THE CLINICAL SIGNIFICANCE OF PROGNOSTIC FEATURES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN SOUTH EGYPT CANCER INSTITUTE

By

Nisreen Mohamed Aly Khalifa

M.B.B.Ch., M.Sc. (Pediatrics)

Assissant lecturer- Pediatric Unit- SECI

Supervised By

Prof. Dr. Salah Saleh Abdel-Hadi

Professor of Pediatric Oncology

National Cancer Institute

Cairo University

Prof. Dr. Mohamed Hamdy Ghazally

Professor of Pediatrics

Faculty of Medicine

Assiut University

Prof. Dr. Farida Hassan Gad-Allah

Professor of Clinical Pathology

National Cancer Institute

Cairo University

٢٠٠٥

Acknowledgments

This study was carried out at South Egypt Cancer Institute, Assiut University, Egypt. I wish to express my sincere gratitude to **Professor Dr. Atef Abdel–Aziz**, the Dean of the Institute for providing the facilities, and inspiring atmosphere for research.

I am grateful to my supervisor, **Professor Dr. Salah Abdel-Hadi**, Professor and Head of the Pediatric Oncology Department, National Cancer Institute, Cairo University, for guiding me through this research project. His profound knowledge in the field of research and pediatric oncology was crucial for this work, his support pushed me through various difficult challenges along the way. Thank you Dr Salah.

I am grateful to Professor **Dr. Mohamed H. Gazally** the General Director of the Pediatrics Hospital and the supervisor of the Pediatric Unit at South Egypt Cancer Institute, who suggested the idea of this work, and his kind support, his great experience has added much to my knowkedge. I stand in great debt for all what he did.

I want to express my gratitude to **Professor Dr. Farida Gad-Allah**, Professor of Clinical Pathology, National Caner Institute, Cairo University, for providing the facility and expertise in cytogenetics. She is aknowledged for the enormous work she did with the G-banding analysis, her stimulating discussions, and valuable comments during preparation of this work.

I wish to thank my colleagues in the clinical pathology department, and **Professor Dr. Mohamed R. Khalef** the Head of the Department, for providing the bone marrow slides and immunophenotyping for my patients.

I express my warmest gratitude to the reviewers of the thesis, **Dr. Mohamed Fawzy**, Lecturer of Pediatric Oncology, National Cancer Institute and **Nabiel N. Mikhail**, Assistant Lecturer of Cancer Epidemiology, South Egypt Cancer Institute, for their valuable comments during the manuscript preparation, and for statistical advice.

I wish to thank all my colleagues and friends at South Egypt Cancer Institute, the personnel working in the pediatric unit, without their help, it would not have been possible to carry out this work. I wish to thank them all and particularly the clinical pharmacy team.

I warmly thank the families and parents of the patients, for their confidence in me, and for their support during these years, sharing with me the most important moments in their children lives, and various moments of enjoyable free time with them.

List of contents

List of Tables

List of Figures

List of Abbreviations

٦-MP:	mercaptopurine
ACTH:	adreno cortico trophic hormone
AIEOP:	Italian Association of Pediatric Hematology and Oncology
ALL:	acute lymphoblastic leukemia
AML:	acute myeloid leukemia
Ara-c :	aracytine
BFM:	Berlin-Frankfurt-Münster
BM:	bone marrow
BMT:	bone marrow transplantation
CALLA:	common acute lymphoblastic leukemia antigen
CCG:	Children Cancer Group
CD:	cluster of differentiation
CGH:	comparative genomic hybridization
CML:	Chronic myeloid leukemia
CNS:	central nervous system
COALL:	Co-operative Study Group for Childhood Acute Lymphoblastic Leukemia
CR:	complete remission
CCR:	continous complete remission
CSF:	cerebro spinal fluid
cyIgM:	cytoplasmic immunoglobulin M
DCLSG:	Dutch Childhood leukemia Study Group
DFCI:	Dana-Farber Cancer Institute
DFS:	disease free survival
DHFR:	dihydrofolate reductase
EFS:	event free survival
FAB:	French-American-British
FACS:	fluorescence activated cell sorter
FISH:	fluorescent *in situ* hybridization
GST:	glutathione-S-transferase

Gy:	gray
HDMTX:	high dose methotrexate
HLA-DR:	major and minor histocompatibilty antigens
HRG:	high risk group
IgM:	immunoglobulin M
IM:	intramuscular
IRG:	intermediate risk group
IT:	intrathecal
IPT:	immunophenotyping
IU:	international unit
IV:	intravenous
KDa:	kilo Dalton
LRP:	lung resistance protein
MPO:	myeloperoxidase
MRC:	Medical Research Council
MRD:	minimal residual disease
MRP:	multiple drug resistance protein
MTT:	methyl –thiazole -tetrazolium
MTX:	methotrexate
NCI:	National Cancer Institute
NOPHO:	Nordic Society of Pediatric Hematology and Oncology
PAS:	periodic acid-Schiff
PB:	peripheral blood
PCR:	polymerase chain reaction
Ph+:	Philadelphia
POG:	Pediatric oncology group
RT-PCR:	reverse transcriptase polymerase chain reaction
SBB:	Sudan black B
SC:	subcutenous
SECI:	South Egypt Cancer Institute
sIg:	surface immunoglobulin
SJCRH:	St Jude Children Research Hospital

SKY: spectral karyotyping

SRG: standard risk group

TBI: total body irradiation

TCCSG: Tokyo Children's Cancer Study Group

TdT: terminal deoxynucleotidyl transferase

TSH: thyroid stimulating hormone

Vs: versus

WBC: white blood count

INTRODCTION

AND

AIM OF THE WORK

Acute lymphoblastic leukemia (ALL) is the most common cancer occurring in children representing ٢٣% of cancer diagnosis among children younger than ١٥ years in western communities (Reis, ١٩٩٦). Approximately ٧٥-٨٠% of children with ALL attain long term survival or even cure (Pui, ٢٠٠٠). As nearly all children with ALL achieve an initial remission, the major obstacle to cure is bone marrow and/or extramedullary relapse. Relapse can occur during therapy or after completion of treatment. While the majority of children with recurrent ALL attain a second remission, the likelihood of cure is then generally poor (Gaynon, ١٩٩٨).

This high cure rate is not reflected on the developing countries, where more than ٥٠% of the world children live, and the incidence of new pediatric cancer including ALL is rising(Agarwal, ٢٠٠٤).

Although there is no standardized staging of ALL analogous to that used for solid tumors and lymphomas, patients with ALL are usually treated according to risk groups defined by both clinical and laboratory features (Pui, ١٩٩٨). A uniform approach to risk classification and treatment assignment was proposed in a special workshop of NCI of USA (Smith et al., ١٩٩٦). For patients with ALL, the standard risk category included patients ١-٩ years of age, who have a WBCs count less than ٥٠,٠٠٠ per microliter. The remaining patients are classified as high risk ALL. In addition to age and WBCs, other important prognostic variables include DNA index (Ito et al., ١٩٩٩), early response to induction chemotherapy (Gaynon et al., ١٩٩٧), and levels of "minimal residual disease (MRD", i.e. leukemia calls that can only be detected by highly sensitive techniques such as polymerase chain reaction (PCR) or

specialized flow cytometry (Cavé, ١٩٩٨). These are significant prognostic variables regardless of initial risk group assignment.

In addition, advances in genetics have led to discovery of new prognostic features such as t(٩;٢٢), t(٤;١١) and t(١;١٩) which are associated with more aggressive disease, some translocation, such as t(١٢;٢١), actually correlate with favorable outcome (Pui, ١٩٩٠).

Risk-based treatment assignment is the key therapeutic strategy utilized for children with acute lymphoblastic leukemia (Smith et al., ١٩٩٦). This approach allows children who have good outcome with modest therapy to be spared more intensive and toxic treatment, while allowing children with higher risk to receive more intensive therapy that may increase their chance to cure (Pui, ٢٠٠٠).

Nearly a quarter of children with ALL who achieve complete remission by standard criteria eventually relapse and die from the disease (Elaine, ١٩٩٨). So numerous important biologic and therapeutic questions remain to be answered in order to achieve the goal of curing every child with ALL (Belyer, ١٩٩٧).

Aim of the Work:

The present study aims at:

١- Evaluating front-line chemotherapy tailored according to risk groups used in the Pediatric Unit, South Egypt Cancer Institute (SECI).

٢- Reviewing the prognostic features commonly used to determine treatment strategies for childhood ALL in SECI.

REVIEW OF LITERATURE

Epidemiology:

ALL is the most common malignancy in children, it accounts for one-fourth of childhood cancers and approximately ٧٥% of all cases of childhood leukemia (Pui, ٢٠٠٠).

The peak incidence of ALL occurs between age ٢ - ٥ years (Swensen, ١٩٩٧). Boys are more commonly affected than girls especially among pubertal children (Fraumeni,١٩٧٤).

Pathogenesis and molecular epidemiology

Acute lymphoblastic leukemia is a clonal disorder of the hematopoietic system arising from mutation in a single cell line that are passed on to all of its descendants (Bhatia,١٩٩٩),٥% of cases of leukemia are associated with inherited genetic syndromes (Taylor,١٩٩٦),e.g.trisomy ٢١,Bloom's syndrome, Fanconi anemia, ataxia-telangiectasia (Linet,١٩٨٥). But the aetiology remain unknown for the vast majority of cases (Gustafsson,١٩٩٩).

The precise pathogenetic events leading to the development of ALL is still unknown, but they are likely to affect genes that control lymphoid cell homoeostasis, resulting in dysregulated clonal expansion of immature progenitor cells(Pui, Campana, Evans, ٢٠٠١). The prenatal origin of some leukemias was established through genetic studies of identical twins with concordant leukemia and backtracking of leukemia-specific fusion-gene sequences(eg *TEL-AML ١)* of neonatal blood spots (Weimels et al., ١٩٩٩).

Fusion-gene sequence has a high concordance rate in identical twins and a very brief latency period, which suggests that this fusion per se may be sufficient for leukaemogenesis or, at least, may be able to provoke a secondary change leading to leukemia development (Greaves, ١٩٩٩).

The similarities between molecular genetics abnormalities in infant leukemias and topoisomerase Π inhibitor-related leukemias suggest that transplacental fetal exposure to substance that inhibit topoisomerase Π might be critical event in generation of leukemias. Flavonoids, quinolone antibiotics, benzene metabolites, catechins, and estrogens can all inhibit topoisomerase Π, both in vivo and in vitro, and may cause mutations that lead to acute leukemias (Biondi, ٢٠٠٠).

A recent case-control study disclosed significant associations between in utero exposure to DNA- damaging drugs, herbal medicines, and material pesticides, and the development of infant leukemia (Alexander et al., ٢٠٠١). It is possible that the activity of enzymes that detoxify carcinogens may be low in infants with leukemia or their mothers, since the functional doses from dietary and environmental exposure are much lower than those from anticancer chemotherapy. In this regard, the deficiency of glutathione-S-transferases (GST-M١ and GST-T١), enzymes that detoxify electrophilic metabolites by catalyzing their conjugation to glutathione, has been associated with infant leukemia (Biondi, ٢٠٠٠). Continued molecular epidemiological studies should provide further insights into the underlying mechanisms of leukemogenesis in children and may lead to the development of effective preventive measures (Pui, Campana, Evans, ٢٠٠١).

Clinical Presentation:

The presenting symptoms and signs of ALL are quite variable. Most cases have acute onset, while in others the initial signs and symptoms appear insidiously and persist for months (Pui, ١٩٩٩).

The presenting symptoms usually reflect the degree of bone marrow failure and extent of extramedullary spread. Fever is the most common finding, occurring in ٥٠%- ٦٠% of patients. In at least one third of these cases fever is due to leukemia and will resolve within ٧٢ hours after the start of induction therapy (Kosmidis, ١٩٨٠). Fatigue and lethargy are common manifestation of anemia, bone pain, arthralagia, or refusal to walk due to leukemic infiltration of the periosteum, bone, joints, or to

expansion of bone marrow cavity is common especially in very young children (Jonson, ١٩٩٠).

Occasionally, patients may present with life threatening infection or bleeding. In rare cases, ALL does not produce early signs or symptoms and is detected in routine examination (Pui, ١٩٩٩).

Physical examination may reveal pallor, petechiae, and ecchymoses in the skin and mucus membranes, bone tenderness. Liver, spleen, and lymph nodes are the most common sites of extramedullary involvement and are enlarged in more than one half of the patients (Pui, ١٩٩٩).

Ocular manifestation as retinal hemorrhage, leukemic infiltration of the orbit, optic nerve, retina, iris, cornea, in association with blurred vision, photophobia and ocular pain occur in ١٠% of the patients, but it become very rare with contemporary treatments (Lo curto, ١٩٩٤).

Painless enlargement of the scrotum can be a sign of testicular leukemia or hydrocele the latter resulting from lymphatic obstruction that is readily diagnosed by ultrasonography. Overt testicular disease is relatively rare at diagnosis occurring only in ٢% of the patients (Gajjar, ١٩٩٦).

Less common presenting features include subcutaneous nodules (leukemia cutis), enlarged salivary gland (Mikulicz syndrome), cranial nerve palsy, and priapism. Finally in some patients, infiltration of the tonsils, adenoids, appendix, or the mesenteric lymph nodes can occur (Pui, ١٩٩٩).

Laboratory Findings:

Blood Picture:

The presenting leukocyte counts range widely, from ٠.١ to ١٥.٠٠×١٠^{٩}/L (median, ١٢×١٠^{٩}/L) and are increased (>١٠×١٠^{٩}/L) in slightly over one half of the patients (Ritter, Schrappe, ١٩٩٩)