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Abstract

Background: Chronic lymphocytic leukaemia (CLL) is diagnosed by integrating persistent lymphocytosis, morphology and flow-cytometric immunophenotyping. Detailed Sri Lankan data remain limited.

Purpose: To describe the clinical, haematological, morphological and flow-cytometric profile of classical CLL and to explore differences across Matutes score groups.

Methods: This descriptive cross-sectional study included 94 diagnosed classical CLL cases at Sri Jayewardenepura General Hospital, Sri Lanka. Demographic and clinical features, full blood count, morphology, lymphocyte subsets, qualitative marker expression, fluorescence-intensity metrics and CD45 indices were analysed. Matutes 3/5, 4/5 and 5/5 groups were compared using Kruskal-Wallis and Fisher-Freeman-Halton exact tests.

Results: Median age was 66.0 years (IQR 59.0–71.8), 57/94 (60.6%) were male, and persistent lymphocytosis was present in 93/94 (98.9%). Morphology was typical, with predominantly small to small-medium lymphoid cells, absent nucleoli and smooth nuclear contours. CD19 and CD5 were expressed in all cases; CD23, CD200 and CD43 were frequent, while FMC7 and CD79b were commonly negative or weak. Matutes scores were 3/5 in 31 (33.0%), 4/5 in 39 (41.5%) and 5/5 in 24 (25.5%). Score groups differed for age, CD19-gated B-cell percentage, CD4/CD8 ratio and CD45 index; hepatomegaly, CD200 positivity and CD38 positivity also differed across groups.

Conclusions: This study showed typical clinical, morphological and immunophenotypic features of classical CLL. Matutes-score groups differed in selected clinical and flow-cytometric parameters, while morphology remained similar. CD45-index variation may reflect immunophenotypic differences, but its diagnostic significance requires further validation in larger, multicentre studies.

Keywords: chronic lymphocytic leukaemia; flow cytometry; immunophenotyping; Matutes score; CD45 index; Sri Lanka

Introduction

Chronic lymphocytic leukaemia (CLL) is a mature B-cell neoplasm defined by persistent clonal B-cell lymphocytosis in peripheral blood, typical small mature lymphocyte morphology and a characteristic flow-cytometric immunophenotype [1-3]. In routine practice, diagnosis integrates clinical findings, full blood count results, blood-film or marrow morphology and multiparameter flow cytometry [1,4-6].

Classical CLL usually shows CD19 and CD5 co-expression, CD23 positivity, weak surface light-chain expression, weak or absent FMC7 and low or absent CD79b. CD200 and CD43 are useful supportive markers, particularly when CLL must be separated from mantle cell lymphoma and other mature B-cell neoplasms [7-14]. CLL scoring systems remain useful where molecular and cytogenetic testing is not routinely available [7,14].

Local data are important for diagnostic practice and flow-cytometry interpretation in Sri Lanka. Fluorescence-intensity (FI) metrics and internally normalized measures such as a CD45 index remain underused in routine interpretation, particularly in resource-limited settings. This study therefore describes 94 patients with classical CLL and evaluates whether FI-based measures, especially a CD45 index, provide additional supportive information. A secondary objective was to determine whether clinical, haematological, morphological and selected flow-cytometric characteristics differed among Matutes score groups (3/5, 4/5 and 5/5).

Materials and Methods

Study design and setting

This descriptive cross-sectional study was conducted at the Department of Haematology, Sri Jayewardenepura General Hospital, Sri Lanka, from July 2023 to December 2025.

Case selection

Newly diagnosed CLL cases confirmed by integrated morphology and multiparameter flow cytometry were included. Patients already receiving treatment and patients with incomplete records were excluded. The analysis was limited to 94 cases classified as classical CLL.

Specimen collection and full blood count analysis

EDTA-anticoagulated peripheral blood was used in most cases. Bone marrow aspirate was analysed when peripheral blood was inadequate or marrow assessment was clinically required. Full blood count (FBC) analysis was performed using the Mindray BC-6800 Plus haematology analyzer.

Flow-cytometric immunophenotyping

Flow cytometry was performed using a BD FACSCanto II flow cytometer with BD FACSDiva v8.0.1 software. Lymphoid events were identified using CD45 versus side-scatter and forward-scatter/side-scatter characteristics, followed by B-cell identification using CD19 gating. The BD OneFlow LST tube was used to identify and differentiate B, T and NK-cell populations. The panel included CD20/CD4 (V450), CD45 (V500), CD8/surface Ig lambda (FITC), CD56/surface Ig kappa (PE), CD5 (PerCP-Cy5.5), CD19/TCR gamma-delta (PE-Cy7), surface CD3 (APC) and CD38 (APC-H7). Additional markers used for B-cell chronic lymphoproliferative disorder classification included CD23, CD10, CD79b, CD200, and CD43. Marker selection and interpretation followed WHO-, EuroFlow- and consensus-based diagnostic practice [2,4-6,16,17].

Fluorescence-intensity standardization

FI data were generated on the same BD FACSCanto II platform using the manufacturer-instructed protocol. CS&T beads, BD OneFlow Setup beads, BD OneFlow FC beads and same-lot, same-conjugate reagents were used to reduce analytical variation. Mean FI and median FI were recorded. The CD45 index was calculated as the CD45 FI of the neoplastic CD19-positive CLL B-cell population divided by the corresponding CD45 FI of the internal T-cell comparator population.

Variables and statistical analysis

The analysis included age, sex, sample type, clinical features, FBC parameters, morphological variables, lymphocyte-subset variables, qualitative immunophenotypic marker categories, FI variables, Matutes score and CD45 indices. Qualitative expression was categorized as strong (++), moderate (+), dim positive (DIM+) or negative (NEG) using internal positive

and negative populations; expression in more than 20% of gated cells was considered positive. Continuous variables were summarized mainly as median (IQR). Categorical variables were summarized as frequencies and percentages. Kruskal-Wallis testing was used for continuous comparisons across Matutes-score groups, and the Fisher-Freeman-Halton exact test with Monte Carlo estimation was used for categorical comparisons because several cells were sparse. Spearman correlation was used for selected relationships. Analyses were performed using SPSS version 20 and R. A two-sided p value <0.05 was considered statistically significant.

Ethical approval

Ethical approval was obtained from the Ethics Review Committee of the University of Sri Jayewardenepura (ERC 08/23).

Results

Demographic and clinical profile: The cohort included 94 patients with classical CLL. Median age was 66.0 years (IQR 59.0–71.8; range 31–93), and 57 (60.6%) were male. Peripheral blood was the main specimen type (90/94, 95.7%). Persistent lymphocytosis was recorded in 93 cases (98.9%). Lymphadenopathy was present in 35 (37.2%), splenomegaly in 13 (13.8%), hepatomegaly in 6 (6.4%), pallor in 20 (21.3%) and fever in 8 (8.5%) (Table 1).

Table 1. Demographic, clinical, haematological and lymphocyte-subset profile of the cohort

Domain / variable	Summary
Age, years	Median 66.0 (IQR 59.0–71.8); mean $\pm$ SD 65.6 $\pm$ 10.4; range 31–93
Male sex	57/94 (60.6%)
Peripheral blood specimen	90/94 (95.7%)
Persistent lymphocytosis	93/94 (98.9%)
Lymphadenopathy	35/94 (37.2%)
Splenomegaly	13/94 (13.8%)
Hepatomegaly	6/94 (6.4%)
Pallor	20/94 (21.3%)
Fever	8/94 (8.5%)
WBC (/ μ L)	24,650.0 (19,207.5–48,780.0)
Absolute lymphocyte count (/ μ L)	19,635.0 (12,925.0–33,002.5)
Haemoglobin (g/dL)	12.8 (11.8–14.3)
Platelet count ($\times 10^9$ /L)	190.5 (130.5–242.0)
Haemoglobin <11 g/dL	17/94 (18.1%)
Platelet count <100 $\times 10^9$ /L	13/94 (13.8%)
CD19-gated B-cell percentage (%)	69.7 (49.7–76.2)
CD3-gated T-cell percentage (%)	22.4 (14.7–33.9)
CD4/CD8 ratio	1.40 (1.12–1.80)

Haematological and lymphocyte-subset profile: Median WBC count was 24,650/ μ L (IQR 19,207.5–48,780.0) and median absolute lymphocyte count was 19,635/ μ L (IQR 12,925.0–33,002.5). Median haemoglobin was 12.8 g/dL (IQR 11.8–14.3) and median platelet count was 190.5 $\times 10^9$ /L (IQR 130.5–242.0). Haemoglobin <11 g/dL was present in 17 (18.1%) and platelet count <100 $\times 10^9$ /L in 13 (13.8%). Median CD19-gated B-cell percentage was 69.7%, median CD3-gated T-cell percentage 22.4%, and median CD4/CD8 ratio 1.40 (Table 1).

Morphological and qualitative immunophenotypic profile: Morphology was typical of classical CLL. Increased N:C ratio was seen in 67 (71.3%), nucleoli were absent in 91 (96.8%), nuclear contours were smooth and round in 89 (94.7%), and all cases showed smudge cells (Figure 1).

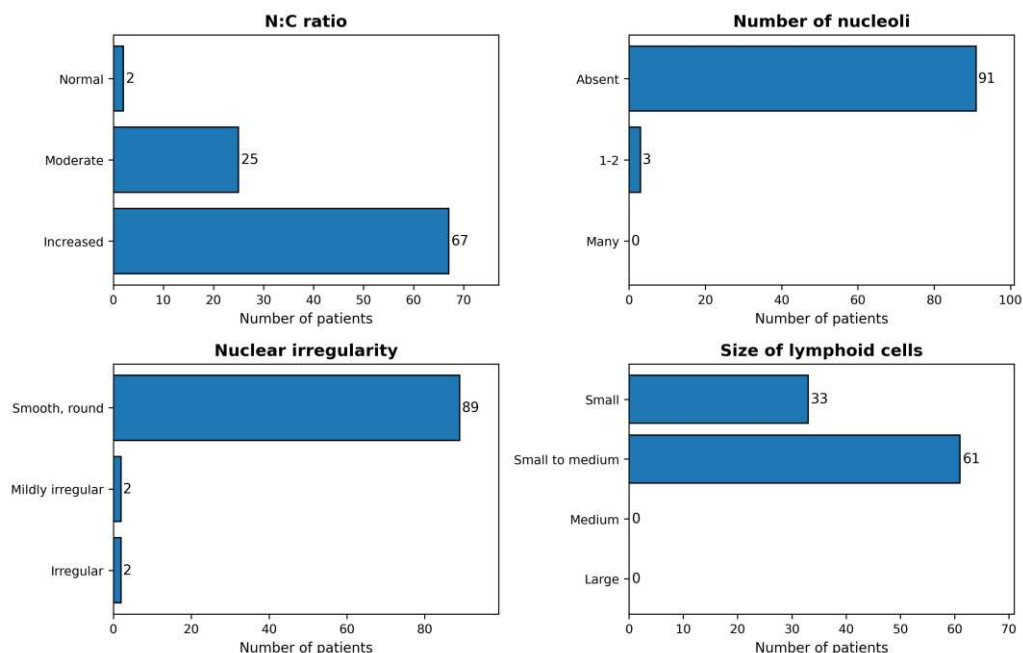


Figure 1: Morphological profile of classical CLL

All cases expressed CD19 and CD5 and were negative for CD10. CD23 was positive in 88 (93.6%), CD200 in 87 (92.6%) and CD43 in 84 (89.4%) (Figure 2). FMC7 was negative in 80 (85.1%) and CD79b was negative in 67 (71.3%). sI kappa restriction was seen in 66 (70.2%) and sI lambda restriction in 28 (29.8%) (Table 2).

Table 2. Morphological and qualitative immunophenotypic profile

Domain	Variable / category	n/N (%)
Morphology	N:C ratio increased	67/94 (71.3)
Morphology	Nucleoli absent	91/94 (96.8)
Morphology	Smooth, round nuclear contour	89/94 (94.7)
Morphology	Small cells	33/94 (35.1)
Morphology	Small-to-medium cells	61/94 (64.9)
Morphology	Smudge cells	94/94 (100.0)
Immunophenotype	CD19 positive	94/94 (100.0)
Immunophenotype	CD5 positive	94/94 (100.0)
Immunophenotype	CD10 negative	94/94 (100.0)
Immunophenotype	CD23 positive	88/94 (93.6)
Immunophenotype	CD200 positive	87/94 (92.6)
Immunophenotype	CD43 positive	84/94 (89.4)
Immunophenotype	FMC7 negative	80/94 (85.1)
Immunophenotype	CD79b negative	67/94 (71.3)
Light-chain restriction	sI kappa-restricted	66/94 (70.2)
Light-chain restriction	sI lambda-restricted	28/94 (29.8)

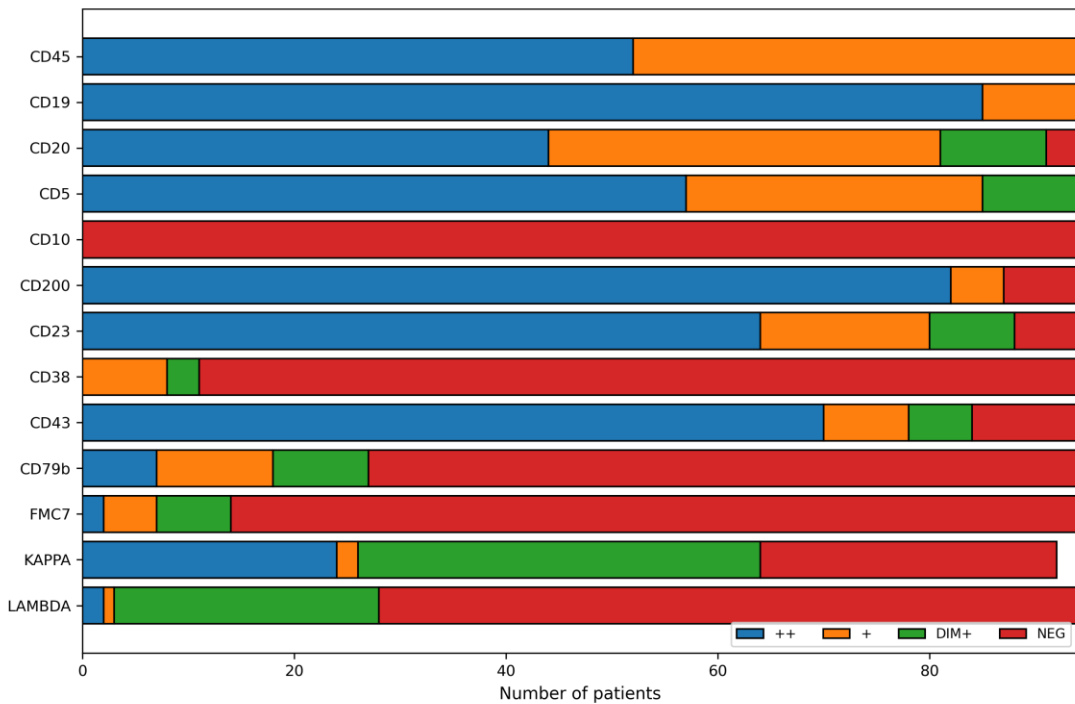


Figure 2: Qualitative immunophenotypic expression profile

Fluorescence-intensity profile and CD45 index: Median CD45 index by mean FI was 0.47 (IQR 0.37–0.60), and by median FI was 0.46 (IQR 0.37–0.63), indicating reduced CD45 intensity on neoplastic B cells relative to internal T cells in many cases (Table 3).

Table 3. Fluorescence-intensity metrics and CD45 index

Marker	Mean-FI median (IQR)	Median-FI median (IQR)
CD45	2840.5 (2258.0–3930.0)	2595.5 (2079.0–3776.0)
CD19	6825.0 (4817.8–8923.0)	6199.0 (4293.5–8048.0)
CD20	3334.0 (2506.2–5736.0)	1956.5 (1265.8–3038.8)
CD5	3282.0 (1657.5–5612.2)	2989.5 (1339.2–4669.8)
CD10	-32.5 (-64.0–5.0)	-44.0 (-63.0–8.0)
CD38	361.5 (255.5–542.0)	293.5 (197.0–388.5)
CD23	1107.0 (539.0–1490.0)	817.0 (460.2–1166.8)
CD79b	377.5 (106.2–509.0)	150.0 (43.0–327.0)
CD200	3934.0 (2607.0–5213.0)	3281.5 (2415.0–4489.2)
CD43	2919.0 (2213.8–4130.0)	2725.0 (1873.5–3682.0)
Kappa	1145.5 (376.5–3916.5)	594.0 (139.2–1929.8)
Lambda	325.5 (149.0–760.0)	137.0 (73.2–418.5)
FMC7	138.5 (75.5–281.2)	103.0 (60.0–184.0)
CD45 T cells (comparator)	6589.5 (4996.0–7621.8)	6023.5 (4610.0–7201.2)
CD45 index	0.47 (0.37–0.60)	0.46 (0.37–0.63)

Matutes-score subgroup analysis: All 94 cases had an updated Matutes score: 31 (33.0%) scored 3/5, 39 (41.5%) scored 4/5 and 24 (25.5%) scored 5/5. Significant differences were observed for age ($p=0.0139$), CD19-gated B-cell percentage ($p=0.0151$), CD4/CD8 ratio ($p=0.0344$), CD45 index by mean FI ($p=0.0010$) and CD45 index by median FI ($p=0.0100$). The median CD45 index by mean FI decreased from 0.57 in the 3/5 group to 0.44 in the 4/5 group and 0.39 in the 5/5 group (Figure 3). Hepatomegaly differed across score groups (exact $p=0.005$), as did CD200 positivity ($p=0.017$) and CD38 positivity ($p<0.001$). The assessed morphological variables did not differ significantly (Table 4).

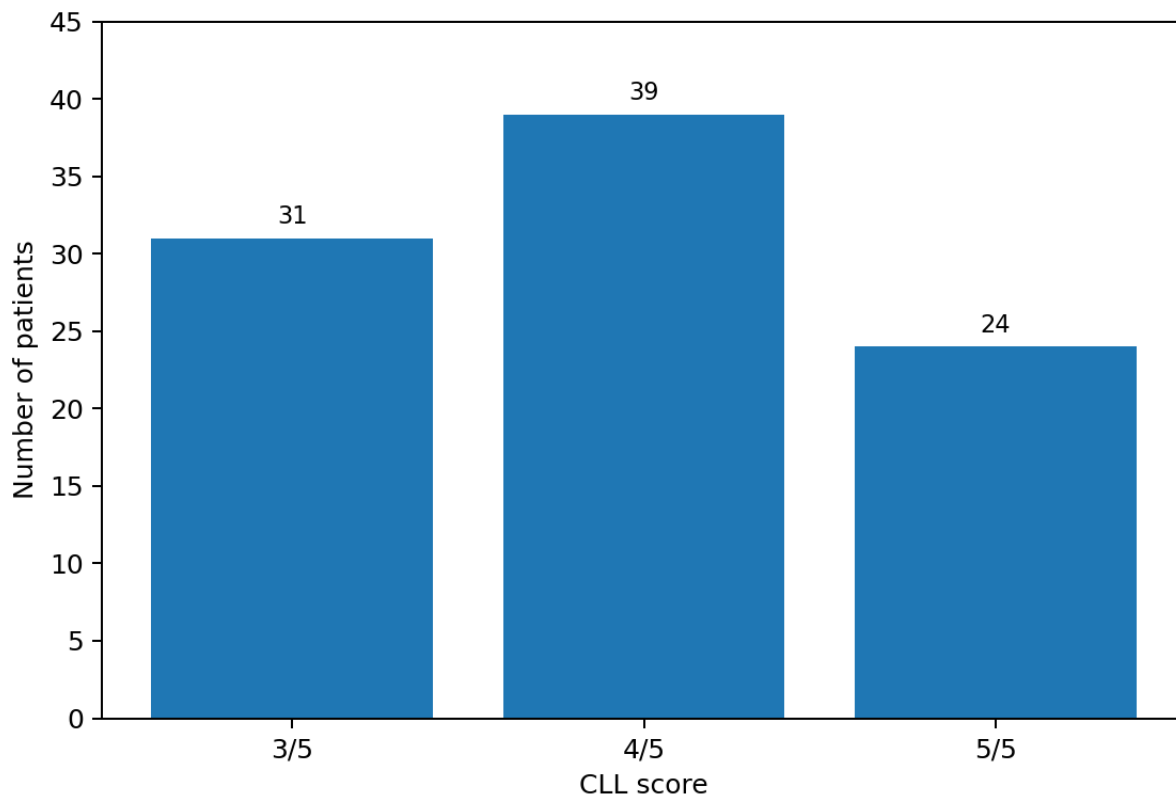


Figure 3: Distribution of CLL immunophenotypic score

Table 4. Clinical, haematological, morphological and selected immunophenotypic characteristics across Matutes-score groups

Variable	Score 3/5 (n=31)	Score 4/5 (n=39)	Score 5/5 (n=24)	p value
Age, years	70.0 (64.0–74.5)	65.0 (62.0–71.0)	59.0 (54.0–66.8)	0.0139
WBC (/μL)	24,730 (16,880–49,600)	29,450 (19,600–78,820)	24,570 (21,407.5–27,580)	0.2110
Absolute lymphocyte count (/μL)	18,550 (12,250–27,250)	23,440 (13,210–70,305)	18,830 (13,222.5–21,985)	0.1900
Haemoglobin (g/dL)	12.9 (12.4–14.7)	12.5 (11.1–14.3)	13.3 (12.1–14.1)	0.3394
Platelet count ($\times 10^9/L$)	213.0 (158.0–271.5)	188.0 (127.5–242.0)	155.0 (85.0–239.8)	0.0931
CD19-gated B-cell percentage (%)	65.4 (49.0–71.7)	74.1 (60.3–76.8)	55.3 (46.4–74.0)	0.0151
CD3-gated T-cell percentage (%)	27.3 (19.2–33.9)	16.6 (15.1–28.2)	20.2 (10.5–42.7)	0.1421
CD4/CD8 ratio	1.50 (1.35–2.10)	1.40 (1.30–1.80)	1.20 (0.85–1.60)	0.0344
CD45 index by mean FI	0.57 (0.52–0.67)	0.44 (0.36–0.55)	0.39 (0.34–0.49)	0.0010
CD45 index by median FI	0.57 (0.45–0.64)	0.49 (0.42–0.68)	0.37 (0.31–0.46)	0.0100
Pallor, n (%)	8 (25.8)	10 (25.6)	2 (8.3)	0.204
Splenomegaly, n (%)	3 (9.7)	4 (10.3)	6 (25.0)	0.210
Hepatomegaly, n (%)	0 (0.0)	1 (2.6)	5 (20.8)	0.005
Lymphadenopathy, n (%)	11 (35.5)	18 (46.2)	6 (25.0)	0.233
N:C ratio increased, n (%)	21 (67.7)	28 (71.8)	18 (75.0)	0.889
Nucleoli absent, n (%)	31 (100.0)	36 (92.3)	23 (95.8)	0.477
Smooth, round nuclear contour, n (%)	31 (100.0)	35 (89.7)	23 (95.8)	0.499
CD200 positive, n (%)	26 (83.9)	39 (100.0)	22 (91.7)	0.017
CD43 positive, n (%)	26 (83.9)	35 (89.7)	23 (95.8)	0.361
CD38 positive, n (%)	9 (29.0)	2 (5.1)	0 (0.0)	<0.001

Selected correlations: WBC correlated strongly with absolute lymphocyte count (ALC) (Spearman rho=0.960, $p<0.0001$), absolute lymphocyte count with CD19-gated B-cell count (rho=0.826, $p<0.0001$), and the mean-FI and median-FI CD45 indices with each other (rho=0.676, $p<0.0001$) shown in Table 5. A representative flow-cytometric case is shown in Fig. 4.

Table 5. Selected Spearman correlations

Variable pair	n	Spearman rho	p
WBC vs ALC	94	0.960	<0.0001
ALC vs CD19-gated B-cell count	94	0.826	<0.0001
CD45 index (mean FI) vs CD45 index (median FI)	94	0.676	<0.0001
CD4+ T-cell count vs CD8+ T-cell count	94	0.628	<0.0001
B-cell count vs T-cell count	94	0.246	0.0167

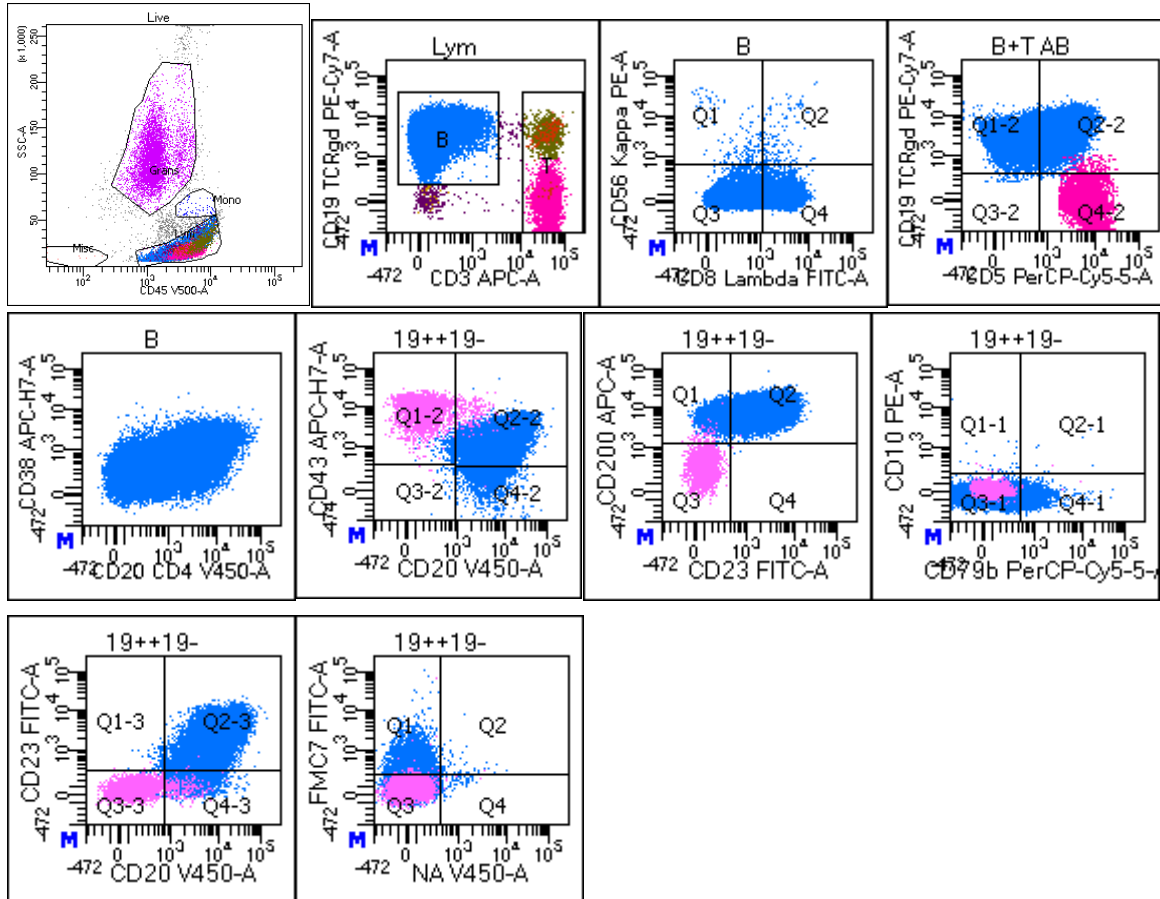


Figure 1: Flow cytometry analysis of a case CLL

Discussion

This study describes 94 patients with classical CLL from a single tertiary-care haematology centre in Sri Lanka. The age distribution and male predominance were consistent with the known epidemiology of CLL [1-3]. Persistent lymphocytosis was nearly universal, and the full blood count pattern was strongly lymphocyte-predominant.

Morphology supported the diagnosis of classical CLL. Most cases showed increased N:C ratio, absent nucleoli, smooth round nuclei, small to small-medium lymphoid cells and smudge cells, consistent with typical CLL morphology [1,2,15]. The qualitative immunophenotype also matched the expected pattern, with universal CD19/CD5 expression, frequent CD23/CD200/CD43 expression, universal CD10 negativity and commonly weak or negative FMC7 and CD79b [4-14].

The CD45-index findings provide an internally normalized measure that may complement raw FI values. A value below 1.0 indicates lower CD45 intensity on neoplastic B cells than on internal T cells. The progressive decrease in the CD45 index

across higher Matutes-score groups suggests a relationship with increasing immunophenotypic typicality, although this observation requires validation and should not be interpreted as a universal diagnostic cut-off.

Among the 94 cases stratified into Matutes 3/5, 4/5 and 5/5 groups, most clinical and morphological variables remained similar. Hepatomegaly was more frequent in the 5/5 group, but this observation was based on only six patients and should be interpreted cautiously. CD200 positivity varied across groups, whereas CD38 positivity was most frequent in the 3/5 group and absent in the 5/5 group.

This study has limitations. It was conducted at a single centre and may be affected by referral bias. Molecular and cytogenetic results were not available, preventing correlation with IGHV mutation status, TP53 disruption, del(17p), del(11q), trisomy 12 or del(13q). Inter-laboratory standardization of FI was not performed; therefore, CD45-index values should be regarded as locally derived supportive metrics rather than transferable cut-offs. The study did not include an independent non-CLL comparator group or an external validation cohort, so diagnostic accuracy, disease discrimination and clinically useful CD45-index cut-offs could not be established.

Conclusion

This study described the clinical, haematological, morphological and immunophenotypic characteristics of 94 patients with classical CLL in Sri Lanka. Matutes-score subgroup analysis showed differences in age, selected lymphocyte-subset parameters, CD45 index, hepatomegaly, CD200 expression and CD38 expression, while morphological characteristics remained largely similar across groups. The CD45 index varied significantly among Matutes-score groups, suggesting a possible association with immunophenotypic patterns of classical CLL. However, these findings are exploratory and require further validation in larger, multicentre studies before their diagnostic significance can be established.

Statements and Declarations

Funding: This study was not supported by any funding.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Review Committee of the University of Sri Jayewardenepura (ERC 08/23).

Informed consent: Informed consent was obtained from all individual participants included in the study.

Author contributions: Conceptualization, methodology, software, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, and visualization: TB and CK. Supervision, project administration, writing—review and editing, and visualization: CM, NF, CG, SJ and CK. All authors read and approved the final manuscript.

Data availability: Data supporting the findings are available within the article or from the corresponding author on reasonable request.

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