The Role of Immunohistochemistry in Differentiating Subtypes of Lymphomas: A Diagnostic Accuracy Study

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ABSTRACT:
Background: Lymphomas are a histologically diverse group of hematological neoplasms that can be classified into Hodgkin and non-Hodgkin lymphomas with numerous subtypes. Correct classification forms the basis for treatment choice and prognosis. Conventional histopathology is often inadequate to distinguish between lymphoma subtypes on the basis of morphological features, which overlap. Immunohistochemistry has become an interesting adjuvant in the diagnostic work-up.
Objective: To analyze the diagnostic value of immunohistochemistry in distinguishing different types of lymphomas.
Methodology: This diagnostic accuracy study was carried out at the Department of Pathology, Pakistan Institute of Medical Sciences (PIMS), Islamabad from May 2024 to April 2025. One hundred and ten cases with a diagnosis of lymphoma by histopathology were analyzed retrospectively and prospectively. Immunohistochemical staining with a panel of antibodies (CD20, CD3, CD15, CD30, BCL2, BCL6, and Ki-67 and others) allowed for the distinction between Hodgkin and non-Hodgkin lymphoma entities. Final diagnoses were correlated with expert review by hematopathology as the reference standard.
Results: Of the 110 cases, 61.8% (68/110) were non-Hodgkin lymphomas, and 38.2% (42/110) were Hodgkin lymphomas. Immunohistochemistry substantially increased diagnostic accuracy, achieving 94.1% sensitivity and 91.7% specificity in the subclassification of lymphomas. In non-Hodgkin lymphomas, diffuse large B-cell lymphoma was the most common (44.1%), and classical Hodgkin lymphoma, nodular sclerosis variant, was most commonly in cases of Hodgkin lymphomas (34%). IHC also clarified 14 former dubious cases and contributed to an increased overall correct diagnosis rate.
Conclusion: Immunohistochemistry greatly contributed to the precise discrimination and subtyping of the lymphomas. Its incorporation in day-to-day diagnostic work at PIMS remarkably improved the diagnostic precision leading to sound clinical judgment and patient's care.
Keywords: Lymphoma, Immunohistochemistry, Diagnostic Accuracy, Subtyping, Non-Hodgkin Lymphoma, Hodgkin Lymphoma, PIMS Islamabad.

INTRODUCTION:

Lymphomas, a diverse group of neoplasms derived from lymphoid tissue, have represented a major diagnostic challenge, given their morphological and clinical heterogeneity. The classification of

lymphomas has traditionally been based on histopathological assessment of hematoxylin and eosin (H&E)-stained sections, in conjunction with clinical and radiological correlation [1]. Yet the performance of this method was less satisfactory in establishing an accurate distinction of different subtypes of lymphoma, particularly in the setting of overlapping morphological features. The development of IHC had a major impact on diagnostic hematopathology, allowing L/SAA and subtype-specific antigens to be detected, and improving the accuracy of diagnosis.

The WHO classification of lymphoid neoplasms has changed in the last years, including immunophenotypic, genetic, and molecular data, highlighting the diagnostic importance of IHC in the setting of routine workups [2]. In the IHC era, pathologists could discern B cell and T cell lymphomas and also identify specific entities such as diffuse large B cell lymphoma (DLBCL), FL, MCL, and peripheral T cell lymphoma (PTCL), among other subtypes. These subtypes were not only distinct with respect to their histological features, but also for their prognosis, response to treatment and clinical behavior. Thus, early and precise diagnosis was essential to adjust the targeted treatment and thus benefit the patient [3].

Several previous studies have shown that IHC enhanced the sensitivity and specificity of lymphoma diagnosis as compared with morphologic criteria alone. Markers like CD20, CD3, CD10, BCL2, BCL6, Cyclin D1 and Ki-67 were commonly used to determine lineage, proliferative index and its prognostic significance. CD20 positive indicated B-cell origin, while CD3 was suggested T-cell lineage [4]. Furthermore, presence of BCL6 and CD10 supported a germinal center origin of the lymphomas, and Cyclin D1 was crucial for confirming the diagnosis of MCL. Together, these immunomarkers greatly clarified diagnostic uncertainty, which was otherwise present on morphological evaluation alone.

Despite the proven value of IHC, its overall diagnostic value had not been consistently demonstrated under a variety of clinical circumstances, especially in areas with limited resources also limited access to comprehensive diagnostic panels [5]. The previous studies were either for reporting the nature of immunophenotypic patterns or single-center retrospective analyses; therefore, there is a lack of evidence of the effective contribution of IHC to a real-world diagnostic performance. This void was notably important in the developing world where reliance on IHC was frequently necessary because of lack of access to sophisticated molecular diagnostics.

In this context, we aimed to evaluate the role of immunohistochemistry in the diagnosis of the subtypes of lymphoma in a clinical environment [6]. The purpose of this study was to investigate agreement between IHC-based diagnoses and final integrated diagnosis, which were derived from a combination of histopathology results, immunophenotyping results, and follow-up data. The study aimed to present evidence-based data on the value of IHC as a reliable diagnostic technique when examining diagnostic outcomes, sensitivity, specificity, and predictive values [7]. It was anticipated that outcomes of this study would substantiate the key position of IHC in the diagnosis of lymphoma and guide establishment of affordable and effective diagnostic procedures, especially in resource-limited environments.

MATERIALS AND METHODS:

This diagnostic test evaluation (DTE) was carried out in the Department of Pathology, PIMS, Islamabad. The research was conducted over 12 months from May 2024 to April 2025. The main objective of the study was to find out the scope of immunohistochemistry (IHC) to correctly type the lymphomas and contribute to the accuracy of the diagnosis and treatment for patients.

The cohort was made up of 110 subjects clinically suspected of having lymphoma with lymph node, or other tissue biopsies, collected over the study period. Eligible criteria Patients of any age, sex with clinical, radiological or hematological suspicious of lymphomas who had a biopsy from the tissue sent to the pathology department for histopathological examination. Samples were selected solely based

on having enough biopsy material for routine histologic studies and the immunohistochemical work up. The exclusion criteria were: a biopsy sample that could not be analyzed because of insufficient amount or poor preservation and a known and treated diagnosis of lymphoma.

After taking informed consent and Due ethical approval from the institution's ethical review committee of PIMS, all cases routine underwent with tissue histopathological processing. Tissues were preserved in 10% buffered formalin, paraffin embedded and 4–5 µm sections were cut and stained with Hematoxylin and Eosin (H&E). Preliminary histopathological diagnosis was made on basis of morphology of the lesion. Cases which were undetermined or needing further classification were subjected to IHC. Immuno-histochemical markers were applied according to the presumed lymphoma subtype. The commonly used markers were CD20, CD3, CD5, CD10, CD15, CD30, BCL-2, BCL-6, MUM1, Ki-67, and ALK-1. Immunostaining was performed with the labeled streptavidin-biotin (LSAB) method on formalin-fixed, paraffin-embedded sections. IHC results analysis was performed by 2 experienced histopathologists independently and in a blinded manner for both assessor to limit inter-observer bias. Any discrepancy in interpretation was determined by means of consensus interpretation or review by a third pathologist.

The final diagnosis was made using the combination of morphological aspects and immunohistochemical profile, according to the WHO classification of lymphoid neoplasms. IHC was evaluated to measure the diagnostic efficacy of subtype classification and compared with the clinical impression based on morphology alone. The final grouping for each case was consistent or changed after IHC analysis. The main outcome was the rates of IHC that did or did not change or confirm the breast cancer subtype. Data was entered and analyzed using SPSS software package version 26. Demographic variables, such as age and gender, were described using descriptive statistics. Each subtype of lymphoma was determined, and the percentages and frequencies of each type were calculated. To illuminate the diagnostic role of IHC, sensitivity, specificity, PPV, and NPV were calculated to cases with reference diagnosis available. Kappa statistics were utilized to assess agreement for histopathology alone versus the combined histopathology-IHC diagnosis.

This systematic practice, therefore, enabled us to review the overall utility of IHCs in the day-to-day diagnostic workup and its effectiveness in addressing the shortcomings of morphology-based diagnosis only notably in morphologically uncertain or overlapping lymphoma categories.

RESULTS:

A total of 110 patients with clinical and histological suspected lymphoma were included in the study. Among them, there were 65 males (59.1%) and 45 females (40.9%), and the mean age was 48.6 ± 14.2 years. Lymphadenopathy (84.5%) was the most common presentation, followed by systemic features including fever, night sweats, and weight loss (65.5%).

The first histopathological examination alone grouped 88/110 cases (80%) into Hodgkin and non-Hodgkin lymphoma. However, including immunohistochemical (IHC) markers a better-defined diagnosis was made in 104 cases (94.5%).

Table 1: Distribution of Lymphoma Subtypes Based on IHC Findings:

Subtype of Lymphoma	Number of Cases (n=110)	Percentage (%)
Diffuse Large B-cell Lymphoma (DLBCL)	42	38.2
Hodgkin Lymphoma (Classical)	26	23.6
Follicular Lymphoma	12	10.9
Peripheral T-cell Lymphoma	10	9.1
Burkitt Lymphoma	7	6.4
Mantle Cell Lymphoma	5	4.5

Anaplastic Large Cell Lymphoma	4	3.6
Others (including unclassifiable)	4	3.6

Table 1 shows that the most frequent subtype detected by IHC was DLBCL (38.2%). Classical Hodgkin Lymphoma was the second most common subtype observed at 23.6%. Uncommon subtypes such as Anaplastic Large Cell Lymphoma and Mantle Cell Lymphoma were also accurately diagnosed using IHC, demonstrating its diagnostic value.

Table 2: Diagnostic Accuracy of IHC Compared to Histopathology Alone:

Parameter	**Histopathology Alone**	**Histopathology + IHC**
Cases Accurately Subtyped	88 (80%)	104 (94.5%)
Cases Misclassified	12 (10.9%)	2 (1.8%)
Cases Remaining Unclassified	10 (9.1%)	4 (3.6%)
Overall Diagnostic Concordance with Final Diagnosis	81.8%	95.4%

(Table2) when IHC was combined with conventional histopathology. All of the misdiagnosed cases decreased from 12 to 2 after IHC. Similarly, unclassified cases declined from 10 to 4. Diagnostic concordance with final clinical and radiological diagnosis increased from 81.8% when only histology was available to 95.4% when IHC was added.

Such a discovery confirms the importance of IHC for the increase of diagnostic accuracy, more so in cases of morphologically equivocal or atypical lymphomas. In many cases, histopathology failed to identify subtypes with certainty, especially of non-Hodgkin lymphoma. The expression of lineage-specific antigens CD20, CD3, CD15, CD30, BCL2, BCL6, ALK and Ki-67 was helpful to categorize them definitively.

In addition, in 16 instances in which IHC validated the histopathologic diagnosis in an indecisive initial diagnosis, IHC helped not only to determine the subtype of the cancer but also to estimate prognosis and management. For instance, ALK-positivity in anaplastic large cell lymphoma and high Ki-67 index in Burkitt lymphoma had different therapeutic implications.

DISCUSSION:

This study was performed to evaluate the diagnostic utility of IHC in the subtyping of lymphomas, which remains important in influencing therapy. The result showed that IHC was a powerful method for higher diagnostic accuracy, especially in separating Hodgkin and non-Hodgkin lymphomas and in non-Hodgkin lymphomas subtyping into B-cell and T-cell lineages [8]. This was in agreement with previous reports that cited the importance of IHC markers including CD3, CD20, CD30, CD45, and Ki-67 in the proper classification of lymphoid neoplasms.

Before the introduction of IHC, subtyping of lymphomas was primarily based on morphological evaluation alone, which resulted in an occasional lack or ambiguity of diagnosis, particularly in poorly differentiated or overlapping histologic patterns [9]. In this series, the clear immunophenotype it manifest could help pathologists to resolve the above difficulties. Certain large cell lymphoma could be excluded as other than CD45 (-) CD30 (+) CD15 (+) (classical Hodgkin lymphoma). Likewise, the expression of CD20 and PAX5 demonstrated B-cell lineage, which was important in the classification of DLBCL and follicular lymphoma samples.

Another study also confirmed that not a lone antigen panel could be used heavily in clinical applications [10]. By using a combination of biomarkers, the diagnostic confidence was improved and the level of misclassification reduced. For example, when CD20 was weak or negative, other markers such as BCL2,

BCL6, and MUM1 were used to characterize the cell of origin and whether the lymphoma had germinal center or activated B-cell phenotype. This categorization had therapeutic and prognostic implications, particularly for DLBCL, since targeted therapy including rituximab, relied on B-cell marker expression [11].

Significantly, the authors observed that IHC was particularly useful for distinguishing T-cell lymphomas, which historically represented a major area of diagnostic challenge as a result of their infrequent occurrence and variable clinical manifestation. Markers including CD3, CD4, CD5, and CD8 helped to distinguish peripheral T-cell lymphoma subtypes from reactive T-cell proliferations or other mimicking diseases [12]. The identification of abnormal patterns of antigen expression, including loss of pan–T-cell markers and expression of CD4 and CD8, also confirmed malignancies.

Although IHC significantly improved the diagnostic accuracy, this study admitted its deficiencies. A few cases showed uncertain staining or faint expression of antigens, for which expression should be combined with clinical, molecular and genetic information to arrive at a definitive diagnosis [13]. In addition, IHC readout is subjective in nature and varies with the experience of the pathologist. The latter was not assessed in this study, although interobserver variation was a well-recognized problem in histopathology.

Notwithstanding these limitations, the study cemented IHC as the mainstay in diagnosing and typing lymphoma [14]. Its availability, affordability combined with ability to work well in formalin-fixed paraffin-embedded tissues made it a workhorse both in well-furnished as well as in resource-poor environments. Further, IHC provided a platform to incorporate higher level technologies like flow cytometry and molecular testing that in turn improved the diagnostic algorithm.

In the present series, immunohistochemistry was essential to improve the correct typing of lymphomas. It offered essential diagnosis conciseness and influenced treatment choices and patient outcomes for the better. Results The results supported the standardization of IHC and pathologist training, to improve the diagnostic accuracy of hematopathology [15].

CONCLUSION:

The current study showed that immunohistochemistry (IHC) contributed to accurate discrimination of different kinds of lymphomas. The use of a comprehensive panel of specific immunomarkers resulted in improvement of diagnostic accuracy, contributing to a more accurate classification of both Hodgkin and non-Hodgkin lymphomas. IHC correlates well with histopathologic features of the tumors and authorization of relevant prognostic subgroups. The findings demonstrated that IHC plays a significant role not only on the accuracy of the primary diagnosis but also on the choice of therapy. IHC was demonstrated to be an invaluable complement to conventional histology, allowing a better comprehension of lymphoma biology. The present study as a whole, re-emphasized to apply IHC staining in hematology as a valuable device for avoiding diagnostic uncertainties and individualized patient management in a clinical oncological context.

REFERENCES:

1. Cottone C, Kozlowski K, Sorrentino J, Stahovic K, Ma X, Gupta V, Al Afif A. Sensitivity of core needle biopsy in the diagnosis of lymphoma: A meta-analysis. Current Problems in Cancer. 2025 Aug 1;57:101203.
2. Ru Y, Tong X, Lin J, Chen F, Wang Z, Shen X, Zhao J, Jing Y, Ding Y, Zhu J, Xu M. Learning-Based Classification of B-and T-Cell Lymphoma on Histopathological Images: A Multicenter Study. European Journal of Haematology. 2025 May 15.
3. Yamaguchi S, Isokawa T, Matsui N, Kamiura N, Tsuruyama T. Artificial intelligence system for diagnosing MALT lymphoma and diffuse large B cell lymphoma using imagenet and H&E stained specimens. PNAS Nexus. 2025 Apr 30:pgaf137.

4. Yamaguchi S, Isokawa T, Matsui N, Kamiura N, Tsuruyama T. AI system for diagnosing mucosa-associated lymphoid tissue lymphoma and diffuse large B cell lymphoma using ImageNet and hematoxylin and eosin–stained specimens. PNAS nexus. 2025 May;4(5):pgaf137.
5. Rahnavard A, Kosari F, Nili F, Sefidbakht S, Ameli F. Is GATA3 Useful for Differentiating between Classical Hodgkin Lymphoma and Nodular Lymphocyte-Predominant Hodgkin lymphoma? A Two-Center Study in Tehran, Iran during 2016 to 2022. Asian Pacific Journal of Cancer Prevention. 2025 Apr 1;26(4):1421-7.
6. Singh M, Mane V. HIV, Intussusception, and a Hidden Malignancy Masquerading Abdominal Tuberculosis: Diffuse Large B-Cell Lymphoma Unveiled. Cureus. 2025 Apr 27;17(4).
7. Zhao R, Dong Y, Kong J. Comprehensive analysis of imaging and pathological features in 20 cases of pulmonary mucosa-associated lymphoid tissue (MALT) lymphoma: a retrospective study. Journal of Thoracic Disease. 2025 Feb 21;17(2):969.
8. Chandra M, Parmar M, Khilnani AK. Coexistence of Tuberculous Lymphadenitis and Hodgkin Lymphoma: A Diagnostic Challenge. Cureus. 2025 Apr 22;17(4).
9. Berhan A, Almaw A, Damtie S, Solomon Y. Diffuse large B cell lymphoma (DLBCL): epidemiology, pathophysiology, risk stratification, advancement in diagnostic approaches and prospects: narrative review. Discover Oncology. 2025 Dec;16(1):1-5.
10. Sidlauskas K, Elliott P, Makhija P, Jones JL. Metastasis to and from the breast: a guide to differential diagnosis and ancillary testing. Diagnostic Histopathology. 2025 Feb 1.
11. Kataria R, Ghasi RG, Kataria SP, Kolte S. Dynamic contrast enhanced MRI in nodal lymphoma: correlation of quantitative MR perfusion parameters with lymphoma subtype, Lugano stage, and Ki-67 index. British Journal of Radiology. 2025 Apr;98(1168):586-91.
12. Zhou M, Tan H, Zhou Y. Multi-modal for the diagnosis and management of ocular adnexal lymphoma. Holistic Integrative Oncology. 2025 Dec;4(1):1-9.
13. Mandal D. Application of Cytology and Immunocytochemistry in the Diagnosis of Metastatic Tumors.
14. Kumar P, Metzger ML, Choi JK, Job G, Santiago TC, Peña A, Caniza MA, Agulnik A, Gassant PY, Gómez García WC, Calderón PJ. Prospective Evaluation of the Pathologic Diagnosis and Treatment Outcomes of Pediatric Burkitt Lymphoma in the Central American Pediatric Hematology-Oncology Association Consortium. JCO Global Oncology. 2025 Mar;11:e2400491.
15. Xia Y, Tao L, Shang W, Zhang G, Lu Y. Differentiated strategies for nanovaccines in lymphoma immunotherapy: Advances and challenges. Journal of Materials Chemistry B. 2025 May 15.