INTRODUCTION

Acute Myeloid Leukemia AML is a clonal disorder of hematopoietic stem cells which is characterized by inhibition of differentiation and accumulation of cells at various levels of maturity. Reduced production of healthy hematopoietic elements results with anemia, neutropenia and thrombocytopenia and gives rise to related clinical situations ***(Ferrara and Schiffer, 2013).***

Patients with acute myeloid leukemia (AML) have approximately 10^{12} malignant cells (blast cells) at the time of diagnosis ***(Suzan et al., 2013).***

The disease is considered to be in complete remission (CR) when fewer than 5% of malignant cells in the bone marrow (BM) are morphologically detectable following chemotherapy ***(Suzan et al., 2013).***

High remission rates (ranging from 50% to 80%) are achieved with modern chemotherapeutic regimens nowadays, Despite these encouraging results, the patients may still harbor as many as 10^{10} leukemic cells, and the level of leukemia cellsis largely unknown ***(Kern et al., 2004)*** a large fraction of AML patients relapse, with 30-40% of young and only 10% of elderly patients being long term survivors ***(Maria et al., 2016)***.

Chemotherapy-resistant blast cells in BM surviving in small amounts are shown to have capacity to trigger future relapses ***(Thörn et al., 2011).***

The sensitivity of morphologic studies can be improved by cytochemical staining, but the detection limit of these methods remains at a level of 1 leukemic cell in a 102 normal cell population ***(Kern et al., 2004).***

The leukemic population undetectable by morphologic methods has been defined as minimal residual disease (MRD). In other words, when there is evidence (immunophenotypic, molecular, or cytogenetic) that leukemic cells remain in the BM but there are insufficient cells to be detected by routine examination under the microscope, the persistence of this residual neoplastic cells below the threshold of conventional morphologic detection is referred to the term "minimal residual disease.

Monitoring of MRD allows for early detection of hematologic relapse and timely therapeutic intervention, and has significantly improved clinical outcome of many hematopoietic neoplasms. MRD assays are capable of producing information on treatment response with more sensitivity than microscopic analysis it can be more than 100 times more sensitive than morphologic examination. Thus, MRD can be used to design risk-based therapies for AML in an effort to minimize toxicity and improve cure rates ***(Ferrara and Schiffer, 2013).***

MRD is the major reason for relapse of leukemia and is detected by molecular methods (polymerase chain reaction

(PCR) amplification of molecular abnormalities) as a golden standard ***(Perea et al., 2006)***, but due to development of technology and the wide availability and conceptual straight forwardness of immunophenotyping, flowcytometry for MRD determination become the most accessible method and are getting developed day-by-day ***(Al-Mawali et al., 2009).***

While studies of MRD by PCR amplification of fusion transcripts are inherently limited to specific leukemic subtypes, studies of MRD by flowcytometry have a potentially much wider applicability. Many studies showed that in adult patients with acute leukemia in morphologic remission, MRD detected in the first bone marrow obtained after induction treatment was highly predictive or relapse ***(David et al., 2014).***

In comparison with molecular methods, advantages of FCM MRD are obtaining results hours after sampling due to technical simplicity and being inexpensive due to low costs (time/cost effectiveness) ***(Thörn et al., 2011).***

In addition FCM has a unique property which is of vital importance in MRD detection; the ability to distinguish viable cells from BM debris and dead cells ***(Buccisano et al., 2012).***

With the great evaluation in recent years, FCM is now accessible for MRD detection, by monitorization of malignant clones and most importantly by exploring viable cells ***(Béné and Kaeda, 2009).***

FCM detection of MRD is based on differential diagnosis of leukemic cells from normal, healthy cells by expressions of aberrant antigens by them ***(Muzzafar et al., 2009).***

The presence of minimal residual disease (MRD) in the bone marrow (BM) of patients with acute myeloid leukemia (AML) following chemotherapy has been established by many studies to be strongly associated with relapse of leukemia. In addition, detection of MRD is the major objective of many of the newer diagnostic techniques used in malignant hematology ***(Cameron, 2012)***.

Many study groups has reported suitability of multi-parametric FCM for MRD detection in AML, which is fast and sensitive. As cytogenetic signature can't always reliably predict the outcome in individual patients, FCM MRD has an important prognostic value in AML. The genuine sensitivity of FCM is claimed to be between 10-4 and 10-5 ***(Buccisano et al., 2012)***.

Monitoring of AML-MRD can be defined by a combination of 4 to 5 surface markers, possibly associated with flowcytometric physical abnormalities that normally are not present in a healthy BM ***(Béné and Kaeda, 2009).***

However the studies about assessment of minimal residual disease in acute myeloid leukemia by flowcytometry are limited, they are in need to be clarified.

Aim of the Work

The aim of this study is to determine the value of MRD monitoring by FCM in adult acute myeloid leukemia especially post induction of remission in order to determine efficacy of treatment, monitor remission status of the patient and predicting impending relapse.

Chapter One

ACUTE MYELOID LEUKEMIA

Definition

Acute myeloid leukemia is defined as a malignant disease that is characterized by abnormal growth and differentiation of haematopoietic stem cells (HSCs), in which immature myeloid precursors (myeloblasts) accumulate in the bone marrow and peripheral blood. The uncontrollable expansion of immature myeloid cells occurs at the expense of the normal production of their terminally differentiated cells, such as red blood cells, platelets and white blood cells (WBCs) ***(Sant et al., 2014).***

Acute myeloid leukemia (AML) 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 quickly if not treated, and would be fatal in a few months. The term “Myeloid” refers to the type of cell this leukemia starts from ***(Appelbaum, 2014).***

In other words acute myeloid leukemia is a clonal hematopoietic disorder that may be derived from either a hematopoietic stem cell or a lineage-specific progenitor cell. AML is characterized by a predominance of immature forms and loss of normal hematopoiesis. Single or multiple

hematopoietic lineages may comprise the leukemic clone. The requisite blast/blast equivalent percentage is 20% in the peripheral blood and bone marrow ***(Abdel-Wahab et al., 2009).***

AML is the most common form of acute leukaemia in adults, being 3–4 times more common in adults than ALL. However, the incidence of AML is lower than several other tumour types. For example, approximately 3,000 new cases of AML are diagnosed every year in the United Kingdom compared with about 40,000 and 13,000 cases of colorectal cancer and non-Hodgkin lymphoma, respectively ***(Sant et al., 2014).***

Mechanisms/pathophysiology:

AML leukemic cells, like other cancer cells, are characterized by abnormal proliferation, survival and differentiation which are caused by various genetic and epigenetic changes in the tumour cells. About half of all AMLs have definitive gross structural cytogenetic changes, defined as events that are visible on a karyotypic level, that include balanced translocations and chromosomal gains or losses ***(Grimwade and Mrózek, 2011).***

Risk factors

AML was regarded as an incurable disorder, Before the 1960s. With improvements in chemotherapy, allogeneic HSCT and supportive care, a small fraction of patients -mainly those <60 years of age- survived in the 1970s. Since then, a continuous

improvement in outcome in younger patients has been reported ***(Burnett et al., 2011).*** This age-related difference explained by the ability of younger patients to tolerate effective therapy and also the accumulation of several risk factors in older patients compared with younger patients ***(Lazarevic et al., 2014).***

Although exposure to DNA-damaging agents, such as benzene, cigarette smoking, ionizing radiation (usually due to therapeutic radiotherapy) and cytotoxic chemotherapy increase the risk of developing AML or MDS, Many patients diagnosed with AML, had no identified predisposing risk factor ***(Bueso-Ramos et al., 2015)***.

AML secondary to ionizing radiation and/or cytotoxic chemotherapy is known as therapy-related AML. Cytotoxics of the alkylating agent class (as, chlorambucil, cyclophosphamide and melphalan) and topoisomerase II inhibitors (as, etoposide, mitoxantrone and anthracyclines) are greatly associated with the development of therapy-related MDS or AML ***(Bueso-Ramos et al., 2015).*** The purine analogue fludarabine, particularly with alkylating agents, is also associated with an increased risk of therapy-related MDS or AML ***(Niparuck et al., 2010).***

There is no strong evidence for familial aggregation of myeloid malignancies (AML and MDS) has been reported with the potential exception of relatives of young patients with AML who, in a limited cohort, were at an increased risk of AML or

MDS. So the risk of all hematological malignancies and of solid tumours among relatives of patients with AML is increased, suggesting that genes for malignancy in general and other environmental factors may be shared ***(Goldin et al., 2012).*** In addition, certain inherited disorders carry a very high risk of AML development. Down syndrome, Fanconi anaemia, Bloom syndrome, ataxia-telangiectasia, Diamond–Blackfan anaemia, Schwachman–Diamond syndrome and severe congenital neutropaenia (also called Kostmann syndrome) all of them known to be Leukemia predisposing conditions ***(Seif, 2011).***

Clinical picture:

AML usually has a rapid onset of symptoms within a few weeks, providing that not been a preceding myelodysplastic phase. The clinical presentation of AML at diagnosis varies from an incidental finding on a routine blood test through to a life-threatening illness that requires immediate intervention ***(Asim et al., 2016)***. AML is characterized by the typical features of bone marrow failure: fatigue and shortness of breath on exertion due to anemia; recurrent infections due to neutropenia; and an increased tendency to bruise and bleed due to thrombocytopenia, In addition to nonspecific symptoms such as tiredness and loss of appetite ***(Asim et al., 2016)***.

Lymphadenopathy (swollen or enlarged lymph nodes) and hepatosplenomegaly can occur. Other extra medullary leukemic infiltration of the gums, skin and other soft tissues (granulocytic

sarcoma), including the meninges, is less common. Hyper leukocytosis, defined as a leukocyte count of >100 × 10^9 per l, that can lead to multi-organ failure and death due to occlusion of the capillaries ***(Röllig and Ehninger, 2015).***

Spontaneous tumour lysis can occur in rare cases of AML with very high tumour burden, leading to hypocalcaemia, hyperkalaemia, hyperuricaemia, hyperphosphataemia, increased plasma levels of lactose dehydrogenase and oliguric renal failure. Such features are often exacerbated with the initiation of treatment. In some patients, a serious bleeding episode can occur, particularly in the early phase of treatment, as the leukemic blasts can activate the coagulation cascade and cause hyper fibrinolysis. This particularly occurs in patients with APL due to the overexpression of tissue factor known as thromboplastin and less frequently, in some cases of monoblastic or monocytic leukaemia due to overexpression of urokinase plasminogen activator receptor. The most serious complication is intracranial hemorrhage, which occurs in around 5% of patients ***(Asim et al., 2016)***.

Screening and prevention:

In AML that does not complicate MDS, the full blood count remains normal until shortly before presentation, limiting the value of simple screening blood tests. The incidence of AML is raised in individuals with clonal hematopoiesis that is detected by exome or whole-genome sequencing analysis, but

is not sufficiently high to justify such screening tests. It is also not clear whether earlier diagnosis would result in considerably improved outcomes. AML has recently been cited as an example of a tumour in which the incidence of the disease can be largely accounted for by the number of cell divisions, during which random mutations arise, which occur in the stem cell pool, implying that environmental or inherited factors have a minor role ***(Tomasetti and Vogelstein, 2015).***

This controversially suggests that, with the exception of phasing out those cytotoxic drugs associated with the development of secondary AML, prevention will rarely be possible, although the validity of this concept has been questioned ***(Löwenberg et al., 2011).***

Differential diagnosis:

The differential diagnosis includes MDS and acute lymphoblastic leukaemia (ALL), which can present in a similar way. By contrast, chronic myeloid leukaemia usually presents with high blood levels of differentiated myeloid cells, splenomegaly and without features of bone marrow failure ***(Asim et al., 2016)***.

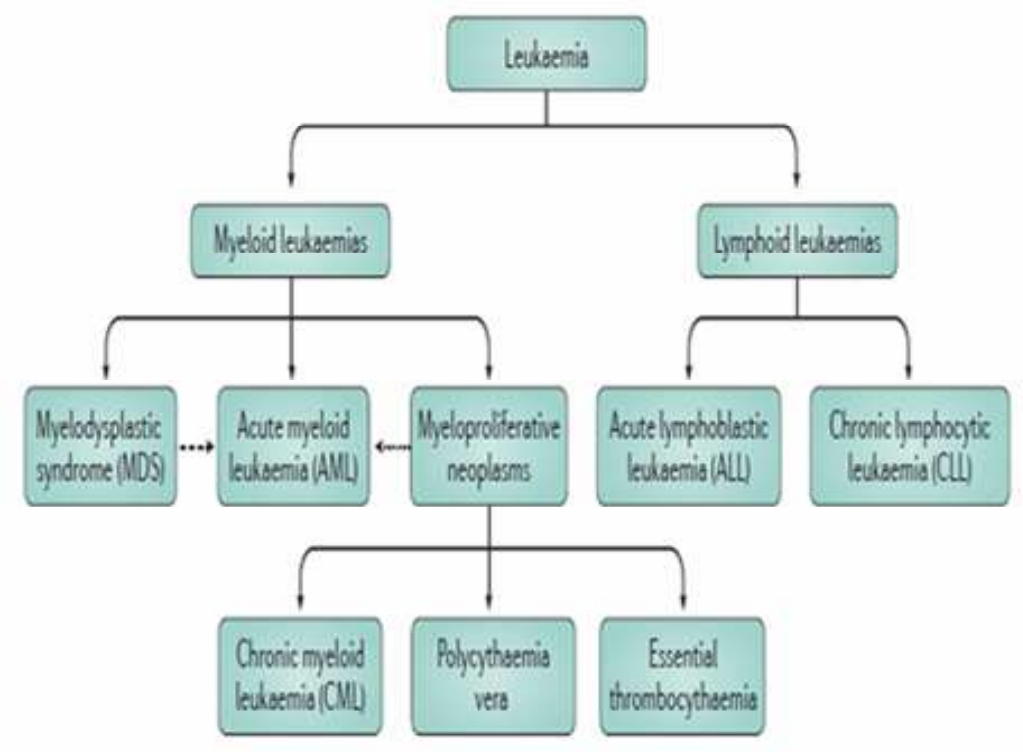

Figure (1): Schematic overview of the main types of leukaemia.

Diagnosis:

AML is diagnosed on the basis of the accumulation of myeloblasts in the bone marrow and blood. In addition, immunophenotyping and the cytogenetic and molecular characterization of myeloblasts are used to distinguish AML from other leukaemias and to define AML subtypes. The different combinations of genetic abnormalities that can accumulate in AML underlie the heterogeneity of the disease ***(Lindsley et al., 2015).***

Table (1): Test/procedures in the initial work-up of a patient with AML.

Test/procedure	General practice	Clinical trial
Tests to establish the diagnosis		
Complete blood counts and differential count	Yes	Yes
Bone marrow aspirate	Yes	Yes
Bone marrow trephine biopsy	Optional[f]	Optional[f]
Immunophenotyping	Yes	Yes
Cytogenetics	Yes	Yes
RUNX1-RUNX1T1, CBCFB-MYH11, PML-RARA, or other gene fusion screening	Optional	Optional
Additional tests/procedures at diagnosis		
Demographics and medical history[a]	Yes	Yes
Performance status (ECOG/WHO score)	Yes	Yes
Analysis of comorbidities	Yes	Yes
Biochemistry, coagulation tests, urine analysis[b]	Yes	Yes
Serum pregnancy test[c]	Yes	Yes
Information on oocyte and sperm cryopresvation	Optional[h]	Optional[h]
Eligibility assessment for allogeneic HSCT	Yes[i]	Yes[i]
Hepatitis A, B, C; HIV-1 testing	Yes	Yes
Chest x-ray, 12-lead ECG; echocardiography (on indication)	Yes	Yes

Lumbar puncture[d]	No	No
Biobanking[e]	Optional	Yes
Prognostic/predictive marker assessment		
NPM1, CEBPA, FL T3 gene mutation	Optional	Yes
WT1, RUNX1, MLL, KIT, RAS, TP53, TET2, IDH1 gene mutation	No	Investigational
ERG, MN1, EVl1, BAALC gene expression	No	Investigational
Detection of minimal residual disease	No	Investigational

(a) Including race or ethnicity, family history, prior exposure to toxic agents, prior malignancy, therapy for prior malignancy, information on smoking.
(b) Biochemistry: glucose, sodium, potassium, calcium, creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase, lactate dehydrogenase, bilirubin, urea, total protein, uric acid, total cholesterol, total triglycerides, creatinine phosphokinase (CPK). Coagulation tests: prothrombin time (PTT), international normalized ratio (INR) where indicated, activated partial thromboplastin time (aPTT). Urine analysis: pH, glucose, erythrocytes, leukocytes, protein, nitrite.
(c) In women with childbearing potential.
(d) Required in patients with clinical symptoms suspicious of central nervous system involvement; patient should be evaluated by imaging study for intracranial bleeding, leptomeningeal disease, and mass lesion; lumbar puncture considered optional in other settings (eg, high WBC).
(e) Pretreatment leukemic bone marrow and blood sample; for further optional storing.
(f) Mandatory in patients with a dry tap (punctio sicca).
(g) Should be performed if chromosome morphology is of poor quality, or if there is typical morphology but the suspected cytogenetic abnormality is not present.
(h) Cryopreservation to be done in accordance with the wish of the patient.
(i) HLA typing and CMV testing should be performed in those patients eligible for allogeneic stem cell transplantation.
(j) Biobanking should also be performed in general practice if at all possible.
(k) Strongly encouraged in AML with normal karyotype.

Morphology

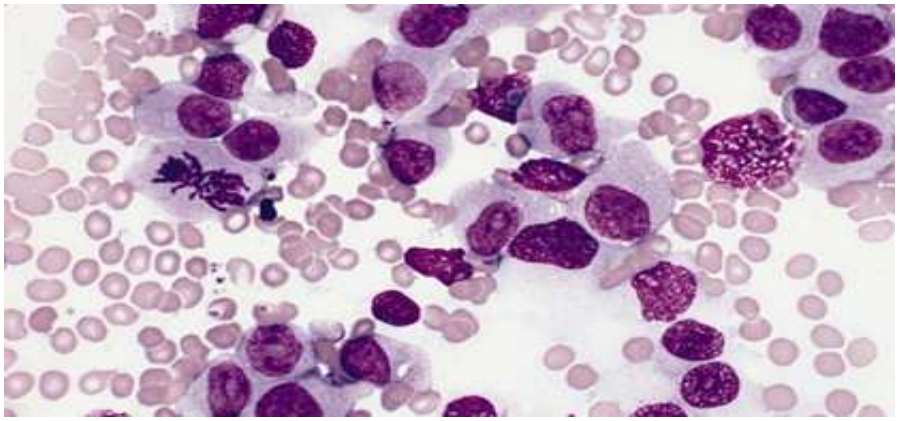

Figure (2): bone marrow aspirate morphology in AML case.

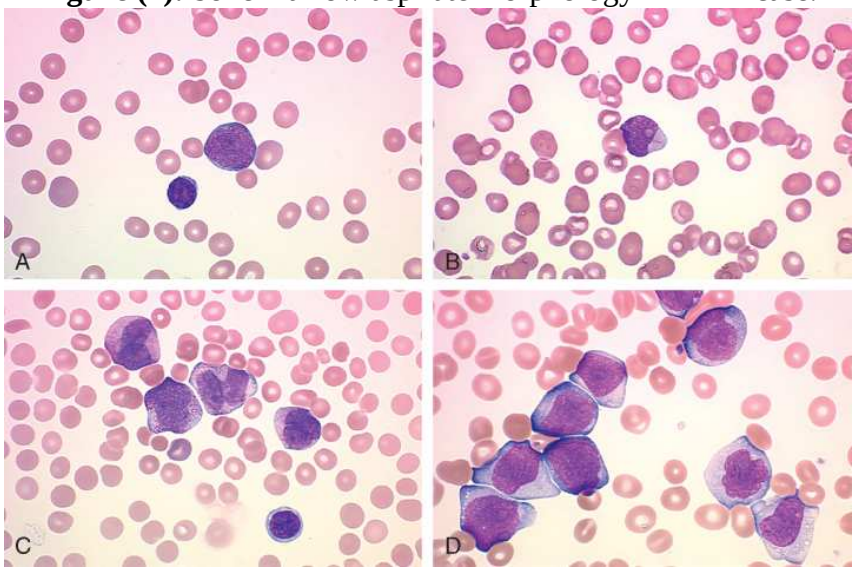

Figure (3): Show that Blast morphology can provide clues about the type of acute myeloid leukemia (AML), but definitive subclassification requires correlation with cytochemistry, flow cytometry, and cytogenetics. (A) Typical myeloid blast morphology not otherwise diagnostic is shown. The blast is larger than a normal lymphocyte and has less condensed chromatin, scant cytoplasm, and a prominent nucleolus. (B) Myeloid blasts may contain cytoplasmic Auer rods. (C) Blasts in AML with t(15;17) are larger, have more abundant cytoplasm containing fine azurophil granules, and have characteristic bilobed, folded nuclei. (D) Blasts in acute monoblastic leukemia have moderate amounts of agranular cytoplasm and may contain cytoplasmic vacuoles (A-D, Wright-Giemsa stains, 3100).