Flow Cytometric Analysis of surface Light Chain Expression Patterns using Monoclonal and Polyclonal Antibodies in Chronic Lymphocytic Leukemia

Thesis

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By

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List of Abbreviations

Abs	: Antibodies
AIHA	: Auto-immune Hemolytic Anemia
AML	: Acute Myeloid leukemia
BCR	: B-Cell Receptor
BM	: Bone Marrow
B-NHL	: B-Non Hodgin Lymphoma
CBC	: Complete Blood Count
CD	: Cluster of differentiation
CLL	: Chronic Lymphocytic Leukemia
DDT	: Dichlorodiphenyltrichloroethane
EBV	: Epstein Barr virus
FAB	: French American British
FCM	: Flowcytometry
FITC	: Flourescien Isothiocyanate
FLCR	: Free Light Chain Ratio
GC	: Germinal Center
HB	: Haemoglobin
HCV	: Hepatitis C virus
iFLC	: Involved Serum Free Light Chain
Ig	: Immunoglobulin
IGH	: Immunoglobulin Heavy Chain
IH	: Immunohistology
IHC	: Immunohistochemistry
IL	: Interleukin
IPT	: Immunophenotyping
IQR	: Interquartile range
ITP	: Immune Thrombocytopenic Purpra

List of Abbreviations *(Cont...)*

κ	:	Kappa
K_2 EDTA	:	Di Potassium-ethylene diamine tetra acetic acid
λ	:	Lambda
LC	:	Light Chain
LNs	:	Lymph Nodes
mAbs	:	Monoclonal Antibodies
MDS	:	Myelodisplastic Syndrome
MRD	:	Minimal Residual Disease
MZ	:	Mantle Zone
pAbs	:	Polyclonal Antibodies
PB	:	Peripheral Blood
PBS	:	Phospate Buffer Saline
PCR	:	Polymerase Chain Reaction
PE	:	Phycoerythrin
PLL	:	Prolymphocytic Leukemia
sFLC	:	Serum Free Light Chain
SLL	:	Small Lymphocytic Lymphoma
SmIg	:	Surface Membrane Immunoglobulin
TCR	:	T-cell Receptor
TdT	:	Terminal Deoxynucleotide Transferase
TNF	:	Tumor Necrosis Factor
WBCs	:	White Blood Cells

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Introduction

Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent, long-lived, mature-appearing, small lymphocytes ***(Provan et al., 2009)***. It is the most common form of leukemia found in adults in Western countries (25-30%) ***(Hoffbrand and Moss, 2011).*** The cells of origin in most patients with CLL are clonal B-cells arrested in the B-cell differentiation pathway, intermediate between pre-B cells and mature B-cells ***(Zenz et al., 2009).***

Immunoglobulin (Ig) light chain (LC) expression analysis has a critical role in the flow cytometric evaluation of mature B-cell neoplasm, including CLL. The presence of a distinct B-cell population expressing only one type of immunoglobulin LC (**κ** or **λ**) essentially establishes a clonal B-cell process, and supports the diagnosis ***(Tomita et al., 2009).***

However, flow cytometry (FCM) does not always detect surface LC expression on lymphoma cells. Few studies have suggested that the choice of anti-LC antibody is one of the main factors determining whether expression of surface LC can be demonstrated ***(Fukushima et al., 1996*)**.

Currently, monoclonal antibodies (mAbs) and polyclonal antibodies (pAbs) are commercially available for the clinical

assessment of LC expression by FCM, but neither has been consistently shown to be superior to the other. The concurrent use of anti-LC mAbs and pAbs in the routine flow cytometric evaluation of B-NHLs increases the sensitivity for demonstrating LC restriction, with neither reagent set showing a clear sensitivity advantage in general, although CLL seems to preferentially show LC expression with pAbs, but the significance of this finding has not been systematically studied ***(Horna et al., 2011)*.**

Aim of the Work

The present study aims at:

- Flow cytometric evaluation of LC expression in CLL patients, using monoclonal and polyclonal antibodies.
- Evaluating and comparing the diagnostic utility, sensitivity and specificity of both monoclonal and polyclonal antibodies.
- Considering the use of one set for screening, with the addition of the other set if LC expression is not identified initially, instead of concurrent use of both surface antibody sets.

Chapter (I)
Chronic Lymphocytic Leukemia (CLL)

I. Introduction

The CLL is the most common adult form of leukemia ***(Xu et al., 2008)***. It is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes ***(Puente and López-Otí, 2013)***. The cells of origin in most patients with CLL are clonal B-cells arrested in the B-cell differentiation pathway, intermediate between pre- B cells and mature B-cells ***(Zenz et al., 2009)***.

The monoclonal B-cells accumulate in the peripheral blood (PB), bone marrow (BM) and secondary lymphoid organs as lymph nodes (LNs) and spleen with the morphology of small mature lymphocytes. Typically, CLL cells exhibit a characteristic immunophenotype, co-expressing CD19, CD5 and CD23, in the absence or low expression of surface CD22, CD79b and FMC7 ***(Gachard et al., 2008).***

The CLL follows an extremely variable clinical course with overall survival times ranging from months to decades. Some patients have no or minimal signs and symptoms during their entire disease course and have a survival time similar to age-matched controls. Other patients experience rapidly deteriorating blood counts and organomegaly, and

suffer from symptoms at diagnosis or soon thereafter necessitating therapy ***(Crowther-Swanepoel et al., 2013)***.

Epidemiology & risk factors

Prevalence and Incidence:

It is the most common leukemia in the western countries (25-30%) with an incidence of 4:100 000/year ***(Hoffbrand and Moss, 2011).*** Incidence rates increase with age reaching up to >30 per 100 000/year at age >80 years, and are higher among men than women ***(Eichhorst et al., 2010).*** Generally, it creates more than 30% of all types of leukemia, with a median age at time of diagnosis of 72 years ***(Panovská et al., 2010).***

Predisposing factors:

a- Environmental:

Environmental factors do not appear to play a role in the pathogenesis of CLL. The incidence of CLL was not associated with exposure to pesticides, sunlight, ionizing radiation or known carcinogens ***(Wierda et al., 2008; Byrd and Flynn, 2013).*** The risk of CLL is not increased in persons exposed to electromagnetic waves ***(Jacobs and Wood, 2002)***.

b- Occupational Factors:

A higher incidence of CLL is seen in some groups of workers in the **rubber industry**. The chemicals used in this industry that are linked to the development of CLL include

carbon tetra-chloride, carbon disulfide, acetone and ethylacetate. The duration and level of exposure to these chemicals appear to correlate with the risk of developing leukaemia ***(Rai and Gupta, 2003).*** **Specific agricultural exposures** linked with elevated risk of CLL include dichlorodiphenyltrichloroethane (DDT), animal breeding and working in flour mills ***(Zheng et al., 2002).***

c- Infections:

Antibodies (Abs) specific for type C hepatitis virus (HCV) and/or viral DNA have been identified in some patients, suggesting a pathogenic role. However, some studies have failed to verify an association between the development of CLL and infection with HCV. The CLL cells are resistant to infection with Epstein Barr virus (EBV), except in unusual cases, making it unlikely that EBV plays a pathogenic role ***(Hsieh et al., 2002; Lichtman et al., 2011).***

d- Hereditary and Genetic Factors:

The CLL exhibits one of the strongest familial tendencies of any malignancy ***(Goldin and Caporaso, 2007)*** First-degree relatives of patients are three times more likely than members of the general population to have CLL or another lymphoid neoplasm ***(Dighiero, 2008)*** No gene has been shown to confer an increased risk of CLL. Some, but not all, studies have indicated that the mean age at diagnosis of off-springs is approximately 10 to 20 years earlier than that of their parents