ORIGINAL RESEARCH PAPER

Pathology

KEY WORDS: Chronic Lymphoproliferative Disorder, Bone Marrow Aspiration, Immunophenotyping

UTILIZATION OF SCORING SYSTEM IN DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA IN INDIAN POPULATION.

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ABSTRACT

CLL is a rare B-lymphoid malignancy in Asian population and very few studies available in indian population . Here we have done prospective study evaluating a scoring system based on the expression of CD5 , CD23, FMC7,CD22 and surface immunoglobulin to distinguish CLL from other types of lymphoid neoplasms in the Indians.

Material and methods: 48 patients samples with a clinical as well as morphological features of chronic lymphoproliferative disorders were analysed by flow cytometry. A score of one was given if the following markers were assigned Cd5+, CD23+,FMC7-,CD22+ and surface immunoglobulins.

Results: Only 26 cases were confirmed as B-CLL(score > 3). In 22 cases with scores < or =3, a variety of non-CLL disorders predominated, such as lymphoplasmacytic lymphoma , hairy cell leukemia, mantle cell lymphoma, and prolymphocytic leukemia.

CONCLUSION: A score of 4 and dual CD5/CD23 expression are essential for the diagnosis of CLL while a score of 2 mostly indicative of non-CLL. Scoring system using CD5, CD23, CD22, FMC7, and SmIg was found to be useful in differentiating CLL from other CLPDs. CD5, CD23, and FMC7 were identified as most sensitive markers in differentiating CLL from other CLPDs.

INTRODUCTION

Chronic lymphoproliferative disorder represent clonal proliferation of morphologically and immunophenotypically mature B or T cells.[1]These disorders having indolent course, variable progression and good control but in most instances low complete cure rates.[2] Other variant of CLPDs should be known and clearly separated from chronic lymphocytic leukemia because their prognosis and treatment is different from that of chronic lymphocytic leukemia.[3]. The incidence in the USA & UK is about 3.9 and 6.5 respectively per 100,000 per year .[4,5]One report from the UK shows that patients of South-Asian origin with CLL have more aggressive disease compared to those among white population.6 In this article we evaluate utilization of scoring system for distinguishing chronic lymphocytic leukemia from other chronic lymphoproliferative disorders in Indian population.

MATERIAL AND METHODS

Newly diagnosed cases of CLPDs were included in the present study. 2ml of peripheral blood sample was collected in EDTA vial for complete blood counts and general blood picture examination with abnormal lymphocyte counts. Bone marrow aspiration was done in patients under local anesthesia using 2% lignocaine solution. Six aspirate smears made for morphological examination.The abnormal lymphocytes were identified on the basis of their size, cytoplasm, cytoplasmic granules, nuclear chromatin, nuclear cleaving and nucleoli(leishman stain). Immunophenotyping was done by a multicolor flowcytometer18. The instrument used was BD FACS Caliber (Becton Dickinson-Fluorescence Assisted Cell Sorter). Co-expression of different lymphoid and myeloid antigens were confirmed by using appropriate pairs of monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin-chlorophyl-protein (PerCP).by using appropriate pairs of monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin-chlorophyl-protein (PerCP).

REAGENTS USED: Fluorochrome labeled antibodies: Chronic lymphoproliferative disorder panel was used which included the following antibodies: PerCP: CD45,CD19

FITC: CD5, CD20, FMC7, Anti lambda,
PE: CD19, CD23, Anti kappa,

DATA ANALYSIS: Data was analyzed using Statistical Package for Social Sciences (SPSS) Version 15.0. Frequency (number), proportions (%), mean and standard deviation was used to represent the data.

RESULTS:

FLOWCYTOMETRIC DIAGNOSIS OF CLL vs Non CLL
CLL Markers (CD5, CD22, CD23, FMC7, SmIg)*

SN	Marker	CLL (n=26) (Score 4-5)			Non-CLL (n=22) (Score ≤3)			Statistical significance	
		No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve	X^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

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.

DISCUSSION

We have, in this study, evaluated 48 newly diagnosed cases of chronic lymphoproliferative disorders. We have use 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, adapted from Matutes E et al. Study[7].

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, slg) 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[8].

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.[9]

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 [10]. In pathological conditions, CD5 is usually expressed in CLL and MCL, in some cases of PLL, diffuse large B- cell lymphoma and HCL[19] . Our results showed that frequency of expression of CD5 antigen as well as CD5+high expession 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 [13,14.]

Comparing our study with Dronca RS, Jevremovic D, et al. study regarding CD5 positive CLL cases.[16]

	Dronca RS et al.[16]	Our study
Number of CD5 positive cases	N=61	N=34
% of CLL cases	44%	76%

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[15].Addressing this issue, Di Raimondo et al. demonstrated that CLL/CD23 negative variant was rare (6%) [15]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).

FMC7 antigen is also considered to be reliable marker for differential diagnosis of CLL, distinguishing CLL from other CLPD [11]. 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%) [9,14].

Comparing our study with study of Ahmed E et al17 study regarding CD23 and FMC7 expression is listed below:

	Ahmed E et al.[17]	Our study
CD 23 positive CLL cases	121	25
CD 23 positive, FMC7 negative CLL cases	105(88%)	24(94%)

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 are equal. Likewise, in the study by Matutes et al.[8] shown that sIg was expressed in 92% of CLL cases with similar distribution of sIgκ+ and sIgλ+ positive cases. Our results showed that the majority of CLL patients had significantly higherfrequency 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, ithas 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), Similarly in the study of Matutes et al. [8]

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