

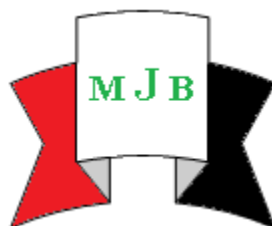
Immunophenotyping of B Cell Chronic Lymphoproliferative Disorders Study of 30 Selected Cases

Khalid A. Mahmood*

Ghassan A. Khaleel**

* Al-Kindy Educational Hospital/ Baghdad, IRAQ.

** Al-Yarmook Educational Hospital/ Baghdad, IRAQ.



Received 29 January 2014

Accepted 3 March 2014

Abstract

Background: B-cell malignancies are clonal expansions of B-cells that express only one type of Ig light chain (L or k) or (rarely) lack surface Ig light chain expression. Multiparameter color flowcytometry allows analysis of light chain expression in total CD19 + B-cell population and also in various subsets of B cells such as CD5+/CD19+, CD23+/CD5+/CD19+ or CD10+/CD19+ positive cells.

Objectives: This study was designed to assess the immune phenotyping of 30 patients with chronic lymphoproliferative diseases using flowcytometry.

Methods: 30 patients with chronic lymphoproliferative disorders has been investigated by flowcytometry. This study done in Al Rawabie laboratory in period between January 2013 till November 2013.

Results: Among 30 patients with chronic lymphoproliferative diseases, 18 patients diagnosed as chronic lymphocytic leukemia, 3 with follicular lymphoma, 6 with marginal zone lymphoma, 2 with splenic lymphoma with villous lymphocyte and one case of multiple myeloma.

Conclusion: Multiple parameter immunophenotyping analysis improves the accuracy of diagnosis in lymphoid malignancies, and can be used in diagnosis, which easily detects infrequent, hematological malignancies differentiated diagnosis, and detection of minimal residue in lymphoproliferative diseases the simultaneous recognition of different cell populations allows the diagnosis of composite cell lymphomas, or double pathologies.

Key Word: Flowcytometry, B- chronic lymphoproliferative disorders, Immunophenotyping.

الخلاصة

التقديم: الاورام الناتجة من تكاثر خلايا اللمفوسايت نوع ب ل تنشأ من الخلايا البائية أحادية النسيلة. إن تشخيص اضطرابات التكاثرية اللمفية يتطلب استخدام تقنيات متعددة بما في ذلك علامات المناعية أشكال الخلايا و ، في بعض الحالات، التشريح المرضي من الأنسجة المتضررة و تحقيقات الجزيئية و الخلوية الوراثية إن عداد الخلايا أثناء الجريان هو أحد التحاليل المخبرية الأساسية في التشخيص الدقيق من هذه الشروط

إن الهدف من هذه الدراسة هو التعرف على المعلمات المناعية التي تحصل في أورام الخلايا نوع ب باستخدام عداد الخلايا أثناء الجريان تم اختيار ٣٠ مريضاً مصاباً باضطرابات التكاثرية اللمفاوية نوع ب وقد تم التشخيص باستخدام عداد الخلايا أثناء الجريان. تمت الدراسة في مختبر الروابي الاهلي للفترة من كانون الثاني ٢٠١٣ الى كانون الاول ٢٠١٣.

النتائج: تبين أن ١٨ مريضاً من مجموع ٣٠ مريضاً مصاباً بالتكاثرية اللمفاوية المزمنة نوع ب و ٣ اللمفوما العقدي و ٦ حول المركزي و ٢ لمفوما الطحال وحاله واحد ورم خلايا البلازما

باستخدام أسلوب التشخيص بواسطة عداد الخلايا اثناء الجريان أدى الى زيادة دقة التشخيص لهذه الامراض كما ازدادت حساسية تشخيص بعض الاورام النادرة والتي تتشابه مع بعضها في طرق التشخيص الاعتيادية كما ساهمت هذه التقنية بالتعرف على عدة انواع من الخلايا خصوصا بحالات الاورام المزدوجة

Introduction

Flow cytometry has become an important tool in the diagnosis and characterization of hematologic and lymphoid neoplasia. The advantages of flow cytometry are largely based on its ability to analyze very rapidly, even in small samples, multiple cell properties simultaneously, including size, granularity, surface and intracellular antigens, and DNA content. The quantitative nature of the data produced, both with regard to cell population distributions and to expression of individual cell antigens, offers objective criteria for interpretation of result. Flow cytometry is able to detect aberrant cells at a frequency of 1/1000 to 1/10,000 cells. [1]

The unique attributes of flow cytometry allow for increased sensitivity in the detection of neoplastic cells and should contribute to improving accuracy and precision in the diagnosis and classification of lymphomas and lymphoproliferative disorders.[2]

B-cell malignancies are clonal expansions of B-cells that express only one type of Ig light chain (L or k) or (rarely) lack surface Ig light chain expression. The clinical presentations and natural histories of chronic lymphoproliferative disorders are extremely heterogeneous.[2] Chronic B-cell lymphoproliferative disorders account for more than 90% of lymphoid malignancies with T-cell and NK-cell neoplasms being relatively uncommon.[3] Immunophenotyping is a key laboratory test in the precise diagnosis of these conditions. [2,3]. CD5 is characteristically expressed in chronic

lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma (MCL).

CD10 is characteristically expressed in acute precursor B- or T-cell lymphoblastic leukemia/lymphoma (ALL) and lymphomas of follicle center cell origin, as follicular lymphoma (FL), Burkitt lymphoma, and a subset of diffuse Large B-cell lymphomas (LBCLs).[3,4]

Classical markers for adverse prognosis of B-CLL are CD38, CD49d and ZAP-70. Most studies consider bad prognosis B-CLL with at least 30% of positive cells for CD38(5). CD38 associated with poor response to therapy and shorter progression free survival its easily measured by flow cytometry and its expression considered as independent poor prognostic factor, this evidence contributes to CD38 is accepted as a dependable marker of unfavorable prognosis and as an indicator of activation and proliferation of cells when tested. Leukemic clones with higher numbers of CD38(+) cells are more responsive to BCR signaling and are characterized by enhanced migration. In vitro activation through CD38 drives CLL proliferation and chemotaxis via a signaling pathway that include ZAP-70 and ERK1/2. Finally, CD38 is under a polymorphic transcriptional control after external signals.[5,6]

From a practical point of view, the immunophenotype should be able to exclude other B-cell lymphoproliferative disorders, specifically those with different prognosis and/or therapy: mantle cell lymphoma (MCL) and hairy cell leukemia (HCL). [6]

The first panel should include the following A pan-B marker: CD20 or CD19 or CD37. The second panel of reagent include CD11c, CD25, cyclin D1 CD103, HC2. Two markers to assess clonality: antiimmunoglobulin light chains: anti-K and anti- λ , with additional Three markers which will help to discriminate between B-CLL and other B cell conditions: CD23, FMC7, and CD22 [2,5,6,7,8]

The aim of the study is to detect the immunophenotyping pattern in thirty patients complain of chronic lymphoproliferative disorders.

Materials and Methods

30 newly diagnosed chronic B-cell neoplasms adult patients .recruited to the study on the basis of clinical, laboratory, and bone marrow aspirate findings and immunophenotypic criteria of chronic B-cell neoplasms. Patients were subjected to clinical sheet details (with special concern to organomegaly ,lymphadenopathy, fever and weight loss) , complete blood count with examination of peripheral blood smears stained with Leishman stain, bone marrow aspiration, ,and flow cytometric immunophenotyping using lymphoproliferative disorders panel (CD19, CD5, CD10 ,CD20, CD22, CD38, kappa and lambda light chains).

Two milliliters peripheral blood samples were collected in a sterile ethylene-diamine- tetraacetic acid (EDTA) containing vacutainer for CBC. Bone marrow (BM) aspiration was

withdrawn, The first few drops were spread on glass slides for morphological examination and 1 ml containing vacutainer for immunophenotyping are collected in anticoagulant (EDTA). Mononuclear cells are obtained from peripheral blood and bone marrow by Ficoll density centrifugation. The cells are washed twice in phosphate buffered saline (PBS) and re suspended in PBS containing 1% bovine serum Albumin (BSA) (Sigma), 0.05% sodium azide , and 2% human AB serum (pH: 7.6) (PBS-BSA- azide -AB buffer) except for the staining of SmIg . For Immunophenotyping by flow cytometry samples were processed within 24 hours. **Statistical Analysis:** The Statistical Analysis System- SAS (2010) was used to effect of different factors in study parameters. T-test was used for significant comparing between means in this study.

Result

Thirty newly diagnosed patients having chronic B-LPDs were studied by Flow cytometry on bone marrow aspirate samples of CLL and infiltrated cases of NHL using a panel of markers: CD20, CD5, CD22, CD19, CD10, CD38, Kappa and lambda light chains.

In current study , using a cut off $\geq 30\%$, the expression of the used markers as follow (CD19 76%, CD20 50% ,CD22 80%, CD5 56%, CD10 10%, CD38 26%, Kappa 86%, Lambda 13%)

there were 15 males and 15 females with a male to female ratio 1:1. Their ages ranged from 45 to 82 years old with mean of 64.3 ± 15.2 years. table1

Table 1 patients characteristics

Number of patients	30
Age/years	Range(45-82) Mean (64 $\pm$ 15.2)
M/F ratio	1/1

The immunophenotyping result shows the diagnosis of : chronic lymphocytic leukemia 18patients with CLL, 6 Mantle cell lymphoma, 3 follicular

lymphoma, 2 splenic lymphoma with villous lymphocyte, one multiple myeloma. As shown in table(2)

Table 2 The distribution of results according to disease frequency .

Disease	Number/30 case	percent
CLL	18	60%
MCL	6	20%
FL	3	10%
SLVL	2	6.3%
MM	1	3.3%

CLL chronic lymphocytic leukemia MCL mantle cell lymphoma FL follicular lymphoma SLVL lymphoma with villous lymphocyte, MM multiple myeloma.

Table 3 the immunophenotyping markers distribution

marker	No, of positive cases	Flowcytometry(%positivity)	P value	Sig
CD19	23	62.06	< 0.01	S
CD20	19	49.66	< 0.01	S
CD22	23	50.6	< 0.01	S
CD5	20	22.76(CLPD) 37.94 (CLL)	< 0.01	S
C10	3	3.5 (CLPD) 35 (FL)	< 0.05	NS
CD38	7	18.4 (CLPD) 30.6 (MM)	< 0.01	S
Kappa	27	62.3	< 0.01	S
Lambda	3	6.86	< 0.01	S

Table 4 Correlation coefficient between CD38 and Kappa with parameters for patients with LDP

Parameters	CD38	Kappa
CD19	- 0.02	0.22
CD20	0.23	0.24
CD22	- 0.07	0.26 *
CD5	- 0.18	0.26 *
CD10	- 0.14	- 0.45 **
Lambda	0.04	- 0.65 **
* (P<0.05), ** (P<0.01).		

Discussion

The annual incidence is approximately 10/100,000 and is increasing in developed countries [1]. Modern hematopathology relies heavily on immunophenotyping both for distinguishing benign from malignant processes as well as for accurate subclassification. The lack of specificity of most of the marker also needs to be considered and results must be interpreted taking into consideration findings with the whole panel of markers investigated and not of a marker in isolation, namely CD5 is a T cell marker but is also positive in B-CLL cells and in a proportion of B cell non-Hodgkin's lymphoma ;CD38 is positive in activated T cells but also in lymphoplasmacytic cells and plasma cells. Except in B-CLL, CD5 and CD19 did not co express on the cells of patients with B-NHL in leukemic phase . The intensity of CD19 and CD22 in typical CLL is lower than other NHL and unlike some reports only few percent of typical CLL shows CD22 negative. In this study all cases of CLL shows CD5 positive, however many studies shows that CD5 expression in B-CLPD is not specific for CLL or MCL and has been reported to occur in 5 to 10% of LPL and 20% or more of MZL [9,10]. The presence of a monotypic B-cell population with dim smIg expression, dim CD20 Expression, uniform CD5 positivity has been described as pathognomonic of CLL 18 Of the 30 cases (60%) in which this FCM phenotype was identified With expression of CD5 in CLL(37.94) compared to all cases of B- CLPD (22.76) and cut off as 30% .(table3) CD10 was expressed in 3 cases (table2). The blood film and bone marrow aspirate diagnosed as CLL but FCM Shows pathognomonic panel of

follicular lymphoma and no case express CD5 +. The prototypic MCL FCM pattern characterized 6 cases (20%) (table2). The all MCL cases shows exhibited one or more of the following: dim smIg, partial CD5 expression, and all with cyclin D1 positive. The observed immunophenotypes in these 6 cases overlapped considerably with those seen in other lymphoma types ,including CLL, LPL, and SMZL, but no case of MCLs were CD10+ or CD5. CD38 expressed in 7cases of CLL and in MM it seems to be an independent prognostic factor in CLL (.p<0.01) in comparison with lambda and CD10, while p<0.05 in comparison with other markers.

A potential shortcoming of this study is that it did not include either FMC7 or CD79b antibodies, both of which have been touted as important in the BCLPD FCM evaluation. Several studies have shown that CLLs are frequently, but not uniformly, FMC7-. FMC7 recognizes an epitope on CD20 that some have argued is present only on cells with high levels of surface CD20 expression.[11]

Conclusion

Multiparametric flowcytometry is highly sensitive and specific in detection and diagnosis of B-cell neoplasia and its Sub classification. However, if the results of FCM are critically evaluated and information regarding both the staining intensity and the presence or absence of certain antigens is recorded, they can provide useful information to the treating physician regarding possible or probable lymphoma types and serve as a guide to determine which cases require further tissue procurement or genetic studies.

References

- 1-FionaE ,Craig and Kenneth A, Foon. Flow cytometric immunophenotyping for hematologic neoplasms. 2008; Blood 15APRIL, Volume 111 number 8 ,3941-3967.
- 2-General Haematology task force of BCSH. Immunophenotyping in the diagnosis of chronic lymphoproliferative disorders. J clinical pathology . 1994;47, 871-875.
- 3-Michael Brown and Carl Wittwer . Flow Cytometry: Principles and Clinical Applications in Hematology. Clinical chemistry (2000); 46 ,8(B),1221-1229.
- 4- Davis BH, Foucark, SzczarkowskiW ,Balle, Witzig T, Foon KA et al, US-Canadian consensus recommendation on the immunophenotypic analysis of haematologic neoplasm by flow cytometry medical indication cytometry 1997; 30(5)249-63.
- 5- Herzenberg LA. B-1 cells: the lineage question revisited. *Immunol Rev*. 2000; 175:9-22.
- 6-Roustron AC,Shingles J,De tuteR, BennettF, JackAS,HillmenP. Flow cytometric immunophenotyping (FCI) of lymphoma Cytometry B clin cytom 2010;78 suppl, 5426.
- 7-Van Bocks, Taele F,Verhasselt B, Philippe J. Prognostic markerin chronic lymphocytic leukemia, Comprehensive review Blood review . 2009; 23(1) ,25-47.
- 8-Maria luz sanchesn et al Heterogeneity of neoplastic cells in B-cell chronic lymphoproliferative disorders: biclonality versus intra clonal evolution of a single tumor cell clone. *Haematologica*,2006 ;91 , 331-339.
- 9-Moric/.e WG, Kurtin PJ, Hodnefield JM, Shanafelt TD, Hoyer JD, Remstein ED, Hanson CA. Predictive value of blood and bone marrow flow cytometry in B-cell lymphomaclassification: comparative analysis of flow cytometry and tissue biopsy in 252 patients. *Mayo Clin Proc*. 2008; 83: 776–785.
- 10-Hunter ZR, Branagan AR, Manning R, Patterson CJ, Santos DD, Tournilhac O, Dorfman DM, Treon SP. CD5, CD10, and CD23 expression in Waldenstrom's macroglobulinemia. *Clin Lymphoma*. 2005 ; 5(4):246–249.
- 11- Delgado J, Matutes E , Morilla AM, et al. Diagnostic significance ofCD20 and FMC7 expression in B-cell disorders. *Am J Clin Pathol*. 2003; 120(5):754-759.