# Minimal Residual Disease in Acute Myeloid Leukemia patient

*Thesis*

*Submitted for Partial Fulfillment of Master Degree in Internal Medicine*

*By*

**Ibrahim Jamal Mahmoud**
*MB.B.Ch, Faculty of Medicine, Ain Shams University*

*Under Supervision of*

**Prof. Dr. Mohamed Mahmoud Metwaly Mousa**
*Professor of Internal Medicine and Clinical Hematology*
*Faculty of Medicine – Ain Shams University*

**Prof. Dr. Emad Abdel Mohsen Abdel Hady**
*Assistant Professor of Internal Medicine and Clinical Hematology*
*Faculty of Medicine –Ain Shams University*

**Dr. Nour Elhuda Hussin Abdullah**
*Lecturer of Internal Medicine and Clinical Hematology*
*Faculty of Medicine-Ain Shams University*

*Faculty of Medicine*
*Ain Shams University*
*2019*


سورة البقرة الآية: ٣٢

# Acknowledgment

*First and foremost, I feel always indebted to* **ALLAH,** *the Most Kind and Most Merciful.*

*I'd like to express my respectful thanks and profound gratitude to* **Prof. Dr. Mohamed Mahmoud Metwaly Mousa,** *Professor of Internal Medicine and Clinical Hematology Faculty of Medicine – Ain Shams University for his keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to* **Prof. Dr. Emad Abdel Mohsen Abdel Hady,** *Assistant Professor of Internal Medicine and Clinical Hematology Faculty of Medicine –Ain Shams University, for his kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

*I am deeply thankful to* **Dr. Nour Elhuda Hussin Abdullah,** *Lecturer of Internal Medicine and Clinical Hematology Faculty of Medicine-Ain Shams University, for her great help, active participation and guidance.*

**Ibrahim Jamal Mahmoud**

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# List of Abbreviations

| Abb. | Full term |
|---|---|
| AL | Acute leukemia |
| ALL | Acute lymphoblastic leukemia |
| AML | Acute myeloid leukemia |
| APL | Acute promyelocytic leukemia |
| ATRA | All trans retinoic acid |
| BM | Bone Marrow |
| CBF | Core-binding factor |
| CBFB | Core-binding factor subunit beta |
| CEBPA | CCAAT/ enhancer binding protein-α |
| CR | Complete remission |
| DIC | Disseminated intravascular coagulation |
| FAB | French-American-British |
| FISH | Fluorescence in situ hybridization |
| FLT3 | FMS-related tyurosine kinase 3 |
| HCT-CI | Haematopoietic cell transplantation Co-morbidity index |
| HR | High risk |
| HSCs | Hematopoietic stem cells |
| HSCT | Hematopoietic Stem Cell Transplantation |
| IPT | Immunophenotyping |
| ITD | Internal tandem duplication |
| LAIPs | Leukemia-associated immune phenotypes |
| LAPs | Leukemia associated immunophenotypes |
| MDS | Myelodysplastic syndrome |
| MFC | Multi-parameter flow cytometry |
| MoAbs | Monoclonal antibodies |
| MPD | Myeloproliferative disorder |
| MPO | Myeloperoxidase |
| MRC | Medical Research Council |

# List of Abbreviations Cont...

| Abb. | Full term |
|---|---|
| MRD | Minimal residual disease |
| MYH11 | Myosin heavy chain11 |
| NGS | Next generation sequencing |
| NK | Normal karyotype |
| NPM1 | Nuclephosmin |
| NSE | Nonspecific esterase |
| OS | Overall survival |
| PAS | Periodic acid-Schiff |
| PB | Peripheral blood |
| PCR | Polymerase chain reaction |
| PML | Promyelocytic leukemia protein |
| RARA | Retinoic acid receptoralpha1 |
| RFS | Relapse free survival |
| RQ-PCR | Real-time quantitative polymerase chain reaction |
| RUNX1 | Runt – related transcription factor1 |
| SBB | Sudan black B |
| SNP | Single-nucleotide polymorphism |
| TKIs | Tyrosine kinase inhibitors |
| TNF | Tumor necrosis factor |
| TRM | Treatment-related mortality |
| WHO | World Health Organization |

# INTRODUCTION

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults. With recent treatment protocols, about 80% of patients will reach complete remission (CR). However, about half of these patients will relapse, reaching a 5 year survival rate of 30-40% ***(Cornelissen et al., 2007).***

In AML patients, morphologic assessment is performed to detect chemotherapy response. By definition, patients are in CR when less than 5% blast cells are present in the Bone Marrow (BM) with normal erythropoiesis, granulopoiesis and megakaryopoiesis ***(Cheson et al., 2003).***

In addition, neutrophils and platelets in peripheral blood (PB) should be at least 1.0 x 109/l and 100 x 109/l, respectively. As about half of the patients in CR will relapse ***(Kern et al., 2008).***

This so-called minimal residual disease (MRD) which is persistence of leukemic cells after chemotherapy treatment and these cells are responsible of relapse. Quantitative MRD assessment could give important prognostic information after chemotherapy treatment ***(Grimwade and Freeman, 2014).***

Two highly sensitive methods for MRD detection in leukemia are multi-parameter flow cytometry (MFC) and real-time quantitative polymerase chain reaction (RQ-PCR). One of

the most frequently used techniques to assess MRD in leukemia is based on assessment of immunophenotypic aberrant antigen expression using flow cytometry. For practical purposes, in most cases, this approach is restricted to cell surface antigen expression ***(Zeijlemaker and Schuurhuis, 2013).***

At diagnosis, so-called leukemia associated immunophenotypes (LAPs) are determined. Such a LAP consists of an aberrantly expressed cell surface marker(s), usually combined with a myeloid marker (CD13/CD33) and with a normal progenitor antigen, i.e. CD34, CD117 or CD133. LAPs are grouped into (1) cross-lineage antigen expression (e.g. expression of lymphoid markers in myeloid blasts),(2) asynchronous antigen expression (co-expression of antigens that are not concomitantly present during normal differentiation), (3) lack of antigen expression and (4) antigen overexpression. Such aberrancies can subsequently be used to detect MRD ***(Kern et al., 2004).***

Due to large heterogeneity of immunophenotypes in AML, determination of LAPs has to be performed for each individual patient. These LAPs are not, or only in very low frequencies, present on normal BM cells in remission BM. Sensitivities have been reported to be in a range of 10-3 down to 10-5 (1 leukemic cell in 1,000 to 100,000 normal cells) Besidesthese relatively high sensitivities, it is also a very rapid technique ***(Zeijlemaker and Schuurhuis, 2013).***

# Aim of the Work

The aim of this study is assessment of measurable minimal residual disease after treatment to identify acute myeloid leukemia patients who are at high risk of poor outcome.

*Chapter One*

# ACUTE MYELOID LEUKEMIA

## Definition

Acute myeloid leukemia is presented as a malignant disease that is characterized by abnormal growth and differentiation of hematopoietic stem cells (HSCs), in which immature myeloid precursors (myeloblasts) invade in the BM and PB. The uncontrollable proliferation of immature myeloid cells occurs at the expense of the normal hematopoiesis ***(Sant et al., 2014).***

It has many other nomenclatures, including acute myelocytic leukemia, acute myelogenous leukemia, acute granulocytic leukemia, and acute nonlymphocytic leukemia. "Acute" means that this leukemia can progress rapidly if not treated, and would be fatal in a short period. The term "Myeloid" refers to the type of cell this leukemia starts from ***(Appelbaum, 2014).***

It is a very heterogeneous disorder with an incidence of 3 to 4 per 100 000 men and women per year. It is characterized by the proliferation of somatically acquired genetic changes in hematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation, and differentiation. Outcome is affected by various factors, including patient features such as age, comorbidities, performance status and disease

characteristics of which the genetic profile of the disease is the most important ***(Richard et al., 2013)*.**

## What are the risk factors of AML:

- ***Genetics:***

Molecular analysis of leukemic blasts from patients with AML have revealed presence of acquired gene mutations and changes in gene and micro ribo nucleo protein (micro RNA) expression. Multiple submicroscopic genetic alterations with prognostic significance have been discovered. Findings are likely to have a major impact on the clinical management of AML ***(Marcucci et al., 2011)***.

From a clinical perspective, there are at least three important aspects with respect to the genetic changes in AML. First, the current World Health Organization (WHO) classification reflects the fact that an increasing number of cases of AML can be categorized on the basis of their underlying genetic defects that define distinct clinicopathologic entities ***(Swerdlow et al., 2008)***.

Second, it has become clear that specific chromosome abnormalities and molecular genetic changes are among the most important prognostic markers and therefore may be used for stratification of patients with AML to risk-adapted therapeutic strategies. Finally, novel therapies are being developed that target some of the identified genetic defects. It is therefore anticipated that these genetic markers will acquire a

predictive value, that is, the ability to predict differential efficacy of a therapy ***(Marcucci et al., 2011).***

To date, only diagnosis of nucleoplasmin 1(*NPM1)*, CCAAT/enhancer-binding protein alpha *(CEBPA)*, and FMS like tyrosine kinase 3 (*FLT3)* mutations has entered clinical practice and affects diagnosis, risk assessment, and also guidance of therapy in the basis of characteristic clinical, pathologic, and biologic features ***(Döhner et al., 2010)***.

AML with *NPM1* mutation and AML with *CEBPA* mutation have been incorporated as provisional entities in the 2008 WHO classification of AML ***(Swerdlow et al., 2008).***

Statistics found that *FLT3* mutations are not considered to define a distinct entity, they provide important prognostic information. Furthermore, they are now being targeted in clinical trials with tyrosine kinase inhibitors (TKIs) ***(Dohner et al., 2010).***

- ***Age:***

Both the nature of AML and the health of the patient change with age. It is axiomatic that older patients are more likely to have more comorbidities and have a poorer performance status than younger patients. When compared with the disease in younger adults, AML in older patients is more likely to be preceded by a myelodysplastic phase, more frequently has unfavorable cytogenetics, more commonly expresses multidrug resistance, and responds less well to chemotherapy ***(Leith et al., 1999).***