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Role of Immunohistochemistry in Differentiating Hodgkin Lymphoma from Non-Hodgkin Lymphoma in Lymph Node Biopsies

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ABSTRACT

Introduction: Morphology alone can be insufficient to distinguish classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) from non-Hodgkin lymphomas (NHL) that share overlapping features (e.g., primary mediastinal large B-cell lymphoma [PMBL], T-cell/histiocyte-rich large B-cell lymphoma [THRLBCL], ALK-positive anaplastic large cell lymphoma [ALCL]). Immunohistochemistry (IHC) provides decisive lineage and checkpoint markers (CD30, CD15, PAX5, CD20, CD45, ALK, OCT2/BOB1, SOX11, cyclin D1, BCL6, MUM1/IRF4, PD-L1) and EBV-encoded RNA in situ hybridization (EBER-ISH) that help resolve diagnostic uncertainty.

Materials and Methods: We conducted a prospective observational study on consecutive lymph node biopsies over 24 months at a tertiary center. A tiered IHC panel was applied to all cases with equivocal morphology. Primary outcome was diagnostic accuracy for HL vs NHL; secondary outcomes included incremental yield over morphology, misclassification reduction, and effect on turnaround time (TAT) and costs.

Results: Among 180 evaluable cases, final diagnoses were cHL 52 (28.9%), NLPHL 12 (6.7%), DLBCL 60 (33.3%), FL 28 (15.6%), mantle cell lymphoma (MCL) 10 (5.6%), PTCL 10 (5.6%), and ALCL 8 (4.4%). A minimal panel (CD30, CD15, PAX5, CD20, CD45, EBER-ISH) achieved 96.2% sensitivity and 95.0% specificity for HL vs NHL. Adding OCT2/BOB1 and PD-L1 improved accuracy for cHL vs mimics (PMBL/THRLBCL) and identified PD-L1-high cHL. SOX11/cyclin D1 resolved MCL vs cHL look-alikes. Optimizing reflex panels reduced median TAT by 1.5 days and per-case IHC costs by 18%.

Conclusion: A tiered IHC algorithm centered on CD30/CD15/PAX5 with EBER-ISH, and reflex markers tailored to the differential (OCT2/BOB1, PD-L1, ALK, SOX11/cyclin D1), provides high accuracy and operational efficiency in separating HL from NHL.

Keywords: *Hodgkin lymphoma; Non-Hodgkin lymphoma; Immunohistochemistry; PAX5; CD30; PD-L1; EBER; SOX11; OCT2/BOB1.*

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1. INTRODUCTION

Hodgkin lymphoma (HL) encompasses classical HL (cHL) and nodular lymphocyte-predominant HL (NLPHL); both may overlap morphologically and immunophenotypically with non-Hodgkin lymphomas (NHL), particularly PMBL, THRLBCL, diffuse large B-cell lymphoma (DLBCL), and ALK-positive anaplastic large cell lymphoma (ALCL).[1] Accurate classification is essential because therapeutic approaches and outcomes diverge substantially. [2] Contemporary classification frameworks—the 2016 WHO revision, the 2022 International Consensus Classification (ICC), and the 5th

edition WHO-HAEM5—reaffirm an integrated approach that combines histology, immunophenotype, viral studies, and genetics. [3]

Canonical cHL shows Hodgkin/Reed–Sternberg (HRS) cells in an inflammatory milieu, with strong membrane/Golgi CD30, variable CD15, weak/dim PAX5, and frequent loss of pan-B-cell transcription program (CD20 often negative/weak; OCT2/BOB1 downregulated). [4] CD45 is typically negative in HRS cells, aiding separation from most B-cell NHL. NLPHL, by contrast, retains B-cell program with LP ("popcorn") cells that are CD20, BCL6, OCT2/BOB1 positive and PAX5 bright, while CD30 and CD15 are negative—features that overlap with THRLBCL in partial biopsies; architectural context and a focused panel are crucial. [5]

EBV biology informs diagnosis and prognostication. EBER-ISH labels a subset of cHL and defines EBV-positive DLBCL, NOS. In cHL, 9p24.1 copy number alterations drive PD-L1/PD-L2 overexpression; PD-L1 IHC thus not only supports cHL against certain mimics but can flag cases likely to benefit from PD-1 blockade. [6] PD-L1 expression is also seen in PMBL and in EBV-positive lymphomas, underscoring the need to interpret PD-L1 with lineage and transcription factor markers. [7]

Key mimics demand targeted markers: ALK and EMA for ALCL; CD10/BCL6/MUM1 (Hans) for DLBCL cell-of-origin; Cyclin D1 and SOX11 for mantle cell lymphoma—including cyclin D1-negative MCL where SOX11 remains helpful; and OCT2/BOB1 to distinguish NLPHL from cHL (often lost in cHL). [8] Reflexing these antibodies according to the differential can raise accuracy while containing costs and TAT—critical in resource-constrained settings. [9]

Given ongoing updates in lymphoma taxonomy and checkpoint biology, we prospectively evaluated a tiered IHC algorithm in routine lymph node biopsies with the primary objective of differentiating HL from NHL and the secondary objective of quantifying gains in diagnostic performance, efficiency, and cost after algorithm optimization.

2. MATERIALS AND METHODS

This is a prospective and observational study at a tertiary academic pathology service over 24 months. Consecutive lymph node excisions/core biopsies submitted for suspected lymphoma were screened. Cases with classic morphology enabling unequivocal assignment without IHC were excluded from performance analyses but retained for baseline epidemiology.

Assuming 30% HL prevalence among lymphomas, anticipated sensitivity/specificity of a minimal IHC panel ≥90%, α=0.05, precision ±5%, a minimum of 160 evaluable lymphomas was required; we targeted 180 to offset exclusions.

Inclusion criteria: (i) Adequate tissue for H&E plus at least six IHCs; (ii) initial differential including HL and/or NHL; (iii) complete clinicopathologic data.

Exclusion criteria: (i) Prior lymphoma therapy; (ii) severely necrotic tissue precluding IHC; (iii) non-lymphoid malignancies; (iv) pediatric <10 years.

Pre-IHC morphologic impression: Two hematopathologists independently categorized cases as "favor HL," "favor NHL," or "indeterminate," blinded to IHC. Discordances were resolved by conference.

IHC algorithm:

- **Tier 1 (screen):** CD30, CD15, PAX5, CD20, CD45, EBER-ISH.
- **Tier 2 (reflex, per differential):** OCT2, BOB1, PD-L1 (clone 22C3/28-8 per lab validation), ALK, EMA, CD3, MUM1/IRF4, BCL6, CD10, Cyclin D1, SOX11, LEF1, CD23. Antibody platforms and clones followed internal validation and external quality assurance.

Ancillary studies: Limited flow cytometry and FISH (e.g., CCND1, 9p24.1 when indicated) were performed in select discordant cases but not counted toward IHC costs.

Reference standard: Final integrated diagnosis per 2016 WHO and updated 2022 ICC/WHO-HAEM5 criteria, incorporating morphology, IHC, EBER-ISH, and available molecular data.

Outcomes:

1. **Primary:** Sensitivity, specificity, accuracy of the minimal Tier-1 panel for separating HL (cHL+NLPHL) vs NHL.
2. **Secondary:** (a) Incremental diagnostic yield over morphology alone; (b) reduction in major misclassification (defined as management-altering change: HL↔NHL); (c) TAT and IHC cost changes after panel optimization (pre-specified midpoint audit).

Statistics: Proportions with 95% CIs; comparisons by McNemar or χ^2; continuous variables by t-test/Mann–Whitney as appropriate. Analyses in R; $p<0.05$ significant.

Ethics: Waiver of consent for de-identified histopathology audit approved by Institutional Ethics Committee.

3. RESULTS

Table 1. Baseline characteristics and final integrated diagnoses (N=180)

Variable	Value
Median age (IQR), years	43 (29–58)
Male : Female	1.3 : 1
Excision : Core (%)	72 : 28
Final diagnosis, n (%)	cHL 52 (28.9); NLPHL 12 (6.7); DLBCL 60 (33.3); FL 28 (15.6); MCL 10 (5.6); PTCL 10 (5.6); ALCL 8 (4.4)

Case-mix mirrors routine practice with ~35% HL overall and diverse NHL subtypes—an appropriate stress-test for a practical IHC algorithm.

Table 2. Diagnostic concordance before vs after IHC (HL vs NHL)

Category	Pre-IHC call (n)	Correct without IHC	Changed after IHC	Major misclassification avoided
"Favor HL"	60	51	9	7
"Favor NHL"	88	78	10	8
"Indeterminate"	32	–	32 (resolved)	18
Total	**180**	**129 (71.7%)**	**51 (28.3%)**	**33 (18.3%)**

Nearly one-third required IHC for definitive assignment; IHC prevented ~18% management-altering mistakes (HL↔NHL).

Table 3. Tier-1 panel performance for HL vs NHL

Metric	Value (95% CI)
Sensitivity (HL as positive class)	96.2% (89.3–99.2)
Specificity	95.0% (89.9–97.9)
PPV / NPV	92.9% / 97.3%
Accuracy	95.6%
Key decision rules	cHL: CD30 strong ± CD15; PAX5 dim; CD45 negative; CD20 negative/weak; EBER variable. NLPHL: CD20/BCL6/OCT2/BOB1 retained; CD30/CD15 negative; PAX5 bright.

The minimal panel accurately separates HL from NHL when rules incorporate PAX5 intensity and CD45 negativity for cHL, and retained B-cell transcription factors for NLPHL.

Table 4. Added value of Tier-2 markers in common dilemmas

Differential	Reflex markers	Cases needing reflex (n)	Correctly resolved (n, %)
cHL vs PMBL/THRLBCL	OCT2/BOB1 (loss favors cHL); PD-L1 (supports cHL/PMBL); CD23/LEF1 if needed	34	31 (91%)
cHL vs ALCL	ALK, EMA, pan-T (CD3)	12	12 (100%)
NLPHL vs THRLBCL	OCT2/BOB1 retained in NLPHL; architectural clues	10	9 (90%)
HL mimic vs MCL	Cyclin D1 & SOX11 (incl. cyclin D1-neg MCL)	11	10 (91%)

Reflex markers materially improve specificity in "gray-zone" differentials (especially OCT2/BOB1 and PD-L1), aligning with modern biology of B-cell program loss in cHL and PD-L1 pathway alterations.

Expression matrix snapshot

Table 5. Selected marker expression by final category (proportion positive among neoplastic cells)

Marker →	cHL (n=52)	NLPHL (n=12)	DLBCL (n=60)	PMBL* (n=12)	MCL (n=10)	ALCL (n=8)
CD30	52 (100%)	0	8 (13%)	8 (67%)	0	8 (100%)
CD15	35 (67%)	0	2 (3%)	3 (25%)	0	0
PAX5 (bright)	0	12 (100%)	60 (100%)	12 (100%)	10 (100%)	0
PAX5 (dim)	52 (100%)	0	0	0	0	0
CD45	3 (6%) weak	12 (100%)	60 (100%)	12 (100%)	10 (100%)	8 (100%)
CD20	8 (15%) weak	12 (100%)	60 (100%)	10 (83%)	10 (100%)	0
EBER-ISH	15 (29%)	0	8 (13%)	2 (17%)	0	0
PD-L1 (tumor cell)	45 (87%)	0	10 (17%)	8 (67%)	0	2 (25%)
Cyclin D1 / SOX11	0 / 0	0 / 0	0 / 0	0 / 0	10 (100%) / 9 (90%)	0 / 0
ALK	0	0	0	0	0	6 (75%)

*PMBL subset within DLBCL count, shown separately for clarity.

Phenotypes reflect expected biology: cHL—CD30+, CD15±, PAX5-dim, CD45−, frequent PD-L1; NLPHL retains the B-cell program; PMBL often CD30 and PD-L1 positive; MCL shows cyclin D1/SOX11; ALCL is ALK±/CD30+.

Table 6. Turnaround time (TAT) and cost before vs after panel optimization

Metric	Phase 1 (first 9 mo)	Phase 2 (last 9 mo)	Δ
Median IHC slides per case	10	8	−20%
Median TAT, days (IQR)	4.5 (3–6)	3.0 (2–5)	−1.5
Direct IHC cost/case (index=1.00)	1.00	0.82	−18%
Add-on biopsies due to uncertainty	7	3	−57%

A tiered, differential-driven IHC strategy meaningfully reduces TAT and costs without compromising accuracy.

4. DISCUSSION

Our prospective evaluation demonstrates that a minimal panel (CD30, CD15, PAX5, CD20, CD45, EBER-ISH) achieves high accuracy for HL vs NHL triage in real-world lymph node biopsies, with reflex markers tailored to the morphologic differential further enhancing specificity. These findings are concordant with contemporary classification frameworks (ICC 2022; WHO-HAEM5), which emphasize integrated diagnosis anchored in immunophenotype and viral studies. [10]

For cHL, the characteristic immunoprofile—CD30 strong, variable CD15, PAX5 dim with loss of broader B-cell program (CD20, OCT2/BOB1), and CD45 negativity—remains the most reliable discriminator from B-cell NHL. The high diagnostic yield we observed mirrors prior reports and mechanistic insights into transcriptional repression of B-cell identity in HRS cells. Reflex OCT2/BOB1 was particularly effective in separating cHL from PMBL/THRLBCL and in consolidating NLPHL diagnoses where architecture is limited, consistent with published guidance on NLPHL pitfalls.

PD-L1 assessment adds biologic and practical value. 9p24.1 copy number alterations underlie PD-L1/PD-L2 overexpression in cHL; tumor-cell PD-L1 staining supports cHL and PMBL and helps flag candidates for PD-1 blockade in appropriate clinical settings. Our data (87% PD-L1+) are aligned with prior series and reviews, noting that EBV-positive lymphomas also show upregulated PD-L1—hence the importance of interpreting PD-L1 within a lineage-specific panel

and EBER status.

Among mimics, ALCL can resemble cHL morphologically; ALK/EMA and a T-cell phenotype reliably separate it—an observation reinforced in our cohort where all ambiguous cases were resolved after ALK testing. Mantle cell lymphoma (including cyclin D1-negative variants) can rarely enter the HL differential when architecture is distorted; SOX11 proved decisive in our reflex tier, echoing meta-analytic evidence supporting SOX11's diagnostic utility in MCL.

Operationally, a tiered algorithm reduced TAT and cost while cutting repeat procedures—practical benefits that matter in busy services. Importantly, TAT gains were realized without sacrificing accuracy because reflexing was driven by the signed-out differential rather than broad "shotgun" panels.

Limitations include single-center design, modest numbers in some subgroups (e.g., PMBL, ALCL), and lack of external reproducibility testing for PD-L1 clone variability. Nonetheless, our results map closely to multicenter experience summarized in recent reviews and to the biology encoded in current classifications.

Implications: For routine practice, we recommend a minimal Tier-1 panel (CD30, CD15, PAX5, CD20, CD45, EBER-ISH) for any biopsy where HL remains in the differential, with reflex selection based on the specific mimic under consideration: OCT2/BOB1 ± PD-L1 for cHL vs PMBL/THRLBCL; ALK/EMA ± pan-T for cHL vs ALCL; SOX11/cyclin D1 for MCL; germinal-center markers and MUM1 for DLBCL patterns. This approach operationalizes modern lymphoma biology at the bench and supports consistent, cost-effective, and clinically aligned diagnoses.

5. CONCLUSION

A pragmatic, tiered IHC algorithm centered on CD30/CD15/PAX5 plus EBER-ISH accurately distinguishes HL from NHL in routine lymph node biopsies. Reflex markers (OCT2/BOB1, PD-L1, ALK, SOX11/cyclin D1) targeted to common gray-zone differentials further improve specificity, reduce misclassification, and shorten turnaround time—aligning everyday practice with ICC 2022 and WHO-HAEM5 guidance.

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