EXPRESSION OF COMPLEMENT REGULATORY PROTEINS ON ADULT ACUTE NON LYMPHOID LEUKEMIA: IMPACT ON INDUCTION OF REMISSION.

A Thesis Submitted In Partial Fulfillment of The Requirements of M.D. Degree In Clinical Pathology And Oncological Laboratory Medicine

By

Nagwa Ramadan Abdel-Wadoud

Supervisors

Prof. Dr. AZZA M. KAMEL

Professor Of Clinical Pathology, NCI, Cairo University

Prof. Dr. HOSSAM K. MAHMOUD

Professor Of Medical Oncology NCI, Cairo University

Dr. NAHLA M. EL-SHARKAWY

Consultant Of Clinical Pathology, NCI, Cairo University

NCI, Cairo University

2009

Acknowledgment

First and foremost, I feel always indebted to "Allah", the most beneficent and merciful.

I am deeply grateful to Prof. Dr. Azza Mahmoud Kamel, Professor of Clinical Pathology, National Cancer Institute, Cairo University, for her supervision, valuable advice and continuous support,and without her this work could not be completed.

Many thanks and gratitude to Prof. Dr. Hossam Kamel Mahmoud Professor of Medical Oncology and President of Cairo University, for his continuous honest guidance and support .

I would like to thank Dr. Nahla Mohammad El-Sharkawy consultant of Clinical Pathology, National Cancer Institute, Cairo University, who gave me much of her time and spared no effort in guiding me in whole of this work.

My profound gratitude to Dr. Raafat Mohammad Abdel-Fattah lecturer of Medical Oncology, National Cancer Institute ,Cairo University, for his supervision, guidance and kind care.

I would like to express my deep gratitude and thanks to all the staff of Clinical Pathology Department, National Cancer Institute, Cairo University for their outstanding assistance and kind care.

Finally, I am greatly indebted to my family, without them this work would not come to light.

EXPRESSION OF COMPLEMENT REGULATORY PROTEINS ON ADULT ACUTE NON LYMPHOID LEUKEMIA: IMPACT ON INDUCTION OF REMISSION

By : Nagwa Ramadan Abdel- Wadoud

Supervisors: Prof. Dr. Azza Mahmoud Kamel. Professor of Clinical and Chemical Pathology. Prof. Dr. Hossam Kamel Mahmoud. Professor of Medical Oncology. Dr. Nahla Mohamed El-Sharkawy Consultant of Clinical Pathology. (NCI, Cairo University, 2009).

Abstract

The aim of this work is to study the expression of complement regulatory molecules: CD35, CD55, CD59, and CD46 in adult acute leukemia, determine the level of expression compared to normal control, correlate the level of expression to other laboratory and clinical parameters with emphasis on findings with prognostic relevance, and correlate the findings to remission induction.

To achieve this aim, this study was performed on 65 adult AML patients presented to the clinic of NCI. Twenty age and sex matched apparently healthy individuals were included as a control group. Samples were analyzed by flow cytometry.

This study documented that CD35, CD55, CD46 and CD59 are expressed on all AML blasts in a higher percentage than controls. This is one of the ways the leukaemic cells protect themselves from the lytic effect of the complement. CD35 and, to some extent, CD55 might have a relation to survival rate. CD59 was the only one which showed a relation to the remission rate.

Key words: Acute myeloid leukaemia, Complement regulatory proteins, CD35, CD55, CD59, and CD46.

INTRODUCTION

Leukemias are a group of disorders characterized by the accumulation of malignant white cells in the bone marrow (BM) and peripheral blood (PB). These abnormal cells cause symptoms because of BM failure (anemia, neutropenia and thrombocytopenia) and infiltration of organs (e.g. liver, spleen, lymph nodes, meninges, brain, skin or tests) ***(Hoffman et al 2005).***

Like all other blood cells, leukemia cells are exposed to blood which contains complement. The complement system is a major fluid phase host defense system that induces immune clearance through enhancement of antibody phagocytosis, potentiation of natural killer activity, enhancement of antibody production and immune cytolysis. Host cells are protected from the lytic effect of the complement system by expressing a number of complement regulatory proteins ***(Huang et al, 2001).***

These proteins include complement receptor 1 (CR1, CD35), decay accelerating factor (DAF, CD55), homologous restriction factor 20 (HRF 20, CD59) and membrane cofactor protein (MCP, CD46). These molecules are largely responsible for the high resistance of nucleated cells to homologous complement attack ***(Guc et al, 2000).***

A number of studies have investigated the expression of these molecules on different hematopoietic malignant cells .The results showed variability in expression of the different molecules from one type to the other and in general reported a lower expression in the less mature form. Variability was also reported from case to case within the same diagnosis. As complement regulatory molecules, basically protect cells from complement lysis which is one of the mechanisms that help to

kill the cells, the lower expression may be a factor contributing to killing and eradication of the cells which may be reflected on response rate in the form of remission induction.

In this work we are going to study the expression of complement regulatory molecules: CD35, CD55, CD59, and CD46 in adult acute leukemia with the aim of:

1- Determine the level of expression compared to normal control.

2- Correlate the level of expression to other laboratory and clinical parameters with emphasis on findings with prognostic relevance.

3- Correlate the findings to remission induction.

ACUTE MYELOID LEUKEMIA (AML)

Definition: AML is a group of malignant disorders resulting from the clonal expansion of myeloid blasts in the PB, BM, or other tissues. It is a heterogeneous disease clinically, morphologically and genetically and involves only one or all myeloid lineages. Most AML subtypes are distinguished from other related blood disorders by the presence of 20% blasts in the bone marrow or peripheral blood ***(Steven et al, 2008).***

Pathophysiology: The malignant cell in AML is the myeloblast. In normal hematopoiesis, the myeloblast is an immature precursor of myeloid white blood cells; a normal myeloblast will gradually mature into a mature white blood cell. However, in AML, a single myeloblast accumulates genetic changes which "freeze" the cell in its immature state and prevent differentiation. Such a mutation alone does not cause leukemia; however, when such a "differentiation arrest" is combined with other mutations which disrupt genes controlling proliferation, the result is the uncontrolled growth of an immature clone of cells, leading to the clinical entity of AML ***(Abeloff et al, 2004).***

Much of the diversity and heterogeneity of AML stems from the fact that leukemic transformation can occur at a number of different steps along the differentiation pathway. Modern classification schemes for AML recognize that the characteristics and behavior of the leukemic cell (and the leukemia) may depend on the stage at which differentiation was halted. Specific cytogenetic abnormalities can be found in many patients with AML; the types of chromosomal abnormalities often have prognostic significance ***(Abeloff et al, 2004).***

The chromosomal translocations encode abnormal fusion proteins, usually transcription factors whose altered properties may cause the "differentiation arrest**.** For example, in acute promyelocytic leukemia, the t (15;17) translocation produces a PML-RARα fusion protein which binds to the retinoic acid

receptor element in the promoters of several myeloid-specific genes and inhibits myeloid differentiation ***(Greer et al, 2004).***

The clinical signs and symptoms of AML result from the fact that, as the leukemic clone of cells grows, it tends to displace or interfere with the development of normal blood cells in the bone marrow**.** This leads to neutropenia, anemia, and thrombocytopenia. The symptoms of AML are in turn often due to the low numbers of these normal blood elements. In rare cases, patients can develop a chloroma, or solid tumor of leukemic cells outside the bone marrow, which can cause various symptoms depending on its location ***(Abeloff et al, 2004).***

Incidence:

The age-incidence of AML is subtly bimodal. Between early childhood and age 45, the annual incidence of (AML) remains constant at 0.8 cases/10^5 population. The incidence rises exponentially after the age of 45, exceeding 15 cases/10^5 populations by age 75 ***(Karen et al, (2006)).***

Frequency:

In the US: Out of approximately 3250 newly diagnosed cases of leukemia in children each year, nearly 20% are AML. While 1 of 3 newly diagnosed infants with leukemia has AML, the ratio of AML to acute lymphoblastic leukemia (ALL) falls rapidly until adolescence, when it increases to account for nearly 50% of all new leukemia diagnoses. Estimates predict 11,960 new cases of AML in the United States in 2005 (6530 men and 5430 women) ***(Karen et al, 2006).***

In Egypt: in the year 2005 there were 20,326 new patients seen at the National Cancer Institute (NCI). Malignant cases were 46.1%; 9.3% of them were leukemic males; of them 2.37% were AML. While 6.3% were leukemic females, of them 2.17% were AML .In children under age of 20, leukemia in girls was

33.27%; of them 9.27% were AML. While in boys it was 34.6%; of them 8.58% were AML ***(Elattar, 2006).***

Mortality/Morbidity:

In 2005, 9000 deaths occurred in the United States. Of these, 5040 occurred in men and 3960 in women. In adults, treatment results were generally analyzed separately for younger (18-60 y) and older (>60 y) patients. With current standard chemotherapy regimens, approximately 25-30% of adults younger than 60 years survive longer than 5 years and are considered cured. Results in older patients are more disappointing, with fewer than 10% of patients surviving long-term ***(Karen et al, (2006)).***

Demography:

AML is more common in whites than in other populations. It is more common in men than in women. The difference is even more apparent in older patients. This is likely because myelodysplastic syndromes (MDSs) are more common in men, and advanced MDS frequently evolves into AML. Some have proposed that the increased prevalence of AML in men may be related to occupational exposures. Prevalence increases with age. The median age of onset is 65 years. However, this disease affects all age groups ***(Karen et al, 2006).***

History of Patients:

Patients present with symptoms resulting from bone marrow failure, organ infiltration with leukemic cells, or both. The time course is variable. Some patients, particularly younger ones, present with acute symptoms over a few days to 1-2 weeks. Others have a longer course, with fatigue or other symptoms lasting from weeks to months. A longer course may suggest an antecedent hematologic disorder (AHD) such as myelodysplastic syndrome (MDS) ***(Abeloff et al, 2004).***

Symptoms of bone marrow failure are related to anemia, neutropenia, and thrombocytopenia. The most common symptom of anemia is fatigue. Patients often retrospectively

note a decreased energy level over past weeks. Other symptoms of anemia include dyspnea upon exertion, dizziness, and, in patients with coronary artery disease, anginal chest pain. In fact, myocardial infarction may be the first presenting symptom of acute leukemia in an older patient ***(Abeloff et al, 2004).***

Patients often have decreased neutrophil levels despite an increased total WBC count. Patients present with fever, which may occur with or without specific documentation of an infection. Patients with the lowest absolute neutrophil counts (i.e, <500 cells/dl and especially <100 cells/dl) have the highest risk of infection. Patients often have a history of upper respiratory infection symptoms that have not improved despite empiric treatment with oral antibiotics ***(Abeloff et al, 2004).***

Patients present with bleeding gums and multiple echymoses. Bleeding may be caused by thrombocytopenia, coagulopathy that results from disseminated intravascular coagulation (DIC), or both. Potentially life-threatening sites of bleeding include the lungs, gastrointestinal tract, and the central nervous system ***(Abeloff et al, 2004).***

Alternatively, symptoms may be the result of organ infiltration with leukemic cells. The most common sites of infiltration include the spleen, liver, and gums. Infiltration occurs most commonly in patients with the monocytic subtypes of acute myelogenous leukemia (AML). Patients with splenomegaly note fullness in the left upper quadrant and early satiety. Patients with gum infiltration often present to their dentist first by gingivitis due to neutropenia which cause swollen gum &thrombocytopenia can cause the gums to bleed. Patients with markedly elevated WBC counts (>100,000 cells/dl) can present with symptoms of leukostasis (i.e, respiratory distress and altered mental status). Leukostasis is a medical emergency that requires immediate intervention. Patients with a high leukemic cell burden may present with bone pain caused by increased pressure in the bone marrow ***(Abeloff et al, 2004).***

Physical Signs:

Physical signs of anemia, including pallor and a cardiac flow murmur, are frequently present. Fever and other signs of infection can occur, including lung findings of pneumonia. Patients with thrombocytopenia usually demonstrate petechiae, particularly on the lower extremities. Petechiae are small, often punctate, hemorrhagic rashes that are not palpable. Areas of dermal bleeding or bruises (i.e., ecchymoses) that are large or present in several areas may indicate a coexistent coagulation disorder such as DIC. Purpura is characterized by flat bruises that are larger than petechiae but smaller than ecchymoses. Signs relating to organ infiltration with leukemic cells include hepatosplenomegaly and, to a lesser degree, lymphadenopathy. Occasionally, patients have skin rashes due to infiltration of the skin with leukemic cells (leukemia cutis). Chloromata are extramedullary deposits of leukemia. Rarely, a bony or soft-tissue chloroma may precede the development of marrow infiltration by AML (granulocytic sarcoma). Signs relating to leukostasis include respiratory distress and altered mental status ***(Abeloff et al, 2004).***

Etiology

A number of risk factors for developing AML have been identified, including:

- "Pre-leukemic" blood disorders such as myelodysplastic or myeloproliferative syndromes can evolve into AML; the exact risk depends on the type of MDS/MPD ***(Karen et al, 2006).***
- Exposure to anti-cancer chemotherapy, in particular alkylating agents, can increase the risk for the subsequent development of AML. The risk is highest about 3-5 years after chemotherapy ***(Karen et al, 2006).***
- Chemotherapy agents, specifically epipodophyllotoxins and anthracyclines, have also been associated with treatment-related leukemia. These treatment-related leukemias are often associated with specific chromosomal abnormalities in the leukemic cells (***Karen et al, 2006).***

- Ionizing radiation exposure can increase the risk of AML. Survivors of Other the atomic bombings of Hiroshima and Nagasaki had an increased rate of AML, as did radiologists exposed to high levels of X-rays prior to the adoption of modern radiation safety practices ***(Karen et al, 2006).***
- Occupational chemical exposure to benzene and other aromatic organic solvents is controversial as a predisposing factor of AML. Benzene and many of its derivatives are known to be carcinogenic in vitro. While some studies have suggested a link between occupational exposure to benzene and increased risk of AML, others have suggested that the attributable risk, if any is slight ***(Karen et al, 2006).***
- Several congenital conditions may increase the risk of leukemia; the most common is probably Down syndrome, which is associated with a 10- to 18-fold increase in the risk of AML**.** Other congenital disorders that predispose patients to AML include Bloom syndrome, congenital neutropenia, Fanconi anemia, and neurofibromatosis. Usually, these patients develop AML during childhood; rarely, some may present in young adulthood ***(Karen et al, 2006).***
- More subtle genetic disorders, including polymorphisms of enzymes that metabolize carcinogens, also predispose patients to AML. For example, polymorphisms of NADPH: quinone oxidoreductase (NQO1), an enzyme that metabolizes benzene derivatives, is associated with an increased risk of AML. Particularly increased risk exists for AML that occurs after chemotherapy for another disease or for de novo AML with an abnormality of chromosomes 5, 7, or both. Likewise, polymorphisms in glutathione S-transferase are associated with secondary AML following chemotherapy for other malignancies ***(Allan et al, 2001).***

Diagnosis:

The first clue to a diagnosis of AML is typically an abnormal result on a complete blood count. The hallmark of leukemia is the reduction or absence of normal hematopoietic element. Anemia is usually normocytic, with a lower than expected

reticulocyte count for the level of the hemoglobin. The decrease in hemoglobin levels can range from minimal to profound. Platelet counts are usually low and are generally commensurate with the degree of bleeding. Patients with spontaneous petechiae usually have platelet counts less than 20,000/m ***(Abeloff et al, 2004).***

White cell counts may be decreased or elevated. On occasion, hyperleukocytosis with white cell counts greater than 100,000 can be observed, with higher numbers conferring a white color to the blood specimen. The white cell differential is usually the key to suspected leukemia, with primitive granulocyte or monocyte precursors observed on peripheral smear.

Mature neutrophils are usually diminished. Auer rods, characteristic cytoplasmic inclusions, can be found in specimens of circulating blood of many AML patients on careful examination of the blood smear. They are particularly prominent in acute promyelocytic leukemia ***(Abeloff et al, 2004).***

The diagnosis of myeloid neoplasms relies on the morphologic, cytochemical and immunophenotypic features of the neoplastic cells to establish their lineage and degree of maturation and to decide whether cellular proliferation is cytologically normal or dysplastic or effective or ineffective . The blast percentage in the peripheral blood, bone marrow and other involved tissues remains of practical importance to categorize myeloid neoplasms and to judge their progression ***(Steven et al, 2008).***

Morphology

Peripheral blood*:* A peripheral blood (PB) smear should be examined and correlated with results of a complete blood count. Freshly made smears should be stained with May-Grunwald-Giemsa or Wright-Giemsa and examined for white blood cell (WBC), red blood cell (RBC) and platelet abnormalities. Evaluation of neutrophil granularity is important when a myeloid disorder is suspected. Manual 200-cell leukocyte differentials of PB smears are recommended in

patients with a myeloid neoplasm when the WBC count permits ***(Arber et al, 2003a).***

Bone marrow aspirate: Bone marrow (BM) aspirate smears should also be stained with May-Grunwald-Giemsa or WrightGiemsa for optimal visualization of cyloplasmic granules and nuclear chromatin. Because the WHO classification relies on percentages of blasts and other specific cells to categorize some entities, it is recommended that 500 nucleated BM cells be counted on cellular aspirate smear . The cells to be counted include blasts and promonocytes, promyelocytes, myelocytes, metamyelocytes, band neutrophils, segmented neutrophils, esinophils, basophils, monocyles, lymphocytes, plasma cells, erythroid precursors and mast cells . If an aspirate cannot be obtained due to fibrosis or cellular packing, touch preparations of the biopsy may yield valuable cytologic information ***(Arber et al, 2003a).***

Bone marrow trephine biopsy: The trephine biopsy provides information regarding overall cellularity, proportion and maturation of haematopoietic cells, and allows evaluation of BM stroma. The biopsy also provides material for immunohistochemical studies that may have diagnostic and prognostic importance. A biopsy is essential whenever there is myelofibrosis. Immunohistochemical staining of the BM biopsy for CD34+ blasts often aids in the correlation of aspirate and trephine biopsy findings, although in some myeloid neoplasms the blasts do not express CD34 ***(Arber et al, 2003a).***

Blasts: The percentage of myeloid blasts is important for diagnosis and classification of myeloid neoplasms, In the PB the blast percentage should be derived from a 200-cell leukocyte differential and in the BM from a 500-cell count of cellular BM aspirate smears. Myeloblasts monoblasts and megakaryoblasts are included in the blast count ***(Arber et al, 2003a).***

Myeloblasts vary from slightly larger than mature lymphocytes to the size of monocytes or larger, with moderate to abundant dark blue to blue-grey cytoplasm. The nuclei are round to oval

with finely granular chromatin and usually several nucleoli, but in some nuclear irregularities may be prominent. The cytoplasm may contain a few azurophil granules ***(Arber et al, 2003a).***

Monoblasts are large cells with abundant cytoplasm that can be light grey to deeply blue and may show pseudopod formation. Their nuclei are usually round with delicate, lacy chromatin and one or more large prominent nucleoli. They are usually strongly positive for nonspecific esterase (NSE) but have no or only weak myeloperoxidase (MPO) activity. Promonocytes are considered as "monoblast equivalents". Promonocytes have a delicately convoluted, folded or grooved nucleus with finely dispersed chromatin, a small, indistinct or absent nucleolus, and finely granulated cytoplasm. Most promonocytes express NSE and are likely to have MPO activity ***(Arber et al, 2003a)***

Megakaryoblasts are usually of medium to large size with a round, indented or irregular nucleus with finely reticular chromatin and one to three nucleoli. The cytoplasm is basophilic, usually agranular, and may show cytoplasmic blebs. Small dysplastic megakaryocytes and micromegakaryocytes are not blasts ***(Arber et al, 2003a).***

In acute promyelocytic leukaemia, the blast equivalent is the abnormal promyelocyte. Erythroid precursors (erythroblasts) are not included in the blast count except in the rare instance of "pure" acute erythroid leukaemia, in which case they are considered as blast equivalents ***(Arber et al, 2003a).***

Cytochemistry and other special stains:

Cytochemical studies are used to determine the lineage of blasts, although in some laboratories they have been supplanted by immunologic studies using flow cytometry or immunohistochemistry. They are usually performed on PB and BM aspirate smears but some can be performed on sections of trephine biopsies of other tissues ***(Harris et al, 1999).***

MPO: Detection of MPO indicates myeloid differentiation but its absence does not exclude a myeloid lineage because early myeloblasts as well as monoblasts may lack MPO. The MPO activity in myeloblasts is usually granular and often concentrated in the Golgi region whereas monoblasts, although usually negative, may show fine, scattered MPO positive granules, a pattern that becomes more pronounced in promonocytes. Erythroid blasts, megakaryoblasts and lymphoblasts are MPO negative ***(Harris et al, 1999).***

Sudan Slack B (SBB): This stain parallels MPO but is less specific. Occasional cases of lymphoblastic leukaemia exhibit SBB positively, in which case light grey granules are seen rather than the deeply black granules that characterize myeloblasts ***(Harris et al, 1999).***

The non-specific esterases, α naphthyl butyrate (ANB) and a naphthyl acetate (ANA), show diffuse cytoplasmic activity in monoblasts and monocytes. Lymphoblasts may have focal punctate activity with NSE but neutrophils are usually negative. Megakaryoblasts and erythroid blasts may have some multifocal, punctate ANA positivity, but it is partially resistant to (NaF) inhibition whereas monocyte NSE is totally inhibited by NaF. The combination of NSE and the specific esterase, naphthol-ASD-chloroacetate esterase (CAE), which stains primarily, cells of the neutrophil lineage and mast cells, permits identification of monocytes and immature and mature neutrophils simultaneously. Some cells, particularly in myelomonocytic leukaemias, may exhibit NSE and CAE simultaneously, while normal eosinophils lack CAE, it may be expressed by neoplastic eosinophils ***(Harris et al, 1999).***

PAS: In acute erythroid leukaemia, a PAS stain may be helpful in that the cytoplasm of the leukemic proerythroblasts may show large globules of PAS positivity ***(Harris et al, 1999).***

Iron stain: Well-controlled iron stains should always be performed on the BM aspirate to detect iron stores, normal sideroblasts and ring sideroblasts, which are defined as erythroid