunohistochemical expression of Galectin-3 in non-neoplastic, benign, and malignant thyroid lesions.
3. To compare the pattern and intensity of Galectin-3 expression among different categories of thyroid nodules.
4. To determine the usefulness of Galectin-3 as an adjunct diagnostic marker in distinguishing benign from malignant thyroid tumors.

MATERIALS AND METHODS

Source of Data: The study included surgically resected thyroid specimens received in the Department of Pathology, Dhanalakshmi Srinivasan Medical College. Clinical details such as age, sex, and type of surgery were obtained from patient case sheets and medical records. Histopathological diagnosis was confirmed using Hematoxylin and Eosin (H&E) stained sections prior to immunohistochemical evaluation.

Study Design: Prospective and retrospective observational study.

Study Location: The study was conducted in the Department of Pathology, Dhanalakshmi Srinivasan Medical College, where all histopathological and immunohistochemical procedures were performed.

Study Duration: The study was carried out over a period of five years, from June 2014 to June 2019.

Sample Size

A total of 50 cases of nodular thyroid lesions were studied:

- 28 non-neoplastic lesions (colloid nodule, multinodular goitre, adenomatoid nodule)
- 8 benign neoplastic lesions (follicular adenoma)
- 14 malignant lesions (papillary carcinoma – 12; follicular carcinoma – 2)

Inclusion Criteria

- Histopathologically confirmed nodular thyroid lesions
- Availability of adequate paraffin blocks for IHC

Exclusion Criteria

- Non-nodular thyroid lesions
- Blocks not retrievable
- Insufficient tissue for immunohistochemistry

Procedure and Methodology

Paraffin blocks were retrieved and H&E slides reviewed. Diagnosis was made based on established histopathological criteria for colloid nodule, multinodular goitre, adenomatoid nodule, follicular adenoma, follicular carcinoma, and papillary carcinoma (classic and follicular variants). Classic papillary carcinoma tissue was used as positive control for Galectin-3.

Sample Processing

H&E Staining

Tissues were fixed in formalin, processed, embedded in paraffin, sectioned at 3–4 µm, and stained with hematoxylin and eosin.

Immunohistochemistry

Sections of 4–5 µm were cut on APES-coated slides. Antigen retrieval was performed using the pressure cooker method with Tris-EDTA buffer (pH 9). Galectin-3 (clone b2c10, pre-diluted) was applied, followed by HRP polymer detection and DAB chromogen. Slides were counterstained with hematoxylin and examined under light microscopy.

Grading of Galectin-3 Expression

- Cytoplasmic ± nuclear staining
- 5% of cells stained considered positive

Intensity

1 = Weak
2 = Moderate
3 = Strong

Proportion

1+ (<5%)
2+ (5–50%)
3+ (>50%)

Statistical Methods: Descriptive statistics were used to calculate frequencies and percentages of Galectin-3 expression among non-neoplastic, benign, and malignant groups.

Data Collection: Demographic details, histopathological diagnosis, and IHC grading were recorded and compiled systematically for analysis of Galectin-3 as a diagnostic marker in thyroid nodules.

RESULTS

This study included **50 cases of thyroid nodules**, comprising 28 (56%) non-neoplastic lesions, 8 (16%) benign neoplasms, and 14 (28%) malignant neoplasms. The results are summarized in the following tables.

Table 1: Histopathological Distribution of Thyroid Nodules (n = 50)

HPE Diagnosis	Frequency	Percentage (%)
Adenomatoid nodule	8	16.0
Colloid nodular goitre	12	24.0
Multinodular goitre	8	16.0
Follicular adenoma	8	16.0
Follicular carcinoma (minimally invasive)	2	4.0
Papillary carcinoma – Classic type	9	18.0
Papillary carcinoma – Follicular variant	3	6.0
Total	**50**	**100.0**

Table 1 shows the histopathological distribution of 50 thyroid nodules included in the study. Non-neoplastic lesions constituted the majority of cases (56%), including colloid nodular goitre (24%), adenomatoid nodule (16%), and multinodular goitre (16%). Follicular adenoma was one of the benign neoplasms that made up 16% of the cases. Malignant tumors accounted for 28% of cases, with minimally invasive follicular carcinoma making up 4% and papillary carcinoma (classic form: 18%, follicular variant: 6%) being the most prevalent kind. According to this distribution, papillary

ISSN (O): 2687-5365; ISSN (P): 2753-6556

carcinoma is the most frequent malignancy, while non-neoplastic thyroid lesions are more prevalent than neoplastic ones.

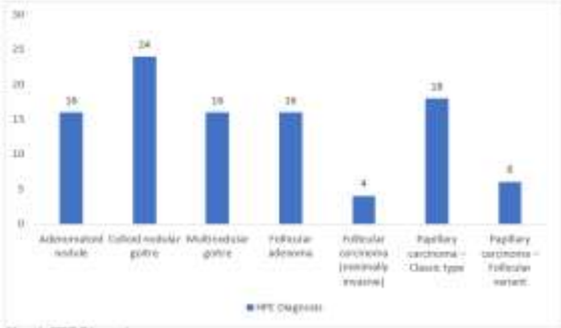

Figure 1: HPE Diagnosis

Table 2. Age-wise Distribution of Thyroid Nodules (n = 50)

Age Group (years)	Frequency	Percentage (%)
< 30	12	24.0
31 – 40	22	44.0
41 – 50	8	16.0
> 50	8	16.0
Total	**50**	**100.0**

Table 2 illustrates the age distribution of thyroid nodules. The age group of 31–40 years old had the highest frequency (44%), followed by individuals under 30 years old (24%). Sixteen percent of cases were in the age categories of 41–50 and above 50. These results imply that in the current investigation, thyroid nodules are more frequently seen in young and middle-aged people.

Table 3: Sex Distribution of Thyroid Nodules (n = 50)

Sex	Frequency	Percentage (%)
Male	5	10.0
Female	45	90.0
Total	**50**	**100.0**

Table 3 presents the sex distribution of cases. A marked female predominance was observed, with females constituting 90% (45 cases) and males only 10% (5 cases), resulting in a female-to-male ratio of 9:1. This demonstrates that thyroid nodules are significantly more common in females in this study population.

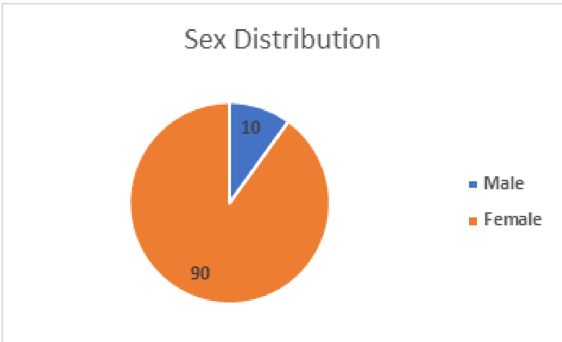

Figure 2: Sex Distribution

Table 4: Expression of Galectin-3 in Thyroid Nodules (n = 50)

HPE Diagnosis	Negative	1+	2+	3+
Adenomatoid nodule (n=8)	6	1	1	0
Colloid nodular goitre (n=12)	8	4	0	0
Multinodular goitre (n=8)	5	3	0	0
Follicular adenoma (n=8)	6	2	0	0
Follicular carcinoma (n=2)	0	1	1	0
Papillary carcinoma – Classic (n=9)	0	1	2	6
Papillary carcinoma – Follicular variant (n=3)	0	0	3	0

Table 4 shows the distribution of Galectin-3 expression across various thyroid lesions. Most non-neoplastic lesions (adenomatoid nodule, colloid nodular goitre, and multinodular goitre) were negative or showed only weak (1+) positivity. Similarly, follicular adenoma predominantly showed negative or weak expression. Malignant tumors, on the other hand, showed more positivity. The follicular type of papillary carcinoma consistently displayed 2+ positive, whereas the classic version demonstrated 3+ positivity in most instances (6/9). Cases of follicular cancer showed no 3+ expression

ISSN (O): 2687-5365; ISSN (P): 2753-6556

but 1+ and 2+ positive. These results confirm Galectin-3's function as a helpful diagnostic marker in distinguishing benign from malignant thyroid nodules, showing that significant expression of Galectin-3 (2+ and 3+) is mostly linked to malignant thyroid tumors.

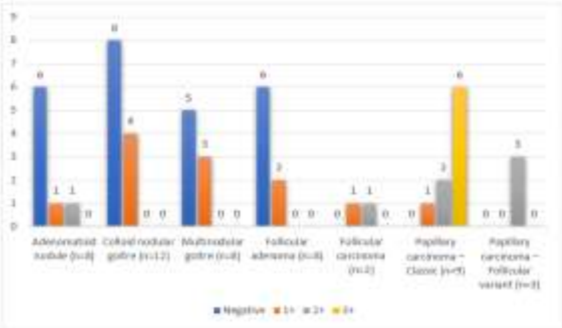

Chart 3: Expression of Galectin-3 in Thyroid Nodules

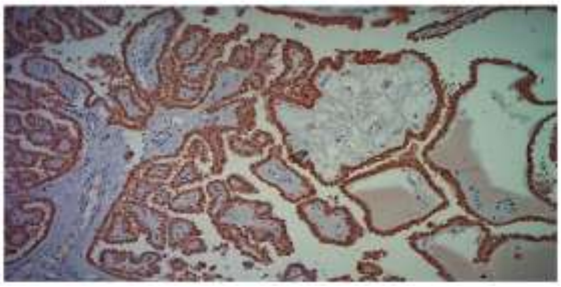

Figure 1: Papillary carcinoma shows Galectin 3 positivity (3+) under10x

Figure 2: Papillary carcinoma, follicular variant H&E (Under 10X)

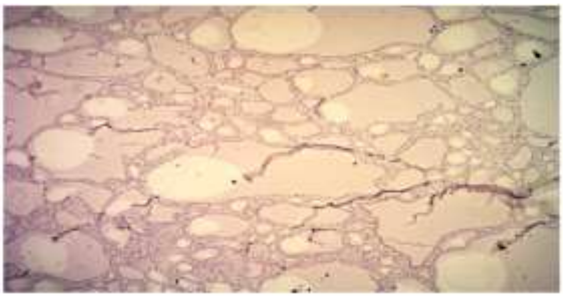

Figure 3: Adenomatoid nodule shows Galectin 3 negative expression under 40x

DISCUSSION

The present study evaluated the immunohistochemical expression of Galectin-3 in 50 cases of thyroid nodules. The majority of cases (56%) were non-neoplastic lesions, followed by benign neoplasms (16%) and malignant tumors (28%), as Table 1 illustrates. Follicular carcinoma made for 4% of malignant tumors, whereas papillary thyroid cancer (classic and follicular variations) accounted for 24%. This pattern is consistent with statistics showing that the most common thyroid cancer is papillary carcinoma.[1]

Thyroid nodules were most prevalent in individuals aged 31 to 40 (44%), followed by those under 30 (24%), according to the age distribution of cases (Table 2). The age categories of 41–50 years and >50 years had the lowest distributions, at 16% each. These results imply that in our research sample, thyroid nodules primarily afflict young and middle-aged people. With 90% of cases being female and 10% being male, the sex distribution (Table 3) revealed a clear female predominance, with a 9:1 female-to-male ratio.[2] This finding is consistent with past research showing that females had a greater prevalence of thyroid nodules and thyroid cancers, most likely as a result of hormonal changes and an immunological susceptibility.

This study's main goal was to evaluate the diagnostic value of Galectin-3 expression. Galectin-3 expression differed across benign, malignant, and non-neoplastic lesions, as seen in Table 4. Most non-neoplastic lesions were either weakly (1+) positive or negative. Six of the eight instances of benign neoplasms (follicular adenoma) were negative, while two merely had a 1+ positive. Malignant tumors, on the other hand, had more pronounced staining patterns. Six out of nine cases had 3+ positive, two cases had 2+ positivity, and one case had 1+ positivity for papillary carcinoma classic variant. In all three instances, the papillary carcinoma follicular variant was 2+ positive. None of the follicular carcinoma patients displayed 3+ staining, but they did show 1+ and 2+ positive.[8]

Consequently, there was a substantial correlation between malignancy and rising levels of Galectin-3 expression. Of the 25 instances in our investigation that had negative expression, 24% were benign tumors and 76% were non-neoplastic lesions. [7] Twelve instances, comprising non-neoplastic lesions (66.66%), benign lesions (16.66%), and malignant lesions (16.66%), had 1+ positive. Crucially, 6 patients had 3+ positive, all of which were malignant, and 7 instances had 2+ positivity, including 85.7% malignant cases. This makes it abundantly evident that malignant tumors are mostly linked to staining grades greater than 2+ and 3+.

According to Herrmann ME et al,[10] goitre and hyperplastic nodules show little to no staining from Galectin-3, but malignant tumors show strong staining. They found that follicular carcinoma had 1+ positive, follicular variant displayed 1+ to 2+, and papillary carcinoma displayed positivity ranging from 1+ to 3+. These results are consistent with what we found in Table 4, where papillary cancer stained more intensely than follicular carcinoma.

Galectin-3 positivity was found in 90% of papillary carcinoma cases, 83.3% of follicular variant cases, and 81.8% of follicular carcinoma cases, according to Saleh HA et al.[11] Benign and non-neoplastic lesions showed lower expression. Sumana BS et al. [12] also observed 91.3% positive in papillary cancer, with classic forms showing significant expression. High Galectin-3 expression in papillary and follicular carcinomas was also shown by Chumila Thinely Bhuti et al.[13] These results corroborate our study's high malignant positive rate.

According to Ajay Kr Singh et al,[14] there was a strong correlation between malignancy and increased Galectin-3 staining intensity. This finding is in line with our research, which found that malignant tumors accounted for the majority of 2+ and 3+ positive (Table 4). The diagnostic utility of Galectin-3 is reinforced by the robust correlation between malignancy and greater intensity staining.

Galectin-3 expression was found in 82% of instances of papillary carcinoma and 33% of cases of follicular carcinoma, according to Article O et al. [15]. According to Al-sharaky DR et al,[16] the sensitivity and specificity for distinguishing between thyroid cancer and adenoma were 96.8% and 70.6%, respectively, with 97% positive in thyroid carcinoma and 30% positivity in follicular adenoma. In contrast, our research showed that the identification of malignancy with a cut-off of 2+ had a sensitivity of 85.7% and a specificity of 97.2%. Additionally, 2+ and above shown outstanding discriminatory capacity with sensitivity of 85.7% and specificity of 100% when comparing benign and malignant neoplasms alone.

Galectin-3 may be a tumor marker that indicates aggressive behavior in papillary thyroid cancer, according to Htwe TT et al.[17] Grade 3 positive can distinguish benign from malignant follicular-patterned neoplasms, according to Article O et al.[18] Its diagnostic utility was further supported by the fact that 3+ positivity was only seen in malignant tumors in our investigation (Table 4). With a statistically significant p value (<0.001) and an area under the curve (AUC) of 0.97–0.98, our investigation demonstrated remarkable diagnostic accuracy. Thus, by comparing the results in Tables 1 and 4, it can be said that there is a high correlation between thyroid cancer and Galectin-3 expression, especially at 2+ and 3+ intensity.

In conclusion, the current study shows that Galectin-3 is an extremely sensitive and specific immunohistochemical marker for distinguishing between benign and malignant thyroid nodules. Tumor type and staining intensity are correlated with its expression, and a cut-off of 2+ and above offers the best diagnostic precision. These results support the function of Galectin-3 as a useful adjuvant in the histological assessment of thyroid nodules and are in line with other research.

CONCLUSION

Galectin-3 is a useful immunohistochemical marker in the assessment of thyroid nodules, as this study shows. Galectin-3 expression was shown to be strongly correlated with thyroid cancer, particularly papillary thyroid carcinoma. The majority of benign and non-neoplastic lesions were either negative or just weakly positive for Galectin-3, while all malignant tumors in this investigation demonstrated positivity, with greater staining intensities (2+ and 3+) mostly observed in malignant lesions. Galectin-3 demonstrated great sensitivity and specificity in differentiating between benign and malignant thyroid nodules when using a cut-off value of 2+ and above. Its diagnostic importance was further supported by the observation that strong (3+) expression was only found in malignant tumors. These results demonstrate the value of Galectin-3 as a supplement to standard histological analysis, especially where morphological overlap makes diagnosis challenging. Apart from its diagnostic precision, Galectin-3 is also reasonably priced and simple to use in standard immunohistochemistry labs. Consequently, Galectin-3 can be regarded as a trustworthy, useful, and economical marker for distinguishing between benign and malignant thyroid nodules, especially when diagnosing thyroid papillary cancer.

Limitations of the Study

1. **Small Sample Size:** The study included only 50 cases, which limits the statistical power and generalizability of the findings to a larger population.
2. **Single-Center Study:** The results may not accurately reflect larger population differences because the study was limited to a single institution and may instead reflect regional demographic and clinical tendencies.
3. **Limited Representation of Malignant Subtypes:** There were only two instances of follicular carcinoma among the bulk of malignant patients, which were papillary carcinomas. The exclusion of other thyroid cancers limited the ability to compare subtypes.
4. **Use of a Single Immunohistochemical Marker:** Galectin-3 was the only one assessed. Adding more markers to a diagnostic panel could have improved comparative analysis and diagnostic precision.
5. **Lack of Follow-Up Data:** The study did not assess long-term clinical outcomes, recurrence, or prognostic significance of Galectin-3 expression.

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