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ORIGINAL ARTICLE

Correlation of Day 8 Steroid Response with Bone Marrow Status Measured on Days 14 and 35, in Patients with Acute Lymphoblastic Leukemia Being Treated with BFM Protocol

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Abstract Berlin–Frankfurt–Munster (BFM) protocol is commonly used in India for treatment of acute lymphoblastic leukemia (ALL). The present study was conducted to correlate day 8 steroid response with bone marrow status (by morphology and flow cytometry) at day 14 and day 35 of treatment. It was a prospective study which included all newly diagnosed ALL patients who visited hospital between March 2013 and February 2015 i.e. 2 years. On 8th day, the number of lymphoblasts was counted in the peripheral blood. Based on the number of blasts patients were classified as good steroid responders and poor steroid responders. Following pre-induction steroids patients were given induction therapy. During this phase on day 14 and day 35 bone marrow (BM) aspiration study was done. Later day 8 steroid response, Day 14 BM status and day 35 BM status were correlated. Results showed that there was a statistically significant correlation between day 8 steroid response and day 14 BM status (both by morphology and flow cytometry). There was no statistically significant correlation between day 8 steroid response and day 35 BM status (both by morphology and flow cytometry). There was no statistically significant correlation between day 14 and day 35 BM status (both by morphology and flow cytometry). Sensitivity and specificity of morphological evaluation of BM was much lower compared to minimal residual disease assessment by flow cytometry. There is a need to incorporate flow cytometry in risk stratification of patients who are being treated with BFM 2002 protocol.

Keywords Acute lymphoblastic leukemia · BFM protocol · Day 8 steroid response · Minimal residual disease

Introduction

Patients with acute lymphoblastic leukemia (ALL) are treated as per risk stratification which are defined by both clinical and laboratory parameters. Risk stratification helps in choosing appropriate therapy, so that those with good prognostic indicators are spared from aggressive and potentially toxic treatment options [1]. Study groups such as Children's Oncology Group (COG) and National Cancer Institute (NCI) recommend treatment regimens based on pretreatment parameters such as age of the patient at presentation, white blood cell (WBC) count at diagnosis, central nervous system (CNS) involvement, testicular involvement etc. However, in 1983, the Berlin–Frankfurt–Munster (BFM) study group found that early treatment response to prednisolone is an independent prognostic factor in the prediction of treatment outcome. Evaluation of bone marrow (BM) at day 14 and day 35 is also important prognostic marker, which determines further mode of treatment [2]. Although BFM 2002 protocol does not advice BM assessment by techniques such as flow cytometry and polymerase chain reaction (PCR), they are considered to be more reliable techniques for assessing minimal residual disease (MRD) [3]. But major disadvantage of this approach is the enormous logistic and

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technological burden, especially when used in large number of patients [2].

In the present study, an attempt was made to correlate day 8 steroid response with measurement of disease status by morphology and flow cytometry at day 14 and day 35 of treatment.

Materials and Methods

The present study was conducted in a tertiary care hospital. It was a prospective study which included all newly diagnosed ALL patients who visited hospital between March 2013 and February 2015 i.e. 2 years. Total of 42 patients were included in the study. Patients with relapsed ALL, patients with ALL who were treated with other protocols such as Hyper-CVAD, and patients of ALL who were partially treated and then referred for further management, were excluded from the study. As per 1995 data, within a population of 882 million, six thousand children will develop acute lymphoblastic leukemia each year in India [4]. Considering prevalence of 0.0006% of ALL in Indian community this sample size was found to be adequate.

Following parameters were collected as soon as diagnosis was of ALL was made (after taking informed consent from the patient),

- BM and Peripheral blood (PB) blast count
- Immunophenotypic profile of individual case
- Prognostic PCR markers of ALL
- Cytogenetics of individual case

Patients were then treated as per BFM 2002 protocol. This protocol included initial pre-induction phase, where steroids were given for 7 days. On 8th day, the number of lymphoblasts was counted in the peripheral blood. Based on the number of blasts patients were classified as good steroid responders and poor steroid responders. Good responders were those who had less than 1000 blasts/cmm, while poor responders were those who had 1000 blasts/cmm or more on day 8 of treatment. Following pre-induction steroids, patients were given induction therapy, which included drugs such as Daunorubicin, Vincristine, L-Asparginase along with steroids. From 8th day onwards along with steroids patients receive Vincristine (1.5 mg/m^2-IV) and Daunamycin (30 mg/m^2) every week for 4 weeks and L-Asparginase (5000 IU/m^2) every 3rd day for 8 doses. (Table 1) Part I of induction phase generally was completed in 1 month. As the number was small, to avoid confounding variables, minimal protocol violations were done. Such violations were done only when clinical condition was inadequate to administer the drug as per the protocol. During this phase I of induction on day 14 and day 35 BM aspirations were done and they were subjected for both morphological evaluation and flow cytometric assessment. On day 36, patients need to be started on phase 2 of induction. Hence day 35 was chosen for 2nd reassessment marrow, instead of routine day 33, so that the procedure and chemotherapy can be completed in single admission. Response in BM was categorized as M1 ($< 5\%$ lymphoblasts), M2 (5–24% lymphoblasts) and M3 ($> 24\%$ lymphoblasts). As there were very few patients in M3 category we had clubbed M2 and M3 marrow status patients into one group and they were categorized as patients not in remission. Complete remission is defined as M1-BM status on day 35 of induction therapy. Similarly flow cytometric analysis was done for MRD, using the previous immunophenotypic markers expressed by the tumor cells. Patients with 0.01% or less number of blasts in BM samples were considered as MRD negative. Later day 8 steroid response, Day 14 BM status and day 35 BM status were correlated. Acceptance from institutional ethical committee was obtained prior to starting the study. Data analysis was done using SPSS (Statistical Package for Social Sciences) Version 21.0. We used Chi square test/Fisher's exact test for finding association between day 8 steroid response with BM status by morphology and flow cytometry of day 14 and day 35 of treatment. Sensitivity and specificity were calculated to find the relation between morphology and flow cytometric analysis.

Results

The present study included total of 42 newly diagnosed ALL patients. Out of 42 patients male patients were 29 (69%) and female patients were 13 (31%). BFM, although a pediatric protocol, it is popularly used in adults as well. Age range in present study was 3 years to 43 years and mean age ($\pm$ SD) was 17.5 ± 10.56 years. Out of 42 patients 8 patients were excluded from further analysis. 7 of them had received steroids prior to presentation, while 1 patient refused treatment due to financial constraints. Of 39 patients whose flow cytometry was available at diagnosis, 32 patients had B cell ALL and 7 patients had T cell ALL. The most common age group of occurrence of ALL in present study was 16–20 years (33.3%) followed by age group of ≤ 5 years (16.7%). With respect to steroid response, of 34 patients, 29 had good steroid response, while 5 patients had poor steroid response. At day 14, BM of 29 patients had shown less than 5% blasts while 5 patients had $\geq 5\%$ blasts. But at day 14 when BM was assessed using flow cytometry 17 patients had blast count of $> 0.001\%$ i.e. MRD positive. At day 35, only 30 patients were available for analysis. Following were the reasons for drop out of 4 patients.

Table 1 Protocol used in phase 1 of Induction

Drug	Treatment method	Single dose	Per day dose	Days of administration
Prednisolone	PO		60 mg/m^2	1–28, then taper over 9 days
Vincristine	IV	1.5 mg/m^2		8, 15, 22, 29
Daunorubicin	IV	30 mg/m^2		8, 15, 22, 29
L-asparginase	IV	5000 IU/m^2		12, 15, 18, 21, 24, 27, 30, 33
Methotrexate	IT	12 mg		1, 12, 33

- Development of pericardial effusion and severe hepatic failure, leading to coma in 1 patient. (Relatives requested for discharge).
- 1 patient was lost to follow up.
- 1 patient refused further treatment as poor prognosis was explained to him. He was p190 positive with M2 BM status in day 14 BM study.
- 1 patient requested discharge against medical advice.

Of remaining 30 patients 26 (86.7%) had M1 BM status and 4 (13.3%) had $\geq$ 5% blasts in BM. By flow cytometry 12 (40%) patients were MRD positive, while 18 (60%) were MRD negative.

When day 8 steroid response was correlated with day 14 BM status by morphology there was significant correlation. This indicated that poor steroid responders most often had $\geq$ 5% blasts in day 14 BM on morphological evaluation. When steroid response was correlated with MRD at day 14, there was a significant association. (p value-0.044).

When day 8 steroid response was correlated with day 35 BM status by morphology no significant correlation was noted. Similarly, there was no significant correlation observed between day 8 steroid response and MRD status by follow cytometry at day 35. When day 14 and day 35 BM status by morphological evaluation were correlated there was no significant correlation. Even by flow cytometric evaluations at day 14 and day 35 did not show any significant correlation.

Compared to flow cytometry, morphological evaluation at day 14 had sensitivity of only 30%, while specificity was 100%. Of 5 patients who had $\geq$ 5% blasts by morphology none were positive by MRD, while 12 patients who had $<$ 5% blasts by morphology were actually positive for MRD. The same analysis at day 35 was not similar. At day 35, the sensitivity of disease evaluation by morphology was 16.67%, while specificity was 88.89%. Out of 4 patients who had $\geq$ 5% blasts by morphology only 2 were positive by flow cytometry. This can be explained by the fact that, in the regenerating BM (which is often present at day 35 of treatment) there is presence of hematogones and regenerating normal blasts, which may be counted as tumor cells by morphology. But these cells are differentiated from tumor cells effectively by flow cytometric technique. Hence misinterpretation on morphology needs to be cleared by flow cytometry as a routine.

Discussion

The prognostic value of MRD detection with flow cytometry has been established across different treatment protocols for childhood and adult ALL. Most of these studies were able to show the potential usefulness of a risk stratification of patients. This risk stratification was based on the quantification of MRD at almost every time point in the treatment protocol. Stratification algorithms included multiple MRD measurements at various time points during or after induction and before consolidation [5]. However, BFM 2002 ALL study protocol did not include MRD for decision making as risk factor but did include it as part of assessment [6].

In the present study 85.3% patients (29 out of 34) had good steroid response. In a study done by Frankova et al. [7], which included 133 children with ALL, 125 children (94%) had a good steroid response. In a similar study conducted in India the overall good steroid response was observed in 82% of cases [8]. In a study by Alfred Reiter et al. [9], which included 998 ALL patients, 91.5% had shown good steroid response.

In a study by Borowitz et al. it was found that 28.6% patients had detectable MRD (by flow cytometry and PCR studies) at the end of induction. Patients with M3 marrow status at day 8 were much more likely to be MRD positive at day 35 than those with M2 or M1 marrows. In the same study MRD status was correlated with NCI risk of the patient. NCI risk group is based on WBC count at diagnosis, CNS/testicular involvement and adverse cytogenetic markers (i.e. E2A-PBX1, BRC-ABL, MLL rearrangement). It was shown that MRD significantly correlated with the risk group. NCI high risk patients were significantly more likely to be MRD positive than standard risk patients. Of high risk patients, 29.8% were MRD positive ($>$ 0.01%) compared with 18.5% of standard risk. At a cut off of 0.1% there were 22.8% high risk patients positive compared with 8.0% standard risk [10]. In another study by Samra et al. various risk factors were correlated with MRD status. Regarding B-ALL, L1 morphology showed significant

association with MRD positivity (80%) than L2 cases (26%). In addition, Ph chromosome positivity showed a significant association with MRD positivity (83%) versus Ph negative cases (35%). Age, gender, immunophenotype, and leukocyte count did not correlate with MRD risk [11]. A study by Reiter et al. [9] which included 998 unselected ALL patients who were being treated as per BFM-86 protocol, they showed that persistence of more than 5% blasts in the marrow on day 35 of induction therapy was the strongest predictor of worse prognosis by Cox regression analysis. Most of these patients were among the prednisolone poor responders. Although a prednisolone poor response was associated with other adverse prognostic parameters such as age less than 1 year and increased leukocyte count, it retained prognostic strength if those parameters were included as co-variables in the Cox regression analysis. This powerful prognostic variable is able to be obtained easily and early in almost every patient, an important attribute for its use as stratification parameter in a large multicentre trial. Compared with the evaluation of reduction of blasts in the marrow as a parameter of treatment response the measurement of blasts in blood is rarely altered by technical problems, Ex-dilution of samples. The shortcoming of the so-defined prednisolone poor response is certainly that "poor response" in the patients with low blast count at diagnosis is missed [9]. In a study by Melchior Lauten et al., it was shown that sensitivity of prednisolone response to predict poor BM response on day 14 or day 35 was low. This was because; only 27.9% of patients with M3 BM status at day 14 and 56.7% of patients with non-remission at day 35 had shown poor prednisolone response before. BM assessment at day 14 allowed a clear separation of 3 different risk groups for patients with M1, M2 and M3 marrow within the subgroups of good and poor steroid responders [2]. In the same study in which patients were followed up for 8 years, prednisolone response lost its significance, where as NCI risk criteria, as well as BM response on day 14 and day 35, retained significance. Hence they had suggested that the prednisolone response could be omitted as stratification parameter for patients with B cell ALL [2].

Compared to morphological evaluation flow cytometry is more sensitive and specific test [12]. Present study showed that at day 14 the morphological evaluation has sensitivity of only 30%, while specificity was 100%. At day 35, sensitivity of morphology was further reduced to 16.67%, while specificity was 88.89%. In a review by Campbell Myriam et al., they stated that morphological assessment of residual leukemia in blood or BM is often difficult and is relatively insensitive. Traditionally a cut off of 5% blasts in the BM (detected by light microscopy) has been used to determine remission status. This corresponds to a level of 1 in 20 malignant cells. If one wishes to detect lower levels of leukemic cells in either blood or marrow specialized techniques such as PCR assays or flow cytometric assays are required. With these techniques, detection of as few as 1 leukemic cell in 1,00,000 normal cells is possible and MRD at the level of 1 in 10,000 cells can be detected routinely [1]. In a study by Ratei et al., the comparison of the flow cytometry MRD negative versus MRD positive patient groups showed that the patients with a negative MRD status had a lower blast count at diagnosis and lower blast count at day 14 and good steroid response. Furthermore, patients with a negative MRD status at day 35 had significantly higher blast reduction rate at both early time points (day 8 and 15) than patients with a positive status [5]. In a study by Frankova et al., the utility of MRD was assessed in patients being treated with BFM-2002 protocol. They also compared PCR-MRD results with disease status assessment by morphological evaluation. Similar to our study, they found a large overlap between different risk groups concerning MRD negativity at the end of induction. Thus they opined that the ALL-IC-BFM criteria are not able to reliably define the low risk group potentially assigned to therapy reduction. During their study the countries involved, implemented the methodology of PCR based MRD testing and were prepared to use the MRD based protocol. They suggested that, great effort should be made to the identification of simpler stratification criteria (for example, flow cytometry MRD assessment), since PCR based MRD monitoring is still unavailable in many countries [7].

Conclusion

In the present study there was a significant correlation observed between day 8 steroid response and day 14 BM status. While similar correlation was not observed between day 8 steroid response and day 35 BM status. Between day 14 and day 35 BM statuses there was no significant correlation. Sensitivity and specificity of morphological evaluation of BM was much lower compared to MRD assessment by flow cytometry. There is a need to incorporate flow cytometry in risk stratification of patients who are being treated with BFM 2002 protocol so that appropriate change in therapy can be planned. But high level logistics and technical skill are needed to interpret MRD properly, which still lack in many of the centers in India.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of

the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Arceci R, Marcus K, Pulsipher M, Ritchey A, Silverman L, Smith M (2013) Childhood acute lymphoblastic leukemia treatment (PDQ). National Cancer Institute at the National Institutes of Health; Bethesda, MD (Updated 2013 Apr 11). www.cancer.gov/cancertopics/pdq/treatment/childALL/HealthProfessional/Page1/AllPages
2. Lauten M, Moricke A, Beier R, Zimmermann M, Stanulla M, Meissner B et al (2012) Prediction of outcome by early bone marrow response in childhood acute lymphoblastic leukemia treated in the ALL-BFM 95 trial: differential effects in precursor B-cell and T-cell leukemia. Hematologica 97(7):1048–1056
3. zur Stadt U, Harms DO, Schluter S, Jorch N, Spaar HJ, Nurnberger W, Volpel S et al (2000) Minimal residual disease analysis in acute lymphoblastic leukemia of childhood within the frame work of COALL study: results of an induction therapy without asparginase. Klin Padiatr 212(4):169–173
4. Chandy M (1995) Childhood acute lymphoblastic leukemia in India: an approach to management in a three-tier society. Med Pediatr Oncol 25(3):197–203
5. Ratei R, Basso G, Dworzak M, Gaipa G, Veltroni M, Rhein P et al (2009) Monitoring treatment response of childhood precursor B-cell acute lymphoblastic leukemia in the AIEOP-BFM-ALL 2000 protocol with multiparameter flow cytometry: predictive impact of early blast reduction on the remission status after induction. Leukemia 23:528–534
6. Stary J, Zimmermann M, Campbell M, Castillo L, Dibar E, Donska S et al (2014) Intensive chemotherapy for childhood acute lymphoblastic leukemia: results of the randomized intercontinental trial ALL-BFM 2002. J Clin Oncol 32(3):174–183
7. Fronkova E, Mejstrikova E, Avigad S, Chick KW, Castillo L, Manor S et al (2008) Minimal residual disease analysis in the non-MRD based ALL IC-BFM 2002 protocol for childhood ALL: Is it possible to avoid MRD testing? Leukemia 22:989–997
8. Arora RS, Eden TOB, Kapoor G (2009) Epidemiology of childhood cancer in India. Indian J Cancer 46(4):264–273
9. Reiter A, Schrappe M, Ludwig WD, Hidderman W, Sauter S, Henze G et al (1994) Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of multicenter trial ALL-BFM 86. Blood 84(9):3122–3133
10. Borowitz MJ, Pullen DJ, Shuster JJ, Viswanatha D, Montogomery K, Willman CL et al (2003) Minimal residual disease detection in childhood precursor B cell acute lymphoblastic leukemia: relation to other risk factors. A children's oncology group study. Leukemia 17:1566–1572
11. Samra M, Mahmoud H, Abdelhamid T, Sharkawy N, Elnahass Y, Elgammal M (2013) The prognostic significance of minimal residual disease in adult Egyptian patients with precursor acute lymphoblastic leukemia. J Egypt Natl Cancer Inst 25:135–142
12. Willemse M, Seriu T, Hettinger K, d'Aniello E, Hop W, Panzer-Grumayer R et al (2002) Detection of minimal residual disease identifies differences in treatment response between T-ALL and precursor B-ALL. Blood 99(12):4386–4393