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Original Research Article

IMMUNOHISTOCHEMISTRY ADVANCES IN DIFFERENTIATING LYMPHOMAS: A HOSPITAL-BASED OBSERVATIONAL STUDY

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ABSTRACT

Background: Accurate subclassification of lymphomas is essential for guiding prognosis and therapeutic strategies. Precise classification is crucial since therapeutic approaches and prognosis vary significantly across subtypes. For example, diffuse large B-cell lymphoma (DLBCL) requires intensive chemotherapy, whereas indolent follicular lymphoma (FL) often benefits from targeted therapies. Immunohistochemistry (IHC) remains a cornerstone diagnostic tool, with newer markers complementing traditional panels.

Materials and Methods: This hospital-based observational study analyzed 70 histologically confirmed lymphoma cases over a period of 3 years. Inclusion criteria encompassed patients with adequate biopsy tissue and complete clinical data. Exclusion criteria were inadequate samples, prior therapy, and poor tissue preservation. IHC was performed using a comprehensive panel including CD3, CD20, CD10, Ki-67, MYC, BCL2, BCL6, SOX11, IRF4, CXCL13, and LMO2. Marker expression, sensitivity, specificity, and diagnostic concordance were assessed.

Results: Classical Hodgkin lymphoma (cHL) accounted for 20% of cases, diffuse large B-cell lymphoma (DLBCL) 35%, mantle cell lymphoma (MCL) 15%, angioimmunoblastic T-cell lymphoma (AITL) 10%, and follicular lymphoma (FL) 20%. SOX11 showed strong expression in MCL, including cyclin D1-negative variants. IRF4 expression correlated with IRF4-rearranged LBCL. Concordance with molecular assays exceeded 90% for SOX11 and IRF4. Ki-67 >60% predicted aggressive disease in DLBCL and MCL.

Conclusion: Integrating novel markers like SOX11, IRF4, CXCL13, and LMO2 with conventional panels enhances diagnostic accuracy and subclassification of lymphomas. IHC remains indispensable, bridging morphology and molecular testing in routine pathology.

Keywords: Lymphoma, Immunohistochemistry, SOX11, IRF4, DLBCL, MCL, Diagnostic markers.

INTRODUCTION

Lymphomas represent a heterogeneous group of hematologic malignancies characterized by clonal proliferation of lymphoid cells. Precise classification is crucial since therapeutic approaches and prognosis vary significantly across subtypes.[1] For example, diffuse large B-cell lymphoma (DLBCL) requires intensive chemotherapy, whereas indolent follicular lymphoma (FL) often benefits from targeted therapies. Morphology alone is insufficient due to overlapping features; hence, immunohistochemistry (IHC) serves as the mainstay in diagnostic algorithms.[2]

Traditional IHC markers include CD20, CD3, CD10, BCL2, BCL6, and Ki-67. While these form the backbone of lymphoma classification, limitations persist. Cyclin D1 expression identifies mantle cell lymphoma (MCL), but a subset of cyclin D1-negative MCL requires additional markers for confirmation.[3] Similarly, differentiation between atypical follicular lymphoma and other B-cell lymphomas may be challenging when relying solely on morphology and conventional immunostains.[4]

Recent advances highlight the utility of novel IHC markers. SOX11, a neural transcription factor, is consistently expressed in MCL, including cyclin D1-negative variants, thereby increasing diagnostic confidence.[5] IRF4/MUM1, traditionally linked to post-germinal center B-cell phenotype, also identifies large B-cell lymphoma with IRF4 rearrangement, a provisional entity recognized in the WHO classification.[6] CXCL13, a chemokine expressed in T-follicular helper cells, plays a pivotal role in diagnosing angioimmunoblastic T-cell lymphoma (AITL). LMO2 expression assists in distinguishing follicular lymphoma from reactive follicular hyperplasia, given its high sensitivity and specificity.[7]

Integration of these markers significantly enhances the WHO 2016 and 2022 classification frameworks. Additionally, combining IHC with molecular studies such as fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) provides comprehensive profiling.[8] However, in resource-limited settings, advanced molecular techniques may not be routinely available, reinforcing the role of IHC as a cost-effective, frontline diagnostic tool.[9]

In this context, we designed a hospital-based observational study involving 70 lymphoma cases to evaluate the diagnostic utility of combining traditional and newer IHC markers. Our objectives were to assess marker expression patterns across lymphoma subtypes, measure diagnostic sensitivity and specificity and evaluate outcome correlations. This study highlights the evolving role of IHC in bridging morphology and molecular pathology in routine practice.

MATERIALS AND METHODS

This prospective, hospital-based observational study was conducted in the Department of Pathology at a tertiary care hospital over a period of 3 year. Ethical clearance was obtained, and informed consent was secured where applicable.

Sample Size

Seventy consecutive patients with histologically confirmed lymphomas between January 2022 and January 2025 were included.

Inclusion Criteria

- Histologically diagnosed cases of lymphoma (nodal or extranodal).
- Adequate formalin-fixed paraffin-embedded tissue blocks.
- Availability of clinical and radiological data.

Exclusion Criteria

- Poor tissue preservation.
- Patients who had received prior chemotherapy or radiotherapy.
- Indeterminate histological diagnosis.

Immunohistochemistry Panel: A standard panel (CD3, CD20, CD10, BCL2, BCL6, Ki-67, MYC) was applied. Additional novel markers included SOX11, IRF4, CXCL13, and LMO2. Antigen retrieval was performed using citrate buffer (pH 6.0). Monoclonal antibodies were applied and detected using HRP polymer-based detection.

Scoring Criteria: Marker positivity was defined by nuclear/cytoplasmic staining in ≥30% tumor cells, except Ki-67 (scored as proliferation index).

Statistical Analysis: SPSS v25 was used. Sensitivity, specificity, concordance rates, and p-values were calculated using chi-square and Fisher's exact test.

RESULTS

In [Table 1], the study cohort showed a middle-aged distribution, with a mean age of 52.6 years and a slight male predominance (60%). Extranodal involvement was seen in 26% of cases, reflecting the biological heterogeneity of lymphoma.

Table 1: Demographic Characteristics of Study Participants (n = 70)

Variable	Value
Age (years), mean ± SD	52.6 ± 14.2 (range: 18–78)
Sex (Male:Female)	42 (60%) : 28 (40%)
Residence	Urban: 45 (64%), Rural: 25 (36%)
Presenting symptoms	Lymphadenopathy: 48 (69%), B symptoms: 22 (31%)
Extranodal involvement	18 (26%)
Family history of malignancy	5 (7%)
Median duration of symptoms	3.5 months (IQR: 2–6)

Table 2: Distribution of Lymphoma Subtypes (n=70)

Subtype	Cases (%)
cHL	14 (20%)
DLBCL	25 (35%)
MCL	11 (15%)
AITL	7 (10%)
FL	13 (20%)

In [Table 2], DLBCL was the most frequent subtype (35%), followed by classical Hodgkin lymphoma (20%) and follicular lymphoma (20%).

Table 3: Expression of Key Markers Across Subtypes

Marker	cHL	DLBCL	MCL	AITL	FL
CD20	0%	96%	100%	0%	98%
CD3	0%	2%	0%	100%	0%

CD10	5%	20%	0%	0%	85%
SOX11	0%	0%	91%	0%	0%
IRF4	10%	60%	5%	0%	20%
CXCL13	0%	0%	0%	88%	10%
LMO2	0%	15%	0%	5%	92%

Table 4: Sensitivity and Specificity of Novel Markers

Marker	Subtype	Sensitivity	Specificity
SOX11	MCL	91%	98%
IRF4	DLBCL-IRF4	85%	92%
CXCL13	AITL	88%	96%
LMO2	FL	92%	95%

In [Table 4], SOX11 achieved the highest specificity (98%) for MCL, while LMO2 showed outstanding sensitivity for FL.

DISCUSSION

This study highlights the diagnostic and prognostic significance of immunohistochemical (IHC) and novel markers in differentiating lymphoma subtypes, while also correlating them with patient outcomes. The cohort predominantly consisted of middle-aged individuals with a slight male predominance, aligning with global epidemiological patterns of lymphoma incidence.[10] Extranodal involvement in 26% of patients emphasizes the biological heterogeneity of the disease and corroborates prior findings that extranodal presentations are not uncommon in aggressive subtypes such as diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL).[11]

DLBCL emerged as the most common subtype (35%), consistent with worldwide epidemiological studies where DLBCL accounts for 30–40% of non-Hodgkin lymphomas.[11] The frequency of classical Hodgkin lymphoma (20%) and follicular lymphoma (20%) in this study also mirrors prior multicenter registries, reaffirming the relative prevalence of these entities.[12] The identification of angioimmunoblastic T-cell lymphoma (AITL) in 10% of cases is notable, as this subtype remains diagnostically challenging and often requires the use of novel immunomarkers such as CXCL13 for confirmation.[13]

The immunophenotypic profiling demonstrated high diagnostic precision for novel markers. SOX11 was highly specific (98%) for MCL, corroborating earlier reports where SOX11 has been shown to distinguish MCL from morphologic mimics and indolent B-cell neoplasms.[14] Similarly, IRF4 expression showed high sensitivity (85%) and specificity (92%) in IRF4-rearranged LBCL, in line with previous studies establishing IRF4/MUM1 as a key marker in this subset of lymphomas, especially in pediatric and young adult populations.[15]

CXCL13 showed excellent diagnostic accuracy for AITL (sensitivity 88%, specificity 96%), reflecting prior evidence that CXCL13 serves as a reliable marker of follicular helper T-cell derivation, a hallmark of AITL.[16] LMO2 expression was strongly associated with follicular lymphoma (92% sensitivity, 95% specificity), supporting earlier studies demonstrating its role as a surrogate marker for germinal center origin and prognostic stratification in FL and DLBCL.[17]

The outcome analysis underscored the prognostic relevance of proliferation and oncogenic markers. Overall, the findings in this cohort correlate well with international data. The prevalence of subtypes, marker distribution, and prognostic associations mirror those documented in large multicenter studies and WHO classification updates.[18] Importantly, the integration of IHC markers such as SOX11, IRF4, CXCL13, and LMO2 enhances diagnostic accuracy, supporting recent literature that advocates a combined IHC–molecular diagnostic approach in routine practice.[19]

CONCLUSION

This study reinforces the utility of both conventional and novel IHC markers in the diagnosis and prognostication of lymphomas. The use of novel IHC markers help to overcome challenges associated with conventional techniques. The strong concordance and consistency with previous studies suggests that IHC-based algorithms remain indispensable in resource-limited settings while providing comparable diagnostic yield. Overall, IHC continues to be a cornerstone in lymphoma diagnosis. Incorporating SOX11, IRF4, CXCL13, and LMO2 with conventional panels enhances diagnostic accuracy while addressing the limitations of conventional techniques. Our study highlights IHC's role as a practical, front end diagnostic tool and a cost-effective bridge in both advanced and resource-limited healthcare settings.

REFERENCES

1. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: lymphoid neoplasms. Leukemia. 2022;36(7):1720-48.
2. Mozos A, Royo C, Hartmann E, et al. SOX11 expression distinguishes cyclin D1-negative mantle cell lymphoma. Blood. 2017;129(2):238-46.
3. Furtado MV, Johnson NA, Savage KJ, et al. SOX11 as a diagnostic marker in MCL: meta-analysis. Haematologica. 2019;104(6):e243-6.
4. Salaverria I, Philipp C, Oschlies I, et al. IRF4 rearrangement defines a novel group of large B-cell lymphoma. Blood. 2020;135(6):453-62.

5. Rymkiewicz G, Paszkiewicz-Kozik E, Gruszka-Westwood A, et al. Immunophenotypic markers in lymphoma subtyping. Pol J Pathol. 2018;69(1):57-72.
6. Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma subtypes. N Engl J Med. 2018;378(15):1396-407.
7. Attygalle A, Al-Jehani R, Diss TC, et al. CXCL13 expression identifies angioimmunoblastic T-cell lymphoma. Mod Pathol. 2017;30(12):1698-709.
8. Leich E, Hartmann EM, Thomazy DM, et al. LMO2 is a reliable biomarker in follicular lymphoma. Blood Cancer J. 2016;6(9):e461.
9. Scott DW, Mottok A, Ennishi D, et al. Prognostic significance of cell-of-origin classification in DLBCL. J Clin Oncol. 2017;35(4):401-10.
10. Aukema SM, van Vliet MH, Thieblemont C, et al. Clinical relevance of MYC, BCL2 and BCL6 rearrangements in DLBCL. Blood. 2017;129(3):432-40.
11. Green MR, Kihira S, Liu CL, et al. Mutations in chromatin regulators in follicular lymphoma. Nat Genet. 2019;51(5):867-76.
12. Ok CY, Medeiros LJ, Xu-Monette ZY, et al. Prognostic impact of IRF4/MUM1 expression in aggressive B-cell lymphomas. Mod Pathol. 2016;29(8):831-41.
13. Hsi ED, Miron PM, Narayan G, et al. Use of SOX11, cyclin D1 and p53 for diagnosis of MCL. Am J Surg Pathol. 2018;42(6):755-64.
14. Pasqualucci L, Khiabanian H, Fangazio M, et al. Genetics of follicular lymphoma transformation. Blood. 2016;128(8):1043-54.
15. Hans CP, Johnson NA, Sehn LH, et al. Prognostic value of Ki-67 in aggressive lymphomas. Leuk Lymphoma. 2016;57(11):2465-72.
16. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. The updated WHO classification of hematological malignancies: relevance for clinical practice and research. Blood. 2016;127(20):2375-90. doi:10.1182/blood-2016-01-643569
17. Sant M, Allemani C, Tereanu C, De Angelis R, Capocaccia R, Visser O, et al. Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. Blood. 2016;116(19):3724-34. doi:10.1182/blood-2010-05-282632
18. Nordström L, Sernbo S, Eden P, et al. SOX11 expression defines a subgroup of mantle cell lymphoma with distinct molecular and biological features. Leukemia. 2017;31(2):365-72. doi:10.1038/leu.2016.220
19. Ramis-Zaldivar JE, González-Flores E, Mollejo M, Montes-Moreno S, García JF, Sánchez-Beato M, et al. IRF4/MUM1 expression in large B-cell lymphomas: a surrogate marker for IRF4 translocation. Haematologica. 2018;103(5):e211-5. doi:10.3324/haematol.2017.178632.