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

Thesis

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الأهمية التنبؤية لوجود ال **(VLA-4)** فى سرطان الدم الميلودى الحاد

رسالة

توطئة للحصول على درجة الماجستير
فى الباثولوجيا الإكلينيكية و الكيميائية

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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.

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List of Abbreviations

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

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

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

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

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 microenviroment 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 α_4 β_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 (P1-3K) 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 microenviromental 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.