To Assess the Correlation between Bone Marrow Morphology and Immunophenotypic Findings in Patients of Chronic Lymphoproliferative Disorders (CLPD's) and to Assess the Role of Flowcytometric Immunophenotyping in Diagnosis and Subclassification of CLPD's

Ekta Dwivedi[1], Rashmi Kushwaha[2], Mili Jain[3], Geeta Yadav[4], S.P. Verma[5], U.S Singh[6], Ashutosh Kumar[7]

ABSTRACT

Introduction: Chronic lymphoproliferative disorder represent clonal proliferation of morphologically and immunophenotypically mature B or T cells characterized by a low proliferation rate and prolonged cell survival. Study aimed to assess the correlation between bone marrow morphology and immunophenotypic findings in patients of Chronic Lymphoproliferative Disorders (CLPD's) and to assess the role of flowcytometric immunophenotyping in diagnosis and subclassification of CLPD's.

Material and Methods: 48 newly diagnosed cases of CLPD were included. After complete clinical evaluation they underwent marrow aspiration, biopsy and immunophenotyping by flowcytometry with selected panel of monoclonal antibodies.

Results: On morphology 47.9% cases were CLL. In 52.1% non CLL cases , 4.2% were PLL , 2% case as LPL and 45.8% cases were CLPD-unclassifiable. Commonest pattern of marrow infiltration noted on trephine biopsy was diffuse in CLL, HCL-V, B-PLL and T-CLPD. On immunophenotyping 95.8% cases were B-CLPD and 4.25% T-CLPD. CD5, CD22, CD23, FMC7 and SmIg were used as first line markers followed by CD 10, CD 25, CD103, CD38, CD138 and Cyclin D1 (on biopsy sections) as second line markers. Final immunophenotypic diagnosis was CLL (54.2%), B-CLPD unclassified (29.2%), 4.1% each of LPL, MCL, T-CLPD and 2% each of B-PLLand HCL-V.

Conclusion: Concordance rate between morphological diagnosis and immunophenotypic diagnosis was 79.17%. Hence, Flowcytometry is necessary for confirmation of diagnosis and to classify the CLPD cases which are unclassifiable by morphology.

Keywords: CLPD, Morphology; CLPD, Immunophenotype; CLPD, Flowcytometry

INTRODUCTION

Chronic lymphoproliferative disorder is most common leukemia (nearly 40%) encountered in adults in the western countries. In India B-cell lymphomas form 79.1% of the NHLs, whereas T-cell lymphomas form 16.2% of the total.[1,2] The advent of immunophenotying of samples from patients with lymphoproliferative disorders has added much for proper diagnosis and classification and for better understanding of the pathogenic mechanisms underlying the development of these disorders. Bone marrow morphology is the first step in distinguishing acute vs chronic, lymphoid vs myeloid, chronic lymphocytic leukemia vs other chronic lymphoproliferative disorders and immunophenotyping is useful adjunct to morphological diagnosis. The histological pattern of lymphoid infiltration in trephine BMB is thought to be prognostic factor in CLL. In patients with Non Hodgkins Lymphoma examination of trephine biopsy is an important step in staging, in assessment of treatment response as well as in assessing the relapse in follow up patients.[3]

So this study was conducted to correlate the bone marrow morphology with immunophenotypic findings and to assess the role of flowcytometric immunophenotyping in diagnosis and subclassification of CLPD's.

The immunophenotyping by flow cytometry as an auxiliary method and in correlation with morphological findings it can make the diagnosis of CLPD faster and more specific.

Study aimed to assess the correlation between bone marrow morphology and immunophenotypic findings in patients of Chronic Lymphoproliferative Disorders (CLPD's) and to assess the role of flowcytometric immunophenotyping in diagnosis and subclassification of CLPD's.

MATERIAL AND METHODS

48 newly diagnosed cases of CLPD were included in this study. A detailed clinical history was taken from the patients followed by systemic examination with special emphasis on

[1]JR, Department of Pathology, K.G.M.U, Lucknow, [2]Professor, Department of Pathology, K.G.M.U, Lucknow, [3]Assistant Professor, Department of Pathology, K.G.M.U, Lucknow, [4]Assistant Professor, Department of Pathology, K.G.M.U, Lucknow, [5]Associate Professor, Department of Clinical Hematology, K.G.M.U, Lucknow, [6]Professor, Department of Pathology, K.G.M.U, Lucknow, [7]Head and Professor, Department of Pathology, K.G.M.U, Lucknow, India

Corresponding author: Prof (Dr) Rashmi Kushwaha, Department of Pathology, K.G.M.U, Lucknow, India

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organomegaly and lymphadenopathy.

Complete blood count with peripheral smear examination was done followed by bone marrow aspiration and trephine biopsy for all the patients.

Bone marrow sample was collected in EDTA vial for flowcytometric immunophenotyping using BD FACS CALIBUR. CLPD panel was used (CD45,CD19, CD5, CD20, FMC7, Anti lambda, CD4, CD25, CD7, CD19, CD38, CD23, Anti kappa, CD10, CD8, CD103, CD22, CD3). Stain –lyse –wash method was used. Data was analysed using Cell Quest Pro software.

This study was approved by institutional ethical clearance committee.

STATISTICAL ANALYSIS

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and Mean±SD.

RESULTS

A total 48 patients were evaluated. The primary outcome measures of the study were correlation between morphology on bone marrow aspirate with immunophenotypic diagnosis. Age of patients ranged from 28 to 80 years. Maximum number of patients were aged between 61-70 years (35.4%) followed by those aged 71-80 years (18.8%). There were only 22 (45.8%) cases aged less than 60 years, 8 (16.7%) each aged 41-50 and 51-60 years, 5 (10.4%) aged 31-40 years and 1 (2.1%) aged less than 30 years. Mean age of patients was 59.1±12.55 years. Majority were males (79.2%) and (20.8%) were females. Male to female ratio of study subjects was 3.8:1. Table 1 is showing the morphological diagnosis on bone marrow aspirate.

CD5, CD22, CD23, FMC7 and SmIg were used as first line markers (Table 2) in our study followed by CD 10, CD 25, CD103, CD38, CD138 and Cyclin D1(on biopsy sections) as second line markers (according to Matutes scoring system).[4,5] (Fig 1, table 2, table 3).

Significantly higher proportion of CLL cases were positive for CD5 and CD23. FMC7 positivity was higher in non-CLL cases as compared to CLL cases.

2 cases were positive for pan T cell marker CD3 and negative for pan B cell markers CD19 and CD20 and were classified as T-Chronic lymphoproliferative disorders.

One case was positive for CLL markers with aberrant CD8 expression (Figure 2).

Among non-CLL cases, a total of 7 cases were CD5 positive and CD23 negative. Immunohistochemistry on biopsy section was done for Cyclin D1 in 2 cases which were positive on biopsy and diagnosed as Mantle Cell Lymphoma.

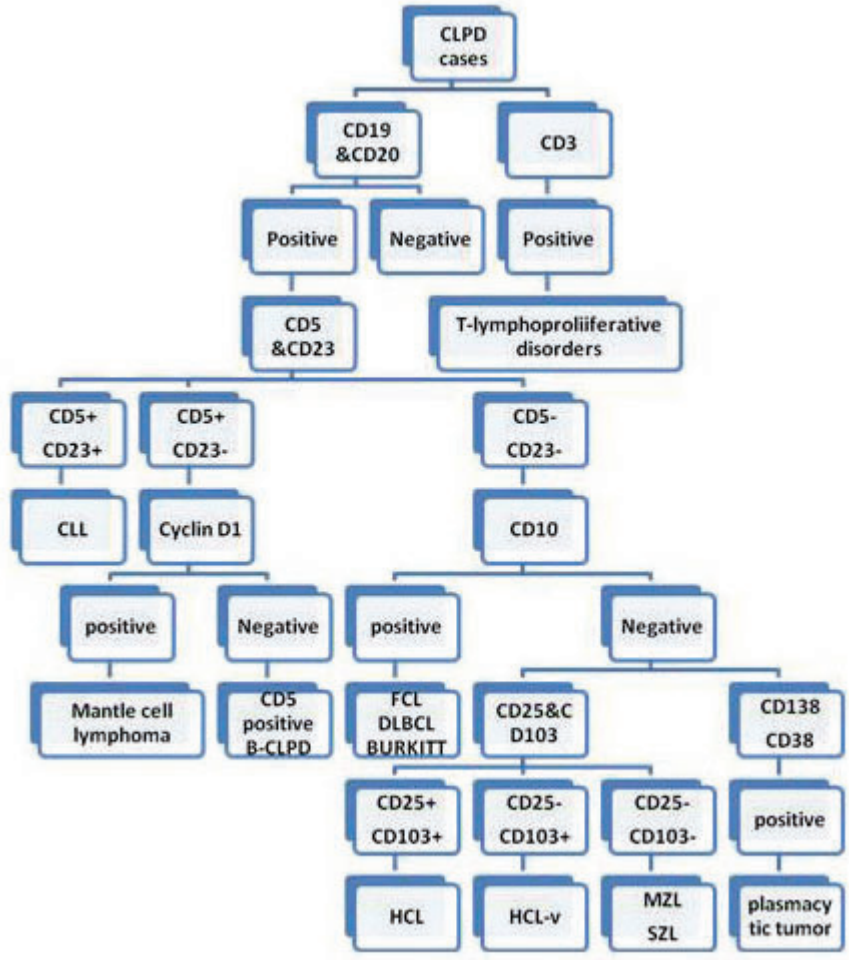

Figure-1: Flow chart of various markers used for diagnosing CLPD's

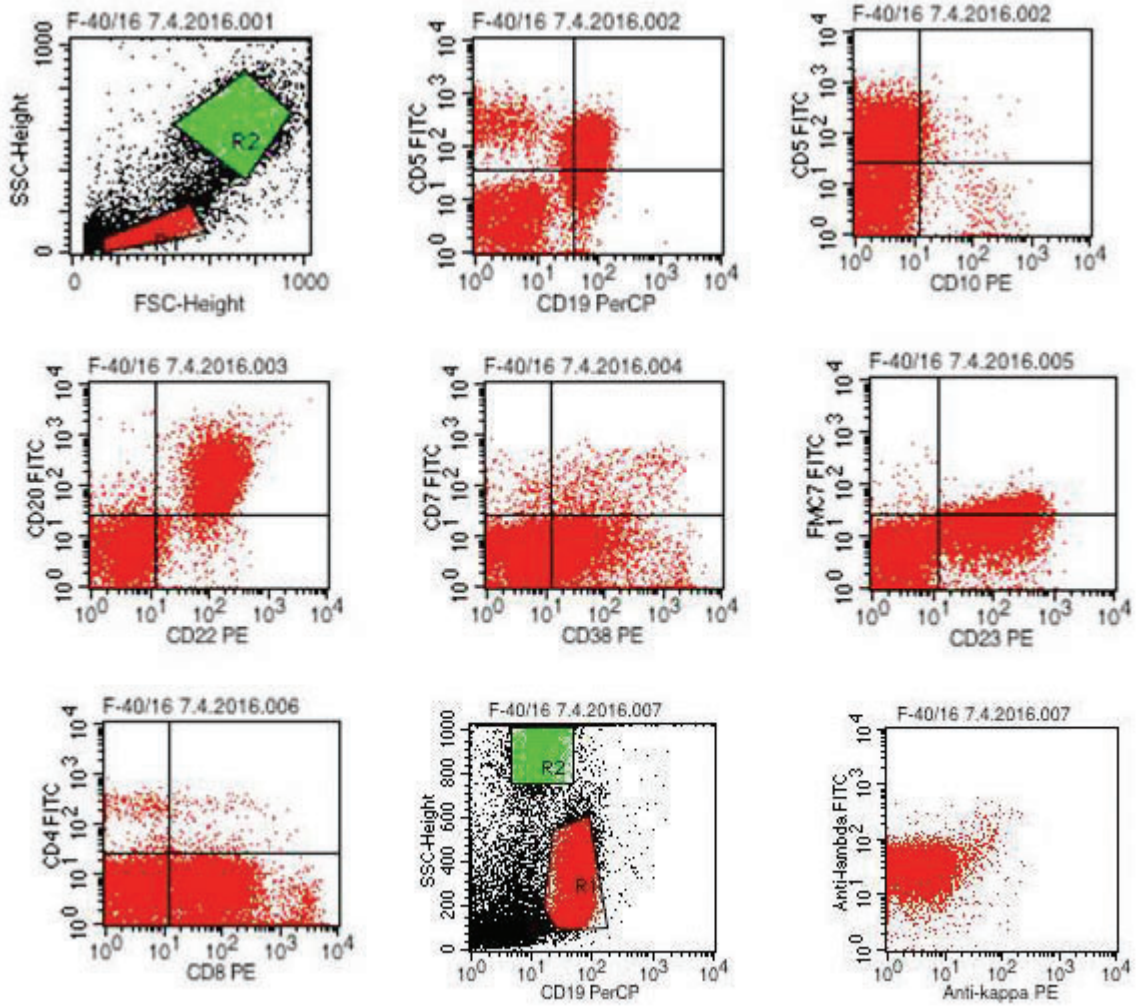

Figure-2: Case of CLL with aberrant CD8 expression: Flow plot showing neoplastic lympocytes (red dot) CD2, CD38, CD8 with lambda clonality are positive for CD5, CD19, CD20, CD22

SN	Diagnosis	No. of cases	Percentage
1.	CLL	23	47.9
2.	CLPD*	22	45.8
3.	PLL	2	4.2
4.	LPL	1	2.1
CLPD*- CLPD cases which were unclassified by morphology			

Table-1: Morphological diagnosis (on Bone Marrow Aspirate/Peripheral blood smear)

SN	Marker	CLL (n=26) (Score 4-5)			Non-CLL (n=22) (Score ≤3)			Statistical significance	
		Number tested	Number +ve	% +ve	Number tested	Number +ve	% +ve	χ^2	'p'
1.	CD5	26	26	100	22	8	36.4	23.36	<0.001
2.	CD22	13	4	30.8	14	3	21.4	0.306	0.580
3.	CD23	26	25	96.2	21	7	33.3	21.10	<0.001
4.	FMC7	26	1	3.8	21	10	47.6	12.42	<0.001
5.	Dim Surface Ig expression	26	26	100	22	16	72.7	8.104	0.004

Table-2: CLL Markers (CD5, CD22, CD23, FMC7, SmIg) (according to Matutes scoring system.)4,5

All the 12 cases that were CD5 and CD23 negative, were also CD10 negative. Among cases with CD5 and CD10 negative status, 3 were tested for CD25 but none were positive, 5 were tested for CD103 of which 1 was positive (20%) and diagnosed as Hairy Cell Leukemia variant (HCL-V) (Fig 3), 10 were tested for CD38 and 6 (60%) were positive and 3 were tested for CD138 and 2 (66.7%) were positive and diagnosed as LPL (Table 4).

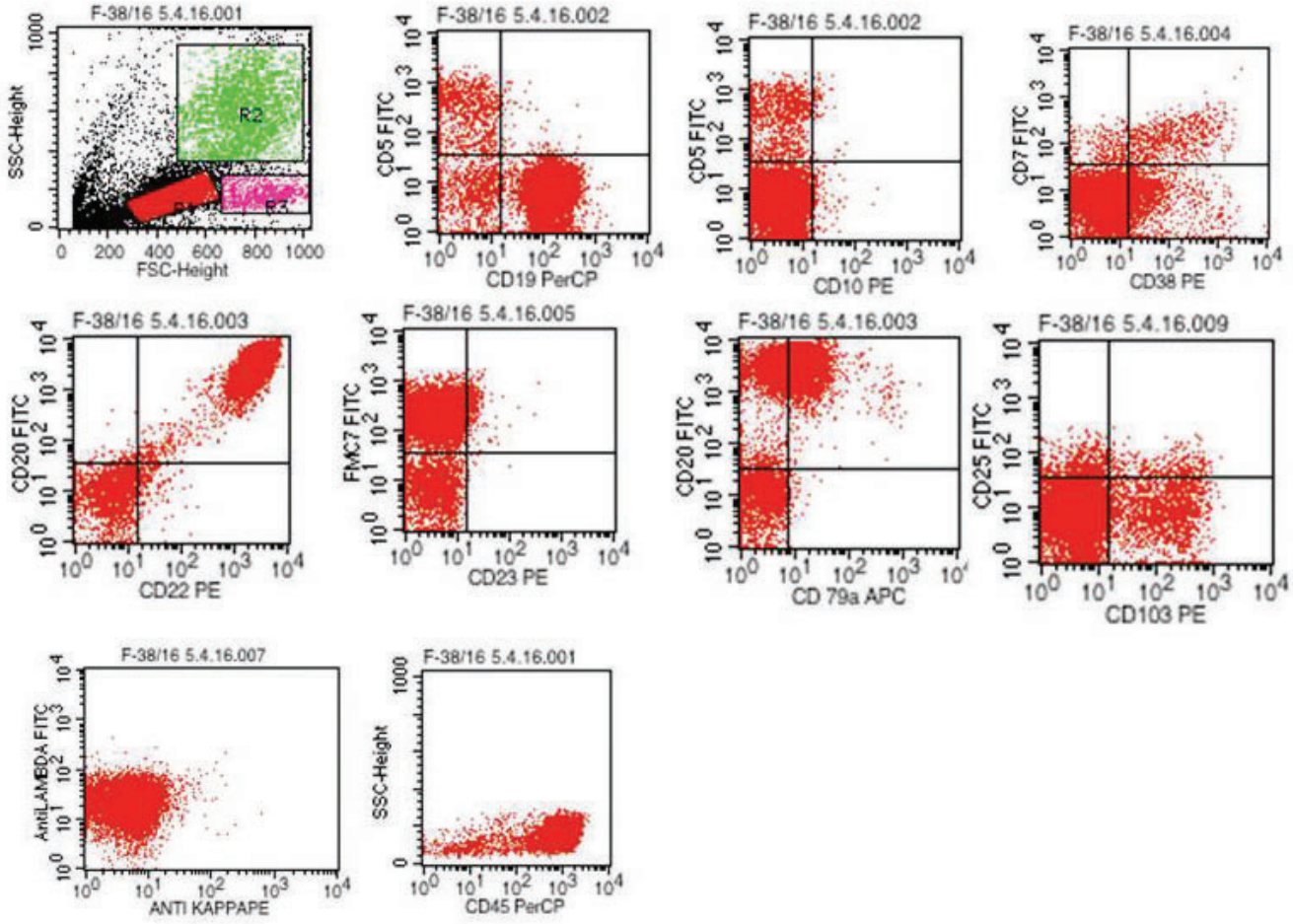

Figure-3: HCL-V: Flow plot showing neoplastic lymphocytes (red dot) are positive for CD19, CD20, CD22, FMC7, CD79a and CD103 with Lambda clonality

SN	State / Test conducted	Number tested	Number of positive cases	Percentage
1.	CD5 positive, CD23 negative			
	Cyclin D1 (on biopsy)	2	2	100
2.	CD5 Negative, CD23 negative			
	CD10	12	0	0
3.	CD5 Negative CD 10 negative			
	CD25	3	0	0
	CD103	5	1	20
	CD38	10	6	60
	CD138	3	2	66.7

Table-3: Exploration of CD5 Positive/Negative Non-CLL cases for further analysis

Diagnosis		No. of patients	Percent
1.	CLL	26	54.2
2.	B-CLPD Unclassified	14	29.2
3.	Others LPL MCL T-CLPD B-Cell PLL HCL-v	8 2 2 2 1 1	16.7
	Total	48	100.0

Table-4: Final diagnosis after flowcytometric immunophenotyping.

Flowcytometric Diagnosis		Morphologic Diagnosis				Total
		CLL	CLPD*	PLL	LPL	
1	CLL	23	3	0	0	26
2	B-CLPD*	0	13	1	0	14
3	LPL	0	1	0	1	2
4	MCL	0	2	0	0	2
5	T-CLPD	0	2	0	0	2
6	B-PLL	0	0	1	0	1
7	HCL-V	0	1	0	0	1
Total		23	22	2	1	48

CLPD-*unclassified, Concordance = 38/48 = 79.17%

Table-5: Concordance between Bone marrow Morphology (Bone marrow aspirate/Peripheral blood smear) and Flowcytometric findings

Marker	Marker Intensity	Score	Marker Intensity	Score
Surface immunoglobulin	Weak	1	Strong	0
CD5	+	1	-	0
CD23	+	1	-	0
CD22/CD79b	Weak	1	Strong	0
FMC7	-	1	+	0

+present; -absent.
NOTE:
Diagnosis of chronic lymphocytic leukemia requires a score of 4 or 5
If <= 3 then exclude mantle cell lymphoma by one or both of:
(i) Immunostaining of bone marrow trephine sections or lymph node for nuclear cyclin D1
(ii) FISH for t(11:14) using blood, BM or lymph node.

Table-6: Scoring system for the diagnosis of CLL4,5

On correlating the bone marrow aspirate findings with flowcytometric immunophenotyping, concordance was maximum for B-CLPD which were unclassifiable (13/14; 92.86%) followed by CLL (23/26; 88.46%). The two single cases of PLL and LPL were diagnosed on morphology as well as on immunophenotyping. Mantle Cell Lymphoma, T-lymphoproliferative disorders and HCL-V were characteristic immunophenotypic diagnosis not possible through morphological assessment. Flow cytometry also subclassified the 22 cases of CLPD diagnosed by morphology as 3 cases of CLL, 1 of LPL, 2 with MCL, 1 with HCL-V and 2 with T-Chronic lymphoproliferative disorders (Table 5).

Age of CLL patients ranged from 37 to 80 years. Majority were above 60 years of age (61.5%). There were 3 (11.5%) patients each aged 31-40 and 41-50 years. Mean age of patients was 60.85±12.23 years.

Fever was the most common symptom (61.5%) in CLL patients followed by weight loss (42.3%) and night sweats (3.8%). Isolated lymphadenopathy (19.2%) and splenomegaly (15.4%) was present in (19.2%) and (15.4%) patients respectively. Lymphadenopathy along with splenomegaly was present in 3 (11.5%) patients.

Bone marrow biopsy showed diffuse pattern of infiltration in 91.7% cases while remaining 8.3% had interstitial pattern.

B-CLPD cases which were not subclassified by flowcytometric immunophenotyping were further subdivided into CD5 positive (5 cases) and CD 5 negative (9 cases). However, in both the categories the maximum age incidence was in 6th decade and diffuse pattern of bone marrow infiltration was found on trephine biopsy.

Mean age incidence in other types of CLPD were 66 years for HCL-v, 54 years for LPL and MCL, 75 years for B-PLL. Mean age of patients was minimum in T-Chronic Lymphoproliferative disorders (31.50 years).

The most common pattern of marrow infiltration noted was diffuse in HCL-V, B-PLL and T-CLPD. Whereas, in MCL it was diffuse and nodular pattern of marrow infiltration on bone marrow trephine biopsy.

DISCUSSION

The identification and the classification of CLPDs is critical for the patient risk assessment and the treatment planning. An accurate diagnosis of CLPD poses numerous challenges and it has to be achieved by a combinatorial diagnostic approach which includes the morphologic examination, the surface marker expression profiling and cytogenetics.

In our study population age of patients ranged from 28 to 80 years with a mean age of 59.1±12.55 years. Maximum number of patients were aged between 61-70 years (35.4%). Male to female ratio of study subjects were 3.8:1.

Peripheral blood film and bone marrow aspirate smears were extensively studied for cellular morphology with reference to cell size, nuclear cleaving, chromatin, nucleoli, cytoplasm and presence of smudge cells. 47.9 % (n=23/48) cases were diagnosed CLL. In 52.1%(n=25/48) non CLL cases, 4.2%(n=2/48) were chronic PLL, 2%(n=1/48) case was LPL and 45.8%(22/48) cases could not be classified and diagnosed as CLPD-unclassifiable by morphology.

For immunophenotyping we have used flow cytometry in all the 48 cases in addition to IHC on biopsy section for cyclin

D1 in few selected cases.

Using pan T- cell marker and pan B -cell markers (CD3,CD19,CD20) we were able to classify 4.2% (2/48) cases as T-Chronic lymphoproliferative disorders and 95.8% (46/48) cases as B-chronic lymphoproliferative disorders. So mature T cell neoplasms are relatively uncommon in comparison to mature B cell neoplasms according to our study.

We have used two B-cell restricted markers (CD23, FMC7), surface immunoglobulin expression and light chain restriction using antikappa and antilambda reagents and assessment of the fluorescence intensity of membrane by CD22, T-cell and B-cell subset marker: CD5. These were the first panel markers basically used to distinguish CLL from other CLPDs.[4]

To estimate the value of each membrane leukocyte antigen in differential diagnosis of CLL, the expression of two B-cell lineage antigens (CD19, CD20) and five antigens from the CLL scoring system (CD5, CD23, FMC7, CD22, sIg) were analyzed. Among lineage B-cell antigens, CD19 was expressed in 46 patients, indicating that it is the only marker consistently expressed on leukemic B- cells. Our results are in the line with literature data which select CD19 as one of the best gating antigen for immunophenotypic analysis of B cell neoplasms, including CLL.[6]

However, other two lineage B-cell antigens, CD20, were less consistently expressed on CLL cells. The probable reason for that is their low expression levels on CLL cells. According to our results, CD20 and CD19 were not important for differential diagnosis of CLL, because these antigens were expressed in virtually all our CLPD cases. Our results are in the line with results obtained by Delgado et al.[5]

One of antigens from CLL scoring system is CD5, which is generally considered as pan T-cell antigen, although some restricted B cells, named B1 cells, also express CD5.[5] In pathological conditions, CD5 is usually expressed in CLL and MCL, in some cases of PLL, diffuse large B- cell lymphoma and HCL.[7,8] Our results showed that frequency of expression of CD5 antigen as well as CD5+ high expression pattern was significantly higher in CLL compared to non-CLL group. Considering these data, CD5 antigen could have important role in differential diagnosis of CLL. Similar results were found in studies by Pangalis et al and Deneys et al, where the frequency of CD5 expression in CLL was very high.[8,9]

Comparing our study with Dronca RS, Jevremovic D, et al study regarding CD5 positive CLL cases.[10]

Considering the CD23 antigen, it was shown that it was expressed in the majority of CLL compared to non-CLL patients, which made it relevant for differential diagnosis of CLL. Our results are in the line with literature data suggesting that CD23 antigen is one of the most important markers for differential diagnosis between CLL and MCL.[9] Addressing this issue, Di Raimondo et al. demonstrated that CLL/CD23 negative variant was rare (6%).[9,11]

In these cases, the diagnosis of MCL has to be confirmed by cyclin D1 immunostaining on biopsy and/or by detection of chromosomal translocation t (11;14).[5]

FMC7 antigen is also considered to be reliable marker for differential diagnosis of CLL, distinguishing CLL from other CLPD.[12] Our results support this finding showing that only3.8% of CLL patients expressed FMC7, whereas it was expressed in 47.6% patients from non-CLL group. Furthermore, some studies have shown wide range of frequency of FMC7 positive CLL cases (12-30%).[6,8]

Another component of the B-cell receptor complex is sIg, which is used to determine monoclonality of B-cells by flow cytometry, defined according to the presence of sIg light chain restriction. Although the monoclonality was detected in 100% of our CLL patients. In our CLL group, the frequency of sIgκ+ and sIgλ+ cases were equal. Likewise, in the study by Matutes et al., it was shown that sIg was expressed in 92% of CLL cases with similar distribution of sIgκ+ and sIgλ+ positive cases.[13] Our results showed that the majority of CLL patients had significantly higher frequency of sIg+low expression pattern compared to the non-CLL patients, what makes this antigen important for differential diagnosis of CLL. Based on our results, which determined the value of each explored antigen for differential diagnosis of CLL, we could define the specific immunophenotypic profile of CLL cells as follows: CD19+ CD20+low CD22+low CD5+high CD23+ FMC7- low sIg+low. Moreover, it is of note that only the combination of the aforementioned antigens can be used for reliable differential diagnosis of CLL, distinguishing it from other CLPDs. By applying the CLL scoring system to all our patients, it has been shown that the majority of patients(88%) with the final diagnosis of CLL had score values 4 and (22%) had score value 5. The majority of our patients with final diagnosis of non-CLL (100%) had lower score values (0 - 3), similar to the study of Matutes et al.[4,13]

One case was of CLL with aberrant CD8 expression although T cell marker is an unusal finding in B-CLL but our study was concordant with Schroers R et al study.[14]

91.7% of CLL cases had diffuse pattern of marrow infiltration while remaining 8.3% had interstitial pattern. Our bone marrow biopsy patterns were comparable to study by Abdel-Ghafar AAA, Mahmoud HM et al.[15]

Whereas in study of Agarwal N et al 67.3% cases had nodular pattern of marrow infiltration.

In our study 100% of morphologically diagnosed CLL cases correlated with flowcytometry findings with score of 4-5. However 3 cases morphologically diagnosed as CLPDs, were finally diagnosed as CLL on the basis of flow cytometry score 4-5. This emphasises the beneficial role of scoring system in CLL cases with variable morphology.

Second line panel was applied selectively, depending on the cell morphology and the results with the first panel.

Of the 7 Cases which were CD5 positive and CD23 negative immunophenotype, cyclin D1 expression was analyzed by immunohistochemistry in 2 cases on the basis of suspicious morphology and diagnosed as mantle cell lymphoma. Cyclin D1 expression is an important criteria for Mantle Cell Lymphoma diagnosis however cases were also reported as Cyclin D1 negative Mantle Cell Lymphoma and t(11,14) should carried out in these cases. As reported by Yatabe Y,

Sujuki R et al and Fu K, Weisenburger DD et al Study.[16,17]
In 12 cases which were CD5 and CD23 negative, CD10 marker was done which was negative.
Further CD25, CD103, CD138 were applied. One case was CD103 positive, 2 cases were CD138 positive and were diagnosed as HCLv and lymphoplasmacytic lymphoma respectively and 14 cases could still not be specifically categorized. However still flow cytometry is helpful in identifying CD20 positive lymphoid cells for targeted anti CD20 (Rituximab) therapy.

CONCLUSION

Use of flowcytometry is necessary for confirmation of diagnosis and to classify the CLPD cases which are unclassifiable by morphology. Scoring system using CD5, CD23, CD22, FMC7, and SmIg is useful in differentiating CLL from other CLPDs.
In a case of CLPD, a screening panel comprising CD19, CD5, CD23, FMC7, CD10, CD20, CD3, kappa and lambda would be successful in the diagnosis of most of CLPDs. This can be followed by a secondary panel as required. CD5, CD23, and FMC7 were identified as most sensitive markers in differentiating CLL from other CLPDs.

Abbreviations

CLPD: Chronic lymphoproliferative disorder, CLL: Chronic lymphocytic leukemia, HCL: Hairy cell leukemia, HCL-V: Hairy cell leukemia variant, PLL: Prolymphocytic leukemia, LPL: Lymphoplasmacytic lymphoma, MCL: Mantle cell lymphoma

REFERENCES

1. Varma S, Varma N. Chronic lymphoproliferative disorders (CLPD's). In, Manoranjan Mahapatra (ed). Recent advances in hematology-3. New Delhi, Jaypee, 2011; 99-109.
2. Naresh KN, Srinivas V, Soman CS. Distribution of various subtypes of non-Hodgkin's lymphoma in India: A study of 2773 lymphomas using R.E.A.L. and WHO Classifications. Ann Oncol 2000;11; 63-7.
3. S P Sah, E Matutes, A C Wotherspoon, R Morilla, D Catovsky A comparison of flow cytometry, bone marrow biopsy, and bone marrow aspirates in the detection of lymphoid infiltration in B cell disorders. J Clin Pathol 2003;56:129–32.
4. Moreau EJ, Matutes E, A'Hern RP, Morilla AM, Morilla RM, Owusu-Ankomah KA et al. Improvement of the chronic lymphocytic leukemia scoring system with the monoclonal antibody SN8 (CD79b). Am J Clin Pathol. 1997;108:378-82.
5. Delgado J, Matutes E, Morrila AM, Morilla RM, Owusu-Ankomah KA, Rafig-Mohammed F, et al. Diagnostic significance of CD20 and FMC7 expression in B-cell disorders. Am J Clin Pathol. 2003; 120:754-9.
6. Béné MC, Nebe T, Bettelheim P, Buldini B, Bumbea H, Kern W, et al. Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European Leukemia Net Work Package 10. Leukemia. 2011; 25:567-74.
7. Montserrat E. Chronic lymphproliferative disorder. Curr Opin Oncol. 1997;9:34-41.
8. Pangalis GA, Angelopolou MK, Vassilakopoulos TP, Siakantaris MP, Kittas C. B-chronic lymphocytic leukemia, small lymphocytic lymphoma and lymphoplasmacytic lymphoma, including Waldemstrom's macroglobulinemia: a clinical, morphologic, and biologic spectrum of similar disorders. Semin Hematol. 1999; 36:104-14.
9. Deneys V, Michaux L, Leveugle P, Mazzon AM, Gillis E, Ferrant A, et al. Atypical lymphocytic leukemia and mantle cell lymphoma immunologically very close: flow cytometric distinction by use of CD20 and CD54 expression. Leukemia. 2001; 15:1458-65
10. Dronca RS, Jevremovic D, Hanson CA, Rabe KG, Shanafelt TD, Morice WG et al. CD5-positive chronic B-cell lymphoproliferative disorders: Diagnosis and prognosis of a heterogeneous disease entity. Cytometry B clin Cytom.2010;78 Suppl 1:S35-41.
11. DiRaimondo F, Albitar M, Huh Y, Obrein S, Montilo M, Tedeschi A, et al. The clinical and diagnostic relevance of CD23 expression in the chronic lymphoproliferative diseases. Cancer. 2002; 94:1721-30.
12. Matutes E, Attygalle A, Wotherspoon A, Catovsky D. Diagnostic issues in chronic lymphocytic leukaemia (CLL). Best Pract Res Clin Haematol. 2010; 23:3-20.
13. Matutes E, Owusu-Ankomah K., Morilla R, Garcia Marco J, Houlihan A, Que T.H. et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia 1994, 1640-5.
14. Schroers R, Pukrop T, Dürig J, Haase D, Dührsen U, Trümper L et al. B-cell chronic lymphocytic leukemia with aberrant CD8 expression: genetic and immunophenotypic analysis of prognostic factors. Leuk Lymphoma 2004;45:1677-81.
15. Abdel-Ghafar AA, El Din El Telbany MA, Mahmoud HM, El-Sakhawy YN. Immunophenotyping of chronic B-cell neoplasms: Flow cytometry vs immunohistochemistry. Hematol Rep 2012; volume 4:e3.
16. Yatabe Y, Suzuki R, Tobinai K, Matsuno Y, Ichinohasama R, Okamoto M et al. Significance of cyclin D1 overexpression for the diagnosis of mantle cell lymphoma: a clinicopathologic comparison of cyclin D1-positive MCL and cyclin D1-negative MCL-like B-cell lymphoma. Blood 2000;95:2253-61.
17. Fu K,weisenburger DD, Greiner TC, Dave S, Wright G, Rosenwald A et al. Cyclin D1–negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling; Blood 2005;106:4315-21.

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