

DUAL COLOURED ANALYSIS OF CORD BLOOD LYMPHOCYTE SUBSETS IN HEALTHY FULL TERM & PRE-TERM INFANTS

M.D. Thesis

Submitted in Partial Fulfillment
of the M.D. Degree in *Clinical Pathology*

By

Ghada Hussein Bashat Mohamed

M.B.B.Ch. (1987), M.S.

Under the Supervision of

Prof. Tarif Hamza Sallam

Prof. of Clinical Pathology
Ain Shams University

Prof. Nevine Ahmed Kassim

Prof. of Clinical Pathology
Ain Shams University

Prof. Dina M. Maged Fakhr

Prof. of Gynaecology. & Obstetrics
Al Azhar University

Dr. Tahany Aly H.El kerdany

Lecturer of Clinical Pathology
Ain Shams University

Dr. Hoda Mohamed El Gindy

Lecturer of Clinical Pathology
Ain Shams University

Faculty of Medicine

Ain Shams University

1996



To My Family

ACKNOWLEDGEMENT

I wish to admit that I am greatly indebted to **Professor Dr. Tarif Hamza Salam** for his encouragement, constant guidance and honest supervision. I am very much obliged for his patience and his precious advises.

I am also sincerely thankful to **Professor Dr. Nevine Ahmed Kassim** for her continuous interest, skilled advice during the practical work and the valuable time she has given me throughout the course of this study. She has been mostly generous and kindly helpful.

I would like to express my deepest gratitude to **Prof. Dr. Diao M. Maged Fakher** for helping me in collecting the samples.

I would like also to thank **Dr. Tahany Aly H. El kerdany** who had spared no effort in guiding me throughout this work. I will always remember her unforgettable sincere help and encouragement.

I would like also to thank **Dr. Hoda Mohamed El Gindy** for her great help.

I would like also to thank **Dr. Gamal Sami Ali**, lecturer of medical childhood studies, Institute of Postgraduate Studies, Ain Shams University Hospital, for helping me in collecting premature infants cord blood samples and providing valuable references in neonatology.

Ghada Hussein Bashat

LIST OF ABBREVIATIONS

- α/β : Alpha / Beta.
- ACD**: Ammonium Citrate Dextrose.
- AIDs**: Acquired Immunodeficiency Syndrome.
- ALLs**: Acute Lymphoblastic Leukaemias.
- AMLs**: Acute Myloid Leukaemias.
- APC**: Antigen Presenting Cells.
- APC**: Allophycocyanin.
- BFU-E**: Burst Forming Unit-Erythroid.
- CALLA**: Common Acute Lymphoblastic Leukaemia Antigen.
- CD**: Cluster Designation.
- CFU-GM**: Colony Forming Unit-Granulocyte Monocyte.
- CGL**: Chronic Granulocytic Leukaemia.
- CLL**: Chronic Lymphocytic Leukaemia.
- CMV**: Cytomegalovirus.
- Con A**: Concanavalin A.
- CR1 & CR2**: Complement Receptors.
- CY**: Cychrome.
- DNA**: Deoxyribonucleic Acid.
- ϵ : Epsilon.
- E-**: Sheep Erythrocyte Rosette Negative.
- EBV**: Epstein Barr Virus.

EDTA: Ethylene Diaminetetraacetate

FAB (M4/M5): French American British (Acute Myelomonocytic Leukaemia / Acute Monocytic Leukaemia).

FCM: Flow Cytometry.

FeR: Fragment Crystalizable Receptor.

FITC: Fluorescein Isothiocyanate.

FS: Forward Scatter.

γ/δ : Gamma / Delta.

HCL: Hairy Cell Leukaemia.

HIV: Human Immunodeficiency Virus.

HLA-DR: Human Leukocytic Antigen.

HTLV-I: Human T Lymphocytic Virus type I.

Ia: Immunoassociated Antigen.

Ig: Immunoglobulin.

IL: Interleukin.

κ -**gene:** Kappa-gene.

λ -**gene:** Lambda-gene.

L-chain: Light-chain.

LCA: Leukocyte Common Antigen.

Leu-8: Inducer suppressor marker.

LFA-3: Lymphocyte function antigen-3

LFS: Log Forward Scatter.

LGL: Large Granular Lymphocyte.

LSM: Lymphocyte Separation Media.

LSS: Log Side Scatter.

MABs or mAbs: Monoclonal Antibodies.

MHC class I & II: Major Histocompatibility Complex Class I & II.

mIg: Membrane Immunoglobulin.

Nacl: Sodium Chloride.

NaH₂PO₄: Monobasic sodium phosphate.

Na₂HPO₄ H₂O: Dibasic sodium phosphate.

NHLs: Non Hodgkin's Lymphomas.

NK: Natural Killer.

PB: Peripheral Blood.

PBS: Phosphate Buffer Saline.

PC1 & PCA-1: Plasma Cell Antigens-1.

PE: Phycoerythrin.

PMT: Photomultiplier Tube.

PTP: Post Thymic Precursors.

PWM: Pokeweed Mitogen.

RBCs: Red Blood Cells.

RD1: Rhodamine.

SLE: Systemic Lupus Erythematosus.

SS: Side Scatter.

T4/T8 ratio: Helper/Suppressor Ratio.

Tc: T Cytotoxic.

TCR: T Cell Receptor.

TDI: Terminal Deoxynucleotidyle Transferase.

Th: T Helper.

Ti: T Inducer.

Tis: T Inducer Suppressor.

Tnk: T like Natural Killer.

VLBWI: Very Low Birth Weight Infant.

WDL: Well Differentiated Lymphoma / Leukaemia.

TABLE OF CONTENTS

I. Introduction and aim of the work	<i>Page</i>
II. Review of literature:	i
1. Development of lymphocyte subsets:	
- B cell ontogeny	1
- T cell ontogeny	8
- Natural killer cells	13
2. Lymphoid antigens and monoclonal reagents:	15
- Leukocyte common markers	18
- T cell markers	21
- B cell markers	34
- Natural killer markers	40
3. Flow cytometry instrumentation and how it aids to immunophenotyping:	44
- Design and operation of a flow cytometry	45
- Multicolour immunofluorescence and multiparameter flow cytometry	63
- Advantages of dual colour analysis	68
- Clinical significance of dual colour T cell analysis	68
- Clinical significance of dual colour B cell analysis	72
- Clinical significance of dual colour natural killer analysis	72
4. Lymphocyte subset values in different age groups:	74
- Lymphocyte subpopulation in normal Egyptian children and neonates.	83
- Effect of labour on lymphocyte subsets in full-term neonates.	84
- Effect of anaesthesia and surgery on lymphocyte populations and function in infants and children.	85

- Effect of sex of the new born and number of pregnancies on the lymphocyte subpopulations in the mother and new born.	85
III. Materials and methods:	
1. Subjects.	88
2. Methods:	88
- Sample collection.	88
- Lymphocyte preparation and isolation of mononuclear cell.	89
- Direct immunofluorescence analysis by FCM:	92
i. Dilution of monoclonal antibodies.	94
ii. Surface marker staining procedure.	97
iii. sample processing on FCM.	97
iv. Data interpretation.	106
IV. Results.	107
V. Discussion.	135
vi. Summery and conclusion.	147
VII. Recommendations.	151
VIII. References.	153
IX. Arabic Summary	

*Introduction
&
Aim of the Work*

