INTRODUCTION

Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common complication of malignancy ***(Heit et al., 2000)***.

Patients with cancer have a four- to six-fold higher relative risk of VTE than age- and sex-matched controls and the overall prevalence of cancer-associated thrombosis is 15%, with rates as high as 50% in advanced metastatic cancer patients ***(Heit et al., 2002)***. As a consequence, VTE represents the second cause of morbidity and mortality in cancer patients ***(Khorana, 2010)***.

The VTE risk in patients with hematological malignancies has been considered lower than that in solid tumors for long time and physicians have been more concerned for bleeding rather than thrombotic complications in such patients ***(Falanga and Marchetti, 2009)***. However, recent studies suggest that the incidence of thromboembolic events in oncohematological patients may be similar, or even higher, to that found in patients with solid cancers ***(Castelli et al., 2010).***

VTE is a common problem in patients with acute leukemia. Depending on the type of leukemia whether acute myeloid leukemia (AML) or acute lymphoid leukemia (ALL), the VTE incidence ranges from 2.1% up to 12.1% ***(Mohren et al., 2006)***. Even in the absence of symptomatic VTE, many

cancer patients present with abnormal blood coagulation tests indicating a hypercoagulable state ***(Caine et al., 2002)***. Dysfunctional alterations of the protein C pathway may be one mechanism responsible for hypercoagulability in these patients. Activated protein C (APC) proteolytically inactivates the activated coagulation cofactors V (FV) and VIII (FVIII). A poor anticoagulant response of plasma to the addition of APC is known as APC resistance ***(Dahlback et al., 1993)***.

APC resistance is most commonly a result of the FV Leiden mutation, which results in a single arginine to glutamine amino acid substitution at the predominant APC cleavage site of FV. APC resistance may also develop because of acquired defects in the protein C system. Both familial and acquired APC resistance are major risk factors for VTE ***(De Visser et al., 1999).*** Previous studies have found that acquired APC resistance is common among patients with solid tumors, and is a more important risk factor for VTE in cancer than in non-malignant conditions ***(Haim et al., 2001)***.

Acquired APC resistance has also been demonstrated in multiple myeloma (MM) ***(Elice et al., 2006)***, but we are not aware of studies that have investigated APC resistance longitudinaly in patients with acute leukemia.

Aim of the Work

To measure acquired activated protein C resistance in patients with acute leukemia and to correlate it with other known standard prognostic factors as well as thrombotic complications.

Chapter One

THE NATURE OF LEUKEMIA

Leukemia is a disease resulting from the neoplastic proliferation of haemopoietic or lymphoid cells. It results from mutation of a single stem cell, the progeny of which form a clone of leukemic cells. Usually there is a series of genetic alterations rather than a single event. Genetic events contributing to malignant transformation include inappropriate expression of oncogenes and loss of function of tumor suppressor genes ***(Bain, 2010).*** Leukemias are broadly divided into: (i) acute leukemia, which, if untreated, leads to death in weeks or months; and (ii) chronic leukemia, which, if untreated, leads to death in months or years. They are further divided into lymphoid, myeloid and mixed lineage (biphenotypic or bilineage) leukemias, the latter showing usual differentiation ***(Bain, 2010).***

I. Epidemiology:

Although the incidence of acute leukemia accounts for less than 3% of all cancers, these diseases constitute the leading cause of death due to cancer in children and persons aged less than 39 years ***(Deschler and Lubbert, 2006).***

AML is the most common type of leukemia in adults, as it accounts for approximately 25% of all leukemias in adults in the Western world ***(Greenlee et al., 2001).*** It continuously shows 2

peaks in occurrence in early childhood and later adulthood, with an incidence of 3.7 per 100,000 persons ***(Deschler and Lubbert, 2006).*** It is slightly more common in males. Little difference in incidence is seen between individuals of African or European descent at any age. A somewhat lower incidence is seen in persons of Asian descent ***(Lichtman and Liesveld, 2007*).**

ALL is the most common malignancy diagnosed in patients under the age of 15 years, accounting for nearly one third of all pediatric malignancy and 76% of all leukemias in this age group ***(Carroll et al., 2003).*** Only 20% of adult acute leukemias are ALL with overall incidence 1 to 1.5 per 100,000 persons ***(Jabbour et al., 2005).*** It was found that ALL occurs more frequently in whites, and affects males more often than females in all age groups ***(Jemal et al., 2004)***.

II. Pathogenesis

In general, leukemia occurs when some blood cells acquire mutations in their DNA. The mutations cause the cell to grow and divide more rapidly and to continue living when normal cells would die. Over time, these abnormal cells can crowd out healthy blood cells, causing the signs and symptoms of leukemia ***(LeViseur et al., 2008).***

A) Pathogenesis of AML:

AML is characterized by acquisition of somatic mutations in hematopoietic progenitors that confer a proliferative and/or survival advantage,impair hematopoietic differentiation and provide properties of limitless self-renewal.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).***

1. 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).***

A number of hematopoietic growth factors have been found to stimulate AML cells, including granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interleukins 1, 3, 4, 6 and 9, Steel factor (KIT ligand), thrombopoietin (MPL ligand) and FLT3 ligand (FLT3L).The effects of growth factors are predominantly proliferative, but they may also be anti-apoptotic ***(Vincent and DeVita, 2012).***

2. 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 CEBPA ***(Licht and Sternberg, 2010).***

Other common chromosomal abnormalities in AML are monosomies or deletions of part or all of chromosomes 5 or 7 (–5/–7 AML) and trisomy 8 ***(Byrd et al., 2002)***. The chromosomal abnormalities also include the long arm of chromosome 11 (11q); balanced translocations between chromosomes 15 and 17 (t(15;17)); chromosomes 8 and 21 (t(8;21)); others such as (q22;q22), (q31;q22), and t(9;11); and inversion such as inv(16) ***(Mrózek et al., 2009).***

Table (1) shows the most frequent chromosomal aberrations and their corresponding fusion genes in AML. The translocation in t(15;17) is always associated with APL and leads to the expression of PML-RARα oncofusion gene in hematopoietic myeloid cells ***(Melnick and Licht, 1999).***

Table (1): Acute Myeloid Leukemia (AML)–Associated Oncofusion Proteins.

Translocations	Oncofusion protein	Frequency of occurance (% of AML)
t(8;21)	AML1-ETO	10 %
t(15;17)	PML-RARα	10 %
Inv(16)	CBF-MYH11	5 %
der(11q23)	MLL-fusions	4%
t(9;22)	BCR-ABL1	2 %
t(6;9)	DEK-CAN	< 1%
t(1;22)	OTT-MAL	< 1%
t(8;16)	MOZ-CBP	< 1%
t(7;11)	NUP98-HOXA9	< 1%
t(12;22)	MN1-TEL	< 1%
Inv(3)	RPN1-EVI1	< 1%
t(16;21)	FUS-ERG	< 1%

Many of the gene rearrangements involve a locus encoding a transcriptional activator, leading to expression of a fusion protein that retains the DNA-binding motifs of the wild-type protein. Moreover, in many instances, the fusion partner is a transcriptional protein that is capable of interacting with a corepressor complex.

A commonly accepted paradigm is that through aberrant recruitment of a corepressor to a locus of active transcription, the fusion protein alters expression of target genes necessary for myeloid development, thus laying the groundwork for leukemic transformation ***(Mitelman et al., 2007).***

Gene mutations in AML are divided into 2 broadly defined complementation groups. One group (class I) comprises mutations that activate signal transduction pathways and thereby increase the proliferation or survival, or both, of hematopoietic progenitor cells. The other complementation group (class II) comprises mutations that affect transcription factors or components of the cell cycle machinery and cause impaired differentiation ***(Mrózek et al., 2007)***

Class I Mutations:

FLT3 mutations Fms-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase that plays a key role in cell survival, proliferation, and differentiation of hematopoietic stem cells. It is frequently over expressed in acute leukemias. FLT3 mutations occur in approximately 30% of AML patients and confer a poor prognosis ***(Stirewalt and Radich, 2003).***

RAS mutations: Mutations in NRAS and KRAS occur in approximately 10% and 5% of AML patients, respectively ***(Tyner et al., 2009).***

Class II Mutations:

Mutations in MLL (Mixed lineage leukemia), brain and acute leukemia gene (BAAL), Wilms tumor gene (WT-1), CCAAT/ enhancer-binding protein α (CEBPα), and nucleophosmin (NPM1) have been observed in AML patients ***(Grisendi et al., 2006).***

Recently, mutations in DNA methyltransferase gene DNMT3A have been identified in one third of patients with de novo AML. DNMT3A represents 1 of 3 human genes that encodes DNA methyltransferase that catalyzes the addition of methyl groups to cytosine within CpG dinucleotide, resulting in repression of nearby genes ***(Ley et al., 2010).***

3. Escape from Programmed Cell Death:

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).***

4. Self-renewal:

Unlike normal progenitor cells, leukemic cells in AML patients can undergo self-renewal rather than lineage-specific commitment. The expression of cytoplasmic nucleophosmin (NPM) variant is associated with expression of genes thought to support maintenance of the stem cell phenotype ***(Alcalay et al., 2005).***

B) Pathogenesis of ALL:

Normal lymphoid cell populations undergo diverse clonal rearrangements of their IG or T-cell receptor (TCR) genes. Cells that successfully complete these genetic changes undergo a highly regulated process of proliferation that results in the production of normal B and T cell populations. Genetic

alteration of a lymphoid progenitor cell through somatic changes results in uncontrolled proliferation and clonal expansion. The leukemic blasts infiltrate the bone marrow and other organs, thus disrupting their normal function and eventually leading to the development of ALL. The leukemic blasts represent a clonal expansion of a single cell. This has been demonstrated by cytogenetics, glucose-6-phosphate dehydrogenase characterization, and analysis of antigen-receptor gene rearrangements and X-linked restriction fragment-length polymorphisms ***(Zhu et al., 2010).***

The leukemic cells duplicate most of the features of normal lymphoid progenitor. Genetic abnormalities in ALL include microscopically evident chromosomal rearrangements or lesions detectable only by molecular analysis. In addition, chromosomal translocations or aneuploidy are found in 75% of ALL cases. These translocations are commonly recurring and are rarely classified as random translocations ***(Strefford et al., 2006).*** Molecular abnormalities seen in ALL can be classified according to the functional consequence of oncogenic mutation. Activation of the ABL protein kinase via rearrangement with the BCR gene is an example of a mutation that results in a proliferative advantage. The most common cytogenetic abnormality in adult ALL results from chromosomal translocation t(9;22)(q34;q11), the Philadelphia chromosome ***(Harewood et al., 2003).***

Other gene rearrangements may result in loss or gain of function mutations involving transcription factors that play a role in haematopoietic development. An example of such gene rearrangement is the t(12;21)(p13;q22) chromosomal translocation that juxtaposes the TEL genes ***(Sulong, 2010).*** Other mechanisms of cancer formation involve loss or inactivation of tumor-suppressor genes via deletions and gene rearrangements. Examples of such mechanisms involve p16 (INK4A) and p53 ***(Graux et al., 2004).***

FLT3 and NOTCH1 have been identified as genes mutated in AML/hyperdiploid and T-ALL, respectively. CREBBP mutations are seen in 18% of relapsed ALL and may confer resistance to therapy ***(Silverman and Sallan, 2003).***

PAX5 gene is mutated in up to 30% of pediatric patients with ALL ***(Esparza and Sakamoto, 2005).*** IKZF1 mutations may be a predictor of relapse ***(Dworzak and Panzer, 2003).***

PHF6 mutations are seen in 38% of adult T-ALL samples. CDKN2A mutations are seen in 42% of cases of T-ALL ***(Swerdlow et al., 2008).***

III. Classification:

Acute leukemia can be classified:

- By morphology and cytochemistry supplemented by immunophenotyping, as proposed by French-American-British (FAB) group ***(Bennet et al., 1976).***
- Proposed World Health Organization Classification of Acute Leukemia ***(Harris et al., 1999).***

The original classification scheme proposed by the French-American-British (FAB) Cooperative Group divides AML into 8 subtypes (M0 to M7) and ALL into 3 subtypes (L1 toL3).

A) Morphologic classification (FAB) type:

Although AML blasts evolve from common myeloid precursors, the 8 subtypes differ in degree of maturation (Table 2).

Table (2): French-American-British (FAB) Classification of Acute Myelogenous Leukemia.

M0	AML with no Romanowsky or cytochemical evidence of differentiation.
M1	Myeloblastic leukemia with little maturation.
M2	Myeloblastic leukemia with maturation.
M3	Acute promyelocytic leukemia (APL).
M3h	APL, hypergranular variant.
M3v	APL, microgranular variant.
M4	Acute myelomonocytic leukemia (AMML).
M4eo	AMML with dysplastic marrow eosinophils.
M5	Acute monoblastic leukemia (AMoL).
M5a	AMoL, poorly differentiated.
M5b	AMoL, differentiated.
M6	"Erythroleukemia".
M6a	AML with erythroid dysplasia.
M6b	Erythroleukemia.
M7	Acute megakaryoblastic leukemia (AMkL).

The FAB classification of ALL includes 3 subtypes (L1 to L3), which are differentiated based on morphology, including cell size, prominence of nucleoli, and the amount and appearance of cytoplasm (Table 3). Approximately 75% of adult ALL cases have blasts with the B –cell phenotype and 25% have blasts with the T-cell phenotype.

Table (3): Morphologic (FAB) Classification of Acute Lymphocytic Leukemia.

L1	Small cells with scanty cytoplasm; nucleoli indistinct and not visible.
L2	Large, heterogeneous cells with moderately abundant cytoplasm; clefting and indentation of nucleus; large and prominent nucleoli.
L3	Large cells with moderately abundant cytoplasm; regular, oval-to-round nucleus; prominent nucleoli; prominent cytoplasmic basophilia and cytoplasmic vacuoles.

B) WHO Classification of Acute Leukemia:

The classification schemes by the World Health Organization (WHO) require the additional evaluation of the leukemic blasts by molecular analysis and flowcytometry ***(Sachdeva et al., 2006).*** The results of these 4 methods of evaluation (i.e, morphology, staining, molecular analysis, flow cytometry) not only differentiate ALL from AML, but also categorize the subtypes of acute leukemia.

1) **The 2008 WHO Classification of AML:**

a) AML 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)
- Acute promyelocytic leukemia (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)