PROMYELCOYTIC LEUKAMIA: MRD DETECTION & ITS CORRELATION WITH CLINICAL OUTCOME

Thesis

Submitted For fulfillment of the M.D. Degree in Clinical pathology and Oncologic Laboratory Medicine

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RECOMMENDATION

- The use of real time RT-PCR reatinely in all cases of acute promylocytic leukemia at diagnosis and follow up to quantify PML/RARα gene and determine MRD.
- Follow up treated cases every 3 months to detect early molecular relapse.
- Using PML-RARα NCN instead of absolute number of transcripts which enables standardization and avoiding differences among laboratories.
- To monitor response to treatment by estimating log reduction in PML-RARα ratio which gives better idea about the efficacy of treatment.

Abstract

The aim of the present work is to study the PML-RARα fusion gene by real-time RT-PCR to quantify the amount of the gene before and after therapy in a trial to asses its prognostic value by monitoring MNRD to improve therapeutic strategies and decision making.

This study carried out on 30 patients at medical oncology department in National Cancer Institute, they were subjected to the following:

At diagnosis:Complete haemogram/BM aspirates examination/

Immunophenotyping of myeloid cells using flow cytometric analysis.

Cytochemistry staining/Cytogenetic investigation and FISH whenever indicated/Real time RT-PCR.

After complete remission:Complete haemogram/ BM aspirates examination/FISH whenever indicated.

After consolidation: Complete haemogram/ BM aspirates examination/Real time RT-PCR.

Our study revealed the following:

- All 30 patients achieved HR and six of the relapsed.
- There was a statistically significant difference between hematological parameters before and after therapy.
- Real time PCR for PML-RARα showed a statistically significant difference between transcripts NCN before and after therapy with marked reduction in PML-RARα after therapy.

- Monitoring patients at short time points (3 months) is valuable and essential for decision making and choice of proper therapy for patients.
- Patients was divided into 2 groups according to level of NCN those with NCN <10 showe3 higher DFS and OS and those with NCN≥ 10 showed shorter DFS.
- Group of patients with 2 or more log reduction had higher DFS.
- Age < 30 years and initial TLC <$10x10^9$/L were the only prognostic parameter which could predict higher DFS.

Finally real time RT-PCR is excellent method for detection of minimal residual disease for patients of acute promyelocytic leukemia.

Key words: MRD- PML-RARα- RT-PCR- APL.

سرطان الدم الميلودى (3q) : أكتشاف الجزء المتبقى من المرض وعلاقته بالحالة الاكلينكية للمريض

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ABBREVIATION

ABL : Abelson

ACT : autologous stem cell transplantation.

AML : acute myeloid leukemia.

APL : acute promyelocytic leukemia.

ATRA : all-trans retinoic acid.

B2M : Beta 2 macroglobulin.

BCR : breakpoint cluster region.

BM : bone marrow.

BMT : bone marrow transplantation.

CBP : CREB binding protein.

CCD : computer-controlled cooled .

CD : clusters of differentiation.

CG :Control gene.

c-DNA :complementary DNA.

Cp45 : cytochrome p45.

GPX : gluta thione peroxidase.

CR : complete remission.

CRABP : cellular retinoid acid binding protein.

CREB : cyclic AMP response element binding protein.

CT : Combinations chemotherapy

C_t : threshold cycle.

CTQ : threshold cycle.

DFs : Disease free survival.

DFS : disease free survival.

DIC : disseminated intravascular coagulation.

DNA : deoxy ribonucleic acid.

Ds-DNA :douplestranded DNA.

EAC : Europeaian against cancer.

FAB : French –American-British

FAM : 6-carboxy fluorescin.

FG : fusion gene.

FISH : fuorescence in situ hybridization.

FRET : fluorescence resonance energy transfer.

G6PD : glucose -6-phosphate dehydrogenase.

GAPDH : glyceraldehydes -3- phosphate dehydrogenase.

G-CSH : Granlocyte colony-stimulating factor

GST : Glutathrin stranoferase.

GUS : Beta glucouonidase.

GVH : graft versus host.

HAT : Histone acetytrandferase.

HDAC : Histone deacety lase.

HKGs : House keeping gene.

MRD : minimal residual disease.

NB : Nuclear body.

NCI : national cancer institute.

N-COR : nuclear receptor co repressor.

NK : Natural killer.

NPM : nucleophsmin.

NR : nuclear receptor.

Numa : nuclear matrix-associated gene.

PLZF : promyelocytic leukemia zinc finger.

PML : promyelocytic leukemia.

POZ : Pox virus and zinc finger.

RAR : Retinoid acid receptor.

RARA : retinoic acid receptor α.

RAS : Retinoid acid syndrome.

REF : Ret finger protein.

RQ-PCR : real time quantitative PCR.

Rt-PCR : reverse transcriptase chain reaction.

RXR : Retinoid x receptor.

SCT : stemcell transplantation.

SMRT : silencing mediator for RARα and thyroid hormone .

SRC : steroid receptor co activator .

t : translocation.

TAMARA : 6-carboxy-tetramethyle rhodamine.

Taq : thermos aquaticus.

TET : tetrachloro-carboxy fluorescin.