

To Evaluate a) the Effectiveness of Immature Reticulocyte Fraction (IRF) as Earliest Predictor of Bone Marrow Regeneration & b) To Test MRD as Strongest Predictor of Relapse in Pediatric Acute Leukemia's in Remission Induction Phase

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Abstract: *Background:* Leukaemia is the most common childhood malignancy accounting for 30% of all cancers. Chemotherapy is the main stay of treatment in acute leukemias & it should be monitored by IRF & presence of blasts in bone marrow (MRD). *Methodology:* Induction remission phase of chemotherapy shows IRF on Sysmex = $(8.3 \pm 1.7)\%$ & ANC = $(0.48 \pm 0.09) \times 10^9/L$ on 14th day & $(1.4 \pm 0.06) \times 10^9/L$ on 29th day. 10% leukemia cases with IRF < 5% & MRD > 0.01% on 14th day & blasts in CSF & bone marrow on 29th day showed relapse. *Conclusion:* Post chemotherapy, IRF is the earliest indicator of haematopoietic recovery in bone marrow much earlier than ANC. The MRD > 0.01% in the remission induction phase on day 29 is the strongest prognostic indicator superior to other commonly used prognostic markers in childhood leukemias and predicts both the early and late relapses. This early laboratory indicator will guide the clinicians to make important therapeutic decisions, which will be economic and life saving for the patients

Keywords: IRF, Chemotherapy, reticulocyte count, ALL, AML, MRD

1. Introduction

Leukaemia's is the most common childhood malignancy. It accounts for 30% of all cancers diagnosed in the pediatric age group in industrialized countries. Acute lymphoblastic leukaemia (ALL) is the most common subtype and accounts for around four-fifths (78%) of all leukaemias.¹

The next most common leukaemia diagnosed in children is Acute myeloid leukaemia (AML). AML accounts for 15% of childhood leukaemias; incidence rates are highest in infants and show little variation with age from 3 years.²

Minimum residual disease (MRD) with hematological parameters like immature reticulocyte fraction (IRF) can predict bone marrow recovery phase over other parameters and forms one of the strongest predictor of prognosis in post chemotherapeutic ALL cases.¹

The aim of this study is to establish the earliest indicator of marrow recovery among the reticulocyte subpopulations in children with leukemias.

2. Materials and Methods

This study was carried out in tertiary care referral pediatric hospital. A total of 50 cases of pediatric leukemias between the age 0 – 16 years were studied

CBC, PBF & Bone marrow aspiration smears were studied to assess absolute neutrophil count (ANC) & erythropoiesis.

An increase in ANC $\geq 0.5 \times 10^9/L$ defines successful myeloid recovery after chemotherapy.^{3,4}

Automated reticulocyte count was done in Sysmex xt-2000i. The measuring principle of the system is based on flow cytometry combined with hydrodynamic focusing. 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 5% of the total number of reticulocytes. It is released into the peripheral blood during periods of intense erythropoietic stimulation. IRF > 5% on Sysmex were taken as bone marrow recovery.⁵

The parameters IRF, ANC & MRD were studied as predictors of prognosis of postchemotherapy bone marrow regenerative changes.

Erythropoiesis and blasts percentage for MRD were assessed and calculated by morphological examination of slides manually. The % of blasts were also confirmed by flow cytometry. A MRD cut off value 0.01 % was taken as the best predictor of 5 year survival rate.⁴

3. Results

A total of 50 leukemic cases on chemotherapy were studied

Table 1: Comparison of recovery of various parameters in IR phase

	Cutoff values B.M recovery	At diagnosis	On 14 th day IR	On 29 th day IR
IRF (symbex) (mean ± SD) %	>5	(2.6 ± 1.2)%	(8.3 ± 1.7)%	(7.9 ± 1.3)%
ANC (PBF) (mean ± SD) X 10 ⁹ /L	≥0.5 x10 ⁹ /L	(0.36±0.08) x10 ⁹ /L	(0.48 ± 0.09)x10 ⁹ /L	(1.4 ± 0.06)x10 ⁹ /L
MRD (B.M flow cyt.) (mean ± SD) %	0.01 % blasts	(59%±8.6)%	(3.2± 0.08)%	(1.6±0.02)%
Erythropoiesis		Markedly reduced – absent	Moderate – markedly Increased	Moderately Increased
Manual retic count	01%	<1%	>1%	>1%

IRF – Immature reticulocyte fraction ANC- absolute neutrophil count

This findings clearly suggests that the IRF & erythropoiesis are earliest predictors of bone marrow recovery phase

B.M – bone marrow IR - Inductionremission MRD – minimal residual disease

Table 2: Various parameters affecting prognosis

Classification of leukemia (50 cases)	ALL (44 cases)		AML(06 cases)		
	B Cell	T Cell	M4	M5	M6
No. of cases	40	04	01	03	02
MRD (blasts > 0.01 % on 29 th day)	12			01	01
CSF (blasts on 29 th day)	01	02	01	01	
29 th day					
IRF< 5 (no. of cases)	01	02	00	00	00
ANC< 0.5 x 10 ⁹ /L (no. of cases)	11	02	00	01	01
prognosis	3 ALL cases showed IRF< 5% & MRD > 0.01% on 14 th day & blasts in CSF & bone marrow on 29 th day Expired		2 AML cases showed IRF< 5% & MRD > 0.01% on 14 th day & blasts in CSF & bone marrow on 29 th day Expired		

This findings suggests that if IRF > 5% ON 14th day & MRD < 0.01% on 29th day of induction remission chemotherapy regime favours good prognosis & no relapse MRD > 0.01% on the 29th day & IRF>5% on 14th day is associated with poor prognosis & relapse. Infact this is the strongest and most powerful parameter to predict relapse & hence bad prognosis.

4. Discussion

The aim of this study is to establish the earliest indicator of marrow recovery among the reticulocyte subpopulations in children with acute leukemia.

Several other 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. Grotto et al. in 1999 used immature reticulocyte fraction (IRF) as a indicator of early recovery.^{6,7}

This present study also evaluated IRF as a earliest predictor of bone marrow recovery in post chemotherapy children in leukemia. This study showed that IRF was the first sign of hematological recovery in 80% of the patients, preceding the rise in ANC on 14th day. Das et al (2006) Study showed, 91.2% IRF recovery occurred before ANC in chemotherapy patients on remission induction phase. Lesesve et al. (1995)

found earlier recovery of IRF in 88.4% cases than ANC by a period of 33.3 days.^{1,5}

In this study ANC recovery initiated during 29th day in 50% of cases but Das et al. (2006) found ANC recovery between 10-35 days (median day 19).

The outcome of hematological cancers in terms of long term survival has greatly improved from 20% to 60% in ALL, from <70% to more than 90% in Hodgkins disease and from 10% to 40% in AML due to better monitoring of the patients

The detection of blasts by morphological analysis of bone marrow smears has a sensitivity of 1- 5%, MRD allows quantitative follow up of individual responses after achievement of complete remission detected by bone marrow cytology. The most reliable method for measuring MRD is flow cytometric profiling and PCR which are 100 times more sensitive than conventional morphological techniques.¹

In other studies patients with MRD of > 0.01 in bone marrow at the end of remission induction therapy has a higher risk of relapse and > 0.1 MRD at the end of continuation phase has the highest risk of relapse

Other studies showed that MRD > 0.01 in the remission induction phase on day 29 is the strongest prognostic

indicators superior to other commonly used prognostic markers in childhood leukemias and predicts both the early and late relapses. In this study 5 patients had MRD > 0.01 on 29th day & IRF > 5%. These children, expired within 2 years of initiation of chemotherapy.

5. Conclusion

Chemotherapy (anti-cancer cytotoxic) drugs is the main stay of treatment in acute leukemias. After chemotherapy blood counts generally fall within a week of treatment and may take some time to recover. At this period extensive monitoring of bone marrow recovery are needed.

Among the hematological parameters IRF, can predict bone marrow recovery over others. So MRD & serial measurement of IRF is useful to monitor bone marrow regenerative function.

In this study IRF parameter showed earlier haematopoietic recovery than ANC for monitoring children with chemotherapy. This early laboratory indicator will guide the clinicians to make important therapeutic decisions, which will be economic and life 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.

The MRD > 0.01% in the remission induction phase on day 29 is the strongest prognostic indicator superior to other commonly used prognostic markers in childhood leukemias and predicts both the early and late relapses.

6. Conflict of Interest

None Declared

7. Funding

No Funding Source

8. Ethical Clearance

Not Required

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