Flow Cytometric Evaluation of CD200 as a Tool for Differention between B-Cell Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma

Thesis

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بسم الله الرحمن الرحيم
وَأَنْزَلَ اللهُ عَلَيْكَ
الْكِتَابَ وَالْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ
تَكُنْ تَعْلَمُ وَكَانَ
فَضْلُ اللهِ عَلَيْكَ
عَظِيمًا
صدق الله العظيم
سورة النساء آية (١١٣)

Dedication

To the soul of my **Father**, may **Allah** rest him in peace.

To my **Mother**, to her I will never find adequate words to express my gratitude.

To my **Brother & Sister**, for their kind and loving help.

To my **Fiancé**, for his continuous support during this work.

I Love You All & Thank You

First thanks to **ALLAH** *to whom I relate any success in achieving any work in my life.*

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Manar Ibrahim

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INTRODUCTION

The B-cell chronic lymphoproliferative disorders (CLPD) are a heterogeneous group of B-cell malignancy of clonal origin with a highly variable clinical course. They are morphologically, immunologically and clinically heterogeneous. They refer to several conditions in which lymphocytes are produced in excessive quantities ***(Hadi et al., 2005)***.

Flow cytometric immunophenotyping (FCIPT) is the preferred method for characterization and classification of many CLPD, allowing rapid and simultaneous analysis of multiple cell parameters, including cell size, complexity, and both surface membrane and intracellular antigens, on large numbers of fresh viable cells ***(Cro et al., 2003)***.

The commonest CLPD is chronic lymphocytic leukemia (CLL), which characteristically expresses CD5 and CD19 on the cell surface. The differential diagnosis of a CD5/CD19 dual positive LPD lies mainly between CLL and mantle cell lymphoma (MCL). MCL has a significantly poorer prognosis than CLL, often requiring more aggressive treatment; thus it is of clinical importance to distinguish the two ***(Matutes et al., 1994; Cabezudo et al., 1999; Swerdlow et al., 2008)***.

Classical CLL is easily distinguished from MCL on immunophenotype alone, as it usually expresses CD23, has weak cell surface expression of immunoglobulin (sIg), and

lacks expression of FMC7 and CD22. These markers form the basis of the CLL score developed by ***Matutes et al. (1994)*** and subsequently revised (with replacement of CD22 by CD79b) by ***Moreau et al. (1997)***. In contrast, MCL most commonly has the phenotype CD5+, CD23-, FMC7+, CD79b+, and sIg strong ***(McCarron et al., 2000)***. In addition CD20 expression is strong in MCL and weak in CLL when compared to normal B lymphocytes ***(Ginaldi et al., 1998)***.

While the revised CLL score enables the identification of the majority of classical CLL, there remains a grey area of 'atypical CLL' that scores less than 4 on the revised CLL score. It has been suggested that absence of CD23 precludes the diagnosis of CLL and indicates MCL ***(McCarron et al., 2000; Deneys et al., 2001)***. These data, however, were largely extrapolated from immunohistochemistry on paraffin embedded tissue sections. With flow cytometry, CD23 positive MCL is a much more frequent finding: estimates of prevalence range from 15% to 55%. In addition to CD23 positive MCL, cases of CLL clearly negative for CD23 are also well described. Thus CD23 alone cannot be relied upon to separate these two conditions ***(Kelemen et al., 2008; Ho et al., 2009)***.

The chromosomal translocation t(11;14)(q13;q32) is the hallmark of MCL in which it can be detected cytogenetically in about 75% of cases. In some instances, histopathologic differentiation between MCL and other low-grade B-cell NHL is difficult. Therefore, detection of the translocation t(11;14) is

of essential diagnostic value for the risk-adjusted management of patients with MCL ***(Siebert et al., 1998)***.

CD200 (previously referred to as OX2) is a membrane glycoprotein, belonging to the immunoglobulin superfamily, that seems to play an immunosuppressive role and regulates myeloid cell activity in a variety of tissues. It is expressed on a subset of T and all CD19+ B lymphocytes but not on NK cells, monocytes, granulocytes or platelets, and highly on central and peripheral nerve tissue. Its expression has also been reported on human myeloma, plasma cells and B-CLL cells ***(Palumbo et al., 2008)***.

Aim of the Work

The aim of this study is to perform a comprehensive flow cytometric immunophenotypic analysis of CLL and MCL with the use of CD200 monoclonal antibody to provide data on the pattern of expression of this marker, trying to resolve the immunophenotypic overlap, hence, clarifying the possible role of CD200 in the precise diagnosis of these overlapping disorders.

Chapter (1)

B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS

The B-cell chronic lymphoproliferative disorders (CLPDs) represent a spectrum of diseases with a broad morphological, clinical, and biological diversity. CLPDs include neoplastic proliferations of peripheral B-cells. These disorders are characterized by being predominantly blood and bone marrow-based disorders having varying degrees of tissue involvement ***(Fred, 2001)***.

The B-cell CLPDs vary widely in their clinical course and presentation. These diseases range from more common, clinically indolent processes to the more aggressive disorders presenting with significant clinical problems. Survival times also vary widely, with the more aggressive disorders typically associated with shorter (1-3 years) survivals as opposed to longer (5-10 years or more) survivals in the clinically indolent processes. Thus, accurate classification is essential in the clinical management of patients with CLPDs ***(Heim and Mitelman, 1987)***.

The diagnosis and classification of B-cell CLPDs have been based on combination of routine morphological evaluation of blood and bone marrow smears and bone marrow biopsy sections. Flow cytometric immunophenotyping has had a very

important impact on the understanding of the various CLPDs ***(Hanson and Curtis, 1993).***

Flow cytometric IPT studies are indispensable for the diagnosis of mature B-cell lymphoid neoplasms through the identification of phenotypically abnormal cells belonging to the B-cell lineage and recognition of phenotypes characteristic of separate disease entities. They are recognized by an immunophenotype that is similar to normal mature lymphoid cells (e.g. surface immunoglobulin [SmIg] on mature B-cells) and lack of antigenic features of immaturity, such as expression of terminal deoxynucleotidyl transferase (TdT), CD34, or weak intensity of CD45 ***(Jaffe et al., 2001).***

In addition, FCM is a useful tool for staging a previously diagnosed hematolymphoid neoplasm, monitoring response to treatment including detection of minimal residual disease (MRD), documenting relapse or progression, and diagnosing intercurrent hematologic malignancy, such as a therapy-related myelodysplastic syndrome (MDS) ***(Stetler-Stevenson et al., 2007).***

B-cell CLPDs include chronic lymphocytic leukaemia, B-cell prolymphocytic leukaemia, non-Hodgkin's lymphoma (including mantle cell lymphoma & follicular cell lymphoma in leukaemic phase), hairy cell leukaemia and splenic lymphoma with villous lymphocytes. Occasionally, a lymphocytic proliferation is encountered that does not satisfy the morphological or immunophenotypical criteria for any of the above categories. These processes are best left unclassified *(Table 1)* ***(Montserrat and Emilio, 1997).***

Table (1): The World Health Organization (WHO) classification of B-cell lymphoid neoplasms:

Precursor Lymphoid Neoplasms

- B lymphoblastic leukemia / lymphoma, not otherwise specified (NOS).
- B lymphoblastic leukemia / lymphoma with recurrent genetic abnormalities.
- B lymphoblastic leukemia/lymphoma with t(9;22) (q34;q11.2); BCR ABL1 (The Philadelphia chromosome).
- B lymphoblastic leukemia / lymphoma with t (v;11q23); MLL rearranged.
- B lymphoblastic leukemia/lymphoma with t (12; 21) (p13; q22); TEL- AML1 (ETV6-RUNX1).
- B lymphoblastic leukemia / lymphoma with hyperploidy.
- Lymphoblastic leukemia / lymphoma with hyperploidy (hyperploidy ALL).
- B lymphoblastic leukemia / lymphoma with t(5;14) (q31 ;q32); IL3-IGH.
- B lymphoblastic leukemia / lymphoma with t(1;19)(q23; p13.3);E2a-PBX1(TCF3-PBX1).
- T lymphoblastic leukemia / lymphoma.

Mature B-Cell Neoplasms

- Chronic lymphocytic leukemia / small lymphocytic lymphoma.
- B-cell prolymphocytic leukemia.
- Splenic marginal zone lymphoma.
- Hairy cell leukemia.
- Splenic lymphoma / leukemia, unclassifiable.
- Lymphoplasmacytic lymphoma.
- Heavy chain disease.
- Plasma cell myeloma.
- Extra nodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT).
- Nodal marginal zone lymphoma.
- Follicular lymphoma.
- Primary cutaneous follicular lymphoma.
- Mantle cell lymphoma.
- Diffuse large B-cell lymphoma, NOS.
- T-cell / histiocyte-rich large B-cell lymphoma.
- Primary Diffuse Large B-cell Lymphoma (DLBCL) of the central nervous system (CNS).
- Primary cutaneous DLBCL, leg type.
- Diffuse large B-cell lymphoma associated with chronic inflammation.
- Lymphomatoid granulomatosis.
- Primary mediastinal (thymic) large B-cell lymphoma.
- Intravascular large B-cell lymphoma.
- ALK positive large B-cell lymphoma.
- Plasmablastic lymphoma.
- Large B-cell lymphoma arrising in HHV8 (human herpes virus 8) associated multicentric Castleman disease.
- Primary effusion lymphoma.
- Burkitt lymphoma.
- B cell lymphoma, unclassifiable, with features intermediate between DLBCL and - Burkitt-like.
- B cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma-like.

(Jaffe et al., 2008)

Table (2): Phenotypic, cytogenetic and molecular characteristics of lymphomas:

Cell type	Phenotype	Typical Cytogenetic Abnormalities	Molecular Abnormality
Follicular	$SmIg^+$, cIg^-, $CD5^-$, $CD10^+$, $CD23^{-/+}$, $CD43^{-/+}$	t(14;18)	Bcl-2 rearrangement
Burkitt	$SmIg^+$, $CD20^+$, $CD10^{+/-}$, $CD5^-$, $CD23^-$, $CD43^-$	t(8;14) (q24;q32); t(8;22) (q24;q11); t(2;8) (p12;q24);	myc rearrange-ment or overexpression
B-cell CLL/SLL	$SmIg^+$, $CD5^+$, $CD10^-$, $CD23^+$, $CD43^+$	Trisomy 12 (30%)	
Mantle cell	$SmIg^+$, cIg^-, $CD5^+$, $CD10^-$, $CD23^-$, $CD43^+$	t(11;14)	Bcl-1 (PRAD-1 or cyclin D1) rearrangement
Nodal marginal zone	$SmIg^+$, $CD5^-$, $CD10^-$, $CD23^-$	Trisomy 3, trisomy 18, rearrangement of 1q21 or 1p34	
Splenic marginal zone	$SmIg^+$, $CD5^-$, $CD10^-$, $CD23^{-/+}$, $CD43^{-/+}$	Chromosome 3 abnormalities	
Diffuse large cell (primary extranodal)	B-cell	3q27 rearrangements with 14q32, 22q11, 2p12	Bcl-6 rearrangement
Ki-1 (CD30) anaplastic large cell	$CD30^+$, $CD15^-$, $CD3^+$	t(2;5) (p23;q35)	ALK fusion gene
MALT	$SmIg^+$, $CD5^-$, $CD10^-$, $CD23^{-/+}$, $CD43^{-/+}$	Trisomy 3 and 18, t(11;18)	

+: indicates usually positive; -: usually negative; +/-: may be positive or negative

(Akpek et al., 2000)