Original Article

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Pathology Section

Flowcytometry in Chronic B-Cell Lymphoproliferative Disorders: A Retrospective Study from a Tertiary Oncology Centre, Trivandrum, India

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ABSTRACT

Introduction: Chronic B-Cell Lymphoproliferative Disorders (B-CLPD) are malignant neoplasms of B lymphocytes characterised by accumulation of mature B lymphocytes in the Bone Marrow (BM), peripheral blood, and lymphoid tissues. Multiparameter flowcytometry has become a powerful tool in diagnosing B-CLPD that identifies a clonal light-chain restricted population expressing B-cell markers.

Aim: To study the morphologic and immunophenotypic profile of B-CLPD by flowcytometry and to determine the incidence of various subtypes.

Materials and Methods: All consecutively diagnosed cases of chronic B lymphoproliferative disorders at Regional Cancer Centre, Trivandrum, Kerala, India from 1st December 2016 to 30th November 2018 were retrospectively analysed and studied. Diagnosis of CLPD was made based on peripheral smear and BM aspiration and immunophenotyping by flowcytometry. Flowcytometry was performed using BD FACS Verse flowcytometer. The results of immunophenotyping by flowcytometry were reviewed and analysed.

Results: During the study period, 231 cases were diagnosed as Chronic Lymphoproliferative Disorder (CPLD), of which 209 cases were B-CLPDs. Male to female ratio (M:F) was 2:1. In the present study, incidence of Chronic Lymphocytic Leukaemia (CLL) was 138 cases (66.03%), Follicular Lymphoma (FL) was 14 cases (6.7%), Hairy Cell Leukaemia (HCL) was 11 cases 5.26% and Mantle Cell Lymphoma (MCL) was 9 cases, (4.31%). Prolymphocytic leukaemia and splenic Marginal Zone Lymphoma (MZL) constituted 4 cases (1.91%) each. Some cases of B-CLPDs had no definite diagnoses which were diagnosed as CD5 positive B-CLPD unclassified 13 cases (6.22%) and CD5 negative B-CLPD unclassified 16 cases (7.66%).

Conclusion: Flowcytometry has helped in definite subtyping of B-CLPD in most cases. Most common subtype in the present study was CLL. CD5+/CD23+ is highly specific for diagnosing CLL. CD200 is revealed to be an excellent marker to distinguish CLL from MCL.

Keywords: Cancer, Diagnosis, Immunophenotyping, Lymphoma

INTRODUCTION

The B-CLPD are a biologically heterogeneous group of malignancies characterised by clonal proliferation of mature B lymphocytes [1,2]. The World Health Organisation (WHO) classification of lymphoid neoplasms 2017 is based on morphological and immunophenotypical characteristics and, in some cases, molecular markers [3]. The characteristic immunophenotypic characteristics of various lymphomas are well-known [4,5]. Distinction between B-CLPD subtypes can be difficult due to overlap in cell morphology and immunologic features. Thus, a variety of clinical, haematological, cytogenetic, and molecular features may be required to be evaluated in combination with morphology and immunophenotyping to accurately subtype B-CLPD [6-8]. The B-CLPD include CLL, B-Cell Prolymphocytic Leukaemia (B-PLL), HCL, MCL, MZL, FL and Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinaemia (LPL/WM) [1,2,6,8]. Discriminating an entity of B-CLPDs from another is of critical importance as there is difference in prognosis and treatment.

The B-CLPD are most often diagnosed by flowcytometric immunophenotyping that identifies a clonal light-chain restricted population expressing B-cell markers [9]. Flowcytometric immunophenotyping has become a powerful tool for ascertaining the ontogeny in B-CLPD by virtue of its high throughput, low turn around time and feasible logistics [10]. Multiparametric flowcytometry is a fast and accurate method for lineage assignment and classification in CPLD [11-14]. It can aid in the diagnosis and prognosis as well as provide relevant information during the treatment follow-up, including the determination of Minimal Residual Disease (MRD) [15-18]. In daily practice, the diagnosis of CPLD is always based on cell morphology and immunophenotype; if tissue sections are studied, the diagnosis is based on histology and immunohistochemistry [16,19,20].

The incidence of B-CLPDs seems to be low in Asian population and only a few studies have described the spectrum of B-CLPDs in Asians [5,21]. Most of these studies are old and the diagnosis of some cases may need to be revised. To the best of our knowledge, there are no studies systematically describing the spectrum and immunophenotyping of B-CLPDs from southern parts of India. A retrospective study was conducted to analyse the morphologic and immunophenotypic profile of B-CLPD by flowcytometry. In addition, the diagnostic values of the immunological markers were also studied.

MATERIALS AND METHODS

A retrospective study was conducted at Regional Cancer Centre, Trivandrum, Kerala, India with the approval of the Institutional Review Board (IRB No:12/2018/03). All consecutively diagnosed cases of B-CLPD by flowcytometry from 1st December 2016 to 30th November 2018 (two years) were retrospectively analysed and studied.

Inclusion criteria: All new cases of B-CLPD diagnosed by flowcytometry done in peripheral blood/ BM aspirate/CSF samples were included in the study.

Exclusion criteria: Relapsed Cases of B-CLPDs were excluded from the study.

Study Procedure

Diagnosis of CLPD was made based on peripheral blood counts, peripheral smear and BM aspiration and immunophenotyping by flowcytometry. Flowcytometry was done in peripheral blood and BM specimens in most cases. In one case, we received Cerebrospinal Fluid (CSF) for flowcytometry. Peripheral blood smears and BM aspirates were air dried and stained with Giemsa stain. Six colour flowcytometry analysis was performed using BD FACS Verse flow cytometer (Becton Dickinson, USA). Standard lyse-wash procedure was used. The cells were stained with various combinations of Fluorescein Isothiocyanate (FITC), Phycoerythrin (PE), Peridinin Chlorophyll Protein (PerCP), phycoerythrin Cyanine 5.5 (PE-Cy 5.5), Allophycocyanin (APC) and Allophycocyanin- H7 (APC-H7) labelled monoclonal antibodies. All the monoclonal antibodies were obtained from Becton Dickinson (San Jose, California, United states). The panel of antibodies used included CD20, CD19, CD5, CD10, CD23, CD14, CD11C, CD200, CD45, FMC7, kappa and lamba. A secondary panel of antibodies were used whenever necessary which included CD25, CD123 and CD103. Briefly, the blood or BM samples were incubated with the relevant antibodies for 30 minutes at room temperature before being washed twice with phosphate buffered saline (pH 7.4). A minimum of 10,000 total events were acquired for each analysis. The sample positivity was based on a finding of ≥20% cells which expressed a particular antigen. Further, the expression levels of the individual antigens were described as strong and dim, depending on the order of the magnitude by which the fluorescence intensity with their cognate antibodies was higher than the corresponding isotype controls [22]. Data was collected from the institutional medical records.

STATISTICAL ANALYSIS

IBM® Statistical Package for Social Science version 24.0 was used for statistical analysis of this study. The categorical variables were reported using frequency and proportion. The continuous variables will be given in terms of mean and standard deviation. Association between two categorical variables was assessed using Chi-square/ Fisher's exact test. A p-value <0.05 was considered to be significant.

RESULTS

Spectrum of B-cell Chronic Lymphoproliferative Disorder

During the study period, 231 cases were diagnosed as CPLD. Among 231 cases, 209 cases were B-CLPD and 22 cases were T-CLPD. Among B-CLPD, M:F ratio was 2:1 and median age at presentation was 62 years. Flowcytometry was done in peripheral blood in 143 cases, BM in 65 cases and CSF in one case.

Chronic lymphocytic leukaemia was the most common subtype accounting for 66.03% (138 cases) followed by FL 6.7% (14 cases), HCL 5.26% (11 cases) and MCL 4.31% (9 cases). Prolymphocytic leukaemia and splenic MZL constituted 1.91% (4 cases) each. Among 209 cases of B- CLPD, 13.88% (29 cases) remained unclassified, which includes CD5 positive B-CLPD unclassified 6.22% (13 cases) and CD5 negative B-CLPD 7.66% (16 cases) [Table/Fig-1].

Diagnosis	Count (n)	Percentage (%)
CD5+5+B-CLPD	13	6.22
CD5-B-CLPD	16	7.66
CLL	138	66.03
FL	14	6.70
HCL	11	5.26
MANTLE	9	4.31
PLL	4	1.91
SMZL	4	1.91
Total	209	100

[Table/Fig-1]: Incidence of B-CLPD by Flowcytometry.
B-CLPD: B-cell Chronic lymphoproliferative disorders; CLL: Chronic lymphocytic leukaemia; FL: Follicular lymphoma; HCL: Hairy cell leukaemia; MANTLE: Mantle cell lymphoma; PLL: Prolymphocytic leukaemia; SMZL: Splenic marginal zone lymphoma

Expression of Immunological Markers

Chronic Lymphocytic Leukaemia (CLL): The CD5 expression was seen in all cases of CLL. All except 3 cases (97.8%) were positive for CD23. The CD200 was positive in all cases of CLL. Out of the 138 cases of CLL, FMC7 was expressed in 22.4% cases. The CD11 expression was seen in 79.7% cases and CD14 in 38.4% cases.

Mantle Cell Lymphoma (MCL): All the 9 cases were positive for CD5. One case showed dim CD23 expression. CD200 was negative in all cases of MCL. All except one case showed FMC7 expression. Immunohistochemistry for Cyclin D1 was done in trephine biopsies of all cases of MCL and was positive in all cases.

Follicular Lymphoma (FL): Ten out of 14 (71.4%) cases were positive for CD10. Loss of CD10 expression was seen in three cases. One case (7.1%) expressed CD5 and 9 cases (64%) showed CD23 expression. Five out of 14 (35.7%) cases had follow-up lymph node biopsy confirming the diagnosis.

Hairy Cell Leukaemia (HCL): All 11 cases were positive for CD200, CD25 and CD103. All except one (90.9%) case showed CD123 expression. CD5 (dim) was expressed in 3 (27.2%) cases. One case (9%) showed CD10 expression in our study.

B-Prolymphocytic leukaemia: CD5+/CD23+/CD200 + expression was present in one case. Rest of the 3 cases showed variable positivity. CD5-/CD23- / CD200- expression was seen in one case. CD5+/CD23-/CD200- expression in one case and CD5+/CD23+/ CD200- expression in one case. The FMC7 was negative in all cases. Two cases expressed CD11C and one case CD14.

Splenic Marginal Zone Lymphoma (MZL): All 4 cases were CD5 negative. Two cases expressed CD23 and CD200. Three cases expressed CD25 and CD123.

Some cases of B-CLPDs had no definite diagnoses which were diagnosed as CD5 positive B-CLPD unclassified (6.22%) and CD5 negative B-CLPD unclassified (7.66%). There were no follow-up biopsies in these cases to arrive at a definite diagnosis.

DISCUSSION

Multiparameter flowcytometry plays a critical role in the diagnosis and characterisation of B-CLPD with peripheral blood and/or BM involvement, contributing to greater accuracy and therapeutic perspectives [23]. In the present study of 209 cases of B-CLPD, CLL remained the most common subtype (66.03%). The proportion of CLL in B-CLPD in the present study was same as compared with the study by Gujral S et al., in India (68.3%) and studies from western countries (61-81.8%) [5,15-17]. This study revealed CD5+, CD23+, CD200+ immunophenotype highly specific for CLL and the CD5+, CD23-, CD200- immunophenotype highly specific for MCL. The CD200 expression helps to improve differentiation between CLL and MCL [24-26]. Study also confirmed CD200 as a marker with high accuracy for differentiating CLL from MCL, which seemed to be superior to CD23. In the study conducted by Kaleem Z et al., 7% showed CD5+/CD23- pattern [14]. Frequency of CD23 negativity in CLL is very low. In some patients, it may be difficult to distinguish between CLL and MCL on FCM and these cases might need a lymphnode biopsy for confirmation [5,14]. The FMC7 can be used as a useful marker to differentiate CLL from non CLL cases. For CLL, the immunophenotypic profiles were similar to other studies except that the FMC7 positivity was little higher than previously reported [5,16,18].

Regarding MCL, the expression of CD23 was similar to other studies from western countries [15-17]. The FMC7 positivity was similar to the study by Gujral S et al., and studies from western countries [5, 15-17]. The CD200 was negative in all cases of MCL and was

positive in most cases of CLL, revealed to be an excellent marker to distinguish CLL from MCL. The proportion of FL in this cohort was also similar to other populations (4.7-8.5%) [5,15,16]. The FL constituted 6.7% of all B-CLPD. The FL is typically a CD10+/CD5-/FMC-7+ B-cell lymphoma with variable CD23 expression [27]. There were 3 cases (21%) of FL which were CD10 negative. The CD10 is a marker for Germinal Center (GC) B cells. FL cells typically express B-Cell antigens and CD10. However, some reports, described the existence of CD10 negative FL, especially in high-grade (grade 3). Absence of CD10 in the leukaemic phase of FL has been observed posing a practical challenge for correct diagnosis [27-29]. The clinical significance of CD23 expression in FL is uncertain. A study by Olteanu H et al., showed that grade 3 FLs are more often CD23- than lower grade FLs [30]. In this series, 9 cases (64%) showed CD23 expression.

The HCL also constituted 5.26% of B-CLPD in the present study. Incidence of HCL in our series was comparable to study by Gujral S et al., in India (5.5%) [5,22,31,32]. The HCL was extremely rare in a study from China by Miao Y et al., with only one patient identified in 653 cases of B-CLPD.CD25 and CD103 was expressed in all cases, which was higher than previously reported figures [5,12,32,33]. CD25 and CD103 were more consistent markers found in all of our 11 cases. Although, HCL is classically thought to be negative for CD10 expression, several studies have reported CD10 positive HCL cases. In the present series one case showed CD10 expression. The frequencies of CD10 expression in HCL in the reported studies ranged from 5 to 26% [34]. The SMZL and B-PLL constituted 1.9% each. Incidence of SMZL and B-PLL in this series was lower than other studies [5,12]. Diagnosis of SMZL by flowcytometry is usually by exclusion.

In current study 29 cases (13.88%) had no definite diagnosis. In that 16 cases (7.66%) were CD5-ve B-CLPD unclassified and 13 (6.22%) were CD5 +ve B-CLPD unclassified.

Limitation(s)

There were a few drawbacks of the present study. Correlation with complete blood counts, clinical and treatment follow-up was not done. Molecular genetics was not available.

CONCLUSION(S)

Diagnosis and subtyping of B-CLPD is a multiparameter approach subclassification may not be possible by flowcytometry alone in all cases. In B-CLPD, a definite subtyping by flowcytometry was possible in most of the cases. Common subtype in this study was CLL. The CD5+/CD23+ is highly specific for diagnosing CLL. The CD200 was expressed in CLL and was negative in MCL, revealed to be an excellent marker to distinguish CLL from MCL.

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