Minimal Residual Disease Detection in Acute Lymphoblastic Leukemia Using Flow Cytometry

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Introduction

- Acute lymphoblastic leukemia is a malignant disease characterized by proliferation and accumulation of early lymphoid precursors (lymphoblasts) that replace the normal hematopoietic cells of the marrow.
- Over the past several years diagnosis and treatment of ALL in children has improved significantly and approximately 80% of children with ALL now survive into adulthood. Only 20-40% of adults with ALL are cured with current treatment regimens.
- Minimal residual disease analysis by flow cytometry at various treatment phases plays an important role in taking timely decisions about changes in therapeutic strategy and hence better outcome.

Aim

To assess the utility of a flow cytometry antibody panel for MRD detection in ALL patients.

Objectives

- **To determine the proportion of ALL patients achieving MRD negativity using the selected flow cytometry antibody panel at different treatment time points.**
- **To compare MRD detection rates between B-ALL and T-ALL subtypes.**
- **To evaluate the time taken to achieve MRD negativity in different ALL subtypes.**
- **To assess the reproducibility and reliability of MRD results obtained with the antibody panel.**
- **To correlate MRD status with patient demographic and clinical characteristics.**

Methods

Design: A prospective study analyzing MRD by flow cytometry in ALL patients.

Setting: Aga Khan University, Pakistan, from October 2023 to December 2024.

Patients: All patients diagnosed with ALL during the study period.

Interventions: The B-ALL panel included CD34-FITC, CD58-PE, CD20-PerCP-Cy5.5, CD10-PE-Cy7, CD19-APC, CD38-V450, CD45-V500-C, CD9-PE, CD22-PerCP-Cy5.5, and Tdt-APC.
The T-ALL panel included CD45-V500-C, cCD3-V450, CD5-PerCP-Cy5.5, CD4-PE-Cy7, CD7-APC, CD8-APC-H7, CD3-V450, CD20-PerCP-Cy5.5, CD10-PE-Cy7, and Tdt-APC.

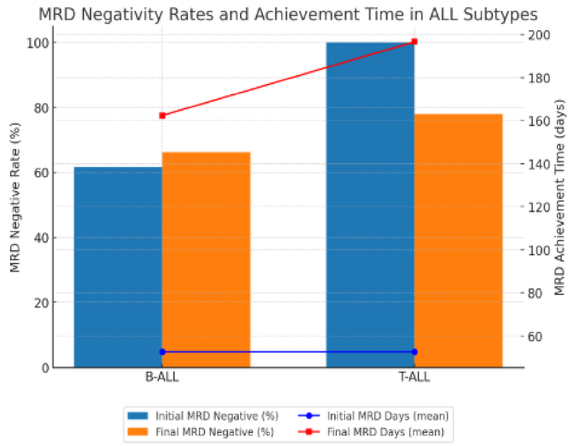
Instrument: BD FACSCanto™ II Flow Cytometer.

MRD Negativity: <0.01% blast cells. Remission status was correlated with morphology, cytogenetics, and molecular studies where available.

Main Outcome Measure: The proportion of patients achieving MRD negativity at different treatment phases.

Results

Total patients:	104
Male-to-female ratio:	2:1
Mean age:	21.54±14.34 years (range: 0.70–76 years)
B-ALL:	n=86, (83%)
T-ALL:	n=18, (17%).
MRD Negativity:	At 52.62±54.24 days 53 (61.6%) B-ALL 18 (100%) T-ALL
Final MRD Analysis:	57 (66.2%) B-ALL 14 (78%) T-ALL at 162.39±120.72 and 196.6±132.4 days, respectively.



Summary

Flow cytometry-based MRD assessment using a standardized antibody panel effectively identified remission status, demonstrating its value in treatment monitoring. A majority of patients achieved MRD negativity, with higher rates in T-ALL, reinforcing MRD analysis for guiding therapy and risk stratification.

Discussion

Flow cytometry-based MRD assessment proved to be a sensitive and reliable tool for monitoring treatment response in ALL, with the standardized antibody panel effectively distinguishing leukemic from normal cells. T-ALL patients showed higher early MRD negativity rates than B-ALL, though they required longer to achieve final negativity. The overall MRD negativity rates were comparable to international data, supporting the robustness of the method in Pakistan's context. Integrating MRD testing into treatment protocols could improve risk stratification and resource optimization. Limitations include the single-center design and lack of long-term survival data, highlighting the need for multicenter studies, extended follow-up, and potential combination with molecular MRD methods.

References

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2. Algamal R, Hassan M, Abdo E, et al. Impact of minimal residual disease detection by next generation flow cytometry on outcome of Egyptian patients with acute lymphoblastic leukemia. *Asian Pac J Cancer Prev*. 2023;24(10):3363-3370.