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Original Research Article

Assessment of hematological toxicity and risk stratification in children aged 1-12 years with acute lymphoblastic leukemia receiving ALL COG protocol: a prospective observational study from a tertiary care center in central India

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy and while standardized chemotherapy protocols have improved survival, treatment-related haematological toxicities remain a significant challenge in low- and middle-income countries.
Methods: A prospective observational study was conducted on 42 children aged 1–12 years with newly diagnosed ALL at a tertiary care center in Central India between 2023 and 2025. Clinical, hematological, immunophenotypic, cytogenetic and Minimal Residual Disease (MRD) data were collected and analyzed.
Results: The cohort comprised 52.4% males, with mean age 7 years. B-cell ALL accounted for 83.3% of cases. Risk stratification revealed 64.3% intermediate-risk, 21.4% high-risk and 14.3% standard-risk. Pallor (85.7%) and fever (73.8%) were the most common presentations. Haematological toxicities particularly neutropenia, anemia and thrombocytopenia were more frequent in higher-risk groups and necessitated frequent transfusions. MRD negativity post-induction was achieved in 78.6% of patients and strongly correlated with remission ($p<0.001$).
Conclusions: Haematological toxicities significantly impact survival in pediatric ALL, particularly in high-risk groups. MRD remains a powerful prognostic marker. Strengthening supportive care, infection prevention and strategies to reduce treatment abandonment are essential to improve outcomes in resource-limited settings.

Keywords: Acute lymphoblastic leukemia, Chemotherapy, Haematological toxicity, MRD, Pediatric oncology

INTRODUCTION

ALL stands as the most prevalent childhood malignancy, accounting for the majority of paediatric leukemia cases globally and in India.[1-4] Advances in chemotherapy protocols have significantly improved survival rates, with 5 years overall survival for Indian children with ALL now ranging from approximately 45% to 81%.[5] However, the intensive multi-agent chemotherapy regimens that underpin these successes are also associated with substantial haematological toxicity, including severe neutropenia, anemia, thrombocytopenia and increased risk of infectious and bleeding complications.[6-8] Haematological toxicity remains a critical concern in the management of paediatric ALL, as it can lead to treatment delays, dose reductions, increased need for transfusions and higher rates of morbidity and mortality.[9-11] The risk and severity of these toxicities are influenced by factors such as treatment phase, protocol intensity, patient age, baseline blood counts and the presence of comorbidities.[8,12,13] Notably, the induction phase of therapy is often associated with the highest incidence and severity of cytopenias, with studies reporting grade 4 neutropenia in up to 60%, grade ≥3 anemia in about 34% and grade ≥3 thrombocytopenia in over half of paediatric

patients receiving standard protocols such as ALL IC-BFM 2009.[14] In India, the burden of ALL is substantial, with an estimated 9,000 new paediatric cases annually.[1,2] Despite improvements in outcomes, challenges persist due to late presentation, variable access to supportive care and the lack of uniform data on treatment-related toxicities across different regions and healthcare settings.[2-4] Prospective observational studies focusing on haematological toxicity are limited, particularly in central India, where institution-based data can provide valuable insights for optimizing supportive care and protocol adaptation.[15]

Given this context, systematic assessment of haematological toxicity in children aged 1–12 years with ALL undergoing treatment as per standardized protocols is essential. Such research can inform risk stratification, guide supportive interventions and ultimately improve both the quality and outcomes of paediatric leukemia care in tertiary centers across India.[14,15] This prospective observational study aims to fill the existing knowledge gap by evaluating the incidence, severity and clinical correlates of haematological toxicity in children receiving ALL protocol-based therapy in a tertiary care center in central India.

METHODS

Study design and setting

This prospective observational study was conducted in the Department of Pediatrics, Government Medical College, Nagpur, over 24 months (January 2023–January 2025). Ethical clearance was obtained from the Institutional Ethics Committee and written informed consent was taken from all guardians.

Study population

Children aged 1–12 years with newly diagnosed ALL were enrolled. Diagnosis was confirmed by morphology and flow cytometry. Exclusion criteria included prior chemotherapy at other institutions or refusal of consent.

Sample size and sampling

A total of 42 patients were included, based on institutional case load and feasibility. Sample size was calculated using the formula.

$n= (z^{(1-a/2)} \times p \times (1-p))/d^2$

z: z score; p: Expected proportion; d: Absolute precision; 1- a/2: Desired Confidence level. Sampling method-convenient method.

Data collection and procedures

Detailed history, clinical examination and laboratory investigations were recorded in a structured proforma. Diagnosis was established with ≥20% blasts in peripheral smear or ≥15% blasts in bone marrow. Flow cytometry was used to classify cases as B- or T-cell ALL, followed by risk stratification. Parents were counseled regarding treatment based on the COG ALL protocol.

Hematological toxicities were assessed by complete blood counts (CBC) using the ASPEN AD 3200 three-part analyzer to minimize observer variation. Patients were assessed on day 8 post-chemotherapy and minimal residual disease (MRD) was evaluated on day 35 post-induction. Risk stratification was repeated after induction.

Statistical analysis

Data were entered in Microsoft Excel and analyzed using SPSS version 25.0. Descriptive statistics were used for baseline variables. Chi-square test or Fisher's exact test was applied for categorical variables. A p-value <0.05 was considered statistically significant.

RESULTS

As per Table 1, In this prospective observational study examining children aged 1–12 years diagnosed with ALL undergoing treatment with the COG ALL protocol, immunophenotypic classification revealed that B-cell lineage ALL was the predominant subtype. Out of 42 total cases, 35 patients (83.3%) were diagnosed with B-cell ALL, while 7 patients (16.7%) had T-cell ALL. This immunophenotypic profile was determined using flow cytometry based on surface markers, in line with diagnostic standards. The preponderance of B-cell ALL aligns with the expected epidemiological trend in pediatric ALL populations.

As per Table 2, the analysis of age distribution among the 42 pediatric patients with ALL enrolled in the study revealed a mean age of 6.98 years with a standard deviation of 2.92, indicating moderate variability around the mean. The standard error of the mean was 0.45, which suggests that the sample mean is a reasonably precise estimate of the population mean. The 95% confidence interval ranged from 6.07 to 7.88 years, signifying that the true mean age of the population is expected to fall within this range. The median age was 7 years, very close to the mean, suggesting a symmetric age distribution. The interquartile range (IQR) was 4 years, indicating that the middle 50% of children fell within a 4-year age span. The range of age was 12 years, reflecting the inclusion of patients from 1 to 13 years of age.

As per table 3, the gender distribution of the 42 pediatric ALL patients enrolled in this study shows a slight male predominance. Out of the total participants, 22 children (52.4%) were male, while 20 children (47.6%) were female. This yields a male-to-female ratio of approximately 1.1:1, suggesting a relatively balanced distribution but with a modest male excess. Such patterns are consistent with known epidemiological trends in

pediatric ALL, which often shows a slight male preponderance across various populations.

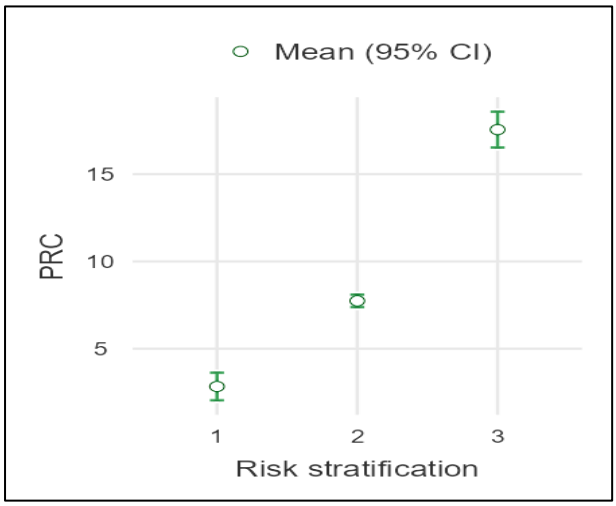

Figure 1: PRC Requirements.

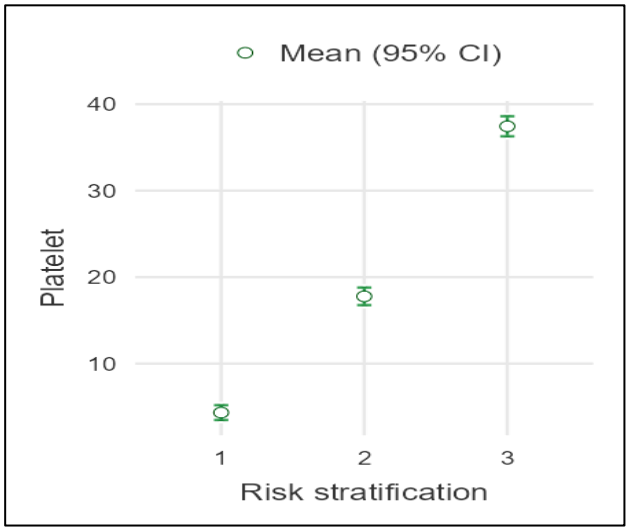

Figure 2: Platelet transfusion needs.

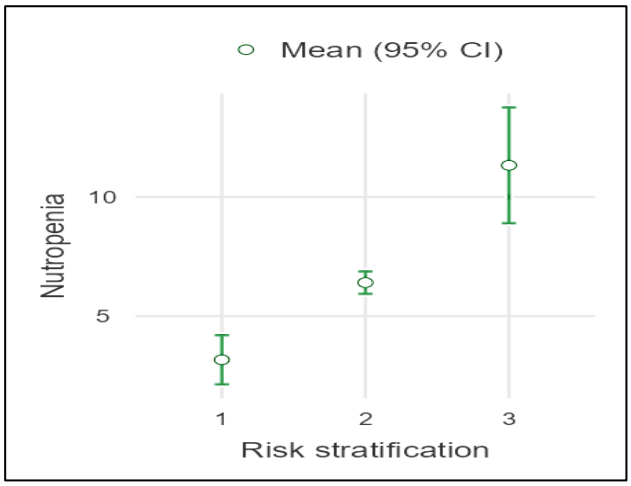

Figure 3: Neutropenia episodes.

As per Table 4, among the 42 children diagnosed with ALL in this study, a substantial majority, 32 patients (76.2%), were from rural areas, while only 10 patients (23.8%) were from urban areas. This stark rural predominance highlights a significant disparity in the geographical distribution of cases presenting to the tertiary care center. The data suggest that the burden of pediatric ALL is notably higher or at least more frequently diagnosed and referred in rural populations within the study region, possibly reflecting underlying demographic, environmental and access-to-care factors.

As per Table 5, Risk stratification revealed that the majority of children, 27 (64.3%), belonged to the intermediate-risk group. High-risk patients accounted for 9 cases (21.4%), while only 6 patients (14.3%) were classified as standard-risk. Risk categorization was based on NCI criteria, clinical features (such as age and WBC count), cytogenetics, MRD levels and involvement of extramedullary sites. This distribution indicates that a significant portion of the study population presented with features necessitating intermediate or high-intensity treatment protocols.

Hematological toxicities showed a clear correlation with risk stratification (Table 6). Children in the high-risk group required the greatest number of transfusions and experienced the most frequent neutropenia episodes. The mean number of packed red cell transfusions was 2.83±0.75 in the standard-risk group, 7.74±0.90 in the intermediate group and 17.6±1.33 in the high-risk group. Similarly, the mean platelet transfusion requirement rose from 4.33±0.82 in the standard-risk group to 17.8±2.6 in the intermediate group and further to 37.4±1.5 in the high-risk group.

Episodes of neutropenia followed a comparable trend, with mean values of 3.17±0.98, 6.41±1.19 and 11.3±3.16 in the standard-, intermediate- and high-risk groups, respectively. Median values with interquartile ranges confirmed this increasing pattern across risk categories. Overall, hematological toxicity burden was five- to six-fold higher in high-risk patients compared to the standard-risk group, underscoring the importance of careful monitoring and intensified supportive care in children stratified to higher-risk categories.

As per Figure 1, Patients in the standard-risk group required a mean of 2.83 PRC units (±0.75), with a median of 3, ranging from 2 to 4. The interquartile range (IQR) was narrow at 0.75, indicating a relatively uniform transfusion burden. In contrast, intermediate-risk patients required 7.74 units on average and high-risk patients had a substantially higher mean requirement of 17.6 units, with a wider standard deviation (±1.33), suggesting increased transfusion needs due to therapy-induced anemia and marrow suppression. The 95% confidence interval for the high-risk group (16.5 to 18.6) was notably distinct from the lower-risk groups, indicating a statistically significant increase.

As per Figure 2, platelet transfusion showed a similar ascending trend across risk groups. The standard group had a mean requirement of 4.33 units, with a median of 4.5. Intermediate-risk patients averaged 17.8 units and high-risk patients averaged 37.4 units, with a median of 37 and a tight IQR of 1. This narrow spread in the high-risk group suggests a consistently high platelet requirement, likely due to intensified chemotherapy causing profound thrombocytopenia. The confidence intervals (36.3–38.6 for high-risk) again confirmed statistical significance between groups.

As per Figure 3, Neutropenic episodes were more frequent in higher-risk groups. The standard-risk group experienced a mean of 3.17 episodes, with a narrow IQR of 1.75. This increased to 6.41 in intermediate and 11.3 in high-risk patients, with standard deviations increasing from 0.983 to 3.16. The maximum number of neutropenic episodes recorded in the high-risk group was 15, further emphasizing the severity of myelosuppression.

As per Table 7, MRD status was evaluated at two key treatment milestones: post-induction and post-consolidation. After induction therapy, 33 patients (78.6%) achieved favorable MRD levels (<0.01%), whereas 9 patients (21.4%) remained MRD-positive (>0.01%). Following consolidation, 30 patients (71.4%) maintained low MRD levels, while 12 patients (28.6%) continued to exhibit MRD positivity. Chi-square analysis revealed a significant association between MRD status at the two stages (χ^2=13.6, df=1, p<0.001), indicating that patients with elevated MRD post-induction were significantly more likely to retain high MRD post-consolidation. These findings underscore the prognostic importance of early MRD clearance as a predictor of achieving deeper remission with subsequent therapy.

Table 1: Acute lymphoblastic leukemia types (n=42).

ALL	Frequency	% of total
B cell	35	83.30
T cell	7	16.70

Table 2: Age distribution (n=42).

	Age
Mean	6.98
Std. error mean	0.45
95% CI mean lower bound	6.07
95% CI mean upper bound	7.88
Median	7
Standard deviation	2.92
Variance	8.5
IQR	4
Range	12

Table 3: Sex distribution.

SEX	Frequency	% of total
Female	20	47.60

Table 4: Place of residence (n=42).

Rural/urban	Frequency	% of total
Rural	32	76.20
Urban	10	23.80

Table 5: Distribution of risk stratification (n=42).

Risk stratification	Frequency	% of total
Standard	6	14.30
Intermediate	27	64.30
High	9	21.40

Table 6: Hematological toxicity parameters.

	Risk stratification	PRC transfusion	Platelet transfusion	Neutropenia
Mean	Standard	2.83	4.33	3.17
	Intermediate	7.74	17.8	6.41
	High	17.6	37.4	11.3
Std. error mean	Standard	0.307	0.333	0.401
	Intermediate	0.174	0.493	0.228
	High	0.444	0.503	1.05
95% CI mean lower bound	Standard	2.04	3.48	2.13
	Intermediate	7.38	16.8	5.94
	High	16.5	36.3	8.9
95% CI mean upper bound	Standard	3.62	5.19	4.2
	Intermediate	8.1	18.8	6.88
	High	18.6	38.6	13.8
Median	Standard	3	4.5	3.5
	Intermediate	8	17	7
	High	17	37	12
Standard deviation	Standard	0.753	0.816	0.983
	Intermediate	0.903	2.56	1.19
	High	1.33	1.51	3.16
Variance	Standard	0.567	0.667	0.967
	Intermediate	0.815	6.56	1.4
	High	1.78	2.28	10
IQR	Standard	0.75	1	1.75
	Intermediate	1.5	3	2
	High	1	1	2
Minimum	Standard	2	3	2
	Intermediate	6	14	5
	High	16	35	4
Maximum	Standard	4	5	4
	Intermediate	9	28	8
	High	20	40	15

Table 7: Distribution of post-induction vs post consolidation MRD.

Post induction vs .post consolidation MRD			
	Post induction MRD frequency	**Post consolidation MRD frequency**	
<0.01	33	30	
>0.01	9	12	
χ^2 tests			
	Value	**df**	**P value**
χ^2	13.6	1	<0.001
N	42		

DISCUSSION

In this study, B-cell ALL accounted for 83.3% of pediatric cases, which is consistent with global and regional epidemiological data. Gaynon et al reported that B-cell ALL constitutes approximately 75–85% of childhood ALL cases, closely aligning with our findings.[16] Similarly, Özdemir et al, observed a predominance of B-cell lineage among children treated with the ALL IC-BFM 2009 protocol, with B-cell ALL occurring at a significantly higher frequency than T-cell variants.[14] The relatively lower proportion of T-cell ALL (16.7%) in this cohort is also comparable to other Indian studies that have reported T-cell prevalence ranging between 10% and 25%.[3,5,13,15] T-cell ALL is frequently associated with older age, male predominance and higher white blood cell counts at presentation, necessitating closer monitoring and more intensive therapy. The predominance of B-cell ALL in this study suggests a favorable prognostic profile, as this subtype generally responds better to treatment and is associated with improved survival outcomes under contemporary risk-

adapted treatment protocols.[8,16] The mean age at diagnosis in this cohort was 6.98 years, which falls within the typical peak incidence window of 2–10 years described in global literature. Gaynon et al similarly reported a median age of approximately 6–7 years among pediatric ALL patients.[16] In addition, Tamer Hassan et al, observed that the majority of ALL cases in their Egyptian cohort were diagnosed between 1 and 10 years of age.[17] The narrow 95% confidence interval (6.07–7.88) and low standard error of the mean (0.45) in this study further support the reliability of these findings.[17] Age at diagnosis is a critical prognostic determinant and children aged 1–10 years are generally categorized as standard-risk under the National Cancer Institute (NCI) criteria, which are integrated into the ALL IC-BFM 2009 protocol.[18]

Therefore, the age profile of this cohort indicates a favorable prognostic distribution. The gender distribution showed a slight male predominance, with 52.4% males and 47.6% females. This finding aligns with existing epidemiological trends, as male-to-female ratios of 1.2:1 to 1.3:1 have been consistently reported across populations.[8,19] Similar trends were reported by Tamer Hassan et al, who also noted a slightly higher male prevalence in pediatric leukemia cohorts.[17] Although this disparity does not alter treatment approaches, it remains an important epidemiological feature, potentially explained by biological factors such as X-linked genetic susceptibility and sex hormone–related immune modulation.

The predominance of rural patients (76.2%) reflects the socio-demographic profile of central India, where government tertiary care centers cater to large rural populations. Comparable findings have been reported by Faruqui N, et al, who observed a higher proportion of rural pediatric oncology cases, attributed both to demographic distribution and healthcare-seeking patterns.[20] Rural children often experience diagnostic delays, nutritional deficiencies and late presentation, which can adversely affect outcomes.[21] Tamer Hassan et al, also highlighted that environmental exposures and inadequate access to early diagnostic services were common challenges in rural cohorts.[17]

hese findings emphasize the importance of strengthening outreach services, early referral networks and community-level awareness programs to reduce disparities in pediatric cancer care. Risk stratification revealed that 64.3% of patients belonged to the intermediate-risk group, a distribution similar to findings from other cohorts using the ALL IC-BFM 2009 protocol. Özdemir et al, reported that most pediatric patients were classified as standard or intermediate risk, with a smaller proportion falling into the high-risk category.[14] In our study, the lower percentage of standard-risk patients (14.3%) may be attributable to delayed diagnosis, unfavorable cytogenetics or persistent MRD positivity, all of which shift patients into higher-risk groups. High-risk patients (21.4%) in this study displayed adverse prognostic features such as CNS/testicular involvement, induction failure or cytogenetic abnormalities including t (9;22) or MLL rearrangements. Since risk stratification directly informs treatment intensity and predicts long-term outcomes, accurate early classification is essential to optimize therapy maximizing cure rates in high-risk patients while avoiding unnecessary toxicity in standard-risk groups.[8,18,19]

This analysis also demonstrated a clear trend of increasing hematological toxicity with advancing risk category, consistent with the biological and therapeutic intensity gradients in ALL.[6-8] The significantly higher number of neutropenic episodes in high-risk patients (mean=11.33) underscores their vulnerability due to intensified chemotherapy and disease burden. These results are comparable to findings by Tamer Hassan et al, who reported a greater frequency of severe and prolonged neutropenia in high-risk pediatric ALL patients.[17]

The increased platelet transfusion requirement in the high-risk group further reflects profound marrow suppression and thrombocytopenia, typical of more aggressive treatment regimens.[22,23] Interestingly, the intermediate-risk group required more packed red cell (PRC) transfusions than both standard and high-risk patients, possibly due to variability in baseline anemia, timing of supportive care or patient-specific marrow responses. Özdemir et al, similarly noted that transfusion requirements do not always follow a linear trend with risk category but may vary depending on individual hematological profiles and induction-related complications.[14] These findings highlight the need for risk-adapted supportive care strategies, including timely transfusions and proactive infection control, to minimize chemotherapy-related morbidity and optimize outcomes.

This study has several limitations. The small sample size (42 patients) limited the statistical power, particularly for subgroup analyses across risk categories and treatment phases. As a single-center study conducted at a tertiary care institution, the findings may not be generalizable to other regions or populations. Attrition across treatment phases could have introduced bias in the evaluation of later outcomes.

The study primarily focused on hematological toxicities and did not assess non-hematological adverse effects, long-term sequelae, overall survival or quality-of-life parameters. Limited assessment of potential confounding factors such as nutritional status, comorbidities and pharmacogenetic variations as well as the absence of comprehensive molecular profiling and comparative treatment approaches, further constrained the scope. Future multicenter studies with larger cohorts and extended follow-up are warranted to validate these findings and enhance strategies for optimizing supportive care in pediatric ALL.

CONCLUSION

This prospective study provides important insights into the hematological toxicity profile and early treatment outcomes of pediatric Acute Lymphoblastic Leukemia (ALL) managed under the COG ALL protocol at a tertiary care center in Central India. Key strengths include its prospective design with real-time data collection, structured data tools, systematic risk stratification and incorporation of Minimal Residual Disease (MRD) evaluation at post-induction and post-consolidation stages.

Importantly, the study reflects real-world clinical challenges in a resource-constrained Indian tertiary care setting, such as treatment abandonment and mortality, making it highly relevant for regional health policy and pediatric oncology practices. This study provides valuable insights into the hematological toxicity profile and early treatment outcomes of children with Acute Lymphoblastic Leukemia treated under the COG ALL protocol in a tertiary care center in Central India. The data demonstrate that higher-risk groups experience significantly more hematological toxicities, including increased need for PRC and platelet transfusions and more frequent episodes of neutropenia. Furthermore, the results confirm the prognostic value of MRD, with early clearance being strongly associated with post-consolidation remission.

These findings highlight the importance of strengthening supportive care systems, enhancing infection management and addressing psychosocial and economic barriers to care. Overall, the study underscores the feasibility of implementing a standardized, protocol-based approach to ALL management in developing regions but calls for context-specific interventions and multicentric collaborations to improve outcomes and ensure equitable access to comprehensive pediatric cancer care.

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