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Case Report

EXPERIENCE OF CHRONIC LYMPHOPROLIFERATIVE DISORDER CASES IN A NEWLY ESTABLISHED FLOW CYTOMETRY LABORATORY IN A TERTIARY CARE HOSPITAL: A SERIES OF 8 CASES

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ABSTRACT

Chronic lymphoproliferative disorder (CLPD) represents a heterogenous group of conditions affecting lymphocytes – especially white blood cells crucial for combating infections. The utilization of flow cytometry for immunophenotyping has significantly improved the diagnosis and differentiation between various CLPDs. The diagnosis of CLPD is based on the findings of peripheral blood smear, bone marrow aspiration and flow cytometry examinations. Here, we report a series of eight cases, diagnosed as CLPD by flow cytometry out of a total 78 cases received for flow cytometry analysis for a period from March, 2022 to March, 2024 after the establishment of a new flow cytometry laboratory.

Keywords: Lymphoproliferative, Lymphocytosis, Immunophenotyping, Neoplasm.

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INTRODUCTION

Chronic lymphoproliferative diseases (CLPD) are recognized as a diverse set of conditions distinguished by the monoclonal expansion and accumulation of seemingly mature lymphocytes. These lymphocytes exhibit a proliferative and/or survival advantage over their normal counterparts in various organs, including the bone marrow, peripheral blood and lymph nodes. This leads to the gradual buildup of clonal cells and their derivatives, resulting in lymphocytosis in the peripheral blood and bone marrow, along with the development of lymphadenopathy, splenomegaly or other forms of organomegaly [1].

Neoplasms within the category of CLPD can originate from both B cells and T cells. Nonetheless, B-cell CLPDs predominate, constituting about 90% of all cases. Among B-cell CLPDs, chronic lymphocytic leukemia (CLL) holds the highest prevalence [2].

A neoplastic illness called CLL is typified by the monoclonal proliferation of mature B cells that divide slowly and are immunologically incompetent. There are 1:2 female to male CLL patients [3]. In the Indian population, the onset of the disease occurs nearly a decade earlier than in the USA, with an incidence of 0.41 cases per lakh. Although this incidence is approximately 10 times lower than that in the USA, where CLL is the most prevalent type of leukemia, the large population size results in an absolute number of new cases and prevalence that is roughly 3 times higher in India [4]. The majority of patients are adults of >70 years. Nevertheless, younger patients (<60 years old) may also exhibit it [5].

While the precise cause of CLL remains unknown, there is evidence linking the disease to environmental factors such as radiation, pathogenic agents and exposure to hazardous substances and medications. In addition, genetic predisposition is significant [3].

The majority of CLL patients are asymptomatic and the diagnosis is frequently incidental, discovered through lymphocytosis observed during a complete blood count (CBC) conducted for an unrelated condition. Other patients may become aware of their condition when experiencing painless swelling of nodes, frequently in the cervical area. At the time of diagnosis, approximately 10% of these patients exhibit B symptoms such as fever, chills, night sweats, and weight loss. [6]. Rare features may also occur, such as severe anemia or bleeding attributed to thrombocytopenia. These manifestations result from the presence of underlying conditions such as autoimmune hemolytic anemia or immune thrombocytopenia [6].

The various diagnostic modalities include CBC, peripheral blood smear (PBS) examination for morphological evaluation, and bone marrow aspiration examination which altogether helped in providing provisional diagnosis and immunophenotyping using flow cytometry for confirmation.

Our flow cytometry laboratory was established in January, 2022 and has since received a total of 78 cases. On examination, eight of these cases were diagnosed with CLPD.

CASE 1

An 89-year-old male patient was admitted in our medicine department with the complaint of fatigue, weight loss, loss of appetite, shortness of breath, abdominal pain, and difficulty in urination since 2 months. On further examination, hepatomegaly with moderate ascites was also present. The CBC revealed as follows: Hb-4.0 g/dL (reference range 11.5–17); WBC-50.3×10^3 cells/μL (reference range 3.5–10); lymphocytes- 42×10^3 cells/μL (reference range 1–3); Platelet-302×10^3 cells/μL (reference range 150–450). On PBS examination, low hemoglobin, hyperleukocytosis with the presence of >90% mature looking small lymphocytes with a differentiated leucocyte count (DLC) of Neutrophil+Band form=06%, Lymphocytes=94% was reported. No opinion could be provided regarding bone marrow aspiration samples, as they were diluted with peripheral blood. Immunophenotypic analysis of blood showed monoclonal B lymphocytes which were approximately~85% of gated lymphoid cells. Neoplastic B lymphocytes were bright positive for CD19; moderate positive for CD45, CD20, CD200, CD23, KAPPA light chain, CD180, CD11c; dim positive for CD5 and negative for LAMBDA light chain, CD38, CD10, CD123, CD103, CD49d, CD4, CD3, CD8, CD7, CD25, CD26, TCR αβ, and T cell receptor (TCR) GD. Findings were suggestive of B- CLL.

CASE 2

A 52-year-old male patient was admitted in medicine department with the complaint of pain in the neck region, difficulty in swallowing, blurring

of vision since 1 week. The results of CBC revealed as follows: Hb-13.1 g/dL; WBC-22.18×10^3 cells/µL; lymphocytes-18.63×10^3 cells/µL; Platelet-193×10^3 cells/µL. On PBS examination-leucocytosis with the presence of~ 84% lymphocytoid cells were seen. The cells were small to medium in size, having high N: C ratio, coarse chromatin and scant cytoplasm. A few showed slightly irregular nuclear membrane. Background showed a few smudge cells (Fig. 1). Bone marrow aspiration findings showed >80% mature looking lymphocytes in DLC (Fig. 2). Both PBS and bone marrow aspiration findings were suggestive of CLPD-? CLL. To confirm, immunophenotypic analysis of blood showed monoclonal B lymphocytes which were approximately ~72.5% of gated lymphoid cells. Neoplastic B lymphocytes were bright positive for CD45, CD19, CD200; moderate positive for CD5, CD180, CD23, CD11c, LAMBDA light chain; dim positive for CD20 and negative for KAPPA light chain, CD38, CD10, CD49d, CD103, CD123, CD4, CD3, CD8, CD7, CD16, CD56, CD25, TCR αβ, and TCR GD. Findings were suggestive of B- CLL.

CASE 3

A 45-year-old male patient was admitted in our medicine department with the complaints of generalized lymphadenopathy, loss of appetite and weight loss since 2 months. On further examination, hepatosplenomegaly was found. The results of CBC revealed as follows: Hb-9.8 g/dL; white blood cell -107.68×10^3 cells/µL; Platelets-80×10^3 cells/µL. On PBS examination, low hemoglobin, and hyperleukocytosis with the presence of >75% of mature looking small lymphocytes and low platelets were seen. Such picture was suggestive of lymphoproliferative disorder. Bone marrow aspiration examination could not be commented as samples were not received. To confirm, immunophenotypic analysis of blood showed monoclonal B lymphocytes which were approximately~ 87% of gated lymphoid cells. Neoplastic B lymphocytes were bright positive for CD19, CD20, CD11c, CD38, CD45; moderate positive for CD5, KAPPA light chain, CD49d; dim to moderate positive for CD23, CD180, CD200 and negative for LAMBDA light chain, CD10, CD123, CD103, CD4, CD3, TCR αβ, TCR GD,CD8, CD7, CD25, and CD26. Findings were suggestive of CLPD-B- CLL.

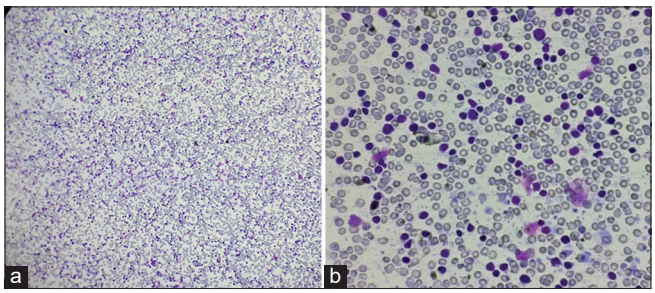

Fig. 1: : Peripheral blood smear picture of B-Chronic Lymphocytic Leukemia (Case 2). (a) shows leucocytosis (Giemsa stain, x100). (b) shows many small to medium sized lymphocytes having high N:C ratio, coarse chromatin, scant cytoplasm along with few smudge cells(Giemsa stain, x400)

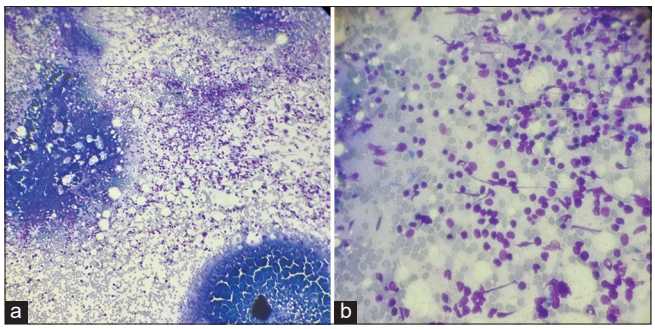

Fig. 2: Bone marrow aspiration picture of B-Chronic Lymphocytic Leukemia(Case 2). (a) shows high cellularity (Giemsa stain, x100). (b) shows many mature looking lymphocytes (Giemsa stain, x400)

CASE 4

A 68-year-old male patient was admitted in medicine department with the complaints of severe anemia, splenomegaly, hepatomegaly, pedal edema, and mild bilateral pleural effusion. The results of CBC revealed as follows: Hb-6.9 g/dL; WBC-229.94×10^3 cells/µL; lymphocytes-213.72×10^3 cells/µL; Platelets-68×10^3 cells/µL. On PBS examination, low hemoglobin, and hyperleukocytosis with the presence of >90% of mature looking small lymphocytes and low platelets were seen. Bone marrow aspiration findings revealed >90% mature looking lymphocytes in DLC. Both PBS and bone marrow aspiration findings were suggestive of CLPD- CLL. Immunophenotypic analysis of blood showed monoclonal B lymphocytes which were approximately~ 96.42% of gated lymphoid cells. Neoplastic B lymphocytes were moderate positive for CD19, CD20, CD200, CD23, CD11c, CD45; dim to moderate positive for CD5, LAMBDA light chain; dim for CD180 and negative for KAPPA light chain, CD38, CD10, CD49d, CD103, CD123, CD4, CD3, CD8, CD7, CD16, CD56, CD25, CD26,TCR αβ, and TCR GD. Findings were suggestive of CLPD-B- CLL.

CASE 5

A 74-year-old male patient was admitted in our medicine department with the complaints of fever, body ache and headache since 7–10 days. The results of CBC revealed as follows: Hb-8.0 g/dL; WBC- 38.19×10^3 cells/µL; Lymphocytes-31.41×10^3 cells/µL; Platelets-67×10^3 cells/µL. On PBS examination, low hemoglobin, leucocytosis with presence of 85% of small mature looking lymphocytes, having a high N: C ratio, coarse chromatin, and scant cytoplasm, along with few smudge cells and low platelets, were seen. Bone marrow aspiration findings showed >80% mature looking lymphocytes in DLC. Both PBS and bone marrow aspiration findings were suggestive of CLPD- CLL. To confirm, immunophenotypic analysis of blood showed monoclonal B lymphocytes which were approximately ~78% of gated lymphoid cells. Neoplastic B lymphocytes were bright positive for CD20, CD38, LAMBDA light chain; moderate positive for CD5, CD19, CD123, CD11c, CD45 (Fig. 2a); dim positive for CD49d, CD180 and negative for KAPPA light chain, CD200, CD23, CD10, CD103, CD4, CD3, CD8, CD7, CD16, CD56, CD25, CD26, TCR αβ, and TCR GD. Findings were suggestive of B- CLL (Fig. 3).

CASE 6

A 93-year-old male patient was admitted in medicine department with the complaints of shortness of breath, generalized swelling, bleeding per rectum since 10–15 days. On further examinations, mild right hydroureteronephrosis was present. The result of CBC revealed as follows: Hb-12.8 g/dL; WBC-58.38 × 10^3 cells/µL; Platelet-176×10^3 cells/µL. On PBS examination, leucocytosis showing ~ 70% mature looking lymphocytes, having high N: C ratio, coarse chromatin and scant cytoplasm along with few smudge cells were seen. No evaluation or assessment could be provided regarding bone marrow aspiration examination, as samples were not received. Immunophenotypic analysis of blood shows monoclonal B lymphocytes which were approximately~ 85% of gated lymphoid cells. Neoplastic B lymphocytes were bright positive for CD20, KAPPA light chain; moderate positive for CD5, CD45, CD180, CD49d; dim to moderate positive for CD19, CD11c; dim positive for CD200 and negative for CD23, CD38, LAMBDA light chain, CD10, CD103, CD4, CD3, CD8, CD7, CD16, CD56, CD25,CD26, CD123, TCR αβ, and TCRGD. Findings were suggestive of B- CLL.

CASE 7

A 65-year-old male patient was admitted in our medicine department with the complaints of fever with chills and rashes since. On further examinations, pallor, petechiae, pedal edema, hepatomegaly, massive splenomegaly, and generalized lymphadenopathy were present. The result of CBC revealed as follows: Hb-7.5 g/dL; WBC-163.52×10^3 cells/µL; lymphocytes-77.46×10^3 cells/µL; Platelet-56×10^3 cells/µL. On PBS examination, leucocytosis showing ~ 70% of small mature looking lymphocytes, having a high N: C ratio,

Table 1: Below table shows analysis of CBC parameters of all 8 cases

SL.NO	Hemoglobin	Platelet	WBC count	PBF-small lymphocytes IN% (approx)
Case 1	Low	Normal	Increased	90
Case 2	Normal	Normal	Increased	72.5
Case 3	Decreased	Decreased	High	87
Case 4	Low	Low	High	96.2
Case 5	Decreased	Low	Increased	78
Case 6	normal	Normal	Increased	85
Case 7	Low	Low	High	70
Case 8	Low	Normal	Increased	86

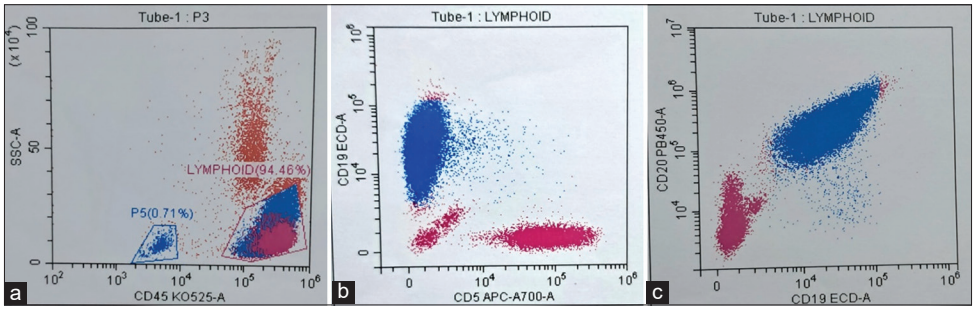

Fig. 3: Flow cytometry result of B-Chronic Lymphocytic Leukemia(Case 5). (a)Neoplastic B lymphocytes show positivity for CD45. (b) Neoplastic B lymphocytes show moderate positivity for CD19 and CD5. (c) Neoplastic B lymphocytes show bright positivity for CD20

fine to coarse chromatin, scanty to moderate cytoplasm, and smudge cells seen (Fig. 4). An opinion or assessment could not be offered for bone marrow aspiration examination as samples were not received. Immunophenotypic analysis of blood shows monoclonal B lymphocytes which were approximately~ 88% of gated lymphoid cells. Neoplastic B lymphocytes were bright positive for CD45, CD20, KAPPA light chain; moderate positive for CD5, CD19, CD180, CD49d and negative for LAMBDA light chain, CD200, CD23, CD38, CD11c, CD103, CD123, CD4, CD3, CD8, CD7, CD16, CD56, CD25, CD26, TCR αβ, TCR GD. Findings were suggestive of mature B-lymphoid neoplasm.

CASE 8

A 77-year-old female patient was admitted in the medicine department with complaints of pain abdomen and body ache since 2 months. On further examination, pallor, massive splenomegaly, and ascites were present. The result of CBC revealed as follows: Hb-7.2 mg/dL; WBC-241.79×10^3 cells/μL; lymphocytes-171.35×10^3 cells/μL; platelet-178×10^3 cells/μL. On PBS examination, hyperleukocytosis with the presence of~86% of small mature looking lymphocytes, having a high N: C ratio, coarse chromatin, and scant cytoplasm along with many smudge cells seen. Bone marrow aspiration findings revealed >85% mature looking lymphocytes in DLC. Both PBS and bone marrow aspiration findings were suggestive of CLPD. Immunophenotypic analysis of blood shows monoclonal B lymphocytes which were approximately~ 86% of gated lymphoid cells. Neoplastic B lymphocytes were bright positive for CD20; moderate positive for CD19, KAPPA light chain, CD45 and negative for CD5, CD200, CD23, LAMBDA light chain, CD38, CD10, CD180, CD11c, CD49d, CD103, CD123, CD4, CD3, CD8, CD7, CD16, CD56, CD25, TCR αβ, and TCR GD. Findings were suggestive of CLPD – Mature B-cell Neoplasm (Fig. 5).

DISCUSSION

In recent times, quantitative flow cytometry has emerged as a compelling tool for both fundamental and clinical research. In our investigation, we incorporated a set of markers including CD19, CD5, CD200, CD20, CD23, KAPPA light chain, LAMBDA light chain, CD180, CD11c, CD49d, CD38, CD45, CD103, CD123, CD4, CD3, CD8, CD7, CD16, CD56, CD25, CD26, TCR αβ, and TCR GD. Half cases had low levels of haemoglobin and adequate platelet count. Additionally, WBC count were increased in all of the cases. Notably, small lymphocytes showed a consistent increase in all cases, with a minimum count of ≥70% were noticed as shown in Table 1.

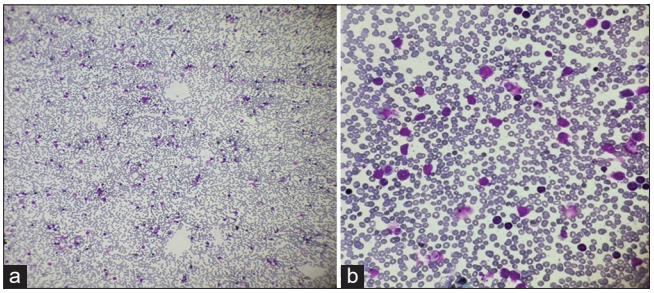

Fig. 4: Peripheral blood smear picture of Chronic Lymphoproliferative Disorder- Mature B cell Neoplasm (Case 7). (a) shows leucocytosis (Giemsa stain, x100). (b) shows small mature looking lymphocytes, having high N:C ratio, fine to coarse chromatin, scanty to moderate cytoplasm and smudge cells (Giemsa stain, x400)

Table 2 shows analysis for intensity of positive immunophenotypic results of all the 8 cases (expressed in number).

In a study led by D'Arena *et al*, encompassing 61 cases of CLL and a few other instances of CLPD, all samples demonstrated the presence of CD19 and CD20 antigens, with CD22 being positive in all cases except one CLL case. CD5, CD23, and CD79b were also expressed [7]. Similarly, in our investigation, CD19, CD5, CD20, and CD23 were predominantly expressed.

In 2017, Falay and Özet pointed up – a positive expression for CD23, CD22, CD79b, and FMC7 was observed in CLL cases. In addition, atypical CLL cases exhibited expressions of CD11c, CD25, CD43, and CD38, among 339 cases [8], findings that align closely with our own research results.

Rawstron *et al*., in a study conducted in 2018, involving 154 cases of CLL, positive expression was observed for CD19, CD23, CD5, CD200, CD43, and ROR1. Weak expression was noted for CD20, CD79b, and CD81, along with weak and limited expression of KAPPA and LAMBDA light chains. The samples tested negative for CD10 [9]. Our study yielded similar results except for CD81 and ROR1.

Likewise, in 2023, Mehrpouri *et al*. study, encompassing 131 patients, including 91 with CLL and 15 with atypical CLL, the study revealed

Table 2: Below table shows analysis for intensity of positive immunophenotypic results of all eight cases (expressed in number). However, CD10, CD103, CD123, CD4, CD3, CD8, CD7, CD16, CD56, CD25, CD26, TCR αβ, and TCR GD exhibit negative results in all eight cases

Markers	Bright	Moderate	Dim to moderate	Dim	Negative
CD19	3	4	1	-	-
CD5	-	5	1	1	1
CD200	1	2	1	1	3
CD20	5	2	-	1	-
CD23	-	3	1	-	4
KAPPA LIGHT CHAIN	2	3	-	-	3
LAMBDA LIGHT CHAIN	1	1	1	-	5
CD180	-	4	1	2	1
CD11c	1	4	1		2
CD49d	-	3	-	1	4
CD38	2	-	-	-	6
CD45	3	5	-	-	-

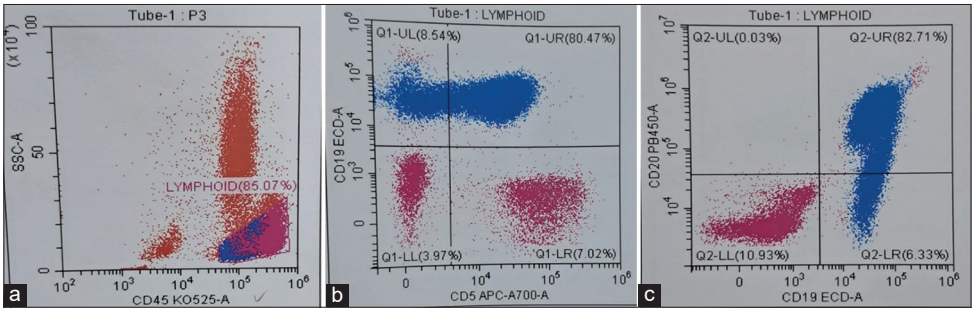

Fig. 5: Flow cytometry result of Chronic Lymphoproliferative Disorder- Mature B cell Neoplasm(Case 8). (a) Neoplastic B lymphocytes show moderate positivity for CD45. (b) Neoplastic B lymphocytes show moderate positivity for CD19 and negativity for CD5. (c) Neoplastic B lymphocytes show moderate positivity for CD20

distinctive expression patterns of CD22, CD23, FMC7, and CD5 in the diagnostic process of B-CLL. Notably, the presence of CD38 and KAPPA light chain was also observed in cases of atypical CLL [10], mirroring our own research findings.

Ozdemir *et al.*'s study similarly highlighted heightened intensity in the expression of markers CD5, CD23, and CD200, alongside weak expression of CD81 [11], aligning with our own findings.

CONCLUSION

CLL is the disease of older age group with a peak incidence of 50–55 years and it affects males twice than females. In this study, we assessed eight cases of which seven were males and one female, with the mean age of 70 years. Flow cytometry with morphological analysis of PBF is a better diagnostic tool. Hence, further evaluation is needed like cytogenetics in patient's treatment management and prognosis.

Consent

This series was reported after obtaining informed consent from all the participants involved.

ETHICAL APPROVAL

Ethical approval was obtained from the Institutional Ethics Committee before the reporting of this series.

AUTHORS' CONTRIBUTION

All authors have equal contribution in the preparation of this manuscript.

CONFLICTS OF INTEREST

Nil.

AUTHORS FUNDING

Nil.

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