CD200 Expression in B-Cell Chronic Lymphoproliferative Disorders

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Background: Flow cytometry immunophenotyping (FCIP) is used for rapid, specific diagnosis of B-chronic lymphoproliferative disorders (BCLPDs). However, cases may deviate from the typical immunophenotype; therefore, there is a need for adding new marker(s) for differentiating BCLPDs.

Lately, few researches highlighted CD200 expression in some BCLPDs. Our aim was to evaluate CD200 expression in different BCLPDs and whether adding CD200 to BCLPD-FCIP routine panels could improve the ability of their differential diagnosis.

Methods: We evaluated CD200 expression in 49 BCLPD patients and 26 age- and sex-matched control subjects. Flow cytometry immunophenotyping first panel included CD5, CD19, sIg, CD23, CD22, CD79b, and FMC7; for BCLPDs other than chronic lymphocytic leukemia (CLL) and mantle cell lymphoma, CD11c, CD103, CD25, and CD10 were evaluated.

Results: Using tricolor FCIP, CD200 showed high bright expression on CD5/19-positive clone in all B-CLL patients (100%), with a mean of 94% (SD, 11%); in the 2 cases of hairy cell leukemia, CD200 was brightly expressed on 96% and 99% of cells. In all other BCLPDs including mantle cell lymphoma, follicular lymphoma and splenic marginal zone lymphoma, CD200 expression (on CD19/22-positive cells) was less than 20% with a mean of 10% (SD, 8%) and a dim pattern. CD200 expression was significantly higher in CLL compared with non-Hodgkin lymphoma groups ($P < 0.001$).

Conclusions: Evaluating CD200 expression has a great impact on accurate BCLPDs diagnosis and could be added to the BCLPD routine panels. The high expression of CD200 in B-cell CLL and hairy cell leukemia could open the option for targeted immune (anti-CD200) therapy.

Key Words: CD200, BCLPDs, flow cytometry

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The World Health Organization (WHO) has set the criteria to distinguish between different B-chronic lymphoproliferative disorders (BCLPDs). However, misdiagnosis is not uncommon because of overlapping disease features.[1] Precise subclassification using WHO criteria requires correlation with the tissue histopathology; however, the demand for rapid, specific diagnosis using minimally invasive approaches has led to a growing tendency to attempt to fully subclassify BCLPDs involving the peripheral blood or bone marrow largely on the basis of flow cytometry immunophenotyping (FCIP) results,[2,3] before proceeding to tissue biopsy.[4]

Flow cytometry immunophenotyping offers advantages over competing laboratory technologies being a fast procedure, analyzes a broader array of antigens compared with those detectable by tissue-based immunohistochemistry (IH), correlates multiple measurements (antigen expression, light scatter) in individual cells, quantitates population frequencies as well as the level of antigen expression, and facilitates the analysis of cells gated on the basis of other parameters.[5]

B-chronic lymphoproliferative disorder immunophenotypes is based on studies of individual disease entities.[3,6] For instance, CD5 and CD23 coexpression by a BCLPD is often considered diagnostic of B-cell chronic lymphocytic leukemia (B-CLL), whereas the absence of CD23 in a $CD5^+$ BCLPD is typically thought of as evidence of mantle cell lymphoma (MCL).[7] Likewise, CD10 positivity favors a diagnosis of follicular lymphoma (FL); CD25, dual CD11c/CD22, and CD103 are all diagnostic markers of hairy cell leukemia (HCL)[8]; however, a recent study held at Mayo Clinic, which included 252 patients with BCLPDs, encountered the following exceptions to standard paradigms of BCLPD-associated FCIP: CD5 expression by disorders distinct from chronic lymphocytic leukemia (CLL) and MCL, lack of uniform CD5 positivity in some CLL and MCL cases, absence of CD10 in approximately 50% of FLs, and expression of CD103 by occasional marginal zone lymphomas.[9] Other studies reported some cases where CD23 is not discriminant.[10,11]

The diagnosis of MCL should be confirmed by demonstration of cyclin D1 positivity by IH or by the presence of the t(11;14) chromosomal translocation detected by fluorescence in situ hybridization (FISH). Immunohistochemistry and FISH represent reliable methods for detection of cyclin D1 expression; however, they are expensive, time consuming, and not available in all centers. Also, cyclin D1 detection can be easily accomplished on tissue biopsy only, whereas its determination in cell suspension by flow cytometry is cumbersome.[12] Working on fixed tissues (especially by B5 solution), CD5 and cyclin D1 analysis can give equivocal or even negative results.[13] On the other hand, cyclin D1–negative MCL does actually exist, and in rare cases, other cyclin D types can be overexpressed.[14] In addition, the presence of the chromosomal translocation t(11;14)(q13;q32) is not pathognomonic for MCL. In fact, MCL without t(11;14) has been reported,[15] and the same translocation can be found in B-CLL and other lymphoproliferative disorders.[16,17]

Therefore, the addition of new marker(s) that help in differentiating BCLPD, especially if they could be evaluated using an easily applied technique, will be of great importance.

CD200 (formerly called OX2) is a transmembrane glycoprotein with immunosuppressive functions. It is expressed on thymocytes, activated T cells, B cells, dendritic cells, endothelial cells, and neurons.[18,19]

Lately, CD200 expression was investigated in some BCLPDs, with few available researches highlighting its relevancy.

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Palumbo et al.[1] reported CD200 expression in B-CLL versus negative expression in MCL. The expression of CD200 was also reported in HCL,[20] multiple myeloma,[21] lymphoblastic lymphoma/leukemia, mediastinal large B cell lymphoma, lymphoplasmacytic lymphoma,[22] angioimmunoblastic T-cell lymphoma,[23] acute myeloid leukemias,[24] and other nonhematologic malignancies.[25]

Starting from these data, we formulated our study to include patients with different BCLPDs to evaluate CD200 expression among them, hypothesizing that the expression of CD200 may be a potential marker for the BCLPD differential diagnosis.

Aim of the Work

The aim of the present work was to study CD200 expression in patients with different BCLPDs and to evaluate whether adding CD200 to the flow cytometry routine panels of BCLPDs could improve the ability of differential diagnosis of these disorders.

PATIENTS AND METHODS

This study was conducted on 49 patients with leukemic phase of BCLPDs as well as 26 age- and sex-matched control volunteers; control subjects attended the outpatients clinic of general surgery for elective repair of hernia. According to immunophenotypic analysis, patients had further diagnoses of CLL (n = 31), MCL (n = 4), HCL (n = 2), and unspecified non-Hodgkin lymphoma (NHL) (n = 12). Unspecified NHL patients could be further classified according to other morphological, histopathologic, and molecular studies into 8 cases with FL and 4 cases with splenic marginal zone lymphoma (SMZL). These patients attended Kasr El Aini Hospital–Cairo University, to be diagnosed in the Clinical Pathology Department between August 2009 and February 2011.

The patients were 34 men (69%) and 15 women (31%), with mean age of 60 (SD, 7) years and 56 (SD, 10) years for CLL and other BCLPD, respectively; control subjects were composed of 18 men (69%) and 8 females (31%), with a mean age of 55 (SD, 10) years.

B-chronic lymphoproliferative disorders were diagnosed by evidence of persistent lymphocytosis greater than 5000/μL (5×10^9/L) for 3 months and immunophenotyping panels; included in the first panel were CD5 (fluorescein isothiocyanate [FITC]), CD19 (phycoerythrin-cyanin 5), sIg (FITC), CD23 (phycoerythrin [PE]), CD22 (FITC), CD79b (PE), and FMC7 (FITC) followed by scoring system according to Matutes score.[26] For BCLPDs other than CLL, immunophenotyping using the second panel included CD11c (phycoerythrin-cyanin 5) for splenic lymphoma, CD103 (FITC) and CD25 (PE) if HCL was expected from morphology of peripheral blood, and CD10 (PE) for FL. In CLL, CD200 (PE) was evaluated on CD5/19-positive clone, whereas in non–CLL-BCLPDs, it was evaluated on CD19/22-positive clone.

Monoclonal anti-CD5, CD19, sIg, CD23, CD22, CD79b, FMC7, and CD10 were supplied by Beckman Coulter (Brea, CA), whereas monoclonal anti-CD11c, CD25, CD103, and CD200 were supplied by BD Biosciences (Franklin Lakes, NJ).

Immunostaining technique, on whole EDTA-anticoagulated blood, was done according to Palumbo et al.,[1] followed by lysing red cells with OptiLyse C (Beckman Coulter), according to manufacturer instructions; immunophenotypic expression on malignant clone cells was evaluated using tricolor FCIP "EPICS XL coulter system flow cytometer."

The proper protocol for triple-color flow cytometry was loaded and used for interpretation of results. Ten thousands cells were analyzed for each sample; the lymphocytes were selectively gated for immunophenotypic analysis. An antigen was considered positive when at least 20% of the cells expressed that antigen.[27] For sIg, the mean fluorescence intensity (MFI) was measured according to Morice et al.,[9] using a logarithmic scale; in brief, a cell population was considered negative when the MFI was not significantly different from that of cells labeled with a matched isotype monoclonal antibody (negative control), dimly positive with an MFI within 1 log of the control, moderately positive with an MFI about 1 log brighter than the control, and brightly positive with an MFI at least 1.5 to 2 logs brighter than the control. Mantle cell lymphoma diagnosis was confirmed by cyclin D1 IH, when bone marrow trephine biopsy was available, and/or FISH analysis on peripheral blood for detection of translocation t(11;14)(q13;q32), whereas HCL diagnosis was confirmed by morphological, cytochemical (staining for tartrate-resistant acid phosphatase) and histopathologic (bone marrow trephine biopsy) patterns. For the NHL cases that could not be assigned to a specific subtype through immunophenotypic analysis; histopathologic examination of lymph nodes and/or spleen together with FISH analysis on peripheral blood for detection of translocation t(14; 18)(q32;q21) could assign these cases.

For CD200 expression, a second gate was done on the neoplastic CD5/$CD19^+$ cells in case of B-CLL or CD22/$CD19^+$ cells in other cases of BCLPDs to detect the percentage of CD200 expression represented in a histogram. CD200 was considered positive when at least 20% of the CD5/$CD19^+$ cells in cases of B-CLL or CD22/$CD19^+$ cells in BCLPDs are positive for CD200.[1]

RESULTS

CD200 showed a bright expression on 60% to 100% of CD5/19-positive clone in all B-CLL patients (100%), with a mean of 94% (SD, 11%); in the 2 cases of HCL, CD200 was brightly expressed on 96% and 99% of the CD19/22-positive cells. On the contrary, in all other BCLPDs including MCL, FL and SMZL CD200 expression, on CD19/22-positive cells, was less than 20%, with a range of 1% to 18%, a mean of 10% (SD, 8%), and a dim pattern (Fig. 1). No statistically significant difference was found by comparing CD200 expression in non–CLL-BCLPDs and control group ($P > 0.05$) as the latter showed CD200 expression of 2% to 11% with a mean of 7% (SD, 2%) on CD19-positive B lymphocytes. A comparative study regarding CD200 expression revealed a significant difference ($P < 0.001$) when comparing B-CLL with both the non–CLL-BCLPDs and control groups. It is worth noting that all of B-CLL cases scored 4 or 5, whereas other BCLPDs scored 0 to 2 according to the Matutes score.

Comparing patients under treatment with untreated patients as regards CD200 expression, the expression was significantly higher in the treated group ($P < 0.05$); this was noted in both CLL and non-CLL groups.

CD200 expression in B-CLL was not correlated with the clinical data, the Rai and the Binet staging systems, laboratory data (eg, hemoglobin level, total leucocytes count, lymphocyte %, absolute lymphocytic count, lymphocyte doubling time, and platelet count), or with the Matutes score. No correlation was found between CD200 expressions in other BCLPDs and clinical or laboratory data or the Matutes score.

As regards the expressions of other immunophenotypic markers, as shown in Table 1, the expressions of CD5 and CD23 were significantly higher in B-CLL patients compared with non–CLL-BCLPDs, whereas the expressions of CD22, CD79b, and FMC7 were significantly lower in B-CLL patients compared with non–CLL-BCLPDs.

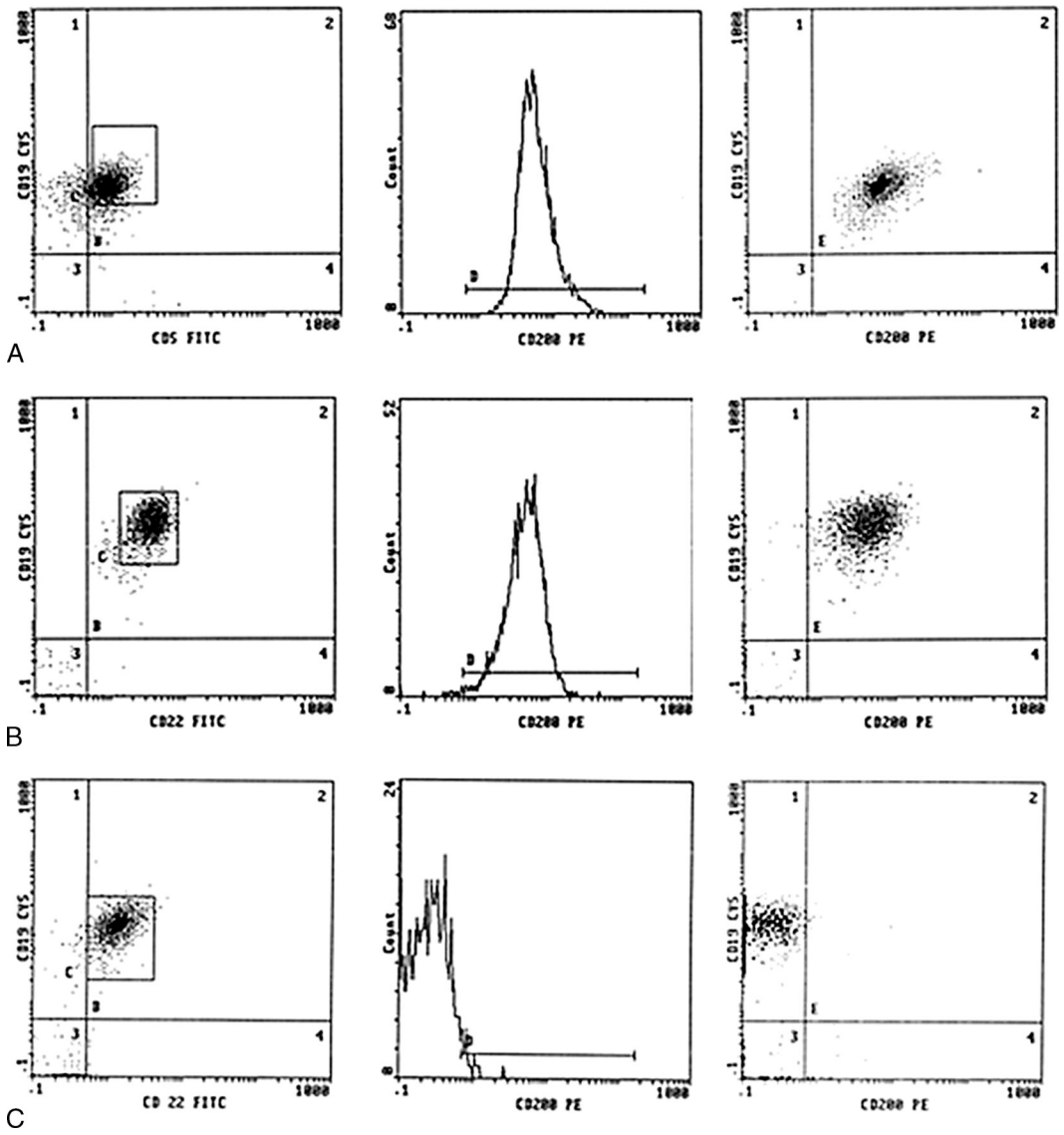

FIGURE 1. A, B-CLL, 1-CD5$^+$/CD19$^+$ clone, 2-CD200 bright expression, 3-CD200$^+$/CD19$^+$ population. B, HCL, 1-CD22$^+$/CD19$^+$ clone, 2-CD200 bright expression, 3-CD200$^+$/CD19$^+$ population. C, MCL, 1-CD22$^+$/CD19$^+$ clone, 2-CD200 negative expression, 3-CD19$^+$/CD200$^-$ population.

In the 2 cases of HCL included in this study, CD25, CD11c, and CD103 showed percentage expressions of 64 and 97, 88 and 65, and 54 and 43, respectively. None of the patients included in this study was positive for CD10 expression.

Among the 49 patients included in this study, 21 patients (43%) had unusual or lacked a usual expression of 1 or more of the routine immunophenotypic markers (Table 2); sIg was brightly expressed in 3 CLL patients (10%) and dimly expressed in 2 non–CLL-BCLPD patients (12.5%); 1 of the 2 patients further received a diagnosis of MCL despite the associated unusual expression of CD23 in this patient (patient 47). However, diagnosis was confirmed by cyclin D1 positivity. CD23 was also unusually expressed in 3 other non–CLL-BCLPD patients (25%). Among patients with CLL, 3 patients (10%) showed positivity for CD22; 2 patients (6.5%) showed positivity for CD79b, whereas unusual negative expression of CD79b was seen in a single patient (6%) of the non–CLL-BCLPDs. FMC7 was exceptionally positive in a single patient with CLL (3%) and negative in a single patient (6%) with other BCLPDs. All of the included FL patients (n = 8) lacked the usual expression of CD10; however, histopathologic examination of lymph nodes and/or FISH analysis for detection of translocation t(14; 18)(q32;q21) confirmed the diagnosis of FL. Also, SMZL patients (n = 4) lacked CD11c expression. In these patients, histopathologic examination of spleen or lymph nodes confirmed the diagnosis.

DISCUSSION

This study addressed the question of whether CD200 expression can be a guiding marker in the differential diagnosis of BCLPDs. From the results of the present work, we can conclude that CD200 is a simply applicable, reliable, nonexpensive, and accurate marker that might be applied for differential diagnosis of BCLPDs.

TABLE 1. Immunophenotypic and Clinical Data of BCLPDs Patients

	CLL (n = 31)			NHL (n = 16)		
	Range	Mean (SD)	Median	Range	Mean (SD)	Median
Age, y	49–76	59.9 (7.4)	57	37–68	55.56 (9.7)	57.5
TLC, 10^9/L	12.8–401	80.3 (76)	57	13–240	44.6 (55.3)	29.5
Lymph, %	62–96	81.6 (9.2)	84	34–91	71.4 (15.9)	73.5
ALC, 10^9/L	11.6–360	66.6 (66.5)	47.8	5.3–192	34 (45)	19.9
CD200, %	60–100	93.7 (10.9)	99	1.2–18	10 (7.7)	6.6
CD19, %	63–99	89 (8.7)	92	50–98	77.9 (15.9)	83.5
CD5, %	52–99	81.1 (11.6)	83	4.5–96.8	31.1 (29.7)	17.2
CD23, %	38–99	57 (17.7)	53	1.2–51	17 (15.4)	14.8
CD22, %	1.8–22	12.2 (5.4)	12	38–92	68.5 (21)	63.6
CD79b, %	1–29.7	9.5 (7.5)	9.2	11.6–86	61.2 (20.4)	61.5
FMC7, %	0.6–21	5.6 (5.4)	3.5	13.7–83	53 (17.6)	55.5
sIg, D/B, n (%)		28 (90%)/3 (10%)			2 (12.5%)/14 (87.5%)	
Sex, F/M, n (%)		8 (26%)/23 (74%)			6 (37.5%)/10 (62.5%)	
PT/UT, n (%)		13 (42%)/18 (58%)			3 (19%)/13 (81%)	

D indicates dim expression; B, bright expression; TLC, total leukocytic count; ALC, absolute lymphocytic count; PT, pretreated; UT, untreated; F/M, female/male.

The evidence is that CD200 was brightly expressed on 60% to 100% of CLL cells in 100% of CLL patients, whereas in all the patients of other BCLPDs, including MCL, FL, and SMZL, the expression was below the cutoff of positivity value (20%) and with a dim pattern, with the exception of HCL, which is considered a special disease entity in the WHO classification of BCLPDs as it has unique clinicopathologic and biological features[28]; in HCL, CD200 was strongly and brightly expressed.

In agreement with these study results as regards CD200 expression in B-CLL, McWhirter et al.[29] reported that B cells from all of 87 CLL patients exhibited 1.6- to 5.4-fold cell surface up-regulation of CD200 relative to normal B cells. Immunohistochemical detection of CD200 expression in CLL was confirmed by Dorfman and Shahsafaei.[22] Another study also stated that CD200 was present on neoplastic cells of all 79 B-CLL patients, with expression of 40% to 100% of $CD5^+$ cells (mean, 96% [SD, 7%]).[1] Compared with Palumbo et al., this study showed a higher percentage of cells expressing CD200 (60%–100%) instead of 40% to 100%. Reviewing their study, CD200 expression was evaluated on total $CD5^+$ cells, which may include residual normal T lymphocytes besides the CLL clone, which is $CD5/19^+$, whereas the current study restricted CD200 evaluation on $CD5/19^+$ clone only. It is to be noted here that CD200 is expressed only on the activated portion of T lymphocytes.[18]

In agreement with the current results as regards MCL, a study on 14 MCL patients in leukemic phase stated that CD200 showed a dim positivity in a small minority (<20%) of $CD5^+$

TABLE 2. Patients With Unusual Immunophenotypic Expression

Case	Diagnosis		sIg	CD23	CD22	CD79b	FMC7	Score	CD200
5	B-CLL	1	Bright					4	+ Ve
10		2	Bright					4	+ Ve
18		3	Bright					4	+ Ve
1		4			+ Ve	+ Ve		4	+ Ve
14		5			+ Ve			4–5	+ Ve
27		6			+ Ve			4–5	+ Ve
6		7				+ Ve		4–5	+ Ve
11		8					+ Ve	4	+ Ve
47	MCL	9	Dim	+ Ve				2	− Ve
41	Non–CLL-BCLPD	10	Dim					1	− Ve
34		11		+ Ve				1	− Ve
37		12		+ Ve				1	− Ve
43		13		+ Ve				1	− Ve
33		14				− Ve		0–1	− Ve
38		15					− Ve	1	− Ve

All of the FL patients (n = 8) lacked the usual expression of CD10; also, SMZL patients (n = 4) lacked CD11c expression.

+ Ve indicates positive; − Ve, negative.

cells in three subjects (4%, 7%, and 16%) and was totally absent in the remaining 11 subjects.[1] The present work included 4 patients with MCL; none of them showed expression of CD200 reaching the cutoff of positivity (2%, 4%, 8%, and 18%); however, CD200 was not totally absent in any of the 4 patients. Perhaps the small sample size explains why this study cannot identify MCL with totally absent CD200 expression.

Our results show that all the patients with FL and SMZL were negative for CD200 expression with no significant difference compared with the control group. In agreement with the current study results, Dorfman and Shahsafaei[22] confirmed CD200 expression in HCL by immunohistochemical analysis. Also, Brunetti et al.[20] reported bright CD200 positivity on all hairy cells from all HCL patients studied, with a large amount of this antigen on neoplastic cells.

Surprisingly, using CD200 expression guidance, we could correctly assign all the patients who showed unusual immunophenotypic expressions in this study to their proper diagnostic groups (Table 2).

In an attempt to find an explanation for the different expression patterns of CD200 in BCLPDs, literature partially referred this to the different activation of the AKT and MEK/ERK pathways in these different disorders. It has recently been reported that CD200 mRNA expression correlates with ERK activation in melanoma.[30] It is worth noting that ERK also activated in B-CLL, which expresses CD200 as well.[31] Strikingly, in MCL, the activated pathway is the AKT, which plays a major role in down-regulation of active ERK,[32] and this could contribute to the absence of CD200 in this disease.

In contrast to our results, which reported that CD200 expression in B-CLL was not correlated with any of the clinical data, laboratory data (eg, total leucocytes count, lymphocyte %, absolute lymphocytic count, lymphocyte doubling time, and platelet count), or the Rai staging system, it is reported that CD200 levels are associated with Rai disease stage and lymphocyte doubling time[33]; however, they applied the correlation with soluble variant of CD200 (sCD200), which is detectable in human serum.

Hematopoietic malignancies are generally considered to be particularly amenable to antibody therapy based on relatively good accessibility of the antibody to target cells. However, despite the success of rituximab (anti-CD20) treatment in a number of lymphomas and leukemias, clinical trials in CLL with single-agent rituximab have demonstrated only partial responses in the majority of patients, with little to no effect on long-term survival.[34] Alemtuzumab (anti-CD52) was the first approved antibody treatment of CLL, but infusion reactions and opportunistic infections limit its use.[35]

The crucial question here is whether blocking CD200 on B-CLL cells by monoclonal antibody might be therapeutically useful. Anti-CD200 antibodies were sufficient for growth inhibition of CD200 expressing tumor in a novel animal model that incorporates human immune cells and human tumor cells (CD200-positive Burkitt lymphoma cell lines).[36]

The effect of targeting CD200 on human CLL or HCL cells by monoclonal antibody and whether this will lead to the lysis of tumor cells need to be explored in future studies.

Our research was based on studying CD200 expression in cases of BCLPDs that presented with leukemic phase. The differential diagnosis of this presentation is restricted to certain types of BCLPDs including CLL/SLL, CLL/PLL, PLL, MCL, FCL, SMZL, HCL, and occasionally lymphoplasmacytic lymphoma, DLBCL, and Burkitt lymphoma. However B-cell tumors that present in leukemic phase can be misdiagnosed as BCLL because of overlapping disease features; conflicting diagnoses usually occur between these disease entities when atypical clinical, morphologic, histochemical, immunophenotypic, histopathologic, and/or molecular presentations are effaced.

The flow cytometry technique is still the most rapid test that could supply the results of chronic lymphoproliferative disorders within a few hours, so we think that addition of this new marker (CD200) to the already available panel will support the definite diagnosis of these cases.

We believe that CD200 will be a guiding marker of special importance in such difficult cases as CLLs that lack CD5 expression; cases of HCL that may lack any of CD11c, CD25, or CD103; and cases of large B-cell lymphoma expressing CD10; in these cases, the positivity of CD200 is crucial in their differential diagnosis. In other difficult cases such MCLs that express CD23 or cases with borderline Matutes scoring (score 3); negative expression of CD200 in such cases may point to MCL.

Unfortunately, our study did not encounter all these variants; however, we hope that this study can be applied on a wider scale of patients and includes more patients with atypical immunophenotypic presentations and that CD200 expression evaluation can be accomplished not only on peripheral blood or bone marrow aspiration samples but also on tissue specimens (bone marrow trephine/lymph nodes/spleen) using immunohistochemical analysis to allow for evaluation of other types of BCLPDs that do not present with leukemic phase.

Thus, from the present study as well as other previous studies, we recommend to add CD200 to the BCLPD flow cytometric routine panels, because the differential diagnosis between different lymphoproliferative disorders, and in particular between B-CLL and MCL, can be difficult using the standard antibodies. We also propose to add CD200 besides the Matutes scoring system after being validated by further studies.

CD200 evaluation by flow cytometry analysis is easy, not expensive, readily usable, and of special great importance when peripheral blood and/or bone marrow samples are available with no tissue sample.

On the other hand, if treatment strategy, in CLL and HCL, would be targeted against CD200 itself (anti-CD200 antibodies), would it damp down the activity of the disease? It seems reasonable to achieve that in the future, whether using anti-CD200 alone or in combination with other B-cell cytotoxins, cancer vaccines, or other immunostimulatory therapies.

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