# INTRODUCTION

Acute myeloid leukemia (AML) is a malignancy originating in a multipotent hematopoietic cell characterized by clonal proliferation of abnormal blast cells in the marrow and impaired production of normal blood cells ***(Hoffbrand et al., 2006).***

AML is the most common type of leukemia in adults, as it accounts for approximately 25% of all leukemias in adults in the western world ***(Greenlee et al., 2001).*** Although the incidence of acute leukemias accounts for less than 3% of all cancers, these diseases constitute the leading cause of death due to cancer in children and persons aged less than 39 years ***(Deschler and Lubbert; 2006).***

Increasingly, recurrent genetic aberrations govern the prognostication and risk assessment in AML, including the large group of patients who present with AML without apparent cytogenetic abnormalities. A steadily growing catalogue of molecular lesions has led to better understanding of molecular lesions in the pathogenesis of AML, thus enabling new AML subgroups and prognostic factors to be defined and the development of outcome-based classification systems, which incorporate information on perturbed pathogenetic pathways and inform about potential therapeutic targets ***(Kuchenbauer et al., 2005).***

In the context of this evolving molecular risk assessment, antigen expression profiles have been identified as surrogates for certain leukemic genotypes. Furthermore, a few single antigens per se have been found to be predictive of clinical response. In most cases, however, the prognostic power of antigens has not been disassociated from underlying genetic determinants ***(Gönen et al., 2012).***

CD25 is the alpha chain of the IL-2 receptor which is a type I transmembrane protein, 55 kda glycoprotein present on activated T cells, activated B cells, some thymocytes, myeloid precursors, and oligodendrocytes that associates with CD122 the beta chain of the IL-2 receptor to form a heterodimer that can act as a high-affinity receptor for IL-2. CD25 is expressed in most B-cell neoplasms, some acute non-lymphocytic leukemia and neuroblastoma. Its soluble form, called sIL-2R may be elevated in these diseases and is occasionally used to track disease progression ***(Wang et al., 2005 & Nakase et al., 2012).***

CD25 expression on B-lineage ALL was found to be associated with adverse outcome independent of established risk factors ***(Paietta et al., 1997).*** Also, recent studies have suggested that CD25 expression on AML blast cells provides prognostic relevance in AML independent of known biomarkers, and is correlated with stem-cell gene-expression signatures associated with adverse outcome ***(Terwijn et al., 2009 & Gonen et al., 2012).***

CD123 is the alpha chain of interleukin-3 receptor (IL3-R), it is known as leukemic stem cell marker, thus enables discrimination between normal hematopoietic stem cells that do not express CD123 ($CD123^{-}$) and leukemic stem cells that are positive for this marker ($CD123^{+}$) ***(Vergez et al., 2011).***

Recent studies have shown that CD123 is highly expressed on leukemia stem cells (with the phenotype $CD34^{+}CD38^{-}$) of patients with AML, and is correlated with tumor load and poor prognosis ***(Riccioni et al., 2004 & Vergez et al., 2011).***

# AIM OF THE WORK

**The aim of this work is to:**

- Investigate CD25 and CD123 expression by flow cytometry technique in AML patients.
- Correlate CD25 and CD123 expression with different cytogenetic subgroups and immunophenotyping profile.
- Detect the effect of CD25 and CD123 expression on the outcome.

***Chapter (1):***

# ACUTE MYELOID LEUKEMIA

## *Definition:*

Acute myeloid leukemia (AML) is characterized by a clonal proliferation of myeloid precursors, with a reduced capacity to differentiate into more mature cellular elements. As a result, there is an accumulation of leukemic blasts or immature forms in the bone marrow (BM), peripheral blood and other tissues, with a variable reduction in the production of normal red blood cells, platelets, and mature granulocytes ***(Campbell, 2011)***.

## *Incidence:*

Most cases of AML occurrence are in early childhood and later adulthood. The peak incidence rate occurs in the first year of life and then decreases steadily up to the age of 4yrs, at this point it remains relatively constant throughout the years of childhood and early adulthood ***(Deschler and Lübbert, 2006)***. AML is therefore primarily a disease of later adulthood with a median age of 65 years old ***(Burnett and Venditti, 2011)***.

AML is the most common type of leukemia in adults, as it accounts for approximately 25% of all leukemias in adult in the Western world ***(Burnett and Venditti, 2011)***.

The mean age of adult Egyptian patients with acute leukemia is (33.4 years), and it is higher in AML patients than in acute lymphoblastic leukemia (ALL) patients. The relatively low age of presentation of acute leukemia among adult Egyptian patients may be due to the low mean age of the general population, or mortality before diagnosis due to the redundancy or lack of accessibility of older adults to health care system ***(Shawkat et al., 2009)*.**

AML in adults has a slight male predominance in most countries ***(Deschler and Lubbert, 2006)*.** Egyptian patients also demonstrated high male/female ratio 1.6:1 ***(Shawkat et al., 2009)*.**

## Etiology and Risk Factors:

Several risk factors have been associated with the development of AML. Generally, known risk factors account for only a small number of observed cases. These include age, genetic disorders, antecedent hematologic disease, exposures to viruses as well as radiation, chemical, or other occupational hazards and previous chemotherapy ***(Deschler and Lubbert, 2006).***

### 1. *Genetic Disorders*:

An increased incidence of AML is seen in patients with disorders associated with excessive chromatin fragility such as Bloom syndrome, Fanconi anemia, Schwachman - Diamond syndrome, Blackfan - Diamond syndrome and Kostmann

syndrome, as well as with Wiskott Aldrich and ataxia telangiectasia syndromes. Other syndromes, such as Down (trisomy of chromosome 21), Klinefelter (XXY and variants), neurofibromatosis and Patau (trisomy of chromosome 13), have also been associated with a higher incidence of AML ***(Jabbour et al., 2006)***.

Inherited familial AML (true non-syndromic familial AML), is a heterogeneous group of disorders, including autosomal recessive forms that become manifest during childhood in association with MDS and monosomy 7, as well as autosomal dominant forms that are preceded by various types of a dysplastic phase and that vary in morphologic subtype. It has been reported in only a few families outside of a syndromic setting such as trisomy 21 or a disorder involving defective DNA repair ***(Smith et al., 2011)***.

Meanwhile, congenital leukemia is a term applied to leukemia diagnosed at birth or within the first month of life. It is a rare entity ***(Prakasha et al., 2008).***

**2. *Antecedent Hematologic Disorder*:**

The most common risk factor for AML is the presence of an antecedent hematologic disorder, the most common of which is myelodysplastic syndrome (MDS) ***(Seiter, 2010)***. Other antecedent hematologic disorders include aplastic anemia, multiple myeloma, myelofibrosis and paroxysmal nocturnal hemoglobinuria (PNH). Aplastic anemia is associated with late

development of AML ***(Gale et al., 2007)***. AML can occur in patients with myeloma who have not received prior chemotherapy or radiation therapy ***(Miller and Pihan, 2009)***. AML secondary to PNH appears to involve the same clone from which the abnormal erythrocytes are derived ***(White et al., 2010)***.

Myeloproliferative disorders e.g., essential thrombocythemia, polycythemia vera, chronic myeloid leukemia (CML) and agnogenic myeloid metaplasia ***(Miller and Pihan, 2009)*** may be associated with AML transformation (t-AML) ***(Seiter, 2010)***.

**3. *Viruses*:**

Viruses may cause disruption of the host genome by insertion, mutation and chromosomal rearrangements. Viruses also result in immune dysfunction, leading to decreased immune surveillance for early tumors. However, it has not been demonstrated that simple infection with either an RNA- or DNA-based virus alone is a cause of acute myeloid leukemia. RNA retroviruses, have been found to cause many neoplasms in experimental animals, including leukemia ***(Deschler and Lubbert, 2006)***. Parvovirus B19 (B19V) was found to play an important role in conversion of pre-leukemic clones to an overt leukemia ***(Yalcin et al., 2009)***.

**4. *Environmental and Chemical Factors*:**

A vast variety of environmental and chemical exposures are assumed to be associated with variable elevated risks of developing AML. Benzene, smoking, dyes, herbicides, pesticides and ionizing radiation (both therapeutic and non-therapeutic) have been implicated as potential risk factor for development of AML ***(Jabbour et al., 2006)***.

***a. Drugs:***

AML arising following exposure to geno-toxic agents has been recognized as a distinctive entity for more than 40 years. It accounts for 10% to 20% of all AML cases **(*Smith et al., 2011).***

The association of AML to treatment of multiple myeloma and lymphoproliferative disorders, and administration of multiple alkylating drugs is well-documented. Treatment of patients with chlorambucil, mustine, melphalan, procarbazine or nitrosourea may predispose to AML especially when these drugs are combined with radiotherapy. AML patients typically present several years after therapy with peak incidence after about 5 years ***(Miller and Pihan, 2009).***

Chloramphenicol, phenylbutazone, and less commonly, chloroquine and methotrexate can result in marrow failure that may evolve into AML ***(De Sanctis et al., 2003*)**. After therapy with agents targeting topoisomerase II, survival of transformed therapy-related acute myeloid leukemia (t-AML) patients has been poor compared with that of patients with de novo AML

and the treatment most likely to cure t-AML is allogeneic hematopoietic stem cell transplantation ***(Döhner et al., 2010)***. Topoisomerase II inhibitors are associated with development of AML after a relatively shorter latent period of 2-3 years **(*Wickremasinghe and Hoffbrand, 2011).***

***b. Benzene:***

Its toxicity is related to cumulative dose and leukomogenic risk is considerable at 124 to 200 part per million ***(Hayes et al., 2008).***

***c. Radiation:***

Ionizing radiation causes radiation-induced genomic instability in hemopoietic cells shown as non-clonal chromosome and chromatid-type aberrations in the clonal progeny of hemopoietic stem cells. The same is caused by Gamma radiation ***(Gowans et al., 2005).***

***d. Smoking:***

AML is 2 to 3 times higher in smokers exceeding 20 packs per year than non-smokers; this could be due to benzene in cigarettes; or the potential leukomogenic chemicals including urethane nitrosamine and radioactive compounds present in tobacco smoke ***(Greer et al., 2009).***

## Pathogenesis:

AML is characterized by acquisition of somatic mutations in hematopoietic progenitors that confer a proliferative and/or survival advantage, impair hematopoietic

differentiation and provide properties of limitless self-renewal ***(Wernig and Gilliland, 2009).*** A single mutation is not sufficient to cause an overt leukemic phenotype, but it likely develops upon the acquisition of further mutations in progenitor cells ***(Döhner and Döhner, 2008).***

***1-<u>Inappropriate Proliferation:</u>***

This abnormal proliferation is often the result of mutations affecting proliferative signaling pathways. Activated kinases have become implicated in the pathogenesis of AML ***(Licht and Sternberg, 2010).***

A number of hematopoietic growth factors have been found to stimulate AML cells, including granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interleukins 1, 3, 4, 6 and 9, steel factor (KIT ligand), thrombopoietin (MPL ligand) and FLT3 ligand (FLT3L). The effects of growth factors are predominantly proliferative, but they may also be anti-apoptotic ***(Vincent and DeVita, 2012).***

***2-<u>Differentiation Blockade:</u>***

Transcription factors are commonly disrupted in AML, either by their fusion as a result of chromosomal translocation, or by point-mutation. Factors affected by chromosomal rearrangement include the core binding factor (CBF) complex and the retinoic acid receptor (RAR). Point mutations in myeloid transcription factors include CCAAT/enhancer-binding protein

alpha (CEBPA) gene ***(Licht and Sternberg, 2010).*** Acute promyelocytic leukemia (APL) is a clear-cut example of differentiation blockade in AML ***(Fufan et al., 2010).***

### *3- Escape from Programmed Cell Death:*

The p53 protein is a focal point in the regulation of apoptotic signaling and cell-cycle regulation. Mutations within p53 are associated with adverse response to chemotherapy in patients with AML ***(Licht and Sternberg, 2010).***

### *4-Self-Renewal:*

Unlike normal progenitor cells, leukemic cells in AML patients can undergo self-renewal rather than lineage-specific commitment. The expression of cytoplasmic nucleophosmin (NPM) variant is associated with expression of genes thought to support maintenance of the stem cell phenotype ***(Alcalay et al., 2005).***

## Classification:

### *1- French-American-British (FAB) Classification:*

When facilities are limited, AML can be classified on the basis of cytology and cytochemistry, as initially proposed by the French-American-British (FAB) group **(*Bennet et al., 1976).*** The FAB classification requires a blast count of 30% or more in the BM for the diagnosis of AML **(*Miller and Pihan, 2009)*** (Table 1).

**Table (1):** The FAB Classification of AML.

| Category | Criteria |
|---|---|
| $M_0$<br>AML with minimal evidence of myeloid differentiation | Blasts ≥30% of BM NEC<br><3% of blasts are MPO or SBB positive<br>Lymphoid markers are negative<br>Immunological or ultrastructural features of myeloid differentiation |
| $M_1$<br>AML without maturation | Blasts ≥90% of BM NEC<br>≥3% of blasts are MPO or SBB positive<br>Maturing monocytic component in BM is ≤10%<br>Maturing granulocytic component is ≤10% |
| $M_2$<br>AML with maturation | Blasts is 30-89% of BM NEC<br>Maturing granulocytic component in BM is >10 % of NEC<br>BM monocytic component is <20 % of NEC and other criteria of M4 not met |
| $M_3$<br>Acute promyelocytic leukemia | MPO and SBB show characteristic heavy staining filling the cytoplasm.<br>Most cells (≥50%) are abnormal promyelocytes with heavy cytoplasmic granulation<br><20% of blasts have basophilic cytoplasm and 90% have multiple Auer rods<br>M3 variant (M3v) is characterized by having microgranules |
| $M_4$<br>Acute myelomonocytic leukemia | Blasts are ≥30% of BM NEC<br>Granulocytic component is ≥20% of BM NEC<br>Monocytic component is 20% to 79% of BM NEC and either PB monocytes ≥$5x10^9$/L or BM like M2 but PB monocytes are ≥$5\times10^9$/L with cytochemical proof of monocytic differentiation<br>M4Eo is an M4 variant characterized by marrow eosinophilia |
| $M_5$<br>M5a (Acute monoblastic leukemia)<br><br><br>M5b (Acute monocytic leukemia | M5a: Blasts are ≥30% of NEC<br>BM monocytic component is ≥80 % of NEC<br>Monoblasts are ≥80% of BM monocytic component<br><br>M5b: Blasts are ≥30 % of NEC<br>BM monocytic component is ≥80% of NEC<br>Monoblasts are <80% of BM monocytic component. |
| $M_6$<br>Acute erythroid leukemia | Erythroid cells are ≥50% of BM cells<br>BM blasts are ≥30% of NEC |
| $M_7$<br>Acute megakaryoblastic leukemia | Blasts are ≥30 % of NEC<br>Blasts are predominantly megakaryoblasts<br>≥50 % megakaryoblasts by morphology or electron microscopy or immunophenotyping $CD41^+$, $CD61^+$. |

The morphologic subtypes of AML also include rare types were not included in the FAB system (accounts for only 1% of all AML cases), such as acute basophilic leukemia, which was proposed as a ninth subtype, M8, by ***Duchayne et al., 1999***.

MPO: myeloperoxidase, SBB: Sudan Black B, BM: bone marrow, NEC: non-erythroid cells, PB: peripheral blood ***(Miller and Pihan, 2009 & Lichtman and Liesveld, 2010)*.**

### *2- Cytochemical Classification:*

The myeloperoxidase (MPO) and Sudan black B (SBB) staining are usually indicative of leukemia of myelocytic origin whereas non-specific esterase (NSE) is indicative of monocytic differentiation. AML blast cells are usually periodic acid Schiff (PAS) negative with the exception of erythroblasts of $M_6$ AML and eosinophils of AML of the $M_4$Eo subclass. However, recently, cytochemistry becomes less important than before as a tool in the diagnosis of AML because of the greater efficiency of immunological methods **(*Vincent and DeVita, 2012).***

### *3- Immunophenotypic Classification (IPT):*

The development of antibodies directed against hematopoietic cell antigens, whether cytoplasmic or cell surface antigens, has greatly facilitated the diagnosis and classification of acute leukemia. It provided prognostic information, a means of detecting residual disease and markers of drug resistance **(*Vincent and DeVita, 2012)*** (Table 2).

**Table (2):** Immunophenotypic markers of AML.

| Marker | $M_0$ | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ | $M_6$ | $M_7$ |
|---|---|---|---|---|---|---|---|---|
| HLA-DR | ++ | ++ | ++ | - | ++ | ++ | + | + |
| CD11b | + | + | + | - | ++ | ++ | - | - |
| CD13 | + | ++ | ++ | ++ | ++ | ++ | - | - |
| CD14 | - | + | + | - | ++ | ++ | - | - |
| CD15 | - | - | + | + | + | ± | - | - |
| CD33 | + | ++ | +++ | +++ | +++ | +++ | + | + |
| CD41,CD61 | - | - | - | - | - | - | - | +++ |
| Glycophorin A | - | - | - | - | - | - | ++ | - |
| TDT | ++ | + | - | - | - | - | - | - |
| CD34 | ++ | + | - | - | - | - | - | + |

***(Tong et al., 2009)***

### *4- Morphologic-Immunologic-Cytogenetic (MIC) Classification:*

It integrated the immunophenotypic (IP) and cytogenetic information with FAB classification, to further describe individual cases of AML and to divide patients into distinct groups with different prognosis ***(Bain et al., 2005)*** (Table 3).