

PROGNOSTIC VALUE OF VERY LATE ANTIGEN-4 (VLA-4)
EXPRESSION IN ACUTE MYELOID LEUKEMIA

Thesis

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

By

Dina Samir Mohamed Atteia

M.B., B.CH

Ain Shams University

Supervised by

Professor/ Hanaa Mohamed Afifi

Professor of Clinical and Chemical Pathology

Faculty of Medicine-Ain Shams University

Doctor/ Mona Ahmed Ismail

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine-Ain Shams University

Doctor/Mahira Ismail Wageeh

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine-Ain Shams University

Faculty of Medicine

Ain Shams University

الأهمية التنبؤية لوجود ال (VLA-4) فى

سرطان الدم الميلودى الحاد

رسالة

توطئة للحصول على درجة الماجستير
فى الباثولوجيا الإكلينيكية و الكيميائية

مقدمة من

طبيبة / دينا سمير محمد عطيه

بكالوريوس الطب و الجراحة العامة

كلية الطب – جامعة عين شمس

تحت إشراف

الأستاذ الدكتور / هناء محمد عفيفى

أستاذ الباثولوجيا الإكلينيكية و الكيميائية

كلية الطب – جامعة عين شمس

الدكتور / منى أحمد إسماعيل

أستاذ مساعد الباثولوجيا الإكلينيكية و الكيميائية

كلية الطب – جامعة عين شمس

الدكتور / مهيرة إسماعيل وجيه

أستاذ مساعد الباثولوجيا الإكلينيكية و الكيميائية

كلية الطب – جامعة عين شمس

كلية الطب - جامعة عين شمس

٢٠١١

SUMMARY

Acute myeloid leukemia (AML) is a clonal hematopoietic stem cell disorder, characterized by arrested differentiation, inappropriate proliferation and survival of immature myeloid progenitors.

The AML has the lowest survival rate of all leukemias. So, assessment of the prognostic factors in AML is very important. Recently, VLA-4 expression was described to have a prognostic impact in AML.

The present study planned to assess VLA-4 expression in patients with AML, and to evaluate its association with the different demographic, clinical and laboratory data, as well as its relation to disease outcome.

The current study was carried out on 50 newly diagnosed AML patients. All patients were subjected to complete history taking, thorough clinical examination and laboratory investigations including: complete blood picture, bone marrow aspiration with examination of Leishman-stained peripheral blood and bone marrow smears, immunophenotyping and detection of the level of VLA-4 expression by flowcytometry. The VLA-4 was expressed in all studied AML patients, but with a highly significant difference than control group.

List of Contents

Title	Page No.
Introduction.....	Error! Bookmark not defined.
Aim of the Work	Error! Bookmark not defined.
Review of Literature	
<i>I- Acute Myeloid Leukemia</i>	<i>Error! Bookmark not defined.</i>
<i>A) Definition.....</i>	<i>Error! Bookmark not defined.</i>
<i>B) Epidemiology and incidence..</i>	<i>Error! Bookmark not defined.</i>
<i>C) Etiology and risk factors</i>	<i>Error! Bookmark not defined.</i>
<i>D) Pathogenesis of AML</i>	<i>Error! Bookmark not defined.</i>
<i>E) Classification</i>	<i>Error! Bookmark not defined.</i>
<i>F) Diagnosis of AML.....</i>	<i>Error! Bookmark not defined.</i>
<i>G) Prognostic criteria of AML</i>	<i>Error! Bookmark not defined.</i>
<i>H) Treatment.....</i>	<i>Error! Bookmark not defined.</i>
<i>II- CD49d.....</i>	<i>Error! Bookmark not defined.</i>
<i>III- Flow Cytometry</i>	<i>Error! Bookmark not defined.</i>
<i>A- Definition.....</i>	<i>Error! Bookmark not defined.</i>
<i>B- Principle of Flow Cytometry...</i>	<i>Error! Bookmark not defined.</i>
<i>C- Components of Flow Cytometers</i>	<i>Error! Bookmark not defined.</i>
<i>D- Advantages of Flow Cytometry</i>	<i>Error! Bookmark not defined.</i>
Subjects and Methods	Error! Bookmark not defined.
Results.....	Error! Bookmark not defined.
Discussion	Error! Bookmark not defined.
Summary	3
Conclusion	Error! Bookmark not defined.
Recommendations	Error! Bookmark not defined.
References	Error! Bookmark not defined.
Arabic Summary	

List of Figures

Fig. No.	Title	Page No.
<u>Fig. (1):</u>	<u>Branching networks of genes involved in chromosome translocations.....</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (2):</u>	<u>Acute Myeloblastic Leukemia without Differentiation (M1 AML) BM aspirate smear</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (3):</u>	<u>Acute Promyelocytic Leukemia (M3-AML)</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (4):</u>	<u>M3 AML with Auer rods- BM aspirate smear....</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (5):</u>	<u>Acute Monoblastic Leukemia (M5a AML) - BM aspirate smear</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (6):</u>	<u>(M5b AML) BM aspirate smear</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (7):</u>	<u>The peroxidase reaction</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (8):</u>	<u>M5 AML (AMoL), non-specific esterase (NSE) stain</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (9):</u>	<u>Analysis of human peripheral blood cells by flow cytometry</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (10):</u>	<u>Correlation between CD49d expression and FAB subtypes.</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (11):</u>	<u>Association between CD49d expression and clinical outcome.</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (12):</u>	<u>Association between CD49d expression and response to chemotherapy.</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (13):</u>	<u>Flowcytometric analysis of AML case (M2).</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (14):</u>	<u>Flowcytometric analysis of AML case (M1)</u>	<u>Error! Bookmark not defined.</u>
<u>Fig. (15):</u>	<u>Flow cytometric analysis of a healthy control</u>	<u>Error! Bookmark not defined.</u>

List of Tables

Table No.	Title	Page No.
Table (1):	Genetic disorders implicated in the pathogenesis of AML Error! Bookmark not defined.	
Table (2):	Environmental factors contributing to AML... Error! Bookmark not defined.	
Table (3):	Morphologic (FAB) classification of AML Error! Bookmark not defined.	
Table (4):	MIC classification of AML: showing the association of morphology (FAB) with cytogenetics and immunophenotyping. Error! Bookmark not defined.	
Table (5):	The WHO classification of AML (2008) Error! Bookmark not defined.	
Table (6):	Panel of MoAbs to differentiate AML and ALL Error! Bookmark not defined.	
Table (7):	Immunologic phenotypes of AML Error! Bookmark not defined.	
Table (8):	Score for biphenotypic acute leukemia Error! Bookmark not defined.	
Table (9):	Clinical correlations of frequent cytogenetic abnormalities observed in AML..... Error! Bookmark not defined.	
Table (10):	Prognostic factors in acute myeloid leukemia... Error! Bookmark not defined.	
Table (11):	Cytogenetic risk group assignments of younger adults with AML Error! Bookmark not defined.	
Table (12):	The common clinical uses of flow cytometry. Error! Bookmark not defined.	
Table (13):	Demographic and clinical data of studied patients Error! Bookmark not defined.	
Table (14):	Cell surface markers of studied patients: Error! Bookmark not defined.	
Table (15):	Characteristics of studied AML patients: Error! Bookmark not defined.	

Table (16): Comparison between control group and AML patients group as regards CD49d expression:**Error! Bookmark not defined.**

Table (17): CD49d expression, outcome and response to therapy in studied AML patients:**Error! Bookmark not defined.**

List of Tables

Table No.	Title	Page No.
Table (18):	ROC study between outcome and CD49d% Died + Relapse Vs Remission	Error! Bookmark not defined.
Table (19):	Sperman Correlation test between CD49d and laboratory parameters.....	Error! Bookmark not defined.
Table (20):	Association between CD49d expression and standard prognostic factors.....	Error! Bookmark not defined.
Table (21):	Association between CD49d expression and FAB subtypes	Error! Bookmark not defined.
Table (22):	Association between CD49d expression and clinical outcome.....	Error! Bookmark not defined.
Table (23):	Association between CD49d expression and response to chemotherapy	Error! Bookmark not defined.

List of Abbreviations

ADC	<i>Analogue-to-digital conversion</i>
Ag	<i>Antigen</i>
Ab	<i>Antibody</i>
ALL	<i>Acute lymphoblastic leukemia</i>
AML	<i>Acute myeloid leukemia</i>
AMMOL	<i>Acute myelomonocytic leukemia</i>
AHD	<i>Antecedent hematologic disorder</i>
AMOL	<i>Acute monoblastic leukemia</i>
AP	<i>Acid phosphatase</i>
APL	<i>Acute promyelocytic leukemia</i>
APL-V	<i>Acute promyelocytic leukemia- variant</i>
ATRA	<i>All-trans-retinoic acid</i>
bFGF	<i>Basic fibroblast growth factor</i>
CAE	<i>Chloroacetate esterase</i>
CAM	<i>Cell adhesion molecule</i>
CBC	<i>Complete blood count</i>
CD	<i>Cluster of differentiation</i>
CGH	<i>Comparative genomic hybridization</i>
CNS	<i>Central nervous system</i>
CR	<i>Complete remission</i>
CRP	<i>C-reactive protein</i>
CML	<i>Chronic myeloid leukemia</i>
CXC	<i>Chemokines</i>
CXCR-4	<i>Chemokine receptor-4</i>
del	<i>Deletion</i>
DFS	<i>Disease free survival</i>
DIC	<i>Disseminated intravascular coagulation</i>
DNA	<i>Deoxyribonucleic acid</i>

DW	<i>Distilled water</i>
ECM	<i>Extracellular matrix</i>
EFF	<i>Efficacy</i>
EGIL	<i>European Group for the Immunological Characterization of Leukemias</i>
EM	<i>Electron microscopy</i>
EPO	<i>Erythropoietin</i>
FISH	<i>Fluorescence in-situ hybridization</i>
FITC	<i>Fluorescein isothiocyanate</i>
FLT-3	<i>Fms-like tyrosine kinase</i>
FN	<i>False negative</i>
FP	<i>False positive</i>
G-CSF	<i>Granulocyte colony-stimulating factor</i>
GM-CSF	<i>Granulocyte Monocyte Colony stimulating factor</i>
GVHD	<i>Graft versus host disease</i>
HLA	<i>Human leukocyte antigen</i>
HM	<i>Hepatomegaly</i>
HS	<i>Highly significant</i>
ICAMS	<i>Intra cellular adhesion molecules</i>
Ig	<i>Immunoglobulin</i>
IL	<i>Interleukin</i>
inv	<i>Inversion</i>
IP	<i>Immunophenotyping</i>
LDH	<i>Lactate dehydrogenase</i>
LFA-1	<i>Leukocyte function-associated antigen-1</i>
LM	<i>Light microscopy</i>
LN	<i>Lymphadenopathy</i>
MDR	<i>Multidrug resistance</i>
MDS	<i>Myelodysplastic syndrome</i>
MIC	<i>Morphologic-immunologic- cytogenetic</i>

MMP s	<i>Matrix metalloproteases</i>
PLT	<i>Platelets</i>
MoAbs	<i>Monoclonal antibodies</i>
MPO	<i>Myelo-peroxidase</i>
MRD	<i>Minimal residual disease</i>
MSC	<i>Mesenchymal stromal cells</i>
NS	<i>Non significant</i>
NSE	<i>Non specific estrases</i>
OS	<i>Overall survival</i>
PAS	<i>Periodic acid Schiff</i>
PB	<i>Peripheral blood</i>
PBS	<i>Phosphate buffer saline</i>
PBSC	<i>Peripheral blood stem cells</i>
PCNA	<i>Proliferating cell nuclear antigen</i>
PCR	<i>Polymerase chain reaction</i>
PE	<i>Phycoerythrin</i>
PNH	<i>Paroxysmal nocturnal hemoglobinuria</i>
PT	<i>Prothrombin time</i>
PTT	<i>Partial thromboplastin time</i>
RD	<i>Remission duration</i>
RNA	<i>Ribonucleic acid</i>
ROC	<i>Receiver operating characteristic</i>
rPM	<i>Round per minute</i>
RT-PCR	<i>Reverse transcriptase-polymerase chain reaction</i>
SBB	<i>Sudan black-B</i>
SCT	<i>Stem cell transplantation</i>
SD	<i>Standard deviation</i>
SDF-1	<i>Stromal cell derived factor-1</i>
SEM	<i>Standard error of the mean</i>
sHGF	<i>Soluble hepatocyte growth factor</i>

Sig	<i>Significance</i>
SKY	<i>Specral karyotyping</i>
SM	<i>Splenomegaly</i>
SN	<i>Significance</i>
SP	<i>Specifity</i>
T	<i>Translocation</i>
TdT	<i>Terminal deoxynucleotidyl transferase</i>
TEM	<i>Transendothelial migration</i>
TLC	<i>Total leukocytic count</i>
TN	<i>True negative</i>
TP	<i>True positive</i>
TSG	<i>Tumor Suppressor Gene</i>
VEGF	<i>Vascular endothelial growth factor</i>
VCAM-1	<i>Vascular cell adhesion molecule-1</i>
WBC	<i>White blood cell</i>
WHO	<i>World Health Organization</i>

INTRODUCTION

Acute myelogenous leukemia (AML) cells are generally chemosensitive, and 70 – 80 % of AML patients undergo complete remission after chemotherapy. However, long term disease free survival remains as low as 30 – 50 %, mainly because of relapse after chemotherapy. The relapse has been ascribed to minimal residual disease (MRD) in the bone marrow (BM) (*Matsunaga et al., 2008*).

Adhesion of acute myeloid leukemia (AML) blasts in the bone marrow microenvironment confers protection from chemotherapy-induced apoptosis. Adhesive properties of leukemic cells are probably responsible for the complication of leukostasis in AML as well as leukemic meningitis, leukemic cutis, extramedullary leukemia and formation of chloromas (*Becker et al., 2009*).

One mechanism for retention of blasts within the bone marrow is adhesion via very late antigen-4 (VLA-4), the $\alpha_4 \beta_1$ integrin heterodimer that binds to its main ligands, fibronectin and vascular cell adhesion molecule-1 (VCAM-1) (*Becker et al., 2009*).

VLA-4 positive cells acquire resistance to anoikis (loss of anchorage) or drug-induced apoptosis through the phosphatidylinositol-3-kinase (PI-3K) 1 AKT/BCL-2 signaling pathway, which is activated by the interaction of VLA-4 and

fibronectin. VLA-4 is involved in the migration of CD34+ cells and AML cells beneath marrow stromal cells (**Becker et al., 2009 and Matsunaga et al., 2003**).

Moreover VLA-4 is known to function not only as an adhesion receptor, but also as a bi-directional signaling molecule for both incoming and outgoing signals in its close interaction with hematopoietic and microenvironmental cells or with soluble signaling factors such as cytokines and chemokines (**Mohlknecht et al., 2008**).

High levels of expression of VLA-4 are seen in all French-American-British classes of AML with averages ranging from 72% to 95%, although there is also a wide range (6%-98%). This throws new light on the molecular pathogenesis of bone marrow minimal residual disease after chemotherapy in patients with acute myelogenous leukemia (**Becker et al., 2009 and Mohlknecht et al., 2008**).

AIM OF THE WORK

The aim of this work is to assess VLA-4 expression by AML blasts and its relation to common prognostic factors and disease outcome.