

IMMUNOPROFILE OF HODGKIN LYMPHOMA AND EXPRESSION OF IMP3 IN CLASSICAL HODGKIN LYMPHOMA, NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA

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ABSTRACT

Background: Accurate distinction among classical Hodgkin lymphoma (cHL), nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), and diffuse large B-cell lymphoma (DLBCL) may be challenging because of overlapping morphologic and immunophenotypic features. This study evaluated the immunoprofile of Hodgkin lymphoma and assessed insulin-like growth factor II mRNA-binding protein 3 (IMP3) expression across cHL, NLPHL, and DLBCL.

Methods: A retrospective and prospective study was conducted over a two-year period at Madras Medical College/Rajiv Gandhi Government General Hospital. Fifty specimens were included: 25 cHL, 2 NLPHL, and 23 DLBCL. Histopathological sections were reviewed and immunohistochemistry was performed using CD3, CD20, CD15, CD30, and IMP3. Categorical variables were assessed using chi-square/Fisher exact tests as appropriate, with $P < 0.05$ considered statistically significant.

Results: The study included 37 males and 13 females; 41 cases were nodal and 9 were extranodal. Among cHL cases, mixed cellularity was the predominant subtype (22/25, 88%). CD15 and CD30 were positive in 22/25 (88%) and 25/25 (100%) cHL cases, respectively. IMP3 showed cytoplasmic positivity in 20/25 (80%) cHL cases, 2/2 (100%) NLPHL cases, and 18/23 (78.3%) DLBCL cases based on the detailed subgroup counts in the study results. These findings indicate that IMP3 positivity occurs in both Hodgkin and large B-cell lymphomas and therefore does not reliably distinguish cHL/NLPHL from DLBCL.

Conclusion: IMP3 may serve as a supplementary immunohistochemical marker because of its cytoplasmic staining in Hodgkin/Reed-Sternberg or lymphocyte-and-histiocyte cells with relatively limited background staining. However, its expression in DLBCL limits its discriminatory value. Larger studies with standardized scoring and validated antibody protocols are required.

KEYWORDS: Hodgkin lymphoma; classical Hodgkin lymphoma; nodular lymphocyte-predominant Hodgkin lymphoma; diffuse large B-cell lymphoma; IMP3; IGF2BP3; immunohistochemistry.

1. INTRODUCTION

Classical Hodgkin lymphoma (cHL) is characterized by a small population of neoplastic Hodgkin and Reed-Sternberg (HRS) cells within a prominent inflammatory microenvironment. The diagnosis depends on the integration of morphology, clinical information, and immunophenotype.^{1,2}

Immunohistochemistry is central to lymphoma diagnosis because no single antigen is completely specific; interpretation requires an appropriate panel in the context of morphology and the expected phenotype of the neoplasm.³ HRS cells are derived from germinal-center B cells but commonly show marked loss of the conventional B-cell program.^{4–6} CD30 is expressed in most cHL cases, while CD15 is positive in a substantial proportion; NLPHL has a different phenotype, with the neoplastic lymphocyte-and-histiocyte cells generally retaining a B-cell phenotype.^{3,6}

IMP3 (IGF2BP3) is an oncofetal RNA-binding protein involved in RNA localization and regulation during development and is expressed in a range of malignant neoplasms.^{7,13–15} Previous studies have reported frequent IMP3 expression in Hodgkin lymphoma, including strong cytoplasmic staining in HRS cells, raising the possibility of a supplementary diagnostic role.^{7,8} However, expression in other large B-cell lymphomas may limit its specificity.⁹

The present study evaluated the immunoprofile of Hodgkin lymphoma using CD3, CD20, CD15 and CD30 and investigated IMP3 expression in cHL, NLPHL and DLBCL, with particular emphasis on its potential utility in distinguishing these entities.

MATERIALS AND METHODS

Study design and setting. This retrospective and prospective study was conducted over a two-year period at Madras Medical College/Rajiv Gandhi Government General Hospital, Chennai, following approval from the Institutional Ethical Committee of Madras Medical College. Fifty specimens comprising cHL, NLPHL and DLBCL from nodal and extranodal sites were included. Blocks with inadequate material were excluded from immunohistochemical analysis.

Case selection. All resection specimens and small biopsies with a histological diagnosis of Hodgkin lymphoma, NLPHL or DLBCL were eligible. Other non-Hodgkin lymphomas and lymphadenectomies performed for tuberculosis or other infectious etiologies were excluded. Clinical details, including age, sex, site, histopathological diagnosis and immunohistochemical findings, were obtained from pathology records.

Histopathology and immunohistochemistry. Hematoxylin and eosin-stained sections from paraffin-embedded tissue blocks were reviewed. Twenty-five of 30 reported cHL cases, two NLPHL cases and 23 DLBCL cases were selected. The Hodgkin lymphoma immunoprofile was evaluated using CD3, CD20, CD15 and CD30, while IMP3 was assessed in all study groups. Four-micrometre sections were cut from formalin-fixed paraffin-embedded blocks. Sections underwent deparaffinization, heat-induced antigen retrieval in Tris buffer, peroxide treatment, primary antibody incubation, HRP-polymer detection, DAB chromogen development and hematoxylin counterstaining. Antibody details and positive controls were recorded in the original study protocol.

Statistical analysis. Categorical variables were summarized as frequencies and percentages. The Kolmogorov-Smirnov test was used for assessment of normality; Student's t-test or Mann-Whitney test was used for continuous variables where applicable, and chi-square/Fisher exact tests were used for categorical comparisons. A P value <0.05 was considered statistically significant. The source study records document the use of MedCalc 7.0.0.2 and SPSS 15.0.

Table 1. Immunohistochemical markers and staining characteristics

Marker	Source/vendor	Species	Staining pattern	Positive control
CD3	Path InSitu	Rabbit monoclonal	Cytoplasmic	Lymph node paracortical areas
CD20	Path InSitu	Rabbit monoclonal	Cytoplasmic	Lymph node follicles
CD15	Path InSitu	Rabbit monoclonal	Membranous and Golgi	Myeloid cells and eosinophils
CD30	Path InSitu	Rabbit monoclonal	Membranous and Golgi	Granulocytes and alveolar macrophages
IMP3	Path InSitu	Rabbit monoclonal	Cytoplasmic	Lymph node follicles

RESULTS

Fifty specimens were included. The study population comprised 37 males (74%) and 13 females (26%). The age distribution was broad, with the largest groups being 31–40 and 51–60 years (11 cases each); the youngest and oldest ages at presentation were 14 and 84 years, respectively, with a reported median age of 35 years. Nodal disease accounted for 41 cases (82%), while 9 cases (18%) were extranodal.

Demographic and clinicopathological profile

Age group (years)	n	%
11–20	10	20.0
21–30	4	8.0
31–40	11	22.0
41–50	10	20.0
51–60	11	22.0
61–70	3	6.0
81–90	1	2.0

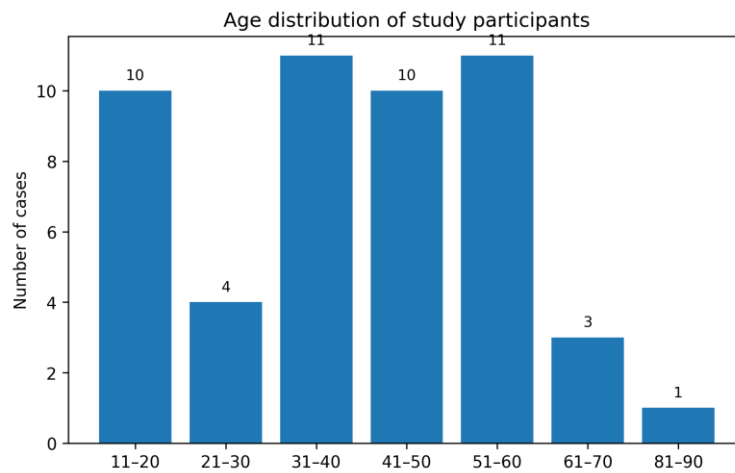


Figure 1. Age distribution of the 50 study participants.

Sex	n	%
Male	37	74.0
Female	13	26.0
Total	50	100.0

Sex distribution of study participants

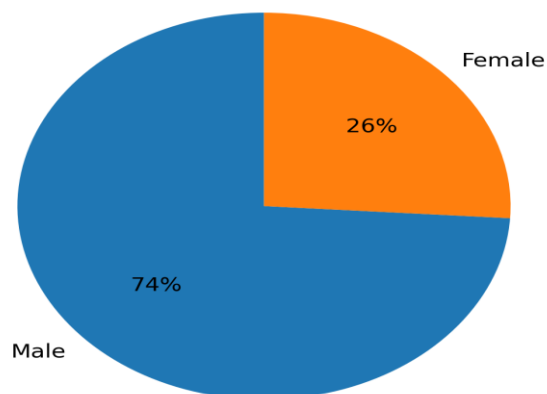


Figure 2. Sex distribution of the study participants.

Site	n	%
Nodal	41	82.0
Extranodal	9	18.0
Total	50	100.0

Site distribution of study participants

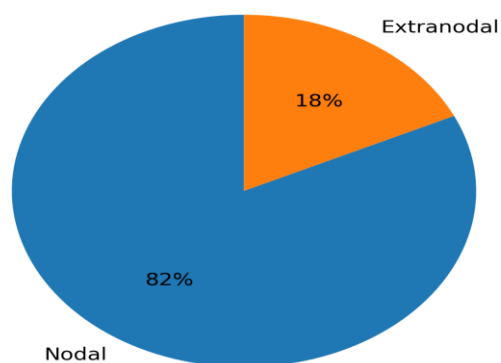


Figure 3. Distribution according to nodal and extranodal site.

Diagnosis	Male n (%)	Female n (%)	Total n (%)
cHL	21 (56.8)	4 (30.8)	25 (50.0)
DLBCL	14 (37.8)	9 (69.2)	23 (46.0)
NLPHL	2 (5.4)	0 (0)	2 (4.0)
Total	37 (100)	13 (100)	50 (100)

The reported association between diagnosis and sex was not statistically significant ($\chi^2=4.036$, df=2, P=0.131).

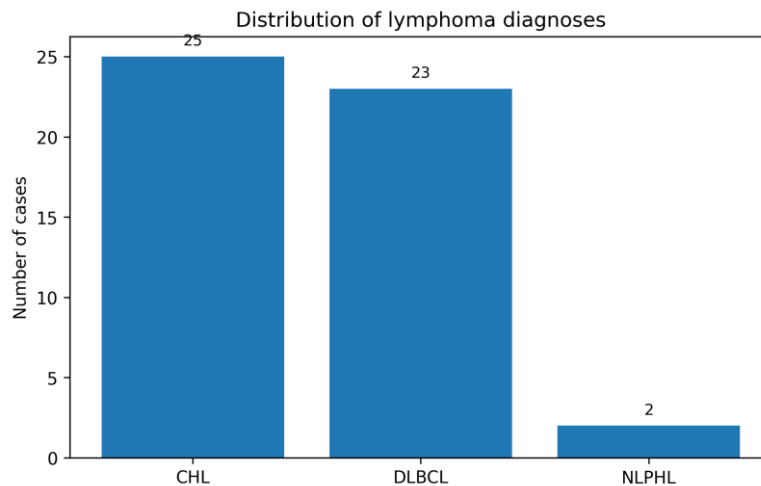


Figure 4. Distribution of cHL, DLBCL and NLPHL.

Histological subtype of classical Hodgkin lymphoma

cHL subtype	n	% of cHL
Mixed cellularity	22	88.0
Lymphocyte-rich	2	8.0
Nodular sclerosis	1	4.0
Total	25	100.0

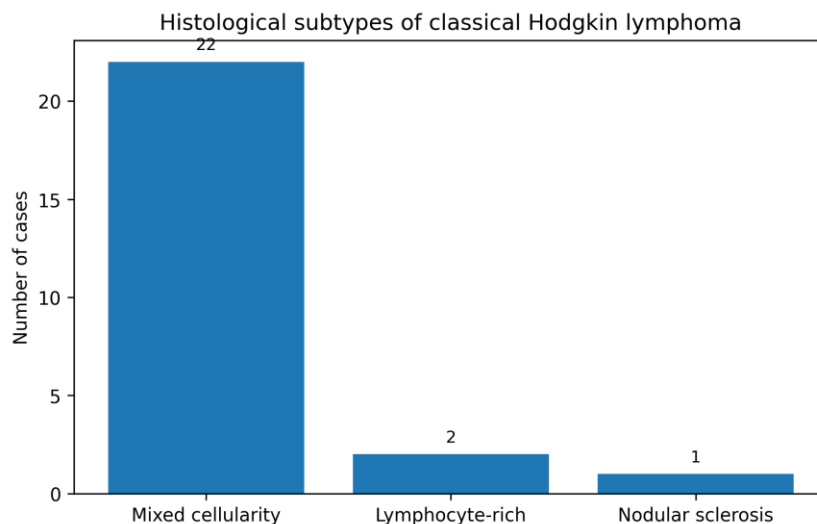


Figure 5. Histological subtypes among the 25 cHL cases.

Immunohistochemical findings

CD3 was positive in 49/50 cases (98%) and CD20 was positive in all 50 cases. In cHL, CD3 and CD20 positivity represented staining of background T and B lymphocytes rather than the neoplastic HRS cells. CD15 was positive in 22/25 cHL cases (88%), while CD30 was positive in all 25 cHL cases (100%).

Marker	Overall finding	Interpretation in cHL
CD3	49/50 (98%) positive	Background T lymphocytes
CD20	50/50 (100%) positive	Background B lymphocytes; diffuse in DLBCL and focal in NLPHL L&H cells

CD15	22/25 (88%) positive	cHL	Membranous/Golgi staining in HRS cells
CD30	25/25 (100%) positive	cHL	Membranous/Golgi staining in HRS cells

IMP3 showed cytoplasmic staining in the study. Based on the detailed subgroup counts reported in the results, positivity was present in 20/25 cHL cases (80%), 2/2 NLPHL cases (100%), and 18/23 DLBCL cases (78.3%). In cHL, staining was described as strong in HRS cells with a largely negative background; in NLPHL, staining was moderate to strong in lymphocyte-and-histiocyte cells; and in DLBCL, staining was diffuse and weak to moderate in most tumor cells. Representative morphology and IMP3 staining are shown in Figures 7 and 8. Figure 7 illustrates mixed-cellularity cHL, with Hodgkin/Reed–Sternberg cells in a polymorphous inflammatory background and corresponding cytoplasmic IMP3 expression. Figure 8 illustrates NLPHL, showing L&H cells in a lymphocyte-rich background and cytoplasmic IMP3 expression in the L&H cells.

Diagnosis	IMP3-positive	IMP3-negative	Positivity
cHL	20	5	80.0%
NLPHL	2	0	100.0%
DLBCL	18	5	78.3%
Total	40	10	80.0%

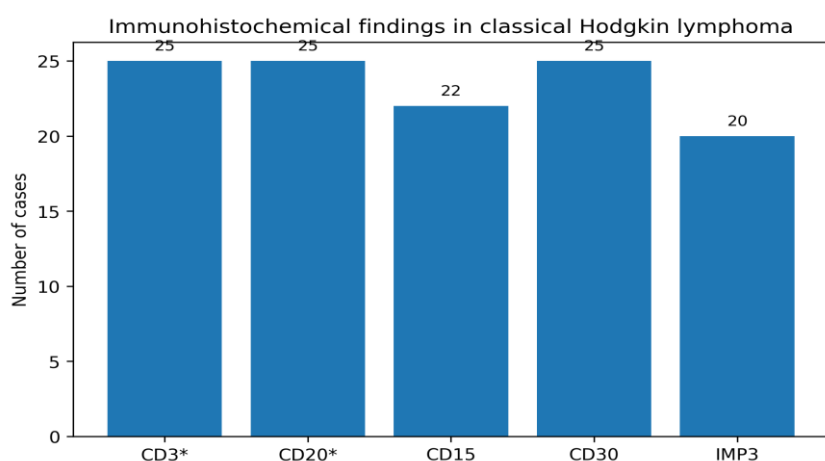


Figure 6. Reported immunohistochemical findings in cHL. *CD3 and CD20 represent background lymphocyte staining.

The source Results file also reports an overall IMP3 positivity of 39/50 (78%) and a DLBCL positivity of 91.3% in separate tables. These figures are internally inconsistent with the detailed sex-by-diagnosis IMP3 counts (cHL 20/25, DLBCL 18/23, NLPHL 2/2), which sum to 40/50 (80%). For this manuscript, the detailed subgroup counts were used for the analytical table rather than reproducing the arithmetic inconsistency.

FIGURE 7. CLASSICAL HODGKIN LYMPHOMA — MIXED CELLULARITY TYPE

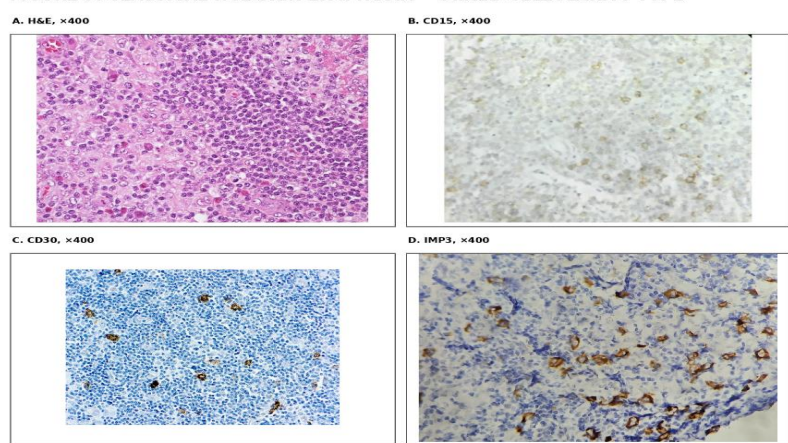


Figure 7. Classical Hodgkin lymphoma, mixed cellularity type. (A) H&E-stained section showing Hodgkin cells scattered in a polymorphous inflammatory background comprising lymphocytes, histiocytes, eosinophils and plasma cells. (B) CD15 immunohistochemistry showing immunoreactivity in Hodgkin/Reed–Sternberg cells. (C) CD30 immunohistochemistry highlighting Hodgkin/Reed–Sternberg cells. (D) IMP3 immunohistochemistry showing cytoplasmic expression in Hodgkin/Reed–Sternberg cells. Magnification: $\times 400$.

FIGURE 8. NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

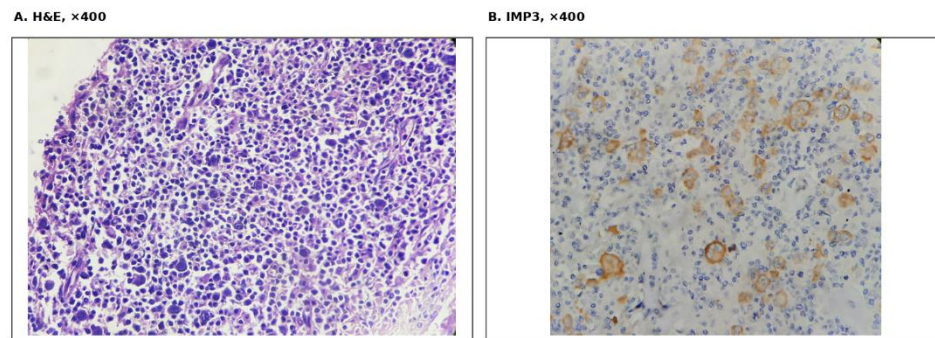


Figure 8. Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). (A) H&E-stained section showing lymphocyte-predominant (L&H, “popcorn”) cells in a lymphocyte-rich background. (B) IMP3 immunohistochemistry showing cytoplasmic expression in L&H cells. Magnification: $\times 400$.

DISCUSSION

The present study evaluated the immunoprofile of 50 lymphomas comprising 25 cHL, 23 DLBCL and 2 NLPHL and examined the expression of IMP3 across these groups. The study population showed a male predominance, with 74% males, and nodal involvement predominated. These findings are broadly compatible with the male predominance and nodal presentation described in published Indian Hodgkin lymphoma series.^{10,11} Among cHL, mixed cellularity was the predominant subtype in the present series (88%). This differs from many Western series in which nodular sclerosis predominates, but mixed cellularity has been reported frequently in Indian cohorts.^{10,11} The small number of cases and institutional case mix should be considered when interpreting this distribution.

The immunophenotypic findings were consistent with the established diagnostic approach. CD30 was positive in all cHL cases in the present series, while CD15 was positive in 88%. Patkar et al. reported CD30 positivity in almost all cHL cases and lower CD15 positivity, while Konkay et al. also demonstrated frequent CD30 and variable CD15 expression.^{10,11} Barakzai and Pervez highlighted that CD20 expression may occur in a subset of cHL and can create a diagnostic challenge.¹² These findings emphasize that individual markers should not be interpreted in isolation.

IMP3 is an oncofetal RNA-binding protein with roles in RNA localization, stability and cellular growth during development.^{13–15} Previous work has demonstrated IMP3 expression in Hodgkin lymphoma. Tang et al. reported selective IMP3 reactivity in 98.8% of Hodgkin lymphomas, with strong diffuse staining in 72.8% of cases.⁷ Masoud et al. reported IMP3 positivity in 83% of Hodgkin lymphoma cases and concluded that it may have a complementary diagnostic role, although combining IMP3 with CD30 did not materially increase sensitivity.⁸ In the present study, IMP3 was positive in 20/25 cHL cases (80%). This is lower than the proportion reported by Tang et al. but close to the frequency reported by Masoud et al.^{7,8} The difference may relate to case composition, antibody clone, technical conditions, scoring criteria and the use of archival tissue. The present study's description of strong cytoplasmic staining in HRS cells with limited background staining supports a possible supplementary role in difficult cases.

However, the major finding of this study is that IMP3 was also expressed in DLBCL. The detailed subgroup counts showed positivity in 18/23 DLBCL cases (78.3%), while both NLPHL cases were positive. This finding limits the discriminatory value of IMP3 when used alone. Zare Mehrjerdi and Ahmadi similarly reported very high IMP3 positivity across Hodgkin and large-cell lymphomas and concluded that IMP3 could not reliably differentiate Hodgkin lymphoma from large-cell lymphoma.⁹ A more recent study evaluating NLPHL and large B-cell lymphoma also found substantial IMP3 expression in both groups and suggested that staining intensity and percentage, rather than simple positivity, may be more informative.¹⁶

The results therefore support the interpretation of IMP3 as an adjunct rather than a lineage-specific or disease-specific marker. Its strong, relatively restricted cytoplasmic staining in HRS cells may be useful when conventional markers are equivocal, but the presence of IMP3 in DLBCL precludes its use as a stand-alone discriminator. Standardized assessment of staining intensity and percentage, together with a comprehensive lymphoma panel and morphologic correlation, is essential. The representative photomicrographs in Figures 7 and 8 demonstrate the cytoplasmic IMP3 staining pattern in HRS cells in cHL and L&H cells in NLPHL, respectively. The study has limitations. It was conducted at a single institution with a relatively small sample, particularly the NLPHL group, which comprised only two cases. The study used selected archival tissue blocks, and the antibody clone and scoring methodology may influence staining results. The statistical documentation contains methods not clearly linked to specific reported comparisons, and the IMP3 results contain an internal numerical discrepancy between summary tables and the detailed subgroup counts. These issues should be addressed in any expanded or prospective validation study.

CONCLUSION

IMP3 was expressed in a substantial proportion of cHL, NLP HL and DLBCL cases in this study. CD30 showed the highest positivity among the conventional markers evaluated in cHL, while CD15 was positive in 88% of cHL cases. IMP3 demonstrated a potentially useful cytoplasmic staining pattern in Hodgkin lymphoma but lacked sufficient discriminatory specificity because it was also expressed in DLBCL. IMP3 may therefore be considered a supplementary marker in diagnostically difficult cases rather than a stand-alone marker for differentiating Hodgkin lymphoma from large B-cell lymphoma. Larger studies with standardized antibody clones, staining thresholds and quantitative scoring are required to define its diagnostic and prognostic utility.

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