Minimal Residual Disease Assessment by Flow Cytometry in B-lineage Acute Lymphoblastic Leukemia to Assess Response to Treatment and Impact on Outcome

Thesis

Submitted for Partial Fulfillment of M.D of Clinical Hematology

Presented by

Ayman Abd Elsalam Mahmoud

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

Under Supervision of

Prof. Dr. Maha Tawfik El Zimaity

Professor of Internal Medicine and Hematology

Faculty of Medicine – Ain Shams University

Prof. Dr. Mohamed Mahmoud Moussa

Professor of Internal Medicine and Hematology

Faculty of Medicine – Ain Shams University

Dr. Raafat Mohamed Abdelfattah

Consultant of Medical Oncology and Hemato-oncology

National Cancer Institute – Cairo University

Dr. Eman Zaghloul Kandeel

Assistant Professor of Clinical Pathology

National Cancer Institute – Cairo University

Dr. Amro Mohamed Sedky El-Ghammaz

Assistant Professor of Internal Medicine and Hematology

Faculty of Medicine – Ain Shams University

Faculty of Medicine

Ain Shams University

2019

Acknowledgement:

First, thanks to Allah, the most compassionate and most merciful as we feel his great care, support and guidance in every step in our life.

I wish to express my most sincere; heart felt appreciation and deep thanks to:

Prof. Dr. Maha Tawfik El Zimaity

Professor of internal medicine and hematology- faculty of medicine- Ain shams university. For generous supervision, valuable instruction, careful reading, beneficial remarks and unlimited encouragement throughout the whole work.

I would like to express my sincere thank deep appreciation to:

Prof. Dr. Mohamed Mahmoud Moussa

Professor of internal medicine and hematology- faculty of medicine- Ain shams university.

For his support, encouragement, cooperation and his devoted time and effort in this study.

I wish to express my special thanks and consideration to:

Dr. Raafat Mohamed Abdelfattah

Consultant of medical oncology and hemato-oncology- National cancer institute- Cairo university.

For his valuable comments and support during the preparation of the study.

I would like to express my sincere thank deep appreciation to:

Dr. Eman Zaghloul Kandeel

Assistant Professor of Clinical Pathology- National cancer institute- Cairo university. For her support, encouragement, cooperation and her devoted time and effort in this study.

I wish to express my special thanks and consideration to:

Dr. Amro Mohamed Sedky El-ghammaz

Assistant Professor of Internal Medicine and Hematology- faculty of medicine- Ain shams university. For generous supervision, valuable instruction, careful reading, beneficial remarks and unlimited encouragement throughout the whole work.

List of contents:

List of abbreviation

ABL: Abelson murine leukemia

A-CGH: array-comparative genomic hybridization

ALL: acute lymphoblastic leukemia

ALLO-SCT: allogenic stem cell transplantation

AML: acute myeloid leukemia

ASO: allele-specific oligonucleotide

BCP: B cell precursor

BCR: B-cell receptor

BCR: breakpoint cluster region

BFM: Berlin-Frankfurt-Münster Group

CALGB: cancer and leukemia group B

CAR: Chimeric antigen receptor

CCR: continuous complete remission

CIR: cumulative incidence of relapse

CMR: complete molecular remission

CNS: central nervous system

COG: children oncology group

CR: complete remission

CRLF2: cytokine receptor-like factor 2

CRS: cytokine release syndrome

CTLA-4: cytotoxic T lymphocyte antigen-4

DFS: disease free survival

DIC: disseminated intravascular coagulation

EFS: event free survival

FISH: fluorescence in situ hybridization

GIMEMA: Gruppo Italiano Malattie EMatologiche dell'Adulto

GMALL: German multicenter acute lymphoblastic leukemia

GRAALL: French Group for Research on Adult ALL

GVHD: graft versus host disease

GVL: graft versus leukemia

HR: high risk

HSCT: hematopoietic stem cell transplantation

HTS: high-throughput sequencing

IPT: immunophenotyping

K–M: Kaplan Meier

LFS: leukemia free survival

MAC: myeloablative conditioning

MDACC: MD Anderson Cancer Center

MFC: multiparametric flow cytometry

MPO: myeloperoxidase

MRD: minimal residual disease

MSD: matched sibling donors

NGS: Next-Generation Sequencing

NILG: Northern Italian Study Group

NRM: non-relapse mortality

NSE: nonspecific esterase

OS: overall survival

PALG: Polish Adult Leukemia Group

PAS: Periodic acid-Schiff

PD-1: programmed cell death-1

PETHEMA: Spanish Programa Español de Tratamientos en Hematologıa

PFS: progression-free survival

RFS: relapse free survival

RI: relapse incidence

RIC: reduced intensity conditioning

RR: relapse rate

RT-qPCR: real time quantitative polymerase chain reaction

SBB: Sudan black B

SNP: single nucleotide polymorphisms

SR: standard risk

TCP: T-cell precursor

TKI: tyrosine kinase inhibitors

TLC: total leucocytic count

URD: unrelated donors

List of tables:

List of figures:

Abstract

The prognostic value of minimal residual disease (MRD) assessed by multi-parameter flow cytometry (MFC) was investigated among 56 adult patients with B-cell acute lymphoblastic leukaemia (B-ALL) treated between 2014 and 2018 using regimens including the Dana Farber and Hoelzer protocols. In this study, 44 (78.6%) achieved complete remission (CR) with a relapse rate of 38.6% (17 cases out of 44 cases) after a median follow up of 15 months. median age was 29.5 years (range18 to 60). Median white blood cell count (WBC) was17.75 x 10^3 (range, 0.38-340 x10^3/µl). MRD by MFC was assessed with a sensitivity of 0.01%, using a 7 marker, 4-colour panel on bone marrow specimens obtained at D14, D28 and post consolidation. MRD ≤0.01 at D14 was associated with improved disease-free survival (DFS) and overall survival (OS) (P <0.001and P <0.001 respectively). Similarly MRD ≤0.01 at D28 and undetectable levels post consolidation was associated with improved DFS (P <0.001and P <0.001 respectively) and OS (P <0.001and P <0.001 respectively). Multivariate analysis including age, WBC at presentation, IPT, cytogenetics, treatment protocol and MRD status at D14, D28 and post consolidation, indicated that MRD negative status was an independent predictor of DFS. Achievement of an MRD negative state assessed by MFC is an important predictor of DFS and OS in adult patients with ALL.

Keyword: acute lymphoblastic leukemia, minimal residual disease.