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CD200 Breaks the Diagnostic Dilemma between Atypical CLL and Other CLPNS

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Case Study

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ABSTRACT

Chronic lymphocytic leukemia (CLL) is characterized by a monoclonal lymphocytosis of small mature-appearing CD5+, CD23+ B lymphocytes. CLL cells arise from the bone marrow. Two subtypes of CLL are morphologically described in the FAB classification including typical and atypical forms, however, no strict morphologic or immunophenotypic criterion is mentioned in the WHO classification. It is further difficult to differentiate atypical CLL from other chronic lympho-proliferative neoplasms (CLPN). Hereby we present a case of a 51 year old male of atypical CLL and the utility of the marker CD200 in the differentiation of aCLL from other CLPNs.

Keywords: Diagnostic dilemma; leukemia; immunophenotype; neoplasm.

1. INTRODUCTION

Chronic lymphocytic leukemia/small lymphocytic leukemia (CLL/SLL) is a neoplasm composed of monomorphic small mature B cells. There must be a monoclonal B-cell count > or equal to $5x10^9$/L with characteristic morphology and phenotype of CLL in peripheral blood. Individuals with a clonal CLL like count less than $5x10^9$/L and without lymphadenopathy, organomegaly, or any other extramedullary disease are considered to have monoclonal B-cell lymphocytosis [1]. Chronic Lymphocytic Leukemia (CLL) is one of the most common diagnoses made by flow

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cytometry laboratories. There is no consensus on which markers need to be used in flow cytometry for accurate immunophenotyping [2].

In the FAB system, two subtypes of CLL have been proposed. First type, having a dimorphic population of small lymphocytes and prolymphocytes in the peripheral blood, the prolymphocyte population constituting more than 10% to less than 55% of the circulating lymphocytes. The second subtype is defined as a spectrum of small to large lymphocytes with less than 10% prolymphocytes in peripheral blood [3-6].

However, data on atypical morphology of CLL and its prognostic implications is limited. Atypical chronic lymphocytic leukemia (aCLL) is a morphologic variant found in approximately 25% of patients with chronic lymphocytic leukemia (CLL). Although aCLL has a more aggressive course compared to typical CLL (tCLL), it is not usually reported [7].

Atypical CLL (aCLL) which is defined as at least 10% circulating lymphocytes resemble circulating lymphoma cells, with nuclear folds and deep clefts [8].

Hereby, we present a case of CLL showing atypical morphology, along with features that can help distinguish it from typical CLL morphologically, clinically, and immunophenotypically. Atypical morphologic features in CLL seem to be a marker of aggressive clinical behaviour.

2. CASE REPORT

A 50 year old male was admitted in the emergency in shock (Systolic Blood Pressure 60mm Hg), deranged liver function test (LFT) and kidney function test (KFT). Complete Blood Analysis showed leukocytosis (total leucocyte count 16,000/microliter $N_{34}L_{66,}$) and thrombocytopenia. The patient had persistently deranged LFTs, KFTs (but no oliguria) and leucocytosis. The patient was a known case of pulmonary tuberculosis 30 years back, and had been incompletely treated. On examination, the patient was in shock, with an elevated pulse rate (102/minute), subconjunctival haemorrhage and icterus. On palpation, there was no hepatosplenomegaly. X-Ray chest was clear. Ultrasound abdomen showed small left kidney with compensatory hypertrophy of right kidney along with mild splenomegaly. Viral titers (HIV, Hepatitis B, Hepatitic C) were negative. CBC with peripheral Blood smear (PBS) examination showed persistent leukocytosis with the presence of many activated lymphocytes on microscopy.

A Clinical Diagnosis of Leptospirosis/Scrub Typhus/Tuberculosis/Leukemoid reaction/CLL was kept and work up was started.

Leptospira IgM was negative. Sputum for Acid Fast Bacilli was negative. The PBS was reviewed. The leukocyte count was 41,860/microliter at day 3 of admission. Smear showed the presence of 78% lymphocytes with atypical morphology. These lymphocytes were 2-2.5 times the size of a small mature lymphocyte having a moderate amount of pale blue cytoplasm, condensed chromatin with high nucleo-cytolpasmic ratio, nuclear membrane showing irregularity at many places, with nuclei showing grooving, indentation and clefting (PBS-1). The Red blood cells were predominantly normocytic normochromic.

A Hemato-pathological diagnosis of mature B cell neoplasm vs T-cell leukemia was considered. Hence a flowcytometric analysis was done. Chronic lymphoproliferative disorder panel was put, comprising of T-cell markers, B-cell markers, immaturity markers, myeloid markers along with kappa and lamba antibodies. Flowcytometry analysis was done on Beckman coulter FC500. Total cell count at the time of IPT (immunophenotyping) was 45,000/microlitre. Gating strategy was side scatter v/s CD45. 55% cells were gated in the CD45 window having low side scatter and moderate to bright CD45 expression. These cells co-expressed CD5 and CD23, had Kappa chain restriction, expressed B cell markers CD19, CD20, CD22, CD79a and were negative for sIgM, FMC-7, CD10, CD138, CD1a, Tdt, CD34, MPO, CD13, CD14,CD15, CD3, CD7 (Figs. 1-16).

A diagnosis of CLL was considered but morphology was not supportive. Also, Mantle Cell Lymphoma (MCL) could not be ruled out as few MCL can show positivity for CD5 and CD23. (Figs. 17-18) To differentiate between both further works up was done, and Cyclin D1 and CD200 were put. FCM analysis revealed high positivity for CD200 and Negative Cyclin D1 thus ruling out MCL.

Considering the morphology and the criterion seen in literature (Table 1), a diagnosis of Atypical Chronic Lymphocytic Leukemia was given.

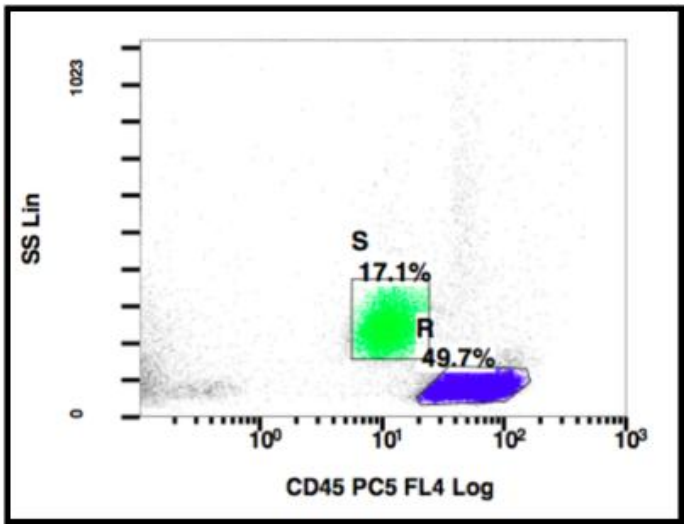

Fig. 1. SSC v/s CD45

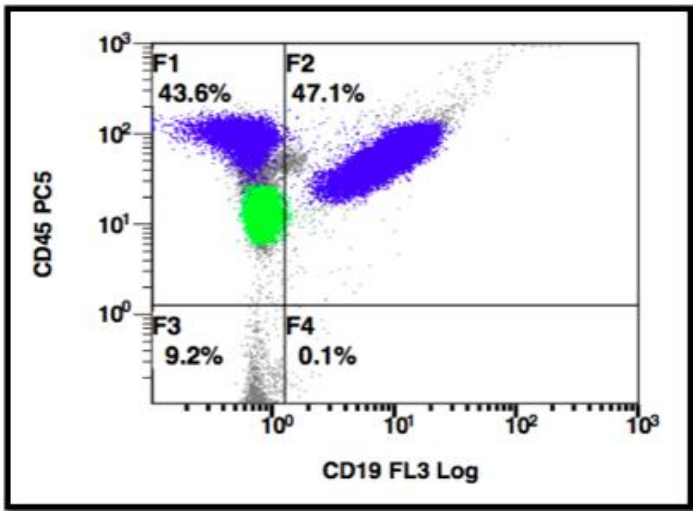

Fig. 2. CD19 v/s CD45

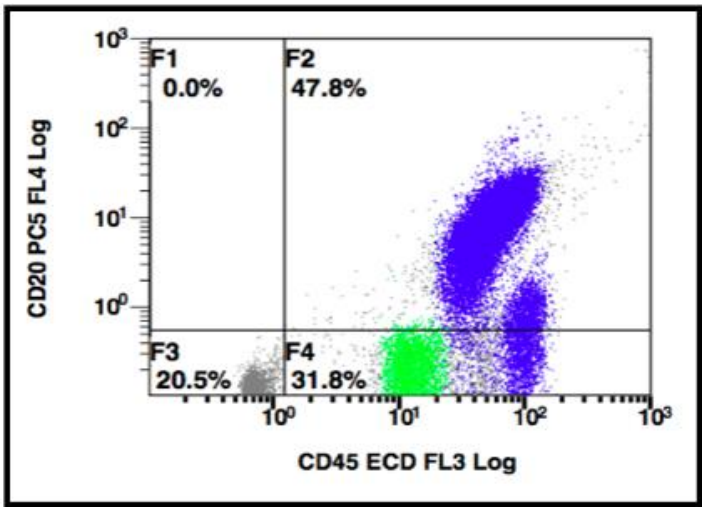

Fig. 3. CD20 v/s CD45

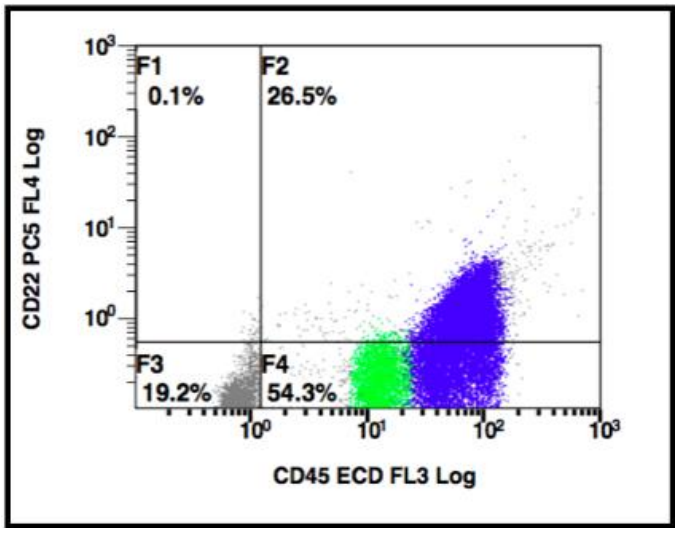

Fig. 4. CD22 v/s CD45

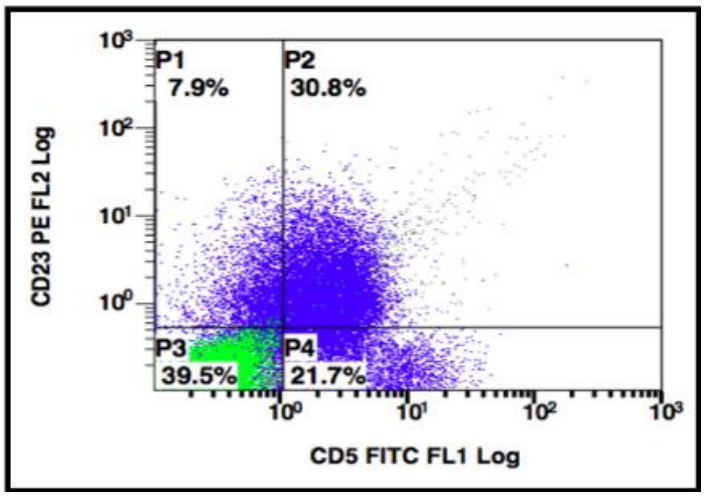

Fig. 5. CD23 v/s CD5

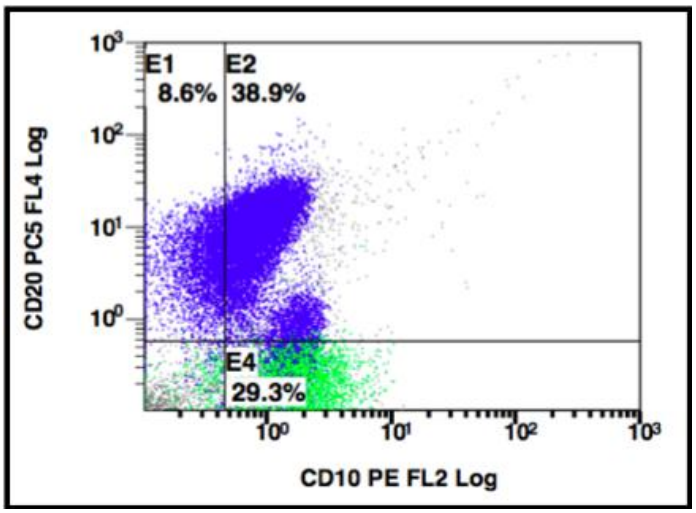

Fig. 6. CD20 v/s CD10

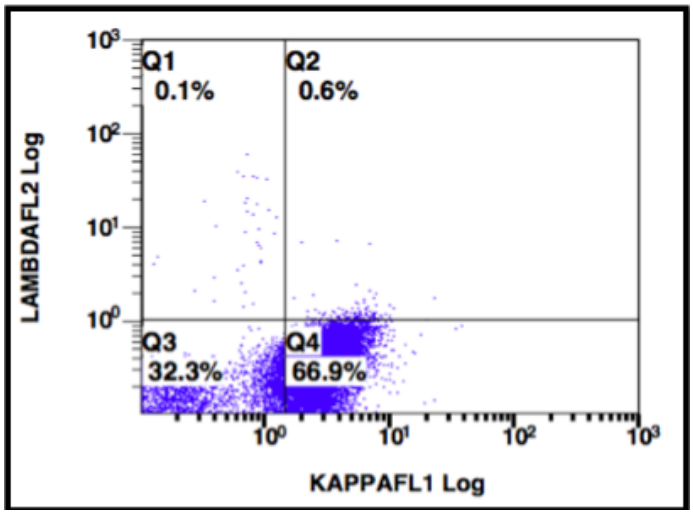

Fig. 7. Kappa v/s Lambda

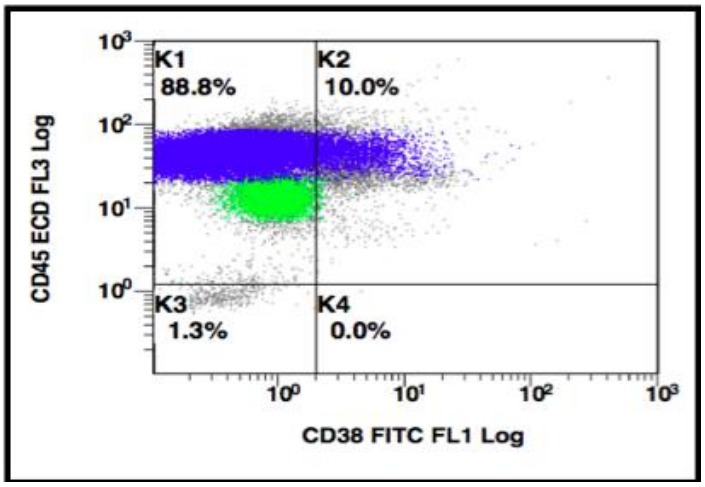

Fig. 8. CD45 v/s CD38

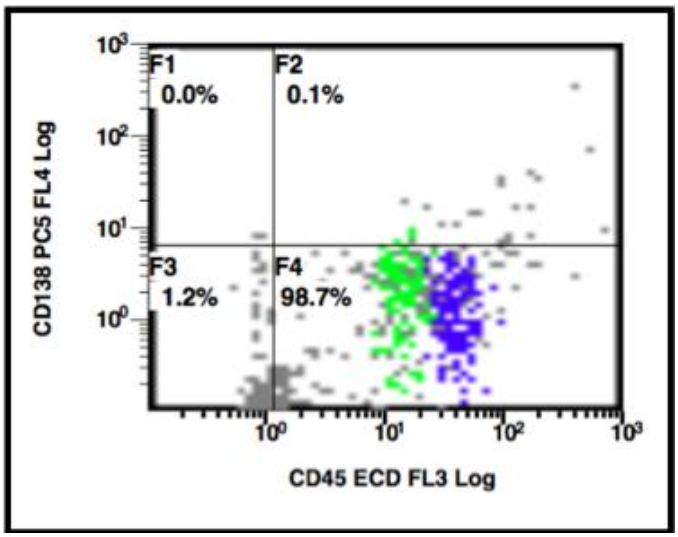

Fig. 9. CD45 v/s CD138

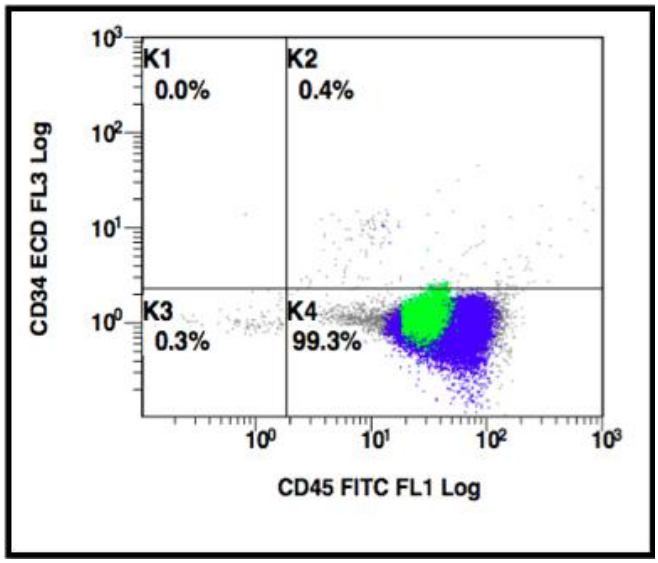

Fig.10. CD34 v/s CD45

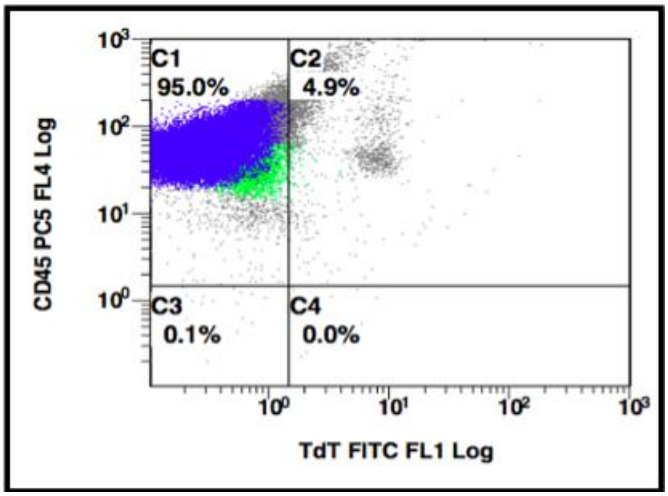

Fig. 11. CD45 v/s TdT

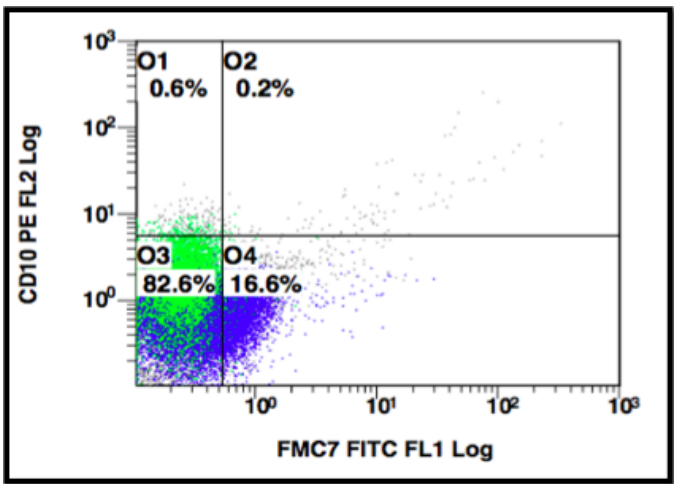

Fig. 12. CD10 v/s FMC7

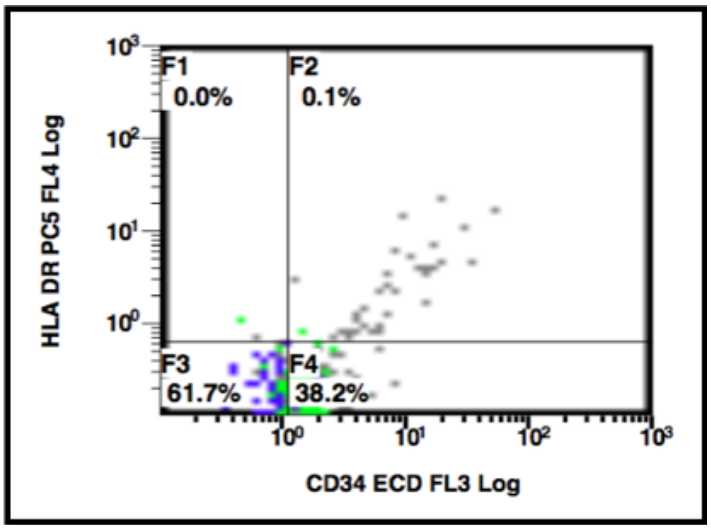

Fig. 13. HLADR v/s CD34

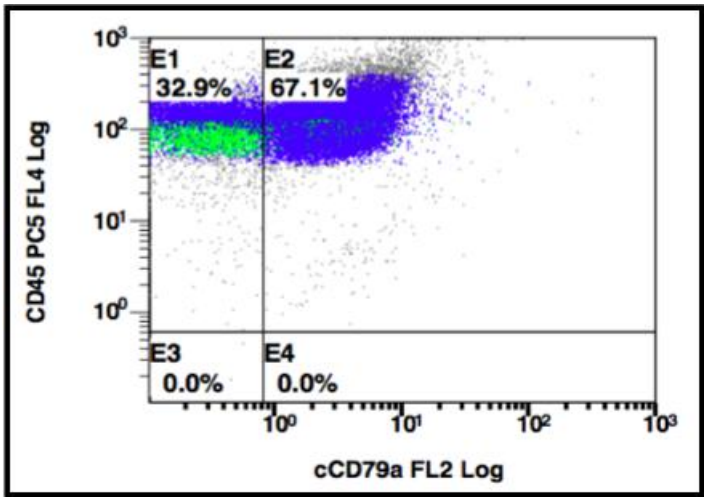

Fig. 14. CD45 v/s CD79a

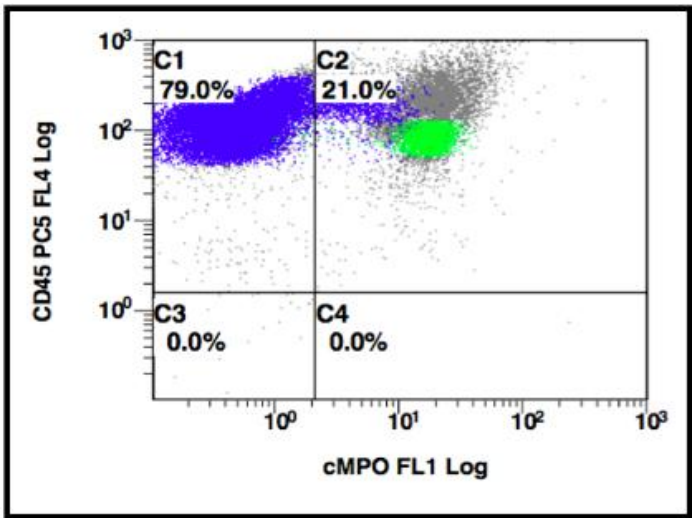

Fig. 15. CD45 v/s MPO

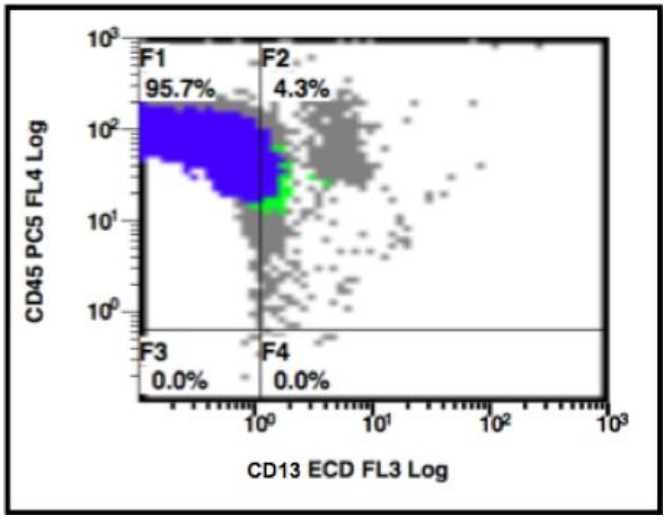

Fig. 16. CD45 v/s CD13

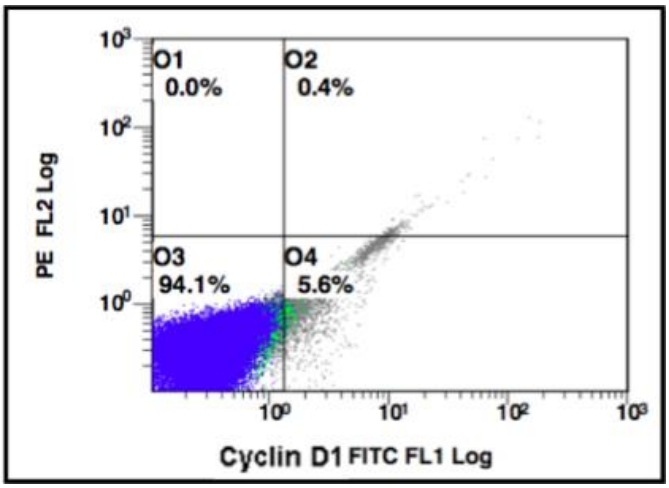

Fig. 17. Cyclin D 1

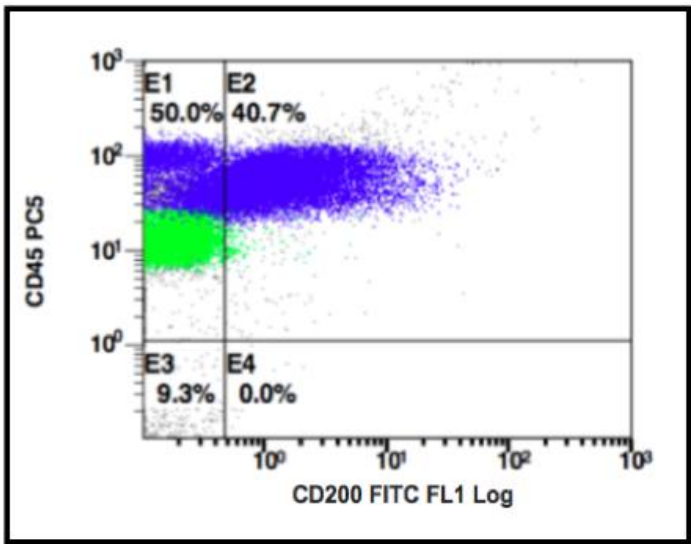

Fig. 18. CD45 v/s CD200

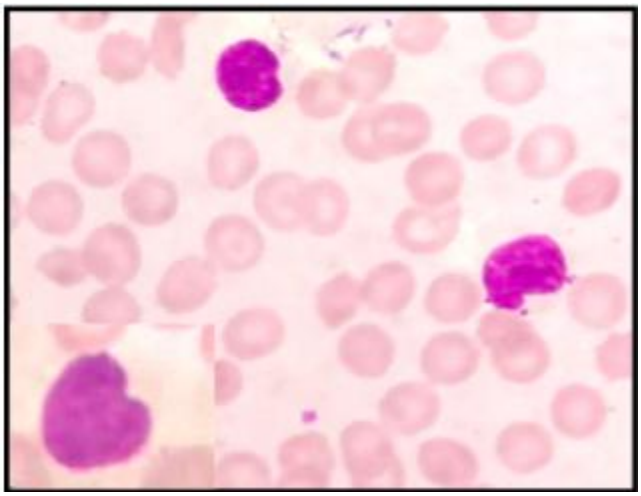

Fig. 19. Wright-giemsa, oil, atypical Lymphoctyes showing clefting(inset)

Table 1. Criteria used for morphologic classification of chronic lymphocytic leukemia CLL

Subclassification	Typical CLL	Atypical CLL
Criterion	90% of lymphocytes are small-to-medium sized with relatively normal morphology, except for a characteristically clumped, chunky chromatin pattern. Prolymphocytes <10% of circulating lymphocytes	Small lymphocytes plus >10% and <55% prolymphocytes; Mixed-cell subtype: >15% lymphoplasmacytoid cells, cells exhibiting nuclear indentations/clefts, or both with; prolymphocytes <10% of cells

3. DISCUSSION

Chronic lymphocytic leukemia (CLL) is a clonal disease of mature and functionally incompetent B lymphocytes representing 30% of all leukemia diagnosed in western countries [9]. The annual incidence rate is 5 per 100000 cases, with a median patient age of 70 years. CLL/SLL accounts for 7% of non Hodgkin lymphomas[1].

CLL can be morphologically classified as typical (tCLL) or atypical (aCLL). 80% of the cases show atypical morphology showing, small-to-medium-sized circulating lymphocytes with a characteristically clumped chromatin pattern and presence of smudge cells on peripheral smear Fig. 19. Prolymphocytes, if present, represent <10% of circulating lymphocytes [10].

The 2008 WHO classification of lymphoid neoplasms stated that in atypical CLL, lymphocytes show less condensed nuclear chromatin and nuclear irregularities and that these findings occur more often in patients with trisomy 12 and other chromosomal abnormalities [11]. The 2016 WHO classification states that cases of CLL having more than 10% but less than 55% prolymphocytes are termed as atypical. These cases usually show trisomy 12, strong surface immunoglobulin positivity, CD20 and FMC7 positivity [1].

Atypical CLL shows morphological and cytogenetic differences as compared to the typical CLL. Lens D et al stated that 17p deletions are common in patients with atypical CLL and associated with a poor prognosis [12]. Schwarz J et al. studied 110 CLL patients retrospectively and found that atypical FAB morphology was shown to correlate with IgVH gene mutation status, trisomy of chromosome 12 and deletion of 17p [13]. Hamblin TJ et al stated that Un mutated V (H) genes were significantly associated with atypical morphology, isolated trisomy 12, advanced stage and progressive disease [14]. Matutes E et al analysed 544 patients and stated that trisomy 12 defines a subgroup of CLL with more frequent atypical morphology, including CLL/PL, stronger SmIg and FMC7 expression, more advanced stages (B and C in 18%) and possibly worse prognosis[15]. Tabernero MD et al studied 104 patients and reported a high incidence of trisomy 12 in patients of atypical CLL [16]. Other markers

including CD38 and CD21 are also more frequently expressed by atypical CLL and these were confirmed by Newman RA et al in a study of 127 patients [17]. The expression of CD79a is seen very frequently in atypical CLL, which has been studied earlier by Anca Bacârea et al. and is seen in our case as well [18].

Atypical CLL is prognostically significant as it is associated with a poor prognosis. Oscier DG et al in their study of 270 CLL patients, saw that atypical lymphocyte morphology is an important poor prognostic factor in stage A CLL, and is independent of trisomy 12 [19].

The diagnosis of CLL requires a flow cytometric immunophenotyping of peripheral blood (PB). Matutes et al had devised a scoring system that helps in the differential diagnosis between CLL and other mature B-cell neoplasms (MBN) [20]. CLL/SLL typically demonstrates low-intensity staining for sIg, low or absent expression of CD22, CD79b and FMC7 and moderate to strong expression of CD5 and CD23. However, this phenotype is not entirely specific and some overlap in exists between CLL and other MBN. The leukemic phase of CD5 positive mantle cell lymphoma (MCL) can be misdiagnosed as CLL.

The addition of CD200 helps in the differential diagnosis between CLL and other mature B-cell neoplasms, especially when the subtype of CLL is atypical which morphologically can cause confusion with mantle cell lymphoma. Martin Spacek et al studied 200 cases of CLL which showed a bright expression of CD200 and all the mantle cell lymphoma cases were CD200 negative. Also, CD200 expression was retained in the atypical morphological variant of CLL [21]. Ting YS et al studied 70 control samples, 63 samples with CLL or atypical CLL phenotype, 6 MCL samples, and 40 samples of other mature B cell neoplasms and stated that All CLL samples (including atypical) were positive for CD200, whereas MCL samples were dim or negative for CD200, establishing CD200 as an important significant marker [22]. El Din Fouad NB et al. further stated in their study that CD200 could be of high value in distinguishing CLL, MCL, and atypical CLL. CD200 expression can also be of prognostic and therapeutic value in CLL cases [23]. R Poongodi et al. studied 77 CLPD cases. Variability in CD200 expression was seen which could help in the differentiation of chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL) from other CLPDs. CD200 was brightly expressed in 100% CLL cases. On the contrary, CD200 was uniformly negative in all Mantle cell lymphoma. Furthermore, all HCL cases showed a bright expression of CD200, thereby making it useful in differentiation from other CLPD with villous lymphocytes [24].

4. CONCLUSION

Atypical CLL is a distinct entity having cytogenetic, immunophenotypic, and morphologic differences from Atypical CLL. Also, it is essential to distinguish a CLL from other chronic lymphoproliferative disorders. A major problem is the lack of a consistent definition for the entity that separates it from other diseases. By applying the above criterion, which requires only standard FCI and morphologic review, subcategorization can be done between typical CLL, atypical CLL and other Chronic lymphoproliferative neoplasms. CD200 becomes an important and significant marker in this regard and should be put up in CLPD panel.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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