

Study of Interleukin 6 (IL-6) in Chronic Lymphocytic Leukemia

Thesis

*Submitted for the Partial Fulfilment of MD
Degree in Internal Medicine*

Presented By

Gehad Hamada Fekry

(M.B.B.Ch - MSc. Internal Medicine)

Under supervision of

Prof.Dr. Essam Abdelwahed Hassan

*Professor of Internal Medicine and Clinical Hematology
Faculty of Medicine, Ain Shams University*

Dr. Emad Abdelmohsen Abdelhady

*Assistant Professor of Internal Medicine and Clinical Haematology
Faculty of Medicine - Ain Shams University*

Dr. Mohamed Tarif Mohamed Hamza

*Assistant Professor of Clinical Pathology
Faculty of Medicine - Ain Shams University*

Dr. Hanaa Fathey Abdelsamee

*Assistant Professor of Internal Medicine and Clinical Haematology
Faculty of Medicine, Ain Shams University*

Dr. Mostafa Kamal El-Razzaz

*Lecturer of Internal Medicine and Clinical Haematology
Faculty of Medicine, Ain Shams University*

*Faculty of Medicine
Ain Shams University*

2018

Acknowledgment

*First and foremost, I feel always indebted to **ALLAH**, the Most Kind and Most Merciful.*

*I'd like to express my respectful thanks and profound gratitude to **Prof. Dr. Essam Abdelwahed Hassan**, Professor of Internal Medicine and Clinical Hematology Faculty of Medicine, Ain Shams University for his keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to **Dr. Emad Abdelmohsen Abdelhady**, Assistant Professor of Internal Medicine and Clinical Haematology Faculty of Medicine - Ain Shams University, for his kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

*I am deeply thankful to **Dr. Mohamed Tarif Mohamed Hamza**, Assistant Professor of Clinical Pathology Faculty of Medicine - Ain Shams University, for his great help, active participation and guidance.*

*I am deeply thankful to **Dr. Hanaa Fathey Abdelsamee**, Assistant Professor of Internal Medicine and Clinical Haematology Faculty of Medicine, Ain Shams University, for her great help, active participation and guidance.*

*I wish to introduce my deep respect and thanks to **Dr. Mostafa Kamal El-Razzaz**, Lecturer of Internal Medicine and Clinical Haematology Faculty of Medicine, Ain Shams University, for his kindness, supervision and cooperation in this work.*

Gehad Hamada Fekry

List of Contents

Title	Page No.
List of Tables	IV
List of Figures	VI
List of Abbreviations	X
Introduction	1
Aim of the Work.....	5
Review of Literature	
▪ Chronic Lymphocytic Leukaemia (CLL).....	6
▪ Management.....	54
▪ Cytokines.....	82
▪ Interleukin-6 (IL-6).....	96
▪ Relation Between IL 6 and CLL	104
Patients and Methods	110
Results	118
Discussion	142
Summary	154
Conclusion.....	156
Recommendations	157
References	158
Arabic Summary	—

List of Tables

Table No.	Title	Page No.
Table (1):	B-cell chronic lymphoproliferative disorders with leukaemic expression.....	6
Table (2):	Immunophenotype and other markers in B-cell chronic lymphoproliferative disorders.	9
Table (3):	The immunophenotype of CLL.....	20
Table (4):	CLL score in B-cell disorders.....	20
Table (5):	The value of bone marrow trephine biopsies in CLL.	21
Table (6):	FISH cytogenetics: biological and clinical correlates.....	25
Table (7):	Differences between CLL, SLL, and MBL.....	27
Table (8):	Most important Outcome Predictors in CLL.....	32
Table (9):	The clinical stage so of Rai and Binet.	35
Table (10):	Main treatment options for patients with CLL not included in trials.	60
Table (11):	Treatments for younger, fit patients.	61
Table (12):	Treatments for older, unfit patients.	65
Table (13):	BTK-targeting agents for CLL therapy.	69
Table (14):	PI3K δ -targeting agents for CLL therapy.....	72
Table (15):	Six cytokine families.....	91
Table (16):	Patients' demographic data as regard age and sex.	118
Table (17):	Patients' demographic data as regard CBC.	118
Table (18):	Patients' demographic data as regard LDH, ESR, Uric Acid, B2 microglobulin and BM Aspirate (% of lymphocytosis).	119
Table (19):	Patients' demographic data as regard classification (Rai and Binet staging systems).....	120

List of Tables Cont...

Table No.	Title	Page No.
Table (20):	Patients' demographic data as regard Coomb's test (direct), B symptoms, cyto 17P deletion, CD 38 and viral markers.....	122
Table (21):	Patients' demographic data as regard outcome.....	123
Table (22):	Comparison between patients and controls as regard serum IL-6 level.	124
Table (23):	Comparison between patient group (pre treatment) and patient group (post treatment) as regard serum IL-6 level.	125
Table (24):	Comparison between CR patients as regard serum IL-6 level pre and post treatment.	126
Table (25):	Comparison between PR patients as regard serum IL-6 level pre and post treatment.	127
Table (26):	Correlation between IL 6 level and other prognostic factors (age, WBC, B2 microglobulin, LDH, ESR, Uric Acid and BM Aspirate (% of lymphocytes).....	128
Table (27):	Correlation between IL 6 level and Sex, B Symptoms, Binet Staging system, Rai Staging system, cyto 17 p deletion, Coomb's test and CD 38.	132
Table (28):	Comparison between Non response, partial response and complete response patients' WBC, LDH, B2 microglobulin, Binet staging system, Rai staging system, cyto 17 p deletion CD 38 and IL 6.....	136

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Blood film of a typical case of CLL.....	14
Figure (2):	Blood film from a case of CLL/PL.....	15
Figure (3):	FISH analysis with a 13q14 probe showing missing red dots in several cells denoting 13q deletion; all the cells are disomic for chromosome 12 (normal pattern).....	16
Figure (4):	FISH analysis with a <i>TP53</i> probe (red) and a control 17 probe.....	16
Figure (5):	Flow cytometry analysis of peripheral blood samples from a case of follicular lymphoma with lymphocytosis (score 0) compared with a case of CLL (score 5) using the antibodies listed in Table 3.....	19
Figure (6):	Low magnification of a trephine biopsy from a patient with CLL and heavy (packed) lymphocytic infiltration.....	21
Figure (7):	Higher magnification of the same case as showing scanty fat spaces and diffuse lymphocytic infiltration	22
Figure (8):	Interstitial lymphocytic infiltration in a case of early CLL with preserved haemopoiesis and abundant fat spaces.....	22
Figure (9):	Nodular pattern of infiltration in CLL, early in the disease	22
Figure (10):	Diffuse infiltration in the bone marrow in a patient with CLL, a large spleen and thrombocytopenia.....	23
Figure (11):	Blood film of a patient with Richter's syndrome with circulating large blast cells that were positive for surface membrane immunoglobulin.....	30

List of Figures Cont...

Fig. No.	Title	Page No.
Figure (12):	CLL patients survival over the years (Hospital Clinic Barcelona).....	31
Figure (13):	Binet “lymphoid” areas	36
Figure (14):	Most immune system cytokines exhibit autocrine and/or paracrine action; fewer exhibit endocrine action.....	86
Figure (15):	Functional groups of selected cytokines.	87
Figure (16):	Overview of the induction and function of cytokines.....	87
Figure (17):	Cytokine attributes of (a) pleiotropy, redundancy, synergism, antagonism, and (b) cascade induction	88
Figure (18):	Effector role of Th1 cells	89
Figure (19):	Effector role of Th2 cells	89
Figure (20):	CD4+ helper T cell	89
Figure (21):	Class I cytokine receptors (hematopoietin).....	92
Figure (22):	GM-CSF receptor subfamily (common β subunit)	92
Figure (23):	IL-6 receptor subfamily (common gp130 subunit)	93
Figure (24):	TNF receptors	93
Figure (25):	Class II cytokine receptors (interferon)	94
Figure (26):	Chemokine receptors.....	94
Figure (27):	Immunoglobulin superfamily receptors.....	95
Figure (28):	Sandwich ELISA.	116
Figure (29):	Patients’ demographic data as regard Binet staging system.	121
Figure (30):	Patients’ demographic data as regard Rai staging system.	121
Figure (31):	Cyto 17p.	122
Figure (32):	Patients’ demographic data as regard outcome.....	123

List of Figures Cont...

Fig. No.	Title	Page No.
Figure (33):	Comparison between patient group (pre treatment) and control group serum IL 6 level.	124
Figure (34):	Comparison between patient group (pre treatment) and patient group (post treatment) serum IL 6 level.....	125
Figure (35):	Comparison between CR patients as regard serum IL-6 level pre and post treatment.	126
Figure (36):	Comparison between PR patients as regard serum IL-6 level pre and post treatment.	127
Figure (37):	Correlation between IL6 level and WBC.....	129
Figure (38):	Correlation between IL 6 level and B2 microglobulin.	129
Figure (39):	Correlation between IL 6 level and LDH.	130
Figure (40):	Correlation between IL 6 level and BM Aspirate (% of lymphocytes).	130
Figure (41):	Correlation between IL 6 level and Uric acid.	131
Figure (42):	Correlation IL 6 level and Binet staging system.	133
Figure (43):	Correlation between IL 6 level and Rai staging system.	134
Figure (44):	Correlation between IL 6 level and CD 38.	134
Figure (45):	Correlation between IL 6 level and B symptoms.	135
Figure (46):	Comparison between Non response, partial response and Complete response patients' as regard LDH.	138

Figure (47):	Comparison between Non response, partial response and complete response patients' as regard B2 microglobulin.....	138
---------------------	---	-----

List of Figures Cont...

Fig. No.	Title	Page No.
Figure (48):	Comparison between non response, partial response and complete response patients' as regard CD38.....	139
Figure (49):	Comparison between non response, PR and CR patients' as regard WBC.....	140
Figure (50):	Comparison between non response, PR and CR patients' as regard IL 6 level.....	140
Figure (51):	ROC for IL-6- pre and post treatment in CLL patients.	141

List of Abbreviations

Abb.	Full term
2CDA	2-chlorodeoxyadenosine
AIHA.....	Autoimmune haemolytic anemia
AML	Acute myeloid leukemia
ATM	Ataxia telangiectasia mutated
BCR.....	B-cell antigen receptor
B-PLL	B-cell prolymphocytic leukaemia
BR	Bendamustine with rituximab
CARs.....	Chimeric antigen receptors
CBC.....	Complete blood count
CFAR	Alemtuzumab with FCR
CIRS	Cumulative Index Rating Scale
CLL	Chronic lymphocytic leukaemia
CMV.....	Cytomegalovirus
CNTF	Ciliary neurotropic factor
CR	Complete response
CRi	Complete response incomplete
CT-1	Cardiotropin-1
DAT.....	Direct antiglobulin test
DIC.....	Disseminated intravascular coagulation
DLBCL.....	Diffuse large B-cell lymphoma
EBV.....	Epstein-Barr virus
ELISA	Enzyme-linked immunosorbent assay
ERIC	European Research Initiative on CLL
FC	Fludarabine plus cyclophosphamide
FCR.....	Fludarabine, cyclophosphamide, rituximab
FISH	Fluorescent in situ hybridization
FR	Fludarabine and rituximab
GVHD	Graft-versus-host disease
GVL.....	Graft-versus-leukemia

List of Abbreviations Cont...

Abb.	Full term
Hb	Hemoglobin
HCL	Hairy cell leukaemia
HHV- 8.....	Human herpes virus 8
HiB.....	<i>Haemophilus influenza B</i>
IgD	Immunoglobulin D
IGHV.....	Immunoglobulin variable region heavy chain
IgM.....	Immunoglobulin M
IGVH.....	Immunoglobulin variable heavy chain
IL	Interleukin
ITP	Immune thrombocytopenia
LDT	Lymphocyte doubling time
LIF	Leukemia inhibitory factor
LO	Lenalidomide + ofatumumab
LR	Lenalidomide + rituximab
MAbs.....	Monoclonal antibodies
MBL	Monoclonal B-cell lymphocytosis
MCL	Mantle cell lymphoma
MDD	Minimum detectable dose
MDR	Minimally deleted region
MDS	Myelodysplasia
MIRs	microRNAs
MRD.....	Minimal residual disease
NHL	Non-Hodgkin lymphomas
NPN	Neuropoietin
O.D.....	Optical density
ORR	Overall response rates
OS	Overall survival
OSM.....	Oncostatin M
PAR.....	Pentostatin and rituximab

List of Abbreviations Cont...

Abb.	Full term
PCO.....	Pentostatin, cyclophosphamide, and ofatumumab
PCR.....	Pentostatin + cyclophosphamide + rituximab
PD	Progressive disease
PFS	Progression-free survival
PL.....	Prolymphocytoid leukaemia
PML.....	Progressive multifocal leucoencephalopathy
PR	Partial response
PRCA.....	Pure red cell aplasia
PRL.....	Partial response with lymphocytosis
PRn	Nodular partial response
RANKL	RANK ligand
RBC.....	Red blood cell
RS.....	Richter's syndrome
SCT	Stem cell transplantation
SD	Stable disease
SLL	Small lymphocytic lymphoma
SLVL.....	Splenic lymphoma with circulating villous lymphocytes
SMZL	Splenic marginal zone lymphoma
SOCS	Suppressor of cytokine signals
SST	Serum separator tube
STAT.....	Signal transducer and activator of transcription
sTK.....	Serum thymidine kinase
TNF.....	Tumor Necrosis Factor
TRM	Transplant-related mortality
TTF	Time to failure
VEGF	Vascular endothelial growth factor
VGEF	Vascular growth endothelial factor
WBC.....	White blood cell

ABSTRACT

Background Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults. These malignant B-CLL cells represent over 99% of peripheral blood mononuclear cells (PBMCs) in CLL patients. In case of an aggressive form, the cell number may quickly double and, as a result, the disease may be fatal within a relatively short period of time. Currently several biomarkers are being used as CLL prognosticators, including: elevated protein levels (e.g. TCL-1, ZAP-70, CD38), elevated RNA levels (e.g. CLLU1, LPL, miRNAs), gene mutations (e.g. TP53, SF3B1, BIRC3, NOTCH1) and epigenetic changes. Early reports implicated IL-6 as pro-tumorigenic factor in various human tumors. IL-6 levels increased significantly in serum of CLL patients and correlated with adverse clinical features and short survival.

Aim of the work: Is to evaluate IL 6 level in patients with CLL at time of presentation and 6 months after chemotherapy and to study its relevance to disease prognosis.

Patients and Methods: This Study is a cross sectional study that was conducted on 55 patients newly diagnosed with chronic lymphocytic leukemia (CLL) in Clinical Hematology Unit in Ain-Shams University Hospitals in Cairo, Egypt. **Result:** The initial serum IL 6 level in the patient group (pre-treatment) ranged from 36-91pg/mL (median 57), and in the control group it ranged from 1-2 pg/mL (median 1). The initial serum IL 6 level in patient group (pre-treatment) range from 36-91 pg/mL (median 57), and in patient group (post treatment) range from 1-32 pg/mL (median 2). There are positive correlations between IL6 level and with WBC, B2 microglobulin, LDH, ESR, B symptoms, Uric Acid, BM Aspirate (% of lymphocytes) and Binet and Rai staging system.

Conclusion: Serum IL 6 is a useful poor prognostic marker in newly diagnosed CLL patients, its prognostic value goes with the other known prognostic markers as the lymphocytic count, ESR and LDH.

Key words: CLL; IL 6; Prognostic markers; cytogenetics

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults, accounting for approximately 30 % of all leukemia cases in European and North American countries with an incidence of 3–5 cases per 100,000 (*Talab et al., 2013*).

CLL is rare under 45 years of age, and its prevalence increases with age. The median age of patients at diagnosis is 70 years and only 10–15% of the patients are diagnosed under 50 years of age. It is characterized by accumulation of non-functional B-cells with a high expression of CD5, CD19, CD20 and CD23 and a low expression of surface immunoglobulins IgM, IgD and CD79a compared to normal B-cells (*Herman et al., 2010*).

These malignant B-CLL cells represent over 99% of peripheral blood mononuclear cells (PBMCs) in CLL patients. Approximately 2–5% of CLL patients show a T-cell phenotype, and these patients have a less favorable prognosis than patients with a B-CLL phenotype (*Shackelford et al., 2006*).

CLL develops through increased proliferation of immature lymphocytes in lymphoid organs, which results from an increased expression of antiapoptotic BCL-2 family proteins (*Willimott et al., 2013*).

As a result, CLL cells can survive for months (unlike normal cells, which only survive for a few days), thereby

decreasing the number of normal lymphocytes and inducing immunodeficiency (*Chen et al., 2008*).

In case of an indolent form, the disease does usually not progress to a severe form, and the patient may survive for years without treatment. In case of an aggressive form, the cell number may quickly double and, as a result, the disease may be fatal within a relatively short period of time (*Chiorazzi et al., 2005; Palomba et al., 2014*).

Currently several biomarkers are being used as CLL prognosticators, including elevated protein levels (e.g. TCL-1, ZAP-70, CD38), elevated RNA levels (e.g. CLLU1, LPL, miRNAs), gene mutations (e.g. TP53, SF3B1, BIRC3, NOTCH1) and epigenetic changes (*Chen et al., 2008*).

Prognostic serum markers that can be used to predict the survival and response to treatment include increased lactate dehydrogenase (LDH) levels, which are associated with a poor prognosis and a likelihood of progressing to Richter's syndrome, increased thymidine kinase (TK) levels, which are associated with aggressive disease, and unmutated immunoglobulin heavy chain variable region (IGHV) genes, which are associated with a high risk for genomic aberrations (*San Jose et al., 2013*).

Evaluation of the IGHV mutation status and FISH are among the most reliable molecular tools used in routine diagnostics