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Role of CD200 in differentiating chronic lymphocytic leukaemia from mantle cell lymphoma and comparing its expression across various B cell chronic lymphoproliferative disorders

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Abstract
Multiparameter flow cytometry is a useful tool for the diagnostic evaluation of chronic lymphoproliferative disorder recently, it has been shown that CD200 may improve the distinction between chronic lymphocytic leukemia and mantle cell lymphoma, but the role of CD200 expression in atypical chronic lymphocytic leukemia and other chronic lymphoproliferative disorder remains to be established.

Methods: A total of 200 samples were evaluated by Flow cytometry over a period of 2 years from august 2017 to October 2019. Cases were diagnosed according to WHO classification of tumours of hematopoietic and lymphoid tissues, 2017.

Results: CD200 was strongly expressed in chronic lymphocytic leukemia and was revealed to be an excellent marker to distinguish chronic lymphocytic leukemia from mantle cell lymphoma, even in cases of atypical chronic lymphocytic leukemia. However, lack of CD200 was not an exclusive finding of mantle cell lymphoma, being also observed in other chronic lymphoproliferative disorder.

Conclusion: CD200 was expressed by all cases of chronic lymphocytic leukemia as well as atypical chronic lymphocytic leukemia cases while it is negative in MCL thus it is particularly useful in distinguishing chronic lymphocytic leukemia/small lymphocytic lymphoma from other CD5+ B-cell neoplasms. CD200 is also expressed in most of the cases of hairy cell leukemia, splenic marginal zone lymphoma, and follicular lymphoma. CD200 is a useful addition to Moreau *et al.* panel for analysis of B cells in lymphoid tissue in Bone Marrow aspirates, and Peripheral Blood.

Keywords: CD200, chronic lymphocytic leukaemia, mantle cell lymphoma

Introduction

The World Health Organisation classification of tumours of hematopoietic and lymphoid tissues, 2017 defines chronic lymphocytic leukemia/small cell lymphoma (CLL/SLL) as an indolent neoplasm of monomorphic small B cells that co express CD5 and CD23 [1]. Its diagnosis relies on the characteristic immunophenotypic profile on flow cytometry based on a scoring system described by Matutes *et al.* with modification by Moreau *et al.* [2] CLL/SLL typically shows moderate to strong co-expression of CD5 and CD23, low-intensity staining for surface immunoglobulin and low or absent expression of CD22, CD79b and FMC7 [2]. This helps to differentiate it from other mature B cell neoplasms especially mantle cell lymphoma (MCL) which is more aggressive and is considered incurable using conventional chemotherapeutic approaches [2] MCL shares CD5 positivity but strongly expresses sCD22, CD79b and FMC7 and is negative for CD23. CLL with increased prolymphocytes (CLL/PL) also called as atypical CLL, which comprises of 5-55% prolymphocytes, displays an immunophenotype (CD5-or CD23-, FMC7+, strong surface immunoglobulin or CD79b+) in between CLL and MCL [1, 3]

The studies have shown high percentage of antigenic inconsistencies in Moreau scoring system and underscore the need for more precise markers in the diagnosis of CLL. CD200 is on the forefront amongst them and has shown better specificity in differentiating CLL from MCL [4].

The present study aimed to analyse CD200 expression by flow cytometry in a series of chronic lymphoproliferative diseases and its diagnostic accuracy in differential diagnosis of

CLL from other lymphomas compared to conventional scoring system.

Material and Methods

This present study included a total of 200 cases of chronic lymphoproliferative disorders (CLPD) diagnosed over a period of 2 years from august 2017 to October 2019. There were 153 male and 47 female patients with age range from 32 to 103 years (median age 59.5 years) (Table 1). Cases were diagnosed according to WHO classification of tumours of hematopoietic and lymphoid tissues, 2017.

Table 1: Demographic features

	CLL (n=170)	MCL (n=13)	HCL (n=7)	SLVL (n=6)	FL (n=4)	Total (n=200)
Male	130(76%)	9(69%)	7(100%)	4(66%)	4(100%)	154(77%)
Female	40(24%)	4(31%)	0	2(34%)	0	46(33%)

CLL, Chronic lymphocytic leukemia; MCL, mantle cell lymphoma; HCL, hairy cell leukemia; vHCL, hairy cell leukemia variant; SMZL, splenic marginal zone lymphoma; FL, follicular lymphoma.

EDTA samples of peripheral blood (19 cases) or bone marrow (181 cases) sent for initial diagnosis or staging. Samples were processed for immunophenotyping using stained–lysed-washed method and gated using CD19 PECy7 vs. side scatter by FACS Canto eight colour flow cytometer with BD Diva software. Primary screening panel was run in three tubes. First tube included florescent labelled antibodies targeting antigens CD19, CD45, CD23, CD200, CD79b, CD10, CD43 and CD20, second tube contained CD19, kappa, lambda and CD5 while third tube contained CD 19, CD45, CD103,CD11C,CD25 and CD103.

Staining of Surface CD antigens or Surface markers

In a tube 5/10 µl antibody is added (as per company's recommendation) to 100 µl bone marrow sample/ peripheral blood. CD19 APCH7 added in each tube for B lymphocytes gating. In tube 1 only CD 19 APCH7 is added to 100 µl bone marrow sample/peripheral blood and is used as unstained control.

After vortexing the sample is incubated for 20 minutes at room temperature. Then 2ml 1 x BD FACS lysing solution (dilution 1:10) is added and incubated for 20 minutes at room temperature in dark. Centrifugation is done at 1500rpm for 5 minutes. Supernatant is discarded and to the pallet 2ml of BD sheath fluid is added. Centrifugation done at 1500rpm for 5 minutes. Supernatant is discarded and pallet is resuspended in 500pl of sheath fluid. For Kappa, Lambda, three washes of sheath fluid are given to 100ml to remove circulating proteins from blood, which interfere with antibody binding. Then 10µl of antibody is added to the sample.

Acquisition and Analysis of Sample on Flow Cytometer BD Diva Software

Normal worksheet is opened the after acquiring the sample. Plots made in the global worksheet (FSC vs SSC, CD19, UFITC vs UPE, UAPC vs UPECy7 and UFITC vs UAPCH7) are gated. Gating is done in unstained tube (Negative control) using CD19, APCH7 vs SSC graph, where CD19 positive lymphocytes are gated and quadrants are made on UFITC vs UPE, UAPC vs UPECy7 and UFITC vs UAPCH7 graphs. Quadrants of unstained tubes are applied in subsequent tubes. Results are generated by software after the quadrant is applied to the sample tubes with the panel of antibodies which are recorded in terms of percentage.

Table 2: Different fluorochromes and respective antibodies.

Fluorochrome	V450	V500	FITC	PE	PERCP CY5.5	PECY 7	APC	APCH
Tube 1	CD20	CD45	CD23	CD200	CD79b	CD19	CD10	CD43
Tube 2			Lamda	Kappa	CD5	CD19		
Tube 3		CD45	CD25	CD103	CD11c	CD19		

Results

Total 200 cases of chronic lymphoproliferative disorder are analysed amongst them 170 cases are of Chronic lymphocytic leukemia (CLL), 13 cases of Mantle cell lymphoma, 7cases of Hairy cell leukemia (HCL) including 1 case of Hairy cell leukemia variant (vHCL), 6 cases of Splenic marginal zone lymphoma (SMZL) and 4 cases of Follicular lymphoma (FL).

All 101 cases of typical CLL (defined by a revised Matutes score ≥4, it includes score point 0 to 1 for each positive marker in CLL that are Surface Immunoglobulin, CD5,CD23,FMC7, and surface CD22) presented moderate to strong expression of CD200 except 3 cases with dim CD200 expression. CD200 was also positive in all 43 cases of atypical CLL (defined by a score <4) with dim positivity in 5 cases. 14 cases of CD 5 negative atypical CLL showed moderate to strong CD200 positivity except in 4 cases with dim positivity for CD200. 9 cases of CD23 negative atypical CLL showed bright to moderate CD200 positivity.

All 13 cases of mantle cell lymphoma including a case that showed dim positivity for CD23 are negative for CD200.

Amongst other CD5/CD23 negative B cell neoplasms, CD200 showed moderate to strong expression in 4 cases of hairy cell leukemia, 2 cases showed dim positivity while a single case of hairy cell leukemia variant was CD 200 negative. Splenic lymphoma with villous lymphocytes (N=6) showed dim to moderate positivity in 5 cases while one was negative.

Variable expression of CD200 in 4 cases of follicular lymphoma with 2 cases being negative, 2 showed dim positivity while only one showed moderate positivity.

Table 3: Distribution of cases by marker positivity in CLPD.

	CLL (n=170)	MCL (n=13)	HCL (n=6)	vHCL (n=1)	SMZL (n=6)	FL (n=4)
CD200	170(100%)	0	6(100%)	1(100%)	4(66%)	1(25%)
CD23	9(5.29%)	0	0	0	0	0
CD10	6(3.5%)	0	1(16%)	0	4(66%)	0
CD79b	25(14.7%)	13(100%)	5(83%)	1(100%)	5(83%)	4(100%)
CD20	6(3.5%)	13(100%)	6(100%)	1(100%)	6(100%)	4(100%)
CD43	156(91.5%)	6(46.15%)	6(100%)	1(100%)	6(100%)	1(25%)
Kappa	24(14.4%)	3(23.07%)	2(15%)	0	0	2(50%)
Lamda	58(34.1%)	4(30.7%)	3(50%)	0	3(50%)	2(50%)
CD5	18(10.5%)	13(100%)	0	0	0	0
Bcl2	0	0	0	0	0	4()
CD19	170(100%)	13(100%)	6(100%)	0	6(100%)	4(100%)
CD38	0	0	0	0	0	0
CD25	0	0	3(50%)	0	3(50%)	0
CD11c	0	1(7%)	3(50%)	1(100%)	4(66%)	0
CD103	0	0	6(100%)	1(100%)	0	0
S Igm	0	1(7%)	0	0	0	0
Fmc7	0	1(7%)	1(7%)	1(100%)	3(50%)	0
CD22	0	1(7%)	0	0	0	0

CLL, Chronic lymphocytic leukemia; MCL, mantle cell lymphoma; HCL, hairy cell leukemia; vHCL, hairy cell leukemia variant;; SMZL, splenic marginal zone lymphoma; FL, follicular lymphoma.

Table 4: CD 200 expression in CLPD

Diagnosis	Cd200 expression	No. of cases	Pattern of cd200 expression
Typical CLL	Positive	101	Moderate to strong
	Negative	0	
Atypical CLL	Positive	43	Dim to strong
(Matutes score <4)	Negative	0	
Atypical CLL	Positive	18	Moderate to strong
(CD5 negative CLL)	Negative	0	
Atypical CLL	Positive	9	Bright to moderate
(CD23 negative CLL)	Negative	0	
MCL	Positive	0	
	Negative	13	
HCL	Positive	6	Dim to strong
	Negative	0	
vHCL	Positive	0	
	Negative	1	
SMZL	Positive	5	Dim to moderate
	Negative	1	
FL	Positive	4	Dim to moderate

CLL, Chronic lymphocytic leukemia; MCL, mantle cell lymphoma; HCL, hairy cell leukemia; vHCL, hairy cell leukemia variant; SMZL, splenic marginal zone lymphoma; FL, follicular lymphoma

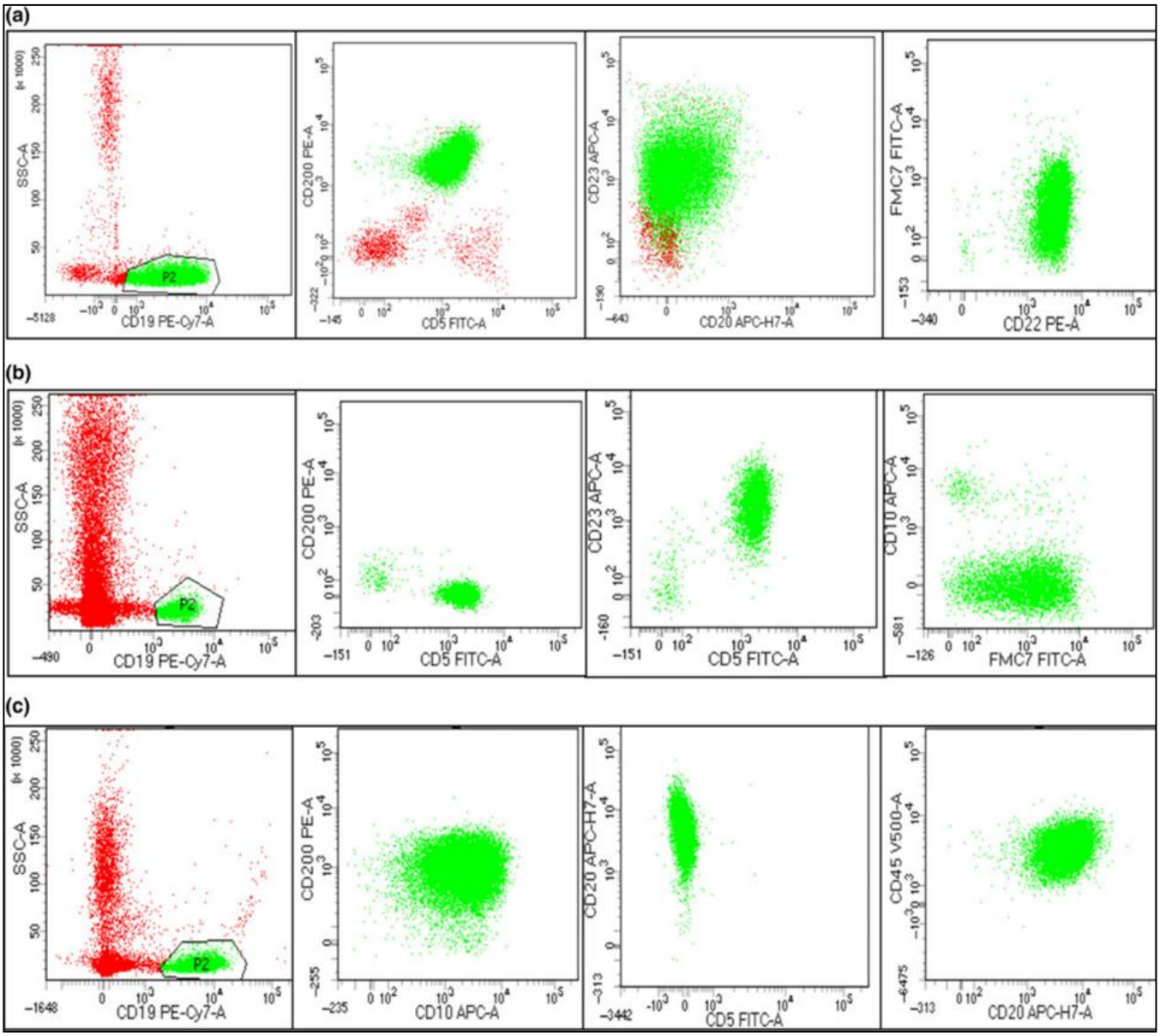

Fig 1: Flow cytometry scatter plots showing expression pattern of CD200 in some representative cases. Chronic lymphocytic leukemia with dim FMC7 expression [Row a], Mantle cell lymphoma [t (11; 14) positive by FISH] with CD23 expression [Row b], follicular lymphoma [Row c].

Discussion

CD200 belongs to type I immunoglobulin superfamily and is composed of a two extracellular variable and constant domains alongwith a transmembrane and a cytoplasmic domains.[5]It is expressed in a subset of T cells and CD19+ B lymphocytes, dendritic cells, thymocytes, endothelial cells and is also highly expressed in central and peripheral nerve tissue [6]. Among the CD5+ B-cell neoplasms, CD200 is uniformly and brightly positive in CLL including atypical CLL whereas, its expression in MCL is negative or very dim. However, 5% (n=3) of cases of mantle cell lymphoma were moderately bright for CD200 in study by Challagundla *et al*. [7] Spacek *et al*. reported dim positivity of CD200 in 4 cases (8.7%) of MCL [8]. None of our cases of MCL showed positivity for CD200. Spacek *et al*. reported CD200 positive with lower intensity in all 21 cases of atypical CLL (defined by a score < 4) (MFI: median 128) than that observed in typical CLL while we reported dim positivity in 9 cases only (with score<4 and CD5/CD23 negative cases) [8]. Challagundla *et al*. concluded dim or absent CD200 expression virtually excludes a CLL diagnosis, but bright expression of CD200 does not completely exclude MCL but in our study three cases of typical CLL in our study showed dim positivity for CD200. Köhnke *et al* showed that CD200 showed high sensitivity (90.6%) and specificity (82.0%) in diagnosing CLL [4]. He suggested "CLL flow score" which is calculated by adding the percentages of CD200+ and CD23+/ CD5+ B cells and then subtracting the percentages of CD79b+ as well as FMC7+ B cells, if the CLL flow score is higher than zero, a diagnosis of CLL is likely [4]. Thus eliminating the interobserver variation due to ill-defined dim expression in Matutes score.

Table 4: Comparison with other similar studies

Studies	Total No of cases	CD200 expression in				
		CLL	MCL	HCL	vHCL	Other neoplasms
D Alapat*et al*., 2012 [10]	117	19/19 (100%)	0/4 (0%)	-	-	36/52 (71%) MM, 3/7 (43%) LPL, 19/20 (95%) AL
Sandes AF *et al*., 2014 [12]	159	56/56 (100%)	0/14 (0%)	13/13 (100%)	-	6/6 (100%) SMZL, 2/4 (50%)LPL, 2/7 (28.6%)PLL, 8/11 (72.7%)FL 21/31 (67.7%)CD5/CD10-neg group
Challagundla P *et al*., 2014 [15]	364	119/119 (100%)	3/61 (5%)	7/7 (100%)	-	Variable expression. (details not mentioned)
El-Sewefy *et al*., 2014 [16]	40	30/30 (100%)	1/10 (10%)	-	-	-
Krehman *et al* 2016	160	98/98 (100%)	0/24 (0%)	6/6 (100%)	0/1 (0%)	3/5 (60%) DLBL 3/6 (50%) SMZL 2/4 (50%) LPL 2/4 (50%) FL 0/1 (0%) PBL 0/1 (0%) BL 4/10 (40%) CD5/ CD10-neg group
Present study 2020	200	170/170(100%)	0/13(0%)	6/6(100%)	0/1(0%)	2/6(85%) SLVL 3/4(50%) FL 0/1(0%)BLL

CLL, Chronic lymphocytic leukemia; MCL, mantle cell lymphoma; HCL, hairy cell leukemia; HCLv, hairy cell leukemia variant; MM, multiple myeloma; LPL, lymphoplasmacytic lymphoma; AL, acute leukemia; SMZL, splenic marginal zone lymphoma; PLL, prolymphocytic leukemia; FL, follicular lymphoma; PBL, plasmablastic lymphoma; BL, Burkitt's lymphoma.

Although role of CD200 in the differential diagnosis of CLL and B-LPD other than mantle cell lymphoma is limited. FLs showed a spectrum of CD200 expression ranging from negative to moderate in study by Challagundla similar to our study [7]. Other studies have shown very bright expression of CD 200 in hairy cell leukemia but we noted dim expression in 2 cases [7].

Conclusion

CD200 was expressed by all cases of CLL as well as atypical CLL cases while it is negative in MCL thus it is particularly useful in distinguishing CLL/small lymphocytic lymphoma (SLL) from other CD5+ B-cell neoplasms. CD200 is also expressed in most of the cases of hairy cell leukemia, splenic marginal zone lymphoma, and follicular lymphoma. CD200 is a useful addition to Moreau *et al* panel for analysis of B cells in lymphoid tissue in Bone Marrow aspirates, and Peripheral Blood.

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