

**Human Myeloid Inhibitory C-Lectin  
(hMICL):  
A Novel Acute Myeloid Leukemia Marker**

Thesis  
Submitted for Partial Fulfillment of Master Degree in  
Clinical and Chemical Pathology

By  
**Amira Mohamed Mohamed Mohy El-Din**  
MB BCh  
Misr University For Science And Technology

Supervised by  
**Professor / Tahany Ali El Kerdani**  
*Professor of Clinical and Chemical Pathology  
Faculty of Medicine, Ain Shams University*

**Doctor / Deena Samir Mohamed**  
*Assistant Professor of Clinical and Chemical Pathology  
Faculty of Medicine, Ain Shams University*

**Doctor / Eman Zaghloul Kandel**  
*Lecturer of Clinical and Chemical Pathology  
National Cancer Institute, Cairo University*

Faculty of Medicine  
Ain Shams University

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رسالة توطئه للحصول على درجة الماجستير  
في الباثولوجيا الإكلينيكية و الكيميائية

مقدمة من

**الطبيبة/ أميرة محمد محمد محي الدين**  
بكالوريوس الطب والجراحة  
كلية الطب- جامعة مصر للعلوم و التكنولوجيا

تحت اشراف

**الأستاذ الدكتور/ تهاني على الكرداني**  
أستاذ الباثولوجيا الإكلينيكية و الكيميائية  
كلية الطب- جامعة عين شمس

**الدكتور/ دينا سمير محمد**  
أستاذ مساعد الباثولوجيا الإكلينيكية و الكيميائية  
كلية الطب- جامعة عين شمس

**الدكتور/ ايمان ز غول قنديل**  
مدرس الباثولوجيا الإكلينيكية و الكيميائية  
معهد الأورام القومي- جامعة القاهرة

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<b>Table of Contents</b>	
<b>Titles</b>	<b>Page</b>
List of Tables	ii
List of Figures	iv
List of Abbreviations	vi
Introduction	1
Aim of Work	3
Review of Literature	
<b>Chapter 1:</b>	
<b>Acute Myeloid Leukemia</b>	
A. Definition	4
B. Epidimiology	4
C. Etiology	5
D. Leukemogenesis	7
E. Classification	9
F. Diagnosis	13
G. Prognostic Factors	28
H. Treatment	29
I. Minimal Residual Disease	33
<b>Chapter 2:</b>	
<b>Human Myeloid Inhibitory C- Lectin</b>	
A. Nomenclature	34
B. Genetics	35
C. Structure	37
D. Expression	40
E. Expression In Other Diseases	41
F. Physiological Functions	42
G. Methods Of Assay	43
Subjects and Methods	49
Results	59
Discussion	75
Summary and Conclusion	83
Recommendations	86
References	90
Arabic Summary	

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## List of Tables

<b>Table</b>	<b>Title</b>	<b>Page</b>
1	Conditions predisposing the development of acute myelogenous leukemia	6
2	Most frequent genetic abnormalities in AML and related oncogenes	8
3	Morphologic (FAB) classification of AML	10
4	The WHO classification of AML and related precursor neoplasms (2008)	12
5	Panel of MoAbs to differentiate AML and ALL	21
6	Immunologic phenotypes of AML	21
7	Acute leukemias of ambiguous lineage according to the WHO classification of tumors of hematopoietic and lymphoid tissues	23
8	Cytogenetic and molecular classification for risk grouping in acute myeloid leukemia	25
9	Genes whose mutations or changes in expression occur recurrently in cytogenetically normal AML and have clinical significance.	26
10	Prognostic factors in acute myeloid leukemia	28
11	The monoclonal antibodies used in diagnosis of Acute Leukemia	53
12	Demographic and clinicopathologic characteristics of studied AML and ALL patients and healthy control subjects	64
13	Comparative study of hMICL percentage expression among AML , ALL and control subjects	65
14	Comparative study of hMICL MFI among AML, ALL and control subjects	65
15	Relationship of hMICL percentage expression to demographic and clinicopathologic characteristics of AML patients	66

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16	Relationship of hMICL MFI expression to demo-graphic and clinicopathologic characteristics of AML patients	67
17	Raw data of studied AML patient	73
18	Raw data of studied ALL patients	74
19	Raw data of studied normal control subjects	74

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<b>List of Figures</b>		
<b>Fig</b>	<b>Title</b>	<b>Page</b>
1	Pyoderma gangrenosum	15
2	Leukemia cutis manifesting as subcutaneous nodules	15
3	BM Aspirate smears of different FAB subtypes of AML	19
4	The peroxidase reaction	20
5	M5 AML (AMoL), non-specific esterase (NSE) stain, bone marrow aspirate smear, 1000x	20
6	C-type lectin receptors encoded by the natural killer gene complex (NKC)	36
7	Schematic view of part of human chromosome 12p13. 1 including MICL ( <i>black arrow</i> ) and several closely related genes	37
8	hMICL structure	37
9	Genomic structure of MICL aligned with the encoded polypeptide and three detected isoforms	39
10	hMICL isoforms structure	39
11	Flowcytometry	46
12	Percentage expression and MFI of hMCIL in studied AML, ALL and control subjects	68
13	Percentage expression and MFI in CD34 <sup>-</sup> versus CD34 <sup>+</sup> blasts in studied AML patients	68
14	Percentage expression and MFI of hMICL in relation to studied AML FAB subtypes	69
15	Percentage expression and MFI of hMICL in relation to studied AML cytogenetic subtypes	69
16	Percentage expression and MFI of hMICL in relation to FLT-3 gene status	70
17	ROC curve for diagnostic cut off of hMICL % among studied AML patients. The diagnostic cut off was set at 9.5%.	70

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18	ROC curve for diagnostic cut off of hMICL MIF among studied AML patients. The diagnostic cut off was set at 2.3	70
19	hMICL expression in AML cases	71
20	hMICL expression in a normal bone marrow sample	72
21	Example hMICL expression in AML and ALL cases	72



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## List of Abbreviations

ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
APL	Acute Promyelocytic Leukemia
ATRA	All-Trans-Retinoic Acid
BAALC	Brain And Acute Leukemia Cytoplasmic Gene
BM	Bone Marrow
BSA	Bovine Serum Albumin
CAE	Chloroacetate Esterase
CBC	Complete Blood Count
CCR7	Chemokine (C-C Motif) Receptor 7
CD	Cluster of Differentiation
CEBPA	CCAAT/Enhancer Binding Protein
CLEC	C-Type Lectin-Like Receptor
CLEC12A	C-Type Lectin Domain Family 12 MemberA
CLL-1	C-Type Lectin-Like Molecule-1
CLR	C-Type Lectin-Like Receptor
CML	Chronic Myeloid Leukemia
CNS	Central Nervous System
CR	Complete Remission
CTLDS	C-Type Lectin-Like Domains
Cy5	Cyanin 5
DC	Dendritic Cells
DCAL-2	Dendritic Cell-Associated Lectin 2
DIC	Disseminated Intravascular Coagulopathy
EDTA	Ethylene Diamine Tetra-Acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
EM	Electron Microscopy
ERG	Erythroblastosis Virus E26 Oncogene-Like (Avian)
FAB	French-American-British
FAV	Favorable
FCM	Flowcytometry
Fig	Figure

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FISH	Fluorescence In Situ Hybridization
FITC	Fluorescein Isothiocyanate
FLT-3	Fms-Related Tyrosine Kinase3
Hb	Hemoglobin
HLA	Human leukocyte antigen
hM1CL	Human Myeloid Inhibitory C-Lectin
HS	Highly Significant
IP	Immunophenotyping
ITIM	Immunotyrosine-Based Inhibition Motif
KLRL1	Killer Lectin Like Receptor 1
LAIPs	Leukemia-Associated Immunophenotypes
LM	Light Microscopy
LOX-1	Low Density Lipoprotein-1
MFI	Mean Fluorescence Intensity
MLL	Myeloid Lymphoid Or Mixed Lineage Leukemia
MoAb	Monoclonal Antibody
MPAL	Mixed Phenotype Acute Leukemias
MPO	Myeloperoxidase
MRD	Minimal Residual Disease
NA	Sodium
NEC	Nonerythroid Cells
NKC	Natural Killer Complex
NKCL	NK-Like C-Type Lectin-Like Receptors
NPM-1	Nucleophosmin-1
NS	Non Significant
NSE	Nonspecific Esterase
p	Probability Of Chance
PAS	Periodic Acid Schiff
PB	Peripheral Blood
PBS	Phosphate Buffered Saline
PE	Phycoerythrin
PLT	Platelets
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
ROC	Receiver Operating Characteristic

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RT-PCR	Reverse Transcription Polymerase Chain Reaction
S	Significant
SBB	Sudan Black B
SCT	Stem Cell Transplantation
SHIP	Src Homology-2 Domain-Containing Inositol Phosphatase
SHP	Src Homology-2 Domain-Containing Tyrosine Phosphatase
SPSS	Statistical Package For Social Science
TdT	Terminal Deoxynucleotidyl Transferase
TLC	Total Leukocytic Count
TSG	Tumor Suppressor Genes
WHO	World Health Organization

## **Introduction**

Acute myeloid leukemia (AML) is the most common acute leukemia affecting adults and its incidence increases with age. The prognosis of AML is poor, primarily because of the relapses occurring on conventional chemotherapy regimens. The overall 5-year leukemia-free survival rate is only 25-35% and even lower in patients over 60 years old (**Zhao et al., 2010**). Therefore, alternative strategies are needed to complement the currently used chemotherapy treatment protocols (**Bakker et al., 2004**).

In the absence of leukemia specific markers, the distinction between leukemic and normal immature cells relies on the expression of antigen combinations defining leukemia-associated immunophenotypes (LAIPs), which are absent or extremely infrequent in normal bone marrow (**Al-Mawali et al., 2008**). However, evidence in the literature has outlined that these LAIPs are very different from patient to patient and they are not necessarily stable over the course of the disease (**Voskova et al., 2004**). Consequently, there is still a need for the identification of new antigens contributing to diagnostic and prognostic information, improving relapse detection, identification and ideally eradication of leukemic stem cells through antibody mediated therapy (**Larsen et al., 2012**).

The Human Myeloid Inhibitory C-Lectin (hMICL) also known as Human C-type lectin-like molecule-1 (CLL-1), or C-type lectin domain family 12 member A (CLEC12A), is a type II transmembrane glycoprotein and member of the large family of C-type lectin-like receptors involved in immune regulation (**Zhao et al., 2010**). The hMICL is a pan-myeloid antigen that is absent on normal uncommitted primitive CD34<sup>+</sup> CD38<sup>-</sup> or CD34<sup>+</sup> CD33<sup>-</sup> stem cells; which aids the discrimination between normal and leukemic stem cells, as well as introduces hMICL as a promising therapeutic target for eradication of antigen-bearing leukemic cells and the subsequent re-establishment of normal hematopoiesis through the remaining normal stem cells (**Bakker et al., 2004**).

### **Aim of Work**

The aim of this work is to determine the diagnostic impact and the applicability of the Human Myeloid Inhibitory C-Lectin (hMICL) in routine clinical flowcytometry for the diagnosis of Acute Myeloid Leukemia (AML).

## Chapter 1

### ACUTE MYELOID LEUKEMIA

#### A) DEFINITION:

Acute myeloid leukemia (AML, also known as acute myelogenous leukemia and, less commonly, as acute non-lymphocytic leukemia) consists of a group of relatively well-defined hematopoietic neoplasms involving precursor cells committed to the myeloid line of cellular development (i.e., those giving rise to granulocytic, monocytic, erythroid, or megakaryocytic elements) (Schiffer et al., 2010).

#### B) EPIDIMIOLOGY:

AML is a highly malignant neoplasm responsible for a large number of cancer-related deaths (Deschler and Lübbert, 2006).

1) Incidence: AML counts for 15 to 20 percent of the acute leukemias in children and 80 percent of acute leukemias in adults (Lichtman and Liesveld, 2006).

2) Age: The AML is the predominant form of leukemia during the neonatal period, but it represents < 15% of cases of leukemia in children under 10 years and 25 to 35% between ages 10 and 15 years. While in adults it represents