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FLOW CYTOMETRIC PATTERN IN DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA, KING HUSSEIN MEDICAL CENTER EXPERIENCE

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ABSTRACT

Background: Flow cytometry has an essential role in diagnosing chronic lymphocytic leukemia (CLL) and distinguishing it from other lymphoproliferative diseases (LPD) according to expression of certain markers called cluster of differentiation (CD). **Aim:** Investigating the pattern of expression of different CD markers in CLL patients at Princess Iman Center for Research and Laboratory Sciences over the period of 1/2011 to 5/2015. **Patients and method:** 214 patients with peripheral blood (PB) lymphocytosis (lymphocytes count > $5x10^9$) diagnosed as CLL by flow cytometry on PB samples using specific panel including the following markers: CD5, CD19, CD23, CD79b, CD25, CD10, FMC7, sIgM. The age range 37-84 years. The Male to Female ratio is 1.8:1. **Results:** The expression of the CD markers in CLL patients was as follow: CD5 expressed in 212 patients (99%), CD19 expressed in 214 patients (100%), CD23 expressed in 199 patients (93%), CD79b expressed in 101 patients (47%), CD25 expressed in 97 patients (45%), FMC7 expressed in 17 patients (8%), sIgM expressed in 48 patients (22%), and CD10 was negative in all patients (0%). **Conclusion:** Flow cytometry is a golden tool for diagnosing CLL. Standardization of the procedure is essential and adding new described monoclonal antibodies related to prognosis and treatment options are recommended.

KEYWORDS: flow cytometry, CLL, CLL panel, LPD, monoclonal antibodies.

INTRODUCTION

Flow cytometry is the most useful method currently used for diagnosis and classification of chronic lymphoproliferative diseases including CLL. CLL is the most common leukemia of adults.[1] The definitive diagnosis of CLL is reached by combining peripheral blood lymphocytosis with the morphology and immunological profile of the lymphocytes.[2] The majority of cases of CLL are easily distinguished from other lymphoproliferative diseases; however some cases show atypical morphology and immunophenotyping that makes diagnosis difficult.[1] In these cases the scoring system which is based on the expression of CD5, CD23, CD79b, FMC7, and sIg may be helpful.[3]

The aim of this study is to show the flow cytometric pattern of CLL cases in our center.

Patients and method

This is a retrospective study which was carried out on 214 patients diagnosed as CLL based on flow cytometry findings using CLL panel at Princess Iman Research and Laboratory Sciences Center over the period from January 2011 to May 2015. All cases that showed peripheral blood lymphocytosis (lymphocytes count > $5x10^9$/L) were included in the study and peripheral blood morphology was seen by our hematopathologist before performing flow cytometry (figure 1). Flow cytometry performed on peripheral blood samples. The age range 37-84 years. The median age at diagnosis for our study patients was 60 years. The Male to Female ratio is 1.8:1

Method

Peripheral blood specimen 2-5 mL EDTA tube incubated at room temperature for 15 minutes with flourochrome conjugated antibodies including CD5, CD19, CD23, CD79b, CD79b, CD25, FMC7, sIgM, CD10. Using lysis buffer, red blood cells lysed, then washing done by PBS with phosphate buffered saline PBS centrifuged for 20 minutes 1000x.100 micron were pipetted into test tube and resuspended in PBS. Tubes then analyzed using BD FACSCANTO II. The results plotted on histograms on a forward and side scatter, then gating done on the lymphocytes area. A certain population of cells is considered positive for a certain marker when more than 20% of that population shows positive staining.[4]

RESULT

The expression of the markers in CLL patients was as the following (figure 2): CD5 expressed in 212 patients (99%), CD19 expressed in 214 patients (100%), CD23 expressed in 199 patients (93%), CD79b expressed in

101 patients (47%), CD25 expressed in 97 patients (45%), FMC7 expressed in 17 patients (8%), sIgM expressed in 48 patients (22%), and CD10 was negative in all patients (0%).

The results showed that there is no specific marker for diagnosis of CLL; the diagnosis is made by using a complete panel containing set of markers including mainly CD5, CD19, CD23, CD79b, FMC7 and sIgM.

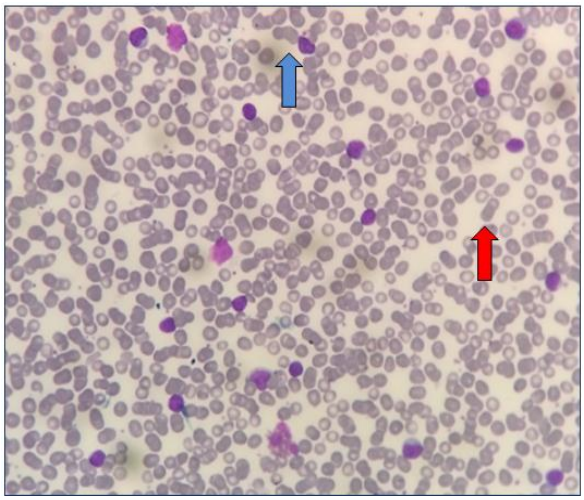

Figure 1 CLL in the peripheral blood film; the lymphocytes (red arrow) are small, round, with distinct clumped chromatin. Smudged cells (blue arrow) are commonly seen.

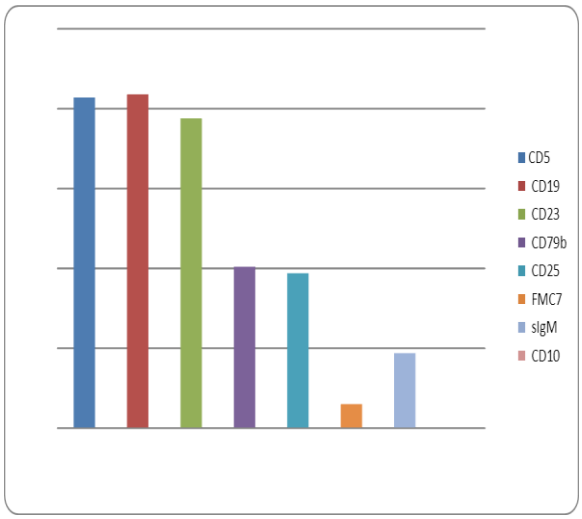

Figure 2 percentage of markers positivity in CLL cases

Table 1 Scoring system for the diagnosis of chronic lymphocytic leukemia (CLL).

Marker	1 point	0 point
sIg	weak	Strong
CD5	positive	negative
CD23	positive	negative
FMC7	negative	Positive
CD79b	weak	Strong

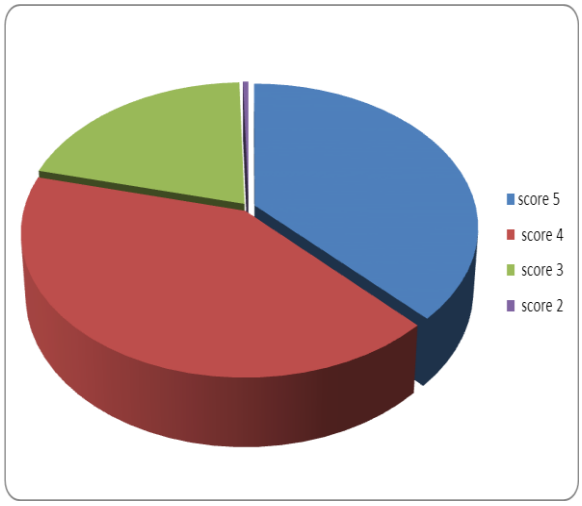

Figure 3 Pie chart showing relative percentages of scores in our CLL cases.

DISCUSSION

CLL is a low grade mature B-cell neoplasm that characterized by peripheral blood lymphocytosis (lymphocytes count > $5x10^9$) with bone marrow involvement. Lymph nodes, liver and spleen may be involved. It occurs primarily in middle-aged and elderly, the mean age at diagnosis is 65 years. The clinical course is usually indolent. The median survival depends on the stage of the disease and the genetic abnormalities.[5]

The incidence rate is about 2-6 cases per 100,000 person per year. It is the most common leukemia in western world.[5] It is 20-30 times more common in Europe, North America and Australia than Asia.

Patients are usually asymptomatic and found accidentally to have lymphocytosis on peripheral complete blood count (CBC). Some patients present with fatigue, splenomegaly, anemia, or lymphadenopathy.[5] For diagnosis and to set for prognostic stratification of CLL, it is essential to integrate CBC, blood film immunuophenotypic and cytogenetics findings.[6] Flow cytometry is the most valuable test to confirm diagnosis.[7] Tumor cells using flow cytometry show dim surface Ig expression, double expression of CD5 and CD23, and positive for CD20, CD22, CD19, CD79a, CD43, and CD11c (weak). CD10 is negative, and FMC7 and CD79b are mostly negative or weakly positive in typical CLL. The immunophenotype has been integrated into a scoring system that may help in differentiation between CLL and other B-cell leukemias. Some cases may have atypical immunophenotypes pattern.[5]

In this study we analyze the pattern of flow cytometry expression in CLL patients in our center. The expression of CD5 was found in 212 out of 214 cases (99%). This result is comparable to other study results carried out in 2002 by Sheikh et al. that reported the expression of CD5 in 98% of CLL cases and Robbins et al who instituted that CD5 expression found in 100% of their CLL patients.[4,8] CD5 is a membrane glycoprotein. It is a pan

T-cell marker that is expressed on long lived B-cells at mantle zone of lymphoid follicles. It promotes B-cell survival through stimulation of IL-10 production. The majority of CLL cases show CD5 expression in addition to some other LPD mainly mantle cell lymphoma.[8]

The expression of CD23 in our cases was 93%; Ahmad et al expression was up to 100% of cases.[10] while, DiRaimondo et al in a study performed at M.D Anderson Cancer Center reported the expression of CD23 of 97% of the studied cases.[9] CD23 is a low affinity receptor for IgE and expressed on B-CLL cells and contribute to accumulation of long-lived B-cells. It is essential in differentiating CLL from other LPD especially Mantle cell lymphoma, it was reported that CD23 expression associated with a favorable prognosis. Typical cases of CLL are rarely CD23 negative, therefore, mantle cell lymphoma should be first ruled out in these cases.[9]

This study showed expression of C19 in all CLL cases (100%), which is the same as other studies.[11-14] CD19 is a type I transmembrane glycoprotein, it is a biomarker for normal and neoplastic B cells. It is the dominant signaling component on the surface of mature B cells.[11] CD19 is considered a lineage specific marker and one of the most reliable B-cell surface marker.[10,11] CD19 is expressed by the majority of B-cell malignancies and its level can be helpful in differentiating some subtypes of lymphomas.[11,13]

This study showed CD79b expression in 101 patients (47%), while in El-Sewefy et al. CLL cases showed CD79b positivity in 37%.[1] CD79b together with CD79a are component of the B-cell receptor complex that is critical for signal transduction following Ig cross linking. It is also important in allelic exclusion of immunoglobulin heavy chain in normal developing B-cells.[15]

In this study the expression of FMC7 was seen in 17 patients (8%). In comparison to other studies, Delago et al show a higher percentage of FMC7 (14%), while in El-Sewefy et al. FMC7 was not seen in any of his CLL cases.[1,2] FMC7 is a glycoprotein expressed in the majority of mature normal B-cells; it is a reliable marker for distinguishing CLL from other B-cell LPD.[10] Typical morphologic CLL cases are usually FMC7 negative.[16]

In this study, the expression of sIgM found in 48 patients (22%), it reached 75 % in Geisler et al. and Lewis et al.[18, 19] Surface immunoglobulin M (sIgM) is B-cell receptor which is critical for the survival of normal B-cells and is retained by the majority of normal B-cell malignancies, including CLL, it is usually weakly expressed in CLL and in cases that express sIgM, the fluorescence intensity is usually low in comparison to other LPD.[17,18]

A scoring system, based on the expression of a group of five surface membrane markers (CD5, CD22, CD23, FMC7, sIgM) [Table1] was found to be helpful in the differentiation between CLL and other lymphoproliferative diseases. Scoring system accuracy reached 91.8% using a cutoff of 4 points or higher.[20] Scores in CLL are usually>3, in other B-cell malignancies the scores are usually<3.[3] In this study(figure 3), 82 cases (38%) score 5, 87 cases (40%) score 4, 44 cases (20.5%) score 3 and 1 case (0.5%) score 1.In comparison to other studies 92% cases score 4 or 5, 6% score 3 and 2% score 1 or 2.[2] Matutes et al. applied the scoring system on a 400 CLL cases and showed that 87% of cases scored 5 and 4 and only 0.4% scored 0 or 1, he found that the difference between high score and low score CLL cases is that cases with higher score are more typical morphologically.[3]

CD38 is a single-chain type II transmembrane glycoprotein is expressed in many hematologic cells in an activation and differentiation-dependent manner, it has a complex ectoenzymatic activity and involved in proliferation and survival of cells. Studies showed that expression of CD38 in CLL is associated with bad prognosis.[21] CD38 expression can be detected by flow cytometry, but in our lab it is not performed routinely in spite of its prognostic significance due to budget limitations.

CD200 is a membrane glycoproteinthat belongs to immunoglobulinsuperfamily and have immunosuppressive effect. It is present on some T and B lymphocytes. Some studies showed that its addition to CLL panel on flow cytometry might be helpful in differentiation between CLL and Mantle cell lymphoma as it is expressed with moderate intensity in the majority of CLL cases and on the opposite expressed on a minority of MCL cases with low intensity.[1]

CONCLUSION

Flow cytometry is a golden tool for diagnosing CLL. Standardization of the procedure is essential and adding new described monoclonal antibodies related to prognosis and treatment options are recommended.

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