Original Article

Role of Histology in Lymphoma Classification

Histology in Lymphoma Classification

Hussain Mohammad Tahir Noorwali

ABSTRACT

Objective: To determine the usefulness of histopathology and immunohistochemistry in the precise identification of lymphomas in a retrospective cohort.

Study Design: Retrospective observational study

Place and Duration of Study: This study was conducted at the Department of Histopathology at King Fahad Specialist Hospital in Dammam, Saudi Arabia from 15.03.2024 to 30.05.2024.

Methods: One hundred and fifty lymphoma cases 2020-2023 were analyzed using histomorphology and immunohistochemistry per World Health Organization classification.

Results: There were 52 (35%) Hodgkin lymphoma and 98 (65%) non-Hodgkin lymphoma. The most common type of non-Hodgkin lymphoma was diffuse large B-cell lymphoma (40%), and follicular lymphoma (18%). In 87%, immunohistochemistry was required to make a definitive classification.

Conclusion: The use of histology and immunohistochemistry cannot be replaced in the correct classification of lymphoma and plays a crucial role in the choice of therapy and prognosis.

Key Words: Lymphoma, Histopathology, Immunohistochemistry, Word Health Organization classification

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INTRODUCTION

Lymphomas are a heterogeneous group of malignant neoplasms arising from B lymphocytes, T lymphocytes or natural killer cells at different stages of maturation. They include biologically distinct diseases that vary considerably in morphology, clinical presentation, molecular abnormalities, response to treatment and prognosis. Contemporary classification therefore does not depend on a single diagnostic feature. The fifth edition of the World Health Organization Classification of Haematolymphoid Tumours and the International Consensus Classification define lymphoma entities through the integrated assessment of tissue architecture, cytological features, immunophenotype, genetic alterations and clinical findings.[1] Despite advances in molecular pathology, conventional histological examination remains the essential first step because it demonstrates the growth pattern, cellular composition, degree of atypia, stromal reaction and relationship between neoplastic cells and their microenvironment.

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Lymphoma represents an important component of the global cancer burden, although its incidence and subtype distribution vary according to age, sex, ethnicity, geographical region, immune status and exposure to infectious agents.[2] Non-Hodgkin lymphoma is generally more frequent than Hodgkin lymphoma, and diffuse large B-cell lymphoma is the most common aggressive subtype in many populations.[3] Saudi cancer-registry data have also identified non-Hodgkin lymphoma as one of the leading malignancies among men, with variations in cancer incidence across different regions of the Kingdom.[4] These epidemiological differences emphasize the importance of institution- and region-specific pathological data because the distribution observed in Western populations may not fully represent patients treated in Saudi Arabian centres.

Adequately processed tissue is fundamental to lymphoma diagnosis. Examination of haematoxylin-and-eosin-stained sections allows the pathologist to assess whether the lymph-node architecture is preserved, follicular, nodular, diffuse or partially effaced. It also helps determine the size and appearance of neoplastic cells and identify diagnostic backgrounds such as granulomas, necrosis, eosinophilia, vascular proliferation or mixed inflammatory infiltrates. Nevertheless, reactive lymphoid hyperplasia, metastatic malignancy and different lymphoma subtypes may show overlapping morphological appearances. Diagnostic-review studies have demonstrated that disagreement frequently involves differentiation between benign and malignant proliferations, determination of lymphoid lineage and assignment of the correct lymphoma subtype.[5]

Immunohistochemistry complements morphology demonstrating lineage, cellular differentiation and abnormal antigen-expression patterns. Pan-leukocyte and lineage-associated markers, including CD45, CD20, PAX5, CD79a and CD3, help distinguish lymphoid neoplasms from non-haematolymphoid malignancies and separate B-cell from T-cell proliferations. CD30 and CD15 support the diagnosis of classical Hodgkin lymphoma when interpreted with characteristic Reed–Sternberg-cell morphology, whereas PAX5 usually shows weak nuclear expression in the neoplastic cells.[6] In follicular lymphoma, the combination of a neoplastic follicular architecture with CD10, BCL6 and abnormal BCL2 expression supports germinal-centre origin and helps distinguish the disease from reactive follicular hyperplasia.[7] In diffuse large B-cell lymphoma, immunohistochemical algorithms employing CD10, BCL6 and MUM1 can provide an accessible approximation of cell-of-origin classification, particularly where gene-expression profiling is unavailable.[8] MYC and BCL2 immunostaining may additionally identify double-expressor disease associated with adverse clinical outcomes, although protein expression should not be considered equivalent to MYC and BCL2 gene rearrangements.[9]

The diagnostic value of immunohistochemistry depends on appropriate antibody selection, technical quality and interpretation within the histological and clinical context. Excessive use of broad panels may increase cost and produce conflicting or nonspecific findings, whereas an insufficient panel can result in incomplete or incorrect classification. A stepwise approach based on the initial morphological differential diagnosis is therefore preferred. Molecular techniques, including fluorescence in situ hybridization, clonality analysis, cytogenetics and next-generation sequencing, are increasingly important for resolving difficult cases and identifying genetically defined entities.[1]

The purposes of this study are to determine the frequency distribution of other subtypes of lymphoma, to determine the diagnostic accuracy of first histological diagnosis, to identify the value of immunohistochemistry in classifying lymphoma and to match histological subtypes with demographic parameters.

METHODS

This retrospective observational study was conducted at the Department of Histopathology at King Fahad Specialist Hospital in Dammam, a major tertiary care and oncology referral center in the Eastern Province of Saudi Arabia vide letter No. 3224 dated February 24, 2024. One hundred and fifty lymphoma cases 2020-2023 were analyzed using histomorphology and immunohistochemistry per World Health Organization classification. Those cases were diagnosed with lymphoma, sufficient tissue to be evaluated histologically, had full work up on immunohistochemistry and full clinical and demographic information were included. The cases where there is a lack of adequate tissue to do the diagnosis, did not have immunohistochemical study, incomplete clinical data cases and cases diagnosed during out of study were excluded.

The following medical records and histopathology reports were analyzed to extract the information, histomorphology characteristics, immunohistochemical panel findings and final diagnosis in WHO classification.

Histopathological assessment of all tissue samples had been fixed in 10% of neutral buffered formalin, routinely processed and embedded in paraffin. Slices of 4-5 μm thickness were cut and stained with Hematoxylin and Eosin (H&E). The histological examination involved examination of Xie et al.[10]

Immunohistochemistry was done by the method of avidin-biotin-peroxidase complex. The list of the antibody panel was chosen according to the primary morphological examination and comprised.[11] A standard antibody panel including B-cell, T-cell, Hodgkin, and proliferation markers was used. Data were evaluated with the help of SPSS-25. Categorical variables were in Chi-square test. A p-value of below 0.05 was taken as statistically significant.

RESULTS

The mean age of the patients was 42.5±16.8 years. The largest age group was 20-40 years, comprising 57 patients (38.0%), followed by those aged 41-60 years, comprising 52 patients (34.7%). Eighteen patients (12.0%) were younger than 20 years, whereas 23 (15.3%) were older than 60 years. The study population included 94 males (62.7%) and 56 females (37.3%), giving a male-to-female ratio of approximately 1.7:1 (Table 1).

There were 98 (65.3%) classified as non-Hodgkin lymphoma, while 52 (34.7%) were classified as Hodgkin lymphoma. Among the non-Hodgkin lymphoma cases, diffuse large B-cell lymphoma was the most frequently reported subtype, accounting for 40.0%, followed by follicular lymphoma, which accounted for 18.0%. The remaining 42.0% comprised other non-Hodgkin lymphoma subtypes (Table 2). Immunohistochemistry was used to confirm B-cell or T-cell lineage in all cases. Among non-Hodgkin lymphomas, immunohistochemistry was required for definitive subtype assignment in 87.0%, whereas histomorphological findings alone were considered sufficient in 13.0% of cases. Immunohistochemistry also contributed to distinguishing reactive from neoplastic lymphoid proliferations in 15.0% of the overall cases and assisted in identifying specific lymphoma entities

Histological examination of classical Hodgkin lymphoma showed scattered large atypical cells within a mixed inflammatory background. The atypical cells demonstrated the characteristic morphology of Reed-Sternberg cells, including abundant cytoplasm, bilobed or multilobated nuclei and prominent eosinophilic nucleoli (Fig. 1).

Table No. 1: Demographic characteristics of patients diagnosed with lymphoma (N = 150)

Variable	No.	%
Age (years)		
<20	18	12.0
20–40	57	38.0
41–60	52	34.7
>60	23	15.3
Gender		
Male	94	62.7
Female	56	37.3

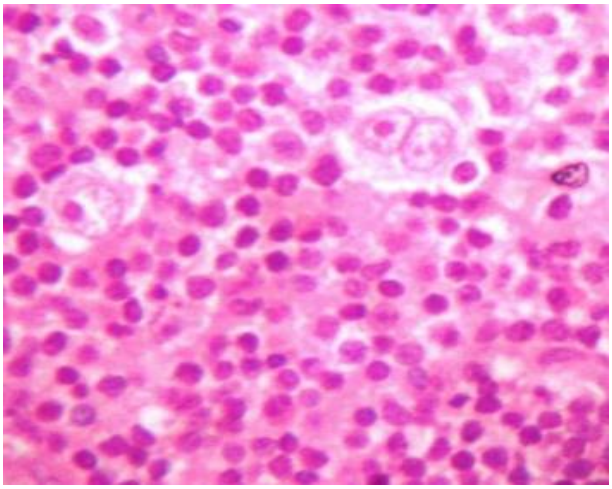

Figure No. 1: High-magnification H&E staining showing the Reed-Sternberg (RS) cells in a mixed inflammatory background. RS cells are characterized by large size, bilobed or multilobated nuclei, prominent eosinophilic nucleoli, and abundant cytoplasm

Immunohistochemical staining demonstrated strong CD30 expression in Reed–Sternberg cells and their variants. The staining showed membranous positivity with accentuation in the paranuclear Golgi region, supporting the diagnosis of classical Hodgkin lymphoma (Fig. 2). The combined morphological and immunohistochemical findings illustrated in Figures 1 and 2 demonstrate the complementary roles of routine histology and CD30 immunostaining in confirming classical Hodgkin lymphoma.

Diffuse large B-cell lymphoma showed diffuse effacement of the normal lymph-node architecture by sheets of large atypical lymphoid cells. These cells had vesicular nuclei, conspicuous nucleoli and a moderate amount of cytoplasm (Fig. 3).

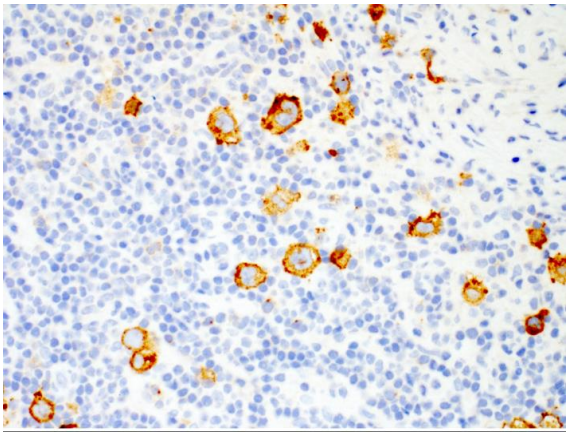

Figure No. 2: CD30 immunohistochemistry showing strong membranous and Golgi-zone positivity in Reed-Sternberg cells and variants, confirming Hodgkin lymphoma

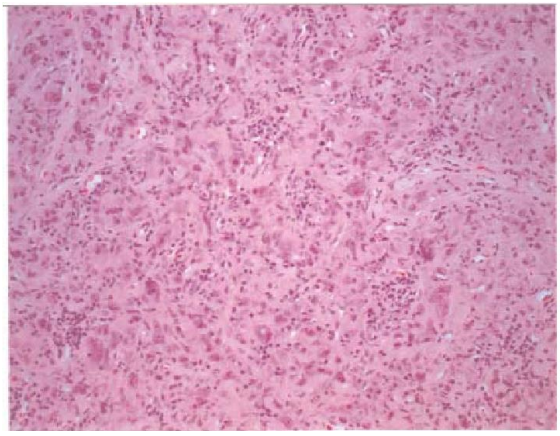

Figure No. 3: H&E staining (40x) demonstrating diffuse proliferation of large lymphoid cells with vesicular nuclei, prominent nucleoli, and moderate cytoplasm, effacing normal nodal architecture

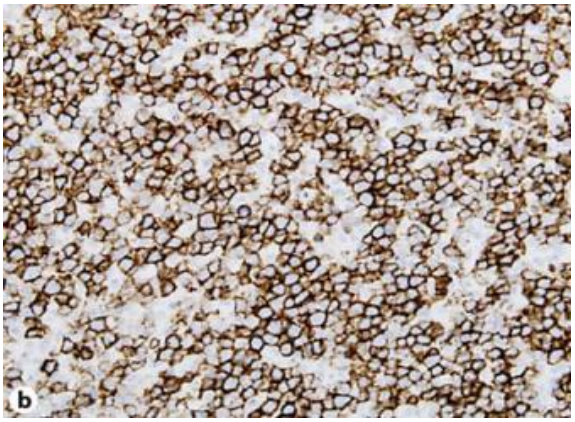

Figure No. 4: Immunohistochemistry (IHC) stain for CD20. The image shows a diffuse infiltrate of large lymphoid cells exhibiting strong, dark-brown membranous positivity for the pan-B-cell marker CD20. This staining pattern is characteristic of a B-cell lineage malignancy, confirming the diagnosis of Diffuse Large B-cell Lymphoma (DLBCL). The background cells are negative, providing an internal control

Table No. 2: Distribution of lymphoma categories and diagnostic contribution of immunohistochemistry

Diagnostic characteristic	No.	%	Denominator
Principal lymphoma classification			
Hodgkin lymphoma	52	34.7	All cases (n=150)
Non-Hodgkin lymphoma	98	65.3	All cases (n=150)
Leading non-Hodgkin lymphoma subtypes			
Diffuse large B-cell lymphoma	Not reported*	40.0	NHL cases (n=98)
Follicular lymphoma	Not reported*	18.0	NHL cases (n=98)
Other NHL subtypes	Not reported*	42.0†	NHL cases (n=98)
Contribution of immunohistochemistry			
Confirmation of lymphoid lineage	150	100.0	All cases (n=150)
Definitive subclassification of NHL	Not reported*	87.0	NHL cases (n=98)
Histomorphology alone sufficient	Not reported*	13.0	NHL cases (n=98)
Differentiation of reactive and neoplastic processes	Not reported*	15.0	All cases (n=150)
Identification of specific lymphoma entities	Not reported*	12.0	All cases (n=150)

*Calculated as the remaining proportion after diffuse large B-cell lymphoma and follicular lymphoma

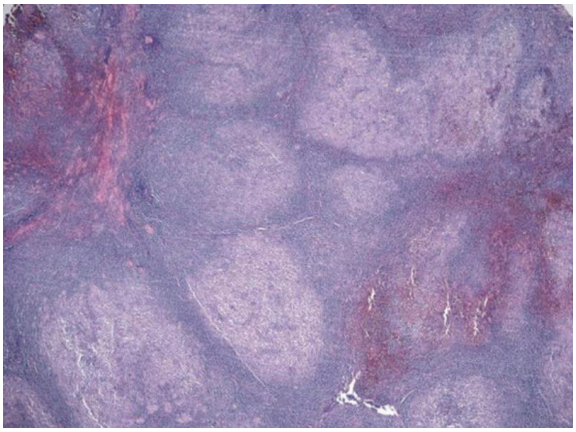

Figure No. 5: H&E staining (10x) showing a nodular growth pattern with closely packed neoplastic follicles lacking normal polarization and tingible body macrophages

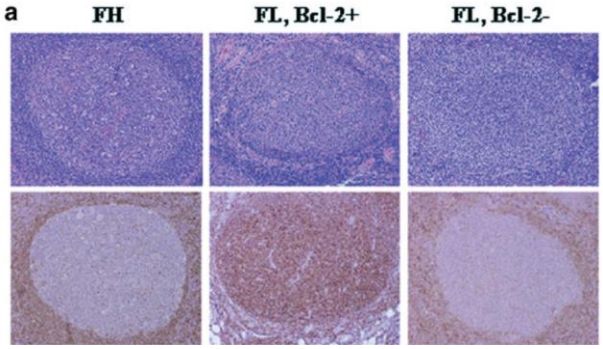

Figure No. 6: BCL2 immunohistochemistry demonstrating strong cytoplasmic positivity in neoplastic follicular centers (abnormal), while reactive follicles would be negative

Immunohistochemical examination showed strong and diffuse membranous CD20 expression in the neoplastic cells, confirming their B-cell lineage. Non-neoplastic background cells showed no comparable staining and served as an internal control (Fig. 4). The morphological features in Figure 3 established the presence of an aggressive large-cell lymphoma, while the CD20 staining shown in Figure 4 confirmed B-cell lineage and supported classification as diffuse large B-cell lymphoma.

Follicular lymphoma demonstrated a predominantly nodular growth pattern composed of closely packed neoplastic follicles. The follicles lacked normal polarization and contained few or no tingible-body macrophages, differentiating them from reactive lymphoid follicles (Fig. 5). BCL2 immunohistochemistry showed cytoplasmic positivity within the neoplastic follicular centres. This abnormal staining pattern supported follicular lymphoma because reactive germinal centres usually show absent or substantially weaker BCL2 expression (Figure 6). Figures 5 and 6 demonstrate that the architectural pattern on routine histology and the abnormal expression of BCL2 provide complementary evidence for diagnosing follicular lymphoma.

DISCUSSION

In the present study, non-Hodgkin lymphoma (NHL) was 65% of all lymphomas and HL 35%. This distribution is consistent with the epidemiological data about NHL in the world, which demonstrates that compared to HL, NHL is more prevalent. Diffuse large B-cell lymphoma (DLBCL) was the most common subtype in NHL (40% of all NHL), as it has been identified as the most common lymphoid malignancy globally.[12,13]

This study showed that immunohistochemistry was critical in the conclusive diagnosis in 87% of the instances. IHC plays several very important roles, CD20/CD79a of B-cells, CD3 of T-cells and CD15/CD30 of Hodgkin lymphoma. This is the most basic step on the classification of lymphoma. Markers specific to the subtypes of lymphoma are used to

identify lymphoma subtypes.[14,15] The subtype markers are cyclin D1 against mantle cell lymphoma, DLBCL subclassification CD10, BCL6, MUM1, BCL2 for follicular lymphoma and CD10 and Ki-67 in Burkitt lymphoma.

In addition to PTCL-NOS and ALCL, immunohistochemistry is indispensable for diagnosing AITL (CD10, CXCL13), ATLL (CD25), MF (CD4, loss of CD7), NK/T-cell lymphoma (CD56, EBER), and EATL (CD8, cytotoxic markers).[16] Proliferation indices such as Ki-67 are prognosticators associated with prognosis. The Hans algorithm (CD10, BCL6, MUM1) further classifies DLBCL into germinal center and non-germinal center with various outcome.

Immunohistochemistry (IHC) panels are used in the case of ambiguous morphology. Indicatively, in differentiating between reactive lymphoid hyperplasia and low-grade lymphoma, it is frequently necessary to show light chain restriction, or aberrant antigen expression.[17,18]

The diagnosis of histology should never be understood out of context. The clinical appearances can be similar histologically observed in various clinical settings. In this study, we have placed special stress on the necessity of comprehensive clinical data to be used in the process of proper classification. The percentage of the DLBCL (40% of NHL) is equal to the international data (30-40%). Distribution of Hodgkin lymphoma (35% of all lymphomas) is greater than that of the Western population (10-15%), but similar to data of developing countries. The male domination and age structure are in line with published epidemiology.

Recommendations

1. Standardized IHC Panels: Establishment of institutional guidelines on the lymphoma work-up according to morphological differentiation diagnosis.
2. Multidisciplinary approach: periodic clinicopathological meetings to compare the findings.
3. Continuous Education: Periodic information about the changes in WHO classification and new entities.
4. Quality assurance: Application of internal and external quality control.
5. Molecular integration: Incremental use of molecular tests in complicated cases.
6. Documentation: Extensive reporting (morphology, IHC, and combined diagnosis).

CONCLUSION

The combination of the molecular methods and the classical histopathology will allow further fine-tuning of the lymphoma types and allow offering personalized medicine options. Nevertheless, a skilled histopathological analysis in conjunction with prudent immunohistochemistry will continue to be imperative in the predictable future.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hussain Mohammad Tahir Noorwali
Drafting or Revising Critically:	Hussain Mohammad Tahir Noorwali
Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

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