**Flow cytometric analysis of Immunophenotypic Characteristics of B-cell lymphoproliferative Disorders: a detailed review**


## Abstract

Mature B-cell neoplasms are diverse disorders with distinct clinical presentations, pathology, and outcomes originating from B-cell lineage, exhibiting clonality, and processing morphological, immunophenotypic and genetic features.

This review evaluates the worldwide differences and the diagnostic challenges as well as the prognostic significance of different forms of B-NHL. Focusing on the significance of the immunophenotypic markers and the modern diagnostic technologies in the personalized treatment of this disease.

The literature search was conducted using key terms like “B cell lymphoproliferative disorders”,” BCLPDs”, “B-NHL” and “Flow cytometric immunophenotyping of B-CLPDs” across multiple databases and google scholar.

Cutting edge diagnostic methods like flow cytometry are mandatory for an accurate subtype designation and treatment plan. For B-NHL, immunophenotypic evaluation is done through measures like CD5 and CD10 expression, but quite simply this is a histologic category that is diagnostically complicated. Aberrant marker expression and bi-clonality highlight the prognostic significance of immunophenotypic characteristics in B-NHL.

Through a comprehensive analysis of the studies worldwide, the review provides insights for clinicians and researches on guiding diagnostic and therapeutic decisions and advanced understanding of B-NHL pathogenesis.



**Keywords: Flow cytometry, Immunophenotyping, Lymphoma, BCLPD, B-NHL**


## Introduction

Mature B Cell neoplasms consists of a diverse group of disorders with distinct clinical presentations, pathology, and outcomes. They originate from B cell lineage, exhibit clonality, and possess morphological, immunophenotypic, and genetic features of matureB-cells[1].Flow cytometric assessment categorizes mature B-cell lymphomas into four main groupsbased onCD5

and CD10 expression: They are: CD5+/CD10-, CD5-/CD10+, CD5+/CD10+, and CD5-/CD10-.The objective of the review is to explore the global variations, diagnostic challenges, and prognostic implications of B- Non-Hodgkin's lymphoma (B-NHL) subtypes, emphasizing the role of immunophenotypic markers and advanced diagnostic techniques in guiding personalized treatment strategies and improving patient outcomes worldwide.

## Methods

The literature search was conducted across multiple databases, namely "Pubmed," "HINARI" database, and the well-established academic search engine "Google Scholar." To effectively narrow down the search, specific key terms were employed, including "B cell lymphoproliferative disorders," "B-CLPD," "B-NHL," and "Flow cytometric immunophenotyping of B-CLPD." These terms were strategically combined using "or" and "and" operators to enhance the precision of the search results.

Inclusion criteria were set to consider only published clinical studies, review papers, and related articles relevant to BCLPDs. Additionally, the search was confined to English-language publications to ensure consistency and accessibility. Each combination of keywords was systematically inputted into the databases to retrieve pertinent literature for the study.

## Results

### 1. CD5+/CD10-group

This group includes Chronic Lymphocytic Leukaemia/Small Lymphocytic Leukaemia (CLL/SLL), Mantle Cell Lymphoma (MCL), some B-cell prolymphocytic leukaemia (B-PLL), and small percentages ofMarginal Zone Lymphoma(MZL), Diffuse Large B-cell Lymphoma (DLBCL), and possibly Lymphoplasmacytic Lymphoma (LPL)(2).

CLL presents with small, mature lymphocytes with dense nuclei lacking discernible nucleoli and partially aggregated chromatin(1). Diagnosis requires confirming monoclonal B cell count >5 x $10^9$/L and demonstrating light chain restriction using flow cytometry(3).

These cells express CD5, CD23, CD19, CD20, CD43, and CD200. They are negative for CD10, and CD79b may be weak. FMC7 is typically negative or weak. Surface CD20, surface Ig, and CD79b levels are low compared to normal B cells(4). Morphology is essential to distinguish CLL/SLL from DLBCL and B-PLL. Fluorescence in situ hybridization (FISH)can offer prognostic insight(5).

Matutes et al. proposed a scoring system (smIg density, CD5, CD79b, CD23, and FMC7) to differentiate CLL from other B-Cell Lymphoproliferative Disorders (B-CLPDs). A score >3/5 indicates "typical" CLL, while in other diseases, it's 0-2(6).In terms of immunophenotype, atypical CLL can be distinguished from classic CLL by the absence of one or fewer surface antigens,typically CD5 and CD23. Additionally, the patient does not fulfill the diagnostic criteria for any other B-cell lymphoid malignancy(7).

Delgado J et al. discovered a link between CD20 and FMC7 expression in patients with B-cell NHL (P.001) but not in CLL. Further, this study compared the current scoring system with a scoring system in which FMC7 was replaced by CD20, and the accuracy of differentiating CLL from other non-CLL disorders fell from 94.4% to 81.5%%[8].Alsor some CD5+, and CD10- neoplasms are challenging to classify using flow cytometry [9,10].

CD200 overexpression distinguishes CLL and HCL from MCL, aiding in prognosis[11]. Sorigue et al. found CLL and CLL-like Monoclonal B cell lymphocytosis (MBL) cases were CD200 positive, but MBL showed lower CD200 levels than CLL[12].

CD38 is a prognostic marker in CLL, with >30% CD38-positive cells indicating shorter survival[13]. Semanaj et al. (2014) noted 70% of CD5+CD19+CD23+ patients with CD38+ belonged to CLL stages III-IV, contrasting with 75% without CD38 belonging to stages 0-I-II[14].

CLL and SLL are related diseases. "SLL" denotes cases with 5 x $10^9$/L circulating lymphocytes and documented nodal, splenic, or extramedullary involvement[15,16]. CLL/SLL comprises 7% of NHL[17].

MCL is a B-cell neoplasm with small-to-medium-sized lymphoid cells with irregular nuclear contours(15). These cells express intense smIg lambda over smIg kappa, CD19, CD5, CD20, CD79b, FMC7, BCL2, and CD43, and are negative/weak for CD23 and negative for CD10, CD200[18].

MCL has a more variable phenotype than CLL/SLL, overlapping with other CD5+ mature B-cell neoplasms. Additional tests like immunohistochemistry for cyclin-D1 protein and cytogenetics for t(11;14) are recommended for diagnosis[19–21]. Distinguishing features include lack of

CD23 expression and presence of plasmacytic differentiation with bright CD38 expression in MZL[2].

B-cell prolymphocytic leukaemia (B-PLL) is a neoplasm of B-cell prolymphocytes affecting peripheral blood, bone marrow, and spleen. Prolymphocytes must account for ≥55% of lymphoid cells in peripheral blood[15]. The key factor distinguishing B-PLL from CLL lies in the proportion of prolymphocytes present in the bloodstream. The FAB (French American British) classification system, widely acknowledged in the medical community, delineates this differentiation as follows: (i) CLL is identified when prolymphocytes constitute equal to or less than 10% of lymphoid cells; (ii) Conversely, B-PLL is diagnosed when prolymphocytes surpass 55% of lymphoid cells[22].B-PLL express bright smIg lambda/kappa, CD19, CD5 (20-30%cases), CD20, CD79b, FMC7, and CD38 (50% cases), and are dimly positive/negative for CD200 and negative for CD10.

Approximately 5% of LPLs are CD5+ (23,24). CD23 is usually negative, and if positive, it's weak. LPL doesn't have CLL/SLL phenotype. Distinguishing LPL from other CD5+ B-cell lymphomas requires morphologic and clinical features.

Some DLBCLs are CD5+ but larger than small CD5+ B-cell lymphomas. Exclusion of the blastoid variant of MCL is recommended through testing for cyclin-D1 overexpression and t(11;14) (q13; q32) gene rearrangement[2].

**2. CD5-/CD10+ group**

DLBCL and Follicular Lymphoma (FL) are common CD10+ and CD5- mature B-cell neoplasms. Swerdlow et al. (2016) suggest CD10+ DLBCL might resemble BL and FL due to their large cell count.

DLBCL and Burkitt Lymphoma (BL) feature large lymphoid cells with moderate to abundant cytoplasm, round nuclei with one to many nucleoli, and fine chromatin. They express pan B cell markers. BL shows bright CD38, positive CD43, and negative CD200[15].

In FL, >40% of lymphocytes display cleaving, scanty cytoplasm, high N/C ratio, and condensed chromatin. They're usually CD5- and CD10+, with positive CD19, FMC7, CD79b, and bright CD20. Most cases show Kappa or Lambda restriction, with variable CD23 expression [15].

**3. CD5+/CD10+ group**

Mature B-cell lymphoid neoplasms co-expressing CD5 and CD10 are rare[25,26]. This group includes DLBCL, FL, MCL, CLL/SLL, BL[27], and rare other B-cell malignancies.

A study found 42 cases with simultaneous expression of CD5 and CD10: DLBCL was reported in 14 (33%), FL in 10 (24%), MCL in 9 (21%), CLL in 4 (10%), acute precursor B-cell lymphoblastic leukemia/lymphoma in 2 (5%), and other low-grade B-cell lymphomas in 3 (7%) cases.All FLs had the expected morphology, were bcl-2+ and bcl-6+, but were CD43– [27].

**4. CD5-CD10-group**

Mature B-cell neoplasms without CD5 and CD10 include DLBCL, MZL, Hairy Cell Leukaemia (HCL), LPL, CD10-FL, and CD5-MCL. HCL consists of small mature lymphoid cells with oval nuclei and abundant cytoplasm with hairy projections in blood and marrow. Most lack CD5 and CD10 but express bright surface immunoglobulin, CD11c, CD103, CD25, CD123, and CD200. The BRAF V600E mutation is a molecular marker of classic HCL, not found in the hairy cell variant (HCL-v) [17,28].

Splenic Marginal Zone Lymphoma (SMZL) comprises small to medium-sized lymphoid cells with regular nuclei, clumped chromatin, and small nucleoli in 20–40% of cells, often with localized cytoplasmic villi. They are typically CD5 and CD10 negative but express CD19, CD20, FMC7, CD79b, and bright smIg lambda/kappa. Some cases lack CD23, CD43, CD200, CD103, CD123, and CD25 [15,29].

LPL consists of small mature B lymphocytes, plasmacytoid lymphocytes, and plasma cells, expressing CD19, CD20, FMC7, CD79b, and CD25. CD23 is positive in 30–60% of cases. Plasma cells express CD19 and CD138[1].

CD10- FL and CD5- MCL are recognized and fit into the CD5- CD10- group. Identifying these variants typically involves combining morphological and immunophenotypic methods.

**5. published data of different types of BCLPDs in different study setups**

A study in Sri Lanka revealed, common WHO lymphoma sub types were DLBCL and FLby immunohistochemistry using biopsy specimens of patients[30].The frequencies of lymphoma subtypes in the Sri Lankan sample (Table 1) were in accordance with the globally observed

variations and similar to those observed in other South Asian countries (Table 2).In this study,all lymphomas were sub classified according to WHO 2007 revised classification of haematopoietic and lymphoid neoplasms[1].

*Table 1: B-CLPDs WHO subtypes in Sri Lanka*

| **Lymphoma sub type** | **Frequency** | **Mean age in years (SD)** | **Number of Males (M)** | **Number of Females (F)** |
|---|---|---|---|---|
| Diffuse large B cell lymphoma | 87 (58.8%) | 53.68 (16.9) | 52 (59.8%) | 35 (40.2%) |
| SLL/CLL | 11(7.4%) | 49.08 (17.15) | 6 (54.5%) | 5 (55.5%) |
| Follicular lymphoma | 26 (17.6%) | 54.7 (13.09) | 11 (42.3%) | 15 (57.7%) |
| Mantle cell lymphoma | 10 (6.8%) | 52.56 (11.94) | 7 (70%) | 3 (30%) |
| Marginal zone lymphoma | 6 (4%) | 61.83 (13.03) | 3 (50%) | 3 (50%) |
| Burkitt lymphoma | 1 (0.7%) | | 1(100%) | - |
| Unclassifiable | 7 (4.7%) | 55.4 (17.11) | 6 (85%) | 1 (15%) |

*Table2: Comparison of B-CLPDs WHO subtypes*

| **Lymphoma WHO sub type** | **Sri Lanka** Waravita T S e al.[31] | **India** Naresh K N et al. [32] | **Pakistan** Mushtaq S et al. [33] | **USA** Morton LM et al. [17] | **Japan** Aoki R et al. [34] | **S. Korea** Yoon SO et al [35] | **China** Yang QP et al, [36] |
|---|---|---|---|---|---|---|---|
| **Sample size** | **148** | **2027** | **151** | **57975** | **1521** | **3482** | **3862** |
| Diffuse large B cell lymphoma | 87 (58.7%) | 937 (46.2%) | 119 (78.8%) | 24246 (41.8%) | 746 (49%) | 1650 (47.2%) | 2288 (59.2%) |
| SLL/CLL | 11 (7.4%) | 155 (7.6%) | 4 (2.6%) | 16984(29.2%) | 32 (21%) | 97 (2.7%) | 256 (6.6%) |
| Follicular lymphoma | 26 (17.5%) | 350 (17.2%) | 9 (5.9%) | 10705 (18.4%) | 413 (27.1%) | 91 (2.6%) | 327 (8.4%) |
| Mantle cell lymphoma | 10 (6.75%) | 95 (4.6%) | 4(2.6%) | 1691 (2.9%) | 61 (4.0%) | 98 (2.8%) | 175 (4.5%) |
| Marginal zone lymphoma | 6 (4%) | 220 (10.8%) | 4(2.6%) | 3247 (5.6%) | 127 (8.3%) | 720 (20.6%) | 355 (9.1%) |
| Nodal | 2 (1.35%) | 52(2.05%) | 0 | 0 | 32 (1.5%) | 54 (1.5%) | 5 (0.1%) |
| Extra nodal | 4 (2.7%) | 168 (8.2%) | 4(2.6%) | 0 | 95 (6.2%) | 661 (18.9%) | 350 (9.0%) |
| Burkitt lymphoma | 1 (0.6% | 50 (2.4%) | 7 (4.6%) | 1102 (1.9%) | 15 (0.98%) | 111 (3.18%) | 106 (2.7%) |

Another study done in China revealed, CLL was the most common B-CLPD, but the overall incidence was lower compared to the West[36]. Proportions of CLL (55.9%), FL (2.6%), and HCL (0.2%) were lower, while LPL (5.4%) was higher in China. CD23 expression in MCL (31.7%) was higher compared to Western cohorts [36,37]. CD200 showed better diagnostic performance (94.6% accuracy) than CD23 in distinguishing CLL from MCL. Some cases remained unclassified as CD5+ BCPLDs (7.7%) and CD5 -BCPLDs (15.8%)[36].HCL was rare in this cohort, with only 1 patient among 653 BCLPD cases[36]. Similarly, Yi et al. found CD5+ BCLPD unclassified (10%) in their study. CD5+ BCLPD unclassified and MCL shared similar immunophenotypes, with CD11c more frequent in CD5+ BCLPD unclassified. However, CD200, usually absent in MCL, was expressed in most CD5+ BCLPD unclassified cases. A USA study proposed non-BM tissue biopsy for definitive diagnosis in CD5+B-CLPD [38].

Miao et al. found a lower proportion of CLL (compared to Gujral et al.) at 68.3%, while LPL was higher at 5.4%(37). Gujral et al. studied mature B-cell NHL at Tata Memorial Hospital, reporting CLL (68.5%), FL (8.5%), MCL (5.5%), SMZL (5%), and HCL (5%). CD5+/CD23+ had 93% PPV for CLL diagnosis, while CD5+/CD23- had 99% NPV for MCL. Some cases (4%) were labeled B NHL Unclassifiable, not fitting WHO categories[39].

In an Albanian study, 2 out of 84 individuals (2.4%) had abnormal T-cells, while 82 (97.6%) showed a pathological B-cell line. 58 (69.1%) had typical CLL markers (CD19+CD5+CD23+), 5 (5.9%) had a non-typical CLL profile (CD19+CD5+CD23-), and 19 (22.6%) had abnormal CD19+CD5- B cells. CD38 positivity correlated significantly with the CLL clinical stage ($p = 0.04$)[14].

In India, Dwivedi et al. (2019) stressed the importance of immunophenotyping, with a 79.17% concordance rate between morphological and immunophenotypic diagnoses. Flow cytometry confirms diagnoses and classifies unclassifiable CLPD cases by morphology[40].

A Brazilian study recommends shifting from traditional four-color to more color (>8) assays for accurate diagnoses, using fewer tubes and markers, and analyzing small cell samples effectively. FL and DLBCL may show reduced CD19 expression, affecting B-cell identification. Neoplastic

B-cells are first gated on CD19+/FSClow/SSClow cells, followed by evaluating other antigens[41].

Another study done in Brazil to study the CD5 expression in leukemic cells from 42 patients with chronic B-cell malignancies by flow cytometry revealed CD5 expression was present in all B-CLL and MCL, and low expression of CD5 was observed in one patient with B-PLL and was negative in all cases of HCL. They demonstrated that CD5 expression can help distinguish between B-CLL from HCL and B-PLL, but it is similarly expressed in MCL [42].

In a study done in Canada, cases of B-cell lymphoproliferation with two B-cell populations were reported. Forty-one of 790 samples (5.1%), from 22 male and 19 female patients, showed two monotypic B-cell populations that differed in immunophenotype. From that, 13 samples had two co-existing aberrant B-cell populations that showed restriction of different sIg, one kappa-positive and one lambda-positive. In 10 of these 13 cases, one population had a B-CLL-related phenotype and two cases also showed a difference in CD38 expression between two populations. In four of the 13 cases[43], an abnormal plasma cell population was accompanied by B-CLPD[44]. It was revealed that patients with positive expression of CD38 had significantly shorter overall survival (mean, 81 months) than patients without CD38 expression (mean, 179 months) ($P = 0.015$) in a study on the Chinese population[44]. A cohort of 413 subsequent patients with de novo leukemic B-cell chronic lymphoproliferative disorders diagnosed in Croatian institutions during 30 months revealed bi-clonality in 16 (3.9%) patients with composite lymphoma. The vast majority (88%) of the cases had one of the clones phenotypically corresponding to CLL or SLL. Bi-clonal cases had an overall B-cell membrane lambda-to-kappa ratio within the normal range, making recognition of malignancy somewhat challenging. For composite lymphoma, the analysis strategy was based on the detection of aberrant B-cell phenotypes, with subsequent confirmation of the monoclonal nature of neoplastic clones about light chain restriction analysis[45].

In a Canadian study[46], they developed a composite diagnostic predictor called the "combined ratio score" (CRS) to distinguish between MCL and SLL. CRS integrated three discriminative ratios (CD20/CD23, FMC7/CD23, CD20/CD11c) using machine-based algorithms. While conventional criteria correctly identified 64% of MCL and 69% of SLL cases, the novel approach accurately assigned 100% of MCL and 97% of SLL cases. The CD20/CD23 ratio was

the most discriminating feature, with 100% sensitivity, specificity, and accuracy. Interestingly, including FMC7 expression reduced diagnostic accuracy (FMC7/CD23-96%, FMC7 alone 80%).

In a Danish study, 503 CD5+ and 37 CD5- B-cell CLL cases were identified. CD5- patients had a slightly shorter survival than CD5+ patients. Most CD5- cases were FMC7+ and CD23+, with strong IgM fluorescence and splenomegaly. However, age, clinical stage, and lymphocyte count didn't differ between CD5+ and CD5- cases[47].

In an Egyptian study on CLL and MCL, 80% of MCL cases showed small cell morphology, with the remaining 20% showing the blastoid variant. Immunophenotypic differences weren't detected between the morphologic variants of MCL. CLL cases were positive for CD19 (100%), CD20 (90%), and CD22 (47%). CD79b negativity was observed in 63% of cases, with low expression in positive cases. CD200 was expressed in 100% of CLL cases with moderate intensity compared to only 10% of MCL cases with low intensity[18].

In 20% of confirmed MCL cases, CD5 expression is absent[48]. In Iraq, CD200 was expressed in 94.9% of 39 CLL patients, while all MCL cases were negative. A significant association between CD200 expression and B-CLL was observed ($p = 0.001$), with only 5.1% of advanced disease cases showing negative expression[49].

The reported frequency of CLL from studies in Hong Kong was variable, ranging from 6.5% to 12%. In a study by Ho et al[50]. on malignant lymphomas, only 12 CLL cases out of 267 cases were observed over 8 years [50]. For instance, in Kwong’s study, up to 20% of CLL cases were retrospective series of CLL cases from Chinese people in Hong Kong, which were CD5-negative[51].

Jain M et al. reported a case of a 50-year-old male with CLL with aberrant CD8 expression. The CD19-gated B cell showed co-expression of CD8 and CD19. Other T cell markers (CD4, CD2, CD3, and CD7) were not expressed by B cells[52]. Inaba et al[53] and Kampalath et al[54]have similarly reported CD2 and CD7 expression in CLL or B-NHL to be associated with poor prognosis and shorter overall survival.

In India, a study found CD3 positive in 10% of CLL cases and CD10 positive in 5%. CD38 was positive in 40% of CLL cases. All atypical CLLs showed CD22 positivity, with two cases

negative for CD5. CD20 was expressed in 85% of CLL cases, while CD79b and FMC7 were expressed in 57.5% and 12.5% of cases, respectively[55]. In Iran, Maljaei et al. showed that lower CD45 density was associated highly with typical CLL ($P < 0.001$) and non-CLL cases had brighter CD45 expression than atypical CLL ($P = 0.014$)[56].

Aberrant expression of immunophenotypic markers is seen in patients with blood cancers. An interesting biological feature is that T-cell antigen expression in B-cell non-Hodgkin's lymphoma (B-NHL) on neoplastic B cells. CD5 is normally expressed by B-CLL and MCL and is considered a diagnostic feature of such diseases[57]. A study revealed that among chronic leukemia patients, aberrant expressions were seen in cases of B-CLL (10/28) with CD11c, CD3, and CD10 as the aberrantly expressed markers[58].

A review article that was written by Zahid Kaleem reported different types of immunophenotypes of B-cell non-Hodgkin's Lymphoma concerning CD5, CD10, and CD23. The following table (table 1) shows these findings[59].

*Table 3: The immunophenotypes of B-NHL, (Kaleem, 2006)*

| The Immunophenotypes of B-cell Non-Hodgkin Lymphoma with Respect to CD5, CD10, and CD23 | | | |
|---|---|---|---|
| **Lymphoma** | **Typical Pattern** | **Less Common Pattern** | **Unusual Pattern** |
| SLL/CLL | CD5+, CD23+, CD10- | CD5+, CD23-, CD10- | CD5-, CD23+, CD10- |
| MCL | CD5+, CD23-, CD10- | CD5+, CD23+, CD10- | CD5+, CD23-, CD10+ |
| FL | CD5-, CD23-, CD10+ | CD5-, CD23+, CD10+ | CD5+, CD23-, CD10+<br>CD5-, CD23+, CD10- |
| DLBCL | CD5-, CD23-, CD10- | CD5-, CD23-, CD10+ | CD5-, CD23+, CD10+<br>CD5+, CD23-, CD10- |
| MZL | CD5-, CD23-, CD10- | CD5-, CD23+, CD10- | CD5+, CD23-, CD10- |
| B-PLL | CD5-, CD23-, CD10- | CD5-, CD23+, CD10-<br>CD5+, CD23-, CD10- | CD5+, CD23+ , CD10- |
| HCL | CD5-, CD10-, CD11c+, CD25+ , CD103+ | CD5-, CD10-, CD11c+ ,<br>CD25-, CD103+ | CD5-, CD10-, CD11c+, CD25-, CD103- |
| BL | CD5-, CD23-, CD10+ | CD5-, CD23-, CD10- | CD5-, CD23+, CD10- |
| LPL | CD5-, CD23-, CD10- | | CD5-, CD23+, CD10- |



## Discussion

Studies from different regions and populations across the world reveal significant variations in the incidence and distribution of B-NHL subtypes. Among them, DLBCL remains the most prevalent subtype across many regions, while others, such as CLL, FL, MCL, SMZL, and BL, exhibit distinct frequency patterns.For instance, studies from India and Pakistan show relatively higher frequencies of DLBCL compared to CLL, whereas studies from the USA and Japan demonstrate more balanced distributions. These discrepancies may reflect regional differences in

genetic predisposition, environmental factors, or healthcare infrastructure affecting disease diagnosis and reporting.

The immunophenotypic markers play a crucial role in subtype classification and diagnosis. CD5 expression, traditionally associated with CLL and MCL, exhibits regional variations and diagnostic implications. Studies from China highlight differences in CD5 expression patterns compared to Western cohorts, influencing disease classification and diagnostic algorithms. Additionally, aberrant expression of markers like CD11c, CD200, and CD38 further complicates subtype identification, emphasizing the need for comprehensive immunophenotyping techniques for accurate diagnosis and prognosis assessment.

However, aberrant marker expression, bi-clonality, and composite lymphoma cases highlight the prognostic significance of immunophenotypic characteristics in B-NHL. Understanding these implications is critical for personalized treatment strategies and patient management.

Moreover, the emergence of novel diagnostic approaches, such as machine-based algorithms and composite diagnostic predictors like the combined ratio score (CRS), showcases advancements in disease classification and subtype differentiation. These innovative methodologies improve diagnostic accuracy and streamline patient management by effectively distinguishing between closely related BCLPD subtypes like MCL and SLL.

## Conclusion

Overall, the data underscores the complex nature of BCLPDs, influenced by both geographical factors and immunophenotypic characteristics. Understanding these nuances is crucial for refining diagnostic strategies, optimizing treatment approaches, and ultimately improving patient outcomes.



## References

1. Swerdlow SH, Campo E, Pileri SA, Lee Harris N, Stein H, Siebert R,. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
2. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood [.67–3941:(8)111;2008 .[: http://dx.doi.org/10.1182/blood-2007-11-120535
3. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H,. iwCLL

guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood. 2018;131(25):2745–60.

4. Rawstron AC, Kreuzer KA, Soosapilla A, Spacek M, Stehlikova O, Gambell P,. Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: An European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. Cytom Part B - Clin Cytom. 2018;94(1):121–8.
5. Dewald GW, Brockman SR, Paternoster SF, Bone ND, O'Fallon JR, Allmer C,. Chromosome anomalies detected by interphase fluorescence in situ hybridization: Correlation with significant biological features of B-cell chronic lymphocytic leukaemia. Br J Haematol. 2003;121(2):287–95.
6. Matutes E, Owusu-Ankomah K, Morilla R, Marco JG, Houlihan A, Que TH,. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia. 1994;8(10):1640–5.
7. Robak T, Krawczyńska A, Cebula-Obrzut B, Urbaniak M, Iskierka-Jażdżewska E, Robak P. Atypical Chronic Lymphocytic Leukemia—The Current Status. Cancers (Basel). 2023;15(18):1–16.
8. Delgado J, Matutes E, Morilla AM, Morilla RM, Owusu-Ankomah KA, Rafiq-Mohammed F, وآخ. Diagnostic Significance of CD20 and FMC7 Expression in B-Cell Disorders. Am J Clin Pathol. 2003;120(5):754–9.
9. DiRaimondo F, Albitar M, Huh Y, O'Brien S, Montillo M, Tedeschi A,. The clinical and diagnostic relevance of CD23 expression in the chronic lymphoproliferative disease. Cancer. 2002;94(6):1721–30.
10. Nelson BP, Variakojis D, Peterson LAC. Leukemic phase of B-cell lymphomas mimicking chronic lymphocytic leukemia and variants at presentation. Mod Pathol. 2002;15(11):1111–20.
11. D'Arena G, De Feo V, Pietrantuono G, Seneca E, Mansueto G, Villani O,. CD200 and Chronic Lymphocytic Leukemia: Biological and Clinical Relevance. Front Oncol. 2020;10(November).
12. Sorigue M, Franch-Sarto M, Sarrate E, Junca J. Usefulness of the CLLflow score. Cytom Part B - Clin Cytom. 2018;94(6):950–2.

13. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL,. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94(6):1840–7.
14. Semanaj V, Pecani A, Dedej T, Barbullushi A, Ylli Z, Curaj T,. The diagnostic value of flow cytometry imunophenotyping in an Albanian patient population with a preliminary clinical diagnosis of chronic lymphocytic leukemia. Maced J Med Sci. 2014;7(1):51–5.
15. Cree IA. The WHO Classification of Haematolymphoid Tumours. Leukemia. 2022;36(7):1701–2.
16. Kalpadakis C, Pangalis GA, Sachanas S, Vassilakopoulos TP, Kyriakaki S, Korkolopoulou P, وآخ. New Insights into Monoclonal B-Cell Lymphocytosis. Biomed Res Int. 2014;2014.
17. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. Blood. 2006;107(1):265–76.
18. El-Sewefy D, Khattab D, Sallam MH, Elsalakawy W. Flow cytometric evaluation of CD200 as a tool for differentiation between chronic lymphocytic leukemia and mantle cell lymphoma. Egypt J Haematol. 2014;39(2):42.
19. Radhakrishnan VS, Lokireddy P, Parihar M, Prakash PS, Menon H. Mantle cell lymphoma: A clinical review of the changing treatment paradigms with the advent of novel therapies, and an insight into Indian data. Cancer Rep. 2022;5(7):1–18.
20. Johnson JG, Terpak LA, Margo CE, Setoodeh R. Extranodal marginal zone B-cell lymphoma of the ocular adnexa. Cancer Control. 2016;23(2):140–9.
21. Ferry JA, Yang WI, Zukerberg LR, Wotherspoon AC, Arnold A, Harris NL. CD5+ extranodal marginal zone B-cell (MALT) lymphoma: A low grade neoplasm with a propensity for bone marrow involvement and relapse. Am J Clin Pathol. 1996;105(1):31–7.
22. Chowdhury Z, Khonglah Y, Sarma S, Kalita P. De novo chronic lymphocytic leukemia/prolymphocytic leukemia or B-cell prolymphocytic leukemia? The importance of integrating clinico--morphological and immunophenotypic findings in distinguishing chronic lymphoproliferative diseases with circulating phas. Autops Case Reports. 2021;11:1–7.

23. Hunter ZR, Branagan AR, Manning R, Patterson CJ, Santos DD, Tournilhac O,. CD5, CD10, and CD23 expression in Waldenström's macroglobulinemia. Clin Lymphoma. 2005;5(4):246–9.
24. Lin P, Medeiros LJ. Lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia: An evolving concept. Adv Anat Pathol. 2005;12(5):246–55.
25. Tiesinga JJ, Wu CD, Inghirami G. CD5+ follicle center lymphoma: Immunophenotyping detects a unique subset of "floral" follicular lymphoma. Am J Clin Pathol. 2000;114(6):912–21.
26. Morice WG, Hodnefield JM, Kurtin PJ, Hanson CA. An unusual case of leukemic mantle cell lymphoma with a blastoid component showing loss of CD5 and aberrant expression of CD10. Am J Clin Pathol. 2004;122(1):122–7.
27. Lin CW, O'Brien S, Faber J, Manshouri T, Romaguera J, Huh YO,. De novo CD5+ Burkitt lymphoma/leukemia. Am J Clin Pathol. 1999;112(6):828–35.
28. Dong HY, Gorczyca W, Liu Z, Tsang P, Wu CD, Cohen P,. B-cell lymphomas with coexpression of CD5 and CD10. Am J Clin Pathol. 2003;119(2):218–30.
29. Arcaini L, Zibellini S, Boveri E, Riboni R, Rattotti S, Varettoni M,. The BRAF V600E mutation in hairy cell leukemia and other mature B-cell neoplasms. Blood. 2012;119(1):188–91.
30. Al-Anizi W, Al-Mashta MR. The frequency of aberrant lymphoid antigens expression in 202 Iraqi patients with de novo acute myeloid leukemia. Iraqi J Hematol. 2017;6(2):49.
31. Waravita TS, Wijetunge TS, Ratnatunga NVI. Pattern of lymphoma subtypes in a cohort of Sri Lankan patients. Ceylon Med J. 2015;60(1):13–7.
32. Naresh KN, Srinivas V, Soman CS. Distribution of various subtypes of non-Hodgkin's lymphoma in India: A study of 2773 lymphomas using R.E.A.L. and WHO classifications. Ann Oncol [11;2000 .[(SUPPL. 1):S63–7.: http://dx.doi.org/10.1093/annonc/11.suppl_1.S63
33. Mushtaq S, Akhtar N, Jamal S, Mamoon N, Khadim T, Sarfaraz T,. Malignant lymphomas in Pakistan according to WHO classification of lymphoid neoplasms. Asian Pacific J Cancer Prev. 2008;9(2):229–32.
34. Aoki R, Karube K, Sugita Y, Nomura Y, Shimizu K, Kimura Y,. Distribution of malignant lymphoma in Japan: Analysis of 2260 cases, 2001-2006. Pathol Int.

2008;58(3):174–82.

35. Yoon SO, Suh C, Lee DH, Chi HS, Park CJ, Jang SS,. Distribution of lymphoid neoplasms in the Republic of Korea: Analysis of 5318 cases according to the World Health Organization classification. Am J Hematol. 2010;85(10):760–4.
36. Yang QP, Zhang WY, Yu JB, Zhao S, Xu H, Wang WY,. Subtype distribution of lymphomas in Southwest China: Analysis of 6,382 cases using WHO classification in a single institution. Diagn Pathol [.77:(1)6;2011 .[: http://www.diagnosticpathology.org/content/6/1/77
37. Miao Y, Cao L, Sun Q, Li XT, Wang Y, Qiao C,. Spectrum and immunophenotyping of 653 patients with B-cell chronic lymphoproliferative disorders in China: A single-centre analysis. Hematol Oncol. 2018;36(1):121–7.
38. Shuhua Y, Zengjun L HW et al. The immunophenotypic characteristics of 260 patients with CD5 + B cell lymphoproliferative disorders. Chin J hematol. 2014;35(04):337–41.
39. Dronca RS, Jevremovic D, Hanson CA, Rabe KG, Shanafelt TD, Morice WG,. CD5-positive chronic B-cell lymphoproliferative disorders: diagnosis and prognosis of a heterogeneous disease entity. Cytom Part B - Clin Cytom. 2010;78(SUPPL. 1):35–41.
40. Gujral S, Polampalli SN, Badrinath Y, Kumar A, Subramanian PG, Nair R,. Immunophenotyping of mature B-cell non Hodgkin lymphoma involving bone marrow and peripheral blood: Critical analysis and insights gained at a tertiary care cancer hospital. Leuk Lymphoma. 2009;50(8):1290–300.
41. Dwivedi E, Kushwaha R, Jain M, Yadav G, Verma SP, Singh U.,. To Assess the Correlation between Bone Marrow Morphology and Immunophenotypic Findings in Patients of Chronic Lymphoproliferative Disorders (CLPD's) and to Assess the Role of Flowcytometric Immunophenotyping in Diagnosis and Subclassification of CLPD's. Int J Contemp Med Res [IJCMR]. 2019;6(7):5–11.
42. Sales MM, Ferreira SIACP, Ikoma MRV, Sandes AF, Beltrame MP, Bacal NS,. Diagnosis of chronic lymphoproliferative disorders by flow cytometry using four-color combinations for immunophenotyping: A proposal of the brazilian group of flow cytometry (GBCFLUX). Cytom Part B - Clin Cytom. 2017;92(5):398–410.
43. Barroso Cavalcanti G, De Farias Sales VS, Kramer Cavalcanti E Silva DG, De Araújo Lopes MC, De Souza Paiva A, Macedo Da Fonseca HE,. Detection of CD5 in B-cell

chronic lymphoproliferative diseases by flow cytometry: A strong expression in B-cell chronic lymphocytic leukemia. Acta Cir Bras. 2005;20(SUPPL. 1):56–62.

44. Mahdi T, Rajab A, Padmore R, Porwit A. Characteristics of Lymphoproliferative Disorders with More Than One Aberrant Cell Population as Detected by 10-Color Flow Cytometry. Cytom Part B - Clin Cytom. 2018;94(2):230–8.
45. Xu W, Li JY, Wu YJ, Yu H, Shen QD, Tian T,. CD38 as a prognostic factor in Chinese patients with chronic lymphocytic leukaemia. Leuk Res. 2009;33(2):237–43.
46. Perković S, Bašić-Kinda S, Aurer I, Ugrina I, Duletić-Načinović A, Lozić D,. Multiparameter flow cytometry is necessary for detection, characterization and diagnostics of composite mature B-cell lymphoproliferative neoplasms. Int J Hematol. 2013;98(5):589–96.
47. Zare H, Bashashati A, Kridel R, Aghaeepour N, Haffari G, Connors JM,. Automated analysis of multidimensional flow cytometry data improves diagnostic accuracy between mantle cell lymphoma and small lymphocytic lymphoma. Am J Clin Pathol. 2012;137(1):75–85.
48. Christensen BE, Nielsen H, Plesner T, Thorling K, Andersen E, Andersen PK. Prognostic Importance of Flow Cytometric Immunophenotyping of 540 Consecutive Patients With B-Cell Chronic Lymphocytic Leukemia. Blood. 1991;78(7):1795–802.
49. Monabati A, Safaei A, Mokhtari M, Boroumand-Noughabi S, Faghih M, Nematollahi P. Mantle cell lymphoma: pathologic and immunophenotyping study of 50 cases and correlation with survival—a single institute experience. J Hematop. 2020;13(3):153–8.
50. Al-mamoori H S. Expression of Cd200 in Cd5 Positive B-Cell Lymphoproliferative Disorder (B-Cll and Mcl) and Its Role in the Discrimination Between These Disorders. Int J Adv Res. 2017;5(1):1155–60.
51. Ho FCS, Todd D, Loke SL, Ng RP, Khoo RKK. Clinico-pathological features of malignant lymphomas in 294 Hong Kong Chinese patients, retrospective study covering an eight-year period. Int J Cancer. 1984;34(2):143–8.
52. Kwong YL, Wong KF, Chan LC, Liang RHS, Chan JKC, Wei D,. The spectrum of chronic lymphoproliferative disorders in Chinese people. Cancer. 1994;74(1):174–81.
53. Jain M, Kushwaha R, Verma SP, Singh US, Tripathi AK, Goel MM,. CD8 expression in B chronic lymphocytic leukemia: A case of immuno-phenotypic aberrancy. Cytom Part B -

Clin Cytom. 2018;94(4):679–82.

54. Inaba T, Shimazaki C, Sumikuma T, Nakagawa M. T-cell associated antigen-positive B-cell lymphoma. Leuk Lymphoma. 2001;42(6):1161–71.

55. Kampalath B, Barcos MP, Stewart C. Phenotypic heterogeneity of B cells in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma. Am J Clin Pathol. 2003;119(6):824–32.

56. Patil Okaly G V., Nargund AR, Venkataswamy V, Jayanna PK, Rao Juvva C, Prabhudesai S. Chronic lymphoproliferative disorders at an Indian tertiary cancer centre - The panel sufficiency in the diagnosis of chronic lymphocytic Leukaemia. J Clin Diagnostic Res. 2013;7(7):1366–71.

57. Seyyed Hadi Maljaei et al. Usefulness of Cd45 Density in the Diagnosis of B-Cell. Indian J Med Sci. 2005;59(5).

58. Harris Nancy L, S. JE, Stein H, Banks PM, Chan JKC, Cleary ML,. A Revised European-American Classification of Lymphoid Neoplasms. Blood J. 1994;84(5):1361–92.

59. Article O, Shahni A, Saud M, Siddiqui S, Mukry SN. Expression of aberrant antigens in hematological malignancies: A single center experience. Pak J Med Sci. 2018;34(2):457–62.

60. Kaleem Z. Flow cytometric analysis of lymphomas: Current status and usefulness. Arch Pathol Lab Med. 2006;130(12):1850–8.