

Reticulocyte count, a surrogate marker of hematopoietic remission in Acute Lymphoblastic Leukemia (ALL).

Rashid Azeem, Fazle Raziq, Sarwar Ali, Tariq Masood, Saba Khan, Fareeha Azeem*

Department of Medicine, Lady Reading Hospital Peshawar, Peshawar, Pakistan

Received: 01-Jun-2023, *Manuscript No.* AACPLM-23-100994; **Editor assigned:** 05-Jun-2023, AACPLM-23-100994 (PQ); **Reviewed:** 20-Jun-2023, *QC No.* AACPLM-23-100994; **Revised:** 31-Jul-2023, *Manuscript No.* AACPLM-23-100994 (R); **Published:** 07-Aug-2023, DOI:10.35841/AACPLM.5.6.176

Abstract

Introduction: Acute Lymphoblastic Leukemia (ALL) is one of the commonest hematological disorders in pediatric age group and more than 50% of all the ALL cases occur in children below 20 years of age. Generally, Absolute Neutrophil Count (ANC) is regarded as one of the sensitive marker of marrow response to chemotherapy. However, seeing the higher frequency of infections in local settings, adversely affecting the ANC, reticulocyte count may be considered as an alternative marker.

Aims and objectives: To determine the correlation of reticulocyte count with bone marrow cytology after induction therapy in ALL patients.

Methodology: This cross sectional analytical study was conducted on ALL patients, Pakistani or Afghani origin, registered at hematology unit of Hayatabad medical complex Peshawar from 25th July 2012 to 25th Jan 2013, after taking informed consent. After completion of induction chemotherapy, reticulocyte count and bone marrow cytology were performed for each patient. The study findings were analyzed using SPSS version 15.

Findings: A sample size of 119 ALL patients, including 88 males and 31 female patients, were enrolled into the study. In 99 (83.19%) cases bone marrow findings correctly correlated with reticulocyte count in apprehending remission or non-remission. The number of blasts is non-significantly correlated at the end of induction chemotherapy ($r=0.158$, -0.158 , $p=0.085$). However, reticulocyte count is negatively significantly correlated with the number of blasts. ($r=-.275$, $p=0.003$).

Conclusion and significance: Reticulocyte count can be used as a surrogate marker of bone marrow response to chemotherapy in a limited resource setting.

Keywords: Hematological disorder, Absolute neutrophil count, Chemotherapy, Bone marrow cytology, Reticulocyte count

Introduction

Acute Lymphoblastic Leukemia (ALL) is one of the commonest hematological disorders in pediatric age group and more than 50% of all the ALL cases occur in children below 20 years of age. Despite the high incidence, during the last five decades the health outcomes of Acute Lymphoblastic Leukemia (ALL) patients have improved markedly. These advances in disease are made possible by the integration of new diagnostic methods, provision of effective and type directed chemotherapeutic agents, and the delivery of improved health care services. The objective for the next 10 years is to increase survival rate further towards maximum and also to reduce side effects related to chemotherapeutic agents [1].

Bone marrow releases reticulocytes as earliest Red Blood Cells (RBC) precursors into peripheral blood circulation, where they transform into mature erythrocytes after circulating for about 24 hours. In this way reticulocytes represent a trustworthy indicator of on-going marrow red cells production. Reticulocytes can be identified by routine staining that is manual reticulocyte count or by automated reticulocyte count

[2]. Immature Reticulocyte Fraction (IRF), which is based on flow cytometry technique, determines the RNA content of RBCs and act as a diagnostic indicator. It gives us a general idea about the marrow RBC production and thus is useful for monitoring therapy by the treating physicians. IRF has a disadvantage that it only shows bone marrow erythropoietic activity and does not provide the details of assimilation of iron in developing RBC's and thus no improvement in the hemoglobin levels. Therefore, IRF if used along with the manual reticulocyte count will be very helpful in monitoring bone marrow recovery after chemotherapy and post-transplant patient recovery as well as monitoring hematinic therapy for anemias [3,4]. Chemotherapeutic drugs severely affect all the cell lines during the induction phases of treatment. A high mortality rate is observed because of the fatal complications of the therapy rather than progression of the disease. Thus, Absolute Neutrophil Count (ANC), reticulocytes count and platelets count can all be used as bone marrow recovery markers. Since reticulocyte count is not affected by underlying infection, it has proved to be a superior marker of marrow recovery in neutropenic patients [5,6].

Various studies support reticulocyte count as a reliable indicator that shows erythropoietic recovery of marrow after induction therapy in leukemic patients as well as a marker of the appropriateness of erythropoietin therapy for treating anemias in ALL patients. A recent study in August 2021, shows that peripheral blood of ALL and Acute Myeloid Leukemia (AML) patients has raised IRF with raised red cell volume and patients having ALL specifically showed high reticulocyte count with raised red cell surface area [7]. Similarly, in another study conducted locally showed 63.5% patients showed early rise of reticulocyte count compared with absolute neutrophil count. Due to higher frequency of infections in local settings, adversely affecting the ANC, the reticulocyte count may be considered as an alternate marker.

The aim of study is to determine the correlation of reticulocyte count with bone marrow cytology after induction therapy in ALL patients.

Materials and Methods

This cross sectional analytical study was conducted on ALL patients, Pakistani or Afghani origin registered at hematology department of Hayatabad medical complex from 25th July 2012 to 25th Jan 2013. The study proposal was approved from the hospital research and ethical committee. Duration of the study was 6 months and detailed history was taken and clinical examination was carried out. Demographic (age, gender) and clinical information e.g., duration and severity of disease and result of last bone marrow examination was also obtained through a Proforma. Peripheral smear, reticulocyte count and bone marrow cytology was performed for each patient after completion of the 4th phase of induction chemotherapy. The

bone marrow aspiration procedure and all the reporting was done by experienced hematologists.

Study data is analyzed using SPSS version 15. For numerical variables that is age and gender, mean and standard derivation was measured while for other categorical parameters frequency and percentages were calculated. Data is presented in the form of tables and charts using MS Excel. Sample size was calculated 119 using WHO sample size calculator, taking 52% proportion of accuracy of reticulocyte count in detecting bone marrow recovery, 9% margin of error and 95% confidence level. Children diagnosed with ALL up to age of 18 years, received induction phase therapy and both gender were included in the study. Patients with disease complications, suffering from other types of leukemias and patients who due to non-compliance cannot be offered the standard treatment regimen or any limitation were excluded from our study.

Results

Out of 119 patients, 88 (73.9%) were males and 31 (26%) were females. In 99 cases (83.2%) bone marrow findings correctly correlated with reticulocyte count in apprehending remission or non-remission while the remaining 20 (16.8%) cases didn't correlate correctly. Pearson *Chi square* test was applied on gender distribution showing an insignificant p value of 0.356 (Table 1).

The number of blasts is non-significantly correlated at the end of induction chemotherapy ($r=0.158$, -0.158 , $p=0.085$). However, reticulocyte count is negatively significantly correlated with the number of blasts ($r=-.275$, $p=0.003$).

Table 1. Accuracy wise distribution of gender.

Gender	Accuracy		Total	p-value
	Yes	No		
Male	72	16	88	0.356
	81.80%	18.20%	100.00%	
Female	27	4	31	
	87.10%	12.90%	100.00%	
Total	99	20	119	
	83.20%	16.80%	100.00%	

Discussion

Acute Lymphoblastic Leukemia (ALL) results a consequence of several genetic alterations in a single bone marrow lymphoid precursor cell. It is grouped into T-cell leukemia and B-cell leukemia on the basis of immunophenotyping [8]. In developing countries with constrained resources like Pakistan, ALL is one of the most common hematological malignancies in childhood. Furthermore, it is most frequently occurring lymphoid leukemia in the pediatric age group of Khyber Pakhtunkhwa (KPK) and in Afghani patients seeking medical care in KPK regions of Pakistan [9].

In hematological malignancies, prolonged treatment regimens, the introduction of high cost novel agents, frequent visits to specialists and the centralized nature of care that usually requires travel from far flung areas make these patients susceptible to high healthcare related financial burden [10]. Therefore, to reduce the financial burden several less costly and easily available investigations must be used by the hematologists for treatment monitoring of leukemia patients, as they are budget friendly as well as less time consuming [11]. Our study suggests that reticulocyte count which is offered at most of the healthcare units and is cost effective as well as reliable can be safely used as a surrogate marker for remission in ALL patients at the end of induction chemotherapy.

Several studies are available on the implication and applicability of the hematological parameter in remission. In a local recently conducted study published in Rawalpindi medical college journal in 2022, a significant increase in reticulocyte count as well as platelet count is seen in remission cases with a p-value (p-0.001). This is in contrast with the non-remission cases where p-value is (p-0.05). Similarly, in another study about 88.9% cases were observed to have early immature reticulocyte fraction recovery relative to ANC recovery. In 9% cases both the IRF and ANC showed recovery on the same day and in just 2.2% ANC recovery was earlier than IRF [12]. Findings of this study are very close to our study findings. A study conducted in clinical pathology and pediatric hematology and oncology department, in Bangladesh showed that among 50 ALL cases, early IRF recovery was observed in 52% of patients supporting the idea that the IRF is an earlier hematopoietic recovery parameter [13]. In another similar study the reticulocyte percentage recovered at 20th day while the Absolute Neutrophil Count (ANC) occurred after a median of 23 days [14].

Most of the modern analyzers in the pathology units are designed in a way that they provide the reticulocyte percentage as well as absolute reticulocyte counts along with complete blood picture details. Furthermore, they also measure hemoglobin content of reticulocytes, percent (%) hypochromic cells, percent (%) microcytic cell, immature reticulocyte fraction, etc. [15]. All this information in a single report is very helpful in categorization of different anemias in different health conditions and can guide in management plans. However, the manual technique of slide examination is usually required for confirmation of CBC parameters and reticulocyte count.

Our study showed significant p value for reticulocyte count in relation to number of blasts. In view of this the reticulocyte count test can be significantly utilized by the diagnostic/therapeutic teams in follow up of ALL patients and selecting further therapy for these patients.

Conclusion

The reticulocyte count can be used as a surrogate marker for remission in ALL patients at the end of induction chemotherapy.

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*Correspondence to

Fareeha Azeem

Department of Medicine,

Lady Reading Hospital Peshawar,

Peshawa,

Pakistan

E-mail: fareeha.azeem@hotmail.com