Value of Reticulocyte Count, Absolute Reticulocyte Count and Platelet Count for Bone Marrow Recovery Status on Remission Induction Phase

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Abstract:

Reticulocyte count performed on a supravitally stained blood film is a initial and common laboratory method of determining early hematological recovery. This method is rapidly replaced by automated flow cytometry methods which have the advantage of much greater precision, before other test become positive after chemotherapy. A prospective observational study was carried out in the Department of Laboratory Medicine in collaboration with Paediatric Haematology and Oncology, Bangabandhu Sheikh Mujib Medical University during a period of one year to evaluate the bone marrow recovery in children with acute lymphoblastic leukemia by automated reticulocyte analysis over platelet count. 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, Ret% and Abs ret count showed early recovery in a median of 6 days before the platelet count recovery and concluded that reticulocyte parameter showed earlier haematopoietic recovery than the platelet count recovery.

Introduction:

Acute lymphoblastic leukaemia (ALL) is the most common malignancy diagnosed in the children aged 2-5 years, representing nearly one third of all pediatric cancers [1]. Chemotherapy is the main stay of treatment in ALL. ALL is a highly curable disease due to chemotherapy responsiveness. The cure rate in western countries lies between 70-80% [2]. Chemotherapy uses anti-cancer (cytotoxic) drugs to destroy the leukaemic cells. In ALL patients, aplasia occurred after chemotherapy and leaves the patient with little or no red cell, white cell or platelet production. As a result, infection may occur which increases the time of aplasia and prolonged the period of hospital stay after chemotherapy[3]. After chemotherapy blood counts generally fall within a week of treatment and may take some time to recovery [4,5,6]. At this period, extensive monitoring of bone marrow recovery is needed. Among the hematological parameters reticulocyte parameter, platelet count can predict the bone marrow recovery over others. So, serial measurement of reticulocyte parameter & platelet count is useful to monitor the bone marrow recovery[7]. Reticulocytes are immature red blood cells. Reticulocytes typically composed about less than 1% of the red cells in the human body[8]. They are released in the peripheral blood after a period of maturation in the bone marrow and undergo further differentiation into mature RBC[8]. The number of reticulocytes in the peripheral blood is a fairly accurate reflection of erythropoeitic activity assuming that the reticulocytes are released normally from the bone marrow and they remain in circulation for the normal time period. An increase in the reticulocyte percent >1% is used as an indicator of erythroid regeneration. Spanish Multicentric Study Group for Haematopoietic recovery defined Absolute reticulocyte count>50×10^9 /L as hematopoietic recovery [5,6]. Platelet count >20×10^9/l defined as a predictor of BM recovery[6]. Platelet recovery acts as a conventional indicator of bone

marrow regeneration. But it is delayed parameter over other haematological parameter[9]. Platelet count may be influenced by frequent platelet transfusions. For platelet recovery a minimum of 4 weeks' time interval is needed[10]. So, Platelet counts are less frequently monitored as a useful predictor[6]. The aim of this study is to compare the earliest indicator of marrow recovery among the reticulocyte parameter and platelet count in children with ALL.

Materials and methods:

This study was carried out in the Department of Laboratory Medicine 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 were included in this study on the basis of inclusion and exclusion criteria. Blood sample (2 ml) was collected in an EDTA tube for complete blood count (CBC), total platelet count, reticulocyte profile (both manual and automated method) and peripheral blood film (PBF) examination. Count was done preferably within 2 hours of collection. Supravital staining of unfixed RBCs was done with new methylene blue (NMB) in 50 patients. Mixing of 100 µl whole blood with 100 µl of 1% NMB solution was done. After incubation at 37° for 15-20 minutes, the dilution was remixed and a wedge smear was performed[19]. 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. 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. Serial hemogram with reticulocyte count, Abs ret and platelet count measurements were 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 was evaluated by Mean, Median, Mode and ANOVA test.

Results:

In this study fifty (50) children with ALL were enrolled. The age limit was between 8 months to 15 years of age and mean age of the patients was 5.5±3.2 years (Table-I). Maximum patients were male. Male and female ratio was 1.5:1(Table-II). In this study, during induction remission phase hemoglobin (Hb) level were gradually decreased up to 12th day (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. This study found mean distribution of Ret %, Ret abs, and platelet count according to recovery of the study patients. In this study Ret % significantly declined up to day 4 and then gradually increased or remain the same with diagnosis day in both automated and manual method. Ret % recovery occurred first day of persistent 3 days' rise of the reticulocyte from the 18th to 28 days. It

recovered at a median of 18 days and the mean ±SD Ret % was 18.5 ±6.8 days,28. ±7.4 days and 24.3±6.7 in early, late and same recovery respectively (Table-III). The absolute reticulocyte concentration significantly declined up to day 4 then it remains the same upto last follow up. The mean ±SD Ret abs was 18.3±4.7 days in early recovery,24.4±5.3 days in late recovery and 20.7±3.5 days in same recovery (Table-III). The platelet count was declined significantly p<0.05 upto 16th days then remain the same with the baseline status. The mean platelet count was 24.1±6.5 days in early recovery,28 ±6.4 days in late recovery and 20.5±5.7 days in same recovery(Table-III). Ret %, Ret abs and platelet recovery were statistically significant p<0.05 in different status. Recovery of Ret % at a median of 20 days, Ret abs 18 days and platelet counts occurred at a median of 24 days, which was 4 day delayed than Ret %,6 days delayed than Ret abs (Table-III)

Age group years	Number of patients	Percentage
≤ 5	29	58.0
6 – 10	18	36.0
11 – 15	3	6.0
Mean ±SD	5.5	±3.2
Range (min – max)	(8 mo	-15 years)

Table 1: Age distribution of the patients (n=50)

Sex	Number of patients	Percentage
Male	30	60.0
Female	20	40.0

Table 2: Sex distribution of the studied patients (n=50)

	Early recovery	Late	Same	p value
Ret				
Mean+-SD	18.5+-6.8	28.4+-7.4	24.3 6.7	0.013ˢ
Median	20	28	24	
Mode	18	24	20	
Range	18-26	20-32	18-32	
Ret abs				
Mean+-SD	18.3 4.7	24.4 5.3	20.73.5	0.001ˢ
Median	18	24	20	
Mode	20	10	20	
Range Min-Max	16-32	20-32	14-32	
Platelet				
Mean+-SD	24.1+-6.5	28+-6.4	20.5+-5.7	0.001ˢ
Median	24	28	22	
Mode	24	24	20	
Range	16-32	20-32	16-32	

Table 3: Comparison of Ret %, Ret abs and Platelet count with recovery in days (n=50)

Discussion:

Few studies were performed to evaluate utility of using flowcytometric analysis of reticulocytes to predict the bone marrow recovery after chemotherapy. Though the high cost of using flowcytometry or third generation automated blood cell counters for automated reticulocyte counts are presently a limiting factor in developing countries such as Bangladesh. But despite the fact, ret%, ret abs can effectively serve as an additional index to indicate bone marrow recovery other than platelet count. Ret %, ret abs and platelet recovery were found statistically significant $p<0.05$ in different status in this study. The mean $\pm$SD Ret % was 18.5 $\pm$6.8 days,28. $\pm$7.4 days and 24.3$\pm$6.7 in early, late and same recovery respectively. Ret% recovered at a median of 20 days. Dekoninck et al (2002) found Ret % recovery from 5 to 33 days (median 19), which was 1 day earlier than this study[11]. But this study found recovery of ret abs was in 18 days and the mean $\pm$SD Ret abs was 18.3$\pm$4.7 days in early recovery,24.4$\pm$5.3 days in late recovery and 20.7$\pm$3.5 days in same recovery. Kuse et al. (1996) confirmed that ARC recovered at a median of 10 days in acute leukaemia which was 8 days earlier than our study[12]. The mean platelet was 24.1$\pm$6.5 days in early recovery,28. $\pm$6.4 days in late recovery and 20.5$\pm$5.7 days in same recovery. Dalal et al found platelet recovery was a median of 29 days in a study that was 5 days delayed recovery from our study[13]. Dekononck showed platelet recovery occurred in a median of 17.2 days compared to ret on 8.7 days that was earlier than our outcome. Bhatnagar et al. in a study found, platelet recovery 1 day delayed than Ret %which was nearly consistent with our study[14]. 85.7% percent of them had thrombocytopenia where platelet was $< 100x\ 10^9/l$. In this study, recovery of Ret% at a median of 20 days, ret abs in 18 days and platelet counts occurred at a median of 24 days; platelet recovery was 4 day delayed than Ret %,6 days delayed than Ret abs. So, our study confirmed, Ret abs was the earlier marker than Ret % and platelet count.

Conclusion:

This study concluded that the reticulocyte parameter showed earlier hematopoietic recovery than the platelet 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 savings and as well as live saving. Nowadays, reticulocyte is offered in most of the third generation haematology analyzer. Moreover, this test is simple, quick, cost effective, reproducible and reliable tool on the automated haematology analyzer. Thus its potential use as a routine test to see the bone marrow recovery is important.

Acknowledgement:

Conflict of interest:

There is no conflict of interest relevant to this paper to disclose.

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