Original Article

Reticulocyte Count and Platelet Count as Predictors of Morphological Remission/Hemopoitic Recovery in Acute Lymphoblastic Leukemia (ALL) after Induction Chemotherapy

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Abstract

Objectives: To determine the predictive values of reticulocyte and platelet count for remission in cases of acute lymphoblastic leukemia after induction therapy.

Materials and Methods: This cross-sectional observational study was conducted in the department of hematology, MTI Hayatabad Medical Complex, Peshawar. All cases of ALL referred to the department for remission after taking induction therapy, irrespective of age and gender were included. Relevant information was collected on a predesigned proforma prepared in accordance with the objectives of the study.

Results: A total of 84 cases referred for remission were included, 56(66.7%) were males and 28 (33.3%) were females. 50(59.5%) cases were in the age range of 5-18 years. The mean with a standard deviation of the age of patients was 15+ 4 years. 75(89.3%) of the cases were classified into ALL-1) by FAB classification. 50(59.5%) of the referred cases had achieved morphological remission by bone marrow aspiration. There was a statistically significant rise in Platelet count of the remission vs non-remission cases (p-0.001). Again there was a statistically significant difference in the retic count of the cases with remission (p-0.05). We observed a statically significant downhill moderate correlation of retic count with remission (in terms of blast count of BM aspiration) (p-0.04, r:-0.32). Platelet count also had an inverse significant correlation with remission (p-0.01, r:-0.37). The diagnostic roles of the peripheral platelet count and retic yielded an area under curves of (0.768 and 0.648 respectively) to predict remission.

We observed that the retic count and platelet count have been shown to have strong predictive values for remission in ALL with interaction values of (R= 0.28**, ΔR^2=0.02, p=0.08). Similarly, an increase in platelet also has a strong predictive value for remission in ALL cases with interaction values of (R= 0.41**, ΔR^2=0.16, p=0.001)

Conclusion: In ALL cases of post-induction therapy, The peripheral blood reading for an increase in Retic and platelet count predicts remission with 95% confidence. These values if strictly observed can reduce the frequency of invasive procedures like bone marrow aspiration.

Keywords: ALL, Remission, Reticulocyte count, Platelet count.

Introduction

Acute Lymphoblastic Leukemia (ALL) is a malignant hematological disorder due to abnormal proliferation of lymphoid progenitors (Lymphoblasts in the bone marrow, peripheral blood, and also in extra medullary sites. ALL occur predominantly in pediatric ages (80%). Its prognosis is good in early ages while poor when occurs in adults. in the United States, its incidence is 1.6/million.[1]

Globally the remission rate of up to 90% is reported for ALL in pediatric ages.[2] Acute Lymphoblastic Leukemia (ALL) prevalence and incidence studies are rarely reported in Pakistan. Similarly, less is reported to highlight the response of ALL to chemotherapy in our population. A local study covering Sindh province reported 70% remission in pediatric age.[3]

Regarding remission, literature has reported the clinical significance of hematological markers (reticulocyte count, ANC, and platelet) to predict remission in cases of ALL. A regular increase in trends of using these markers has dramatically decreased the frequencies of invasive procedures like bone marrow aspiration and trephine.[4.]

Furthermore immature reticulocyte count as a fraction of new parameters added in advanced hematology analyzers can predict the hemopoitic remission before other tests become positive after induction therapy in cases of ALL. A study reported that 52% of patients showed retic fraction improvement in follow-up cases much before than improvement in ANC in cases of ALL to document hemopoitic recovery.[5] A study from Turkey also concluded that immature reticulocyte fraction detects remission in ALL in pediatric age earlier than ANC.[6]

Platelet recovery or time to platelet recovery (TPR) has been reported to predict disease-free survival (DFS) and also the overall survival rate (OSR) in ALL. Similarly, TPR can also be used as a prognostic factor in leukemia.[7] We could not find literature from Pakistan documenting the predictive roles of these hematological parameters in remission cases of ALL, therefore present study was designed to determine the predictive values of reticulocyte, ANC, and platelet count in remission of acute lymphoblastic leukemia after induction therapy to avoid expensive (PCR, Immuno-histochemistry) and invasive (Bone marrow aspiration and trephine) procedures.

Materials and Methods

This was an observational study conducted in the department of Hematology / Pathology Hayatabad Medical Complex, Peshawar from January 2020 to March 2021.

Using Open-Epi software for sample size calculation, a sample size of 84 was selected to represent the true population based on the anticipated proportion of 70% remission in ALL cases, keeping a 95% confidence level and 6% absolute precision due to feasibility issues.[3]

A total of 84 diagnosed patients of Acute lymphoblastic leukemia (ALL) undergoing induction of remission were included. The diagnosis in all these patients was based on cytological including Giemsa stained peripheral blood smear, bone marrow aspirate smears, and trephine biopsy, immune-phenotypic and cytogenetic criteria.

Patients of all ages and both gender were included. Patients with complications and patients in whom standard treatment protocol could not be observed due to any limitation were excluded from our study.

All the patients were subjected to a detailed history and clinical examination. On day 07th of the end of the 4th cycle of induction chemotherapy. 3 ml venous blood was collected into a vacuotainer containing K_3 EDTA (tripotassium ethylene diamine tetra acetic acid) and platelet counts were performed within 02 hours of sample collection by five parts Sysmex automated haematology Analyzer (XE-3000).

A supravital stain of unfixed red blood cells was done with new methylene blue by mixing 100 ul of the patient's blood with 100ul methylene blue, after incubation for 20 minutes at a temperature of 37 ˚ C, the mixture was remixed and wedge blood smear was performed to obtain reticulocyte count, after which the procedure of bone marrow aspiration and trephine biopsy was performed.

All the haematological procedures as well as reporting were performed by experienced hematologists.

Data was entered in the SPSS version 25 for analysis. The normality of data distribution was determined by Shapiro wilk test. The quantitative variables (Age, Platelet count, retic count) were presented with Mean + SD. The categorical variables were presented with frequency and percentages. An Independent T-test was used to determine the difference between quantitative variables in remission vs non-remission cases. The Pearson correlation test was used to determine the quantitative correlation between continuous variables. For the diagnostic role of the peripheral platelet count and retic, Receiver operating curve (ROC) was used in SPSS to determine the Area under the curve (AUC) to determine the relationship of clinical sensitivity of different hematological

indicators (Retic and platelet count) and to predict remission.

Results

A total of 84 cases referred for remission were included, 56(66.7%) were males, and 28 (33.3%) were females. Out of the total, 50(59.5%) cases were in the age range of 5-18 years. The mean with a standard deviation of the age of patients was 15±4 years. 75(89.3%) of the cases were classified into ALL-1) by FAB classification. 50(59.5%) of the referred cases had achieved morphological remission by bone marrow aspiration. (Table 1)

We observed a statistically significant rise in Platelet count of the remission vs non-remission cases (p-0.001). Again there was a statistically significant difference in the retic count of the cases with remission (p-0.05) using the Student t-test as a test of significance. The difference in absolute Neutrophil count in both categories was not statistically significant. (Table 2)

We observed a statically significant downhill moderate correlation (Pearson correlation test) of retic count with remission (in terms of blast count of Bone marrow aspiration) (p-0.04, r:-0.32). Platelet count also had an inverse significant correlation with remission (p-0.01, r:-0.37). (Table 3)

The diagnostic roles of the peripheral platelet count and retic yielded an area under curves of (0.768 and 0.648 respectively) to predict remission. (Figure 1)

Table 1: Descriptive statistic of patients

a. Gender wise distribution of patients		
	Frequency	Percent
Male	56	66.7
Female	28	33.3
Total	84	100
b. Age wise distribution of patients		
	Frequency	Percent
<5 years	10	11.9
5.1-18 years	50	59.5
18-39 years	21	25
>39years	3	3.6
Total	84	100
c. Morphological diagnosis		
	Frequency	Percent
ALL-1	75	89.3
ALL-2	6	7.1
ALL3	3	3.6
Total	84	100
d. Remission status of patients		
	Frequency	Percent
Yes	50	59.5
No	34	40.5
Total	84	100

Table 2: The impact of platelet and reticulocyte count on remission in Acute Lymphoblastic Leukemia (ALL)

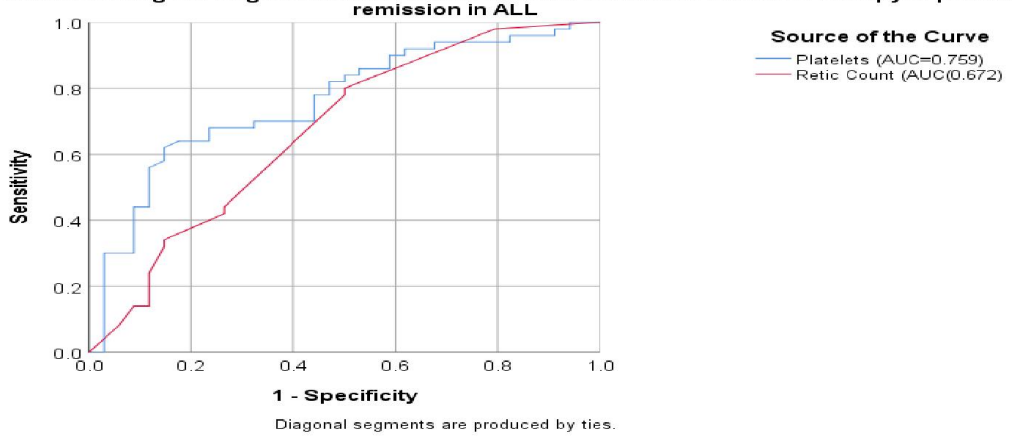
Hematological indices	*Remission status*	*N*	*Mean±SD*	*P-value*
Platelet count	Yes	50	332.1±136	0.001
	No	34	207.9±135	
Reticulocyte count	Yes	50	1.7±1.4	0.049
	No	34	1.4±1.2	
ANC	Yes	50	5255±37	0.067
	No	34	3286±58	

Table 3: Correlations of reticulocyte count and platelet count with the number of blast cells in remission cases after induction therapy

		Morphological remission	*Reticulocyte count*
Reticulocyte count	$\mathbf{r_s}$	-.312**	
	p	0.004	
	N	84	
Platelet Count	$\mathbf{r_s}$	-.367**	.327**
	p	0.001	0.002
	N	84	84

** *Correlation is significant at the 0.01 level*

Fig 1. ROC curve showing the diagnostic role of Platlet and retic count after induction therapy to predict remission in ALL



Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Confidence Interval	
				Lower Bound	Upper Bound
Platelets	0.759	0.053	0.000	0.655	0.864
Retic	0.672	0.062	0.008	0.551	0.793

The test result variable(s): platelets, retic has at least one tie between the positive

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 1:

Discussion

Survival in cases of acute lymphoblastic leukemia mainly depends on complete remission. Complete remission can be defined based on certain variables in different procedures/investigations to be more accurately defined remission. However, we hypothesized that a reticulocyte count of more than 2.5% and a platelet count of more than 250000/cmm3 is essential to label a case as remission before going into the invasive procedures.

In the present study, the mean age of patients was 15±4 years with male gender predominance (66.7%). 89.3% of the cases were classified into ALL-1 by FAB classification. The correlation strength of retic count and platelet count with remission was (p-0.04, r:-0.32), (p-0.01, r:-0.37) respectively.

A study on the bio-clinical and demographic (age & gender) characteristics of a large cohort of 5000 ALL patients concluded that the peak incidence of ALL was reported at 1-5 years of age followed by another spike in age 6-10 years, with a progressive decrease in cohort 11-30 years, and disappear in the 50-60 years cohort. Furthermore, they reported a lower incidence in the female gender which supports our findings.[8] Rauf et al[6] also reported the mean age of children with ALL as 12±5 years in their study conducted in the department of hematology, Armed force institute of Pathology Rawalpindi which matches the age of occurrence of ALL with our population.

The rate of morphological remission was 59.5% in the present study which is lower than as reported by Vallacha A et al[7] from Karachi (70%). Another study from malysia[5] reported 63% remission which is in concordance with our findings. In European countries, a remission rate of up to 90-95% is reported in pediatric age.[9]

Regarding the clinical significance of the hematological parameter in remission, a statistically significant rise in Platelet count and retic count was observed in cases with remission vs no remission (p-0.001) and (p-0.05) respectively. While no such difference in absolute Neutrophil count in both the categories was noted in groups.

A study published in "Leukemia" journal reported an increase in reticulocyte count in all patients who achieved remission /recovery after chemotherapy (ATCO) (p<0.001) that matches our findings.[10]

Reticulocyte count is used by many clinical centers to assess hemopoitic recovery in ALL cases. Microscopically quantification is done by hematologists and flowcytometry or advanced hematology analyzers can give you accurate immature reticulocyte fractions (IRF). According to a study performed by Rawalpindi AFIP to show remission success in ALL, they reported 78% sensitivity for IRF

to predict remission which is higher than the present study.[11]

Replication analysis has also confirmed the prognostic value of platelet count on treatment day 33 and has a strong inverse correlation with the number of blast cells after induction therapy (P<0.001) [12] which is consistent with our findings. we did not measure and analysed the platelet indices to see the correlation of the individual platelet indices with remission, a study from Bangladesh reported that Platelet count and platelet-crit (PCT) were significantly higher in the post-therapy remission cases versus non-remission cases (p<0.05) [13] still in term of platelet count it correlates with our findings.

Total leukocyte count (TLC) and ANC are easily ascertainable hematological parameters that reflect the plasma levels of chemotherapy to achieve remission in ALL. However, we could not appreciate the difference in absolute Neutrophil count in remission cases versus no remission groups and our findings are in concordance with the study of Lucas K et al.[14] However they have reported an inverse relationship of post-induction therapy with TLC and ANC. A study reported mean TLC in relapsed and non-relapsed groups respectively (p = 0.03) and the same was for mean ANC (x 10(3)/mm3) were 3.0 +/- 0.9 and 2.5 +/- 0.6 in both groups (p = 0.05).[13] Authors have reported ANC with 96% sensitivity even to predict the hemopoitic recovery in ALL.[15]

On the ROC curve, the platelet count and retic yielded an area under curves of (0.768 and 0.648 respectively) to predict remission. Another study from China reported using the Receiver Operating Curve (ROC) and regression analysis with findings that platelet count can have a predictive value for the prognosis of patients with acute leukemia.[16]

Conclusion

The higher reticulocyte and platelet count in cases of post-induction therapy can predict remission in acute lymphoblastic leukemia (ALL). There is a statistically significant inverse correlation of reticulocyte and platelet count with the number of lymphoblasts in ALL after induction therapy. The diagnostic values of reticulocyte and platelet count on the ROC curve as acceptable to be used in clinical setup to reduce the frequency of invasive procedures like (bone marrow aspiration and trephine biopsy) and expensive procedures (immune histochemistry and PCR).

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