Leukemia

Thesis

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Introduction

Hematological malignancies are often associated with hemostatic disturbances which seem, at least partly, to be associated with von willebrand factor (vWF) defects. Both increased activity of the coagulation system, as well as, hyperfibrinolysis have been described. In addition, patients often experience low platelet counts (<40 × 10^9/L) which may cause life-threatening hemorrhages. Other pathophysiological mechanisms that may add to an increased bleeding tendency include a massive proteolytic state, trigged by pro-coagulant substances, plasminogen activators and proteinases released into the circulation from leukemic cells together with proteolytical degradation of the vWF. A vWF multimeric pattern resembling type 2A vWF mediated by an IgG antibody directed against the GPIb binding site has been described. Furthermore, an abnormal vWF multimeric pattern has been associated with elevated platelet, as well as, leukocyte count ***(Nilsson et al., 2012).***

Acute myeloid leukemia (AML) patients are at an increased risk of bleeding. Besides disease-related thrombocytopenia and platelet dysfunction, a systemic coagulopathy may contribute to the hemorrhagic diathesis, which causes bleeding complications in 40-70% of AML patients at presentation. The coagulopathy, in AML, has been attributed to disseminated intravascular coagulation (DIC), excessive fibrinolysis, and the action of non-specific proteases. The DIC is especially common in acute promyelocytic leukemia (APL) [FAB-M3], with an estimated prevalence of 70-90%. However, various degrees of DIC have been reported in other subtypes, such as M1, M2, M4, and M5 ***(Langer et al., 2004).***

Therefore, the clinical manifestations of bleeding show special features specific to the form of acute leukemia***.*** This hazard has a significant negative impact on the morbidity and mortality of patients with this disease. Recognition of these characteristics is important in the diagnosis and management of acute leukemia ***(Kwaan and Huyck, 2010).***

Recently, ***Nilsson et al., (2012)*** suggested that coagulation is activated in patients with *de novo* AML, with a peak during induction treatment, and decline in activation subsequently. Increased thrombin

generation occurs before consumption of clotting factors is apparent in global hemostatic tests (e.g. prolonged APTT).

Aim of the Work

The aim of this work is to:

- Investigate the vWF-Ristocetin cofactor (vWF-RCO) activity pattern in newly-diagnosed (*de novo*) AML patients, prior to the start of induction treatment and after two weeks of therapy.
    - Correlate vWF-Ristocetin cofactor (vWF-RCO) activity to haemostatic complication, in order to explore the potential role of vWF-Ristocetin cofactor in the prevalent phenomenon of bleeding in association with AML patients.
    - Correlate between vWF-Ristocetin cofactor (vWF-RCO) activity and other haemostatic parameters, and the known prognostic factors in patients with AML.

Chapter (1) Acute Myeloid Leukemia

Definition:

Acute myeloid leukemia (AML), is a cancer where an overgrowth of abnormal white blood cells prevents the production of normal blood cells. The AML is characterized by a clonal proliferation of myeloid precursors, with a reduced capacity to differentiate into more mature cellular elements. As a result, there is an accumulation of leukemic blasts or immature forms in the bone marrow (BM), peripheral blood (PB) and other tissues, with a variable reduction in the production of normal red blood cells, platelets and mature granulocytes ***(Kennedy et al., 2013).***

Epidemiology:

The AML is the prominent form of leukemia in the neonatal period and three present 15-20% of the cases during childhood and early adulthood. An early peak occurs at birth and in the first 4 weeks of the life (congenital leukemia), a rare entity with reported incidence between 4.3 and 8.6 per million live births ***(Prakasha et al., 2008).*** Afterwards, the incidence of AML remains constant till the age of 10.

The AML is the most common acute leukemia in adults, and accounts for approximately 80 percent of cases of adult and geriatric leukemia ***(Siegel et al., 2013).*** A slight send peak occurs in late adolescence, then the incidence remains stable till the age of 55 ***(Abaza, 1988)***.

In adults, the median age at diagnosis is approximately 65 years. The incidence increases with age with approximately 1.3 and 12.2 cases per 100,000 population for those under or over 65 years, respectively ***(Siegel et al., 2012).*** Afterwards it increases progressively till it reaches about 15/ 100,000 persons in the 8^{th} and 9^{th} decades of life ***(Abaza, 1988).***

The male: female ratio is approximately 5:3. This incidence is similar among persons of different races ***(Siegel et al., 2012)***.

Etiology and Risk Factors:

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

and Lubbert, 2006).

- **Genetic Disorders:**

An increased incidence of AML is seen in patients with disorders associated with excessive chromatin fragility such as Bloom syndrome, Fanconi anemia, Schwachman - Diamond syndrome, Blackfan - Diamond syndrome and Kostmann syndrome, as well as with Wiskott-Aldrich and ataxia telangiectasia syndromes. Other syndromes, such as Down (trisomy 21), Klinefelter (XXY and variants), neurofibromatosis and Patau (trisomy13), have also been associated with a higher incidence of AML ***(Jabbour et al., 2006).***

- **Secondary AML**

The most common risk factor for AML is the presence of an antecedent hematologic disorder, the most common of which is myelodysplastic syndromes (MDS) ***(Seiter, 2010)***. Other antecedent hematologic disorders include aplastic anemia, multiple myeloma, myelofibrosis and paroxysmal nocturnal hemoglobinuria (PNH). Aplastic anemia is associated with late development of AML ***(Gale et al., 2007).*** AML can occur in patients with myeloma who have not received prior chemotherapy or radiation therapy ***(Miller and Pihan, 2009).*** AML secondary to PNH appears to involve the same clone from which the abnormal erythrocytes are derived ***(White et al., 2010).***

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

- **Viruses:**

Viruses may cause disruption of the host genome by insertion, mutation and chromosomal rearrangements. Viruses also result in immune dysfunction, leading to decreased immune surveillance for early tumors. Parvovirus B19 (B19V) plays an important role in conversion of pre-leukemic clones to an overt leukemia ***(Yalcin et al., 2009).***

- **Environmental and Chemical Factors:**

A vast variety of environmental and chemical exposures are

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

a. Drugs:

The AML arising following exposure to genotoxic agents has been recognized as a distinctive entity for more than 40 years. Secondary or therapy-related AML accounts for 10%-20% of all AML cases ***(Smith et al., 2011).***

Treatment of patients with lymphoproliferative disorders with alkylating agents as chlorambucil, mustine, melphalan, procarbazine or nitrosourea may predispose to AML, especially when these drugs are combined with radiotherapy. The AML patients typically present several years after therapy with peak incidence after about 5 years ***(Miller and Daoust, 2009)***. Topoisomerase II inhibitors are associated with development of AML after a relatively shorter latent period of time (2-3 years) ***(Wickremasinghe and Hoffbrand, 2011).***

Chloramphenicol, Phenylbutazone, and less commonly, chloroquine and methoxypsoralen can result in BM failure that may evolve into AML ***(De Sanctis et al., 2003).***

b. Benzene:

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

c. Radiation:

Therapeutic radiation increases AML risk, particularly if given with alkylating agents ***(Jabbour et al., 2006).*** The primary carcinogenic effect of ionizing radiation is causing radiation-induced genomic instability in hemopoeitic cells. However, the relationship of inducible instability and AML induction is still unexplained ***(Larson, 2007).*** However, the current use of diagnostic x-ray imaging does not appear to be associated with any increased leukemia risk in patients. Meanwhile, fetal exposure to intrauterine x-rays increases the risk of subsequent childhood leukemia ***(Larson et al., 2007).***

The AML is also common in workers in the nuclear industry but

not in people living near nuclear power plants. Exposure to electromagnetic fields (such as living near power lines), some dyes, herbicides and pesticides, have been implicated as another potential risk factor for AML ***(Konoplev and Bueso-Ramos, 2006).***

d. Smoking:

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

Pathogenesis:

The AML is characterized by acquisition of somatic mutations in hematopoietic progenitors that confer a proliferative and/or survival advantage, impairing hematopoietic differentiation and providing properties of limitless self-renewal ***(Wernig and Gilliland, 2009).*** A single mutation is not sufficient to cause an overt leukemic phenotype, but it likely develops upon the acquisition of further mutations in progenitor cells ***(Dohner and Dohner, 2008)***.

- **Inappropriate proliferation:**

This abnormal proliferation is often the result of mutations affecting proliferative signaling pathways. Activated kinases have become implicated in the pathogenesis of AML ***(Licht and Sternberg, 2010).*** Leukemic cells exhibit a proliferative response to many of the endogenous hematopoietic growth factors critical for normal hematopoiesis such as G-CSF, GM-CSF, M-CSF, CSF, IL-3, IL-4, IL-5, IL-6, IL-7, FLT3 and KIT ligand which are mediated through specific growth factor receptors that are frequently expressed on the surface of AML cells ***(Rosenfeld and List, 2001).***

It appears that a combination of these factors can produce a synergistic growth response. In particular, stem cell factor can enhance by some of 10-20 folds the proliferation of leukemic blasts induced by G-CSF, GM-CSF and IL-3 ***(Giles et al., 2002).***

- **Differentiation blockade:**

Transcription factors are commonly disrupted in AML, either by

their fusion as a result of chromosomal translocation, or by point-mutation. Factors affected by chromosomal rearrangement include the core binding factor (CBF) complex and the retinoic acid receptor (RAR). Point mutations in myeloid transcription factors include CCATT/enhancer-binding protine alpha (CEBPA) ***(Licht and Sternberg, 2010)***. Acute promyelocytic leukemia (APL) is a clear-cut example of differentiation blockade in AML ***(Fufan et al., 2010)***.

- **Escape from programmed cell death:**

The ability to evade apoptosis is critical to the development of a malignancy. Protein tyrosine kinase activation can have the dual effect of promoting cell proliferation and, in addition, enhancing cell-survival by activating phosphatidyl-inositol 3-kinase (PI 3-kinase) signaling. The phospholipid products of PI 3-kinase activate the AKT serine/threonine kinase, and this kinase- in turn- phosphorylates BAD and releases the BCL-2 pro-survival molecule.

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

- **Self-Renewal:**

Unlike normal progenitor cells that are committed to a particular hematopoietic lineage, leukemic cells from patients with AML can undergo self-renewal rather than lineage-specific commitment. Moreover, the leukemic stem cell population in AML is functionally heterogeneous with differing capacities for self-renewal ***(Hope et al., 2004).***

Nuclophosamine (NPM) is mutated in approximately one-third of newly diagnosed AML, and the expression of this cytoplasmic NPM variant is associated with expression of genes thought to support maintenance of the stem cell phenotype ***(Alcalay et al., 2005).***

The FLT3-ITD mutant of AML, which activates proliferative and survival pathways, also confers the property of self-renewal in human CD34+ cells. Thus, the expression of mutated and fusion genes in AML

seems to underlie some aspects of enhanced self-renewal, although such findings do not exclude the possibility that the progenitor cell in AML might itself have intrinsic self-renewal properties independent of a leukemogenic insult ***(Chung et al., 2005).***

Classification:

- **French-American-British (FAB) classification:**

Since 1976, AML has been classified according to the criteria of the FAB group. This classification is based strictly on morphology and cytochemistry, and although it includes two categories linked to chromosomal abnormalities (M3 and M4EO), cytogenetic abnormalities play no part in FAB classification ***(Volger et al., 1992).*** The FAB classification requires a blast count of 30% or more in the BM for the diagnosis of AML (Table 1) ***(Miller and Pihan, 2009).***

Table (1): The FAB Classification of AML.

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

	M5b: Blasts are ≥30 % of NEC BM monocytic component is ≥80% of NEC Monoblasts are <80% of BM monocytic component.
M_6 Acute erythroid leukemia	Erythroid cells are ≥50% of BM cells BM blasts are ≥30% of NEC
M_7 Acute megakaryoblastic leukemia	Blasts are predominantly megakaryoblasts

MPO: Myeloperoxidase; **SBB:** Sudan Black B; **NEC:** Non-erythroid cells,

(Miller & Pihan, 2009 and Lichtman & Liesveld, 2010).

- **Cytochemical classification:**

The MPO and SBB stain positivity are usually indicative of leukemia of myelocytic origin, whereas non-specific esterase (NSE) is indicative of monocytic differentiation. The AML blasts are usually periodic acid Schiff (PAS) negative with the exception of erythroblasts of AML- M_6 and eosinophils of AML- M_4Eo subclass. However, recently, cytochemistry becomes less important as a tool in the diagnosis of AML because of the greater efficiency of immunological methods ***(Vincent and DeVita, 2012).***

- **Immunophenotypic classification (IPT):**

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

Table (2): Immunophenotypic markers of AML

Marker	M_0	M_1	M_2	M_3	M_4	M_5	M_6	M_7
HLA-DR	++	++	++	-	++	++	+	+
CD11b	+	+	+	-	++	++	-	-
CD13	+	++	++	++	++	++	-	-
CD14	-	+	+	-	++	++	-	-
CD15	-	-	+	+	+	±	-	-
CD33	+	++	+++	+++	+++	+++	+	+
CD41,CD61	-	-	-	-	-	-	-	+++
Glycophorin A	-	-	-	-	-	-	++	-
TDT	++	+	-	-	-	-	-	-
CD117	+	+	+	_	+	+	+	+
CD34	++	+	-	-	-	-	-	+
MPO	+	+	++	++	+	+	–	–

(Tong et al., 2010).

- **World Health Organization (WHO) Classification:**

The WHO classification defines biological and clinical entities within AML and the relationship between morphology, IPT and genetic abnormalities (Table 3) ***(Schoch and Haferlach, 2002).*** The most significant difference between the WHO and FAB classifications is that WHO recommends the requisite blast percentage of at least 20% in the PB or BM (versus at least 30% in the FAB scheme) for the diagnosis of AML ***(Brunning et al., 2001).*** In addition, patients with the clonal, recurring cytogenetic abnormalities t(8;21)(q22;q22), inv(16) (p13q22) to have AML regardless of the blast percentage ***(Vardiman et al., 2002).***

Table (3): WHO Classification of AML and related neoplasms

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); MLLT3-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: • AML with minimal differentiation • AML without maturation • AML 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

Myeloid sarcoma
Myeloid proliferations related to Down syndrome: Transient abnormal myelopoiesis Myeloid leukemia associated with Down syndrome
Blastic plasmacytoid dendritic cell neoplasm

(Vardiman et al., 2009 and Weinberg et al., 2009).

Diagnosis of AML

- **Clinical Diagnosis of AML**

  - **Non-specific manifestations:**

Pallor, fatigue, weakness, palpitation and dyspnea on exertion reflect the development of anemia. Anorexia and weight loss are frequent finding. Fever is present in many patients at the time of diagnosis ***(Lichtman and Liesveld, 2010).***

  - **Haemorrhagic manifestations:**

Easy bruising, petichae, epistaxis and other bleeding tendencies, reflecting thrombocytopenia are frequent early manifestations of the disease ***(Lichtman and Liesveld, 2010).*** Coagulopathy is discussed in details in Chapter 2.

  - **Skin manifestations:**

Discrete leukemic cells involving the skin may be the first manifestation of acute myeloid tumor, or it may be presented in the form of non-specific lesions: leukemia cutis or granulocytic sarcoma of skin (Figure 1). Lesions are in the form of macules, papules, vesicles, pyoderma gangrenosa or vasculitis. Skin involvement preceding BM and PB involvement is rare. Cryptococcosis most commonly occurs in immunosuppressed patients ***(Yonal et al., 2011)*.**

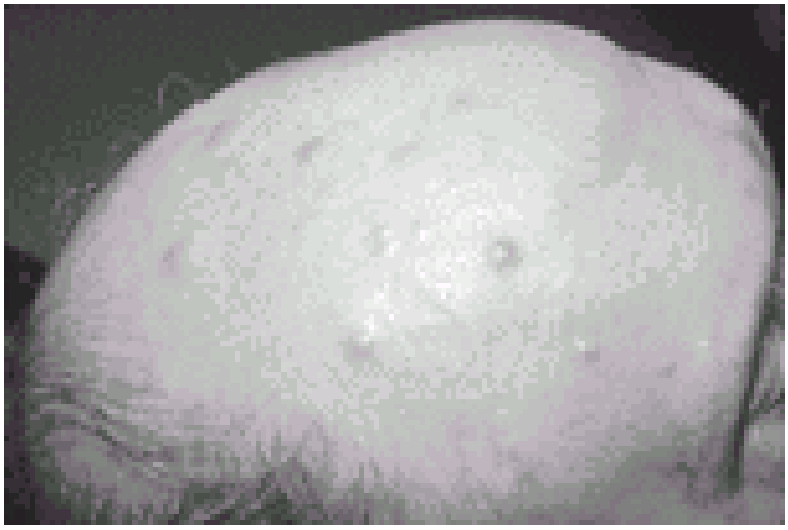

Fig. (1): Leukemia cutis manifesting as subcutaneous nodules ***(Greer et al., 2009)*.**

- **Cardio-respiratory manifestations:**

Pneumonia is the most common pulmonary problem, while pulmonary leukostasis and chemotherapy-related toxicities may occur subsequent to therapy. Cardiac dysfunction, including murmurs, heart failure and dysrythmia, is most often secondary to anaemia ***(Larson et al., 2007).***

- **Gastrointestinal tract manifestations:**

Dysphagia, oral candidiasis, gingival hypertrophy, perirectal infection and fulminant necrotizing colitis related to granulocytopenia

and cytotoxic therapy are frequent ***(Lichtman and Liesveld, 2010).***

- **Organomegaly:**

Palpable splenomegaly or hepatomegaly occurs in one third of patients. Lymphadenopathy is extremely uncommon except in the monocytic variant of AML ***(Lichtman and Liesveld, 2010)***. Extramedullary involvement is most common in monocytic or myelomonocytic leukemia ***(Hejmadi et al., 2008).***

- **Central Nervous System (CNS) manifestations:**

The CNS involvement in AML is uncommon but it may be manifested either as myeloblastoma (which is rare), or as typical meningeal infiltration with or without cranial nerve palsy. The most common presenting symptoms are consequences of increased intracranial pressure and usually consist of constant headache, sometimes associated with lethargy or other mental changes ***(Schiffer et al., 2004).***

- **Ocular manifestations:**

Retinal hemorrhages are most often caused by thrombocytopenia, however, patients with extreme hyperleukocytosis may develop so-called cotton-wool spots as a result of retinal ischemia. The conjunctivae may be pale, according to the magnitude of the anemia ***(Larson et al., 2007).***

- **Urogenital manifestations:**

Proctitis, especially is common in the monocytic variant of AML. It can be a presenting sign or a difficult problem during periods of severe granulocytopenia and diarrhea ***(Larson et al., 2007).***

• Laboratory Diagnosis of AML

- **Blood Picture:**
  - ***Red blood cells (RBCs)****:*

Anemia is a constant feature. Red cell morphology is mildly abnormal with exaggerated variation of cell-size and occasional poikilocytes. Nucleated red cells or stippled erythrocytes may be