Journal of the Egyptian National Cancer Institute (2013) **25**, 135–142

Cairo University

Journal of the Egyptian National Cancer Institute

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www.sciencedirect.com

Full Length Article

The prognostic significance of minimal residual disease in adult Egyptian patients with precursor acute lymphoblastic leukemia

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Received 19 January 2013; accepted 20 May 2013
Available online 29 June 2013

KEYWORDS

Adult ALL;
Minimal residual disease;
Flowcytometry

Abstract *Background:* Minimal residual disease (MRD) studies in adult acute lymphoblastic leukemia (ALL) give highly significant prognostic information superior to other standard criteria as age, gender and total leucocytic count (TLC) in distinguishing patients at high and low risk of relapse.

Objectives: We aimed to determine the value of MRD monitoring by flowcytometry (FCM) in predicting outcome in adult Precursor ALL patients.

Patients and methods: Bone marrow (BM) samples were analyzed by 4-color FCM collected at diagnosis and after induction therapy (MRD1) to correlate MRD positivity with disease free survival (DFS) and overall survival (OS).

Results: Study included 57 adult ALL patients (44 males and 13 females) with a median age of 22 years (18–49). DFS showed no significant difference with age, gender and initial TLC (p = 0.838, 0.888 and 0.743, respectively). Cumulative DFS at 2 years was 34% for B-lineage ALL (n: 35) and 57% for T-lineage ALL (n: 18) (p = 0.057). Cumulative DFS at 2 years was

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Peer review under responsibility of The National Cancer Institute, Cairo University.

ELSEVIER Production and hosting by Elsevier

 http://dx.doi.org/10.1016/j.jnci.2013.05.004

7% for MRD1 positive (high risk, HR) versus 57% for MRD1 negative patients (Low risk, LR) ($p < 0.001$). Cumulative DFS at 2 years was 29% for HR patients (n: 26) versus 55% for LR (n: 27) according to GMALL classification ($p = 0.064$). Cumulative OS did not differ according to age, gender and TLC ($p = 0.526$, 0.594 and 0.513, respectively). Cumulative OS at 2 years was 36% for B ALL (n: 39) versus 77% for TALL (n: 18) ($p = 0.016$) and was 49% for Philadelphia chromosome (Ph) negative patients versus 0% for Ph-positive patients ($p < 0.001$). Regarding MRD1, OS at 2 years was 18% for MRD1 HR (n: 17) versus 65% for MRD1 LR (n: 38) ($p < 0.001$). OS was 35% for high-risk patients (n: 30) and 62% for low-risk patients (n: 27) classified according to GMALL risk stratification ($p = 0.017$).

Conclusion: MRD by FCM is a strong independent predictor of outcome in terms of DFS and OS and is a powerful informative parameter in guiding individual treatment in ALL patients.

Introduction

Based on retrospective analyses of large cohorts of patients, conventional pre-therapeutic risk criteria including age, elevated total leucocytic count (TLC) at diagnosis, adverse immunophenotypic features and cytogenetic as well as molecular aberrations provide the basis for upfront risk stratification in current treatment protocol [1]. The classical definition of remission in ALL based on cytomorphology provides only superficial information about the effectiveness of the treatment because, within the patient group that achieves remission, morphology is unable to discriminate between patients at high risk of relapse and those with excellent prognosis. Therefore, sensitive techniques for Minimal residual disease (MRD) detection were developed for detection of lower frequencies of malignant cells during and after treatment [2]. MRD measurement by flow cytometry (FCM) is based on the detection of leukemia associated immunophenotypes (LAP) that can be used to distinguish them from normal hematopoietic cells [1].

The source of relapse in adult precursor ALL patients is the persistence of MRD that is undetectable by standard diagnostic techniques. Several studies have shown that detection of MRD in childhood and adult ALL is an independent risk parameter of high clinical relevance, both in de novo and relapsed ALL as well as in ALL undergoing hematopoietic stem cell transplantation [3–5] and suggest that detection of MRD at an early time point during/following induction or consolidation therapy has emerged as a powerful and independent predictor of prolonged event free survival (EFS) in children and adults with ALL [6,7]. Consequently, an increasing number of treatment protocols use MRD as a tool for treatment stratification. However, the decisions for selection of one MRD methodology over another are complex and dependent upon a number of factors in our institution especially time to deliver results, expertise and resources.

In this study, we aimed to determine the value of MRD monitoring by FCM in adult precursor ALL patients especially post-induction of cytoremission in order to predict impending relapse to start preemptive salvage treatment in time.

Patients and methods

All eligible adults diagnosed as de novo precursor ALL patients who presented to the Medical Oncology Department of Egyptian National Cancer Institute (NCI), Cairo University, in the time period from April 2006 to April 2007 were recruited in this study. The study was approved by the IRB of the NCI.

Pretreatment evaluation included thorough history and full clinical examination, complete blood count (CBC), bone marrow (BM) aspiration for morphology and cytochemistry and FCM immunophenotyping. Liver and kidney functions tests, uric acid level, serum electrolytes, and cerebrospinal fluid (CSF) examination were also done in addition to cytogenetics for Ph' chromosome, chest radiographs, abdominal ultrasound, ECG and echocardiography. Informed consents were obtained from all patients before inclusion into the study.

Eligibility criteria included (1) age from 18 to 50 years, (2) all FAB subtypes except L3, (3) all immunophenotypes except Mature B subtype, (4) ECOG performance status $\leqslant 2$, (5) no other malignancy, (6) no prior chemotherapy or radiotherapy, and (7) no medical contraindications.

MRD assessment FCM was done at diagnosis to detect LAP and for detection of MRD after induction therapy (MRD1) and during maintenance therapy (MRD) using a Coulter EPICS XL-MCL flow cytometer system *(Coulter Corporation, Hialeah)* and a reagent system *(Coulter Diagnostics, Hialeah)*. Surface staining fluorescent labeled mouse monoclonal antibodies against human T, B, myeloid antigens and isotypic controls were obtained from Becton Dickenson *(Mountain View, California)*. Intracellular staining was done using IntaPrep permeabilization reagent from the Beckman Coulter by which cells were fixed with reagent 1(fixation reagent using formaldehyde), after washing, permeability was induced with reagent 2 (using Saponine for permeability) and remaining erythrocytes were lysed [8]. The following monoclonal antibodies were used for four color combinations for the detection of MRD [9]

- Precursor B-ALL: TdT/CD10/CD19/CD 45; CD10/CD20/CD19/CD 45; CD34/CD38/CD19/CD 45; CD34/CD22/CD19/CD 45; CD19/CD34/CD45; CD10/CD20/CD22/CD 45.
- T-ALL: TdT/CD1/ cyt CD3; TdT/ cyt CD3/ CD7; CD4/CD8/ CD3/CD45.

At least 3×10^5 ungated events were collected and analyzed [9]**.** Minimum target sensitivity for quantifying MRD was defined as the ability to detect 30 clustered MRD events in 3x 10^5 total cellular events (0.01%).Cut off point of MRD1 was $< 10^{-3}$ (0.1%) and for MRD at any time point was $< 10^{-4}$ (0.01%) [10,11]**.** Risk groups were defined as MRD low risk (MRD-LR) for patients with MRD $< 10^{-4}$ at all examined time