IMMUNOPHENOTYPING IN THE DIAGNOSIS OF CHRONIC LYMPHOPROLIFERATIVE DISORDERS

ESSAY

Submitted for Partial Fulfilment of

Master Degree in

Clinical and Chemical Pathology

BY

LAMIAA KAMAL EL-DIN EL-KHARBOTLI

Supervisors

Prof. Dr. NADIA MOHAMED ABDUL-MONEIM MOWAFY

Professor of Clinical Pathology

Faculty of Medicine, Ain Shams University

Dr. SALWA SAAD MOSTAFA KHODAIR

Lecturer of Clinical Pathology

Faculty of Medicine, Ain Shams University

Dr. HODA MOHAMED EL-GINDI

Lecturer of Clinical Pathology,

Faculty of Medicine, Ain Shams University

1995

ACKNOWLEDGEMENT

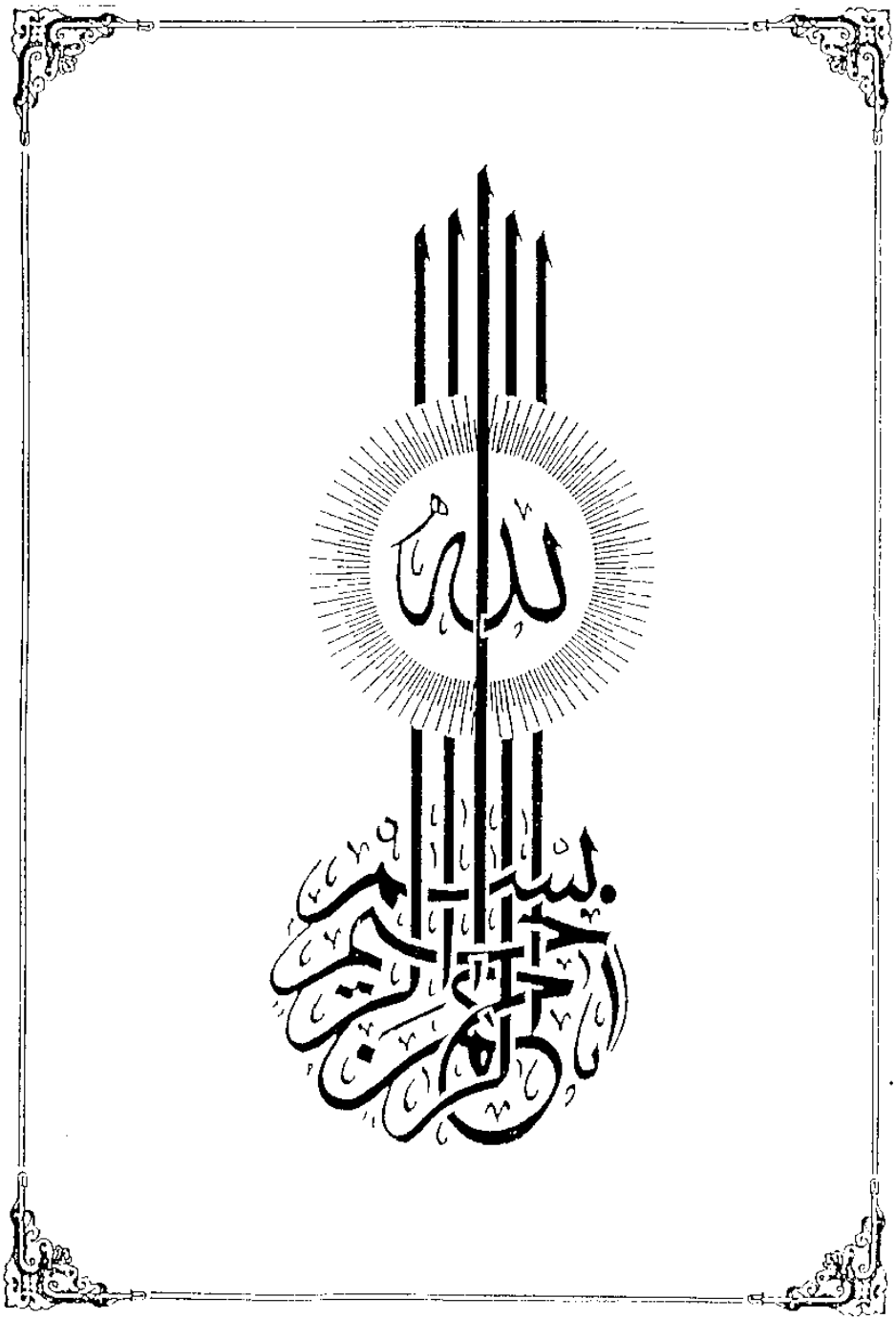
Thanks to God, creater of all, for helping me to complete this work.

A very special debt of gratitude is extended to Dr. NADIA MOHAMED MOWAFI, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for giving me the privilege of working under her super-vision, for her encouragement and unfailing guidance.

A great amount of appreciation must be extended to Dr. SALWA SAAD KHODAIR, Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University, who kindly supervised and motivated the performance of the study with interest and indispensable advice.

I would like to express my deepest gratitude and sincerest thanks to Dr. HODA MOHAMED EL-GENDI, Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her unforgettable help and tremendous efforts during this work.

Finally, thanks must be extended to my family and to every one who helped me in the preparation of this work even with a word of encouragement.



CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION AND AIM OF WORK

INTRODUCTION

The chronic lymphoproliferative disorders are a heterogeneous group of diseases that are clonal expansions of malignantly transformed cells whose differentiation has been interrupted and arrested early in lymphocyte ontogeny. The diagnostic approach in these disorders has changed dramatically in recent years. Technological advances uncovered new complexities and made in possible to firmly and clearly establish the lineage and stage of differentiation. Although morphology, cytochemistry and cytogenetics may play an important role in the diagnosis of lymphoproliferative disoders, immunophenotyping assumes a much more important role.

Aim of Work:

The aim of this essay is to present in short an account of the role of immunophenotyping in the diagnosis of chronic lymphoproliferative disorders.

BLOOD

LYMPHOCYTES

BLOOD LYMPHOCYTES

The immune system has evolved to protect us from the numerous potential pathogens which are present in the environment. The basis of immunity is the immune system's ability to recognize foreign molecules (antigens) and react to them, while at the same time tolerating the molecules of the body's own tissues ***(Male et al., 1987)***.

The lymphocytes which are the essential component of the immune system, constitute 20-45% of blood leucocytes. They are of two main types, namely B cells which develop in the bone marrow or fetal liver and differentiate into antibody producing plasma cells and T-cells which differentiate in the thymus and serve a number of functions. These include helping B cells to make antibody, killing virally infected cells, and stimulating the microbicidal and cytotoxic activity of other immune effector cells, including macrophages. Communication between the cells is affected either by cell/cell contact or by soluble factors ***(Male et al., 1987)***. The use of monoclonal antibodies to some of the cell surface antigen or markers allows the characterization of B and T-lymphocytes ***(Brooks et al., 1980)***.

Life span of circulating lymphocytes:

The circulating lymphocytes can be divided into two groups according to their life span. The first includes the long lived lymphocytes (life span from few months to five years). and these constitute about 65% - 85%: they are mainly T cells in the resting phase ***(Baserga, 1981)***. The second short lived category (Life span from few hours to five days) constitute the remaining 15 - 35 % of circulating lymphocytes and include the remaining T, B and non T, non B cells ***(Milstein, 1987)***.

Classification of surface markers:

Surface markers can be classified into two general groups: receptors and antigens ***(Koepke et al., 1984)***.

Receptors are molecules on cells which have particular affinity for a specific compound or group of compounds. The best example of receptors on cells are hormone receptors whose function in the mechanism of action of hormone is well known. In contrast, haemopoietic cell receptors don't have clearly defined functions.The best known lymphocyte receptor is probably the sheep erythrocyte receptor (E-rosette receptors) on T-cells ***(Kaplan and Clark; 1974)***.

Other receptors include Fc portion of immunoglobin molecule *(Christensson and Biberfield, 1978)*, Complement components *(Cossman and Jaffe, 1981)* and surface immunoglobulin of B lymphocytes (SIg) *(Gathings et al., 1977)*. Although it acts as a receptor, SIg is considered surface antigen because it is always detected with an anti immunoglobulin antibody *(Warner, 1974)*.

Surface antigens are defined as any molecule on the surface of cells to which antibodies can be made. These antibodies when labelled, serve as probes used to recognize the antigen and thus a particular types of cells. It should be recognized that the antigens presence may not have anything to do with the ability of cells to carry out a specific function.

In contrast to receptor, antigen is defined by its antibody reactivity and not by its function *(Koepke et al., 1984)*.

Surface membrane markers of blood lymphocytes:

Normal blood lymphocytes possess many membrane antigens or markers which allow the differentiation and characterization of B and T lymphocytes using monoclonal antibodies.

The international workshops on leukocyte differentiation antigens have grouped the available monoclonal antibodies into many clusters of differentiation (CD units) *(Milstein, 1987)*.

Table (1): Principle features of known cluster differentiation (CD) molecules.

(Hoffbrand and Pettit, 1993)

Cluster	Main cellular distribution	Comments/function/diagnostic value
CD1a	Thymocytes, dendritic cells	Ligand for some $\gamma\delta$ T cells
CD1b	Thymocytes, dendritic cells	Ligand for some $\gamma\delta$ T cells
CD1c	Thymocytes, dendritic cells	Ligand for some $\gamma\delta$ T cells
CD2	Pan T cell, NK cells	SRBC receptor, adhesion (LFA-2) binds LFA-3
CD3	Pan T cell	Signal transduction from the T-cell receptor
CD4	T helper subset	Adhesion (binds class II MHC)
CD5	Pan T cell, B-cell subset	B CLL expresses
CD6	Subset of T cells	
CD7	Subset of T cell	
CD8	T suppressor cell	Adhesion (binds class I MHC)
CD9	Pre-B cells, monocytes, platelets	
CD10	Precursor B and some mature B cells	Expressed in c-ALL,kidney,intestine neural tissue
CD11a	Leucocytes	Adhesion (combines with CD18 to form LFA-1 integrin)
CD11b	Granulocytes, monocytes, NK cells	Adhesion (combines with CD18 to form Mac-1 integrin)
CD11c	Granulocytes, monocytes, NK cells	Adhesion (combines with CD18 to form P.150.95 integrin)

Cluster	Main cellular distribution	Comments/function/diagnostic value
CD12	Monocytes, granulocytes	
CD13	Monocytes, granulocytes	
CD14	Monocytes	
CD15	Granulocytes	X hapten (carbohydrate epitope)
CD16	NK cells, granulocytes, macrophages	FcR III
CD17	Granulocytes, monocytes, platelets	
CD18	Leucocytes	Adhesion (ß-chain of LFA-1 integrin family)
CD19	B cells	
CD20	B cells	
CD21	Mature B cells	C3dR, receptor for EBV
CD22	B cells	
CD23	Activated B cells, macrophages, FDC	IgE - FcR
CD24	B cells, granulocytes	
CD25	Activated T cells, B cells macrophages	IL-2 receptor
CD26	Activated T cells, B cells macrophages	
CD27	T cells, plasma cells	
CD28	T cells	
CD29	Broad	Adhesion (VLA-integrin ß-chain) associates with CDw49
CD30	Activated T and B cells	Reed-Sternberg cells express; Ki detects
CD31	Monocytes,platelets,B cells, endothelium	GPIIa
CDW32	Monocytes, platelets	FcR II (receptor for aggregated Ig)

Cluster	Main cellular distribution	Comments/function/diagnostic value
CD33	Monocytes, myeloid progenitors	
CD34	Precursors of haemopoietic cells	Marrow progeniors
CD35	Granulocytes, monocytes, B cells	C3b receptor
CD36	Monocytes, platelets	Platelet GP IIIb
CD37	Pan-B, some T cell, FDC	
CD38	Thymocytes,activated T cells plasma cells	Plasma cell tumours
CD39	B cells	
CD40	B cells	
CD41	Platelets	Platelet GP IIb (forms complex with GP IIIa = CD61)
CD42a&b	Platelets	Form GP Ib (platelet adhesion to Von Willebrand factor
CD43	Leucocytes	
CD44	Leucocytes, erythrocytes	
CD45	Leucocytes	Leucocyte common antigen (LCA)
CD46	Leucocytes, epithelial cells fibroblasts	Regulates complement activation
CD47	Broad	
CD48	Leucocytes	Adhesion (associates with CD29 to form VLA-1)
CDw49a	T cells,monocytes, platelets	Adhesion (assocites with CD29 to form VLA-2)
CDw49b	Platelets, cultured T cells	
CDw49c	Leucocytes	Adhesion (assocites with CD29 to form VLA-3)
CDw49d	T cells, monocytes, B cells	Adhesion (assocites with CD29 to form VLA-4)