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Immature reticulocyte fraction as a predictor of bone marrow recovery in children with acute lymphoblastic leukaemia on remission induction phase

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Abstract

Immature reticulocyte fraction a new routine parameter in the hematology analyzer can give the idea of the earliest morphologic change of bone marrow recovery before other test become positive after chemotherapy. A prospective observational study was carried out in the Department of Clinical Pathology in collaboration with Paediatric Haematology and Oncology, Bangabandhu Sheikh Mujib Medical University during a period of one year starting from October 2009 to September 2010 to evaluate the bone marrow recovery in children with acute lymphoblastic leukaemia by automated reticulocyte analysis. Total fifty patients were enrolled in this study on remission induction phase. All patients were between 8 months to 15 years age range with a mean age of 5.5 ± 3.2. At the end of the study out of 50 cases, 52% patients showed early immature reticulocyte fraction recovery and concluded that the immature reticulocyte fraction parameter showed earlier haematopoietic recovery than the current practice of absolute neutrophil count recovery.

Introduction

Acute lymphoblastic leukaemia (ALL) is a malignancy of lymphoid tissue[1]. ALL occurs most frequently in the children[2]. After diagnosis chemotherapy is the main stay of treatment in ALL. Chemotherapy uses anti-cancer (cytotoxic) drugs to destroy the leukaemic cells. After chemotherapy blood counts generally fall within a week of treatment and may take some time to recover[3,10,19]. At this period extensive monitoring of bone marrow recovery are needed. Among the hematological parameters immature retculocyte fraction can predict bone marrow recovery over others. So, serial measurement of immature reticulocyte fraction (IRF) is useful to monitor bone marrow regenerative function. Reticulocytes are immature red blood cells. They are released in the peripheral blood after a period of maturation in the bone marrow and undergo further differentiation into mature RBC[6]. Flow cytometric reticulocyte analysis is more precise and more sensitive than manual reticulocyte counting[7]. Besides this, the measured fluorescence intensity allows the quantification of reticulocyte maturity[20]. In automated flowcytometry method, reticulocyts have been classified morphologically into three maturational stages: low fluroscent reticulocytes (LFR), middle fluroscent reticulocytes (MFR) and high fluroscent reticulocytes (HFR)[3]. Immature Reticulocyte Fraction (IRF) is defined as the ratio of immature reticulocytes to the total number of reticulocytes. They are larger, having the greatest light scatter properties due to the highest level of ribonucleic acid (RNA). Immature reticulocytes normally constitute less than five percent (5%) of the total number of reticulocytes[5]. It is released into the peripheral blood during periods of intense erythropoietic stimulation. An increase in the reticulocyte percent >1% used as an indicator of erythroid regeneration[3,8]. Spanish Multicentric Study Group for Haematopoietic recovery defined IRF >5% as recovery[8]. Absolute neutrophil count (ANC) is defined as the number of mature neutrophils plus bands per unit of volume generally accepted as a primary indicator of successful bone marrow recovery. An increase in ANC $\geq 0.5 \times 10^9$/L defines successful myeloid recovery after chemotherapy[4,9,10]. The aim of this study is to establish the earliest indicator of marrow recovery among the reticulocyte subpopulations in children with ALL.

Materials and Methods

This study was carried out in the Department of Clinical Pathology and Department of Paediatric Haematology and Oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka during the period of October' 2009 to September' 2010. 50 Children upto 18 years of age irrespective of sex with acute lymphoblastic leukaemia attended in Paediatric Hemato-Oncology outpatients and

inpatients department for treatment included in this study. Blood sample (2 ml) was collected in an EDTA tube for complete blood count (CBC), absolute neutrophil count (ANC), reticulocyte profile (both manual and automated method) and peripheral blood film (PBF) examination. Count was done preferrably within 2 hrs of collection.

Supravital staining of unfixed RBCs was done with new methylene blue (NMB) in 50 patients and 40 controls. Mixing of 100 µl whole blood with 100 µl of 1% NMB solution was done in a 10x75- mm tube. After incubation at 37^0 for 15-20 minutes, the dilution was remixed and a wedge smear was performed[11]. The number of reticulocytes per 1000 RBCs determined microscopically on x100 objective. Then percentage of reticulocyte was done. A reticulocyte was defined as a RBC containing at least 2 granules of reticulum (Figure-1). Absolute reticulocyte count was calculated from RBC count obtained from automated hematology analyzer.

Automated reticulocyte count was done in 50 patients in Sysmex xt-2000i haematology analyzer. The measuring principle of the system is based on flow cytometry combined with hydrodynamic focusing. EDTA blood (100 µl) is aspirated and intracellular RNA is stained by auramine O, which is fluroscent under argon laser light[12]. The reticulocyte population is further subdivided into LFR, MFR and HFR (Figure-2). The percentage of reticulocyte is given as the sum of LFR, MFR and HFR. The IRF was manually calculated by MFR plus HFR. IRF>5% were taken as bone marrow recovery[3]. ANC was calculated from total leucocyte count and differential count of WBC. Serial hemogram with reticulocyte count, IRF and ANC measurements was done in every 4 days interval upto 32 days of the therapy.

All necessary and relevant data were processed. Data were evaluated by standard statistical methods. Analysis was done by Statistical package for social science (SPSS) 16 by applying appropriate formula. Bone marrow recovery were evaluated by Mean, Median, Paired t test, Co-efficient of variance test, ANOVA test.

Results

During study randomly selected 40 healthy subjects were taken to establish the reference values for reticulocyte profile in the laboratory. Fifty children with ALL were 8 months to 15 years of age range and mean age of the patients was 5.5±3.2 years. Maximum patients were male. Male and female ratio was 1.5:1. In this study, during induction remission phase hemoglobin (Hb) level were gradually decreased upto 12th day ($p<0.05$). However from 16^{th} day onwards the Hb level remain the similar with baseline status which was not significant ($p>0.05$) (Paired t-test). At diagnosis mean total count of WBC was 14.74±25.47 ($x10^9$/L). During induction remission total count of WBC was sharply declined at day 8 and continued up to last follow up. Statistically significant differences were observed between different follow-ups day ($p<0.05$) (Paired t-test).

At diagnosis mean total count of RBC was 4.0±0.9 ($x10^{12}$/L). During induction remission total count of RBC was significantly declined upto 20^{th} day ($p<0.05$) (Paired t-test). In this study ret% significantly declined upto day 4 and then gradually increased or remain the same with the diagnosis day in both automated and manual method (Paired t-test). The absolute reticulocyte concentration significantly declined upto day 4 then it remain the same upto last follow up.

This study showed IRF declined gradually in 24 cases and reaches to low-level mean of 12.1±7.1 % with a median of 6 days (days 4-8). But IRF showed high range at diagnosis before the chemotherapy (Paired t-test). Recovery day was ranged from 16.6±4.6 (median day 16) (Table-I) (ANOVA test). In this study the children were profoundly neutropenic (<500/ml) in day 12 and day 16. The ANC were remain the same with the day of diagnosis from day 20 to 32 day of the therapy. The ANC were significantly ($p<0.05$) declined upto day 16 of therapy (Paired t-test). The range of ANC recovery was 10-32 days (median 23 day) (Table-I) (ANOVA test). This study found that 52 % cases recovery of IRF occurred earlier than ANC and the median difference of IRF preceding ANC was days 7. Late recovery occurred in 22% cases. It was on the same day in 26% cases. Thus the similar or earlier recovery was in 78% cases.

Table I: Comparison of IRF and ANC recovery in days (n=50)

Parameter	Recovery in days	P Value
IRF		
Mean+SD	16.6+4.6	0.016*
Median	16	
Range (Min-max)	(10-30)	
ANC		
Mean+SD	23.3+5.7	0.001*
Median	23	
Range (Min-max)	(10-32)	

* Significant

Table II: IRF recovery versus ANC recovery

Parameter	Earlier IRF recovery	Same IRF recovery	Later IRF recovery
No. of cases (50 cases)	26 (52%)	13 (26%)	11 (22%)
Median difference (Day post CT)	7 (10-30 days)	-	8 (14-32 days

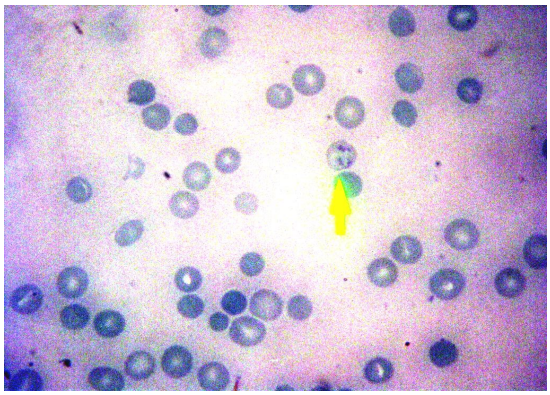

Fig. 1: Photomicrograph of reticulocytes in children with ALL on day 8 induction of remission phase. Stained supravitally with new methylene blue (x1000)

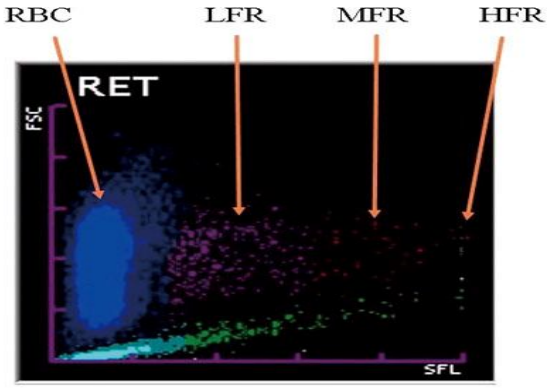

Fig-2: IRF scattergram in Sysmex XT-2000i hematology analyzer

Discussion

Several studies showed that the younger reticulocytes detected by the flow cytometric method are better indicators of recovery than the detection of ANC in post chemotherapy patients[5,13,17]. Grotto et al. in 1999 used immature reticulocyte fraction (IRF) as a indicator of early recovery. This study also evaluated IRF as a predictor of bone marrow recovery in post chemotherapy children with ALL and it was compared with other hematological indicators of bone marrow recovery.

This study showed that IRF was the first sign of haematological recovery in 52% of the patients studied preceding the rise in ANC. The IRF-signaled recovery appeared on a median of 7 days when compared to the ANC. Das et al. (2006) showed earlier IRF recovery at a median 21 days. This study found ANC recovery at a median of 23 days but Das et al. (2006) found ANC recovery between 10-35 days (median day 19). In this study IRF showed 78% similar or early recovery. A study showed, 91.2% IRF recovery occurred before ANC in chemotherapy patients on remission induction phase[8]. Lesesve et al. (1995) found earlier recovery of IRF in 88.4% cases than ANC by a period of 33.3 days. IRF recovery was 72.1% similar or earlier than ANC[13]. In other studies, IRF were also used as a marker of recovery and showed similar results[3,16].

In this study IRF parameter showed earlier haematopoietic recovery than the current practice of ANC recovery for monitoring in children with acute lymphoblastic leukemia after chemotherapy. This early laboratory indicator will guide the clinicians to make important therapeutic decisions, which will be economic and live saving for the patients. This study concluded that reticulocyte counting with IRF can be routinely and widely used in the laboratory to evaluate the bone marrow erythropoietic activity after chemotherapy.

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