

The Role of CD20 in the Diagnosis, Classification, and Management of Lymphomas: A Narrative Review

Leen Naif Almohammadi

Al-Rayan National College of Medicine, Madinah, Saudi Arabia

Abstract: CD20 is a non-glycosylated transmembrane phosphoprotein encoded by the MS4A1 gene and expressed on the surface of B lymphocytes from the late pro-B-cell stage through the memory B-cell stage, but not on early progenitors or terminally differentiated plasma cells. Because of this restricted and stable expression pattern, CD20 has become the single most widely used immunohistochemical marker for confirming B-cell lineage in the diagnostic work-up of lymphoid neoplasms, and it forms the basis of the classic CD3/CD20/CD45 triage panel used to separate T-cell, B-cell, and non-lymphoid processes. This narrative review summarizes current evidence on the biology of CD20, its diagnostic role across B-cell non-Hodgkin lymphoma subtypes and classic versus nodular lymphocyte-predominant Hodgkin lymphoma, the diagnostic pitfalls posed by CD20-negative and CD20-aberrant lymphomas, the prognostic implications of reduced or lost CD20 expression, and the marker's central role as the target of rituximab and other anti-CD20 monoclonal antibodies. Across the reviewed literature, CD20 consistently emerges as the most reliable single marker for B-lineage assignment, while also functioning as a direct link between diagnosis and immunotherapy, underscoring the value of accurate, standardized CD20 assessment in routine pathology practice.

Keywords: CD20, lymphoma, immunohistochemistry, B-cell markers, non-Hodgkin lymphoma, rituximab

1. Introduction

Lymphomas comprise a biologically and clinically heterogeneous group of malignancies arising from cells of the lymphoid lineage, and accurate subclassification is essential because treatment intensity, drug selection, and prognosis differ substantially between subtypes. Morphology alone is frequently insufficient to distinguish these entities, so immunophenotyping by immunohistochemistry (IHC) or flow cytometry has become an indispensable extension of routine histopathological diagnosis. Among the panel of cluster of differentiation (CD) antigens used in this setting, CD20 occupies a special position: it is the most consistently positive pan-B-cell marker available and, together with CD3, forms the backbone of the initial lineage-assignment step in most diagnostic algorithms. This review focuses specifically on CD20, tracing its molecular biology, its established and emerging diagnostic roles, the well-recognized pitfalls of CD20-negative disease, its prognostic weight, and its unique dual identity as both a diagnostic marker and a therapeutic target.

2. Methods

This narrative review was based on a targeted, non-systematic literature search of PubMed/MEDLINE, Scopus, and Google Scholar, supplemented by hand-searching the reference lists of retrieved articles for additional relevant sources. The search covered the period from January 2000 to August 2026, in order to capture both foundational immunohistochemical and clinical studies and more recent (2022–2025) reviews and classification updates. Search terms were combined using Boolean operators and included: "CD20," "MS4A1," "B-cell lymphoma," "non-Hodgkin lymphoma," "Hodgkin lymphoma," "immunohistochemistry," "immunophenotyping," "CD20-negative," "rituximab," and "anti-CD20 monoclonal antibody."

Articles were considered for inclusion if they were published in English, reported original data or a structured review on CD20 biology, diagnostic use, prognostic significance, or therapeutic targeting in lymphoid neoplasms, and were accessible as full text. Case reports were included selectively where they illustrated a recognized diagnostic pitfall (e.g., CD20-negative or CD20-aberrant lymphomas) rather than isolated clinical curiosities. Studies were excluded if they addressed CD20 exclusively in non-neoplastic or non-lymphoid contexts (e.g., isolated autoimmune disease cohorts without a lymphoma comparison), were available only as conference abstracts without peer-reviewed full text, or were not accessible in English. Because this is a narrative rather than a systematic review, no formal risk-of-bias assessment, PRISMA flow diagram, or meta-analytic synthesis was performed; instead, the identified literature was thematically organized around the biological, diagnostic, prognostic, and therapeutic roles of CD20, prioritizing recent high-impact studies, widely cited historical work, and one regional (Sudanese) dataset relevant to the authors' practice setting.

3. Molecular Biology of CD20

CD20 is a tetra-transmembrane phosphoprotein of 297 amino acids encoded by the MS4A1 gene located on chromosome 11q12.2 (2). It is expressed on the surface of essentially all B cells from the late pro-B-cell stage through the memory B-cell stage, with the notable exception of the earliest pro-B-cell progenitors and terminally differentiated plasma cells, which downregulate B-cell surface antigens as part of normal plasmacytic differentiation (2). Functionally, CD20 participates in B-cell development, activation, and calcium-channel signalling through its association with Src-family tyrosine kinases such as Lyn, Fyn, and Lck (2). A biologically important feature is that once an antibody binds CD20, the antigen–antibody complex remains fixed on the cell membrane rather than being internalized or shed, a property

that underlies both the reliability of CD20 as an IHC target and its suitability as a therapeutic target for monoclonal antibodies (2).

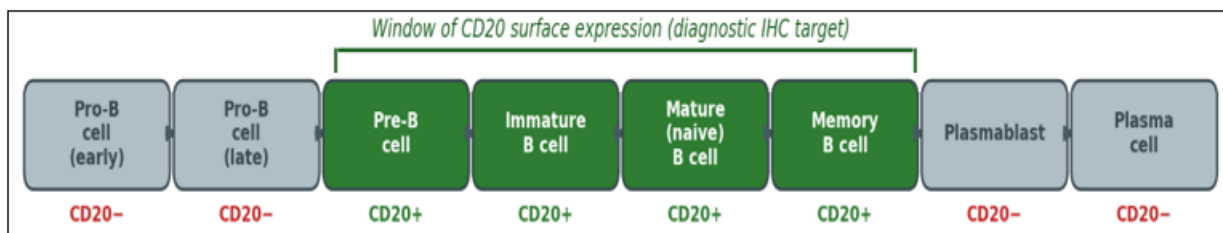


Figure 1: CD20 surface expression across the stages of B-cell maturation.

4. CD20 in the Initial Lineage Triage of Lymphoid Neoplasms

The first step in the immunophenotypic evaluation of a lymphoid proliferation is determining whether the dominant population is of B-cell, T-cell, or non-lymphoid origin. This step is conventionally achieved with a limited panel comprising CD20, CD3, and CD45 (4). Typical T-cell processes are CD3-positive and CD20-negative, whereas B-cell processes are CD3-negative and CD20-positive; both compartments are usually CD45-positive, while non-lymphoid infiltrates such as leukemia cutis may be negative for both CD3 and CD20 despite retaining CD45 positivity (4). Because CD20 is expressed across nearly the entire spectrum of mature B-cell maturation, it functions as the most dependable single indicator of B-lineage differentiation, and its combination with CD3 allows pathologists to rapidly triage a lymphoid infiltrate before deploying more extensive, subtype-specific panels (4, 8).

5. CD20 Expression Across B-Cell Non-Hodgkin Lymphoma Subtypes

CD20 is expressed, albeit with variable intensity, across the great majority of B-cell non-Hodgkin lymphomas (NHL), which collectively account for roughly 85% of all NHL cases (2). In diffuse large B-cell lymphoma (DLBCL), not otherwise specified, tumour cells are typically large, CD3-negative, and strongly CD20-positive, often together with a high Ki-67 proliferation index (4). Flow-cytometric studies have shown, however, that CD20 expression intensity is heterogeneous both between and within lymphoma subtypes: small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) characteristically shows dim CD20 staining, whereas follicular lymphoma (FL) typically shows bright expression, a difference that has downstream implications for response to CD20-directed immunotherapy (1). Machine-learning-based marker-selection studies of lymphoma classification workflows have likewise confirmed that CD20 remains one of the most informative single markers when hierarchical diagnostic algorithms are built from immunohistochemical panels, reflecting its central and reproducible role across diverse B-cell entities (6). Table 1 summarizes typical CD20 expression patterns across the major lymphoma subtypes discussed in this review.

Table 1: Typical CD20 expression across major lymphoma subtypes discussed in this review

Lymphoma subtype	CD20 status	Typical intensity	Key distinguishing markers
Classic Hodgkin lymphoma	Negative (HRS cells)	Negative / focal weak	CD30+, CD15+, PAX5 (weak)
Nodular lymphocyte-predominant HL	Positive (L&H cells)	Strong, diffuse	Oct-2+, CD30-, CD3+ rosettes
DLBCL, NOS	Positive	Strong (usually)	High Ki-67, CD3-
Follicular lymphoma	Positive	Bright	CD10+, BCL2+, BCL6+
CLL / SLL	Positive	Dim	CD5+, CD23+
Mantle cell lymphoma	Positive	Moderate-bright	CD5+, Cyclin D1+, SOX11+
Burkitt lymphoma	Positive	Strong	CD10+, Ki-67 ~100%, MYC+
Plasmablastic lymphoma	Negative / very weak	Negative	CD138+, MUM1+, CD38+
Primary effusion lymphoma	Negative	Negative	HHV8+, CD30 variable
CD20-positive T/NK-cell lymphoma (rare)	Positive (aberrant)	Variable, often dim	CD3+, pan-T markers+

6. CD20-Negative and CD20-Aberrant Lymphomas: A Diagnostic Challenge

Despite its overall reliability, a clinically important minority of B-cell lymphomas either lack CD20 expression from the outset or lose it during the course of disease. CD20-negative B-cell NHL is estimated to account for only 1–2% of B-cell lymphomas but is disproportionately associated with extranodal involvement, atypical morphology, aggressive clinical behaviour, and inferior response to standard chemoimmunotherapy (2). The principal subtypes in this

group include plasmablastic lymphoma, primary effusion lymphoma, ALK-positive large B-cell lymphoma, and HHV8-associated large B-cell lymphoma arising in multicentric Castleman disease; these entities typically show downregulation of mature B-cell antigens (CD20, CD79a, PAX5) alongside acquisition of plasma-cell-associated markers such as CD138, MUM1, and CD38, reflecting a terminally differentiated B-cell phenotype (5). In this setting, CD19, CD79a, and PAX5 become the essential alternative B-lineage markers, supplemented where necessary by Oct-2, Bob-1, and SOX11 (2). A related but distinct phenomenon is

secondary CD20 loss after rituximab-containing therapy: relapsed DLBCL and other B-cell lymphomas can show reduced or absent CD20 expression at relapse, an event linked to MS4A1 gene downregulation or, less commonly, structural gene loss, and one that carries direct implications for the choice of salvage immunotherapy (12). Rare aberrant phenotypes have also been documented in the opposite

direction, including CD20-positive T-cell and NK/T-cell lymphomas, which can create a diagnostic pitfall if CD20 positivity is interpreted in isolation without confirmatory pan-T-cell markers (7, 9). Table 2 outlines the complementary marker panel that should be considered whenever CD20 immunostaining is negative, equivocal, or discordant with the morphologic impression.

Table 2: Complementary immunohistochemical panel to resolve CD20-negative, equivocal, or discordant results

Marker	Primary lineage association	Diagnostic notes	Typical use
CD20	B cells (late pro-B – memory)	Lost in plasmablastic/plasma-cell differentiation and occasionally after rituximab	First-line B marker
CD3	T cells (pan-T)	Rare aberrant expression in some CD3+ B-cell lymphomas (e.g., EBV+ DLBCL)	First-line T marker
CD45 (LCA)	Leukocytes (broad)	May be weak/negative in some plasmablastic and anaplastic processes	Confirms hematolymphoid origin
CD19	B cells (broad)	Often retained when CD20 is lost; useful backup B marker	CD20-negative work-up
CD79a	B cells (broad, incl. plasma cells)	More retained than CD20 in terminally differentiated B-cell tumours	CD20-negative work-up
PAX5	B cells (nuclear)	Frequently retained in classic HL and CD20-negative DLBCL	CD20-negative work-up; confirms HRS B-cell origin
CD30	Activated lymphocytes / HRS cells	Positive in classic HL and ALCL; not B- or T-lineage specific	Hodgkin lymphoma work-up
CD15	Myeloid / HRS cells	Classic marker of HRS cells in classic HL	Hodgkin lymphoma work-up

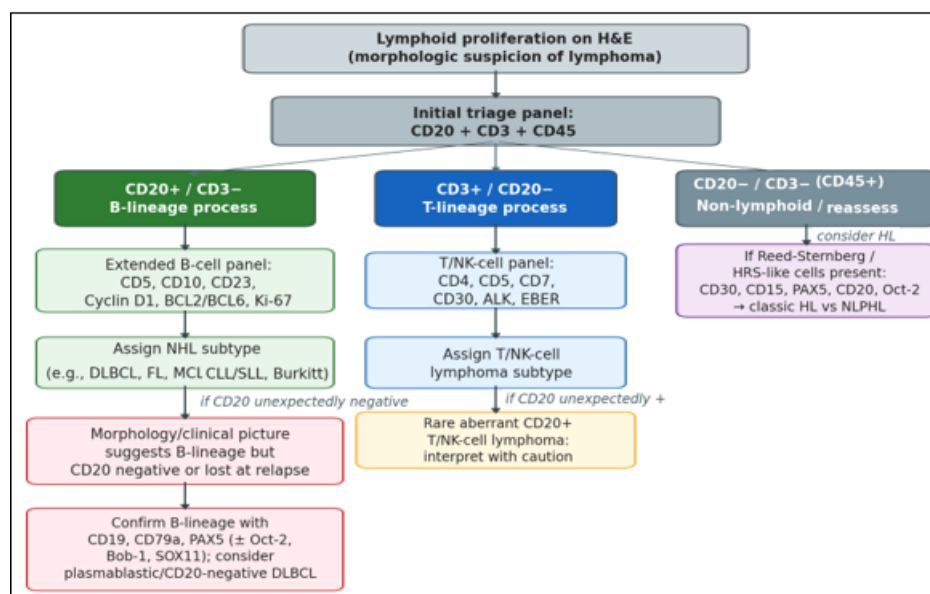


Figure 2: Proposed diagnostic algorithm for the use of CD20 in lymphoma lineage triage and subclassification

7. CD20 in Hodgkin Lymphoma

CD20 also plays a discriminating role within Hodgkin lymphoma (HL). In classic HL, the malignant Hodgkin and Reed–Sternberg (HRS) cells are typically CD20-negative or, at most, weakly and focally positive, while being positive for CD30 and CD15; PAX5 usually shows weak nuclear positivity, reflecting the B-cell origin of HRS cells despite their loss of most B-cell surface antigens (4). By contrast, nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is immunophenotypically distinct: its lymphocyte- and histiocyte (L&H, or "popcorn") variant cells are strongly and diffusely CD20-positive and Oct-2-positive, while typically negative for CD30, and are surrounded by a rosette-like rim of CD3-positive reactive T cells (4). This CD20 positivity pattern is one of the key features that separates NLPHL from classic HL, with direct implications for treatment strategy, since some NLPHL protocols incorporate

rituximab given the CD20 positivity of the neoplastic cells (4).

8. Prognostic Significance of CD20 Expression

Beyond its diagnostic value, the level of CD20 expression carries independent prognostic weight in DLBCL. In a landmark flow-cytometric and molecular study of 272 diagnostic DLBCL samples, roughly 16% of cases showed reduced CD20 expression, and the subset with concurrently bright CD19 expression experienced markedly inferior survival when treated with CHOP or rituximab-CHOP, independent of the International Prognostic Index (1). Subsequent semi-quantitative immunohistochemical studies have corroborated that lower CD20 expression intensity identifies a subgroup of DLBCL patients with poorer outcomes on R-CHOP, suggesting that CD20 assessment could eventually be incorporated into risk stratification rather

than used solely as a binary positive/negative diagnostic criterion (11). Loss of CD20 at relapse after rituximab-containing therapy has similarly been associated with resistance to further CD20-targeted treatment, reinforcing the recommendation that relapsed lymphomas be rebiopsied to reassess CD20 status before selecting salvage therapy (12).

9. CD20 as a Therapeutic Target

The stability of the CD20 molecule on the B-cell membrane, together with its broad expression across mature B-cell neoplasms, made it an ideal target for monoclonal antibody therapy (2). Rituximab, a chimeric anti-CD20 monoclonal antibody, depletes malignant and normal B cells through complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and direct induction of apoptosis, and it has become a cornerstone of treatment for CD20-positive B-cell lymphomas, most commonly in combination regimens such as R-CHOP (2, 3). In mantle cell lymphoma, single-agent rituximab produces objective responses in a substantial proportion of both untreated and previously treated patients, although complete responses remain uncommon with single-agent use, and the combination of rituximab with cytotoxic chemotherapy backbones such as R-CHOP or bendamustine-rituximab is generally required for durable disease control (3). The diagnostic and therapeutic roles of CD20 are therefore tightly linked: accurate assessment of CD20 status at diagnosis, and reassessment at relapse, directly informs whether CD20-directed immunotherapy remains a rational treatment option, or whether alternative targets (e.g., CD19- or CD79b-directed agents) should be considered instead (12).

10. Regional Data and Clinical Relevance

Population-based immunohistochemical data from the region reinforce the broader literature. A Sudanese cross-sectional study of 82 patients with palpable lymphadenopathy that evaluated CD3, CD5, CD10, CD19, and CD20 by immunohistochemistry found that CD20 had the strongest and most statistically significant association with both the Hodgkin/non-Hodgkin distinction and with individual lymphoma subtypes among the five markers tested, and the authors concluded that CD20 was the most consistent and useful marker for differentiating lymphoma subtypes in their cohort (10). Such regional data are valuable because access to extended immunohistochemical panels, flow cytometry, and molecular testing can be limited in resource-constrained laboratory settings, making a single highly reliable marker such as CD20 disproportionately important for practical diagnostic decision-making in these environments (10).

11. Discussion: Synthesis, Research Gaps, and Future Directions

Taken together, the reviewed literature converges on a consistent picture: CD20 is the most dependable single immunohistochemical marker for B-cell lineage assignment, but its diagnostic reliability is not absolute, and its expression is more heterogeneous and biologically dynamic than a simple positive/negative readout suggests. Several apparent discrepancies across the literature are worth reconciling. First, studies quantifying CD20 by flow cytometry (mean

fluorescence intensity or MESF units) versus routine chromogenic immunohistochemistry are not directly comparable, since flow cytometry can detect graded reductions in expression that a qualitative IHC read may still classify as simply "positive"; this likely explains why the prognostic impact of "reduced" CD20 expression is better established in flow-cytometry-based cohorts (1, 11) than in routine diagnostic IHC series. Second, the reported prevalence of CD20-negative disease varies between series (approximately 1–3% in most DLBCL cohorts), which may partly reflect differences in antibody clones, fixation protocols, and the specific threshold used to call a case "negative" versus "weakly positive," underscoring the value of standardized cutoffs such as the 10% (1+) threshold historically used in regional immunohistochemistry studies (10).

A second point of tension concerns the interpretation of CD20 in aberrant contexts. Case-level reports of CD20-positive T-cell and NK/T-cell lymphomas (7, 9) and of CD3-positive B-cell lymphomas describe genuinely rare but recurring phenomena rather than laboratory artifact; because these reports are individually small, their true population frequency and clinical significance remain uncertain, and pooled or registry-level data would be needed to determine whether such aberrant expression carries independent prognostic weight or simply reflects a diagnostic curiosity. Similarly, while reduced or lost CD20 expression after rituximab-containing therapy is well documented (12), the relative contribution of transcriptional downregulation versus structural MS4A1 gene alterations, and the extent to which this phenomenon is reversible, remain incompletely resolved across the reviewed studies.

Several research gaps follow from this synthesis. There is a need for larger, prospective, multi-institutional cohorts that directly compare quantitative (flow cytometry or digital image analysis) versus qualitative (chromogenic IHC) CD20 assessment, ideally using a harmonized scoring system, to determine whether a validated intensity cutoff should be incorporated into routine diagnostic and prognostic reporting rather than used only in specialized research settings. Regional data from the Middle East and North Africa, including Saudi Arabia, remain comparatively sparse relative to data from North America, Europe, and East Asia; the Sudanese cohort discussed above (10) is a useful regional reference point, but larger multicenter studies from Gulf and broader MENA populations would help determine whether lymphoma subtype distribution and marker performance in these populations parallel those reported elsewhere. Finally, as CD20-directed and CD20-independent immunotherapies (e.g., CD19- or CD79b-targeted agents, bispecific T-cell engagers) continue to diversify, future work should clarify how post-treatment CD20 loss should be systematically monitored and how quickly biopsy-confirmed CD20 status should be reassessed at relapse to guide the choice of salvage immunotherapy.

Looking forward, digital pathology and machine-learning-assisted immunohistochemistry scoring, as illustrated by recent hierarchical classification frameworks (6), may help standardize CD20 interpretation across institutions and reduce inter-observer variability in borderline or

heterogeneous cases. Likewise, wider adoption of quantitative flow-cytometric or image-analysis-based CD20 measurement, rather than binary positive/negative reporting, could allow CD20 intensity to be incorporated more formally into prognostic indices alongside established clinical scores. These directions, together with expanded regional data collection, represent the most promising avenues for translating the diagnostic strengths of CD20 described in this review into more precise, individualized patient management.

12. Conclusion

CD20 remains the single most useful immunohistochemical marker for establishing B-cell lineage in the diagnostic work-up of lymphoid neoplasms, owing to its broad, stable expression across most stages of B-cell maturation and its retention on the cell membrane after antibody binding. Its diagnostic value extends from the initial CD3/CD20/CD45 triage step, through subtype-specific classification of B-cell non-Hodgkin lymphomas, to the distinction between classic and nodular lymphocyte-predominant Hodgkin lymphoma. At the same time, CD20-negative and CD20-aberrant lymphomas represent a recognized diagnostic blind spot that requires a broader marker panel (CD19, CD79a, PAX5) to avoid misclassification, and reduced or lost CD20 expression carries independent prognostic and therapeutic significance, particularly after rituximab-based treatment. Given its unique position at the intersection of diagnosis and CD20-directed immunotherapy, standardized, reproducible assessment of CD20 expression should remain a routine and closely scrutinized component of lymphoma work-up.

Conflicts of Interest

The authors declare no conflicts of interest.

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