Differential Expression Patterns of Bcl-2, D2-40, β-Catenin and E-Cadherin in Thymomas: Correlation with Clinical Stages and Subtypes

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Abstract: Thymomas are rare mediastinal tumors exhibiting heterogeneous behavior. Although histologic subtypes and stages serve as prognostic factors, the molecular mechanisms of thymoma progression are unclear. Immunohistochemical markers like Bcl-2, D2-40, β-catenin, and E-cadherin offer insights into thymoma biology, but their predictive value for clinical outcomes remains uncertain. This study evaluated the expression of these markers across thymoma subtypes and stages, aiming to assess their prognostic significance. A retrospective analysis was conducted on 66 thymoma cases resected at a single center between 2005 and 2023. Immunohistochemical staining was performed to assess the expression of Bcl-2, D2-40, β-catenin, and E-cadherin. Clinicopathological characteristics were correlated with immunohistochemical findings using statistical analysis. Differential expression patterns of Bcl-2, D2-40, β-catenin, and E-cadherin were observed across thymoma subtypes and clinical stages. Bcl-2 displayed cytoplasmic positivity predominantly in type A and B thymomas, while E-cadherin showed membranous staining in type B thymomas and cytoplasmic staining in type A and AB thymomas. β-catenin demonstrated membranous staining in type B thymomas and cytoplasmic staining in type A and AB thymomas. D2-40 expression was localized to peripheral regions and invasive nests of thymomas, with higher expression in type B2 thymomas and early-stage tumors. Our findings indicate that immunohistochemical markers may provide valuable insights into thymoma biology and prognosis. Further validation in larger, multicenter cohorts is warranted to confirm the prognostic significance of these markers and their potential utility in guiding clinical management.

Key Words: thymoma, Bcl-2, D2-40, β-Catenin, E-cadherin

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Thymomas, rare mediastinal tumors originating from thymic epithelial cells, exhibit significant morphologic and clinical heterogeneity. Although thymomas are generally considered low-grade malignancies, some cases show aggressive features such as local invasion, pleural dissemination, and, rarely, distant metastasis.[1] Clinical staging remains a key factor in prognosis and treatment, with increasing recognition of histologic subtypes based on the 2021 WHO classification.[1,2] Type B2 and B3 thymomas tend to be more aggressive, while types A and AB are usually confined to early stages and have better outcomes. Type B1 thymomas are seen as having intermediate malignant potential. Encapsulated thymomas, although infrequent, may occasionally demonstrate infiltrative behavior, particularly in types A and AB.[3–5] Given the variability in thymoma behavior, understanding the mechanisms of tumor progression is crucial.

Immunohistochemistry has enhanced the morphologic classification of thymomas, with studies identifying specific markers to aid in differentiating subtypes.[6] Cytokeratin profiles and lymphoid cell immunophenotyping have shown promise, though their clinical application remains limited.[3,4,7–10] In tru-cut biopsy samples, where subtype identification is critical, immunohistochemical markers can offer diagnostic value.[10,11]

Our study aimed to evaluate the utility of monoclonal antibodies against Bcl-2, β-catenin, E-cadherin, and D2-40 across all histologic subtypes and stages, based on the WHO classification. These markers, linked to processes like epithelial-mesenchymal transition (EMT) and anoikis, play key roles in tumor progression. Bcl-2, especially in types A and AB thymomas, may serve as a biomarker for less aggressive behavior. D2-40, associated

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All data generated or analyzed during this study are included in this published article.

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with invasiveness in various malignancies, could provide insights into metastatic potential in thymomas. β-catenin's expression pattern might indicate invasive characteristics, while E-cadherin could reflect varying levels of aggressiveness between thymoma subtypes. By analyzing resected specimens, we sought to correlate the expression of these markers with tumor malignancy and clinical stage, and compare our findings with the literature.

MATERIALS AND METHODS

Study Design

We reviewed electronic records of 323 mediastinal mass cases from the Pathology Department archives (2005 to 2023), and selected 66 thymoma cases with available histologic sections and tissue blocks for analysis. Cases with thymic hyperplasia, carcinoma, or tumors of nerve of lymphoid origin were excluded. Ethical approval was obtained (KU GOKAEK-2023/07.02, Project No: 2023/83). Tru-cut biopsies were obtained from at least 3 distinct areas of each mass to ensure homogeneity, followed by mass resection in all thymic neoplasia cases. Three to 15 histologic sections per case were analyzed, and tumor classification followed WHO guidelines. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections, including both biopsy and resection material, to assess whether subtyping could be reliably performed with tru-cut biopsy.

Immunohistochemistry Technique

Four micrometer tissue sections were immunostained with Bcl-2 (clone 124), E-cadherin (clone 36), β-catenin (clone 14), and D2-40 (clone D2-40), all diluted 1:100. Standard immunohistochemical techniques were used, with positive and negative controls included. Staining intensity (0 to 3) and percentage of positively stained tumor cells were evaluated, focusing solely on epithelial cells, excluding areas of lymphocytic infiltration. Positive staining was defined as antigen-specific staining above background levels, with nonspecific or aberrant staining documented but excluded from the analysis.

Statistical Analysis

All data were analyzed using GraphPad Instat. Descriptive statistics included mean, SD, median, minimum, and maximum values for continuous variables, and percentages for categorical variables. Normality was assessed with the Kolmogorov-Smirnov test. Parametric data were analyzed using the Student t test, while nonparametric data were analyzed with the Mann-Whitney U test. Multiple group comparisons were conducted using the Kruskal-Wallis test and Dunn post-hoc test. Categorical variables were compared using the χ^2 test, and correlations were assessed with Spearman correlation. Bonferroni correction was applied to account for multiple comparisons. A P-value of <0.05 was considered statistically significant.

RESULTS

Comparison of demographics and clinicopathologic characteristics of thymoma according to the clinical stage is presented in Table 1. The age, sex, presence of Myasthenia Gravis, the tumor diameter did not differ among tumor stages. However, the adjacent visceral surgical margin positivity of Stages II and III was observed in higher ratio compared with the Stage I ($P<0.0001$). Considering the radiologic findings, calcification, irregular shape and contour were observed in 66.7%, 91.7%, and 100% of cases at stage III, respectively, which were the highest ratios among all stages ($P<0.05$). Total mediastinal fat obliteration was detected mostly in the cases at stage II-A. Necrosis, invasiveness and heterogeneous enhancement pattern were observed in all cases at stage III ($P<0.001$), but not in other stages. Pleural/pericardial thickened/effusion was present in 91.7% of cases at stage III which was the highest ratio among stages ($P<0.0001$). The percentages of higher subtypes of thymoma increased significantly among the advanced stages ($P=0.0444$). The number of cases received chemotherapy, radiotherapy, the recurrence rate and the follow-up duration did not differ among stages ($P>0.05$). The mortality rate was 6.1% in the cases at stage I, 41.7% at stage II-A, 33.3% at stage II-B and 16.7% at stage III ($P=0.0278$).

The histologic images of cytoplasmic and membranous Bcl-2, E-cadherin, β-catenin, D2-40 immunoreactivities were presented in Figures 1–4. The correlation between the positive cases for Bcl-2, E-cadherin, β-catenin, D2-40 expression with the demographics and clinicopathological findings of thymoma was presented in Table 2 and the correlation analysis for the staining percentage and intensity were given in Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/AIMM/A533 and Supplementary Table 2, Supplemental Digital Content 1, http://links.lww.com/AIMM/A533. The expression patterns of all proteins did not differ significantly according to demographics. The most notable difference in staining patterns among the different histologic subtypes with the antigens utilized in this study was detected for Bcl-2 (Fig. 1) and D2-40 (Fig. 4) and difference in staining patterns among the clinical stages was detected for E-cadherin (Fig. 2) and D2-40 (Fig. 4).

Bcl-2 showed strong and consistent cytoplasmic positivity in the majority of the tumor cells (Fig. 1A). 13.8% of cases with Bcl-2 positive thymoma had Myasthenia Gravis disease ($P=0.0498$). Bcl-2 expression was higher in the cases with thymoma subtypes A and B compared with the other subtypes and there was a significant correlation between distribution of subtypes and the cases with Bcl-2 positive thymoma ($P<0.0001$). The staining pattern for E-cadherin in type A and AB thymoma was cytoplasmic (Fig. 2A) and in types B1–B3 it was membranous (Fig. 2B). 39.3% of cases with E-cadherin positive thymoma had a capsule invasion ($P=0.0309$). E-cadherin expression was highest in the cases at Stage I (54.1%, $P<0.0001$). There was a significant correlation between distribution of clinical stages

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TABLE 1. Correlation Between Clinical Stage and Clinicopathologic Characteristics of Thymoma

Parameters	Total patients (N = 66), N (%)	Stage I (N = 33), N (%)	Stage II-A (N = 12), N (%)	Stage II-B (N = 9), N (%)	Stage III (N = 12), N (%)	*P*
Age, mean ± SD	58.1 ± 13.7	60.4 ± 12.5	53.3 ± 11.1	57.0 ± 17.0	57.1 ± 16.6	0.479
< 50 y	18 (27.3)	6 (18.2)	5 (41.7)	2 (22.2)	5 (41.7)	0.262
≥ 50 y	48 (72.7)	27 (81.8)	7 (58.3)	7 (77.8)	7 (58.3)	
Sex						
Male	40 (60.6)	17 (51.5)	7 (58.3)	8 (88.9)	8 (66.7)	0.224
Female	26 (39.4)	16 (48.5)	5 (41.7)	1 (11.1)	4 (33.3)	
Presence of myasthenia gravis	18 (27.3)	8 (24.2)	4 (33.3)	1 (11.1)	5 (41.7)	0.622
Tumor diameter, mean ± SD	5.8 ± 2.9	5.9 ± 2.7	6.3 ± 3.4	4.3 ± 3.0	6.2 ± 2.7	0.217
< 5 cm	30 (45.5)	13 (39.4)	5 (41.7)	7 (77.8)	5 (41.7)	
5-10 cm	30 (45.5)	17 (51.5)	6 (50.0)	1 (11.1)	6 (50.0)	0.521
> 10 cm	6 (9.0)	3 (9.1)	1 (8.3)	1 (11.1)	1 (8.3)	
Surgical margin positivity	20 (30.3)	1 (3.0)	7 (58.3)	5 (55.6)	7 (58.3)	**< 0.0001**
Radiologic findings						
Position						
Middle	27 (40.9)	11 (33.3)	6 (50.0)	4 (44.4)	6 (50.0)	0.648
Right/left	39 (59.1)	22 (66.7)	6 (50.0)	5 (55.6)	6 (50.0)	
Calcification	24 (36.4)	7 (21.2)	4 (33.3)	5 (55.6)	8 (66.7)	**0.0232**
Shape						
Oval	29 (43.9)	16 (48.5)	8 (66.7)	4 (44.4)	1 (8.3)	
Irregular	37 (56.1)	17 (51.5)	4 (33.3)	5 (55.6)	11 (91.7)	**0.0297**
Contour						
Smooth	24 (36.4)	14 (42.4)	7 (58.3)	3 (33.3)	0 (0.0)	
Lobulated	18 (27.3)	12 (36.4)	4 (33.3)	2 (22.2)	0 (0.0)	
Irregular	24 (36.4)	7 (21.2)	1 (8.3)	4 (44.4)	12 (100)	**< 0.0001**
Mediastinal fat obliteration						
Absent	38 (57.6)	15 (45.5)	6 (50.0)	8 (88.9)	9 (75.0)	
Partial	21 (31.8)	16 (48.5)	3 (25.0)	1 (11.1)	1 (8.3)	**0.0316**
Total	7 (10.6)	2 (6.1)	3 (25.0)	0 (0.0)	2 (16.7)	
Presence of necrosis	38 (57.6)	18 (54.5)	2 (16.7)	6 (66.7)	12 (100)	
Presence of invasiveness	24 (36.4)	5 (15.2)	1 (8.3)	6 (66.7)	12 (100)	**0.0006**
Enhancement pattern						**< 0.0001**
Homogeneous	26 (39.4)	15 (45.5)	10 (83.3)	1 (11.1)	0 (0.0)	
Heterogeneous	40 (60.6)	18 (54.5)	2 (16.7)	8 (88.9)	12 (100)	**0.0001**
Presence of pleural/pericardial thickened/effusion	18 (27.3)	4 (12.1)	1 (8.3)	2 (22.2)	11 (91.7)	**< 0.0001**
Thymoma subtype						
A	9 (13.6)	5 (15.2)	0 (0.0)	2 (22.2)	2 (16.7)	
AB	10 (15.2)	6 (18.2)	4 (33.3)	0 (0.0)	0 (0.0)	
B1	27 (40.9)	17 (51.5)	1 (8.3)	5 (55.6)	4 (33.3)	**0.0444**
B2	16 (24.2)	4 (12.1)	6 (50.0)	1 (11.1)	5 (41.7)	
B3	4 (6.1)	1 (3.0)	1 (8.3)	1 (11.1)	1 (8.3)	
Chemotherapy	2 (3.0)	0 (0.0)	1 (8.3)	0 (0.0)	1 (8.3)	0.307
Radiotherapy	21 (31.8)	6 (18.2)	5 (41.7)	3 (33.3)	7 (58.3)	0.064
Recurrence	10 (15.2)	3 (9.1)	4 (33.3)	1 (11.1)	2 (16.7)	0.244
Chemoradiotherapy after recurrence	2 (3.0)	1 (3.0)	1 (8.3)	0 (0.0)	0 (0.0)	0.614
Follow-up, month, median (min-max)	72 [1–192]	72 [1–192]	70.5 [1–108]	72 [1–72]	72 [2–143]	0.604
Mortality	12 (18.2)	2 (6.1)	5 (41.7)	3 (33.3)	2 (16.7)	**0.0278**

Bold values are in Statistical significance ($P < 0.05$).

and the cases with E-cadherin positive thymoma ($P = 0.0215$, Supplementary Table 2, Supplemental Digital Content 1, http://links.lww.com/AIMM/A533).

β-catenin was characterized by a strong membranous pattern of staining in type B1–3 thymoma (Fig. 3B) but showed only cytoplasmic staining in type A and AB (Fig. 3A). None of the cases tested showed nuclear staining for β-catenin. The expression pattern of β-catenin did not differ significantly according to any clinicopathological findings of thymoma ($P > 0.05$). The endothelial cell positivity of lymphatic vessels was regarded as the internal control for D2-40 immunoreactivity. In the normal thymus, D2-40 was expressed in the cells present along the inner surface of the thymic capsule and interlobular septa (ie, subcapsular epithelium) (Fig. 4). In the involuted thymus, strands and islands of small epithelial cells embedded in fatty tissue were also positive for D2-40. In the positive cases, D2-40 was expressed on the cell membrane of the tumor cells, localized to the peripheral areas and invasive nests of thymomas with no expression in the central areas. 62.5% of cases with D2-40 positive thymoma had a capsule invasion ($P = 0.0415$). D2-40 expression was highest in the cases at Stage I (37.5%, $P = 0.0014$). D2-40 expression was highest in the cases

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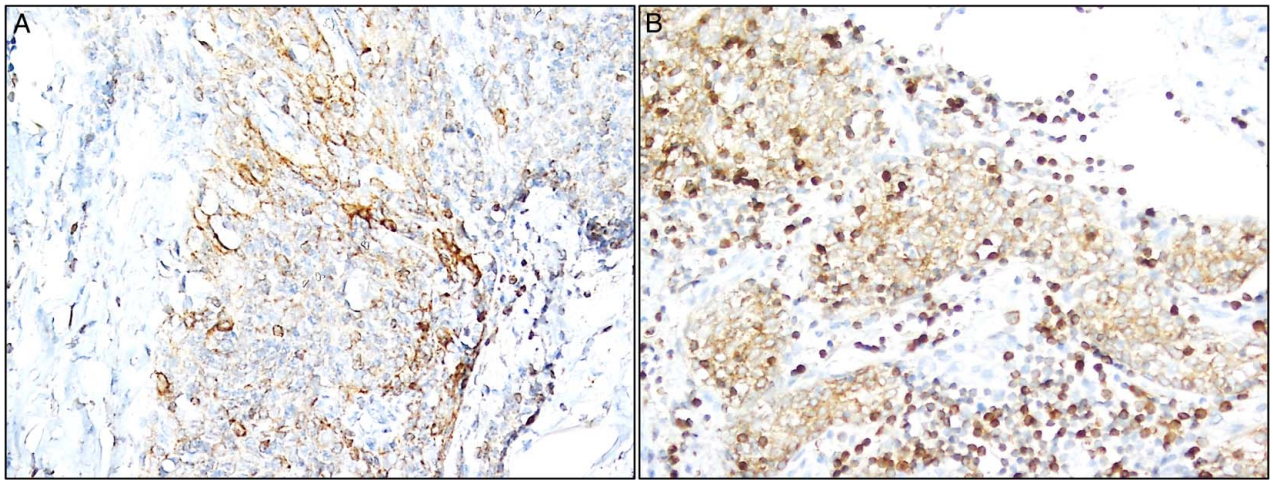


FIGURE 1. Light microscopic images of cytoplasmic staining (A) and membranous staining (B) for Bcl-2 detected in tumor cells (×100, ×200).

with thymoma subtype B2 compared with the other subtypes (45.8%, $P = 0.0401$). Half of the cases with D2-40 positive thymoma received radiotherapy ($P = 0.0338$) (Table 2). There was no significant correlation of D2-40 expression percentage and intensity with any demographics and clinicopathological findings of thymoma (supplementary Table 1 and 2, Supplemental Digital Content 1, http://links.lww.com/AIMM/A533).

DISCUSSION

Thymomas pose a clinical challenge due to their rarity and diverse behavior.[2,5] In our study, we explored the expression of Bcl-2, D2-40, β-catenin, and E-cadherin in various histologic subtypes and clinical stages to assess their prognostic value. We found that reduced E-cadherin was linked to capsular invasion, suggesting its role in EMT and tumor aggressiveness. Bcl-2 expression, notably higher in less aggressive subtypes (types A and AB), highlights its potential for distinguishing thymoma subtypes. D2-40, associated with lymphatic involvement, was more prominent in invasive nests, particularly in B2 thymomas, supporting its role in local invasion. These findings highlight the significance the importance of immunohistochemical markers in understanding thymoma biology and their potential in clinical decision-making.

Bcl-2, known for regulating apoptosis, has been reported in higher stages of thymomas (III/IV) and all thymic carcinomas.[12–14] Khoury reported Bcl-2 positivity in 57% of type A and 33% of type AB thymomas, while Alexiev found it in 12% of B3 thymomas but not in types A, AB, B1, or B2.[15,16] Despite some conflicting findings, our study confirmed consistent Bcl-2 positivity in type A and B2 thymomas, suggesting its role as a biomarker for less aggressive subtypes. Further research is warranted to explore its prognostic significance.

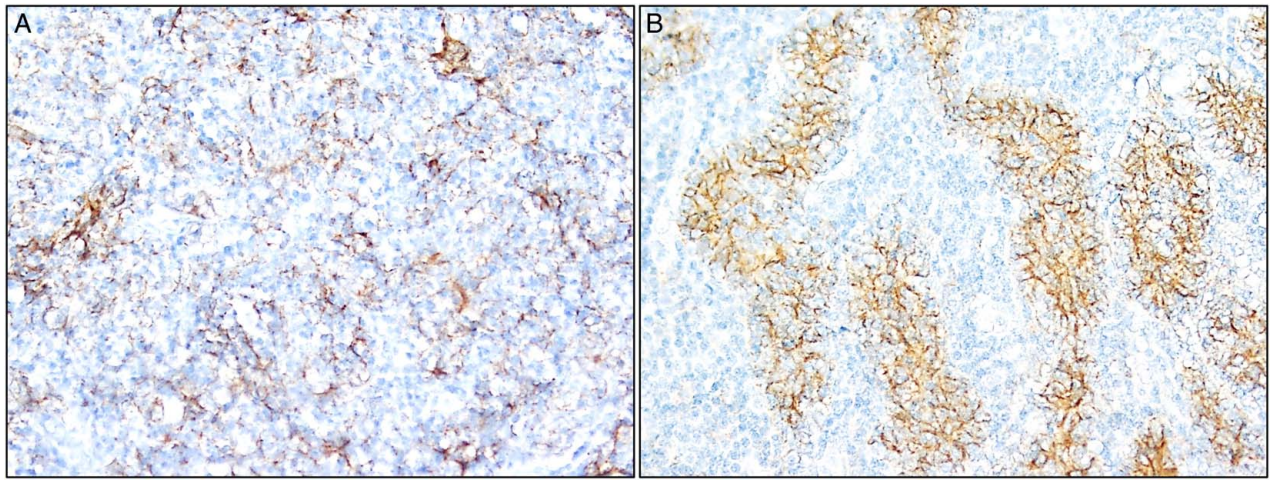


FIGURE 2. Light microscopic images of cytoplasmic staining (A) and membranous staining (B) for E-cadherin detected in tumor cells (×100, ×200). full color online

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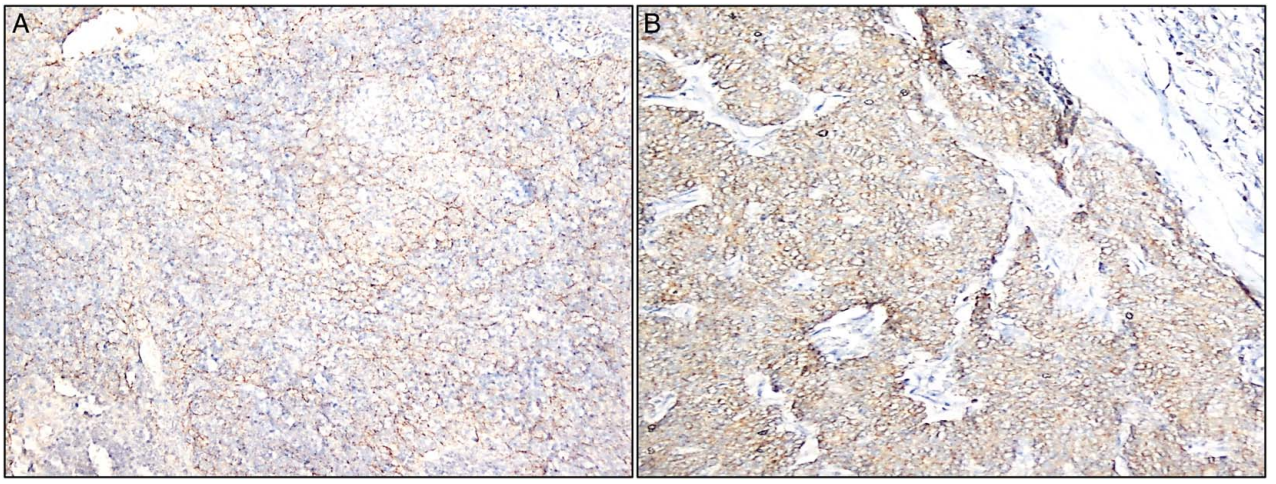


FIGURE 3. Light microscopic images of cytoplasmic staining (A) and membranous staining (B) for β-catenin detected in tumor cells (×100, ×200). full color online

The loss of E-cadherin, a key cell-cell adhesion protein, is crucial for tumor invasiveness, as it weakens intercellular adhesion and promotes cell motility. This process, central to EMT, allows epithelial cells to acquire mesenchymal traits, increasing their ability to invade. Reduced E-cadherin expression has been linked to greater tumor invasiveness in various cancers, including thymomas. Studies show that restoring E-cadherin reduces tumorigenicity and metastasis, highlighting its role as a biomarker for assessing invasive potential.[11,16–20] In thymomas, we found that lower E-cadherin expression was associated with capsular invasion, supporting its role in aggressive tumor behavior. E-cadherin expression patterns have also been used to differentiate between thymoma subtypes, with type A/AB thymomas showing strong cytoplasmic staining, while type B thymomas exhibit membranous staining. Despite its broad reactivity, limiting its diagnostic utility, higher E-cadherin expression was associated with early-stage thymomas, suggesting its potential as a prognostic marker for favorable outcomes. Our findings emphasize the importance of E-cadherin as a marker for EMT and tumor invasiveness in thymomas, warranting further studies into its functional role and interaction with other EMT-related markers.

β-catenin, a key adhesion molecule, has been studied for its role in distinguishing thymoma subtypes. While animal studies highlight its role in thymic epithelial growth and tumor progression, human studies have not established a clear link between β-catenin expression and invasiveness.[18] Immunohistochemistry shows β-catenin expression across all thymoma subtypes, with variations in localization. In type A thymomas, β-catenin is predominantly cytoplasmic, while in type B thymomas, it appears membranous, especially in invasive regions of type B3 and the periphery of type B2 thymomas.[17,18] Strong cytoplasmic staining is common in type A/AB thymomas (97% to

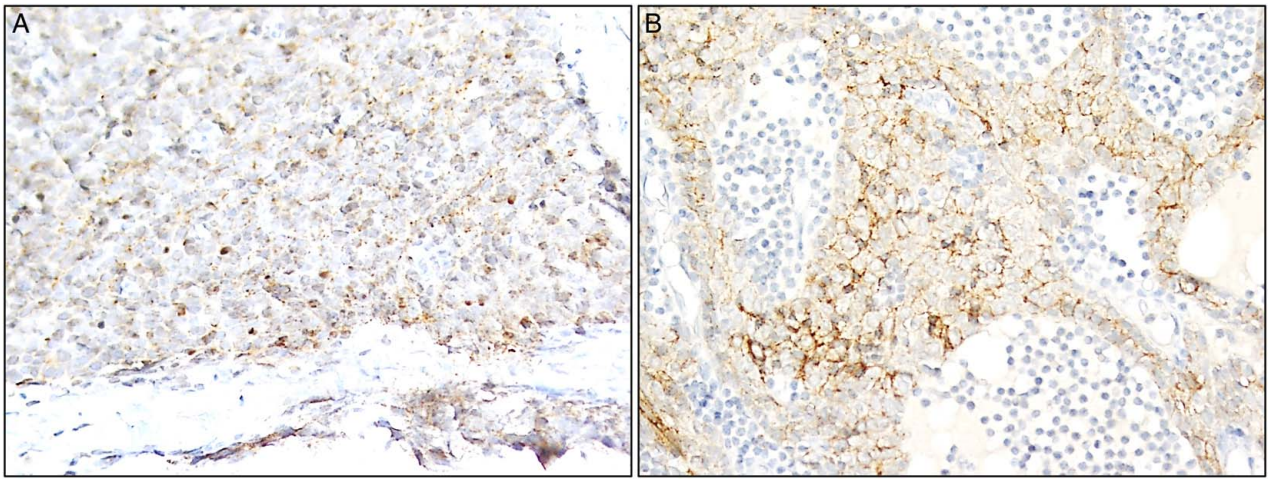


FIGURE 4. Light microscopic images of cytoplasmic staining (A) and membranous staining (B) for D2-40 detected in tumor cells (200, ×200). full color online

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TABLE 2. Correlation Between the Positive Cases for Bcl-2, E-Cadherin, β-Catenin, D2-40 Expression and Clinical Findings and Clinical Stage and Subtype of Thymoma

Parameters	Bcl-2 (N = 29) N (%)	E-cadherin (N = 61) N (%)	β-catenin (N = 39), N (%)	D2-40 (N = 24) N (%)
Age, < 50 y (N = 18)	9 (31.0)	16 (26.2)	10 (25.6)	9 (37.5)
Age, ≥ 50 y (N = 48)	20 (69.0)	45 (73.8)	29 (74.4)	15 (62.5)
P	0.742	0.887	0.939	0.261
Sex				
Male (N = 40)	14 (48.3)	37 (60.7)	24 (61.5)	14 (58.3)
Female (N = 26)	15 (51.7)	24 (39.3)	15 (38.5)	10 (41.7)
P	0.119	0.977	0.852	0.981
Presence of myasthenia gravis (N = 18)	4 (13.8)	16 (26.2)	13 (33.3)	7 (29.2)
P	**0.0498**	0.608	0.295	0.794
Tumor diameter				
< 5 cm (N = 30)	15 (51.7)	29 (47.5)	18 (46.2)	13 (54.2)
5–10 cm (N = 30)	12 (41.4)	27 (44.3)	16 (41.0)	10 (41.7)
> 10 cm (N = 6)	2 (6.9)	5 (8.2)	5 (12.8)	1 (4.2)
P	0.634	0.421	0.391	0.416
Presence of capsule invasion (N = 29)	13 (44.8)	24 (39.3)	16 (41.0)	15 (62.5)
P	0.898	**0.0309**	0.748	**0.0415**
Surgical margin positivity (N = 20)	9 (31.0)	17 (27.9)	12 (30.8)	8 (33.3)
P	0.909	0.319	0.921	0.899
Clinical stage				
I (N = 33)	14 (48.3)	33 (54.1)	20 (51.3)	9 (37.5)
II-A (N = 12)	8 (27.6)	12 (19.7)	9 (23.0)	6 (25.0)
II-B (N = 9)	2 (6.9)	9 (14.8)	5 (12.8)	3 (12.5)
III (N = 12)	5 (17.2)	7 (11.5)	5 (12.8)	6 (25.0)
P	0.231	**< 0.0001**	0.417	**0.0014**
Thymoma subtype				
A (N = 9)	7 (24.1)	7 (11.5)	3 (7.7)	3 (12.5)
AB (N = 10)	10 (34.5)	10 (16.4)	8 (20.5)	3 (12.5)
B1 (N = 27)	4 (13.8)	26 (42.6)	14 (35.9)	6 (25.0)
B2 (N = 16)	8 (27.6)	14 (23.0)	10 (25.6)	11 (45.8)
B3 (N = 4)	0 (0.0)	4 (6.6)	4 (10.3)	1 (4.2)
P	**< 0.0001**	0.284	0.103	**0.0401**
Chemotherapy (N = 2)	0 (0.0)	1 (1.6)	1 (2.6)	1 (4.2)
P	0.584	0.344	0.791	0.684
Radiotherapy (N = 21)	10 (34.5)	19 (31.1)	12 (30.8)	12 (50.0)
P	0.885	0.683	0.826	**0.0338**
Recurrence (N = 10)	4 (13.8)	9 (14.8)	7 (17.9)	3 (12.5)
P	0.785	0.753	0.680	0.923
Mortality (N = 12)	6 (20.7)	12 (19.7)	9 (23.1)	5 (20.3)
P	0.884	0.622	0.360	0.928

Bold values are in Statistical significance ($P < 0.05$).

100%), while type B1 and B2 also show consistent positivity.[19] Our findings align with the literature, with β-catenin exhibiting membranous staining in type B thymomas and cytoplasmic staining in type A/AB. Notably, no nuclear β-catenin staining was observed, indicating a lack of canonical Wnt signaling activation in our thymoma samples.

DD2-40, an antigen expressed in lymphatic endothelium and various tissues, is a biomarker linked to tumor proliferation. Studies show D2-40 expression across thymoma subtypes, with correlations between positive expression and invasiveness or poor prognosis.[21–23] Cytoplasmic D2-40 reactivity is more common in AB and B1 thymomas, while B2 and B3 types often show membranous expression.[24] However, the distribution of D2-40 in combined B1B2 and B2B3 thymomas remains unclear.[23] In our study, D2-40 was primarily localized in peripheral areas and invasive nests, with higher expression in type B2 thymomas and early-stage tumors. Notably, half of the D2-40-positive cases received radiotherapy, suggesting a possible link between D2-40 expression and treatment.

Accurate thymoma classification is challenged by tumor heterogeneity, especially in biopsy samples. To minimize sampling bias, we took tru-cut biopsies from at least 3 different areas of the tumor and performed full mass resection in confirmed cases. Between 3 and 15 histologic sections were analyzed per case, with homogeneous tumor regions selected for immunohistochemical staining. By utilizing multiple sections and markers such as Bcl-2, D2-40, β-catenin, and E-cadherin, we aimed to provide a more accurate assessment of thymoma subtypes and their correlation with clinical stages.

The retrospective, single-center design of this study may introduce selection bias and limit the generalizability of the findings. The small sample size reduces statistical power, and the histopathologic heterogeneity of thymomas could lead to inconsistencies in subtype classification, despite adherence to WHO criteria. Variability in patient demographics, treatment protocols, and histopathologic practices across institutions may affect result interpretation. Immunohistochemical staining patterns, while standardized, are subject to interpretation variability, which could impact accuracy. This study focused on the expression of Bcl-2, E-cadherin, β-catenin, and D2-40, without exploring their functional roles. Larger, multicenter studies are needed to confirm these findings and validate the prognostic value of these markers. In addition, future studies with functional assays and molecular profiling, as well as longer follow-up periods, are required to better understand thymoma progression and refine therapeutic strategies.

CONCLUSIONS

Our study demonstrates the potential of immunohistochemical markers, such as Bcl-2, E-cadherin, β-catenin, and D2-40, in shedding light on thymoma biology and progression. While significant correlations were identified between marker expression and thymoma subtypes and stages, the study's reliance on correlation data without multivariate analysis limits its predictive value. Clinical and methodological biases, including sample selection and the retrospective design, must also be considered. Given thymoma variability and treatment complexity, these findings should be considered preliminary. Multidisciplinary approaches involving oncologists, pathologists, and surgeons remain critical for treatment

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decisions. Future research with larger cohorts, multivariate analyzes, and mechanistic studies is needed to validate the prognostic significance of these markers and explore potential therapeutic targets for precision medicine.

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