Assessment of CD49f Expression in Acute Lymphoblastic Leukemia

Thesis

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INTRODUCTION AND AIM OF THE WORK

Acute lymphoblastic leukemia (ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow **(Pui et al., 2008)**.

Acute lymphoblastic leukemia (ALL) may be distinguished from other malignant lymphoid disorders by the immunophenotype of cells. Immunochemistry, cytochemistry, and cytogenetic markers may also aid in categorizing the malignant lymphoid clone **(Seiter, 2009)**.

Although prognostic factors for response and disease-free survival depend on the treatment regimen, however, the most important prognostic ones are clinical parameters, age, white blood cell count (WBC), immunophenotype, cytogenetic, and molecular genetics determined at diagnosis of ALL. Recent researches aim to improve treatment outcome by optimizing dosage and scheduling of anti leukemic agents based on the individual patient's leukemic cell phenotypic and genetic features, in addition to host normal cell pharmacogenetics characteristics **(Hattori et al., 2006 and Borowitz et al., 2008)**.

In ALL patients; immunophenotypic assessment at diagnosis and measurements of minimal residual disease (MRD) during therapy provide crucial information about

the response to treatment and the risk of relapse **(Parab et al., 2007)**.

Flow cytometry is a practical and widely applicable tool for initial diagnosis and monitoring MRD in patients with ALL. This approach is based on the identification of immunophenotypes expressed by leukemic cells but not by normal lympho-haematopoietic cells in bone marrow and peripheral blood. These phenotypes can identify one leukemic cell among 10, 000 normal cells and are currently applicable to at least 90% of patients with ALL. A strong correlation between flow cytometric measurements at initial diagnosis, during clinical remission and treatment outcome has been demonstrated, suggesting that these assays should be incorporated into treatment protocols (**Vaskova et al., 2005).**

Recent studies have showed a preponderance of cells displays dim or un- detectable CD49f in normal B - lymphopoiesis, whereas in most cases of precursor B-ALL, CD49f expression is moderate to bright. The latter finding suggests that CD49f expression may be up-regulated during treatment, as has been suggested previously for several other antigens whose expression changes during remission induction. On a more practical level, CD49f may be a potentially useful marker of MRD, even in cases with dim or undetectable CD49f at diagnosis **(DiGiuseppe et al., 2009).**

AIM OF THE WORK

The purpose of this study is to assess CD49f expression in 30 patients with acute lymphoblastic leukemia by flow cytometry; and correlate its expression with standard prognostic factors in such cases. In addition evaluate the CD49f expression as useful marker for detection of minimal residual disease .

Chapter (I)

ACUTE LYMPHOBLASTIC LEUKEMIA

Acute lymphoblastic leukemia (ALL) is a malignant disorder of lymphoid progenitor cells that proliferate and replace the normal hematopoietic cells of the bone marrow. These lymphoblasts replacing the normal bone marrow elements result in a marked decrease in the production of normal blood cells ***(Whitlock et al., 2010).***

It is a rapidly progressing form of leukemia that is characterized by the over production and continuous multiplication of malignant and immature white blood cells (referred to as lymphoblast) in the bone marrow. It is fatal if left untreated as ALL spreads into the bloodstream and other vital organs quickly ***(Marshall et al., 2006)*** However it is among the most curable of the pediatric malignancies ***(Alison et al., 2006).***

I-Epidemiology

Incidence

The ALL is the most common childhood malignancy, accounting for approximately one third of pediatric cancers ***(Satake, 2010).***

It represents about 80% of childhood acute leukemia with peak prevalence between the ages of 2 and 5 years; however, it makes only up to 20% of adult

leukemias ***(Jabbour et al., 2005*; *Pui, 2006, Pui et al., 2008)***.

The overall incidence of ALL varies among different racial groups, Whites are more frequently affected than black (**Satake, 2010)**. In adults there is a slight male predominance with a male to female ratio of 1.3:1.0. Geographic differences in the incidence of ALL are reflected by higher rates in North America and Europe and lower rates in African and Asian populations **(*Cao and Coutre, 2004*).**

II- Pathogenesis and Aetiology:

The precise pathogenetic events leading to development of ALL are unknown ***(Bunin, 2004; Lightfoot and Roman, 2004)***. Epidemiologic studies have examined a number of possible risk factors (e.g., environmental, genetic, immunological, or infectious) in an effort to determine the etiology of the disease ***(Martin et al., 2006)***.

A-Environmental Factors:

Only one environmental risk factor (ionizing radiation) has been significantly linked with acute leukemia; most environmental risk factors as, electromagnetic fields (EMFs), or cigarette smoking, have been weakly or inconsistently associated with either form of childhood leukemia ***(Martin et al., 2006)***.

B-Genetic Factors:

A higher incidence of ALL has been noted among monozygotic and dizygotic twins of patients with ALL, reflecting possible genetic predisposition ***(Jabbour et al., 2005)***. Only a few cases (<5%) are associated with inherited, predisposing genetic syndromes, such as Down's syndrome, Nijmegen breakage syndrome ***(Pui et al., 2008)***, and inherited diseases with excessive chromosomal fragility such as Fanconi anemia. Bloom syndrome, and ataxia-telangiectasia have a higher risk of developing ALL ***(Jabbour et al., 2005).***

A positive family history of hematopoietic malignancies among first- or second-degree relatives has been associated with a small increased risk for childhood ALL. Furthermore, the risk for ALL is not increased in children with a family history of cancers other than hematopoietic malignancies ***(Infante-Rivard and Guiguet, 2004)*.**

C- Infectious Agents:

The human T lymphotropic virus I and II (HIVI and II) are retroviruses that are implicated in some cases of adult T-cell leukemia/lymphoma ***(Foon and Fisher, 2001).*** The main feature of retroviruses is that they contain RNA, direct DNA synthesis from RNA template by the enzyme reverse transcriptase after invading the host. This virus can be transmitted from mother to child ***(Yatgin et al., 2001)***.

Herpes viruses, as Epstein Barr virus (EBV) are highly associated with the development of leukemia L3 subtypes of ALL ***(Annino et al., 2002)***.

Parvovirus is incorporated into the DNA of the nucleus; where it is replicated by the cell along with host nuclear DNA ***(Harrison et al., 2002)***. The host response during parvovirus B19 infection associated acute leukaemia may provide insights into possible mechanisms by which the virus may at least precipitate the overt leukaemia. The chemokine MCP-1 is released by leukaemic cells; it is chemotactic for monocytes and induces the tumoricidal activity of monocytes, and is thus a possible host defence against neoplasia. B19 virus infection is associated with immune cell proliferation and upregulation of key mediators, such as GM-CSF, IL-6, TNFα and IFNγ. Such mechanisms may play an important role in the conversion of preleukaemic clones to an overt leukaemia. BM examination usually reveals suppression of erythroid elements **(Kerr et al., 2003).**

D-Immunodeficiency:

Acute lymphoblastic leukemia may also occur in children with various congenital immunodeficiencies (ie, Wiskott-Aldrich syndrome, congenital hypogammaglo-bulinemia, and ataxia-telangiectasia) that have an increased predisposition to develop lymphoid malignancies **(Satake, 2010).** Abnormally low serum immunoglobulin levels have been observed in 30% of

newly diagnosed acute leukemia patients. It is unclear whether such abnormalities precede the development of leukemia or are a consequence of the disease ***(Broder et al., 1998).***

E-Other Important Variables:

Reproductive history and birth charac-teristics: The risk for childhood ALL has been shown to be significantly higher among children who were born when their parents were older; significant trends in ALL incidence have been related to increasing mother's (>35 years) ***(Martin et al., 2006)***.

Several studies have described an association between increased birth weight and childhood ALL, particularly ALL developing early in life. Increased birth weight has been associated with a high rate of cell proliferation and with an increase in the number of precursor cells that are at risk for malignant transformation. However, birth weight tends to increase with maternal age, which is also a known risk factor for ALL in offspring ***(Martin et al., 2006)***.

III-Pathophysiology:

The development of ALL is believed to involve a transformation event that occurs in a single progenitor cell that has the capability for indefinite clonal expantion. The leukemogenic event may occur in committed lymphoid cells of B- or T-cell lineages or in early precursors, which gives rise to the different subtypes of

ALL based on the stage of lymphoid differentiation of the cell in which the event occurred **(Esparza and Sakamoto, 2005).**

A-Genetic Abnormalities:

Cytogenetic abnormalities occur in approximately 70% of cases of acute lymphoblastic leukemia (ALL) in adults **(Seiter, 2011).** In general, hematological malignancies are characterized by recurrent chromosomal aberrations that lead to the formation of gene fusions and the subsequent expression of chimeric proteins with unique properties **(Brassesco et al., 2011).** These include the aberrant expression of proto-oncogenes, chromosomal translocations that create fusion genes encoding active kinases and altered transcription factors, and hyperdiploidy involving more than 50 chromosomes. These genetic alterations contribute to the leukemic transformation of hematopoietic stem cells or their committed progenitors by changing cellular functions ***(Hanahan and Weinberg, 2000).***

Altered cellular functions include an enhanced ability of self-renewal, a subversion of control of normal proliferation, a block in differentiation, and an increased resistance to death signals or apoptosis ***(Moorman et al., 2007).***

Table (1) Common Cytogenetic Abnormalities in ALL:

Abnormality	Genes Involved	3-Year Event-Free Survival
t(10;14)(q24;q11)	*HOX11*/*TCRA*	75%
6q	Unknown	47%
14q11	*TCRA*/*TCRD*	42%
11q23	*MLL*	18-26%
9p	Unknown	22%
Chromosome 12	*TEL*	20%
t(1;19)(q23;p13)	*PBX1*/*E2A*	20%
t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)	c-*myc*/*IGH* *IGK*/c-*myc* c-*myc*/*IGL*	17%* 80%† 5-10%*
t(9;22)(q34;q11)	*bcr-abl*	66%‡
t(4;11)(q21;q23)	*AF4-MLL*	0-10%

(Seiter, 2011)

*Traditionalregimens.†Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin[Adriamycin], dexamethasone) with rituxan.
‡Hyper-CVAD with imatinib.

1. Chromosomal Translocations:

Altered self-renewal and differentiation of hematopoietic stem cells can also result from chimeric transcription factors, which arise from genetic translocations that fuse portions of two different transcription factors. These chimeric transcription factors

activate diverse transcriptional cascades that, at least in part, converge to modify the normal pattern of expression of members of the important family of HOX genes, which encode the HOX transcription factors ***(Buske and Humphries, 2000; Ferrando and Look, 2003)***. The HOX transcription factors bind to DNA and regulate genes involved in the differentiation of both the embryo and the hematopoietic stem cell; they are also important in the self-renewal and proliferation of hematopoietic stem cells ***(Pui et al., 2004 b).***

a-The TEL-AML1 Fusion gene:

The fusion gene translocation TEL/AML1, resulting from the translocation t(12; 21) (p13;q22), is present in 20–30% of childhood ALL ***(Schmidt et al., 2009).***

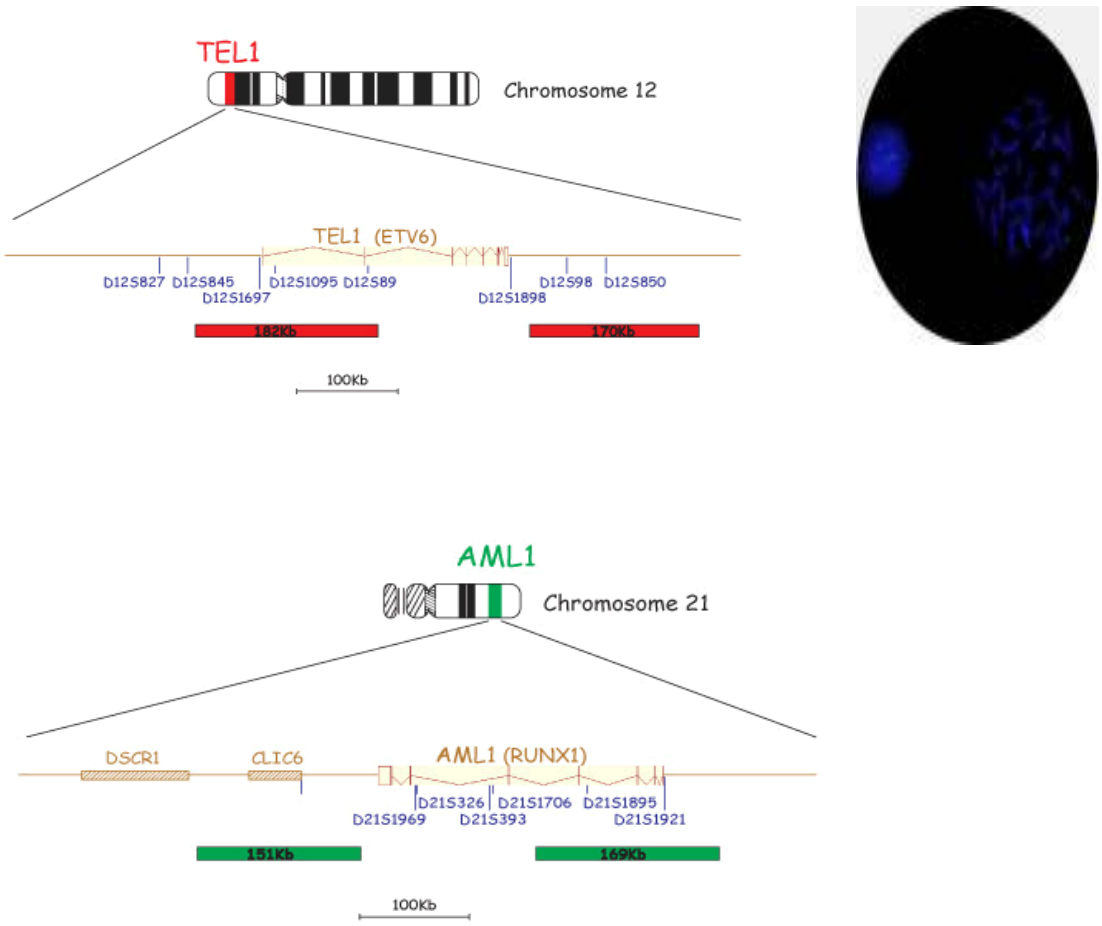

Fig (1): The TEL/ AML1 fusion gene is brought about by the cytogenetically invisible t(12;21) translocation. using FISH in around 21-37% of ALL pediatric patients **(cytocell)**

Tel gene is an important regulator of haemopoietic-cell development, essential for definitive haemopoiesis ***(Hock et al., 2004).*** It is almost the entire coding region of another transcription factor gene, and is required for the homing of hematopoietic progenitor cells to the bone marrow. AML1, encodes the α subunit of core binding factor (also called CBFα), a master regulator of the formation of definitive hematopoietic stem cells ***(Pui et al., 2004)***. The chimeric TEL-AML1 transcription factor retains an essential protein–protein interaction domain of TEL and the DNA-binding and transcriptional regulatory sequences of AML1 ***(Loh and Rubnitz, 2002; Speck and Gilliand, 2002)***.

A prominent effect of the TEL-AML1 fusion protein is inhibition of the transcriptional activity that is normally initiated when AML1 binds to a DNA region termed the core enhanced sequence ***(Hiebert et al., 1996).*** The binding of AML1 to the core enhanced sequence recruits other transcription factors and coactivators to this region, and the resulting protein complex regulates transcription. These changes in the normal AML1-mediated transcriptional cascade alter both the self-renewal capacity and the differentiation capacity of hematopoietic stem cells ***(Speck and Gilliland, 2002; Downing, 2003).***

TEL-AML1 occurs as a 1st hit already in utero and leads to the expansion of a pre leukemic clone. A 2nd hit, usually postnatal, is required to fully transform these cells

leading to the clinical manifestation of the leukemia. The pre leukemic clone may survive treatment thereby providing the reservoir for other secondary mutations that give rise to subsequent leukemia - clinically diagnosed as a relapse ***(Grümayer, 2007).***

b-*Translocations involving the MLL gene:*

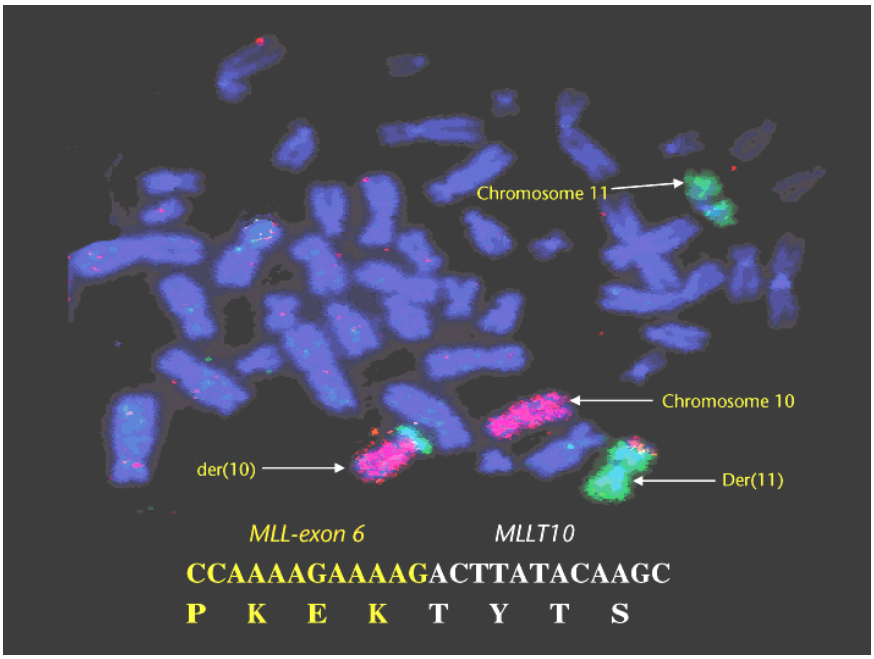

Fig 2: Metaphase spread of an acute myeloid leukemic cell, showing a balanced translocation between chromosomes 11 (green) and 10 (pink). All other chromosomes are in blue. In this case the translocation resulted in an in-frame fusion of the 5□ end of the MLL gene on 11q23 with the 3□ end of the MLLT10 gene on 10p12 **(Saha and Kathleen, 2006)**

A second component of the HOX regulatory pathway is the mixed-lineage leukemia (MLL) protein ***(Ernst et al., 2002; Ayton and Cleary, 2001)***, a nuclear protein that maintains the expression of particular members of the HOX family. Leukemia-associated