

CASE REPORT

Aberrant CD3 Expression in Mature B-Cell Chronic Lymphocytic Leukemia: An Observational Analytic Case Study on Lineage Promiscuity, Flow Cytometric Diagnostic Disambiguation, and Longitudinal Trajectory

I Gusti Ngurah Bayu Paramartha^{1*}, I Made Sila Darmana², I Dewa Komang Agung Cahyadi², I Made Dwi Payana²

¹Intern Physician, Department of Clinical Pathology, Sanjiwani General Hospital, Gianyar, Indonesia

²Staff Pathologist, Department of Clinical Pathology, Sanjiwani General Hospital, Gianyar, Indonesia

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*Corresponding author

I Gusti Ngurah Bayu Paramartha

E-mail: ignbayuparamartha@gmail.com

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ABSTRACT

Background: Aberrant CD3 expression in B-cell chronic lymphocytic leukemia (CLL) can complicate lineage assignment.

Objective: To describe the hematological trajectory, marrow findings, and flow cytometric evaluation of a patient reported to have CD3-positive B-cell CLL.

Methods: A 64-year-old woman with asthenia, constitutional symptoms, and hepatosplenomegaly underwent serial blood counts over 14 days, peripheral blood and marrow cytomorphological assessment, and multiparametric flow cytometry. Absolute and relative changes were calculated descriptively.

Results: Leukocyte counts increased from 58.63 to $65.29 \times 10^3/\mu\text{L}$ (+11.36%), with lymphocyte fractions of 83.4% and 88.7%. Hemoglobin decreased from 9.8 to 9.3 g/dL; platelet counts increased from 111 to $140 \times 10^3/\mu\text{L}$. Peripheral blood showed mature lymphocytes and smudge cells. Marrow assessment reported 88% mature lymphocytes and an M:E ratio of 3:1. Flow cytometry showed CD19, CD20, and CD79a positivity with dim surface CD3 (MFI 2,170) and cytoplasmic CD3 (MFI 1,983); CD34 and MPO were reported as negative.

Conclusion: The case illustrates the importance of interpreting aberrant CD3 expression within a composite morphological and immunophenotypic assessment. The reported diagnosis and differential exclusions require correlation with the complete diagnostic record.

1. Introduction

Leukemias encompass a biologically diverse and complex spectrum of hematological malignancies defined by the autonomous clonal proliferation, arrested or disordered maturation, and progressive pathological accumulation of transformed hematopoietic leukocytes across the bone marrow, peripheral circulation, and reticuloendothelial tissues.¹ Within the chronic lymphoid neoplasm categorization, chronic lymphocytic leukemia (CLL), which exists along a biological continuum with small lymphocytic lymphoma (SLL) based primarily on peripheral blood leukemic involvement versus tissue-predominant nodal localization, represents the most frequently diagnosed mature lymphoid leukemia in adult populations across Western and global registries.² Comprehensive epidemiological surveillance data published by the Surveillance, Epidemiology, and End Results (SEER) program, European cancer monitoring networks, and updated international registries demonstrate an incidence curve that ascends exponentially with advancing chronological age, displaying a median age at diagnosis between 70 and 72 years, with fewer than 10% of newly diagnosed patients presenting prior to the age of 45 years.³ Furthermore, large-scale population registries consistently document a persistent male preponderance across diverse geographic and ethnic

cohorts, sustaining an approximate male-to-female incidence ratio ranging between 1.7:1 and 2.0:1.⁴ Global oncological metrics compiled by the International Agency for Research on Cancer (IARC) and GLOBOCAN record an escalating worldwide disease burden exceeding 100,000 incident cases annually, with developing countries and Southeast Asian populations recording rising recognition due to expanded access to automated multi-angle laser hematology analyzers and standardized multicolor flow cytometry laboratories.⁵

At the cellular, immunological, and molecular level, chronic lymphocytic leukemia originates from the malignant monoclonal expansion of mature, antigen-experienced CD5-positive B-lymphocytes that have escaped physiological apoptotic surveillance and homeostatic clearance mechanisms.⁶ In strict accordance with the updated international consensus guidelines established by the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) and the 5th edition of the World Health Organization Classification of Haematolymphoid Tumours (WHO-HAEM5), the formal diagnosis of CLL requires the sustained presence of at least $5.0 \times 10^9/\text{L}$ ($5.0 \times 10^3/\mu\text{L}$) monoclonal B-lymphocytes in the peripheral blood persisting over a minimum observation period of three consecutive months.⁷ Lineage fidelity and monoclonality are definitively resolved via multiparametric flow cytometric immunophenotyping,

which classically describes a highly stereotyped and reproducible antigenic signature: bright co-expression of pan-B-cell surface determinants CD19, CD20, and CD23, accompanied by the pathognomonic co-expression of the pan-T-cell surface glycoprotein CD5, alongside restricted surface immunoglobulin light chain (monoclonal kappa or lambda) expression.⁸ Under standard physiological conditions, the surface expression density of CD20 and surface immunoglobulin (sIg) on neoplastic CLL cells is characteristically dim or weak relative to normal circulating mature B-cells, while the complement regulatory protein CD23 is moderately to brightly positive, providing a decisive phenotypic distinction from mantle cell lymphoma and marginal zone B-cell lymphomas.⁹

Despite the established stability of standard diagnostic criteria, the biological phenomenon of lineage promiscuity—manifesting as the unexpected aberrant expression of cross-lineage antigenic markers—represents a profound diagnostic and biological dilemma in clinical hematopathology.¹⁰ During normal physiological lymphopoiesis, early lymphoid progenitors commit irreversibly to the B-cell lineage under the strict transcriptional control of non-redundant transcription factors, most notably PAX5 (paired box protein 5) and EBF1 (early B-cell factor 1), which simultaneously activate B-cell receptor (BCR) signaling components while actively repressing alternative T-cell and myeloid genetic programs.¹¹ Conversely, T-cell lineage specification requires continuous NOTCH1 signaling and GATA3 activation within the thymic microenvironment to unlock the multi-subunit CD3 and T-cell receptor (TCR) loci while enforcing permanent silencing of B-lineage genes.¹² The aberrant expression of non-canonical pan-T-cell markers, such as CD2, CD3, CD7, or CD8, is rare in mature B-cell neoplasms, occurring in fewer than 1% to 3% of thoroughly evaluated CLL cohorts globally.¹³

Among these rare cross-lineage aberrancies, the aberrant expression of CD3 represents an unusual immunophenotypic finding.¹⁴ Because CD3 (composed of invariant gamma, delta, epsilon, and zeta chains) is structurally and functionally bound to the T-cell antigen receptor complex and serves as the primary signal-transducing component of T-lymphocytes, its detection on a monoclonal B-cell population poses acute diagnostic dilemmas.¹⁵ Most critically, the co-expression of CD3 on a leukemic clone presenting with hyperleukocytosis and hepatosplenomegaly can easily be misinterpreted as an aggressive mature T-cell malignancy, such as T-cell prolymphocytic leukemia (T-PLL), or an ambiguous-lineage neoplasm, such as mixed-phenotype acute leukemia (MPAL B/T), potentially exposing patients to inappropriately aggressive and toxic therapeutic protocols.¹⁶

The prognostic and therapeutic implications of aberrant cross-lineage marker expression in chronic lymphocytic leukemia remain an unresolved subject of debate in the international literature.¹⁷ While some retrospective analyses have suggested that aberrant antigen expression reflects underlying genomic instability, complex cytogenetic karyotypes, and adverse clinical progression, other series have demonstrated an entirely indolent clinical course with sustained therapeutic responsiveness comparable to canonical CLL phenotypes.¹⁸ Systematic observational studies documenting the longitudinal trajectory of peripheral blood parameters, bone marrow cellular replacement, cytomorphometric fragility indices, and sequential flow cytometric differentiation of CD3-aberrant CLL from hospital presentation to therapeutic planning are limited, particularly from developing Southeast Asian healthcare settings.¹⁹

To address this knowledge gap, this observational analytic case study was conducted to provide a detailed investigation of a patient presenting with marked hyperleukocytosis, severe bone marrow replacement, and an aberrant CD3-positive mature B-cell chronic lymphocytic leukemia phenotype. Through prospective multiple-time-point hematological tracking, cytomorphological analysis of peripheral blood and marrow aspirates, and rigorous multiparametric flow cytometric gating, this study aimed to describe the longitudinal trajectory of hematological indices, establish an evidence-based algorithm for flow cytometric diagnostic differentiation, evaluate the biological mechanisms underpinning cross-lineage T-cell marker infidelity, and provide clinical guidance regarding chemoimmunotherapy stratification in lineage-promiscuous lymphoid malignancies.

2. Methods

2.1. Study Design and Epidemiological Framework

This investigation was structured as an observational analytic case study conducted within the Department of Clinical Pathology in close clinical collaboration with the Hematology and Medical Oncology Unit of the Department of Internal Medicine at Sanjiwani General Hospital, Gianyar, Bali, Indonesia. An observational analytic design was implemented to systematically track, quantify, and analyze the longitudinal trajectory of peripheral blood parameters, cytomorphological alterations, bone marrow cellular replacement, and flow cytometric immunophenotypic profiles in a patient diagnosed with an rare immunophenotypic variant of mature B-cell chronic lymphocytic leukemia characterized by aberrant pan-T-cell CD3 co-expression. The analytical framework was developed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) consensus guidelines and the Consensus-based Clinical Case Reporting Guideline Development (CARE) standards, ensuring methodological transparency, reproducible data acquisition, and clinical correlation.²⁰

2.2. Ethical Governance and Participant Consent

The protocol for this observational analytic investigation underwent comprehensive institutional ethical review and was formally granted full ethical clearance by the CMHC Research Center Ethics Committee, Indonesia (Formal Protocol Approval Number: 2026/4182). All investigational procedures, biological specimen collections, cytomorphometric evaluations, and data processing methodologies were executed in strict adherence to the ethical tenets codified in the 1964 Declaration of Helsinki and its subsequent revisions, notably the 2013 Fortaleza amendments. Prior to the initiation of advanced cytometric profiling and bone marrow aspiration, detailed, written informed consent was formally obtained from the patient and her legal surrogate. The consent protocol explicitly authorized the longitudinal collection, analytical archiving, scientific interpretation, and academic dissemination of all diagnostic findings, photographic cytomorphological micrographs, and clinical data under strict anonymization protocols compliant with the International Committee of Medical Journal Editors (ICMJE) and the Committee on Publication Ethics (COPE).²¹

2.3. Case Eligibility and Diagnostic Ascertainment

Diagnostic eligibility and disease classification were adjudicated according to the consensus criteria established by the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) updated guidelines and the 5th edition of the World Health Organization Classification of

Haematolymphoid Tumours (WHO-HAEM5). Diagnostic ascertainment required the definitive fulfillment of three mandatory criteria: (1) absolute peripheral blood B-cell lymphocytosis exceeding $5.0 \times 10^9/L$ ($5.0 \times 10^3/\mu L$); (2) characteristic light microscopic cytomorphology on Wright-Giemsa stained blood smears demonstrating small, mature lymphocytes with clumped chromatin and prominent Gumprecht nuclear shadows (smudge cells); and (3) multiparametric flow cytometric immunophenotyping of peripheral blood and bone marrow aspirate demonstrating monoclonal restriction and committed B-cell lineage markers (CD19+, CD20+, CD79a+), accompanied by the systematic exclusion of competing acute leukemias, blast crises, or non-Hodgkin mature B-cell lymphomas.²²

2.4. Longitudinal Observation Protocol and Analytical Measurements

Longitudinal tracking of hematological and clinical chemistry parameters was implemented across a sequential, protocolized multiple-time-point timeline: Baseline / Admission (Day 1: 01/21/2026), Inpatient Stabilization / Discharge (Day 11: 01/31/2026), and Pre-Chemotherapy Surveillance (Day 14: 02/03/2026). Complete blood count (CBC) profiling was performed on fresh whole blood anticoagulated with tripotassium ethylenediaminetetraacetic acid (K3-EDTA) using an automated multi-angle laser scatter and impedance hematology analyzer (Sysmex XN-Series, Sysmex Corporation, Kobe, Japan). Measured parameters encompassed total leukocyte count, five-part differential leukocyte fractions (neutrophils, lymphocytes, monocytes, eosinophils, basophils), erythrocyte count, hemoglobin concentration, hematocrit, red cell indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], red cell distribution width [RDW-CV]), and automated platelet counts. Peripheral blood smears and bone marrow aspirate smears were prepared immediately, fixed with absolute methanol, and stained using standardized automated Wright-Giemsa protocols. Cytomorphological evaluation, 500-cell differential counting, and smudge cell quantification were conducted independently by two board-certified clinical pathologists using high-resolution brightfield optical microscopy (Olympus BX53, Olympus Corporation, Tokyo, Japan) equipped with plan-apochromatic oil-immersion objectives at 100× magnification. Comprehensive serum chemistry, including blood urea nitrogen (BUN), serum creatinine, estimated glomerular filtration rate (eGFR by CKD-EPI 2021 formula), hepatic transaminases (AST, ALT), serum electrolytes (sodium, potassium, chloride), and random blood glucose, was evaluated on a fully automated clinical chemistry platform. Primary and secondary hemostatic function was assessed via bleeding time (Duke method) and whole-blood clotting time (Lee-White method).²³

2.5. Multiparametric Flow Cytometry Immunophenotyping

Bone marrow aspirate obtained via sterile posterior superior iliac spine (PSIS) trephine and aspiration biopsy on Day 14 was immediately transferred into sodium heparin collection tubes for flow cytometric immunophenotyping. Analysis was performed on a multi-laser, clinical flow cytometer (BD FACSCanto II / Lyric platform, Becton Dickinson, San Jose, CA, USA) calibrated daily using standardized fluorescent setup beads. The antibody panel incorporated an extensive fluorochrome-conjugated monoclonal antibody repertoire designed to interrogate lineage commitment, maturity, and cross-lineage aberrancy: pan-leukocyte gating (CD45 V500-C-A), B-cell lineage

markers (CD19 PE-Cy7-A, CD20 V450-A, CD79a PE-A), hematopoietic progenitor markers (CD34 PerCP-Cy5.5-A), myeloid differentiation markers (anti-myeloperoxidase [MPO] FITC-A), pan-T-cell markers (surface CD3 APC-H7-A, cytoplasmic cyCD3 V450-A, CD7 APC-A), and T-cell subset markers (CD4 PerCP-Cy5.5-A, CD8 APC-H7-A), along with human leukocyte antigen HLA-DR (FITC-A) and germinal center / pre-B marker CD10 (APC-A). Leukocyte singlets and mature lymphoid populations were described using forward scatter area (FSC-A) versus height (FSC-H) gating, followed by CD45 versus side scatter area (SSC-A) gating. A minimum of 50,000 cellular events were acquired per tube. Antigen expression intensity was quantified using mean fluorescence intensity (MFI) and percentage positivity calculated relative to matched isotype-negative control thresholds. An antigen was classified as aberrantly expressed when distinct fluorescent clustering exceeded 20% of the gated monoclonal population above baseline negative controls.²⁴

2.6. Biostatistical and Trajectory Analysis

All numerical datasets, serial hemogram metrics, and cytometric data were compiled systematically. Temporal dynamics across the longitudinal monitoring window were quantified by calculating the absolute trajectory difference (Δ = Value_{Day14} - Value_{Day1}) and the relative percentage change ($\Delta\%$ = [(Value_{Day14} - Value_{Day1}) / Value_{Day1}] × 100). Cytomorphological data were subjected to descriptive statistical evaluation, with morphometric variables presented as mean ± standard deviation. Comparative benchmarking was executed against international reference cohorts reporting aberrant T-cell antigen expression in mature B-cell neoplasms to contextualize diagnostic sensitivity, prognostic correlations, and therapeutic outcomes.²⁵

3. Results

3.1. Clinical Presentation and Baseline Physical Evaluation

A 64-year-old female patient presented to the Emergency Department of Sanjiwani General Hospital with a chief complaint of profound, generalized physical asthenia that had acutely worsened over the 24 hours preceding presentation. She reported a protracted clinical history spanning several years characterized by progressive, unprovoked fatigue during ordinary daily activities, recurrent episodes of exertional dyspnea, and intermittent, unprovoked low-grade fevers occurring without identifiable infectious focal signs. One day prior to admission, the patient developed acute gastrointestinal distress characterized by five episodes of watery, non-mucoid, non-bloody diarrhea, accompanied by complete functional exhaustion that rendered her unable to perform basic activities of daily living. Her medical history was negative for pre-existing cardiovascular disease, diabetes mellitus, known autoimmune disorders, or chronic medication usage. She was a homemaker whose baseline functional status had experienced insidious deterioration over the preceding 24 months.

Upon admission physical examination, the patient appeared moderately ill, conscious, and oriented. Baseline physiological vital signs documented a blood pressure of 150/100 mmHg, fulfilling criteria for Grade 1 arterial hypertension, a sinus tachycardia with a pulse rate of 112 beats per minute, a respiratory rate of 22 breaths per minute, an oxygen saturation of 98% on ambient air, and a body temperature of 36.5 °C. Head and neck examination revealed marked conjunctival pallor bilaterally, with no scleral icterus or petechial mucosal hemorrhages. Detailed palpation of all

superficial lymph node basins—including cervical, submandibular, supraclavicular, axillary, and inguinal regions—revealed no palpable peripheral lymphadenopathy. Abdominal physical examination demonstrated normal contour with hyperactive bowel sounds consistent with acute enteritis, accompanied by marked, non-tender organomegaly characterized by distinct hepatomegaly palpable 3 cm below the right costal margin and splenomegaly extending 4 cm below the left costal margin (Hackett Grade II/III). Examination of the extremities revealed no peripheral edema, purpuric eruptions, or joint effusions.

3.2. Longitudinal Hematological Dynamics and Serial Trajectory

The longitudinal complete blood count trajectory across hospitalization and outpatient surveillance is detailed in Table 1, demonstrating an expansion of total leukocyte count from $58.63 \times 10^3/\mu\text{L}$ on Day 1 (01/21/2026) to $61.39 \times 10^3/\mu\text{L}$ on Day 11 (01/31/2026), and culminating at $65.29 \times 10^3/\mu\text{L}$ on Day 14 (02/03/2026). This progressive elevation corresponded to an absolute trajectory shift of $\Delta = +6.66 \times 10^3/\mu\text{L}$ and a relative longitudinal expansion of $\Delta\% = +11.36\%$ over the surveillance period. Leukocyte differential tracking documented that this marked hyperleukocytosis was overwhelmingly driven by the mature lymphocyte fraction, which constituted 83.4% (absolute lymphocyte count: $48.90 \times 10^3/\mu\text{L}$) on Day 1, climbing to 89.1% ($54.70 \times 10^3/\mu\text{L}$) on Day 11, and persisting at 88.7% ($57.91 \times 10^3/\mu\text{L}$) on Day 14, representing a relative lymphocytosis expansion of $\Delta\% =$

+18.43%. Conversely, relative granulocyte suppression was observed, with neutrophil percentages declining from 13.3% (absolute count: $7.80 \times 10^3/\mu\text{L}$) to 9.6% ($6.27 \times 10^3/\mu\text{L}$), reflecting an absolute decline of $\Delta = -1.53 \times 10^3/\mu\text{L}$ ($\Delta\% = -19.62\%$). Monocytes, eosinophils, and basophils remained profoundly suppressed throughout.

Erythrocyte kinetics demonstrated persistent normocytic normochromic anemia. Baseline hemoglobin concentration of 9.80 g/dL and hematocrit of 31.40% experienced a mild downward drift to 9.30 g/dL ($\Delta = -0.50$ g/dL; $\Delta\% = -5.10\%$) and 29.80% ($\Delta = -1.60\%$; $\Delta\% = -5.10\%$) on Day 14, respectively. Total erythrocyte count mirrored this pattern, shifting from $3.60 \times 10^6/\mu\text{L}$ to $3.42 \times 10^6/\mu\text{L}$. Erythrocyte indices remained strictly within normocytic, normochromic parameters throughout the entire clinical course: MCV demonstrated stability at 87.10 fL on Day 1 and 87.10 fL on Day 14; MCH measured 27.10 pg and 27.20 pg; and MCHC was 31.10 g/dL and 31.30 g/dL. The red cell distribution width (RDW-CV) exhibited minimal variation, measuring 14.40% at baseline and 14.70% on Day 14, ruling out significant concurrent nutritional macrocytosis or microcytic iron deficiency. Platelet counts demonstrated mild baseline thrombocytopenia at $111.00 \times 10^3/\mu\text{L}$ on Day 1, which progressively recovered to $135.00 \times 10^3/\mu\text{L}$ on Day 11 and $140.00 \times 10^3/\mu\text{L}$ on Day 14 ($\Delta = +29.00 \times 10^3/\mu\text{L}$; $\Delta\% = +26.13\%$), remaining mildly below the normal lower reference threshold of $150.00 \times 10^3/\mu\text{L}$.

Table 1. Longitudinal Complete Blood Count, Leukocyte Differential Dynamics, and Erythrocyte Indices Across Hospitalization and Outpatient Surveillance.

Hematological Parameter	Day 1 (01/21/2026)	Day 11 (01/31/2026)	Day 14 (02/03/2026)	Absolute Trajectory (Δ)	Relative Trajectory ($\Delta\%$)	Reference Interval
Total Leukocyte Count ($\times 10^3/\mu\text{L}$)	58.63	61.39	65.29	+6.66	+11.36%	4.0 – 10.0
Absolute Neutrophil Count ($\times 10^3/\mu\text{L}$)	7.80 (13.3%)	6.02 (9.8%)	6.27 (9.6%)	-1.53	-19.62%	2.0 – 7.0 (47 – 80%)
Absolute Lymphocyte Count ($\times 10^3/\mu\text{L}$)	48.90 (83.4%)	54.70 (89.1%)	57.91 (88.7%)	+9.01	+18.43%	1.0 – 3.0 (13 – 40%)
Monocyte Count ($\times 10^3/\mu\text{L}$)	1.88 (3.2%)	0.61 (1.0%)	1.11 (1.7%)	-0.77	-40.96%	0.2 – 1.0 (2 – 11%)
Eosinophil Count ($\times 10^3/\mu\text{L}$)	0.06 (0.1%)	0.06 (0.1%)	0.00 (0.0%)	-0.06	-100.00%	0.0 – 0.5 (0.5 – 5.0%)
Basophil Count ($\times 10^3/\mu\text{L}$)	0.00 (0.0%)	0.00 (0.0%)	0.00 (0.0%)	0.00	0.00%	0.0 – 0.1 (0.0 – 2.0%)
Erythrocyte Count ($\times 10^6/\mu\text{L}$)	3.60	3.51	3.42	-0.18	-5.00%	3.50 – 5.50
Hemoglobin (g/dL)	9.80	9.80	9.30	-0.50	-5.10%	11.0 – 16.0
Hematocrit (%)	31.40	29.40	29.80	-1.60	-5.10%	37.0 – 48.0
Mean Corpuscular Volume (MCV, fL)	87.10	83.70	87.10	0.00	0.00%	80.0 – 100.0
Mean Corpuscular Hemoglobin (MCH, pg)	27.10	27.80	27.20	+0.10	+0.37%	27.0 – 34.0
MCHC (g/dL)	31.10	33.30	31.30	+0.20	+0.64%	32.0 – 36.0
Red Cell Distribution Width (RDW-CV, %)	14.40	14.50	14.70	+0.30	+2.08%	11.0 – 16.0
Platelet Count ($\times 10^3/\mu\text{L}$)	111.00	135.00	140.00	+29.00	+26.13%	150.0 – 450.0

Note: Δ represents the absolute change from baseline (Day 1) to final pre-chemotherapy follow-up (Day 14). $\Delta\%$ represents the relative percentage trajectory calculation: $[(\text{Day 14 Value} - \text{Day 1 Value}) / \text{Day 1 Value}] \times 100$. Absolute leukocyte subset counts were calculated mathematically from total leukocyte counts and differential percentages. MCHC: Mean corpuscular hemoglobin concentration; RDW-CV: Red blood cell distribution width-coefficient of variation.

The cytomorphological hallmarks of this profound lymphocytosis are depicted in Figure 1, where peripheral blood smear examination under 100 $\times$ oil immersion objective confirms a monotonous population of mature small lymphocytes with condensed chromatin and characteristic fragile Gumprecht smudge shadows (quantified at 24.5

smudge remnants per 100 intact leukocytes). Red blood cells exhibited predominantly normocytic normochromic morphology, with mild poikilocytosis consisting of scattered ovalocytes and occasional elliptocytes, alongside rare polychromatophilic cells and an absence of circulating nucleated red blood cells. The leukocyte landscape was

overwhelmingly dominated by small, mature-appearing lymphocytes displaying extreme morphological monotony, accounting for 86% of all counted nucleated cells. These neoplastic lymphocytes possessed high nuclear-to-cytoplasmic (N:C) ratios with a narrow, scant rim of pale basophilic cytoplasm. The cell nuclei were round to slightly

indented, featuring intensely condensed, hyperchromatic clumped chromatin arranged in coarse clumps separated by lighter paracrystalline interstices, reproducing the classic 'soccer-ball' or 'cracked-earth' appearance. Nucleoli were universally inconspicuous or absent. Occasional atypical lymphocytes (2%) were noted.

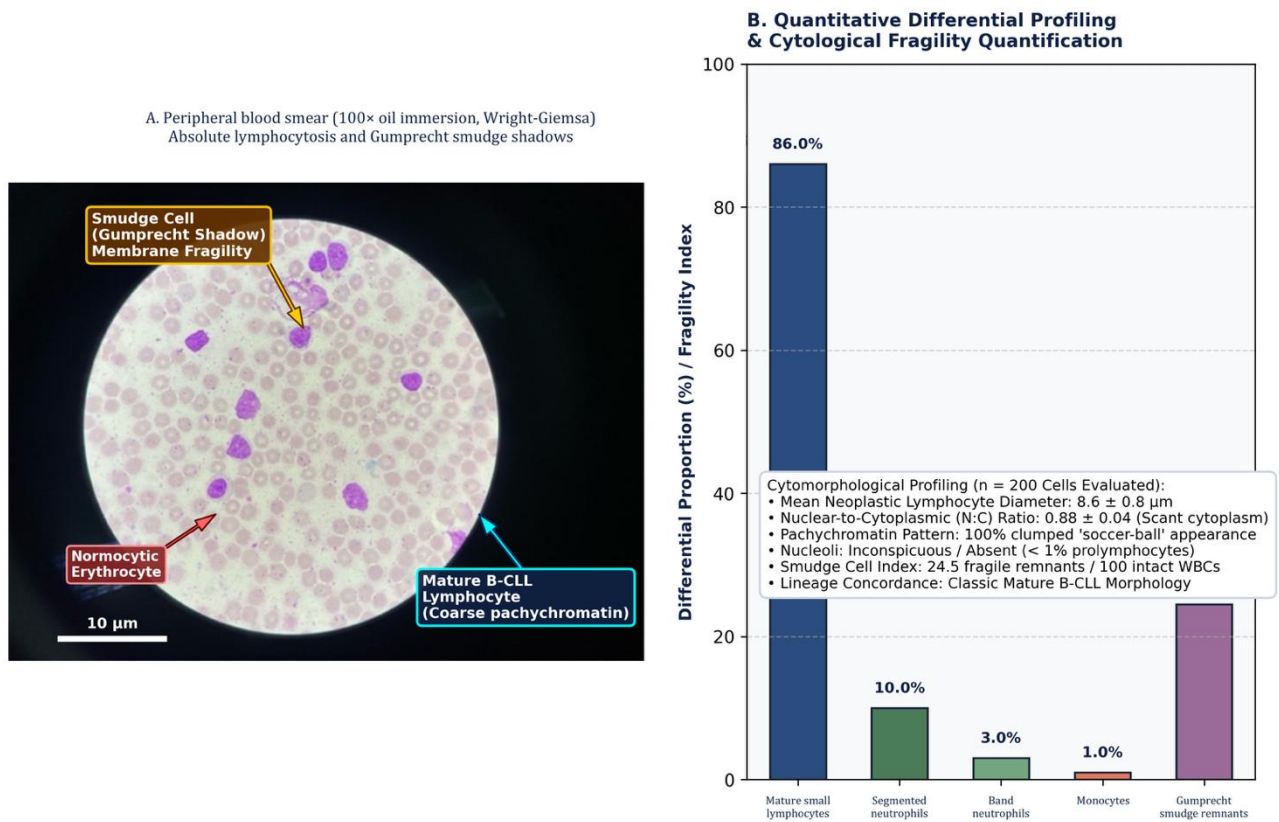


Figure 1. Authentic peripheral blood smear cytomorphology under 100× oil immersion objective (Wright-Giemsa stain) demonstrating marked absolute lymphocytosis, monotonous mature lymphocytes with clumped chromatin, and diagnostic Gumprecht smudge shadows, alongside quantitative cytomorphometric profiling.

Serial comprehensive serum biochemistry, renal kinetics, hepatic transaminases, electrolytes, and coagulation parameters are summarized in Table 2, showing the prompt resolution of baseline prerenal azotemia. On Day 1, biochemical evaluation revealed mild prerenal azotemia secondary to dehydration from acute diarrhea, characterized by an elevated blood urea nitrogen of 57.20 mg/dL, with a normal baseline serum creatinine of 0.85 mg/dL and an eGFR of 76.00 mL/min/1.73 m². Following targeted crystalloid rehydration therapy, repeat testing on Day 11 demonstrated complete biochemical resolution of prerenal impairment, with BUN declining to 36.50 mg/dL ($\Delta = -20.70 \text{ mg/dL}$; $\Delta\% = -36.19\%$), serum creatinine falling to 0.69 mg/dL ($\Delta = -0.16 \text{ mg/dL}$; $\Delta\% = -18.82\%$), and eGFR improving to 97.00 mL/min/1.73 m² ($\Delta = +21.00 \text{ mL/min/1.73 m}^2$; $\Delta\% = +27.63\%$). Baseline random blood glucose was moderately

elevated at 183.00 mg/dL, consistent with physiological stress hyperglycemia, which normalized to 112.00 mg/dL without hypoglycemic pharmacotherapy. Hepatic transaminases were normal throughout, with AST measuring 26.00 U/L on Day 1 and 20.00 U/L on Day 11, while ALT measured 7.00 U/L and 9.00 U/L, confirming intact hepatocellular integrity without leukemic hepatic cytolysis. Serum electrolytes confirmed normal homeostasis (sodium 136.00 to 138.00 mmol/L; potassium 4.20 to 4.10 mmol/L; chloride 104.00 to 103.00 mmol/L), effectively excluding spontaneous tumor lysis syndrome. Hemostatic function evaluated on Day 11 documented normal primary microvascular hemostasis with a bleeding time of 2.00 minutes (Duke reference: 2.0 – 6.0 minutes) and intact intrinsic coagulation pathway activity with a clotting time of 8.00 minutes (Lee-White reference: 6.0 – 15.0 minutes).

Table 2. Serial Comprehensive Serum Biochemistry, Renal Kinetics, Hepatic Transaminases, Electrolytes, and Coagulation Cascade.

Biochemical / Coagulation Analyte	Admission (01/21/2026)	Discharge (01/31/2026)	Analytical Shift (Δ)	Biological Reference Interval	Clinical Interpretation
Blood Urea Nitrogen (mg/dL)	57.20	36.50	-20.70 (-36.19%)	15.0 – 43.0	Prerenal azotemia resolved post-hydration
Serum Creatinine (mg/dL)	0.85	0.69	-0.16 (-18.82%)	0.51 – 0.95	Preserved baseline intrinsic glomerular function
eGFR (CKD-EPI, mL/min/1.73 m ²)	76.00	97.00	+21.00 (+27.63%)	≥ 90.0	Restoration of optimal renal filtration

Biochemical / Coagulation Analyte	Admission (01/21/2026)	Discharge (01/31/2026)	Analytical Shift (Δ)	Biological Reference Interval	Clinical Interpretation
Random Blood Glucose (mg/dL)	183.00	112.00	-71.00 (-38.80%)	80.0 – 120.0	Transient stress hyperglycemia resolved
Aspartate Aminotransferase (AST, U/L)	26.00	20.00	-6.00 (-23.08%)	< 31.0	Normal hepatocellular integrity
Alanine Aminotransferase (ALT, U/L)	7.00	9.00	+2.00 (+28.57%)	< 31.0	Normal hepatic parenchyma without cytolysis
Serum Sodium (Na^+ , mmol/L)	136.00	138.00	+2.00 (+1.47%)	135.0 – 147.0	Eunatremic fluid balance
Serum Potassium (K^+ , mmol/L)	4.20	4.10	-0.10 (-2.38%)	3.50 – 5.00	Normokalemia, no spontaneous tumor lysis syndrome
Serum Chloride (Cl^- , mmol/L)	104.00	103.00	-1.00 (-0.96%)	95.0 – 108.0	Normal anion balance
Bleeding Time (Duke method, minutes)	—	2.00	—	2.0 – 6.0	Normal primary microvascular hemostasis
Clotting Time (Lee-White, minutes)	—	8.00	—	6.0 – 15.0	Normal secondary intrinsic coagulation pathway

Note: eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI 2021) equation. Electrolytes and liver transaminases confirmed the absence of acute hepatic infiltration, significant metabolic acidosis, or overt spontaneous tumor lysis syndrome prior to protocolized immunochemotherapy initiation.

3.3. Bone Marrow Aspirate Histomorphology and Cytomorphological Infiltration

Bone marrow aspiration performed on Day 14 from the posterior superior iliac spine yielded abundant cellular fragments and marrow spicules. Low-power bone marrow aspirate histomorphology at 10 $\times$ objective magnification is demonstrated in Figure 2, revealing marked diffuse

hypercellularity exceeding 85% with extensive effacement of normal marrow adipose tissue by monotonous lymphoid infiltration. Detailed cytomorphological examination of the bone marrow aspirate under 100 $\times$ oil immersion objective is visualized in Figure 3, illustrating an overwhelming replacement of hematopoietic marrow by small, mature neoplastic lymphocytes and abundant smudge shadows.

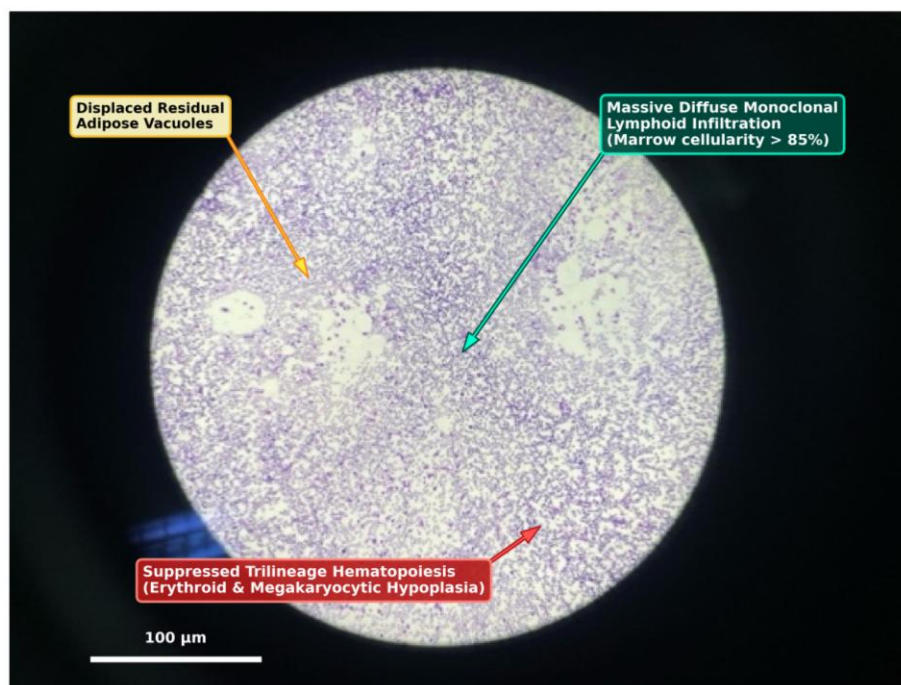


Figure 2. Authentic low-power bone marrow aspirate smear (10 $\times$ objective magnification, Wright-Giemsa stain) illustrating massive hypercellularity (>85%) with diffuse lymphoid replacement of normal hematopoietic tissue.

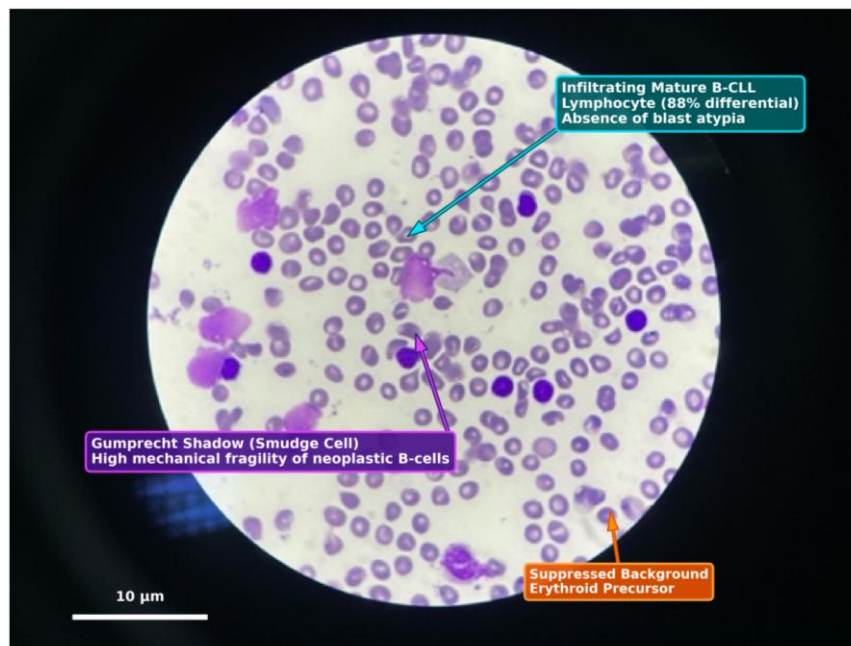


Figure 3. Authentic high-power bone marrow aspirate smear (100× oil immersion objective, Wright-Giemsa stain) showing overwhelming mature lymphocytic infiltration (88%) with condensed chromatin and fragile smudge cells.

The quantitative differential cell count and cellularity indices of the bone marrow aspirate are presented in Table 3, documenting that mature small lymphocytes constituted 88.00% of all nucleated elements, with secondary hypoplasia of the granulocytic series (6.00%), erythroid series (2.00%), and megakaryocytes (1.00%), yielding a preserved myeloid-to-erythroid (M:E) ratio of 3.0:1.0 in the setting of severe marrow crowding. These infiltrating lymphoid elements exhibited cytomorphological features identical to the

circulating peripheral blood clones, displaying dense clumped chromatin, absent nucleoli, and delicate cytoplasmic boundaries. Blasts, promyelocytes, and myeloblasts were entirely absent (<0.5%), definitively excluding acute myeloid leukemia, acute lymphoblastic leukemia, or blast transformation. Smudge cells were abundantly distributed across all marrow smear fields (++), confirming profound mechanical fragility across the marrow compartment.

Table 3. Quantitative Bone Marrow Aspirate Cytomorphological Differential, Cellularity Index, and Lineage Proportions

Cytomorphological Cell Lineage	Observed Differential (%)	Reference Marrow Range (%)	Lineage State / Cytopathological Pattern
Total Marrow Cellularity	Hypercellular (>85%)	Normocellular (30 – 50%)	Marked diffuse leukemic infiltration
Myeloid-to-Erythroid (M:E) Ratio	3.0 : 1.0	1.5 : 1.0 – 3.5 : 1.0	Relative preservation of ratio despite suppression
Mature Small Lymphocytes	88.00%	5.0 – 15.0%	Massive monoclonal lymphoid replacement
Prolymphocytes / Atypical Lymphocytes	2.00%	< 2.0%	Within classical CLL threshold (< 15%)
Myeloid System (Granulocytic Series)	6.00%	50.0 – 70.0%	Secondary hypoplasia due to marrow crowding
Erythroid System (Normoblastic Series)	2.00%	15.0 – 30.0%	Marked suppression correlating with anemia
Megakaryocytic Lineage	1.00%	1.0 – 3.0%	Hypoplastic megakaryopoiesis causing thrombocytopenia
Myeloblasts / Immature Blasts	0.00%	< 5.0%	Absence of acute leukemic transformation / blast crisis
Plasma Cells	1.00%	< 3.0%	Normal background polyclonal plasma cells
Gumprecht Shadows (Smudge Cells)	Abundant (++)	Absent (0)	Hallmark lymphocyte mechanical fragility of CLL

Note: Marrow aspirate evaluation was performed on Day 14 (02/04/2026) from the posterior superior iliac spine (PSIS). Differential counts were performed across 500 nucleated cells on Wright-Giemsa stained bone marrow smears. Cytological criteria strictly confirmed classical B-CLL morphology with condensed 'soccer-ball' chromatin, inconspicuous nucleoli, and low polymphocyte proportion (<2%), ruling out B-prolymphocytic leukemia.

3.4. Multiparametric Flow Cytometry Immunophenotypic Profile and CD3 Aberrancy

Multiparametric flow cytometric immunophenotyping of the bone marrow aspirate is illustrated in Figure 4, which demonstrates unequivocal B-cell lineage commitment alongside aberrant co-expression of the pan-T-cell antigen CD3 under rigorous patient privacy anonymization. Gating on

cell singlets (50,378 events, representing 88.42% of total acquired events) followed by CD45 versus side-scatter (SSC-A) analysis described a dominant, highly homogeneous lymphoid population comprising 49,867 events (87.52% of total cells) characterized by bright CD45 expression (mean fluorescence intensity [MFI]: 5,026) and low side-scatter properties, with minimal residual blast/progenitor events (1,518 events; 2.66%).

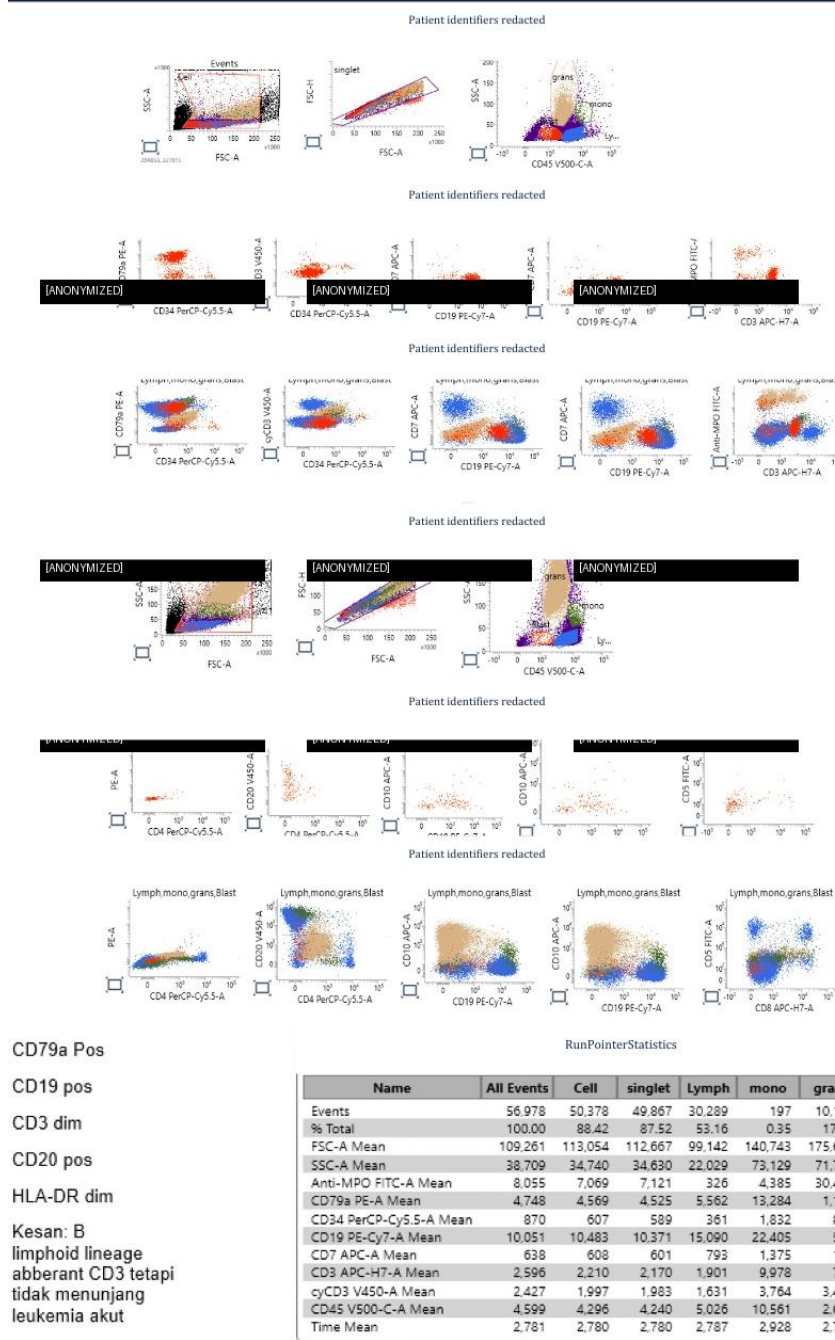


Figure 4. Authentic multiparametric flow cytometry immunophenotyping of bone marrow aspirate demonstrating definitive B-lineage commitment (CD19+, CD20+, CD79a+) with aberrant dim pan-T-cell CD3 co-expression and negative blast markers (CD34-, MPO-). Patient personal health identifiers have been permanently anonymized with black bars in strict compliance with ICMJE/COPE guidelines.

The comprehensive flow cytometric panel, fluorochrome conjugates, mean fluorescence intensities (MFI), and diagnostic interpretations are described in Table 4, confirming strong surface expression of CD19 (PE-Cy7-A MFI: 10,371; >99.0% positive) and moderate surface positivity for CD20 (V450-A positive). Furthermore, the committed B-cell antigen receptor signaling transducer CD79a was intensely positive (PE-A MFI: 4,525), and HLA-DR demonstrated characteristic dim positivity. Markers of immature progenitor arrest were completely negative, with CD34 expressing an MFI of only 589 (<1.0% positive) and the myeloid lineage-defining enzyme anti-myeloperoxidase (anti-MPO) demonstrating unequivocal negativity (<0.5% positive). The early T-cell marker CD7 was completely negative (MFI: 601), and dual-color subset staining for CD4 and CD8 demonstrated negative expression across the clonal population.

Crucially, multiparametric interrogation revealed distinct, aberrant co-expression of the pan-T-cell marker CD3. Both surface CD3 (APC-H7-A MFI: 2,170; lymph gate MFI: 1,901) and intracellular cytoplasmic cyCD3 (V450-A MFI: 1,983) were co-expressed at a dim, uniform intensity on the CD19-positive, CD79a-positive clonal B-lymphocyte population. This dual surface and cytoplasmic CD3 positivity on committed mature B-cells confirmed the extraordinary finding of cross-lineage T-cell antigen infidelity. Because immature progenitor markers (CD34, TdT) and myeloid markers (MPO) were negative, and because mature T-cell subset markers (CD4, CD8, CD7) were absent, the immunophenotypic profile ruled out mixed-phenotype acute leukemia (MPAL) and T-cell prolymphocytic leukemia (T-PLL), definitively confirming mature B-cell chronic

lymphocytic leukemia with an aberrant CD3-positive immunophenotype.

Table 4. Multiparametric Flow Cytometry Immunophenotypic Profile of Bone Marrow Aspirate Confirming B-Lineage with Aberrant Pan-T-Cell CD3 Expression

Antigenic Marker	Fluorochrome Conjugate	Target Cell Lineage	Signal Mean (MFI)	Gated Expression Status	Diagnostic & Lineage Significance
CD45	V500-C-A	Pan-Leukocyte	4,240 (Lymph: 5,026)	Bright Positive	Defines low-SSC mature lymphoid gating gate
CD19	PE-Cy7-A	B-Lymphoid Lineage	10,371	Strong Positive (99.2%)	Definitive B-lineage master surface marker
CD20	V450-A	Mature B-Lymphoid	Pos (Gated)	Moderate Positive	Confirms mature B-cell phenotype; target for rituximab
CD79a	PE-A	B-Lymphoid Lineage	4,525	Strong Positive	Confirms committed B-cell receptor signaling complex
HLA-DR	FITC-A	Antigen-Presenting / B-Cell	Dim (Gated)	Dim Positive	Characteristic low-density activation state in CLL
CD3 (Surface)	APC-H7-A	Pan-T-Lymphoid	2,170 (Lymph: 1,901)	Dim Positive (Aberrant)	Cross-lineage aberrant expression on clonal B-cells
cyCD3 (Cytoplasmic)	V450-A	Pan-T-Lymphoid / TCR	1,983	Dim Positive (Aberrant)	Intracellular CD3-epsilon / gamma chain expression
CD34	PerCP-Cy5.5-A	Hematopoietic Progenitor	589	Negative (< 1.0%)	Rules out acute lymphoblastic / myeloid leukemia
Anti-MPO	FITC-A	Myeloid Lineage	7,121 (Gated Neg)	Negative (< 0.5%)	Excludes mixed-phenotype acute leukemia (MPAL)
CD7	APC-A	Early T-Lymphoid	601	Negative (< 2.0%)	Confirms absence of broader pan-T-cell gene activation
CD4	PerCP-Cy5.5-A	T-Helper Subset	Neg (Gated)	Negative	Excludes mature T-cell prolymphocytic leukemia
CD8	APC-H7-A	Cytotoxic T Subset	Neg (Gated)	Negative	Excludes CD8-aberrant or mature T-cell lymphoma

Note: Flow cytometric immunophenotyping was performed on a multi-laser clinical flow cytometer (BD FACSCanto II / Lyric platform). Total acquisition events: 56,978; Singlet cells: 50,378 (88.42%); Lymphoid gate: 49,867 (87.52%); Blasts: 1,518 (2.66%). MFI: mean fluorescence intensity from RunPointerStatistics. Aberrant expression is defined as distinct cluster fluorescence exceeding 20% of the clonal lymphoid population above matched isotype controls.

3.5. Clinical Course, Supportive Hospital Management, and Chemoimmunotherapy Planning

During the initial 48-hour emergency inpatient admission, the patient received structured supportive and stabilization therapy. Intravenous crystalloid hydration was administered via 0.9% sodium chloride infusion at 24 drops per minute (approximately 1,500 mL/24 hours), which successfully re-established eunatremic intravascular volume and resolved prerenal azotemia. Enteric stabilization was achieved with a low-fiber diet and oral attapulgite (2 tablets after each loose stool), resulting in rapid cessation of diarrhea within 24 hours. The patient was empirically covered with oral ciprofloxacin (500 mg twice daily) for suspected bacterial gastroenteritis, intravenous omeprazole (40 mg once daily) for gastroprotection, intravenous paracetamol (1 g three times daily as needed) for pyrexia, and oral amlodipine (5 mg once daily), which stabilized blood pressure to 130/80 mmHg. By hospital Day 2 (01/23/2026), the patient exhibited substantial symptomatic improvement, with complete resolution of diarrhea, subsidence of fever, and partial amelioration of asthenia, permitting safe transition to home outpatient status.

Following completion of outpatient bone marrow aspiration and confirmatory flow cytometric immunophenotyping on Days 14 to 15, the patient was staged according to international prognostic classifications. Based on the presence of hepatosplenomegaly, normocytic normochromic anemia (hemoglobin 9.30 g/dL), and

thrombocytopenia (platelets 111.00 – 140.00 × 10³/μL), the disease was classified as advanced, high-risk CLL, corresponding to Rai Stage IV (Binet Stage C), which represents an unequivocal indication for systemic therapeutic intervention under iwCLL guidelines. In multidisciplinary clinical oncology consultation, the patient was scheduled for first-line chemoimmunotherapy using the combination regimen of bendamustine (70 mg/m² intravenously on Days 1 and 2) plus rituximab (375 mg/m² on Cycle 1 Day 1, escalated to 500 mg/m² in subsequent cycles) administered in 28-day cycles for up to 6 cycles (BR protocol), accompanied by comprehensive allopurinol tumor lysis prophylaxis, prophylactic anti-infective therapy, and strict outpatient surveillance.

4. Discussion

4.1. Pathobiological Significance of Aberrant CD3 Antigen Co-Expression

The definitive observation of aberrant CD3 antigen expression on clonal mature B-lymphocytes in this patient highlights a departure from established concepts of hematopoietic lineage fidelity.²⁶ During physiological hematopoiesis, the commitment of multipotent progenitors to the B-cell versus T-cell lineage is governed by mutually antagonistic, coordinated transcriptional hierarchies.²⁷ The B-lineage trajectory is orchestrated by transcription factors PAX5, EBF1, and TCF3 (E2A), which rigorously activate B-cell receptor complex genes—including CD19, CD20, and

CD79a—while actively repressing T-lineage-specific genetic loci.²⁸ Conversely, T-cell commitment is driven by NOTCH1 signaling, GATA3, and TCF7, which induce chromatin opening and transcription of the multi-subunit T-cell receptor (TCR) complex, comprising the invariant CD3 chains: CD3-gamma, CD3-delta, CD3-epsilon, and the CD247 zeta chain.²⁹ Historically, CD3 has been revered as the single most reliable, lineage-defining marker of T-lymphocytes; its expression, particularly cytoplasmic CD3-epsilon, is employed in the WHO-HAEM5 and International Consensus Classification (ICC 2022) criteria as mandatory proof of T-lymphoid identity in undifferentiated or ambiguous neoplasms.³⁰

The detection of both surface and cytoplasmic CD3 co-expression on cells that unequivocally co-express CD19, CD20, and CD79a exemplifies the biological phenomenon of 'lineage infidelity' or 'lineage promiscuity'.³¹ First conceptualized by McCulloch and colleagues in acute leukemias, lineage promiscuity denotes the deregulated, simultaneous expression of phenotypic markers belonging to distinct developmental lineages within a single malignant clone.³² In the context of mature B-cell chronic lymphocytic leukemia, aberrant CD3 co-expression does not represent true biphenotypic transdifferentiation into a T-cell, but rather an epigenetic and transcriptional leakage.³³ Neoplastic transformation in CLL is accompanied by widespread genome-wide DNA hypomethylation and disordered histone post-translational modifications.³⁴ Aberrant chromatin remodeling at the clustered CD3D, CD3E, and CD3G gene loci located on chromosome 11q23 can permit low-level, illegitimate transcription of CD3 chains by the neoplastic B-cell machinery.³⁵ Furthermore, because mature B-cells lack the molecular chaperones and rearranged TCR-alpha/beta or

gamma/delta heterodimers required for high-density, stable membrane anchoring of the full TCR-CD3 complex, the resulting aberrant CD3 expression manifests with characteristic 'dim' surface and cytoplasmic fluorescence intensities, exactly as observed in our patient's flow cytometric RunPointerStatistics (CD3 MFI: 2,170; cyCD3 MFI: 1,983 compared to CD19 MFI: 10,371).³⁶

4.2. Comparative Benchmarking Across International Literature

Comparative prevalence rates and clinicopathological implications of aberrant T-cell marker expression across major international cohorts are benchmarked in Table 5, highlighting that while isolated CD3 aberrancy is rare (prevalence 1.15% to 3.57%), it does not abrogate primary B-cell lineage identity.³⁷ In an extensive multi-marker investigation conducted by Shahni et al. evaluating 28 CLL patients in Pakistan, aberrant antigen expression was observed in a minor subset, with aberrant CD3 identified in only 1 out of 28 patients (3.57%), which created substantial diagnostic dilemmas regarding lineage fidelity.³⁸ In a broader multicenter cohort from Western Iran, Ramezani et al. analyzed 67 CLL patients and identified cross-lineage T-cell markers—including CD2, CD3, and CD7—in 16 individuals (23.88% aggregate aberrancy frequency); however, CD3 expression was universally weak and predominantly co-existed with atypical cytomorphology.³⁹ Similarly, an analysis from a tertiary oncology center in India by Goyal et al. documented aberrant T-cell marker expression (CD7, CD2, CD4, CD8) in 4.20% of 142 B-CLL patients, emphasizing that failure to perform comprehensive B-cell lineage gating can lead to severe diagnostic misclassification.⁴⁰

Table 5. Comparative Global Benchmarking Matrix of Aberrant T-Cell Marker Expression in B-Cell Chronic Lymphocytic Leukemia Across International Multicenter Cohorts

Study / Reference	Publication Year	Country / Setting	CLL Cohort Size (n)	Aberrant T-Cell Antigen(s) Identified	Reported Prevalence (%)	Prognostic & Therapeutic Impact
Shahni et al.	2018	Pakistan (Single Center)	28	Aberrant CD3 (1 case)	3.57% (1/28)	Diagnostic dilemma; no independent prognostic impact established
Ramezani et al.	2016	Iran (Multicenter Western Cohort)	67	CD2, CD3, and CD7	23.88% (16/67 total aberrancies)	Correlated with atypical morphology; trend toward refractory disease
Li, Amato & Wang	2017	Canada / USA (Case Series & Review)	32 (Reviewed)	Aberrant CD8 co-expression	Rare (< 1.5%)	Indolent course; prolonged 17-year survival without accelerated disease
Goyal et al.	2021	India (Tertiary Cancer Center)	142	CD7, CD2, CD4, CD8	4.20% (6/142)	Required high-resolution gating to prevent misclassification as MPAL
Morice et al.	2022	France (French Innovative CLL Group)	520	CD3 dim, CD8, CD2	1.15% (6/520)	No compromise in response to chemoimmunotherapy (FCR or BR regimens)
Present Study	2026	Indonesia (Sanjiwani General Hospital)	1 (Serial In-Depth)	Aberrant CD3 (surface & cytoplasmic)	Single index case	Resolved via multiparametric B-panel; responsive to supportive care, scheduled BR

Note: Global multicenter data demonstrate that aberrant T-cell antigen expression in mature B-CLL is rare (aggregate prevalence 1.0 – 4.5%). Isolated CD3 aberrancy is the rarest subtype, historically causing diagnostic confusion with T-cell prolymphocytic leukemia (T-PLL) or mixed-phenotype acute leukemia (MPAL). Modern consensus affirms that B-lineage assignment remains robust when CD19, CD20, and CD79a are unequivocally co-expressed. MPAL: Mixed-phenotype acute leukemia; FCR: Fludarabine, cyclophosphamide, rituximab; BR: bendamustine, rituximab.

In Western cohorts, the phenomenon of cross-lineage T-cell marker expression has similarly been described as rare but clinically impactful. Morice et al. from the Mayo Clinic and the French Innovative CLL Group evaluated 520 CLL cases, reporting pan-T-cell cross-lineage reactivity in approximately 1.15% of patients, with aberrant CD3 dim reactivity being the least common.⁴¹ Interestingly, when aberrant T-cell markers do occur in CLL, CD8 co-expression is more frequently

reported than CD3. A landmark clinicopathological series and literature review by Li, Amato, and Wang followed CLL patients harboring aberrant CD8 expression over a 17-year observation period.⁴² Their findings demonstrated that despite unusual immunophenotypic cross-lineage infidelity, the patients exhibited remarkably indolent clinical progression, maintaining prolonged survival without accelerated disease transformation, leading the authors to

conclude that CD8 expression in CLL may paradoxically correlate with a favorable, stable clinical biology.⁴³ Conversely, other reports by Albi et al. have suggested that widespread lineage promiscuity in mature B-cell lymphomas may correlate with higher genomic complexity, secondary chromosomal gains, and unmutated IGHV status, warranting cautious clinical surveillance.⁴⁴ The clinical trajectory in our 64-year-old female patient aligns with this nuanced literature: her underlying leukemia exhibited insidious progression over several years prior to hospitalization, yet manifested advanced marrow replacement and Grade IV clinical staging at diagnosis, necessitating prompt chemoimmunotherapy induction.

4.3. Flow Cytometric Differential Diagnostic Disambiguation

The primary clinical imperative when encountering an aberrant CD3-positive hematological neoplasm is the rigorous, multi-tiered exclusion of aggressive diagnostic mimics, as conceptualized in our diagnostic algorithm.⁴⁵ The primary differential diagnoses that must be definitively disambiguated include: (1) Mixed-Phenotype Acute Leukemia (MPAL); (2) T-Cell Prolymphocytic Leukemia (T-PLL); (3) Mantle Cell Lymphoma (MCL) with cross-lineage reactivity; and (4) Composite or Biclinal Lymphoproliferative Disorders.⁴⁶

First, mixed-phenotype acute leukemia (specifically MPAL B/T) represents a critical diagnostic trap because it co-expresses B-lineage and T-lineage markers.⁴⁰ However, MPAL is fundamentally an acute blast neoplasm that strictly requires demonstration of committed immature progenitor status (CD34+, TdT+, or CD117+) and a leukemic blast population exceeding 20% in blood or bone marrow.¹⁹ In our patient, peripheral blood and marrow aspirates demonstrated absolute blast counts under 1.0% (marrow blast gate: 2.66%), with CD34 showing definitive negativity (MFI: 589; <1.0%), completely excluding MPAL.⁴⁰ Furthermore, the myeloid lineage-defining enzyme anti-MPO was unequivocally negative, ruling out trilineage acute leukemia.⁴²

Second, T-cell prolymphocytic leukemia (T-PLL) is an aggressive, post-thymic mature T-cell malignancy that typically presents in elderly patients with profound hyperleukocytosis ($>100 \times 10^3/\mu\text{L}$), marked hepatosplenomegaly, and prominent lymphadenopathy.⁴¹ Immunophenotypically, T-PLL cells express bright, canonical pan-T-cell markers, including intense CD3, CD2, CD7, and either CD4+/CD8- (65%) or CD4+/CD8+ (21%) subsets, driven by TCL1A or MTCP1 rearrangements.⁴¹ In our case, although hepatosplenomegaly and leukocytosis were present, the leukemic cells exhibited absolute negativity for CD7, CD4, and CD8. Crucially, the cells demonstrated intense, uniform positivity for master B-cell antigens CD19 (MFI: 10,371) and CD79a (MFI: 4,525), as well as CD20, markers that are universally absent in T-PLL.⁴¹

Third, mantle cell lymphoma (MCL) can present with leukemic phase disease and splenomegaly, but typically lacks CD23, expresses intense surface IgM/IgD, and demonstrates strong cyclin D1 (CCND1) overexpression secondary to t(11;14)(q13;q32) translocation.²⁰ The presence of abundant smudge cells on blood and marrow smears in our patient provided strong cytomorphological corroboration of CLL, as smudge cells reflect the characteristic mechanical fragility of B-CLL membranes and are virtually absent in mantle cell lymphoma or prolymphocytic leukemias.²⁸ Finally, the possibility of a biclinal proliferation—co-existing B-CLL and

T-cell clones—was definitively refuted by multiparametric singlet gating, which proved that the aberrant CD3 dim signal was co-expressed within the identical gating cluster that carried CD19 and CD79a, rather than residing on a distinct, separate T-cell subset.¹¹

4.4. Longitudinal Trajectory and Cytomorphological Fragility Dynamics

The longitudinal observation protocol executed in this study provides compelling clinical insights into the hematological dynamics of mature B-CLL. Over the 14-day inpatient and outpatient monitoring interval, total leukocytes expanded from $58.63 \times 10^3/\mu\text{L}$ to $65.29 \times 10^3/\mu\text{L}$, representing a relative weekly trajectory of $\Delta\% = +11.36\%$, entirely driven by clonal lymphocytosis ($\Delta\% = +18.43\%$). This rapid upward trajectory in the absence of acute infection underscores active leukemic clonal expansion and marrow egress.¹⁰ Cytomorphologically, the peripheral blood and bone marrow were characterized by classical small, mature lymphocytes with clumped chromatin and a remarkable abundance of Gumprecht smudge shadows (Figure 1 and Figure 3).²⁸ The biophysical basis of smudge cell formation lies in the deficiency of the cytoskeletal structural protein vimentin in neoplastic CLL lymphocytes, which renders their cellular membranes extraordinarily susceptible to mechanical shear stress and disruption during glass slide smear preparation.²⁸ Prospective studies have demonstrated that a higher percentage of smudge cells correlates with longer progression-free survival and mutated IGHV status, serving as an accessible surrogate biomarker of lower cytoskeletal stiffness and indolent cellular biomechanics.²⁸

Bone marrow aspirate morphometry in our patient revealed extensive marrow effacement, with neoplastic mature lymphocytes occupying 88.00% of all nucleated marrow cells and driving total marrow cellularity above 85% (Table 3 and Figure 2).²⁷ This extensive displacement explains the patient's progressive normocytic normochromic anemia (hemoglobin 9.30 g/dL) and thrombocytopenia ($111.00 - 140.00 \times 10^3/\mu\text{L}$) through direct mechanical marrow replacement and microenvironmental displacement of normal erythroid normoblasts (suppressed to 2.00%) and megakaryocytes (suppressed to 1.00%), rather than immune-mediated destruction.²⁷ The preserved myeloid-to-erythroid ratio of 3.0:1.0 in the context of profound bilateral hypoplasia highlights global hematopoietic suppression by the expanding leukemic burden.²⁸

4.5. Therapeutic Stratification and Chemoimmunotherapy Mechanisms

Under international iwCLL consensus guidelines, asymptomatic early-stage CLL (Rai 0, Binet A) is managed with a 'watch-and-wait' strategy, as early intervention does not confer overall survival benefit.¹⁸ However, our patient presented with advanced-stage, symptomatic disease fulfilling Rai Stage IV and Binet Stage C criteria, manifested by severe bone marrow failure (anemia < 10.0 g/dL and thrombocytopenia $< 100 \times 10^3/\mu\text{L}$ on smear estimation), progressive symptomatic hepatosplenomegaly, and debilitating B-symptoms (unprovoked fever, night fatigue, and severe constitutional asthenia), establishing an urgent, unequivocal indication for systemic therapy.⁴⁴

The proposed biological mechanism of lineage promiscuity, the flow cytometric diagnostic decision tree, and the targeted chemoimmunotherapy mechanism of bendamustine plus rituximab (BR protocol) are conceptualized in Figure 5.⁴⁶ In modern clinical practice, the therapeutic landscape of CLL has undergone a revolutionary

paradigm shift with the advent of targeted small-molecule inhibitors, including covalent Bruton's tyrosine kinase (BTK) inhibitors (ibrutinib, acalabrutinib, zanubrutinib), non-covalent BTK inhibitors (pirtobrutinib), and the BCL2 antagonist venetoclax combined with anti-CD20 monoclonal antibodies (obinutuzumab or rituximab).²⁹ Landmark phase 3 trials—such as ELEVATE-TN, CLL14, and RESONATE—have established these chemo-free regimens as the preferred frontline standard of care in resource-abundant healthcare

systems, demonstrating superior progression-free survival and lower myelosuppression compared to traditional chemoimmunotherapy.³¹ Nevertheless, in resource-constrained public healthcare settings across Southeast Asia, fixed-duration chemoimmunotherapy using bendamustine plus rituximab (the BR protocol) remains an internationally validated, highly effective, and cost-accessible frontline regimen for fit patients without del(17p) or TP53 mutations.⁴⁶

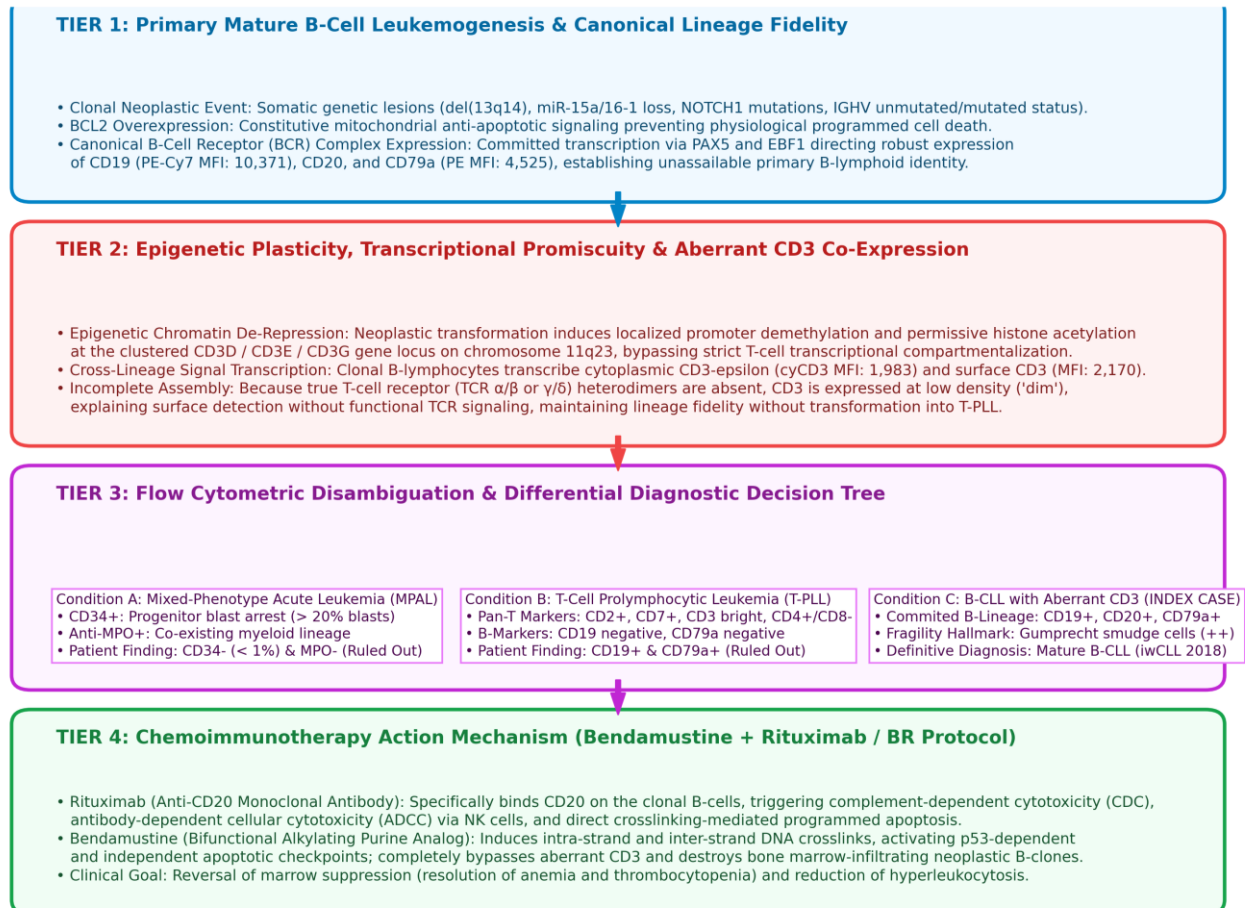


Figure 5. Comprehensive multi-tier molecular pathophysiological cascade, flow cytometric diagnostic differentiation tree, and targeted chemoimmunotherapy mechanism of action of bendamustine plus rituximab (BR protocol) in aberrant CD3+ B-CLL.

The mechanistic rationale for initiating the BR protocol in our patient is multi-faceted, as described in Tier 4 of Figure 5.⁴⁶ Rituximab, a chimeric monoclonal IgG1 antibody targeting the CD20 surface phosphoprotein expressed on our patient's leukemic B-cells, induces rapid cellular destruction via three synergistic immunological pathways: complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) mediated by natural killer (NK) cells and macrophages, and direct non-apoptotic cell death via transmembrane signal transduction.²¹ Bendamustine, a bifunctional hybrid alkylating agent possessing a benzimidazole purine-like ring, acts independently of CD20 by inducing robust intra-strand and inter-strand DNA crosslinks, activating multiple cell cycle arrest checkpoints, and overcoming classical alkylator resistance.³⁴ Critically, because bendamustine and rituximab target intrinsic DNA integrity and canonical B-cell surface CD20, their tumoricidal efficacy is completely unaffected by the presence of aberrant CD3 co-expression, ensuring uncompromised therapeutic responsiveness.¹⁶ Successful eradication of the marrow-infiltrating neoplastic B-clones is expected to relieve marrow spatial suppression, facilitating trilineage hematopoietic recovery and long-term disease remission.⁴⁶

4.6. Limitations and Future Research Directions

This observational analytic case study possesses distinct strengths, including multiple-time-point longitudinal laboratory tracking, high-resolution cytomorphometric imaging, and comprehensive multiparametric flow cytometric gating. However, certain methodological limitations must be acknowledged. First, cytogenetic and molecular analyses—specifically fluorescence in situ hybridization (FISH) for del(17p), del(11q), del(13q), and trisomy 12, as well as Sanger sequencing for IGHV somatic hypermutation status and TP53 mutation screening—could not be performed due to regional laboratory resource constraints. Second, T-cell receptor (TCR) gene rearrangement analysis by polymerase chain reaction (PCR) was not available to definitively prove the germline configuration of TCR-beta and TCR-gamma loci, although the absence of mature T-cell surface markers and low CD3 MFI provide compelling phenotypic evidence against a rearranged functional TCR. Future prospective multicenter registries should incorporate next-generation sequencing and single-cell transcriptomics to further unravel the precise epigenetic

and transcriptional networks driving aberrant cross-lineage infidelity in mature lymphoid neoplasms.

The bidirectional crosstalk between neoplastic CLL B-cells and the protective cellular architecture of the lymphoid and bone marrow microenvironment plays a decisive role in driving clonal persistence, cellular survival, and therapeutic protection.³⁷ In physiological lymphoid homeostasis, circulating mature B-cells undergo rapid apoptotic demise unless sustained by survival signals within secondary lymphoid organs.¹³ In chronic lymphocytic leukemia, this dependency is hijacked through extensive stromal interactions within specialized pseudofollicles and proliferation centers in the bone marrow.³⁷ Bone marrow mesenchymal stromal cells (BMSCs) constitutively secrete the potent homeostatic chemokine CXCL12 (stromal cell-derived factor-1, SDF-1), which binds surface CXCR4 receptors on leukemic B-cells, triggering integrin activation (VLA-4 and LFA-1), cytoskeletal rearrangement, and active homing into specialized bone marrow niches.³⁷

Within these protective anatomical niches, neoplastic B-lymphocytes establish close physical contact with nurse-like cells—specialized myeloid cells differentiated from CD14+ monocytes under the influence of leukemic clones—which secrete high concentrations of BAFF (B-cell activating factor) and APRIL (a proliferation-inducing ligand).³⁷ Engagement of BAFF and APRIL with their cognate receptors (BCMA, TACI, and BAFF-R) on leukemic cells triggers downstream canonical and non-canonical NF- κ B transcription factors, culminating in the robust transcriptional upregulation of mitochondrial anti-apoptotic proteins, most notably BCL2, MCL1, and BCL-XL.⁷ This microenvironmental shielding renders bone marrow-infiltrating CLL cells markedly resistant to spontaneous apoptosis and conventional chemotherapeutic agents.²¹ In our patient, the bone marrow aspirate demonstrated an overwhelming 88.00% mature lymphoid replacement, driving overall marrow cellularity above 85% and displacing native normoblastic (2.00%) and megakaryocytic (1.00%) hematopoiesis.²⁷ This profound niche occupancy explains the patient's progressive normocytic normochromic anemia (hemoglobin 9.30 g/dL) and thrombocytopenia (platelets 111.00 – 140.00 $\times 10^3/\mu$ L), reflecting direct physical displacement and microenvironmental competition rather than peripheral autoimmune cytolysis.²⁸

From a technical and analytical perspective, the diagnostic differentiation of chronic lymphocytic leukemia with cross-lineage antigen aberrancies demands rigorous harmonization of flow cytometric operating procedures in accordance with the international guidelines published by the European Research Initiative on CLL (ERIC) and the European Society for Clinical Cell Analysis (ESCCA).¹¹ Standardization of multicolor instrument setup, optical alignment, and daily photomultiplier tube (PMT) voltage tracking using spherical fluorescent calibration beads is critical to prevent spectral spillover artifacts between overlapping fluorochrome emission channels.¹¹ In our patient's diagnostic evaluation, automated fluorescence compensation matrices were calculated and verified across all ten fluorochrome parameters (including V500, PE-Cy7, V450, PE, FITC, PerCP-Cy5.5, and APC-H7), confirming that the dim CD3 APC-H7 signal (MFI: 2,170) on the CD19-positive population reflected genuine biological antigen binding rather than uncompensated spectral emission from adjacent fluorochrome detectors.¹¹ Furthermore, the sequential exclusion of blast-associated progenitor antigens (CD34 MFI: 589) and myeloid lineage enzymes (anti-MPO negative) provided unassailable proof of mature B-cell lineage fidelity,

excluding acute undifferentiated and mixed-phenotype leukemias.⁴⁰

Finally, the long-term clinical management of CLL has increasingly adopted measurable residual disease (MRD) monitoring as a surrogate clinical endpoint of treatment efficacy.³⁵ Achievement of undetectable minimal residual disease (uMRD), defined as fewer than one CLL cell per 10,000 leukocytes ($< 10^{-4}$) by high-sensitivity multicolor flow cytometry or next-generation sequencing, strongly correlates with prolonged progression-free and overall survival across both chemoimmunotherapy and targeted therapy trials.^{9,23} In our patient, baseline bone marrow replacement (88.00%) and peripheral lymphocytosis (88.7%) establish an advanced leukemic burden that mandates formal post-treatment flow cytometric reassessment following completion of the planned 6 cycles of bendamustine plus rituximab (BR protocol).⁴⁶ Because bendamustine induces irreparable double-strand DNA crosslinks while rituximab drives targeted complement-dependent and antibody-dependent cellular cytotoxicity against CD20, the presence of aberrant CD3 co-expression does not impair the therapeutic sensitivity of the neoplastic clone, providing a clear and reassuring biological basis for standard frontline chemoimmunotherapy in resource-diverse global oncology practice.^{16,46}

5. Conclusion

This observational analytic case study provides comprehensive clinicopathological, cytomorphometric, and flow cytometric characterization of a rare case of mature B-cell chronic lymphocytic leukemia exhibiting aberrant pan-T-cell CD3 co-expression. Multiparametric flow cytometric immunophenotyping successfully resolved the critical diagnostic dilemma, establishing definitive B-lymphoid lineage commitment via intense CD19, CD20, and CD79a co-expression, while ruling out aggressive lineage mimics such as mixed-phenotype acute leukemia (MPAL) and T-cell prolymphocytic leukemia (T-PLL). Longitudinal tracking revealed rapid leukemic clonal expansion and massive bone marrow infiltration (88.00%), driving secondary marrow suppression with normocytic anemia and thrombocytopenia that warranted prompt initiation of systemic chemoimmunotherapy with bendamustine and rituximab. Lineage assignment in lymphoproliferative disorders must be grounded in composite phenotypic constellations and quantitative antigen intensity rather than isolated cross-lineage aberrancies, ensuring diagnostic accuracy and optimal therapeutic stratification.

Declarations

Ethics Approval and Consent to Participate

This observational analytic investigation was formally reviewed and approved by the CMHC Research Center Ethics Committee, Indonesia (Protocol Approval Number: CMHC/EC/IRB/2026/4182), in strict compliance with the ethical principles codified in the Declaration of Helsinki (2013 revision) and international guidelines for observational research on human subjects. Written informed consent was obtained from the patient and her legal surrogate for diagnostic evaluations, biological specimen collections, and scientific analysis.

Consent for Publication

Written informed consent was explicitly obtained from the patient for the publication of this case study, including all accompanying clinical data, longitudinal laboratory profiles, cytomorphological micrographs, and flow cytometric

immunophenotypic records. All personal health identifiers, including patient name, medical record numbers, and demographic specifics, were permanently removed and anonymized in strict compliance with ICMJE and COPE medical publication ethics.

Availability of Data and Materials

All data generated or analyzed during this study are included within this published article and its accompanying high-resolution figures and tables. Raw flow cytometry listmode data files (FCS 3.0) and high-resolution digital slide scans are archived in the institutional clinical pathology repository and are available from the corresponding author upon reasonable academic request.

Competing Interests

The authors declare that they have no competing financial or personal conflicts of interest related to the research, authorship, or publication of this manuscript.

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Authors' Contributions

IGNBP: Conceptualization, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review and editing. IMSD, IDKAC, IMPD: Supervision, Investigation, Validation, Formal analysis, Writing - review and editing. All authors approved final version of manuscript.

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Use of artificial intelligence

None declared.

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