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The Role of CD200 in Differentiating B-Chronic Lymphocytic Leukemia from Mantle Cell Lymphoma and its Prognostic Value

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ABSTRACT

Background: There is a wide range of disease feature overlap between B-cell lymphomas as Mantle Cell Lymphoma (MCL), especially when presented in leukemic phase, and B-Chronic Lymphocytic Leukemia (B-CLL). Both arise from CD5+ve B-cells; their distinction is critical as MCL is a more aggressive neoplasm.

Objective: To determine whether there is a role for CD200 in the differentiation between B-CLL and MCL andits prognosticvalue.

Patients and Methods: A retrospective study was conducted onarchival material of 61 patients (48 with B-CLL and 13 with MCL) with a well defined diagnosis with fifteen age and sex matched healthy subjects as control group. Detection of t(11:14) was done by Fluorescent in Situ Hybridization (FISH) and CD200 was done by Immunohistochemistryon stored fixed paraffin embedded bone marrow biopsies.

Results: CD200 expression was significantly higher in B-CLL patients (47.4±28.9) compared to MCL patients (6.2±5.3) (p=<0.001).

Conclusion: Adding CD200 in routine monoclonal antibody panels could be of diagnostic utility in differentiating B-CLL from MCL. Also, anti CD200 targeted therapy may carry a promising treatment option to CD200 expressing cancers in the future.

Key Words: *B-cell lymphomas – B-Chronic lymphocytic leukemia – Mantle Cell Lymphoma – CD200.*

INTRODUCTION

There is a wide range of B-cell tumors present in leukemic phase that may be misdiagnosed as B-Chronic Lymphocytic Leukemia (B-CLL) because of overlapping disease features especially in the presence of CD5 positive lymphocytosis **[1]**. So, it is important to differentiate between B-CLL and Mantle Cell Lymphoma (MCL) by immunophenotypic analysis-particularly CD23 because of its positivity in B-CLL and negativity in MCL; however some cases of B-CLL are CD23 negative **[2-4]**.

The diagnosis of MCL should be confirmed by demonstration of cyclin D1 or by the presence of the t(11:14) detected by cytogenetics, fluorescence in situ hybridization (FISH) and Western blot or polymerase chain reaction (PCR) analysis. Even though these represent reliable methods, they are expensive, time-consuming and not available in all Centers **[5-8]**.

Cyclin D1 negative MCL do actually exist **[9]**, also MCL without t(11:14) have been reported and the same translocation can be found in B-CLL and other lymphoproliferative disorders **[10,11]**. Therefore, there is a need for new markers that allow an easier differential diagnosis between CLL and MCL.

CD200 is a transmembrane glycoprotein with inhibitory immunoregulation role. It has a relatively broad distribution as it is expressed on thymocytes, activated T cells, B cells, dendritic cells, endothelial cells, and neurons **[12-15]**.

El Desoukey et al., **[16]** reported that CD200 is expressed in B-CLL and Hairy Cell Leukemia (HCL) versus negative expression in MCL, follicular lymphoma (FL), and splenic marginal zone lymphoma (SMZL). The expression of CD200 was also reported in multiple myeloma, lymphoblastic lymphoma/leukemia, mediastinal large B cell lymphoma, lymphoplasmacytic

lymphoma, angioimmunoblastic T-cell lymphoma, acute myeloid leukemias, and other non-hematologic malignancies **[17-20]**.

The different expression of CD200 in B-CLL than in MCL could be explained by the different activation of the AKT, also known as Protein Kinase B (PKB), which is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration; also, by activation of Mitogen-activated protein kinases, originally called ERK, Extracellular signal-regulated kinases (MEK/ERK), pathways. It has been reported in melanoma that CD200 mRNA expression correlates with ERK activation **[21,22]** and this pathway is also activated in B-CLL **[23,24]** where CD200 is in fact present. While, in MCL the well-known activation of AKT might lead to a downstream down regulation of active ERK **[25,26]** and this could contribute to the absence of CD200 in this disease.

Starting from these data, we addressed the question of whether CD200 expression can be a guiding marker in the differential diagnosis of CLL and MCL.

PATIENTS AND METHODS

The retrospective study included 61 patients, 48 patients with B-CLL (21 males and 27 females) and 13 patients with MCL (9 males and 4 females) with fifteen age and sex matched healthy subjects as control group. Cases have been selected from patients who were attendingthe Clinical Hematology Unit of Assiut University Hospital and South Egypt Cancer Institute between years 2005 and 2012. The study was approved by the Ethics committee of the Faculty of Medicine, Assiut University. Patients with liver dysfunction, other CD5+ve lymphoproliferative disorders and autoimmune diseases were excluded from the study population. Diagnosis of B-CLL was made by flow cytometry according to the latest version of the Matutes score **[27]**. Diagnosis of MCL was based on morphology and immunohistochemical detection of CD5+ve lymphocytes and FISH detection of t(11:14) in bone marrow biopsies.

The collected retrospective data were: Full history with clinical examination stressing on anemic manifestations, bleeding tendency, B-symptoms, presence of hepatomegaly, splenomegaly and lymphadenopathy; also, the laboratory results of peripheral hemogram, Coomb's test, lactic dehydrogenase, uric acid and bone marrow aspirate with immunophenotyping.

Bone marrow biopsies were selected from stored fixed paraffin embedded samples of study-included patients for detection of t(11;14) by Fluorescent in situ hybridization (FISH) technique in 5μ thick paraffin sections cut on positive charge slides. While, detection of CD200 was done by immunohistochemistry using a rabbit anti-human CD200 IgG affinity purified polyclonal antibody (SIGMA, U.S.A.) number HPA03119 and K5361/EnVision™ G/2 Doublestain System, Rabbit/Mouse (DAB+/Permanent Red) (Dako, USA) using 3μ thick paraffin sections.

Statistical analyses:

The collected data were analyzed by using SPSS/PC (version 17). Descriptive statistics: Mean, standard deviation, frequencies, percentage were calculated. Test of significances: Chi square test was used to compare the difference in distribution of frequencies among different groups. One-way Analysis of Variance (ANOA) was calculated to test the mean differences in continuous variables between groups. A significant *p*-value was considered when it is <0.05. Correlation analysis between CD200 and different variables of CLL was calculated. Validity statistics for CD200 test was calculated with a cutoff of 20%.

RESULTS

The study included 61 patients, 30 males (49.2%) and 31 were females (50.8%) with 15 halthy matched controls 8 males (53.3%) and 7 females (46.7%). The patient's age ranged from 35-85 with a median of 55 years while that of the controls was 31-78 with a median of 44 years. Forty eight patients (78.7%) were diagnosed as chronic lymphocytic leukemia through detection of (CD5+ve) by flow cytometryand 13 patients (21.3%) were diagnosed as mantle cell lymphoma by immunohistochemical detection of CD5+ve lymphocytes and FISH detection of t(11:14) in bone marrow biopsies.

Table (1); summarize the clinical findings and peripheral Hemogram data of B-CLL and MCL patientswith only statistically significant difference in WBCs count in B-CLL in relation to MCL patients (p<0.01). While, Table (2), illustrates the percent of positivity in different diagnostic markers in both patient's groups including CD200 activity that appears positive in 72.9% of B-CLL patients and only in 7.6% of MCL patients (p<0.001). The ability of the test to detect positive cases (sensitivity) was 72.9% and the ability to detect negative cases (specificity) was 92.3%. The ability to predict positive cases (PPV) was 97.2% and that to predict negative cases (NPV) was 48% with 77% accuracy.

CD200 expression was significantly higher (p<0.001) in B-CLL patients compared to MCL and control with a Mean±SD in CLL (47.4± 28.9), in MCL (6.2±5.3) and in control (8.7±2.1) with insignificant difference between MCL and control group (Fig. 1).

Also, CD200 expression in B-CLL was not correlated with any of the clinical data (anemia, bleeding tendency, B-symptoms and organomegaly), laboratory data (total leucocytic count, lymphocyte percentage in bone marrow aspirate, hemoglobin level, or platelet count).

Table (1): Clinical and peripheral hemogram criteria of booth B-CLL and MCL patients.

Item	B-CLL (n=48)	MCL (n=13)	*p* value
Clinical Findings:			
Bleeding	8 (16.3%)	1 (7.7%)	
Pallor	17 (35.4%)	3 (23.1%)	
Fever	15 (31.2%)	2 (15.4%)	NS
Hepatomegaly	18 (37.5%)	4 (30.4%)	
Splenomegaly	34 (70.8%)	10 (76.9%)	
Lymphadenopathy	39 (81.2%)	11 (84.6%)	
Peripheral Hemogram:			
Hb	9.9±201	9.7±201	NS
WBCs	95.8±104.9	19.7±21.9	<0.01
Platelets	139.5±70	113.7±56.2	NS

B-CLL: B- Chronic Lymphocytic Leukemia.
MCL : Mantle Cell Lymphoma.
Hb : Hemoglobin.
WBCs : White Blood Cells.
p<0.05 = *
p<0.01 = **
p<0.001 = ***
NS = Not significant.

Table (2): Percent of diffferent diagnostic criteria of booth B-CLL and MCL patients.

Diagnostic markers	B-CLL (n=48)	MCL (n=13)	*p* value
CD23	48 (100%)	0 (0.0%)	<0.001
FMC7	2 (4.2%)	12 (92.3%)	<0.001
SIg	28 (58.3%)	11 (84.6%)	<0. 05
t(11:14)	0 (0.0%)	13 (100%)	<0.001
CD200	35 (72.9%)	1 (7.6%)	<0.001

B-CLL: B- Chronic Lymphocytic Leukemia.
MCL : Mantle Cell Lymphoma.
CD : Cluster of differentiation.
p<0.05 = * p<0.01 = ** p<0.001 = ***

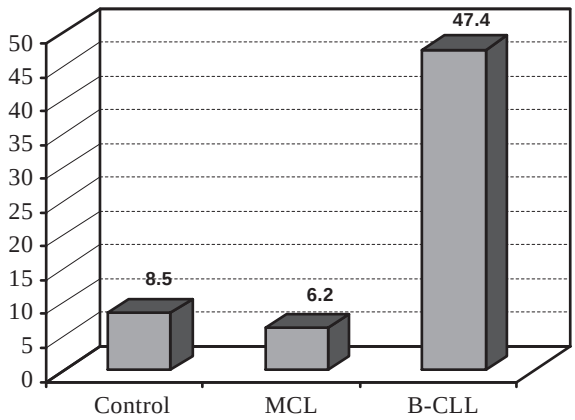

Fig. (1): Mean CD 200 expression in B-CLL, MCL patients and controls.

B-CLL: B- Chronic Lymphocytic Leukemia.
MCL : Mantle Cell Lymphoma.

DISCUSSION

It is important to differentiate B-CLL from MCL as the latter is a more aggressive disease and generally treated differently than B-CLL **[13]**. Differential diagnosis between B-CLL and MCL is assessed by immunophenotyping analysis on freshly isolated cells and in this respect CD23 is considered a reliable marker because of its positivity in B-CLL and negativity in MCL **[28,29]**.

The diagnosis of MCL should be confirmed by demonstration of Cyclin D1 positivity or by presence of chromosomal translocationt (11:14) **[30]** which is not pathgnomonic for MCL. In fact some MCL cases have been reported without t(11:14) **[31]**. The same translocation can be found in B-CLL and other lymphoproliferative disorders **[10,32]**.

In the current study, the frequency of CD200 positivity in B-CLL patients is significantly higher ($p<0.001$) than in MCL patients. The frequency of CD200 positivity in B-CLL patients is less when compared to the results of Palumbo et al., **[13]** who reported a statistically highly significant value in the expression of CD200 between CLL and MCL. The antigen was expressed in the neoplastic cells of all the B-CLL patients (100%) they studied but not in MCL. This is especially true for immunohistochemistry technique used in the current study that probably has a lower sensitivity when compared to flow cytometry as the flow cytometry can detect CD200 positive cells in a freshly prepared blood sample while immunohistochemical technique depends on staining of stored fixed tissue samples.

Also, CD200 expression is significantly higher in CLL than control group; this is in agreement with the results of Mc-Whirter et al., 2006 **[33]** who reported that CLL patients exhibited 1.6 to 5.4 fold up-regulation of CD200 relative to normal B cells. The up-regulation of CD200 may be a mechanism used by CLL tumors to evade eradication by the immune system.

The different expression of CD200 could be explained by the different activation of the Mitogen-activated protein kinases, originally called ERK, Extracellular signal-regulated kinases (MEK/ERK) pathways in these two diseases. It has been reported that CD200mRNA expression correlates with ERK activation in melanoma as reported by **[21,22]**. This pathway is also activated in B-CLL as confirmed by **[23,24]**, where CD200 is in fact present. On the contrary, in MCL the well known activation of AKT might lead to a down regulation of active ERK and this could contribute to the absence of CD200 according to **[25,26]**.

In this study the optimum cut-off point of CD200 detection was selected at 20%, with a sensitivity of 72.9%, specificity 92.3%, positive predictive value 97.2%, negative predictive value 48% and accuracy of 77% to differentiate the B-CLL cases from the MCL cases. On the other hand Bhatnagar et al., 2010 **[34]** reported that at the same cutoff sensitivity was 100%, specificity was 98.7%, and positive predictive value was 98.7%. These differences may be attributed to the difference in the technique as they used flow cytometry and we used immunohistochemistry on archived paraffin-embedded blocks.

In the current study, CD200 expression in B-CLL was not correlated with any of the clinical and laboratory data; this is in agreement with El Desoukeyand his colleagues in 2012 **[16]** as they found that CD200 expression in B-CLL was significantly higher than other lymphoproliferative disease and was not correlated with any of the clinical and laboratory data.

CD200 could be very useful for the differential diagnosis between B-CLL and MCL especially in patients with CD5 positive lymphocytes. Although the B-CLL and MCL have very different behavior towards therapy and prognosis, they share many immunophenotyping and morphological features that make the differentiation exceedingly difficult as described by Bosch and his colleagues in 1998 **[31]**. So we recommend adding CD200 in immunohistochemistry and flow cytometry routine panels as it would be of great diagnostic value especially in cyclin D1 negative MCL cases as those reported by Fu et al., 2005.

Performing CD200 by immunohistochemistry might be useful in revising old cases or those referred by peripheral centers when fresh cells are not available. Also, further studies are needed to prove CD200 usefulness in differentiating B-CLL from other B-cell low grade lymphomas as it is considered a simple applicable, reliable, non expensive and accurate marker.

We recommend further studies of CD200 expression levels on B-CLL cells in relation to treatment and prognosis and we hope that anti-CD200 targeted therapy in CD200 expressing cancers would be promising treatment in the future.

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