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CD200 as a Valuable Marker for Differentiating between CLL and Other B-cell Lymph Proliferative Disorders

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ABSTRACT Chronic lymphocytic leukaemia (CLL) being the commonest subtype of mature B-cell lymphoid neoplasms need to be differentiated correctly from other B-chronic lymphoproliferative disorders (BCLPD). CD200 is being an essential marker for prognosis and diagnosis of CLL. Sixty patients diagnosed with BCLPD were evaluated for CD200 expression with eight-colour flow cytometry. An integration of modified Matutes score and CD200 were applied for CLL diagnosis. The overall immunophenotype of CLL patients in this study showed significant, frequent expression of CD5, CD22, CD23, CD43 and CD200. Atypical versus typical CLL patients' phenotype comparison revealed significant lower expression of CD5 and CD43 with higher expression of CD79b in atypical CLL patients. CD200 was expressed significantly higher in CLL patients (typical and atypical) than in MCL patients who showed dim CD200 expression in only twenty-five percent of patients. The inclusion of CD200 within the diagnostic markers can empower the modified Matutes score accuracy for CLL.

INTRODUCTION

Mature B-cell lymphoproliferative disorders (BCLPD) is derived of mature B-lymphocytes and can include arterial peripheral blood, secondary lymphoid organs, as lymph nodes, spleen and bone marrow. World Health Organization (WHO) in 2016 classified these disorders, according to their histopathological characteristics, immunophenotype, genotype and genetic alterations (Mora et al. 2019; Elsaid et al. 2019; Swerdlow et al. 2016). Chronic lymphocytic leukaemia (CLL) is the most common form of Mature B-cell lymphoproliferative disorders (BCLPD). The clinical identification of CLL is done according to presence of more than 5,000 monoclonal B-cells' characteristic immunophenotype (that is, weak SmIg, CD5+CD19+, weak CD20, and CD23+) and morphology of small-sized lymphocytes and atypical looking lymphocytes (Köhnke et al. 2017).

Matutes et al. (1994) designed an score system derived from immunophenotyping called Matutes score or MS for the identification of CLL, which was later modified and CD79b replaced CD22. In this scoring system, score for immunophenotypic was granted, which includes CD5 positive, CD22 weak or negative, CD23 positive, FMC7 negative, and SmIg weak. According to this score system, typical CLL cases usually score 4, or other mature B-cell lymphoid neoplasms usually score 3 or less (Moreau et al. 1997). Currently, the criteria for CLL diagnosis have been updated by the International Working Group on CLL (IWCLL), WHO, and the European Research Initiative on CLL (ERIC) (Hallek 2017). In the last few years, many haematopathologists found different immunophenotypic patterns in CLL that overlapped with other BCLPD that necessitates further investigations and the use of lengthy techniques such as cytogenetics and molecular techniques (Gill 2020).

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The use of CD200 as a diagnostic marker in addition to Matutes score features may enable the distinguishing of CLL from other BCLPD without delay. Although CD200 assessment is not required by the WHO for CLL diagnosis, it is introduced in the EuroFlow guidelines, and the published data details the high diagnostic value of CD200 as well (van Dongen et al. 2012).

CD200 [OX-2 antigen] is a transmembrane glycoprotein encoded by the *MOX2* gene at 3q12, which may enhance the discrepancy between CLL (CD200 positive) and MCL (CD200 negative) cases. High expression of CD200 has also been shown in HCL cases. Most of these results are based on typical CLL cases, and do not differentiate it from atypical CLL and MCL. Therefore, CD200 expression in other LL disorders are scarce and remains to be established. To study the accurate role of CD200 on the differential analysis of B-cell lymphomas (Sandes et al. 2014) CD200 is expressed by B cells, activated T cells, neurons, and endothelial cells. It binds to CD200 receptor that is expressed on granulocytes, monocytes, dendritic cells, and macrophages. The CD200-CD200 receptor interaction has a despotic effect on T cell-mediated immune responses. CD200 showed to be up-regulated in some malignancies and there are some trials for modulating the CD200-CD200 receptor interaction to treat them (David et al. 2017; Dorfman and Shahsafaei 2010).

Objectives

The present study aimed at studying the impact of adding CD200 to the MS for diagnosing CLL and clarifying if a special pattern of cytogenetics abnormalities is linked to the CD200 positive CLL patients. Study also evaluates CD200 expression and its diagnostic role in CLL other B-cell lymphoproliferative disorders.

METHODOLOGY

This study included the assessment of cases presented to the outpatient department with initial suspicion of B-CLPD at King Abdullah Medical City (KAMC)-Makkah, Saudi Arabia. The study included 60 patients that were enrolled from December 2018 to January 2021. All the participants of the study were in the adult aged group, comprising patients aged between 34-91 years old with a median of 63 years, wherein 44 (73.3%) were males and 16 (26.7%) were females, with a male-to-female ratio of 2.7:1. The included patients were newly diagnosed and had full investigations needed for diagnosis including morphology, flow cytometry (FC), and immunohistochemistry (IHC), and cytogenetics, corresponding to the World Health Organisation's (WHO) 2016 guidelines and International Working Group on CLL (IWCLL). Patients who had received previous treatment were excluded.

Patients' specimens were freshly collected in sodium EDTA and sodium heparin tubes, stained and analysed within 24-36 hours from collection. Specimens were lysed over 5 minutes by BD FACS Lysing solution (BD) and washed in Phosphate Buffer Solution (PBS). Subsequently, cells were re-suspended in 500µL of PBS. A total of 1×10^6 cells from whole blood/bone marrow aspirate specimens were incubated for 15 minutes in the dark at 37 °C. Flowcytometry Immunophenotyping analysis was performed with BD (Becton Dickinson) three lasers with the eight colours FACS Canto II Flow Cytometer. All monoclonal antibodies were purchased from BD company.

The monoclonal antibodies (MAbs) were targeted against the following antigens of CD5, CD23, CD19, CD20, CD10, FMC7, CD45,CD79b, CD200, sIgM, surface Kappa light chain, surface Lambda light chain, CD38, CD103, CD11c, CD25, and CD123. CD45 was used for lymphocytes gating/selection. CD19 was used for B-lymphoid cells selection. Other markers were used for further definition of B-cell lymphoproliferative disorders immunophenotype. Calibration was performed daily using the 7-colours beads analysis, and other daily performance was checked by BD CST beads to ensure reproducibility of the fluorescence intensity. Software analysis was performed using FACs DIVA8 Software. A minimum of 50,000-gated events was acquired per tube, and the lymphocyte population was outlined through CD45 high/sideways scatter (SSC) low location, and B-lymphocytes were subsequently defined through CD19 positive cells selection. The percentage of positive cells for each marker was defined (positive is more than 20%). Additionally, the expression level of CD200 (weak, moderate, or high) was evaluated as per log scale of the fluorescence axis

on B-cell population. The cut-offs used, were weak expression when the positive peak lied within the first logarithmic percentile, moderate expression in the second percentile, and strong expression when over the second percentile.

Ethical Considerations

The study was approved by the ethical committee at KAMC-Makkah. No informed consents were needed, as this is a retrospective data collection study (Approval number 20-738).

Statistical Analysis

Data from results of this study were evaluated in Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 20. Quantitative data were tabulated in form of median (minimum minus maximum). Frequency and percentage were used to present categorical data. To make comparisons between qualitative data a chi-square or Fisher's exact test were used. To see significant of data set a two-sided alpha value was set at 0.05. Probability (P-value) < 0.05 and < 0.001 were considered significant and highly significant, respectively.

RESULTS

The current study involved 60 patients of suspected BCLPD. Out of those patients, 38 (63.3%) proved to be CLL patients, 12 (20%) were diagnosed as mantle cell lymphoma (MCL), and the remaining 10 (16.6%) had other BLCPD as following, that is, 2 Burkitt's Lymphoma (BL), 2 Diffuse Large B-cell Lymphoma (DLBCL), 2 Follicular Lymphoma (FL), 2 hairy cell leukaemia (HCL), one patient diagnosed as lymphoplasmacytic lymphoma (LPL) and one patient diagnosed as marginal zone lymphoma (MZL). The demographic and basic peripheral blood data were presented in Table 1. The average age of patients in the two groups was comparable, and male percentage varied between sixty to eighty-five percent. Total lymphocyte count was quite more in CLL patients, but percentage was significantly high in CLL group.

The overall immunophenotype of CLL patients in this study showed significant, frequent expression of CD5, CD22, CD23, CD43, CD200, weak membranous IgM, and significantly lower expression of CD10, FMC7 and CD79b/CD22 compared to MCL and other B-LPDs (Table 2).

Implementing the Matutes score to the study patients, the scoring of the CLL patients was as following, that is, 9 patients (23.7%) had a score of 5, 20 patients (52.6%) had a score of 4, and 9 patients (23.7%) had a score of 3. The patients who had score 4 and 5 were considered typical CLL and the patients who had score 3 were diagnosed as atypical CLL. There were no morphological variations between typical and atypical CLL patients. Furthermore, all the atypical CLL patients were cyclin D1 negative by immunohistochemistry and/or chromosomal studies so MCL was excluded. Comparing the CLL patients (typical and atypical) phenotype to MCL patients' phenotype, CD5 expression revealed to be lower/dimmer in atypical CLL patients than typical CLL and MCL patients. CD23, CD43, and CD200 showed lower expression in MCL than CLL patients. CD79b showed significantly lower expression in typical CLL patients than both atypical CLL and MCL (Table 3).

Table 1: Demographic and basic peripheral blood data of the study group

	CLL(Number=38)	*MCL(Number =12)*	*Other BCLP(Number =10)*
Age (years)	67 (34-91)	62.0 (39-69)	46.0 (35-66)
Sex: malesFemales	26 (68.4%)	10 (83.3%)	8 (80%)
	12 (31.6%)	2 (16.7%)	2 (20%)
Total Leucocytes count	58.0 (6.0-555.0)	49.0 (13-79)	4.0 (2.0-44.0)
Lymphocyte %	87.5 (42-98)	47.0 (12-85)	40.0 (11-77)
Prolymphocytes %	6.0 (3-9)	0	0
Atypical lymphocytes %	0	14.0 (8-15)	8.5 (5-52)

All the quantitative parameters were presented as median (min-max) and the qualitative data were presented as number and percentage.

Table 2: Frequency of entire CD markers expression by flowcytometry in the study patients

	CLL(38patients)	*MCL(12 patients)*	*Other BCLPD (10 patients)*	*P value*
CD5	34 (89.5)	12 (100)	0 (0)	< 0.001
CD10	1 (2.6)	0 (0)	6 (60)	< 0.001
CD11c	11 (28.9)	5 (41.6)	2 (20)	0.08
CD19	38 (100)	12 (100)	10 (100)	-
CD 20	37 (97.4)	12 (100)	10 (100)	0.33
CD22	28 (73.7)	12 (100)	9 (90)	0.018
CD23	37 (97.4)	2 (16.7)	2 (20)	< 0.001
CD43	35 (92.1)	2 (16.7)	2 (20)	< 0.001
FMC7	0 (0)	2 (16.7)	2 (20)	0.09
CD79b	7 (18.4)	9 (75)	5 (50%)	< 0.001
CD200	36 (94.7)	3* (25)	2 (20)	< 0.001
Membranous IgM	15 (39.5)	7 (58.3)	3 (30)	0.001
sKappa light chain	20 (52.6)	8 (66.7)	5 (50)	0.5
sLambda light chain	18 (47.4)	4 (33.3)	5 (50)	0.5

All the data were presented as number and percentage.*CD200 expression in MCL was weak.5 patients of MCL show Dim CD 11c.

Table 3: Matutes' score CD markers expression in CLL subgroups and MCL

Positive marker expression	*Typical CLL (number =29)*	*Atypical CLL (number =9)*	*MCL (number =12)*	*P value*
CD5	29 (100)	5 (55.5)	12 (100)	< **0.001**
CD 20	28 (96.5)	9 (100)	12 (100)	0.416
CD22	22 (75.8)	6 (66.6)	12 (100)	0.132
CD23	29 (100)	8 (88.8)	2 (16.7)	< **0.001**
CD43	29 (100)	6 (66.6)	2 (16.7)	< **0.001**
FMC7	0 (0)	0 (0)	2 (16.7)	0.037
CD79b	1 (3.4)	6 (66.6)	9 (75)	< **0.001**
CD200	29 (100)	7 (77.7)	3 (25)	< **0.001**
Membranous IgM	10 (34.48)	5 (55.5)	7 (58.3)	0.428
sKappa light chain	18 (62.1)	4 (44.4)	8 (66.7)	0.308
sLambda light chain	11 (37.9)	5 (55.6)	4 (33.3)	0.346

CD200 and CD43 Evaluation in the Study Group

CD200 expression in CLL patients, whether typical or atypical, was moderate to strong. All the typical CLL and 77.7 percent of atypical CLL patients expressed CD200. No significant difference was seen in expressions of CD200 between the atypical and typical CLL patients. CD200 expression was significantly higher in CLL patients (typical and atypical) than in MCL patients who showed dim CD200 expression in only twenty-five percent of the patients. CD43 was expressed in 66.6 percent of atypical and one hundred percent of the typical CLL patients with moderate to strong expression in typical CLL patients. On the other hand, CD43 expression (dim to moderate) was apparent in MCL patients with a percentage of 16.7 percent ($p<0.001$) (Table 4).

Table 4: Marker expression in CLL subgroups

Positive marker expression	*Typical CLL (29 patients)*	*Atypical CLL (9 patients)*	*p value*
CD5	29 (100)	5 (55.5)	<**0.001**
CD 20	28 (96.5)	9 (100)	0.763
CD22	22 (75.8)	6 (66.6)	0.44
CD23	29 (100)	8 (88.8)	**0.237**
CD43	29 (100)	6 (66.6)	**0.010**
FMC7	0 (0)	0 (0)	-
CD79b	1 (3.4)	6 (66.6)	<**0.001**
CD200	29 (100)	7 (77.7)	**0.051**
Membranous IgM	10 (34.48)	5 (55.5)	0.48
sKappa light chain	18 (62.1)	3 (44.4)	0.17
sLambda light chain	11 (37.9)	5 (55.6)	0.29

Atypical versus typical CLL patient's phenotype comparison revealed significant lower expression of CD5 and CD43 with higher expression of CD79b in atypical CLL patients. The expression of other markers (CD23, CD20, CD22, FMC7, CD200, Membranous IgM, Kappa light chain, Lambda light chain) showed no significant difference (Table 4).

Cytogenetic Analysis in the CLL Group

Five patients (three typical and two atypical CLL patients) had no cytogenetic analysis data. There was no significant difference between the typical and atypical CLL patients regarding the cytogenetic analysis (Table 5).

Table 5: Cytogenetic analysis in typical versus atypical CLL patients

	Typical CLL (29 patients)	*Atypical CLL (9 patients)*	*p value*
13q deletion	7 (25.9)	0 (0)	0.054
11q deletion	3 (11.1)	0 (0)	0.091
17p deletion	2 (7.4)	3 (50)	0.075
IgH chain mutation	2 (7.4)	3 (50)	0.075
Complex (more than one genetic abnormality)	5 (18.5)	2 (33.3)	0.094
Normal cytogenetics	6 (22.2)	2 (33.3)	0.107

Cytogenetic data was not available for 5 CLL patients, 2 typical patients and 3 atypical patients.

DISCUSSION

Diagnosis and prognosis of BCLPD is based on IMP (Van Dongen et al. 2012; Ivancevic et al. 2014). The major issue is that chronic lymphocytic leukaemia (CLL) is sometimes confused with other lymphoproliferative disorders such as Mantle cell lymphoma (MCL) and hence requires intensive diagnostic protocol to differentiate among other lymphoproliferative disorders. Few studies have evaluated CD200 role and expression in the discrepancy diagnosis of various lymphoproliferative disorders. Previous results indicate that CD200 is consistently expressed in CLL, and hairy cell leukaemia B cells and at the same same time no significant correlations were seen between CD200 expression and response to treatment. Therefore, CD200 could be of elevated prognostic and therapeutic value in differentiating CLL from other lymphoproliferative disorders. However, its correlation with the prognosis and treatment outcome is still not properly investigated. This current study aims to illustrate the function of CD200 in the diagnosis of CLL and whether CD200 adds discriminative power to the Matutes Score.

Several reports has been studied the CD200 expression in several lymphoproliferative disorders as a percentage of positive cells or MFIR and have established the role of CD200 expression in several lymphoproliferative disorders (Köhnke et al. 2017). Flow cytometry immunophenotyping is a fundamental tool for the diagnosis of B-cell CLPDs, including CLL (Jalal 2021). The Matutes score for flowcytometry diagnosis of BLCPDs and CLL was introduced around 20 years back and was based on the expression of CD5, CD23, FMC7, SmIg, and CD22/CD79b on the malignant cells. Matutes score continues to be helpful and is still applied by many flowcytometry laboratories (Mora et al. 2019). Recently, CD200 (a member of the Ig receptors membrane proteins superfamily) was identified as an adjunctive powerful marker that helps in supporting CLL diagnosis.

Despite it was proposed to use MFIR for getting technical reproducibility among different laboratories, some other studies found that MFIR and the percentage of positive cells for CD200 had similar accuracies to distinguish different categories within B-CPLD. Furthermore, MFIR is not convenient in daily routine, clinical work, and is not usually mentioned on flowcytometry reports (4, 20). The researchers demonstrated that CD200 positivity shows a constant expression pattern in CLL patients, including atypical phenotype CLL patients. In this study, CD200 expression level is significantly higher in CLL than in other B-CLPD, matching with previous studies findings, and this supports the addendum diagnostic role of CD200 to detect CLL phenotype (Mongeau-Marceau et al. 2016; Sorigue et al. 2020; Ting et al. 2018).

Other studies mainly discussed the role of CD200 for discriminating CLL and MCL, as CD200 is almost consistently positive in CLL and usually absent in MCL (Palumbo et al. 2009; Lesesve et al. 2015; Fan et al. 2015; D'Arena et al. 2020). In this study, most of the MCL patients

were CD200 negative and few MCL cases showed lower or dimmer positivity of CD200 compared to CLL (Challagundla et al. 2014; Wright et al. 2001; Spacek et al. 2014). Moreover, the researchers also observed negative expression of CD200 in most of BCLPD (non-MCL). Only a minority of BCLPD (non-MCL) patients showed CD200 positivity (dimmer than CD200 in CLL).

This minor, relatively dimmer CD200 positivity in non-CLL, non-MCL BCLPD patients could have been more detailed with higher number of enrolled patients, but overall, these findings are coping with other studies that highlighted heterogeneous pattern of CD200 expression, ranging from negative to moderate. Therefore, CD200 is not individually a marker that can distinguish different B-CLPD (20, 24). In this study, MS showed to be effective in segregating CLL from other B-CLPD. MS was high (4 or 5) in typical CLL patients, 3 in atypical CLL cases, 3 or less in MCL patients, and 2 or less in minority of other BCLPD. Including CD200 in MS allowed clear distinction of CLL (typical and atypical) from MCL and from other B-CLPD, increased the CLL diagnostic accuracy of MS to one hundred percent. Regarding the cytogenetics studies results, they showed no relevant variation between typical and atypical CLL patients.

CONCLUSION

As far as the researchers investigated, no adequate studies highlighted the Saudi Arabian oncology centres experience regarding the CD200 utility in CLL diagnosis. The study supports the other similar studies, and also addresses a Saudi Arabian Centre experience. The present study also confirms that inclusion of CD200 in routine flow cytometry work-up can facilitate the differentiation between CLL and MCL and has a beneficial impact on accurate diagnosis in patients with atypical phenotypes. In conclusion, CD200 is a valuable CLL diagnostic marker, as when CD200 is integrated to the Matutes Score, the score diagnostic accuracy sharply increases.

RECOMMENDATIONS

The results from the study indicate that CD200 can be a valuable, but not specific, diagnostic marker for differentiating lymphoid disorders in corporation with other regular diagnosis to increases its accuracy. This CD200 simple tool can help with rapid, cost-effective diagnosis, before retrieving cytogenetics results, or in limited resources centres where cytogenetics may be non-accessible.

ABBREVIATIONS

CLL:	Chronic lymphocytic leukaemia
BCLPD:	B-chronic lymphoproliferative disorders
BL:	Burkitt's Lymphoma
DLBCL:	Diffuse Large B-cell Lymphoma
FL:	Follicular Lymphoma
FC:	Flow cytometry
HCL:	Hairy cell leukaemia
IHC:	Immunohistochemistry
IWCLL:	International Working Group on CLL
LPL:	Lymphoplasmacytic lymphoma
PBS:	Phosphate Buffer Solution
MAbs:	Monoclonal antibodies
MCL:	Mantle cell lymphoma
MZL:	Marginal zone lymphoma
SSC:	Sideways scatter
WHO:	World Health Organisation

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