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Flow cytometry immunophenotyping in diagnosed cases of B-cell chronic lymphoproliferative disorders

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ABSTRACT

Objectives: Mature B-cell neoplasms consist of a diverse group of disorders with distinct clinical presentations, pathology, and outcomes. This article aims to describe the clinical, morphological, and flow cytometry immunophenotypic findings of 30 diagnosed patients with B-cell lymphoproliferative disorders (B-CLPDs) in a tertiary care hospital in Sri Lanka.

Material and Methods: A descriptive cross-sectional study, including 30 cases of BCLPDs diagnosed over a period of 6 months, was analyzed. Diagnosis of BCLPDs was made by morphology of peripheral blood, bone marrow, and the immunophenotypic analysis by multiparametric flow cytometry on bone marrow aspirates or peripheral blood.

Results: CD5−/CD10− BCLPD with negative hairy cell markers was the most common subtype (30%), followed by chronic lymphocytic leukemia (CLL) at 26.6% and mantle cell lymphoma (MCL) at 20%. Persistent lymphocytosis was the most frequent clinical finding across BCLPD cases, while hepatomegaly was common in the cases of the CD5−/CD10− BCLPD with negative hairy cell markers. This category appears distinct from other known subtypes like splenic marginal zone lymphoma (SMZL) or hairy cell leukemia (HCL), suggesting a unique clinical profile.

Conclusion: The study highlights distinct immunophenotypic and clinical profiles across BCLPD subtypes, with CD5−/CD10− BCLPD subtype with negative hairy cell markers emerging as the most prevalent and showing unique marker patterns that may aid in differential diagnosis.

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

INTRODUCTION

According to the World Health Organization (WHO) classification in 2017, B-cell lymphoproliferative disorders (B-CLPDs) encompass a diverse group of diseases. This group includes chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), mantle cell lymphoma (MCL), B-cell prolymphocytic leukemia (B-PLL), follicular lymphoma (FL), splenic marginal zone lymphoma (SMZL), lymphoplasmacytic lymphoma (LPL), Burkitt lymphoma (BL), and diffuse large B-cell lymphoma (DLBCL).[1] B-CLPDs are characterized by the clonal proliferation

Table 1: Frequencies of different BCLPDs.

Flow Diagnosis		
BCLPD subtype	**Frequency**	**Percent (%)**
DLBCL	1	3.3
CD5−/CD10− BCLPD with negative hairy cell markers	9	30
CLL	8	26.6
MCL	6	20
LPL	2	6.6
B-PLL	1	3.3
HCL-V	1	3.3
SMZL	2	6.6
Total	30	100

DLBCL: Diffuse large B-cell lymphoma, BCLPD: B-cell lymphoproliferative disorders, CLL: Chronic lymphocytic leukemia, MCL: Mantle cell lymphoma, LPL: Lymphoplasmacytic lymphoma, B-PLL: B-cell prolymphocytic leukemia, HCL-V: Hairy cell leukemia-variant, SMZL: Splenic marginal zone lymphoma.

of mature B-lymphoid cells in the peripheral blood or bone marrow. Flow cytometry-based immunophenotyping plays an important role in the diagnosis of B-CLPDs. The characteristics of cluster of differentiation (CD) markers are an important factor in the accurate diagnosis and classification of B-CLPDs.[1,2]

As per the World Health Organization global statistics for 2020, a total of 544,352 new cases of non-Hodgkin's lymphoma (NHL) were reported, with a total of 259,793 new deaths across all ages and genders. NHL has emerged as the predominant hematological cancer worldwide, with an annual incidence of approximately 5.8 cases per 100,000 population and an annual mortality of 2.6 cases per 100,000 population.[3]

CLL is the most common leukemia in adults in Western countries. The disease is rare in Asian countries, with a low incidence maintained among emigrant populations. The worldwide annual incidence is between 1 and 5.5 per 100,000 people, with a higher prevalence in men compared to women. The incidence significantly rises with age, exceeding 20 cases per 100,000 individuals in those over 70 years old. The incidence of CLL is approximately 4.2 cases per 100,000 people in the Western world.[4] In certain Asian countries (e.g., China, India, and Japan), the percentage of CLL cases is much lower (4%–10% of leukemias).[4] The median patient age at diagnosis of CLL is approximately 70 years, but CLL also presents in younger adults.[5] There is a male predominance, with a male-to-female ratio of 1.5:2.1.[6]

MCL accounts for about 3%–10% of NHL. It occurs among middle-aged to older individuals, with a median age of about 60 years. There is a variably marked male predominance, with a male-to-female ratio of 2:1.[7]

B-PLL is an exceptionally rare condition, making up about 1% of lymphocytic leukemias. The majority of patients are over 60 years old, with a median age ranging from 65 to 69 years. The incidence rates are comparable between males and females.[1]

HCL is an uncommon condition, representing 2% of lymphoid leukemias. It primarily affects middle-aged to older adults, with a median age of 58 years. While there have been rare cases of HCL diagnosed in individuals in their 20s, it is extremely rare in children. The ratio of males to females affected by this disease is 4:1[1] Hairy cell leukemia-variant (HCL-v) is about one-tenth as common as HCL, with an annual incidence of approximately 0.03 cases per 100,000 people. Middle-aged to elderly patients are affected.[1]

SMZL is recognized as a rare condition, representing only 2% of lymphoid neoplasms.[1] The majority of patients diagnosed with SMZL are over 50 years old, with a median age ranging from 67 to 68 years. The incidence rates are similar for both males and females. In contrast, LPL primarily affects adults, with a median age of 65 years, and approximately 50%–60% of those affected are male.[1] The highest incidence rates of FL are observed in the United States and Western Europe, while Eastern Europe, Asia, and developing nations report significantly lower rates.[8] FL accounts for about 20% of all lymphomas and predominantly affects adults, with a median age of 60 years and a male-to-female ratio of 1:1.7.[8,9]

The aim of this article is to describe the clinical, morphological, and flow cytometry immunophenotypic findings of 30 diagnosed patients with B-CLPDs at a tertiary care hospital in Sri Lanka.

MATERIAL AND METHODS

A descriptive cross-sectional study was carried out in the Department of Hematology of the Sri Jayewardenepura General Hospital, which is a prominent blood cancer diagnostic center. Ethical approval was obtained from the ethical committee of the University of Sri Jayewardenepura. The hospital receives samples from many areas of the country. Consecutively diagnosed 30 cases of both male and female patients with B-CLPDs were included in the study.

Patients currently undergoing treatment for B-CLPDs were excluded from participation. Additionally, individuals with insufficient clinical information and other pertinent details were excluded from the study.

Peripheral blood or bone marrow samples used for this study were collected and sent to Sri Jayewardenepura General Hospital Laboratory for diagnostic purposes.

A full blood count (FBC) of peripheral blood or bone marrow was performed using BC-6800 Fully Automated Hematology

Table 2: Clinical symptoms of different types of BCLPDs.

Clinical details	Yes/ No	CD5(–), CD10 (–) BCLPDs with negative hairy markers	CLL	MCL	LPL	SMZL	B-PLL	DLBCL	HCL-v
Pallor	Yes	6/9	-	2/6	1/2	2/2	-	-	-
	No	3/9	8/8	4/6	1/2	-	1/1	1/1	1/1
Persistent lymphocytosis	Yes	9/9	7/8	6/6	2/2	2/2	1/1	1/1	1/1
	No	-	1/8	-	-	-	-	-	-
Splenomegaly	Yes	5/9	-	2/6	2/2	2/2	-	-	1/1
	No	4/9	8/8	4/6	-	-	1/1	1/1	-
Hepatomegaly	Yes	9/9	1/8	1/6	1/2	2/2	-	-	1/1
	No	-	7/8	5/6	1/2	-	1/1	1/1	-
Lymphadenopathy	Yes	4/9	5/8	4/6	1/2	1/2	1/1	1/1	-
	No	5/9	3/8	2/6	1/2	1/2	-	-	1/1
Fever	Yes	2/9	3/8	5/6	2/2	2/2	-	1/1	-
	No	7/9	5/8	1/6	-	-	1/1	-	1/1

CLL: Chronic lymphocytic leukemia, MCL: Mantle cell lymphoma, LPL: Lymphoplasmacytic lymphoma, SMZL: Splenic marginal zone lymphoma, B-PLL: B-cell prolymphocytic leukemia, DLBCL: Diffuse large B-cell lymphoma, HCL-V: Hairy cell leukemia-variant, BCLPD: B-cell lymphoproliferative disorders.

Table 3: FBC findings in CLL cases.

FBC findings in CLL				
	Unit	Minimum	Maximum	Mean
WBC count	Cells/µl	17,810	106,460	46,430
Absolute neutrophil count	Cells/µl	2,930	58,970	18,179
Neutrophil percentage	%	10.9	57	31.625
Absolute lymphocyte count	Cells/µl	11,095	45,211	25,377
Lymphocyte percentage	%	37	87.3	63.5
HGB	g/dl	10.3	15.7	12.7
Platelet count	Cells/µl	107×10^3	371×10^3	167.62×10^3

FBC: Full blood count, CLL: Chronic lymphocytic leukemia, HGB: Hemoglobin.

Table 4: FBC findings in MCL cases.

FBC findings in MCL				
	Unit	Minimum	Maximum	Mean
WBC count	Cells/µl	15,060	41,220	26,835
Absolute neutrophil count	Cells/µl	3,810	9,550	5,928
Neutrophil percentage	%	16.0	34.3	24.0
Absolute lymphocyte count	Cells/µl	8,720	30,090	19,081
Lymphocyte percentage	%	57.9	78.7	70.5
HGB	g/dL	8.9	12.6	10.6
Platelet count	Cells/µl	70×10^3	174×10^3	174×10^3

FBC: Full blood count, HGB: Hemoglobin, MCL: Mantle cell lymphoma.

Analyzer. Immunophenotyping was performed using a BD FACS CANTO™ II Flow Cytometer and FACS Diva Software was used for analysis. Cell morphology of the peripheral blood and bone marrow was confirmed after staining with Leishman's stain.

The flow cytometry immunophenotyping was performed on bone marrow aspirate or peripheral blood samples. The reagents used to detect expression of CD markers were selected following the guidelines[9] and Euro Flow eight-color antibody panels for immunophenotyping of hematological malignancies and the validated protocols given by Becton Dickinson (BD) Bioscience, India.[10–12] The BD One Flow LST was used to identify and differentiate neoplastic T cells, B cells, and NK cells from normal blood cells. The BD One Flow LST tubes were pre-coated with monoclonal antibodies, including CD20/CD4 (V450), CD45 (V500), CD8/smlg lambda (FITC), CD56/smlg kappa (PE), CD5 (PERCP CY 5.5), CD19/TCR gamma delta (PE CY 7), smCD3 (APC), and CD38 (APC H7).

Table 5: FBC findings in CD5(−),CD10(−) BCLPDs.

CD5(−), CD10(−), BCLPDs with negative hairy markers				
	Unit	**Minimum**	**Maximum**	**Mean**
WBC count	Cells/µl	2,720	327,000	56,589
Absolute neutrophil count	Cells/µl	1,350	5,050	9,628
Neutrophil percentage	%	6	57	30.8
Absolute lymphocyte count	Cells/µl	1,670	36,370	11,624
Lymphocyte percentage	%	36.7	83.7	57.5
HGB	g/dL	9.0	10.1	9.4
Platelet count	Cells/µl	101×10^3	209×10^3	131×10^3

FBC: Full blood count, BCLPDs: B-cell chronic lymphoproliferative disorders, HGB: Hemoglobin.

T lymphocytes were identified by CD4 (V450), CD8 (PERCP CY 5.5), CD5 (PERCP CY 5.5), TCR gamma delta (PE CY 7), and smCD3 (APC). B lymphocytes were identified by CD20 (V450), CD19 (PE CY 7), CD38 (APC H7), SMLG kappa (PE), and SMLG lambda (FITC). NK cells were identified by the positivity of CD56 and the negativity of smCD3. Additionally, CD20 (V450), CD45 (V500), CD23 (FITC), CD10 (PE), CD103 (PE), CD79b (PERCP CY 5.5), CD11c (PERCP CY 5.5), CD19 (PE CY 7), CD200 (APC), CD123 (APC), BCL-2 (PE), and CD43 (APC H7) surface staining markers were used to identify B-CLPDs.

Data were analyzed using SPSS version 20, descriptive statistical methods, Pearson's correlation test with *p*-value, and independent sample *t*-test.

RESULTS

Table 1 represents the distribution of subtypes as diagnosed through immunophenotyping by flow cytometry. Each subtype is listed along with its corresponding frequency and percentage within the cohort of 30 diagnosed cases.

DLBCL is observed in one such case constituting 3.3% of the total. CD5(−), CD10(−) BCLPDs with negative hairy cell markers (CD5 - CD10 - BCLPD) account for the highest proportion, with nine cases representing 30% of the total. CLL follows closely behind with eight cases, making up 26.6% of the total. MCL is observed in six cases, comprising 20% of the total. LPL and SMZL each have two cases constituting 6.6% of the total for each subtype. B-PLL and HCL-V each have one case constituting 3.3% of the total.

All 30 cases underwent clinical assessment [Table 2] based on the presence or absence of specific clinical symptoms, including pallor, persistent lymphocytosis, splenomegaly, hepatomegaly, lymphadenopathy, and fever.

Clinical Findings

The clinical features of these cases were distributed as follows: [Table 3]: The prevailing clinical manifestation identified within the CLL cases was persistent lymphocytosis, observed in 7 out of the 8 diagnosed instances. Clinical findings of CD5(−), CD10(−) BCLPDs and MCL indicate that persistent lymphocytosis is universally present in all cases, while hepatomegaly is observed in all cases of CD5(−), CD10(−) BCLPDs, pallor, splenomegaly, lymphadenopathy and fever thus exhibiting varying degrees of prevalence among the cases.

Table 3 outlines the findings from FBC analysis in individuals diagnosed with different BCLPDs. The data includes the minimum, maximum, and mean values for various parameters.

These findings provide insights into the hematological profile of individuals with CLL, including the characteristic elevation in WBC count, associated with lymphocytosis, and potential abnormalities in other blood cell types such as neutrophils, platelets, and hemoglobin.

MCL tends to have a lower WBC count ($p = 0.2$) and absolute neutrophil count ($p = 0.185$) compared to CLL. MCL showed a higher mean lymphocyte percentage ($p = 0.407$) and absolute lymphocyte count ($p = 0.298$) compared to CLL, indicating a greater lymphocytic involvement. HGB levels in MCL appear to be significantly lower compared to CLL ($p = 0.035$) [Table 4].

Platelet counts did not show a significant difference between MCL and CLL ($p = 0.887$). Overall, these comparisons highlight distinct hematological characteristics between MCL and CLL.

Table 5 shows a notable finding, including a wide range of white blood cells (WBC) counts from 2,720 to 327,000 cells/µl, with a mean of 56,589 cells/µl, indicating significant leukocytosis. The absolute lymphocyte count ranges from 1,670 to 36,370 cells/µL, with a mean of 11,624 cells/µL, and lymphocyte percentage varies from 36.7% to 83.7%, with a mean of 57.5%. Hemoglobin levels range from 9.0 g/dL to 10.1 g/dL, with a mean of 9.4 g/dL, while platelet counts range from 101,000/µL to 209,000/µL, with a mean of 131,000/µL.

When comparing FBC findings between CLL and CD5 negative CD10 negative BCLPDs with negative hairy markers, significant differences were found in absolute lymphocyte count ($p = 0.033$) and hemoglobin levels ($p = 0.001$) showing higher mean values of both in CLL.

However, no significant differences were found in WBC count ($p = 0.788$), neutrophil count ($p = 0.365$), neutrophil percentage ($p = 0.934$), lymphocyte percentage ($p = 0.519$), or platelet count ($p = 0.302$).

Table 6: Morphological findings of different types of BCLPDs.

Morphological findings		CD5(−), CD10(−)	CLL	MCL	LPL	SMZL	B-PLL	DLBCL	HCL-v
Nuclear/cytoplasmic ratio of lymphoid cells	Normal	-	1/8	-	-	-	-	-	-
	Moderate	1/9	7/8	3/6	1/2	2/2	1/1	-	-
	Increased	8/9	-	3/6	1/2	-	-	1/1	1/1
The number of nucleoli	Absent	-	8/8	1/6	-	1/2	-	-	-
	1–2	9/9	-	5/6	1/2	1/2	1/1	1/1	-
	Many	-	-	-	1/2	-	-	-	1/1
Nuclear irregularity	Smooth, round	-	8/8	-	-	-	-	-	-
	Mildly irregular	-	-	-	1/2	1/2	-	-	-
	Irregular	9/9	-	6/6	1/2	1/2	1/1	1/1	1/1
Size of the lymphoid cells	Small	-	7/8	-	-	-	-	-	-
	Small to medium	2/9	1/8	2/6	-	1/2	-	-	-
	Medium	4/9	-	1/6	1/2	1/2	1/1	-	-
	Large	3/9	-	3/6	1/2	-	-	1/1	1/1

BCLPDs: B-cell chronic lymphoproliferative disorders, CLL: Chronic lymphocytic leukemia, MCL: Mantle cell lymphoma, LPL: Lymphoplasmacytic lymphoma.

Table 7: Qualitative CD marker expression of CD5(−), CD10(−) BCLPDs with negative hairy cell markers.

	Qualitative CD marker expression of CD5(−), CD10(−) BCLPDs with negative hairy cell markers				
CD Marker	+++	++	DIM+	Negative	Not applied
CD45	9/9	-	-	-	-
CD19	9/9	-	-	-	-
CD20	7/9	2/9	-	-	-
CD5	-	-	-	9/9	-
CD10	-	-	-	9/9	-
CD38	1/9	-	1/9	7/9	-
KAPPA	7/9	-	-	2/9	-
LAMBDA	-	2/9	-	7/9	-
CD23	-	2/9	1/9	6/9	-
CD79b	5/9	3/9	1/9	-	-
CD200	2/9	1/9	1/9	5/9	-
CD43	-	1/9	1/9	7/9	-
FMC7	1/9	-	1/9	7/9	-

BCLPDs: B-cell chronic lymphoproliferative disorders, +++: Strong, ++: Moderate, DIM+: Less bright.

Table 8: Qualitative expression of CD markers in CLL.

	Qualitative expression of CD markers in CLL				
CD Marker	+++	++	DIM+	Negative	Not applied
CD45	8/8	-	-	-	-
CD19	7/8	1/8	-	-	-
CD20	1/8	5/8	1/8	1/8	-
CD5	5/8	3/8	-	-	-
CD10	-	-	-	8/8	-
CD38	-	-	2/8	6/8	-
KAPPA	2/8	-	3/8	3/8	-
LAMBDA	-	-	3/8	5/8	-
CD23	6/8	2/8	-	-	-
CD79b	-	2/8	-	6/8	-
CD200	8/8	-	-	-	-
CD43	4/8	1/8	1/8	2/8	-
FMC7	-	-	-	8/8	-

CLL: Chronic lymphocytic leukemia, +++: Strong, ++: Moderate, DIM+: Less bright.

When comparing FBC findings between MCL and CD5-negative, CD10-negative BCLPDs with negative hairy markers, there were no significant differences in WBC count ($p = 0.414$), neutrophil count ($p = 0.316$), neutrophil percentage ($p = 0.366$), lymphocyte percentage ($p = 0.08$), platelet count ($p = 0.288$), absolute lymphocyte count ($p = 0.175$), and hemoglobin levels ($p = 0.111$). FBC findings for lymphomas such as DLBCL, B-PLL, LPL, SMZL and HCL-V are not compared as reported cases are minimal. The study report will be presented in future as more data becomes available.

Morphological Findings

Each category within Table 6 indicates the proportion of cases exhibiting the specified morphological characteristic within each lymphoid malignancy type.

Flow Cytometry Immunophenotypic Findings

The following tables provide a qualitative assessment of CD marker expression. Each CD marker is listed along with the observed intensity of expression (+++ indicating strong, ++ indicating moderate, DIM+ indicating dim expression, and NEGATIVE indicating absence of expression) and the frequency of occurrence among the cases.

Table 7 outlines the qualitative expression of CD markers in CD5-negative, CD10-negative lymphomas with negative hairy markers.

They typically express CD45, CD19, CD20 (often strongly), KAPPA (often strongly), and CD79b (often strongly). However, they commonly lack expression of CD5, CD10, CD38, CD23, CD43, and FMC7. Additionally, they display variable expression of CD200. This expression profile suggests a non-specific B-cell lymphoma subtype which cannot be categorized under CD5-negative, CD10-negative lymphomas such as HCL, SMZL, or HCL-V.

Table 8 outlines the qualitative expression of CD markers in CLL cases.

Expression of CD45 was universally observed in all CLL cases (8/8), indicating a consistent presence. CD19 expression was noted in 7 out of 8 cases, predominantly as a strong expression (+++), with a minority exhibiting a moderate expression (++).

CD20 expression varied, with five cases showing moderate expression (+), one case showing strong expression (+++), one case showing dim expression, and one case showing absence of expression. CD5 expression was detected in all eight cases, with five exhibiting a moderate signal (++), and the remaining cases exhibiting a strong signal (+++). CD10 expression was exclusively absent in all CLL cases (8/8). CD38 expression was absent in the majority of cases (6/8), while two cases exhibited dim expression.

Strong Kappa light chain restriction was observed in two cases, with three cases showing weak expression and three cases with negative expression [Figure 1]. Lambda light chain expression was absent in the majority of cases (5/8), while three cases exhibited weak expression.

CD23 expression was detected in six out of 8 cases, predominantly strongly (+++), while two cases revealed moderate expression (++).

CD79b expression was detected in two cases, both exhibiting DIM+ expression, while majority of cases (6/8) were negative.

Table 9 outlines the qualitative expression of CD markers in MCL cases.

Table 9: Qualitative expression of CD markers in MCL.

	Qualitative expression of CD markers in MCL				
CD Marker	+++	++	DIM+	Negative	Not applied
CD45	6/6	-	-	-	-
CD19	6/6	-	-	-	-
CD20	6/6	-	-	-	-
CD5	3/6	2/6	-	1/6	-
CD10	-	-	-	6/6	-
CD38	-	-	-	6/6	-
KAPPA	5/6	-	-	-	-
LAMBDA	1/6	-	-	5/6	-
CD23	1/6	1/6	1/6	3/6	-
CD79b	4/6	1/6	-	1/6	-
CD200	3/6	-	-	3/6	-
CD43	-	2/6	-	4/6	-
FMC7	-	2/6	-	4/6	-

MCL: Mantle cell lymphoma, +++: Strong, ++: Moderate, DIM+: Less bright.

These cases are characterized by strong expression of CD45, CD19, and CD20, along with variable expression of CD5. The absence of CD10 and CD38 is also consistent with the MCL phenotype.

Qualitative expression of CD markers in other lymphomas such as DLBCL, B-PLL, LPL, SMZL, and HCL-V will be described in future reports as more data becomes available.

Table 10 presents the mean fluorescence intensity (FI) values for B cells, T cells, and NK cells in CLL, CD5(−), CD10(−) BCLPDs, and MCL cases, measured across forward scatter (FSC-A) and side scatter (SSC-A) parameters.

CD5 negative CD10 negative lymphomas with negative hairy cell markers, all three cell types (B cells, T cells, and NK cells) tend to have higher mean Fluorescence Intensity (FI values for both FSC-A and SSC-A compared to those in CLL. Mean Fluorescence Intensity (FI of B cells for both FSC-A ($p = 0.008$) and SSC-A ($p = 0.016$) are statistically significant. This suggests potential differences in cell size and granularity between the two conditions. Specifically, in negative CD5 negative CD10 negative lymphomas with negative hairy cell markers, B cells, T cells, and NK cells exhibit larger size and higher granularity compared to CLL.

There are no significant differences in the mean fluorescence intensity of B cells, T cells, and NK cells between CLL and MCL, showing FSC-A ($p = 0.124$) and SSC-A ($p = 0.679$) for B cells.

There are no significant differences in the mean fluorescence intensity of B cells, T cells, and NK cells between CD5(−) and

Table 10: Mean FI of B, T, NK cells.

	Mean fluorescence intensity of B, T, NK cells								
	In CLL			In CD5(−), CD10(−) BCLPDs with negative hairy markers			In MCL		
	B cells	T cells	NK cells	B cells	T cells	NK cells	B cells	T cells	NK cells
FSC-A	62,221	75,282	81,536	81,485	79,960	88,385	72,504	70,694	78,595
SSC-A	10,852	14,549	16,120	15,314	15,795	18,149	11,792	13,479	18,248

FI: Follicular lymphoma, B: B lymphocytes cells, T: T lymphocytes cells, NK: Natural killer cells, CLL: Chronic lymphocytic leukemia, BCLPDs: B-cell chronic lymphoproliferative disorders, MCL: Mantle cell lymphoma, FSC-A: Forward scatter- A, SSC-A: Side scatter- A.

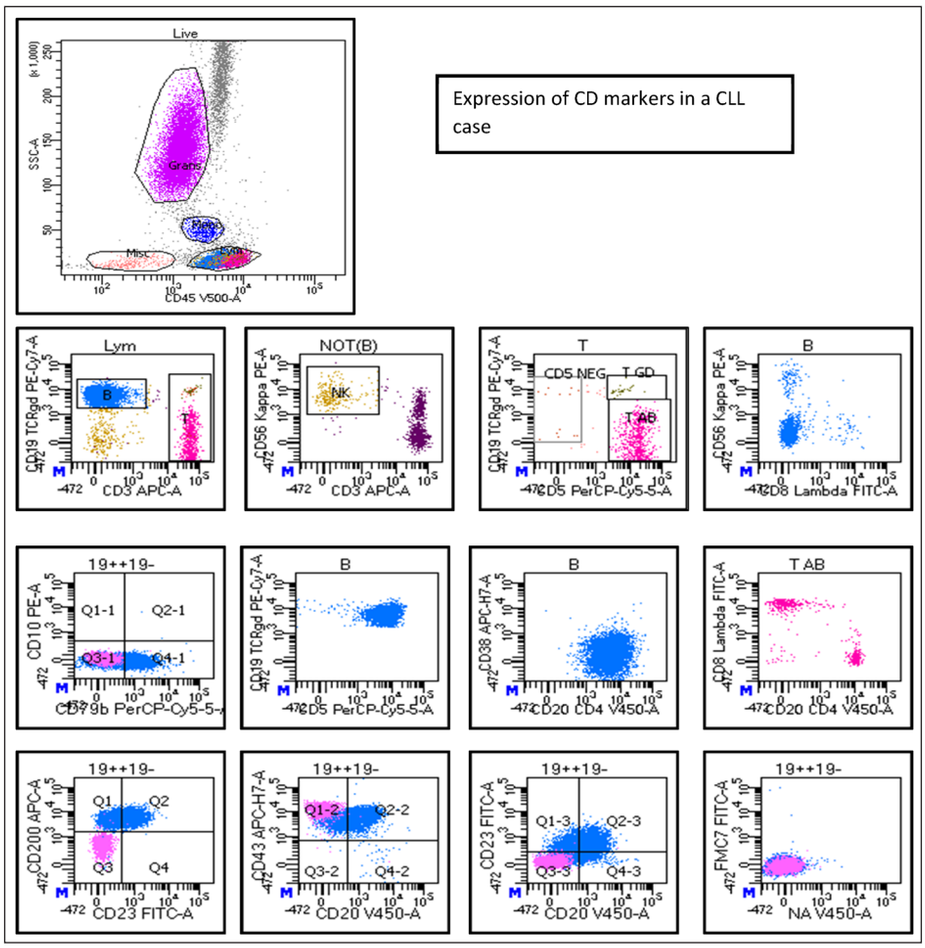

Figure 1: Expression of CD markers in a case of chronic lymphocytic leukaemia. 19++: CD19 positive B cells, 19−: CD19 negative, FITC-A: Fluorescein isothiocyanate.

CD10(−) BCLPDs with negative hairy cell markers and MCL, indicating FSC-A ($p = 0.213$) and SSC-A ($p = 0.145$) for B cells.

DISCUSSION

Distribution and immunophenotypic characteristics of B-CLPDs vary in the different studies. In Sri Lanka, studies identified DLBCL and FL as predominant subtypes among lymphomas, consistent with global patterns and reflecting similarities with South Asian nations.[13–15]. DLBCL represents 58.8% of cases in Sri Lanka, while FL accounts for 17.6%, showing comparable prevalence to the studies in India[14] and Pakistan[15] but differing from Western countries[16], where SLL/CLL is more frequent. Other notable subtypes include MCL and MZL, which appear in lower frequencies but similarly

align with South Asian observations. Moreover, CD5-negative and CD10-negative lymphomas with negative hairy markers are more common in Asia than the West, indicating regional diagnostic challenges and differing B-CLPD presentations.[17–19]

Comparatively, Chinese studies reveal lower CLL and higher LPL incidences, with unique immunophenotypic traits. Chinese MCL cases exhibit higher CD23 expression, and CD200 has proven highly effective (94.6% accuracy) for distinguishing CLL from MCL.[20,21] CLL in China often features lower CD23 positivity than observed in Western settings, highlighting unique regional immune markers that could refine diagnostic protocols.

Further, research from Gujral S et al.[22] and Ibrahim et al.[23] underscores the utility of advanced diagnostic markers. CD38 positivity is frequently associated with shorter survival times in B-CLL cases across multiple regions,[23,24] whereas the combined ratio score (CRS) reliably distinguishes MCL from SLL with high specificity, emphasizing the potential for diagnostic innovation using multi-parameter flow cytometry.[25]

In the Middle East, a study in Iraq[26] showed high prevalence of CD200 expression in CLL (94.9%), reinforcing its diagnostic relevance in B-CLL classification. A Brazilian study also recommends shifting to eight-color flow cytometry, enabling more precise differentiation of B-CLPDs by efficiently assessing a broader range of antigens.[27]

Global literature study highlights the complexities of B-CLPD diagnosis, particularly with atypical or overlapping immunophenotypes like CD5+/CD23+ in CLL or cases with aberrant CD8 and T-cell antigen expressions in B-CLPD. These cases often require non-bone marrow biopsies and advanced immunophenotypic assessments for accurate categorization, as demonstrated in U.S. and Canadian research.[28]

Regional studies indicate that while the WHO classification provides a standard framework, immunophenotypic marker prevalence and diagnostic tools can vary significantly by region, necessitating adaptive diagnostic criteria. Advanced flow cytometry, incorporating multi-color panels and refined biomarkers like CD200, CD20/CD23 ratios and CRS algorithms may enhance diagnostic accuracy and support more personalized treatment approaches for B-CLPD across diverse populations.

CONCLUSION

This study reveals diverse subtype distributions, with CD5−/CD10− BCLPD with negative hairy cell markers (30%), CLL (26.6%) and MCL (20%) as the most common, highlights persistent lymphocytosis as a key feature and identifies a unique CD5−/CD10− BCLPD subtype with negative hairy cell markers differing from SMZL and HCL.

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