INTRODUCTION

Myeloid malignancies are cancers that affect one family of cells in the blood and bone marrow. They are clonal disorders that are characterized by acquired somatic mutation in hematopoietic progenitors ***(Fröhling et al., 2005)***. They include acute myeloid leukaemia (AML), myeloproliferative disorders (MPDs), and myelodysplastic syndromes (MDS) ***(Brent, 2007).***

Myeloid leukaemias are a cell autonomous or intrinsic disorders in which the genetic events lead to malignant transformation of a hematopoietic cell that is necessary and sufficient for the generation of leukaemia ***(Lane et al., 2009).***

Myeloid leukaemias are life-threatening disorders of hematopoietic stem cells, depending on the subtype and phase of the disease, type of affected progenitor and deregulated genes. The clinical picture and prognosis vary in these patients ***(Hehlmann et al., 2005).***

Thus, despite recent progress in clinical hematology, the prognosis in most patients with advanced disease is poor ***(Bennett, 2003).*** During the past few years, our knowledge about factors regulating growth and survival of leukaemia cells has increased significantly ***(Sattler et al., 2003).***

Angiogenesis is the formation of new blood vessels from a preexisting network, with continuing expansion of vascular tree in response to an increase in tissue mass ***(Carmeliet, 2005)***. Increased angiogenesis contributes to pathophysiology of solid tumors and other non-malignant diseases (rheumatoid arthritis, diabetic retinopathies, psoriasis, etc…) ***(Shibuya, 2008).***

Recent studies show that angiogenesis and angiogenic factors play an important role in hematological malignancies ***(Li et al., 2008)***. Angiogenesis plays a role in the pathogenesis of high-risk myeloid malignancies and in the mechanisms of disease progression. Secretion of cytokines and growth factors modulates angiogenesis in the marrow leading to increased vascularity and sustenance of the clonal population ***(Estey, 2004).***

In myeloid leukaemia, the increased microvessel density (MVD) is the results of the action of several angiogenic growth factors secreted by leukaemic blasts ***(Loges et al., 2006)***. Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and the angiopoietins (Ang) are recognized as the key players in this process ***(Schliemann et al., 2006).***

Vascular endothelial growth factor (VEGF) is a pleiotropic cytokine and key regulator of angiogenesis that has recently been implicated in the pathogenesis of various malignancies and the formation of metastases ***(Schoenleber et al., 2009).***

Numerous studies have reported high levels of VEGF expression in a wide variety of tumors that have been associated with poor prognosis ***(Podar and Anderson, 2005)***. This evidence has led to the principle that inhibition of VEGF can block tumor angiogenesis, remove the main nutrient supply, and thereby incapacitate tumor growth ***(Kerbel, 2008).***

AIM OF THE STUDY

The aim of this work is to study the expression of vascular endothelial growth factor in myeloid leukaemias and to correlate its level with other prognostic criteria.

MYELOID LEUKAEMIAS

The Nature of Leukaemia

Leukaemia is a disease resulting from the neoplastic proliferation of haemopoietic or lymphoid cells. It results from mutation of a single stem cell, the progeny of which form a clone of leukaemic cells. Usually there is a series of genetic alterations rather than a single event. Genetic events contributing to malignant transformation include inappropriate expression of oncogenes and loss of function of tumour suppressor genes ***(Bain, 2010).***

Leukaemias are broadly divided into: (i) acute leukaemias, which, if untreated, lead to death in weeks or months; and (ii) chronic leukaemias, which, if untreated, lead to death in months or years. They are further divided into lymphoid, myeloid and mixed lineage (biphenotypic or bilineage) leukaemias, the latter showing usual differentiation ***(Bain, 2010).***

World Health Organization (WHO) Classification Myeloid Neoplasms

In 2001, the World Health Organization (WHO), in collaboration with the Society for Hematopathology and the European Association of Haematopathology, published a Classification of Tumors of the Hematopoietic and Lymphoid Tissues as part of the 3rd edition of the series, WHO Classification of Tumors as shown in ***tab.(1) (Jaffe et al., 2001; Vardiman et al., 2009a).***

The classification reflected a paradigm shift from previous schemes in that, for the first time, genetic information was incorporated with morphologic, cytochemical, immunophenotypic, and clinical information into diagnostic algorithms for the myeloid neoplasms ***(Swerdlow et al., 2008).***

In the WHO classification, the term "myeloid" includes all cells belonging to the granulocytic (neutrophil, eosinophil, basophil), monocytic/macrophage, erythroid, megakaryocytic and mast cell lineages. The WHO criteria for myeloid neoplasms apply to initial diagnostic peripheral blood (PB) and bone marrow (BM) specimens obtained prior to any definitive therapy for a suspected hematologic neoplasm. Morphologic, cytochemical, and/or immunophenotypic features are used for establishing the lineage of the neoplastic cells and for assessment of their maturation ***(Vardiman et al., 2009a).***

The blast percentage remains a practical tool for categorizing myeloid neoplasms and judging their progression. In the WHO scheme, a myeloid neoplasm with 20% or more blasts in the PB or BM is considered to be acute myeloid leukemia (AML) when it occurs de novo, evolution to AML when it occurs in the setting of a previously diagnosed myelodysplastic syndrome (MDS) or myelodysplastic/ myeloproliferative neoplasm (MDS/MPN), or blast transformation in a previously diagnosed myeloproliferative neoplasm (MPN). In some cases associated with specific genetic abnormalities, however, the diagnosis of AML may be made regardless of the blast count in the PB or BM. The 20% blast threshold is not a mandate to treat the patient as having AML or blast transformation; therapeutic decisions must always be based on the clinical situation after all information is considered ***(Vardiman et al., 2009a).***

Table (1): WHO classification of myeloid neoplasms.

Myeloproliferative neoplasms (MPN)
Chronic myelogenous leukemia, BCR-ABL 1-positive
Chronic neutrophilic leukemia
Polycythemia vera
Primary myelofibrosis
Essential thrombocythemia
Chronic eosinophilic leukemia, not otherwise specified
Mastocytosis
Myeloproliferative neoplasms, unclassifiable
Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalities of PDGFRA, PDGFRB, or FGFR1
Myeloid and lymphoid neoplasms associated with PDGFRA rearrangement
Myeloid neoplasms associated with PDGFRB reaqrrangement
Myeloid and lymphoid neoplasms associated with FGFR1 abnormalities
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
Chronic myelomonocytic leukemia
Atypical chronic myeloid leukemia, BCR-ABL 1-negative
Juvenile myelomonocytic leukemia
Myelodysplastic/myeloproliferative neoplasms unclassifiable
Provisional entity: refractory anemia with ring sideroblasts and thrombocytosis
Myelodysplastic syndrome (MDS)
Refractory cytopenia with unilineage dysplasia
Refractory anemia
Refractory neutropenia
Refractory thrombocytopenia
Refractory anemia with ring sideroblasts
Refractory cytopenia with multilineage dysplasia
Refractory anemia with excess blasts
Myelodysplastic syndrome with isolaged del(5q)
Myelodysplastic syndrome, unclassifiable
Childhood myelodysplastic syndrome
Provisional entity: refractory cytopenia of childhood

(Vardiman et al., 2009a).

Acute Myeloid Leukemia

Definition

Acute myeloid leukemia (AML) is a group of neoplastic disorders characterized by an increase in the number of immature myeloid cells in the bone marrow with or without involvement of the peripheral blood, resulting in bone marrow failure syndrome. AML can be divided into two subtypes: de novo, when it is not caused by chemotherapy or another preceding haematological condition, and secondary, when it is derived from such a condition ***(Villela & Bolanõs-Meade, 2011).***

Epidemiology

AML is more frequently seen in older adults. The incidence in the US is 3.5 cases per 100 000, being higher in patients over the age of 65 years compared with younger patients (15.9 vs 1.7, respectively), and causes approximately 2.1% of all cancer deaths in the US, with an annual death rate of 3.2 per 100 000 in 2007 ***(Altekruse et al., 2007; Surveillance Research Program, 2010).***

Risk Factors

For most types of leukemia, the risk factors and possible causes are not known. Leukemia risk factors include:

- Exposure to very high levels of radiation.
- Exposure to certain chemicals such as benzene and formaldehyde.
- Smoking cigarettes increases the risk of acute myelogenous leukemia.
- Receiving chemotherapy as alkylating agent.
- Down syndrome and other genetic conditions.
- Viruses - HTLV-1 (human T-lymphotropic virus) and HIV (human immunodeficiency virus).
- Certain blood disorders, such as myelodysplastic syndromes ***(Lichtman & Liesveld, 2007).***

Pathogenesis

AML results from a series of somatic mutations in either a hematopoietic multipotential cell or, occasionally, a more differentiated, lineage restricted progenitor cell. Somatic mutation results from a chromosomal translocation in the majority of patients ***(Kelly & Gilliland, 2002).*** The translocation results in

rearrangement of a critical region of a proto- oncogene. Fusion of portions of two genes usually does not prevent the processes of transcription and translation; thus, the fusion gene encodes a fusion protein that, because of its abnormal structure, disrupts a normal cell pathway and predisposes to a malignant transformation of the cell as shown in ***fig. (1) (Lichtman & Liesveld, 2007).***

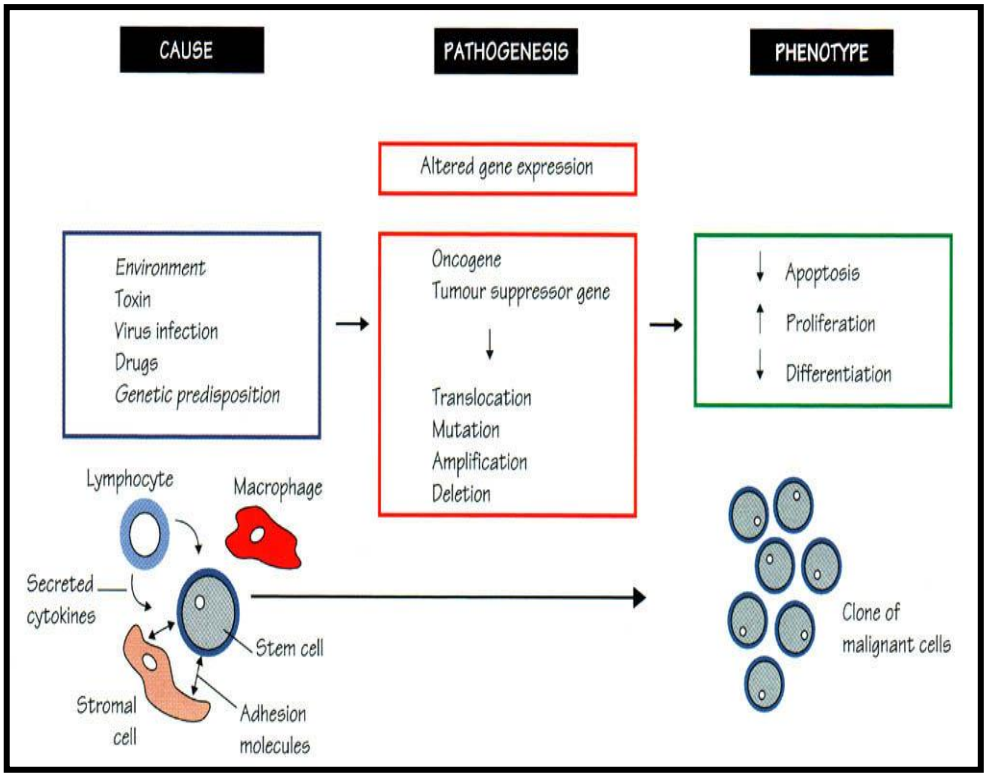

Figure (1): Risk factors and pathogenesis of haematological malignancies *(Maheta & Hoffbrand, 2000).*

Classification

The acute leukemias have been traditionally classified by the FAB (French-American-British) classification system as shown in ***tab. (2).*** The FAB classification is based largely on morphology and a few cytochemical stains and has limited significance in terms of prediction of prognosis and choice of therapy .

Table (2): FAB classification of AML (1985).

M0- AML with minimal differentiation
M1- Myeloblastic leukemia without maturation
M2- Myeloblastic leukemia with maturation
M3- Acute promyelocytic leukemia
M4- Acute myelomonocytic leukemia
M5- Acute monoblastic leukemia
M6- Acute erythroblastic leukemia
M7- Acute megakaryoblastic leukemia

(Shah & Agarwal, 2008).

Over the last decade the FAB classification has been increasingly supplemented or replaced by the World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues as shown in ***tab. (3) (Swerdlow et al.,2008).***

Table (3): WHO classification.

Acute Myeloid Leukemia with Recurrent Genetic Abnormalities
Acute myeloid leukemia with t (8; 21) (q22; q22), (AML1/ETO)
Acute myeloid leukemia with abnormal bone marrow eosinophils and inv (16) (p13q22) or t (16; 16) (p13; q22), (CBFβ/MYH11)
Acute promyelocytic leukemia with t (15; 17) (q22; q12), (PML/ RARα) and variants
Acute myeloid leukemia with 11q23 (MLL) abnormalities
Acute Myeloid Leukemia with Multilineage Dysplasia
Following MDS or MDS/MPD
Without antecedent MDS or MDS/MPD, but with dysplasia in at least 50% of cells in 2 or more myeloid lineages
Acute Myeloid Leukemia and Myelodysplastic Syndromes, Therapy Related
Alkylating agent/radiation-related type
Topoisomerase II inhibitor-related type (some may be lymphoid)
Others
Acute Myeloid Leukemia, Not Otherwise Categorized
Acute myeloid leukemia, minimally differentiated
Acute myeloid leukemia without maturation
Acute myeloid leukemia with maturation
Acute myelomonocytic leukemia
Acute monoblastic/acute monocytic leukemia
Acute erythroid leukemia (erythroid/myeloid and pure erythroleukemia)
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma

(Shah & Agarwal, 2008).

Genetic Abnormalities in AML

Mutations in a number of genes that confer a proliferative and/or survival advantage to cells but do not affect differentiation (Class I mutations) have been identified in AML, including mutations of FLT3, ALM, oncogenic Ras and PTPN11, and the BCR/ABL and TEL/PDGFbR gene fusions. Similarly, gene mutations and translocation-associated fusions that impair differentiation and apoptosis (Class II mutations) in AML include the AML/ETO and PML/RARa fusions, MLL rearrangements, and mutations in CEBPA, CBF, HOX family members, CBP/P300, and co-activators of TIF1 ***(Shah & Agarwal, 2008).***

AML results when hematopoietic precursor cells acquire both Class I and Class II genetic abnormalities. Although only one cytogenetic or molecular abnormality has been reported in many cases of AML, new molecular tools now are identifying multiple genetic mutations in such cases ***(Rubnitz et al., 2010).***

The abnormalities t(8;21), inv(16)(p13q22) or t(16;16) (p 1 3;q22), t(15;17)(q22;q11-12), t(9;1 1)(p22;q23) are associated with a high complete remission rate while del(5q), +13, +8, -7, inv 3, del(12p), t(9;22), or complex abnormalities are associated with a lower rate of complete remission (CR) ***(Rubnitz et al., 2008).***

Translocations of the mixed lineage leukemia (MLL) gene at 11q23 are frequent in childhood AML (14%) and are associated with 65% of infant AML. They confer an intermediate risk. Alterations in receptor tyrosine kinases, tyrosine phosphatases and in oncogenes such as RAS have been implicated in the pathogenesis of AML ***(GJL Kaspers & Zwaan, 2007).***

Another novel mutation of significant importance discovered is the frameshift mutation in exon 12 of the nucleophosmin gene (NPM1) results in aberrant cytoplasmic localization of the NPM protein (NPMc(+)). It occurs in 25% to 35% of adult AML, but is relatively less frequent in childhood AML. There is a favorable impact of NPMc(+) on survival in children lacking FLT3/ITD, which is similar in magnitude to the favorable impact of t(8; 21) and inv (16) ***(Brown et al., 2007).***

In addition to NPM1 mutations and FLT3/ITDs, several new genetic abnormalities have been identified in normal karyotype AML, such as CCAAT/enhancer binding protein alpha gene CEBP α, the neuroblastoma RAS viral oncogene (NRAS), and brain and acute leukemia cytoplasmic (BAALC) gene. CEPBα is associated with a favorable prognosis. Other abnormalities including BAALC genes are associated with poor prognosis ***(Marcucci et al., 2008; Schlenk et al., 2008).***