Abstract

Background: Age, presenting total leukocyte counts, steroid response and cytogenetics are known prognostic markers for acute lymphoblastic leukemia (ALL). Measurable Residual Disease (MRD) (or minimal residual disease) after induction chemotherapy is well accepted prognostic markers in childhood leukemia. In resource constrained countries evaluation of MRD either not widely available or increases the cost of treatment.

Methods: This is a retrospective analysis of children with acute lymphoblastic leukemia, who were treated with non-MRD based protocol. The correlation was tested between known risk factors and risk groups with end of induction MRD.

Results: Day15 bone marrow morphology and risk groups were significantly associated with MRD level. All standard risk patients except one had MRD negative. statistically significant number of intermediate risk group and half of high-risk group had positive MRD.

Conclusion: In resource constrained settings, MRD can be avoided in standard risk, but cannot be avoided in higher risk groups for optimization of therapy.

Main text

Introduction: Minimal Residual Disease (MRD) (or measurable residual disease) after induction chemotherapy is a well-known prognostic marker in childhood leukemia. Owing to its compelling evidence, most recent protocols include MRD as a guide to escalate or deescalate further therapy[1].Post induction, negative MRD (<0.01%) predicts long term survival whereas positive MRD indicates aggressive biology of disease and relative resistance to chemotherapy drugs which warrants more aggressive strategies like hematopoietic stem cell transplant or immunotherapy[2].Around the world different groups have proposed different criteria for risk stratification of childhood Acute Lymphoblastic Leukemia (ALL) and some of them include MRD as one of the criteria. As per National Cancer Institute (NCI) criteria, risk stratification is based on presenting counts and age[3]. Berlin Frankfurt Munster (BFM) group considers Age (<1yr or >6yr), presenting count (less than or more than 20000/cu.mm), ph t(9:22) and MLL translocation status as baseline risk factors[4]. Newer studies use MRD at various time points during chemotherapy to re-classify patients in various risk groups.As most MRD data in ALL

comes from western countries with scarcity of data in Indian children, we retrospectively analyzed our data of MRD in children with ALL and correlated MRD findings with established risk factors.

Material and Method: Retrospective analysis of all newly diagnosed Pediatric (1-16 years) ALL patients who received their induction chemotherapy between September 2017 to August 2020 was done. Patients were treated with IC-BFM 2002 protocol[5]. Blood samples were taken at baseline for complete blood counts, peripheral smear, liver function test, renal function test, serum LDH as routine practice. Bone marrow aspiration was done for flow-cytometry, cytogenetic and karyotyping. Peripheral smear was sent on D8 for steroid response. Bone marrow morphology done on Day 15. Post induction marrow aspiration was done and assessed for morphology and MRD on day 33. Patients were risk stratified into three risk groups as per IC-BFM2002 protocol: (1) Standard Risk (SR) defined as prednisolone good response (PGR), age more than 1 year to less than 6 years, initial WBC less than $20x10^9$ /L, andM1 (< 5% blasts) or M2 (≥ 5% to < 25% blasts) marrow on day 15, M1 marrow (less than 5% blasts)on day 33 (all criteria must be fulfilled); (2)Intermediate risk (IR), defined as PGR, age less than 1 year or more than 6 years, and/or WBC >$20x10^9$/L ,M1 or M2 marrow on day 15 and M1 marrow on day 33, or SR criteria but M3 (≥ 25% blasts) marrow on day 15 and M1 marrow on day 33; (3)High risk(HR), defined as at least one of the following: PPR (poor prednisolone response),IR and M3 marrow on day 15, M2 or M3 marrow on day 33, t(9;22) (BCR-ABL), or t(4;11) (MLL-AF4). Aberrant marker defined as abnormal expression or loss of expression of cell specific lineage marker not associated with specific cell type[6].Frequency of aberrant marker calculated and studied in relation to MRD.For B cell ALL, Immunophenotyping was done on 10 color Beckman Coulter's Navios EX flow-cytometry. Antibodies used for B cell typing and for BMRD were CD10, CD19, CD20, CD34, CD38, CD58, CD73, CD86, CD123 and CD45 was used as gating marker to gate the blasts. 1 lac cells were acquired for diagnostic flow and 16 lacs for BMRD. Percent of BMRD positive blasts was calculated using viable cells as the denominator. For T cell ALL markers used were CD3, CyCD3, CD45, CD5, CD16/CD56, CD4, CD34, CD7, CD8 and CD387. As per established criteria for MRD assessment by flow-cytometry, value <0.01% taken as negative and more than or equal to 0.01% taken as positive MRD[2].

Treatment: As per IC BFM 2002 risk stratification, patients were stratified as standard risk,intermediate and high risk. Standard risk T ALL, intermediate and high risk (both T and B cell ALL got the same induction chemotherapy), while B cell ALL standard risk received less intense chemotherapy. If D8 prednisolone response was poor they were shifted to intermediate/high risk protocol5.

Statistical Analysis: Data was entered in MS Excel, coded and analyzed in statistical software STATA, version 10.1, 2011. Data analysis included both Descriptive and Inferential statistics. Descriptive statistics were used to summarize quantitative variables with mean, standard deviation (SD), or median, range. Frequency and percentages were used to summarize categorical (qualitative) variables. Inferential statistics mainly included Chi-square test or Fisher's exact test (for small frequencies) for assessing statistical significance of difference in various parameters expressed as proportions in two comparison groups. Significance of difference in means in two groups was assessed by a two-independent sample t-test with equal variances. Binary Multiple Logistic Regression (MLR) analysis was performed for assessing effect of baseline characteristics like age and cytogenetic on dichotomous (MRD) outcome.

A p-value <0.05 was considered statistically significant for all the comparisons.

Results: Total 68 children were included over a 3-year period in this study. Baseline patient characteristics and relevant investigations entered in Table 1. Median age was 6 years (range 1-16 years) with male preponderance (male: female = 1.83). Median total leukocyte count at presentation was 14560/cu.mm (range 272390-330/cu.mm). Presenting hemoglobin ranged from 2.4 to 13.1 gm/dl (median: 7.9 gm/dl).Twenty three percent of total patients had aberrant markers. Table 2shows distribution of patients as pertype of ALL and CNS status. Majority i.e. 83.8% (57/68) were B cell ALL and 16%(11/68) were T cell ALL. In B cell ALL, cytogenetic analyses were done in 54 out of 57 patients for t(1:19), t(12:21), MLL translocation and t(9:22) Table 3. Translocation (12:21) was present in 8(15%) patients and was the commonest abnormality. Three (3.7%) were positive for t(9:22). Thirty-seven children (68%) had normal cytogenetics.Day 15 marrow was done for 53 children, 51 had M1, 1had M2 and 1 had M3 marrow status.Table 4 shows baseline risk groups and re-risk stratification after seven days of steroid. Day 8 steroid response was available for all 68 patients. At presentation, based on age, presenting counts and cytogenetics,21 patients (30.8%) were in standard risk, 43 (63.2%) were in intermediate risk and 4 were in high risk group. One patient with intermediate risk had poor response to steroids on day 8 (i.e. more than 1000 blast/cu.mmin peripheral smear) and was re-stratified to high risk group. Seven out of Sixty-Eight children (10%), had positive MRD after 1 month of induction. All were pre- B ALL. All standard risk except 1had negative MRD post induction chemotherapy. However,among intermediate,7.1% (3/42)and in high-risk 60% (3/5), had positive MRD post completion of induction chemotherapy. In high risk group, 2/3 ph positive patients had positive MRD post induction by flow-cytometry though all of them had negative BCR/ABL byRT PCR. These patients received Imatinib from Day 15 of Induction. One patient who had poor steroid response had positive MRD post induction chemotherapy. In univariate analysis (Table 6) there was no significant association betweenMRD results and type of leukemia i.e.

B and T cell ALL (P=0.58). We also found no significant association between cytogenetic (p=0.13) and aberrant markers (P=0.50) with MRD.Ageand presenting counts were not significantly associated with level of minimal residual disease, (P= 0.22 for age, P=0.62 for presenting counts). However, the odd ratio for age>10 years was 3.5, which suggest higher the age, more are the chances of getting MRD positive. There was no significant association of Day 8 steroid response(P=0.103), but D15 marrow morphology(P=0.015)and risk groups (P=0.001)had statistically significant association with MRD level post induction.

Discussion: MRD is the best-known predictor of disease outcome[1]. MRD is a result of biological nature of blast and effectiveness of treatment regimen. Most of the treatment protocols include MRD as a guide for risk assessment and treatment plan. MRD can be doneby PCR or flow-cytometry. PCR is not easily available in developing countries in contrast to flow-cytometry which is widely available. This retrospective analysis was done to see correlation of known risk factors and risk groups with MRD and to see if MRD can be avoided in risk groups, based on morphology.ICBFM 2002 protocol which we followed is a non MRD based protocol. Mini risk study[5] from same group which correlated MRD with known risk factors found that negative MRD at day 33 was associated with following factors - age of 1–5 years, WBC<20 000 /µl, non-T immunophenotype, good prednisone response and non-M3 morphology at day 15.In another study[9], NCI criteria i.e. age <1yr and >10years and TLC more than 50000/cumm had no association with level of MRD post induction which is in contradiction to what was found in mini risk study. In our study, we foundage more than six years was not statistically associated with positive MRD(P=0.22). When this association was checked with patients more than 10 years of age, the odd ratio was high (3.5). Presenting countswere also not associated significantly with MRD. This might be due to small sample size.Day 8 steroid response is a strong predictor of treatment outcome and most new protocols take this criterion to risk stratifies patients[7,10]. In our study, one patient with poor steroid response on D8 had positive MRD at the end of induction. D8 responsewas not significantly associated with MRD level, this may be due to small number of subjects with poor response. With regards to day 15 marrow status, 5/51 patients with M1, 1/1 with M2 and 1/1 M3 status on day15 had positive MRD at the end of induction. D15 marrow morphology was significantly associated with MRD results (P=0.015). Though the sample size is small, it still has good correlation to MRD. This is in accordance with what has been described by mini risk study where M1 marrow on D15 had low MRD as compared to non M1 marrow.We had only 3 patients with ph positive ALL which fall in the high-risk category as per risk stratification. Post induction, all of them were negative for BCR-ABL by PCR though two had positive MRD by flow-cytometry. Again, in view of small numbers it is not feasible to consider them for statistical analysis.In our cohort, value of MRD is statistically associated with various risk groups (p<.001).Only one child in standard risk group (4.7%)

had positive MRD. This is in contrast to BFM study[7] where they found 33% standard risk patients had positive MRD at day 33. This difference may be due to their larger cohort used by BFM group. Based on these findings, avoidance of MRD in standard risk group can be suggested in resource strain setup, though larger study is needed to confirm this finding.Three out of Forty-two (7%) patients in the intermediate risk group had positive MRD thus need more intensive protocol to prevent possible relapse but if treated on morphology-based criteria would receive lesser treatment .Thus it is difficult to avoid MRD in this subgroup as the plan of further treatment is different in MRD positive patients.Two out of five patients (40%)in high-risk cohort become MRD negative post induction. Monitoring of MRD is essential for re risk stratification in this group to redefine therapy[11,12].Our study had a few limitations; first of all, this was a retrospective study, done on a small sample of institutionalized patients with some having incomplete or missing data (especially Day 15 marrow morphology was not available for all children).Many intended associations of MRD with known factors could not be established. Hence findings of the study might have limited generalizability to a larger ALL patient population. However, considering limited research from resource limited regions in this area, our study does provide a one piece of evidence to support that monitoring MRDcan change risk stratification defined by morphology alone.

Conclusion: In resource constrained settings, MRD can be avoided in patients with standard risk ALL. Though morphology-based risk-group stratification identifies high-risk patients to some extent, still significant number of intermediate and high-risk patients had positive MRD which were not identified by conventional risk stratification. MRD cannot be avoided in these risk groups, who require optimization of therapy to prevent relapse based on their MRD status.

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