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CONTRIBUTION OF CD200 IN THE DIAGNOSTIC ACCURACY OF MATUTES SCORE FOR THE DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA IN LIMITED RESOURCE LABORATORIES

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ABSTRACT

Background: Objective: CD 200 is a type I immunoglobulin super family membrane glycoprotein and is expressed in many B-chronic lymphoproliferative disorders (B-CLPDs). This study aimed at analyzing the expression pattern of CD200 by flow cytometry immunophenotyping (FCI) and to evaluate its utility in narrowing down the differential diagnosis of B-CLPDS.
Material and methods: A total of 38 patients of CLPD were enrolled in the study which included 23 cases of Chronic lymphocytic leukemia (CLL) 09 cases of Mantle cell lymphoma (MCL) and 06 cases of Hairy cell leukemia (HCL). Provisional diagnosis was made based on peripheral blood examination, imaging and bone marrow aspiration findings. All the 23 cases of CLL expressed CD200 with moderate-to- bright intensity (median MFI: 1174). None of the 09-mantle cell lymphoma (MCL) cases, two being CD23-positive, expressed CD200 (median MFI: 10). All six hairy cell leukemia (HCL) cases expressed CD200. CD200 expression in HCL was brightest among all the CLPDs with a median MFI of 5050.
Conclusion: CD200 has an important role in differentiating CLL from MCL, especially in patients where an immunophenotypic overlap exists. HCL has a consistent and very bright expression of CD200. It would be prudent to include CD200 in the primary panel of antibodies for CLPD analysis in low resource settings.
Key words: Chronic Lymphocytic Leukemia (CLL), Matutes Score. CD200 Marker, Immunophenotyping. Flow Cytometry.

INTRODUCTION

CD 200 (membrane MRC OX–2) is a type I immunoglobulin super family membrane glycoprotein and its gene is located at chromosome 3q12 (1). CD 200 is expressed in a variety of human cells including B cells, a subset of T cell endothelial cells, dendritic cells, and neuronal cells of peripheral and central nervous system.[2] CD200 has the limited capacity to transmit either activatory or inhibitory signals, but on interaction with CD200 is capable of downregulating or inhibiting the exaggerated activity of the immune system. CD 200 is expressed in a number ofseveral hematologic malignancies including B-CLPDS, lymphoblastic leukemia/ lymphoma, lymphoma (B-ALL), Hodgkin lymphoma, acute myeloid leukemia, and Plasma cell myeloma.[3-5] Flow cytometric immunophenotyping (FCI) is the method of choice for diagnosing and classifying of hematolymphoid malignancies. There are no consensus guidelines in panel designing and more so in a resource constrained set up like most centrescenters in our country and thus, varied panels of antibodies are used in different laboratories to reach a final diagnosis. Chronic lymphocytic leukemia diagnosis requires the presence of ≥ 5 x109/l monoclonal B-cells exhibiting characteristic immunophenotype initially described by Estella

Matutes" Matutes scores MS" surface membrane immunoglobulin (SmIg) weak, CD5+, CD19+, CD23+, CD22 weak/-, and FMC7 –, that was modified later by replacing CD22 by CD79b. In Matutes scoring system, a value of 0 or 1 is assigned according to the expression of the above-mentioned five markers.

The majority of CLL cases have a score of 4 or 5 whereas non-CLL cases score below 4. Some atypical CLL cases may score 3 where the addition of more markers such as ROR1, CD81, CD43 and most importantly CD200 would be very helpful. Currently, CD200 is not included in the primary panel of antibodies being used for the diagnosis and classification of CLPDS in majority of the centers and it does not form a part of the Matutes score for diagnosing Chronic lymphocytic leukemia (CLL). The diagnosis of MM is largely based on demonstration of clonality, and an aberrant immunophenotype of the plasma cells characterized by several well-known markers There are a limited number of studies which look at the expression of CD200 in B-CLPDs and MM. Data on CD200 expression in B cell neoplasms and plasma cell myelomas is scarce especially in an Indian population. In addition, there is paucity of the literature regarding the diagnostic utility and the prognostic impact of this biomarker alone. We thus aim to evaluate the expression pattern of CD200 by FCI in B-CLPDS and PCM and ascertain its utility in narrowing down the differentials.

MATERIALS AND METHODS

This was a noninterventional study carried out over a period of 2 years. Clinical, hematologic, and flow cytometry data of all the non-Hodgkin's lymphoma patients diagnosed during this period was collected. The study was approved by the Institutional research committee. Samples for FCI were collected in ethylene diamine tetra acetic acid (EDTA) vacutainers and both peripheral blood (PB) and bone marrow aspirate (BMA) samples were used. Additionally, 10 control samples were evaluated for CD200 expression in normal reactive B-lymphoid cells. These included bone marrow aspirate samples of patients with idiopathic thrombocytopenic purpura, nutritional anemias, and postinduction marrows in cases of acute myeloid leukemia.

Sample Processing

Peripheral blood and BMA samples were processed using stain-lyse-wash protocol. Before the acquisition of the samples, calibration and fluorochrome compensation of the FCM were performed according to the manufacturer's instructions. Based on the cell count of the sample, dilution was done to achieve a total nucleated cell count of 10000/µl. Panels used were as shown in Table1 and Table 2.

Table 1: Panel of Antibodies with their fluorochrome conjugates used in the B-CLPD tubes

Tube No.	FITC	PE	ECD	PC5	PC7
1	Neg	Neg	Neg	CD45	
2	CD5	CD23	CD19	CD38	
3	CD103	CD11c	CD19	CD34	
4	FMC7	CD23	CD19	CD22	
5	CD20	CD45	CD19	CD138	
6	CD16	CD56	CD3	CD45	
7	SMIg	CD27	CD19	7AAD	
8	CD19	CD10	CD45	TCR alpha and beta	
9	Kappa	Lambda	CD19		CD200

Interpretation

Gating on lymphoid cells was based on CD45 versus side scatter and CD 19 versus side scatter. Plasma cells were identified based upon CD 138 versus side scatter analysis. CD 200 expression was evaluated semi-quantitatively by comparison with the isotype PE control antibody. Expression of CD200 was considered positive if there were more than 20% CD 200 positive cells. CD200 expression was evaluated semi-quantitatively by comparison with the isotype PE control antibody and designated as either negative or 1+(<1 log shift in mean fluorescence intensity [MFI] compared to isotype control), 2+(1-2 log shift in MFI) or 3+(more than 2 log shift in MFI).

Acquisition and analysis

Samples were acquired using a BD FACS Canto II instrument and analyzed with FACS Diva version 8 software. The instrument QC was run daily using the cytometer setup and tracking (CST) beads. A minimum of 50,000 events were acquired for every tube. The clinical, morphological, and immunophenotypic findings including which included flow cytometry and immunohistochemical findings were used to arrive at the diagnosis.

RESULTS AND DISCUSSION

A total of 38 patients of CLPD were enrolled in the study which included 23 cases of CLL, 09 cases of MCL and 06 cases of HCL. Cases were evaluated prospectively. Provisional diagnosis was made based on peripheral blood examination and bone marrow aspirate was made. Further confirmation of diagnosis and characterization of subtype was done by flowcytometry, bone marrow biopsy and immunohistochemistry.

Statistical Analysis: Statistical analysis was done by using descriptive and inferential statistics using

Pearson chi square test for categorical data, Frequency and percentage were calculated for presentation. P-value less than 0.05, considered as significant at 95% confidence level. The statistical software SPSS 18.0 used in the analysis. The age group of patients ranged from 46 to 78 years (median, 60years of age) with clustering of the cases in the age group 51-60yrs. This is in concordance with the studies done by Smedby KE et al.[22] This is in concordance with with the studies done by Smedby KE et al,[22] 10 patients (28.5%) were ≤55 years of age and 04 (11%) patients were ≥70 years of age. Out of 38 patients there were 28 males and 10 females with M:F ratio of 2.8:1.

The clinical presentation of our patients was variable, and the most common presenting complaints were weakness, easy fatigability, fever and evidence of infection. Other complaints were pallor, loss of appetite and weight loss. Only 01 of our patients were asymptomatic, as he was undergoing routine annual medical examination and diagnosed on with routine clinical investigations. This contrasts with the study done by Hodgson K et al,[9] who had mostly asymptomatic patients diagnosed with routine clinical examination and investigations. Most common presentation of our patients was weakness and fatigue which was in concordance with study done by Hodgson K et al,[9] 33 out of 38 patients (86%) presented with weakness and fatigue. The second common clinical feature was pallor associated with weakness and was noted in 26 patients (68%). Fever along with evidence of infection most commonly involving respiratory tract was noted in 18 patients (47%). Less common presentations included lymphadenopathy, splenomegaly, and abdominal pain. Bleeding was noted in 03 patients and all of them were ladies who reported the history of menorrhagia.

In our study we had 23 cases of CLL (60%), 09 cases of MCL (24%) and 06 cases of HCL (16%). CLL being the most common of all CLPD's as studied by D Alapat et al.[6] CD200 expression pattern in various CLPDs were noted. In CLL group only 02 cases were CD200 negative. 14 cases were showing moderate expression, 05 cases showed bright expression, and 02 cases showed dim expression. Overall positivity of CD200 was noted in 21 cases (91%), which is in concordance with D Alapat et al.[6] In our study we could analyze that the level of expression of CD200 is variable with some being dimly positive.

In HCL group none of the case was CD200 negative. 05 cases showed moderate to bright expression and 01 case showed dim CD200 expression which is in concordance to the Pillai V et al (88). Expression of CD200 in hairy cell leukemia cases was studied by Pillai V et al,[7] in which 180 cases of B- CLPD were included in his study and stated that all the hairy cell leukemia cases exhibit high level staining for CD200.

In MCL group all 06 cases were CD200 negative. Confirming the previous studies, like D Alapat et al,[6] CD200, were uniformly negative in all the MCL cases. Although most CLL's and MCL's manifest immunophenotypes that permit their reliable distinction from each other, but aberrant phenotype is noted in few cases making definitive diagnosis impossible on the basis of flowcytometry.[8] In such cases CD200 is evaluated which enhances diagnostic accuracy by FCM as studied by D Alapat et al.[6]

Serum lactate dehydrogenase level increased in 14 out of 23 cases (60%) of CLL, 01 out of 6 cases (16.6) of HCL and 7 out of 09 cases (77%) of MCL.

In our study 12 patients had interstitial patterns of infiltration, 15 patients had nodular patterns of infiltration, and 11 patients had diffuse patterns of infiltration.

Correlation with known prognostic markers-

In our study we have correlated expressions of CD 200 with various known prognostic markers and as far as we are aware this is the first ever kind of study correlating CD 200 expression with these undermentioned parameters. Blood investigations revealed anemia in 11 out of 23 cases (47%) in CLL, 8 out of 09 cases (88%) in MCL and all patients (100%) of HCL cases. The hemoglobin level was correlated with CD200 expression pattern and in our study no significant association (p-value 0.54) between hemoglobin level and CD200 expression was noted. As per study done by Binet et al,[10] hemoglobin level of ≤10gm/dl was noted as poor prognostic factor. Anemia is primarily due to the decreased production of red blood cells by marrow because of infiltration by neoplastic lymphoid cells. Thrombocytopenia was noted in 9 out of 23 cases (39%) in CLL, 2 out of 6 cases (33%) in HCL and out of 9 cases (66%) in MCL. Thrombocytopenia was correlated with CD200 expression and in our study no significant correlation was noted between presence of thrombocytopenia and CD200 expression pattern in any of the subclassifications. It is a well-known fact that anemia and thrombocytopenia are known factors that increase the risk stratification as per Binet et al (10) and Hodgson K et al.[9] Hepatosplenomegaly was noted in 8 out of 23 cases (34%) in CLL, 5 out of 6 cases (83%) in HCL and 8 out of 9 cases (88) in MCL. Presence of hepatosplenomegaly was correlated with CD200 expression and in our study no significant correlation was noted between presence of hepatosplenomegaly and CD200 expression pattern in any of the subclassifications. Hepatosplenomegaly is a known factor that increases the risk stratification as per Binet et al,[10] and progression is based on presence of splenomegaly of >3cm as studied by Xavier Troussard et al.[11] Lymphadenopathy was noted in 3 out of 23 cases (13%) in CLL, 3 out of 6 cases (50%) in HCL and 7 out of 9 cases (77%) in MCL. Presence of lymphadenopathy was correlated with CD200 expression and in our study no significant correlation was noted between presence of lymphadenopathy and CD200 expression pattern in any of the subclassifications. Lymphadenopathy was a frequent finding in cases of MCL in our study which is concordant with the studied done by Bosch F et al,[12] and Campo E et al.[13] Lymphadenopathy is a known

factor that increases risk stratification as per Binet et al.[10] Estimated survival in each group has been done in age matched controls and has been found similar in low-risk staging, shorter survival in intermediate stage and poor survival in high-risk staging. Serum lactate dehydrogenase level was found to be increased in 14 out of 23 cases (60%) of CLL, 01 out of 6 cases (16.6) of HCL and 7 out of 09 cases (77%) of MCL. The serum lactate dehydrogenase level was correlated with CD200 expression pattern and in our study no significant association was noted between serum LDH level and CD200 expression pattern. High serum lactate dehydrogenase level was noted in 77% of MCL cases. Serum LDH waswas found to be increased in diseases with high turnover and is related to the high tumor burden. Patients with high level LDH are known to have short survival as it is a well-known poor prognostic marker. In our study 12 patients had interstitial patterns of infiltration, 15 patients had nodular patternpatterns of infiltration, and 11 patients had diffuse patterns of infiltration. Bone marrow biopsy infiltration pattern was correlated with CD200 expression pattern and in our study significant association (p-value <0.001) of negative CD200 expression pattern with diffuse sheet pattern of bone marrow biopsy was seen. The pattern of bone marrow involvement is directly related to the tumor burden and is an important prognostic factor. Diffuse patterns have poor outcomeoutcomes while as nodular and interstitial pattern have good outcome as studied by Xavier T et al,[11] and Rozman C et al.[14]

Table 2

CD maker	No of Cases positive
SMIg:	21 weak positive 02 strong positive
CD5	CD5- dim expression 06 -moderate expression 01 - negative (atypical CLL.)
CD23:	CD23 -mod to bright expression
FMC7:	21 - negative 02 cases dim positive
CD22:	23 -dim to moderate positiv3

Correlation of various other markers used for CLPD panels

Table 3: Correlation with other studies

	Total cases	CD 200 expression in CLL MCL HCL HCLv			
D Alapat et al., 2012 (6)	117	19/19 (100%)	0/4(0%)	-	-
Sandes AF et al., 2014(15)	159	56/56(100%)	0/14(0%)	13/13(100%)	-
Challagundla P et al.,2014(16)	364	119/119(100%)	3/61(5%)	7/7(100%)	-
El – Sewefy et al., 2014(17)	40	30/30(100%)	1/10(100%)		
P.Divya et al.,2022 (1)	52	23/23(100%)	0/2(0%)	2/2(100%)	1/2(50%)
K -Rahman et al.,2016 (18)	160	98/98 (100%)	0/24 (0%)	6/6 (100%)	0/1 (0%)

Weakness of our study was lack of correlation of CD 200 with molecular markers, short follow up, small sample size. Due to the unavailability of molecular investigations at present, CD 200 expression was not correlated with them. Due to paucity of time and the study being prospective in nature long follow up couldn't be done which could have given us additional findings like progression of disease, overall survival. On follow up of 01 year none of our patient had disease progression nor faced any mortality. Weakness of our study was lack of correlation of CD 200 with molecular markers, short follow up, small sample size. Due to the unavailability of molecular investigations at present CD 200 expression was not correlated with them. Due to paucity of time and the study being prospective in nature long follow up couldn't be done which could have given us additional findings like progression of disease, overall survival.

Flow cytometric immunophenotyping is an important diagnostic modality for the CLPDS with peripheral blood or bone marrow involvement. The differential diagnosis is narrowed based on the expression of CD5, CD23 and CD10. Three broad subgroups: CD5 +/CD10 +, CD +/CD10-, and CD5-/CD10-. Two common differential diagnoses for the CD5+/CD10 CLPDS are the CLL and MCL, which can usually be distinguished based on their typical immunophenotypic profile. But there can be instances where atypical immunophenotypic expression can make this differentiation difficult which has significant impact on patient management. In such instances CD200 will come to the rescue.

CONCLUSION

CD200 expression is useful in diagnosis and classifying B-CLPDs especially in low resource settings. The addition of CD200 to flow cytometry marker panels may be particularly helpful in differentiating some disease entities, in particular CLL and MCL, which differ considerably in their clinical behavior and prognosis. There is some evidence that serum levels of soluble CD200 may be related to disease progression and prognosis in patients with CLL. Wong et al (20) showed that CD200 can be released from CD200+ neoplastic cells by ectodomain shedding. Of interest, increasing

evidence indicates that anti-CD200 treatment might be therapeutically beneficial for treating CD200-expressing malignancies, such as CLL.[21]

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