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Adult Acute Myeloid Leukemia – A Possible Relation to Disease Invasion and the Impact of Independent Prognostic Markers Associated with Survival Outcome

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Additional information is available at the end of the chapter

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Abstract

Over the past decade, leukemia exists and frequently occurs in adults. Radiation exposure, hereditary syndromes, smoking, age, and many other unknown factors are generally the major risk factors for leukemia. Acute myeloid leukemia (AML) is a hematological malignancy that is dispersed from its beginning and may be perceived as a prototype of metastatic cancer, yet leukemia is considered a highly malignant neoplasms responsible for a large number of cancer-related deaths. In addition, to uninhibited proliferation, leukemic cells disperse early from the bone marrow into the peripheral blood, followed by an infiltration of various organs such as lymph nodes, liver, spleen, lungs, intestinal tract, skin, or mucous membranes. Several studies are concerned with the critical role of angiogenesis in the development and growth of solid tumors and hematological malignancies. Moreover, angiogenic mediators created by AML cells act through external or internal autocrine loops, thereby directly inducing cell survival, spread, and disease development. In recent years, many researchers focus on angiopoietins (Ang), an innovative family of angiogenic mediators, which have shown to be vital regulators of angiogenesis and vascular stability. Ang-1 and its antagonist Ang-2 act via the receptor tyrosine kinase sTie 2, which is expressed in endothelial cells (ECs) of the vasculature and in subset of hematopoietic stem cells. Binding of Ang-1 causes phosphorylation of sTie2 and ensures the integrity of the vasculature by stimulating interactions between ECs and endothelial support cells. This chapter reviews the incidence, mortality, pathogenesis, and diagnostic procedures of AML. As well as aims at evaluating serum levels of endostatin, MMP- 9, and uPAR in acute myeloid leukemia patients before chemotherapy and after achieving complete remission. At the same time, the chapter also assesses the pretreatment levels of plasma Ang-1, Ang-2, and sTie2, and the calculated ratio of Ang2/sTie2 receptor in a cohort of AML patients also studies their impact on the AML patients' overall survival.

Keywords: AML, endostatin, MMP- 9, uPAR, Angi-1, Angi-2, sTie2

1. Introduction

Cancer is considered as one of the major causes of mortality in the world. Despite the recent advances in science, cancer has not been cured yet [1]. Healthy cells are different from cancer cells. Cancer cells can do what others cells cannot such as: become resistant to growth inhibition; evade apoptosis; invade, metastasize, duplicate without limits; and support angiogenesis [2]. Cancer death rates remained approximately the same in the United States from 1975 through 2002, unlike heart disease. It is predicted that there will be about 15 million new cancer cases worldwide diagnosed and roughly 12 million cancer patients could die by 2020 [3].

Leukemia is a cancer of the body's blood-forming tissues. It's caused by the rapid production of abnormal white blood cells. The high number of abnormal white blood cells are not able to fight infection. The white blood cells can debilitate the ability of the bone marrow to produce red blood cells and platelets, figure 1 [4]. Leukemia develops when blood stem cells in the bone marrow change. The bone marrow will no longer grow and behave abnormally. The cells that are abnormal are called leukemia cells, figure 2. Leukemia cells will then start to crowd out the normal blood cells, causing the normal blood cells to not do their normal jobs over time [5].

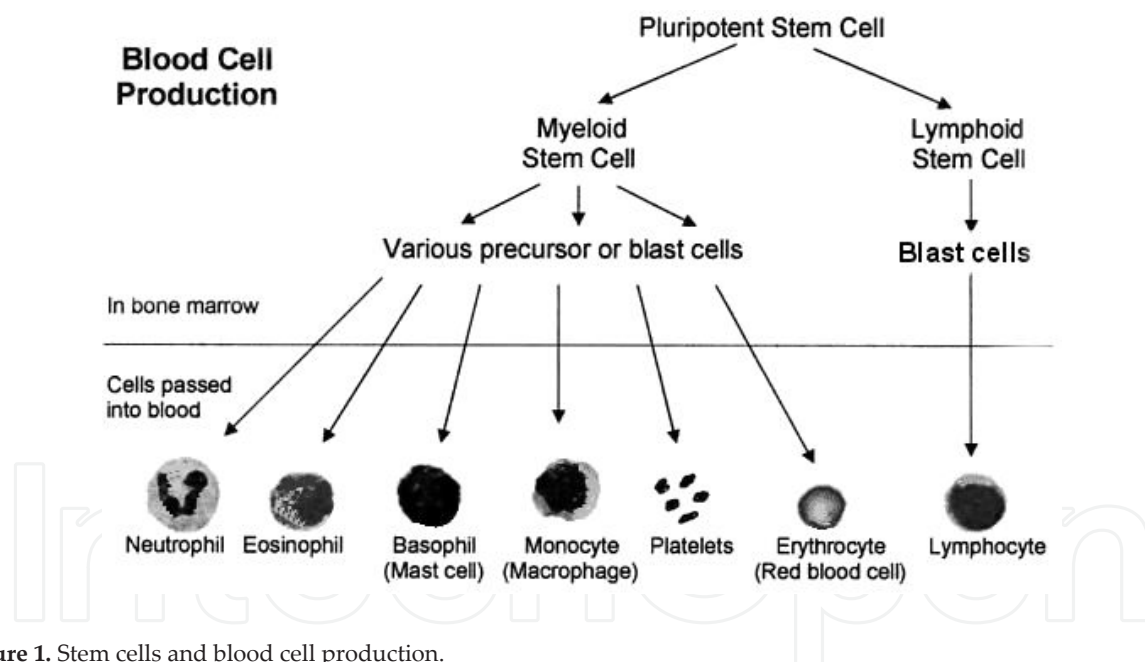


Figure 1. Stem cells and blood cell production.

There are many types of leukemia that exist. There are forms of leukemia that are more common in children, while other forms of leukemia only occur in adults. There are many types of risk factors for leukemia and they include, among others, age, hereditary syndromes, radiation exposure, smoking, and other unknown factors [6]. Furthermore, depending on the type of leukemia the signs and symptoms will vary. The type of blood that the stem cell leukemia develops determines the type of leukemia. Abnormal lymphoid stem cells develop lymphocytic leukemias, also known as lymphoblastic leukemias. Abnormal myeloid stem cells develop myelogenous leukemias [7, 8].

A genetically heterogeneous clonal disorder called acute myeloid leukemia (AML) is characterized by the accumulation of somatic genetic alterations in hematopoietic progenitor cells that transform mechanisms of self-renewal, differentiation, and proliferation [9]. Approximately 55% of adults with AML are detected by non-random clonal chromosome aberrations (i.e., balanced translocations, deletions, inversions, monosomies, and trisomies). These chromosome changes have been recognized as the most important prognostic factor of complete remission, risk of relapses, and long-term survival and have contributed to disease designation [10, 11]. A number of gene mutations and deregulated expression of genes have been identified in recent years, clarifying the immense heterogeneity of cytogenetically-defined AML subsets, especially the larger subsets of AML showing normal karyotype [12, 13].

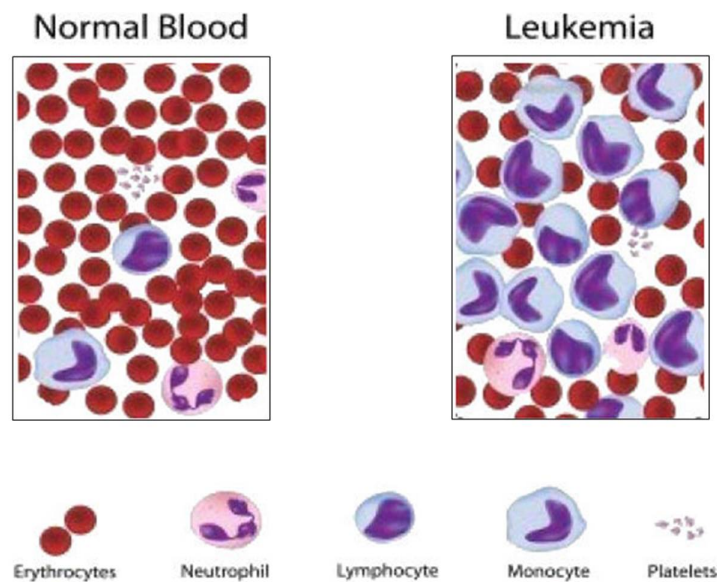


Figure 2. The normal and leukemic cells in the bone marrow.

Numerous studies have shown that angiogenesis is a crucial part in the growth and development of hematological malignancies and solid tumors [14]. Furthermore, there are several angiogenic mediators produced by AML cells that act through internal and external loops; thus promoting cell survival, spread of the disease, and proliferation [15]. It has been proven that basic fibroblast growth factor (b FGF) and vascular endothelial growth factor (VEGF) are major regulators of tumors with angiogenesis in AML; cellular VEGF represents an adverse prognostic factor [16].

In addition, many studies have focused on angiopoietins (Ang), which are a family of novel angiogenic mediators. Ang have been shown to be important regulators of angiogenesis and vascular stability [17]. Ang-1 and its antagonist, Ang-2, act through the tyrosine kinase sTie 2 receptor. These are expressed in endothelial cells (ECs) of the vasculature and in a subset of hematopoietic stem cells [18].

Acute leukemias are known to be a rare disease, but have a disproportionately large effect on cancer survival statistics [19]. They are the most common type of leukemia in adults, yet they

continue to have the least survival rate of all other leukemias. Although, the rates have improved remarkably in the younger age groups, the prognosis in older patients continues to be very poor [20, 21].

2. Incidence and mortality

Incidence of acute leukemia accounts for <3% of all cancers. Leukemia constitutes as the leading cause of death due to cancer in children and persons <39 years old [22]. The most frequent form of leukemia is AML. AML accounts for about 25% of all leukemias in the Western world in adults [23]. U.S., Australia, and western Europe have the highest AML incidence worldwide [24]. AML in the U.S. during the years 1975–2003 was approximately 3.4 occurrences per 100,000 persons (= 2.5 per 100,000 persons when age-adjusted to the world standard population) [25].

The mortality associated with AML, just like its incidence, varies with factors such as age, gender, and race. The age-adjusted mortality rate in the U.S. seem to increase with age and peaks at 17.6 per 100,000 persons in people aging between 80 to 84. The age adjusted mortality rate for females in the years between 2000–2003 was 2.2 per 100,000, where it appears in 3.5 per 100,000 in males in the same period. Estimates show that approximately 7,800 adults will die annually of AML in the U.S. [26, 27]. However, an estimated 54,270 new cases of leukemia are expected in 2015 [28].

3. Etiology

Several risk factors have been associated with AML. Known risk factors only account for a small number of cases that were observed [29]. This will include age, antecedent hematologic disease, and genetic disorders, as well as exposures to chemical or other occupational hazards, radiation, viruses, chemical, and previous chemotherapy [30, 31]. There are several congenital conditions that may increase the risk of leukemia; the most common is most likely Down syndrome, which is associated with a 10- to 18-fold increase in the risk of AML [32].

4. Pathogenesis

A repercussion of specific chromosome translocations of the pathogenesis of AML are associated with the appearance of oncogenic fusion proteins. One of the fusion proteins is generally a transcription factor where the other partner is a variable in function and often involved in the control of apoptosis and cell survival. AML-associated fusion proteins function as aberrant transcriptional regulators that interfere with the process of myeloid differentiation, which determines the stage-specific arrest of maturation and enhance cell survival in a cell-type specific manner as a consequence [33].

The French-American-British (FAB) system (Table 1) described AML subtypes as M0 through M7. However, AML has been reclassified by the World Health Organization (WHO) into four categories in an attempt to predict the prognosis and biologic properties of AML subcategories more accurately and to enhance the clinical relevance of the system [34].

French - American - British classifications
M0: Myeloblastic without maturation
M1: Myeloblastic with minimal maturation
M2: Myeloblastic with maturation
M3: Promyelocytic; M3v: Promyelocytic ("microgranular")
M4: Myelomonocytic; M4 Eo: Myeloblastic with abnormal eosinophils (Eo)
M5: Monocytic: poorly (M5a) or well differentiated (M5b)
M6: Erythroleukemia
M7: Megakaryoblastic

Table 1. AML classification.

5. WHO classification

According to WHO, AML classifies depending on morphology, immunophenotype, genetics, and the combination of clinical features [35]. In the hopes that future work will elucidate molecular pathways that may be amenable to targeted therapies, the classification tries to identify biologic entities (Table 2) [36, 37]. The subgroup "AML with recurrent genetic abnormalities" amounts to some primary AML entities. "AML with t (8;21) (q22;q22); RUNX1-RUNX1T1" and "AML with inv (16) (p13. 1;q22) or t (16;16) (p13. 1;q22); CBFβ-MYH11" are considered as AML regardless of bone marrow blast counts. In "APL with t (15;17) (q22;q12); PML-RARA", RARA translocations with other partner genes are recognized separately. The former category "AML with 11q23 (MLL) abnormalities" was redefined as "AML with t (9;11) (p22;q23); MLLT3-MLL" and is now a unique entity; balanced translocations other than that involving MLLT3 should be specified in the diagnosis. Three new cytogenetically defined entities were incorporated: "AML with t (6;9) (p23;q34); DEK-NUP214"; "AML with inv (3) (q21;q26. 2) or at (3;3) (q21; q26. 2); RPN1-EVI1"; and "AML (megakaryoblastic) with t (1; 22) (p13;q13); RBM15-MKL1", a rare leukemia most common in infants. Two new provisional entities defined by the presence of gene mutations were added, "AML with mutated NPM1 [nucleophosmin (nucleolarphosphoproteinB23, Numatrin)]" and "AML with mutated CEBPA[CCAAT/enhancer binding protein (C/EBP), alpha]". There is growing evidence that these two gene mutations represent primary genetic lesions (so-called class II mutations) that impair hematopoietic differentiation.

Categories**Acute myeloid leukemia with recurrent genetic abnormalities**AML with t (8;21) (q22;q22); *RUNX1-RUNX1T1*AML with inv (16) (p13.1q22) or t (16;16) (p13.1;q22); *CBFB-MYH11*APL with t (15;17) (q22;q12); *PML-RARA**AML with t (9;11) (p22;q23); *MLL3-MLL+*AML with t (6;9) (p23;q34); *DEK-NUP214*AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*AML (megakaryoblastic) with t (1;22) (p13;q13); *RBM15-MKL1**Provisional entity: AML with mutated NPM1**Provisional entity: AML with mutated CEBPA***Acute myeloid leukemia with myelodysplasia-related changes†****Therapy-related myeloid neoplasms§****Acute myeloid leukemia, not otherwise specified (NOS)**

Acute myeloid leukemia with minimal differentiation

Acute myeloid leukemia without maturation

Acute myeloid leukemia with maturation

Acute myelomonocytic leukemia

Acute monoblastic/monocytic leukemia

Acute erythroid leukemia

Pure erythroid leukemia

Erythroleukemia, erythroid/myeloid

Acute megakaryoblastic leukemia

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute myelosclerosis)

Myeloid sarcoma (syn.: extramedullary myeloid tumor; granulocytic sarcoma; chloroma)**Myeloid proliferations related to Down syndrome**

Transient abnormal myelopoiesis (syn.: transient myeloproliferative disorder)

Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm**Acute leukemias of ambiguous lineage**

Acute undifferentiated leukemia

Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged

Mixed phenotype acute leukemia, B/myeloid, NOS

Mixed phenotype acute leukemia, T/myeloid, NOS

*Provisional entity: Natural killer(NK)-cell lymphoblastic leukemia/lymphoma***Table 2.** Acute myeloid leukemia and related precursor neoplasms and acute leukemias of ambiguous lineage (WHO).

6. Diagnostic procedures

6.1. Morphology

AML is first described by its morphology figure 3, or what the cancer cells look like when put under a microscope. The type of normal, immature white blood cell most closely resembles classified AML. The cancer that is in the cells that normally produce neutrophils is a subtype called myeloid leukemia in most patients with AML.

Using a May-Grunwald-Giemsa or a Wright-Giemsa stain, blood and marrow smears are morphologically examined. Using this method, they can give information about blood diseases (e.g., anemia, leukemia) that will change the aspect, leukocytes, number, size, or shape of erythrocytes and platelets [38]. 200 leukocytes on blood smears and 500 nucleated cells on marrow smears, with the latter containing spicules, is recommended to be counted. A marrow or blood blast count of 20% or more is required to be a diagnosis of AML, except for AML with t(8;21), t(15;17), t(16;16), or inv(16) and in a few cases of erythroleukemia. Included in the blast count are myeloblasts, monoblasts, and megakaryoblasts. AML with monoblasts, monocytic, or myelomonocytic differentiation and promonocytes, however, not abnormal monocytes are counted as blast equivalents. Only rare instances of pure erythroid leukemia can erythroblasts be not counted as blasts. Occasionally, the cytoplasm of the immature cells may contain abundant basophilic cytoplasm, containing variable numbers of indistinct coalescent granules. The diagnosis is M1 in case the immature cells are <10%, and the diagnosis will be AML-M2 if the immature cells are >10%. AML M2 baso showed a higher number of basophils along with the typical M2 morphology and was sometimes correlated with the t(6;9) [39].

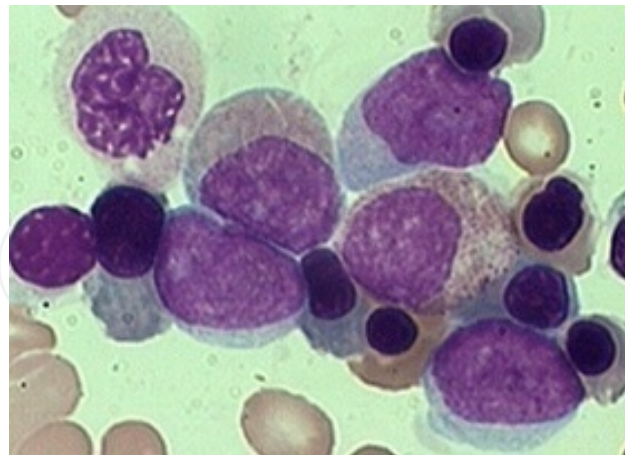


Figure 3. AML-M2 morphology. Note the presence of myeloid maturation.

6.2. Immunophenotyping

Immunophenotyping was performed by using a direct immunofluorescent technique and flow cytometry on peripheral blood specimens anti-coagulated by heparin of a newly diagnosed

acute leukemia. For most markers, a commonly used criterion is 20% or more of leukemic cells expressing the marker [40], whereas for selected markers (e.g., cytoplasmic CD3, MPO, TdT, CD34, and CD117) a lower cutoff has been applied (10%). Phenotype acute leukemia (MPAL) diagnoses lineage assignment, and Measurement of Minimal Residual Disease is used to detect aberrant immunophenotypes (MRD), which is necessary for quantification of expression patterns of several surfaces and cytoplasmic antigens [41]. For morphological evaluation flow cytometry, determination of blast count should not be used as a substitute.

Immunophenotyping establishes the diagnosis of AML with minimal differentiation, acute megakaryoblastic leukemia, and acute leukemias of ambiguous lineage [42]. An AML with minimal differentiation is without morphologic and cytochemical evidence of myeloid differentiation [43].

Acute megakaryoblastic leukemia has 20% or more blasts, of which 50% or more are of megakaryocytic lineage; megakaryoblasts express less commonly CD42 one or more platelet glycoproteins CD41 and/or CD61. Acute Undifferentiated Leukemia (AUL) or those with blasts that express markers of more than one lineage (i.e., MPAL), are acute leukemias of ambiguous lineage, are rare leukemias, and comprise cases that show no evidence of lineage differing. AULs often express CD34 and/or CD38 and/or HLA-DR but lack lineage associated markers. Distinct blast populations or one blast population with markers of different lineages on the same cell or a combination of both can be contained in MPAL. WHO defines MPAL and encompasses subsets that are with or without genetic abnormalities [44].

6.3. Cytogenetic and molecular genetics

Chromosome preparation from peripheral blood and/or bone marrow was done according to standard techniques after culturing for 24 or 48 hours [45]. Colcemid treatment, hypotonic shock, and 3:1 methanol: acetic acid fixation and chromosome analysis were carried out on G-banded metaphase and were included in routine methods for metaphase spread preparations. According to the 1995 International System for Human Cytogenetic Nomenclature at least four metaphase were karyotyped and described [46]. In order to confirm or exclude the presence of leukemia-associated gene rearrangements, molecular genetic studies were performed. Thus, either fluorescence with LSI Dual Color Break Apart Rearrangement probe for Mixed Lineage Leukemia (MLL) or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for detecting AML rearrangements were used [47].

6.4. Genome-wide studies

The major ways to distinguish AML subtypes involve hematopathologic diagnosis with flow cytometry and cytogenetic/molecular analyzes. Essential in the distinction among the subclasses of AML is the notion that different subtypes of AML express different proteins, either at the cell surface, defining AML types distinguishable based on flow cytometry, or as a result of chromosomal rearrangements or gene mutations. Therefore, it is logical that a technique that can define a transcriptional gene expression globally should be able to distinguish among AML subtypes [48]. Identifying novel genetic abnormalities and the promise of making the

systematic characterization of cancer genomes feasible has resulted in progress in genomics technology. For example, gene- and micro RNA-expression profiling have proven valuable for the discovery of novel leukemia subgroups and of prognostic signatures [49]. Uniparental disomy (UPD) is when a genome-wide single nucleotide polymorphism (SNP)-based mapping arrays, giving both copy number and allele-specific information, led to the identification of a novel mechanism involved in the pathogenesis of AML [50]. UPD is due to a mitotic recombination event and may render a cell homozygous for a pre-existing mutation positioned in the affected genomic region. The power of SNP genotyping as a tool for gene discovery is shown by several recent studies [51]. Hopefully, high-throughput DNA sequence analysis will become possible at an affordable cost, while analyses of genomic copy number will continue to be informative with regard to the selection of candidate leukemia genes. This may ultimately result in the development of comprehensive, disease- and allele-specific oncogene mutation profiling strategies [52].

6.5. Additional diagnostic tests

A patient with AML may need additional diagnostic tests and procedures that can confirm the diagnosis. Leukemia usually does not form tumors, so imaging tests are not used for diagnosis and are often used to check infections or other problems that may occur. X-rays routinely looks for suspected lung infection. Moreover, imaging in a few cases may be done to determine disease extent if it is thought to be spread beyond the bone marrow and blood.

6.5.1. *Computed tomography (CT)*

The computed tomography (CT) scan is usually needed if it is suspected that leukemia is growing in an organ, such as the spleen. Unlike a regular x-ray, CT scans can illustrate in detail soft tissues. In some cases, a CT scan can be used to guide the biopsy needle into areas of abnormality, such as an abscess [53].

6.5.2. *Magnetic resonance imaging (MRI) scan*

Asymptomatic patients are diagnosed with acute leukemia after the identification of abnormal peripheral blood counts. However, magnetic resonance imaging (MRI) has found that abnormal bone marrow signals appear in patients who have not been previously diagnosed with leukemia. The differentiation between benign marrow edema and tumorous involvement of the bone marrow is the measurement of the tissue microstructure the diffusion-weighted imaging reflects the random motion of water protons. MRI enables precise assessment of bone marrow infiltration early and before osteolytic changes become visible by conventional radiology imaging or CT scans [54].

6.5.3. *Ultrasound*

Ultrasound uses sound waves and ultrasound echoes and then produces a picture of internal organs or masses. Usually for this test, a small, microphone-like instrument called a transducer is placed on the skin. These transducers emit sound waves and pick up echoes as they bounce

off organs in the body. The echos are then converted into an image that is displayed on a computer. Ultrasounds are used to look at the lymph nodes near the surface of the body or look at the enlarged organs inside the abdomen such as the kidneys, liver, and spleen [55].

7. Prognostic factors

The patient characteristics and general health condition of those related to characteristics particular to the AML clone are prognostic factors that may be subdivided. Patient characteristics and general health condition usually predict treatment-related mortality (TRM) and becomes important as patient age increases. Characteristics particular to the AML clone predicts resistance to at least conventional therapy. The following studies are aimed at assessing the pretreatment levels of plasma Ang-1, Ang-2, and sTie2, and the calculated ratio of Ang-2/sTie2 receptor in AML patients. Moreover, it aims to evaluate serum levels of endostatin, MMP- 9, and uPAR in AML patients before chemotherapy and after achieving complete remission. At the same time, it also studies the impact in the lives AML patients and their overall survival.

7.1. Circulating angiopoietin-2 is a strong prognostic factor in AML

The prognostic significance and over expression of cellular angiopoietin in the isolated peripheral AML blast and AML bone marrow is not demonstrable. Loges (2005) [56] showed that patients with high cellular Ang-2 had extended overall survival compared to those with low Ang-2 expression. Previously, we assessed the pretreatment levels of plasma Ang-1, Ang-2, and sTie2, and the calculated ratio of Ang-2/sTie2 receptor in a cohort of 71 AML patients in order to evaluate the impact in the lives of AML patients and their overall survival.

7.1.1. Materials

Seventy-one newly-diagnosed AML patients were tested in this study. Table 3 shows the patients' characteristics. All 71 patients were tested and followed up to 24 months (Table 3) or up until their deaths. Patients were followed in the oncology department at Mansoura Cancer Institute in Egypt where they were treated with approved protocols. 3+7 protocols (Daunorubicin 45 mg/m² iv days 1–3; Cytarabine 100 mg/m²/day continuous infusion for 7 days) were used to treat the AML patients. Upon post remission, there was a high dose of cytarabine. Salvage therapy (HAM protocol) was applied (Cytarabine 3 gm/m² bid iv 3 hours infusion days 1–3; Mitoxantrone 10 mg/m² iv days 3–5) for patients who did not respond to the induction therapy. Complete hematological remission patients (bone marrow blast cells <5% in bone marrow) were submitted to consolidation therapy that contained a high dose cytarabine containint regimine (2 g/m²/2hours/day × 4 days) + Daunorubicin 45 mg/m²/day ×3 days. The M3 patients received all trans retinoic acid and went through chemotherapy. Nineteen normal, healthy subjects made up the normal control group.

Parameters	AML Patients
NO	71
Median age / years (range)	34 (16–55)
Male	40
Female	31
Mo	2
M1	12
M2	19
M3	3
M4	18
M5	10
M6	4
M7	3
Karyotypes	
Favorable; t(8:21), t(15-17), inv(16)	21
Intermediate; normal, +8, +22, others	41
Poor complex, -5, -7	9
Peripheral WBCs × 103/cmm	16.0 (9.5–89.2)
Peripheral blast cells %	20 (12.0–70.0)
Bone marrow Blast cells%; median(range)	55% (12–88%)
Follow up	24 months

Table 3. Patients' characteristics.

7.1.1.2. Methods

In a sterile tube with Ethylenediaminetetraacetic acid (EDTA), 6 ml of peripheral blood were collected from each AML patient at presentation and before the start of induction chemotherapy. For ten minutes in a refrigerated centrifuge, the plasma was separated by centrifugation at 1500 Xg. The separated plasma was reserved at -70°C, thawed in use. Using commercially available kits from R&D systems (Minneapolis, MN, USA) and according to the manufacturer's instructions, Enzyme-linked immunosorbent assay (ELISA) were performed. Briefly, patient samples using the anticoagulant were collected with Ethylenediaminetetraacetic acid (EDTA) and stored at -80°C. Plasma samples were transferred to separate microplates, each containing a specific antibody for Ang-1, Ang-2, or sTie2. At room temperature, the mixtures were incubated for 2 hours. Plates were washed 4 times to remove unbound antigen. Enzyme-linked polyclonal antibodies specific for each angiogenic factor were then added, then incubated for 2 hours, followed by another washing step. At color development, adding of the substrate was

stopped and a standard curve was used to compare the intensity of the color measure. Optical density of each well was determined at 570 nm.

7.1.3. Statistics

The software package SPSS version 10 has been used in this study. Mann-Whitney rank sum test for independent groups were used to analyze the differences in angiogenic factor level between AML and control groups. Furthermore, the Spearman rank correlation coefficient (R_s) was applied to assess the correlations between continuous variables. The Kaplan-Meier method was used for survival curves estimation. Overall, survival was the primary outcome of the studies and was calculated from the date of the first diagnosis to the death of the patient from any cause. In order to evaluate the predictive effect of each angiogenic factor, the univariate and multivariate Cox regression analysis was performed (figure 4, 5). Optimal cut-off points depend on 50 percentile of each angiogenic factor.

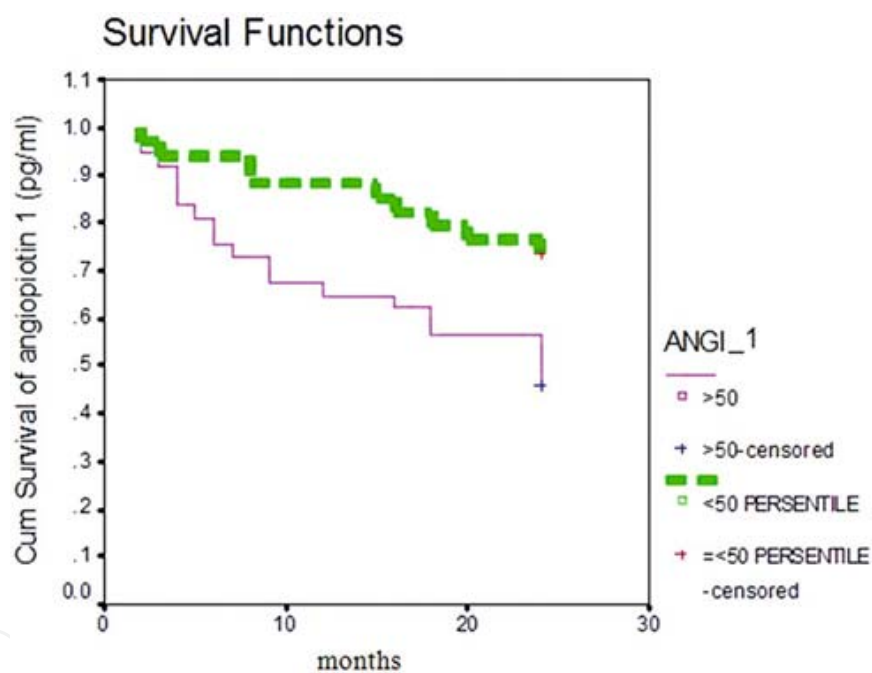


Figure 4. Kaplan-Meier survival analysis of AML patients according to Ang-1 levels. AML patients with high Ang-1 levels (≥ 260) displayed significantly poor survival rates than those with low Ang-1 levels (≤ 260) ($P=0.018$).

7.1.4. Results

7.1.4.1. Comparison of plasma levels of Ang-1, Ang-2, sTie2, and Ang-2/sTie2 ratio in AML patients versus healthy controls

The plasma levels (median and range) of Ang-1, Ang-2, and sTie2 in pre-therapeutic AML patients and healthy volunteers are illustrated in Table 3. Circulating levels of Ang-2 and the calculated Ang-2/sTie2 ratio are decidedly higher in AML patients compared with controls

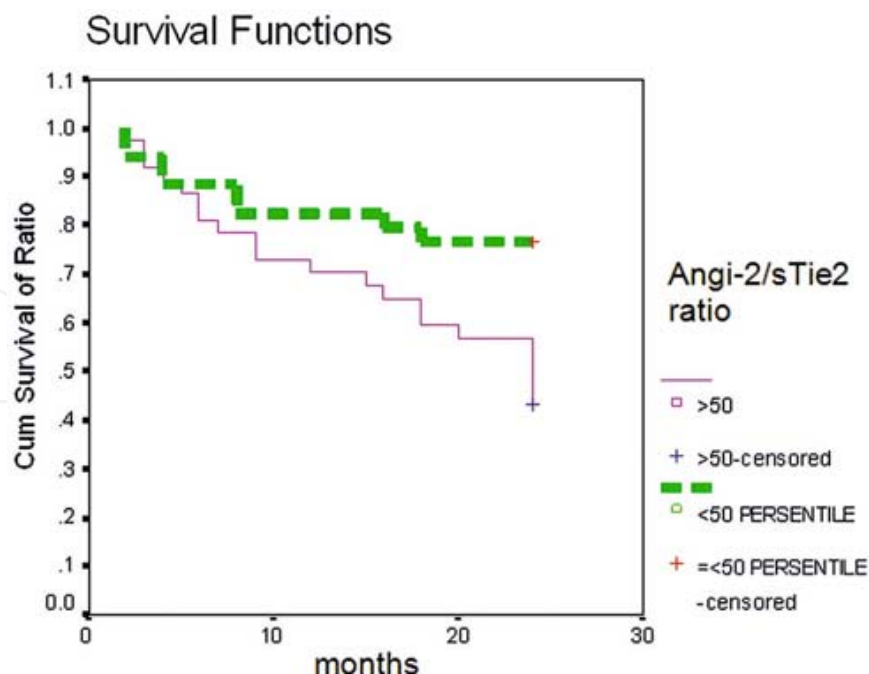


Figure 5. Kaplan-Meier survival analysis of AML patients according to Ang-2/sTie ratio. AML patients with a high Ang-2/sTie ratio (>279) displayed significantly poor survival rates than those with low Ang-2/sTie ratio (≤ 279) ($P=0.004$).

($P=0.002$, $P=0.015$ respectively). In AML patients, the Ang-1 and sTie2 levels were not significantly compared with the controls (Table 4).

	Ang-1 (pg/ml)	Ang-2 (pg/ml)	sTie2 receptors ng/ml	Calculated Ang-2/sTie
Patients (n=71)	260(0–2250)	1400(133–4800)	3.9(1.0–35)	279 (32.4–1233)
Control (n=19)	180 (0–2195)	392 (185–1520)	3.1 (2–3.9)	139 (78–471)
P value	0.573	0.002**	0.057	0.015*

Table 4. Plasma angiopoietins levels in AML patients as compared to controls.

7.1.4.2. The association between plasma levels of Ang-1, Ang-2, sTie2, calculated Ang-2/sTie2 ratio, and clinico-pathological features

A significant correlation was obtained between Ang-2, determined Ang-2/sTie2 ratio, as well as patients' age with P value <0.05 . On the other hand, no significant correlation was seen with sTie2 receptor that gives a P value of 0.786. Moreover, significant correlation was observed with Ang-2, sTie2, and WBCs with $r=0.338$, $P=0.004$, $r=0.263$, $P=0.027$. At the same time, no significant correlation has been seen in Ang-1 and calculated Ang-2/sTie2 ratio. The cell blast percentage in peripheral smears were significantly correlated to Ang-2 and sTie2 receptors with the following data obtained ($r=0.365$ $P=0.002$, $r=0.387$ $P=0.001$); no significant correlation with Ang-1 or Ang-2/sTie2 ratio (Table 5). Significant correlation appears clearly between LDH and Ang-1, Ang-2, and sTie2, contrary with Ang-2/sTie2 ratio. A positive correlation between

Ang-2 and Ang-2/sTie2 ratio with cytogenetic grades was found. On the other hand, a negative correlation was obtained with sTie2 receptor levels ($P>0.1$).

	Ang-1	Ang-2	sTie2 receptors	Ang-2/sTie2 ratio
Age (years)	0.254 0.030*	0.336 0.004*	-0.033 0.786	0.287 0.015*
WBCs ($\times 10^3$)	0.170 0.155	0.338 0.004*	0.263 0.027*	0.205 0.086
Peripheral blood	0.183 0.127	0.365 0.002*	0.387 0.001*	0.169 0.158
Blast in BM%	0.137 0.254	0.305 0.010*	0.317 0.007*	0.187 0.119
LDH	0.513 0.000*	0.362 0.002*	0.262 0.027	0.146 0.225
Cytogenetic	0.258 0.030*	0.426 0.000*	0.170 0.157	0.333 0.005*

Table 5. Correlation angiopoietins and other prognostic markers.

7.1.4.3. Association between plasma levels of Ang-1, Ang-2, sTie2, calculated Ang-2/sTie2 ratio, and overall survival

In the study, univariate Cox proportional hazard analysis was dependent on 50% as a cutoff. This was done to assess the effect of circulating Ang-1, Ang-2, sTie2 levels, and Ang-2/sTie2 ratio on AML overall survival. The clinic-pathological variable as cytogenetic settled that a significant effect on the overall survival ($p<0.005$) variable as cytogenetic (intermediate vs. good vs. poor) and LDH (≤ 450 vs. >450).

AML survival was significantly associated with angiogenic factors Ang-1 (≤ 260 vs. >260), Ang-2 (≤ 1400 vs. >1400), Ang-2/sTie ratio (≤ 279 vs. >297) but sTie2 showed no effect. The death of Ang-2 was higher for the relative risk (RR) when the base line >1400 pg/ml (RR 5.7, with 95% confidence interval (CI) (0.061–0.50, $p=0.001$) had high significance with this ratio. CIs were 0.084–0.653, $P=0.004$ and the RR was 4.1 95%. No significant role of sTie2 >3.9 ng/ml (RR 1.24 95%, CI 0.481–3.220, $p=0.652$) table 6. Additionally, we performed multivariate Cox regression analysis incorporating all variables that were in significant effect on univariate analysis. The calculated Ang-2/sTie2 ratio was identified to be as the most prognostic factor with significant independent impact on survival ($p = 0.000$) (Table 7).

The findings from this study did not coordinate with Loges et al. (2005), wherein cellular Ang-2 was identified as the predictor of AML patients with favorable prognosis [56]. On the basis that the source of circulating Ang-2 from not only leukemic blasts, but also from other cell types such as endothelial cells [57,58], show the differences between soluble Ang-2 and cellular expression of Ang-2. Other hematological malignancies or solid tumors show limited studies considering prognostic relevance. Moreover, angiosarcoma [59], breast cancer [60], multiple myeloma, chronic myeloid leukemia [61], and recently in acute myeloid leukemia [58], higher levels of Ang-2 have been detected. An attractive therapeutic target when introducing anti-angiogenic strategies in the treatment of AML could be strategies in the treatment of AML for Ang-2.

Variable	No	RR(oddis ratio)	95%	P value
Age (years)				
≤ 25	33	0.894	0.346–2.311	0.817
≥ 25	38	1.12		
Sex				
Male	38	1.11	0.37–1.16	0.818
Female	33	0.88		
WBCS(x 10 ³ /l)				
≤ 16.2 x 10 ³	36	0.104	0.034–0.320	0.000
> 16.2 x 10 ³	35	3.62		
Bone Marrow				
infiltrate	38	0.202	0.073–0.561	0.002
≤ 50	33	4.95		
> 50				
Karyotype				
Good	21	2.34	1.11–4.17	0.014
Moderate	41	1.00	0.02–2.19	0.235
Poor	9	0.27		
LDH (U/L)				
≤ 450	36	0.143	0.049–0.417	0.0000
> 450	35	6.99		
Ang-1(pg/ml)				
260	34	0.306	0.113–0.831	0.0180
> 260	37	3.27		
Ang-2 (pg/ml)				
≤ 1400	34	0.177	0.061–0.510	0.001
> 1400	37	5.65		
S Tie				
receptor(ng/ml)	32	1.2	0.481–3.22	0.652
3.9	39	44.81		
> 3.9				
Ratio				
279	34	0.234	0.064–0.6530	0.004
> 279	37	4.27		0

Table 6. Univariate analysis of overall survival in AML patients.

95% confidence interval for B				
Model	Lower bound	Upper bound	T	P
Ang-1	0.000	0.000	-0.068	0.946
Ang-2	0.000	0.000	1.829	0.072
Ang-2/sTie ratio	-0.002	-0.001	-3.939	0.000**
WBCs	-0.007	0.002	-1.092	0.279
Peripheral Blast	-0.016	0.004	-1.266	0.210
Cell %	-0.010	0.008	-0.299	0.766
Blast in BM %	0.000	0.000	-0.732	0.467
LDH				

**Multivariate regression analysis of angiogenesis markers with other variants clarified that the ratio is the most foretelling predictor (p=0.000).

Table 7. Multivariate regression analysis of all variants.

Ang-2 blocks the therapeutic efficacy that has been manifested in solid tumors [62] and a recombinant Fc fusion protein against the action of angiopoietins is now being studied in a phase I study with patients that have advanced solid tumors. This study has concluded that the calculated ratio between Ang-2/sTie is a factor that should be a part of the decision-making process when choosing to use anti-angiogenic therapy.

7.2. Endostatin levels associated with favorable outcome

Inhibiting angiogenesis and tumor growth have been highly effective using endostatin [63, 64]. By inhibiting proliferation-inducing apoptosis in endothelial cells, it may mediate these biological effects [65]. Endostatin has been shown to induce regression of metastatic tumor in an animal model. The relation between endostatin levels and patients' outcome is scarce and the data surrounding endostatin levels in AML patients are controversial [66]. Tumor derived proteases generate the extracellular matrix protein collagen XVIII and is the C terminal anti-angiogenic fragment. It is not clear in AML patients the levels of prognostic relevance of serum endostatin. The study took serum levels of endostatin before chemotherapy and after complete remission in acute leukemia patients. The study also took the patients' outcome and correlated the endostatin levels.

7.2.1. Patients serum sample

Samples from 8 females and 22 males, with a median age of 37 within a range of 19 to 66 years old, with AML had been taken before chemotherapy. Also, 20 out of 30 patients were tested again once they were in complete remission (CR). From the healthy normal person group, ten

samples were taken and matched with the same age and sex and were evaluated as the control group for reference. Enzyme linked immunosorbent assay (ELISA) were determined using serum endostatin (sE) levels.

7.3. Study results

7.3.1. Endostatin levels in AML

In the control group, endostatin levels ranged from 5–20 ng/ml and have a median of 11.8 ng/ml. Pre-treatment serum endostatin (sE) levels ranged from 3–70 ng/ml and have a median of 14.8. The post-treatment sE levels ranged from 15.8–78 gm/ml with a median of 35 ng/ml figure 6. There was no statistically significant differences that were found between pre-treatment sE levels and normal controls. The post-treatment levels were statistically higher in pre-treatment controls. CR and sE and the relationship between them were then evaluated. Twenty out of the 30 AML patients reached CR. The patients who achieved CR had higher sE levels shown from the Wilcoxon tests.

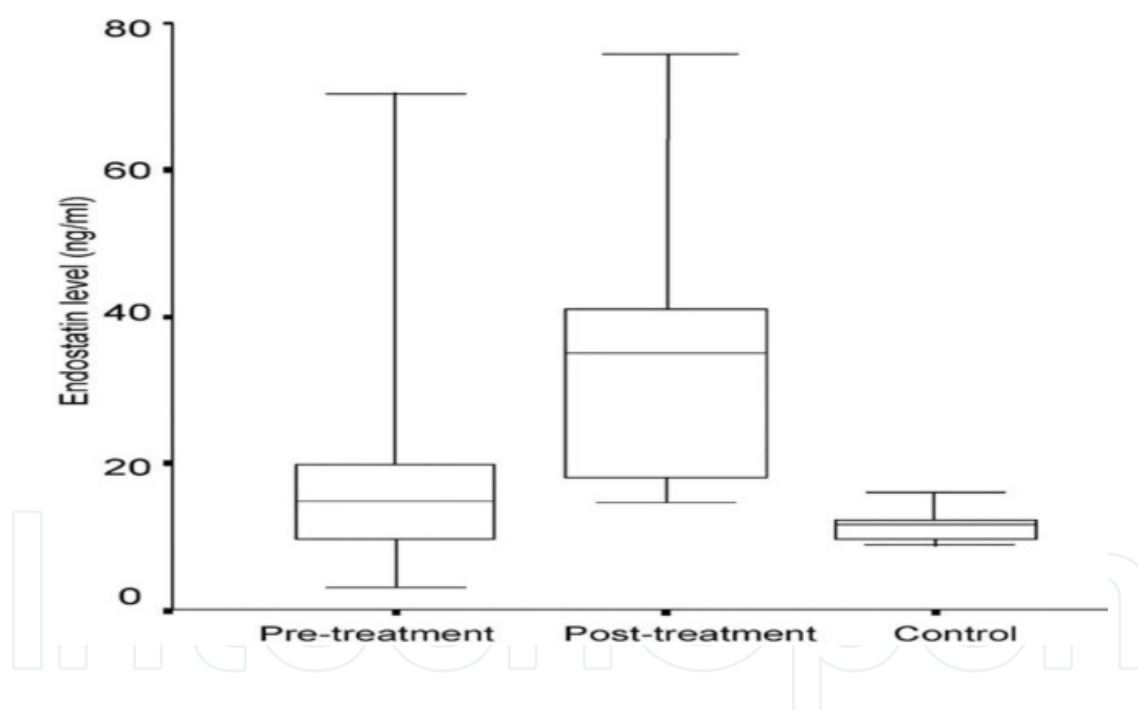


Figure 6. The Box-plot diagram showing the serum endostatin range levels in AML patients pre- and post-treatment compared with controls. Both 25th and 75th percentiles are illustrated with the upper and lower lines of each box. The median indicated with the line appears in each box. Pre-treatment compared to control ($P>0.05$); post treatment compared to control ($P=0.000$); and pre-treatment compared to post treatment ($P= 0.001$).

At the time of diagnosis as illustrated in Table 8, the sE levels increased markedly in the survived group when compared to those who died; the survived AML patients and control groups had a range of 9.6–70 ng/ml and median 38.15 compared with the levels of the died group that had a range of 3–25.5 ng/ml and median 14.8 ($P= 0.04$) and control group with a range 5–20 ng/ml and median 11.8 ($P=0.026$).

Group	Mean \pm SD	Median	Range	P value
Died (n=19)	12.87 \pm 6.07	14.5	(3-25.5)	
Survived (n=6)	56.58 \pm 60.39	38.15	(9.6-70)	0.026
Control (n=10)	11.73 \pm 4.28	11.8	(5-20)	

Table 8. Baseline serum endostatin levels in died AML patients as compared to the survived group as well as normal controls.

The baseline sE level was not significantly correlated to age, hemoglobin level, peripheral WBCs counts, platelet counts, blast cells percentage in bone marrow, and BCDR ($P > 0.05$) (Table 9).

Features	Endostatin	
	r	P
Age	0.166	0.444
Hemoglobin g/dl	0.068	0.745
WBCs $\times 10^3$ /cmm	0.112	0.593
Platelet count $\times 10^3$ /cmm	0.101	0.630
Bone Marrow Blast cell %	0.029	0.889
BCDR	- 0.01	0.98

BCDR (blast cell distribution ratio) = peripheral blast cell % / Bone marrow blast cell %

Table 9. Correlation between baseline endostatin levels and some clinical and laboratory parameters.

The prognostic value of sE was then evaluated by dividing AML patients into low and high sE groups using the 75 percentile level of AML group (i.e., 20.5 ng/ml) as the cut off. As illustrated in Table 10 and Figure 7, high sE patients survived for a significantly longer time than low sE patients ($P = 0.02$).

Endostatin level	Total	Died	% Censored	Mean Survival time	P value
Below 75%	19	17	10.53%	18.84	0.02
Above 75%	6	8	66.67%	36.33	

Table 10. Forty-eight weeks disease-free survival of the studied cases in relation to the pre-treatment serum endostatin level.

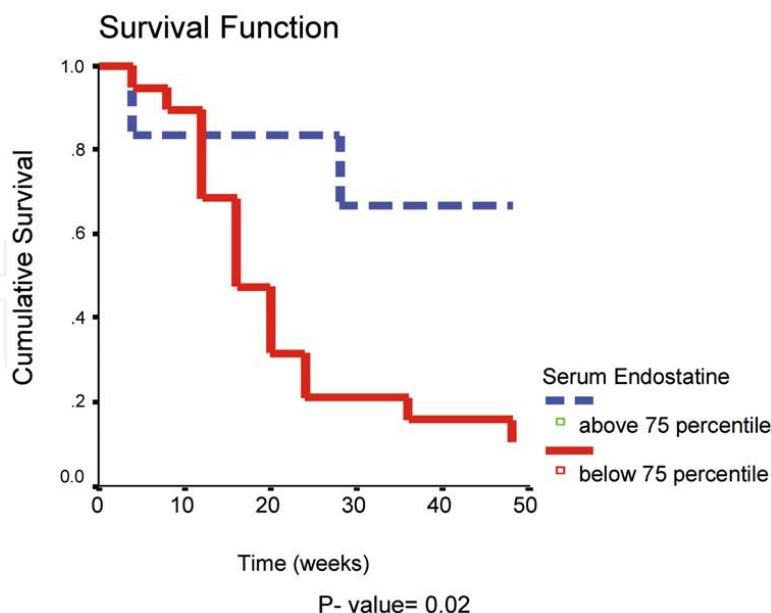


Figure 7. The overall survival of AML patients, according to pre-treatment concentrations of serum endostatin. The 75% concentration level of serum endostatin was used as cut off value.

The study showed that there was not much difference in the endostatin serum levels of the pre-treatment AML patients compared to the normal control group. The baseline for AML patients was lower than at CR. There was no correlation between pre-treatment endostatin levels, age, platelet counts, bone marrow blast cell counts, peripheral white blood cell counts, and blast cell distribution ratio. AML patients were divided into high and low sE groups using the 75 percentile of sE levels of the patient group and showed the prognostic value of sE. The study showed that the group with the high sE levels survived a longer time than the patients that had a low sE levels. The results conclude that the elevated endostatin levels in AML diagnosis is a good prognostic marker for patients' outcome. Wide-scale study is recommended in order to establish the clinical value of this study.

7.4. Role of metalloproteinase and urokinase in acute myeloid leukemia

An important role of the matrix metalloproteinase (MMPs) and urokinase may be important when trying to find tumor invasion and metastasis. An anchored membrane protein that promotes generation of plasmin on the surface of cell types is the Urokinase-type plasminogen activator receptor (uPAR-CD87), which is a glycosyl phosphatidyl inositol (GIP) and facilitates cellular tissue invasion and extravasation. Inflammatory cells or inaction of neoplastic promotes invasion from UPAR by proteolysis of urokinase [67, 68].

The collagens elastin and gelatin are a family of enzymes with the common ability to degrade various components of ECM [69]; they are also called MMPs. This family of enzymes all help the physiological processes that occur when the tissue is remodeling and repairing. The determination of these parameters in the blood are recommended as non-invasive tools in cancer diagnosis and monitoring may be reflected in body fluids called Cellular Concentration of MMPs [70].

Components of ECM, cytokines, hormones, growth factors, or a variety of biochemical stimuli all modulate the transcription of MMPs [71]. Various extracellular proteinases such as plasmin and urokinase are catalyzed by the activation of pro-MMP. Crucial roles of MMPs and soluble urokinase-type plasminogen activator receptor (suPAR) in the invasiveness of many malignant disorders have been accumulated in evidences from recent studies. The studies assessed the levels of MMP-9, uPAR in AML patients and compared them with previous clinicopathological status [72].

7.4.1. *Methods and Patients*

Twenty-five males and 18 females who were recently diagnosed with AML were used in the study. The ages ranged from 26 to 73 years old, with a mean of 46.8. The AML patients were studied at the time they were diagnosed, after starting chemotherapy, and during any relapses. During the induction of chemotherapy, seven patients died. The French-American-British (FAB) study group and immunophenotypic studies performed the diagnosis [73]. There were no patients that had a history of chemotherapy or radiotherapy that were diagnosed with hematological disorders. The criteria proposed by Cheson et al.[74] is the Morphologic CR. Peripheral blood counting was obtained through WBC and a percentage of blast cells. Blast cell distribution ratio (BCDR) was equal to the ratio between peripheral absolute blast cell counts to absolute bone marrow blast cell count.

The following FAB subtypes 2 M7, 4 M3, 4 M4, 4 M6, 6 M1, 7 M5, and 16 M2 were included in the AML patients. The normal subject group was made up of 10 normal subjects that matched the age and sex of the patients were used as the control group. A high-dose combination chemotherapy containing idarubicin, cytarabine, mitoxantrone, and etoposide were treated in the AML patients. Remission induction therapy included one or two courses of cytarabine, etoposide, and idarubicin. The patients that achieved morphological CR received one course of intensive therapy with cytarabine and mitoxantrone. And lastly, all patients got consolidation treatments. All AML patients were then followed around for up to a year. EDTA tubes were used to collect blood samples and kept in ice before the plasma was separated.

The plasma was then removed within 2 hours by centrifugation for 30 minutes at 4°Celsius at 1800 g and stored frozen at -70°Celsius until the examination. Ficoll-Hypaque centrifugation was used to separate the mononuclear cell fraction. Most of the mononuclear cells in AML patients were blast cells that ranged from 0–96%. 1% Triton X-100 and protease inhibitors that were contained in cells were lysed in PBS. Lysates were centrifuged at 14,000 g for 10 minutes and 4°Celsius and the supernatants were stored at -70°Celsius until it was examined. The colorimetric assay kit determined the total protein. UPAR and ELISA were used to analyze the same amount of protein from each sample.

7.4.2. *Immunophenotypic by flow cytometry*

7.4.2.1. *Preparation samples*

Erythrocyte-lysed BM samples were used to diagnose all immunophenotyping study cases. A microscope adjusted to 1×10^6 in each tube quantified the number of cells. The following

monoclonal antibodies were used to analyze antigen expression with fluorochrome-conjugated:

CD34

CD33

CD14

CD11b

CD45

CD10

CD19

CD22

CD3

CD5

CD7

Direct immunofluorescence was performed by first incubating 1×10^6 cells with the specific monoclonal antibody for 15 minutes in the dark at room temperature. To assess background fluorescence intensity, an isotype-matched negative control was used. Cells were lysed (FACS lysis solution) for 5 minutes and centrifuged at 250 g for 5 minutes. Before being re-suspended in PBS and examined, the cells were washed twice with phosphate-buffered saline (PBS). The EGIL recommendations were followed for immunologic criteria for lineage assignment [75].

7.4.2.2. Data analysis

EPICS flow cytometry was measured. For data acquisitions, the Cell quest software program was used. Over 10,000 events/tubes were measured..

Thresholds for positivity were based on isotype negative controls. Analytical gates were set on desired viable cells based on forward light scatter and side light scatter combined with exclusion of normal cells using a CD45 tube. The positivity threshold was 20% for all markers except for cytoplasmic or intra-nuclear antigens for which a 10% threshold was used [76].

7.4.2.3. Assay of suPAR and uPAR in cell lysates

As previously described [77], ELISA and suPAR were used. Immuno-plates were coated overnight with polyclonal antihuman uPAR antibodies. The wells were incubated with standard dilutions of purified recombinant suPAR or with 1:10 dilutions of plasma after blocking and washing. The volume equal to 20 ug of protein was put into each well for the cell lysates to determine protein concentrations. A mixture of monoclonal antihuman upper antibodies were rinsed and then incubated after antigen binding and then followed by alkaline phosphatase-conjugated antibodies. P-nitrophenyl phosphatase substrate was allowed to

develop at room temperature to get a color reaction. Absorbance was read at 405 nm. The assay is 0.03 ng/ml lower detection limit. To estimate the total uPAR load in the cells in the circulation, the exact amount of uPAR in lysates was multiplied by the mononuclear cell count in the peripheral blood. This was tested without specific monoclonal antibodies.

7.4.2.4. Total MMP-9 enzyme-linked immunosorbent assay of plasma

Using a linked immunosorbent assay kit for human total MMP 9 according to the manual was used to determine total MMP-9 activities in the plasma. The diluted plasma samples or MMPs standards were mixed with 100 μ l of 50 μ l anti-MMP immunoglobulin G labeled with horseradish peroxidase in 10 mmol/l ethylenediaminetetra acetic acid. A 100- μ l aliquot of the mixture was transferred to each well that was previously coated with an anti-MMP immunoglobulin G. The horseradish peroxidase-bound activity was detected by adding 100 μ l volume of 0.15 mol/l citric acid sodium phosphate buffer at pH 4.9, containing 2.0 g/l of o-phenylenediamine and 0.02% (v/v) hydrogen peroxide, followed by incubation at room temperature for 20 minutes. Adding 100 μ l sulphuric acid 1 mol/l will stop the action. The micro-plate reader was adjusted to 492 nm for absorbance measurement. The human MMPs values were obtained from the standard curve.

7.4.3. Results

7.4.3.1. AML patients suPAR, cellular uPAR and MMP-9 levels versus control

At diagnosis the AML patients' suPAR and cellular PAR levels that were considered in mean were highly significant compared to the healthy controls with $P=0.001$ for both. However, during AML remission, the suPAR levels declined, achieving levels close to the control levels. On the contrary, during relapse, the levels increased again and came close to diagnosis levels (Table 11, Figure 8).

	suPAR (ng/ml)			cellular uPAR (ng/ mg protein/ 10 ⁹ cells/l)	MMP-9 (ng/ml)		
	At diagnosis	Remission	Relapse		At diagnosis	Remission	Relapse
AML patients	3.01 \pm 0.38	0.7 \pm 0.05	2.1 \pm 0.17	2.1 \pm 0.17	11.21 \pm 1.71	44.5 \pm 0.76	13.56 \pm 1.07
Controls	1.02 \pm 0.14			0.26 \pm 0.02	42.05 \pm 2.18		
P value	0.001	0.02	0.003	0.001	0.001	>0.05	0.001

Table 11. suPAR, cellular uPAR and MMP-9 in AML patients vs. controls.

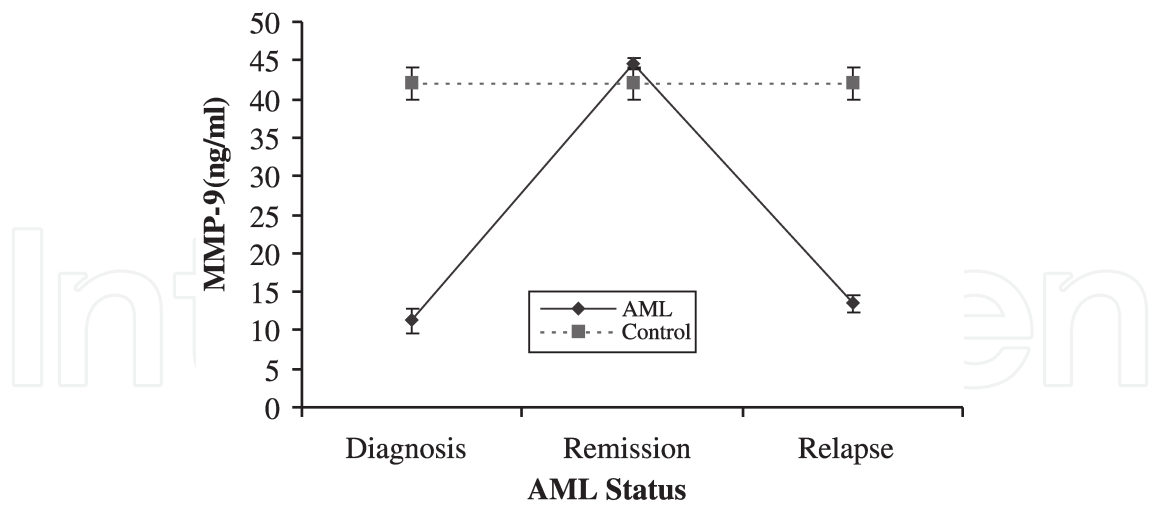


Figure 8. Soluble MMP-9 concentration levels at different AML status.

On the one hand, soluble MMP-9 levels were significantly lower in AML patients at diagnosis as compared to normal control, and elevated during AML remission and declined again during relapse (Table 11, Figure 9).

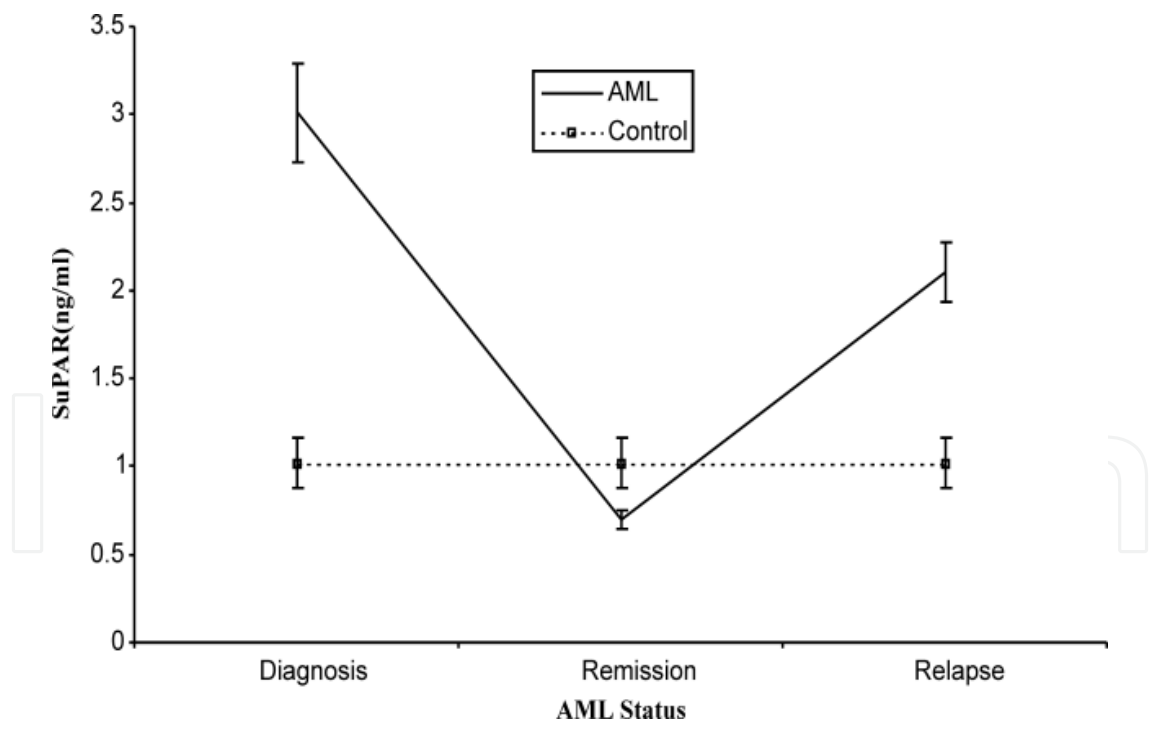


Figure 9. suPAR levels in different AML status.

On the other hand, AML patients who achieved complete induction remission have lower levels for both MMP-9 and suPAR as compared to patients who resist remission ($P=0.001$ for both, Table 12).

AML achieved remission	suPAR (ng/ml)	MMP-9(ng/ml)
Yes (27)	1.72±0.19	14.47±1.15
No (13)	3.27±0.20	34.02±5.2
P	0.001	0.001

Table 12. suPAR and MMP-9 levels at diagnosis in AML patients who achieved complete remission vs. who did not.

The levels of sMMP-9 and suPAR were significantly different according to AML status (Table 13). The MMP-9, cellular uPAR and suPAR diagnosis levels were uneven when classified according to FAB subtypes being highest among M5 (P<0.05 for all) (Tables 13 and 14).

suPAR (ng/ml)			MMP-9(ng/ml)		
Diagnosis	Remission	Relapse	Diagnosis	Remission	Relapse
(n=43)	(n= 36)	(n=10)	(n=43)	(n= 36)	(n=10)
3.01± 0.38	0.07±0.05	2.1±0.17	11.12±1.7	44.5±0.76	13.56±1.07

Table 13. suPAR and MMP-9 levels in different AML states.

	M1(n=6)	M2(n=6)	M3(n=4)	M4(n=4)	M5(n=7)	M6(n=4)	M7(n=2)	P
suPAR	1.9±0.01	3.31±0.57	2.1±0.43	3.6±0.41	5.3±0.4	0.85±0.4	0.80±0.1	0.03
cellular uPAR	0.33±0.1	0.8±0.15	0.4±0.11	0.96±0.14	1.26±0.21	0.53±0.1	0.11±0.11	<0.05
MMP-9	16.2±1.8	18.2±2.5	18.7±4.0	16.1±0.3	28.1±7.1	9.1±2.3	7.7±0.95	<0.05

Table 14. suPAR, cellular uPAR, MMP-9 levels in different FAB subtypes.

7.4.3.2. suPAR, MMP-9, and cellular uPAR levels and extramedullary involvement

In order to study whether MMP-9 and uPAR have significant effects in the AML blast cell invasion, the AML patient's serum levels of MMP-9, suPAR, and cellular uPAR were compared in the presence and absence of extramedullary involvement (Table 15). The AML subgroup with extramedullary involvement exhibit a significant elevation in MMP-9, cellular uPAR, and suPAR with a value P<0.05; P=0.001; 0.001, respectively.

7.4.3.3. Peripheral blast cells count, BCDR, cellular uPAR, suPAR, and MMP-9 correlation

A significant correlation was noticed between suPAR and MMP-9 and the peripheral blast cells counts ($r=0.88$; $P=0.001$; $r=0.65$; $P=0.001$), as well as BCDR ($r=0.84$; $P=0.001$; $r=0.65$; $P=0.001$). At the same time, there is a significant correlation between suPAR and cellular uPAR ($r=0.88$; $P=0.001$). Moreover, MMP-9 is correlated significantly with suPAR ($r=0.84$; $p=0.001$) as shown in Table 16.

	AML patients without extramedullary infiltration	AML patients with extramedullary infiltration	P value
suPAR (ng/ml)	4.48±0.65	1.94±0.31	0.001
Cellular uPAR (ng/mg protein/10 ⁹ /cells/1)	1.14±0.11	0.41±0.08	0.001
MMP-9 (ng/ml)	21.96±3.23	15.24±1.64	<0.05

Table 15. suPAR, cellular uPAR, and MMP-9 in AML patients with extramedullary infiltration vs. those without.

	Peripheral blast cells count	BCDR	cellular uPAR	MMP-9
suPAR	r = 0.881	r = 0.839	r = 0.881	r = 0.61
	p = 0.001	p = 0.001	p = 0.001	p = 0.001
MMP-9	r = 0.65	r = 0.65	r = 0.844	–
	p = 0.001	p = 0.001	p = 0.001	–

Table 16. Correlation between suPAR, MMP-9, and peripheral blast cells count, BCDR, and cellular uPAR.

The levels of suPAR, cellular uPAR, and MMP-9 at diagnosis were significantly higher in died AML patients group as compared to the survived group (Table 17).

	Survivors	Non-survivors	P value
suPAR	2.06 ± 0.17	7.86 ± 0.68	0.001
Cellular uPAR	0.54 vs. 0.07	1.61 ± 0.10	0.001
MMP-9	14.2 ± 0.96	37.87±4.47	0.001

Table 17. suPAR, cellular uPAR, and MMP-9 in AML survivors vs. AML non-survivors.

Elevated WBC count and suPAR levels had no correlation in a study in patients with chronic myeloid leukemia in chronic phase; it was also found that all suPAR levels in patient populations fell within the normal range. Moreover, it shows evidence that serum suPAR and MMP-9 are strong prognostic variables in AML patients. Levels were significantly higher in AML non-survivors when compared to AML survivors in suPAR and MMP-9 diagnostic levels. suPAR and MMP-9 derived from AML blast cells could be a possible explanation for the prognostic value. suPAR and MMP-9 levels are higher in patients with extra-medullary infiltration according to the hypothesis. In conclusion, MMP-9 and suPAR levels might be used as a marker for disease activity and might contribute to blast cell dissemination. MMP-9 and suPAR may be target molecules in the strategy of treating AML.

8. Treatment

AML has traditionally been considered a medical emergency, delaying induction chemotherapy until molecular testing results return; it may benefit some patients but harm others. Advanced age and adverse cytogenetics would show a lower CR rate and that risks associated with giving immediate intensive therapy to patients in whom poor prognostic characteristics with the risk of waiting to initiate treatment for additional test results to return [78]. Therapy for AML includes remission induction followed by post-remission chemotherapy for most patients. The aim of induction therapy in new patients diagnosed with AML is to lay down complete remission (CR), which in turn could optimize the cure and disease-free survival rate [79].

8.1. Remission induction therapy

The backbone of remission induction therapy consists of an anthracenedione or anthracycline combined with cytosine arabinoside (cytarabine, Ara-C), a regimen that has not changed since it was first introduced 30 years ago [80]. Daunorubicin is given at a dose of 45 mg/m²/d×3 days, or mitoxantrone or idarubicin are given at doses of 12 mg/m²/d×3 days in combination with cytarabine, which is administered as a continuous infusion at 100 or 200 mg/m²/d×7 days (7+3 chemotherapy). Increasing the doses of cytarabine or the anthracycline, compares different anthracyclines or anthracenedione adding more drugs or using growth factors that prime agents or support care [81]; improved CR rates and disease-free survival commonly come at the price of increased treatment-related mortality than offsetting potential survival advantages. The median survival for older AML patients that follow these intensive approaches are typically 10 to 12 months, with higher median of survival for those entering CR, compared to non-responders or those achieving CR with incomplete platelet recovery (CRi).

8.2. Post-remission therapy

Usually, approaches to therapy for older AML patients who are in post-remission involve cytarabine and it is administered for a few days and then introduced in the remission setting. However, this is done alone or in combination with anthracene dione or anthracycline for 1 to 2 cycles. Severe neurological toxicity in one-third of patients is associated with high doses of postremission cytarabine. There is no added benefit to survival from more intense post-remission therapy or adding other agents. There is no added benefit from maintenance therapy through a more protracted course or post-remission therapy [82]. No randomized study shows that despite recommendations, no post-remission therapy or over post-remission therapy have any survival advantages. Post-remission therapy that is becoming more common is Stem Cell Transplantation (SCT) is being considered more. SCT is done at the cost of high treatment mortality and offers the chance of a cure. SCTs have limited uses to the population and the limited matched donors who are related to patients have limited applicability. Ongoing non-myeloablative approaches studies have demonstrated the feasibility of more survival rates. Ablative approaches may not provide advantages over non-myeloablative regimens for older AML patients [83].

8.3. Role of transplantation

Although associated with an improved anti-leukemic effect compared with chemotherapy, the oral solution (OS) did not consistently improve as shown from several prospective trials of evaluated autologous SCT. There remains a debate about what patients benefit from OS and in what type of patient an aggressive treatment should be reserved for and how SCT from a matched sibling donor has been part of standard care for 25 years [84]. Balancing the relapse risk that the patient faces with chemotherapy compared to the risk of the procedure itself is a decision that the patient faces. If a patient has a low relapse risk, they have a higher chance of responding if they relapse, so holding off on SCT to second CR is more practical. The risk factors that affect SCT could be influenced by many items such as the cytomegalovirus status of the donor and host, the age of the recipient and the donor, and the parity of a female donor. The degree of matching and the comorbidities present in the patient can be evaluated in a risk score [85]. A further complication in assessing the data for SCT in first CR is the method of assessment.

8.4. Molecular genetic implications for diagnosis and therapeutics

Molecular revelations have recently defined further prognostic cohorts. FMS-like tyrosine kinase 3 (FLT3) is important in the development of myeloid and lymphoid lineages may occur in 25% to 30% [86] for internal tandem duplications and domain or mutations of kinase activating loop. FLT3 ITD has proliferative advantage and anti-apoptotic signals and predicts shorter CR duration. FLT3-ITD to FLT3 wild-type has a high allele ratio and long length of duplication and location of insertion [87]. This information can help with the decision to go through allogeneic SCT even though the benefits may not be unanimous. FLT3 inhibitory activity has had little effect as monotherapy and at least three randomized trials are going in combination with chemo, which should also take account for molecular subsets. Fifty percent of cytogenetically normal acute myeloid leukemia (CN-AML) of the other intermediate group has nucleophosmin 1 (NPM1) mutations that result in delocalizing in the cytoplasm [88]. NPM1 mutations in the absence of FLT3-ITD are favorable outcomes that are similar to CBF leukemia in younger AML patients [89]. These types of patients can benefit from consolidated chemo and do not require CR after SCT. There are suggestions that NPM1 patients should receive additional all-trans-retinoic acid therapy, but this has not been proven. The prognostic value of the molecular biomarkers (e.g., NPM1) could be useful in predicting the outcome in older patients [90].

9. Conclusion and outcomes

AML in older adults is considered a difficult disease to be cured, representing one of the most challenging groups to be treated in oncology. However, advances in AML treatment have resulted in improved remission (an absence of signs and symptoms) and cure rates. This improvement may benefit treatment with intensive or alternative chemotherapy that is appropriate for the patient, enhanced by supportive performance status and cytogenetics.

Remission rates in adult AMLs are universally related to age, with an expected remission rate of more than 65% for those younger than 60 years. Earlier, the median survival of adult patients with AML at the beginning of treatment was 40 days. Recently, AML patients younger than 60 years have complete response rates of 70% to 80% after induction chemotherapy. Overall, survival is only about 50% for those who go into complete remission. Given the desperate nature of survival outcomes, clinical trials should be considered at diagnosis, along with considering the aggressiveness of therapy and patient-oriented treatment goals.

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