



Implication of Flow Cytometry-Based Maturity Score in Risk Stratification of Acute Myeloid Leukemia In Adult Egyptians

Thesis

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Presented By

Esraa Adel Mahmoud El Debaky

M.B., B.Ch

Supervised By

Prof. Dr. Hanaa Mohammed El Sayed Afifi

*Professor of Clinical Pathology
Faculty of Medicine, Ain Shams University*

Dr. Shaimaa Abdelmalik Pessar

*Assistant Professor of Clinical Pathology
Faculty of Medicine, Ain Shams University*

Dr. Alia Mohammed Saeed Ahmed

*Lecturer of Clinical Hematology & Hemato-oncology Unit
of Internal Medicine Department
Faculty of Medicine, Ain Shams University*

Faculty of Medicine - Ain Shams University

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

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To:

My parents

*for their endless love, support,
and continuous care*

*My Husband
&
My Family*



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List of Abbreviations

Abb.	Full term
<i>ALL</i>	<i>Acute lymphoblastic leukemia</i>
<i>AML</i>	<i>Acute Myeloid Leukemia</i>
<i>AML-MRC</i>	<i>AML with myelodysplasia-related changes</i>
<i>APL</i>	<i>Acute promyelocytic leukemia</i>
<i>ATRA</i>	<i>All-trans-retinoic acid</i>
<i>BM</i>	<i>Bone Marrow</i>
<i>CART</i>	<i>chimeric antigen receptor-T</i>
<i>CBC</i>	<i>Complete blood count</i>
<i>CBF</i>	<i>Core Binding Factor</i>
<i>CD</i>	<i>Cluster of Differentiation</i>
<i>CEPBA</i>	<i>CCAAT/enhancer-binding protein alpha</i>
<i>CI</i>	<i>Confidence interval</i>
<i>c-kit</i>	<i>Mast / stem cell growth factor receptor (SCFR)</i>
<i>CNS</i>	<i>Central nervous system</i>
<i>CR</i>	<i>Complete Remission</i>
<i>DIC</i>	<i>disseminated intravascular coagulopathy</i>
<i>DNA</i>	<i>Deoxyribonucleic acid</i>
<i>DNMT3A</i>	<i>DNA-Methyltransferase 3A</i>
<i>EFS</i>	<i>Event free survival</i>
<i>ELN</i>	<i>European Leukemia Net</i>
<i>FAB</i>	<i>French American British classification</i>
<i>FCM</i>	<i>Flow Cyto-metry</i>
<i>FISH</i>	<i>Fluorescence in-situ hybridization</i>
<i>FITC</i>	<i>Fluorescein isothiocyanate</i>
<i>FLT3</i>	<i>FMS-like tyrosine kinase 3</i>
<i>GvL effect</i>	<i>Graft-versus-leukemia effect</i>
<i>HLA-DR</i>	<i>Human leukocyte antigen-antigen D related</i>
<i>HSCT</i>	<i>Hematopoietic stem cell transplantation</i>

List of Abbreviations *cont...*

Abb.	Full term
<i>iCR</i>	<i>incomplete remission</i>
<i>IDH</i>	<i>Isocitrate dehydrogenase</i>
<i>INV</i>	<i>Inversion</i>
<i>IPT</i>	<i>Immuno-phenotyping</i>
<i>IQR</i>	<i>Interquartile range</i>
<i>ITD</i>	<i>internal tandem duplications</i>
<i>K-EDTA</i>	<i>K-Ethylene Diamine Tetra-Acetic Acid</i>
<i>LAIP</i>	<i>Leukemia associated immunophenotype</i>
<i>LAPs</i>	<i>Leukemia-associated aberrant</i>
<i>MDR</i>	<i>Multi-drug resistant</i>
<i>MDS</i>	<i>Myelodysplastic syndrome</i>
<i>MLL</i>	<i>Mixed lineage leukemia</i>
<i>MPAL</i>	<i>Mixed phenotype acute leukemia</i>
<i>MPNs</i>	<i>Myeloproliferative neoplasms</i>
<i>MPO</i>	<i>Myeloperoxidase</i>
<i>MRD</i>	<i>Minimal residual disease</i>
<i>NCCN</i>	<i>National Comprehensive Cancer Network</i>
<i>NOS</i>	<i>Not otherwise specified</i>
<i>NPM</i>	<i>Nucleophosmin</i>
<i>NSE</i>	<i>Non- specific esterase</i>
<i>OS</i>	<i>Overall survival</i>
<i>p</i>	<i>Short arm of chromosome</i>
<i>PB</i>	<i>Peripheral Blood</i>
<i>PBS</i>	<i>Phosphate buffered saline</i>
<i>PCR</i>	<i>Polymerase chain reaction</i>
<i>PE</i>	<i>Phycoerythrin</i>
<i>PR</i>	<i>partial remission</i>
<i>q</i>	<i>Long arm of chromosome</i>
<i>RBCs</i>	<i>Red blood cells</i>

List of Abbreviations *cont...*

Abb.	Full term
<i>RNA</i>	<i>Ribonucleic acid</i>
<i>SBB</i>	<i>Sudan black B</i>
<i>SCF</i>	<i>Stem cell factor</i>
<i>SCT</i>	<i>Stem cell transplantation</i>
<i>SD</i>	<i>Standard deviation</i>
<i>STAT</i>	<i>Signal transducer and activator of transcription</i>
<i>t</i>	<i>Translocation</i>
<i>TAM</i>	<i>Transient abnormal myelopoiesis</i>
<i>TdT</i>	<i>Terminal Deoxynucleotidyl transferase</i>
<i>TET2</i>	<i>ten eleven translocation 2</i>
<i>TK</i>	<i>Tyrosine kinase</i>
<i>TKD</i>	<i>tyrosine kinase domain</i>
<i>TKI</i>	<i>Tyrosine kinase inhibitor</i>
<i>TLC</i>	<i>Total leucocytic count</i>
<i>TP53</i>	<i>tumour protein p53</i>
<i>TRM</i>	<i>Treatment-related mortality</i>
<i>WBCs</i>	<i>White blood cells</i>
<i>WHO</i>	<i>World Health Organization</i>

INTRODUCTION

Acute myeloid leukemia (AML) is a malignant tumor of hemopoietic progenitor cells of non-lymphoid lineage, arising in the bone marrow (BM) (*Provan et al., 2015*). Cytogenetic and molecular genetic abnormalities are thought to drive clonal expansion of early hematopoietic progenitor cells, which leads to rapid progressive suppression of normal bone marrow hematopoiesis. Subsequently, patients suffering from AML develop symptoms attributed to granulocytopenia, anemia, and thrombocytopenia (*Estay et al., 2006*).

The diagnosis of acute leukemia is established by the presence of 20% or more blasts in the bone marrow or peripheral blood. AML is further diagnosed by demonstrating the myeloid origin of these cells through testing for myeloperoxidase activity or documenting the presence of Auer rods, immunophenotyping, presence of an extra-medullary tissue infiltrate, or a documented t(8;21), inv(16) or t(15;17) in the appropriate clinical setting, regardless of the blast percentage (*Vardiman et al., 2009*).

The leukemic lineage and evolution processes can be characterized by examining a variety of differentiation antigens, and cellular immune-phenotypic identification by Flow cytometry (FCM). The FCM has become an integral part of the laboratory diagnosis and classification of acute leukemia. Flow cytometric analysis of leukemia should include panels of

antibodies against differentiation antigens for hematopoietic lineage and differential stage assignment (*Liu et al., 2014*).

In 2009, the European Leukemia Net (ELN) proposed a standardized reporting system that risk stratifies patients according to their genetic subgroup. Nowadays, it is well established for early prognostic assessment in AML patients (*Mrózek et al., 2012*).

In order to establish immunophenotypic features that predict prognosis, many studies over the past two decades have been providing relevant information at the role of various cellular phenotypes assessed at initial diagnosis in predicting therapy response. The associations of these phenotypes generally have been strong and are clearly predictive when coupled with several factors such as age, sex, initial hemoglobin level, and total leucocytic and platelets counts (*Vaskova et al., 2005*).

The expression of single AML blast cell antigens has been evaluated with partly conflicting results; however, the influence of immunophenotypic blast maturity is largely unknown. **In 2015, Schneider et al.** proposed a flow cytometric maturity score based on the quantitative expression of three markers of immaturity; CD34, CD117, and TdT with a score from 0 to 5; a score of 5 indicates maximal immaturity and a score of 0 indicates maturity. They claimed that AML blast maturity can predict clinical outcome and correlated well with survival rates even within the different ELN cytogenetic risk groups.

AIM OF THE WORK

To determine the influence of immunophenotypic maturity, via application of the flow cytometric maturity score based on quantitative expression of the three markers of immaturity; CD34, CD117, TdT with a score from 0 to 5, on clinical outcome and laboratory parameters of patients with acute myeloid leukemia within the different cytogenetic risk groups.