RESEARCH PAPER

Role of Mid-induction Peripheral Blood Minimal Residual Disease Detection in Pediatric B-Lineage Acute Lymphoblastic Leukemia

KARTHIK BOMMANNAN, MAN UPDESH SINGH SACHDEVA, NEELAM VARMA, PARVEEN BOSE AND *DEEPAK BANSAL

*From Department of Hematology and *Pediatric Hematology-Oncology Unit, PGIMER, Chandigarh, India.*

Correspondence to: Dr Man Updesh Singh Sachdeva, Additional Professor, Department of Hematology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India. drmanupdeshpgi@yahoo.co.in

Received: September 30, 2015; Initial review: February 08, 2016; Accepted: September 02, 2016.

Objective: To study the role of mid-induction (day 15) peripheral blood minimal residual disease (PB-MRD) detection in pediatric B- lineage acute lymphoblastic leukemia (B-ALL).

Design: Prospective.

Setting: Tertiary-care center.

Patients: 40 consecutively-diagnosed treatment-naive, pediatric B-ALL patients.

Intervention: National Cancer Institute (NCI) standard risk patients were given three drug induction regimen comprising vincristine, L-asparginase and prednisolone; NCI high-risk patients were supplemented with daunorubicin.

Main outcome measure: Day 15 PB-MRD and bone marrow MRD (BM-MRD) analyzed by six color flow cytometry.

Results: The sensitivity of day 15 PB-MRD to identify concurrent day 15 BM-MRD positivity was 64%, with 100% specificity. The positive and negative predictive values were 100% and 62.5%, respectively. PB-MRD was positive in 67% of relapsed patients.

Conclusion: BM-MRD is a well-established prognostic factor in B-ALL. We suggest, day 15 PB-MRD could be considered as an early, minimally invasive and easily accessible MRD screening option.

Keywords: *Childhood Cancer, Bone marrow, Diagnosis, Prognosis*.

Published online: November 05, 2016. PII:S097475591600024

Minimal residual disease (MRD) detection at end-induction in bone marrow aspirate samples is an independent prognostic tool in childhood and adult B-ALL patients [1-4]. Recently, the concept of MRD detection in peripheral blood samples (PB-MRD) has come to the lime-light [5]. There are a few studies comparing the MRD levels in paired PB and bone marrow (BM) samples collected at varying time points of treatment [5,6]. In addition, some groups have attempted MRD detection in peripheral blood samples at day 15 of induction and have proven its role in early treatment modifications [6,7]. PB-MRD analysis, specifically done on day 15 of induction had better prognostic correlation than the PB-MRD analysis done at later time points (day 33, week 12, pre-maintenance and post-maintenance)[6]. In pediatric B-ALL management, day 15 PB-MRD could be an early and minimally invasive tool to assess treatment response. With this hypothesis, we planned a prospective study to estimate the sensitivity and specificity of mid- induction (day 15) PB-MRD in predicting day 15 BM-MRD.

METHODS

The patients were enrolled from the department of Pediatrics (Hematology-oncology unit) and the samples were processed and analyzed in the flow cytometry facility of a tertiary-care hospital in India. With institutional ethical clearance, patients were enrolled from July 2012 to May 2013. Under informed consent from the parents, all pediatric B-ALL patients with a diagnostic immunophenotype of bright CD19, CD10 dual expression, and variable (dim to bright) surface CD34 expression (CD19+CD10+CD34+/-) were included. All National Cancer Institute (NCI) standard risk patients underwent three drug induction comprising vincristine, L-asparginase and prednisolone, while the NCI-high risk patients were given daunorubicin in addition. As per the treatment protocol followed in our institute (adapted from UKALL 2003, version 7), a single-routine bone marrow examination was carried out on day 15 of induction (mid-induction) for morphological assessment of treatment response. The first pull bone marrow sample along with a peripheral blood sample were used to evaluate MRD.

Accompanying Editorial: Pages 1063-64.

Day 15 PB and BM samples were processed by lyse-stain-wash technique. An antibody-flurochrome

combination of CD34PE, CD20PerCP, CD19PECy7, CD10APC and CD45APCH7 along with Syto13, a nucleic acid binding dye were used. A minimum of one million events were acquired in BD FACS Canto II flow cytometer (Becton Dickinson. San Jose, USA) and analyzed with BD FACS Diva software. MRD of ≥0.01 % was considered positive [8,9].

The comparison of means between two groups was done using paired t-test or Wilcoxon signed rank test. Comparison of means between more than two groups was done using one way ANOVA and in case of significant result, Bonferroni correction was applied to compare the means of two groups at a time and the significance of each pair of groups was determined. For determination of correlation between two groups Spearman's rho was used. The tests were considered statistically significant at P <0.05 and were calculated using and SPSS version-17.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Forty patients were enrolled in the study, among which 30 (75%) were NCI standard risk and the rest 10 (25%) were NCI high risk. The cohort had 33 males and 7 females with a male to female ratio of 4.7:1. The mean (SD) age was 5 (2.8) years. All the patients were negative for *BCR-ABL1* (p190 and p210), *AF4-MLL*, *ETV6-RUNX1* and *E2A-PBX1* by qualitative polymerase chain reaction (PCR).

Of 40 mid-induction BM samples, MRD was positive in 25 samples (62.5%). Of these BM-MRD positive patients, there were 21 males and 4 females with a mean (SD) age of 5.6 (3.0) years. The mean (SD) BM-MRD was 1.17 (1.56) %. The BM-MRD negative patients had a mean (SD) age of 4 (2.1) years comprising 12 males and 3 females. The difference in BM-MRD among the standard risk and high risk patients were insignificant (P=0.20).

Of 40 mid-induction PB samples, 16 (40%) were MRD positive. The PB-MRD positive patients had a mean (SD) age of 5.1 (2.9) years, comprising 14 males and 2 females. The mean (SD) PB-MRD was 0.21 (0.34)%. PB-MRD negative patients comprised 19 males and 5 females with a mean (SD) age of 4.9 (2.8) years. Similar to BM-MRD, the difference in PB-MRD was insignificant (P=0.10) between the standard risk and high risk patients.

There were no statistically significant differences in the baseline and day-15 parameters like, hemoglobin (P=0.39), leukocyte count (P=0.12) and platelet counts (P=0.63), between the BM-MRD positive and negative patients and also between the PB-MRD positive and negative patients (P values of 0.66, 0.49 and 0.97 for hemoglobin, leukocyte count and platelet counts, respectively) **(*Table* I)**.

On analyzing PB and BM as sample pairs, among 40 pairs analysed, 16 sample pairs were MRD positive in both PB and BM. Isolated BM-MRD positivity was seen in 9 sample pairs and 15 sample pairs did not show any MRD in both the samples. However, none of the pairs showed isolated PB-MRD positivity.

Among 25 BM-MRD positive patients, 16 had concurrent PB-MRD positivity (64% of BM-MRD positive patients). The PB and BM-MRD of these 16 patients showed a significant direct correlation

TABLE I COMPARISON BETWEEN BASELINE AND DAY 15 PARAMETERS IN PERIPHERAL BLOOD AND BONE MARROW MRD POSITIVE AND NEGATIVE PATIENTS

Parameters	*Peripheral blood, Mean (SD)*			*Bone marrow, Mean (SD)*		
	Positive	*Negative*	*P value*	*Positive*	*Negative*	*P value*
*Age (y)	5.1 (2.9)	4.9 (2.8)	0.85	5.6 (3)	4 (2.1)	0.10
Male sex	14	19	0.82	21	12	0.74
Baseline						
*TLC × 10^9 /L	22 (18)	53.3 (48)	0.12	34.4 (29)	51 (37)	0.42
PB-blast (%)	62 (34)	60 (37)	0.87	58 (36)	66 (35)	0.50
BM-blast (%)	93 (4)	94 (19)	0.18	86 (18)	90 (13)	0.53
Day-15 TLC	2.1 (1.4)	2.9 (2.1)	0.49	1.9 (1.2)	3.6 (2.4)	0.36
BM-blast (%)	2.1 (1)	2 (1)	0.98	1.9 (1)	2.5 (1)	0.14

**NCI risk parameters. Positive MRD ≥0.01%; Negative MRD < 0.01%. TLC: total leukocyte count; PB: Peripheral blood; BM: Bone marrow. None of the cases in the cohort had day 15 peripheral blood blast by morphologic analysis. All the patients were negative for BCR-ABL1 (p190 and p210), AF4-MLL, ETV6-RUNX1 and E2A-PBX1 by qualitative polymerase chain reaction (PCR).*

WHAT IS ALREADY KNOWN?

- Bone marrow MRD at the end of induction is an independent prognostic factor in B- ALL management.

WHAT THIS STUDY ADDS?

- Mid induction peripheral blood MRD could be considered as a screening procedure for treatment response assessment.

(Spearman's rho=0.670, *P*=0.007). These 16 PB-MRD positive patients had a mean (SD) BM-MRD of 1.47 (1.84)%, whereas, the mean (SD) BM-MRD in PB-MRD negative patients was only 0.23 (0.52)%. This indicates a 6.3 times higher residual medullary leukemic load in PB-MRD positive patients. Of the PB-MRD positive patients, 75% had BM-MRD of >0.1% and 25% had BM-MRD ranging from 0.01 to 0.1%. A minimum medullary MRD of 0.3% was seen in 81.2% of PB-MRD positive patients. The sensitivity of PB-MRD to identify BM-MRD positivity was 64% with 100% specificity. The positive and negative predictive values were 100% and 62.5%, respectively.

During follow-up, two standard-risk patients died before completing induction and the data of one patient was not traceable. These three patients were day 15 BM-MRD negative. Of the remaining thirty seven patients, the mean (SD) follow-up period was 101(27) weeks. None of the BM-MRD negative patient relapsed. At a mean (SD) of 79 (23) weeks, six BM-MRD positive patients (25%) had relapsed. Three patients had isolated central nervous system (CNS) relapse, one had isolated medullary relapse; concurrent CNS and medullary relapse was seen in one patient while another patient had an initial unilateral testicular relapse at week 51, followed by CNS relapse at week 71. Four out of six patients (67%) with disease relapse had day 15 PB-MRD positivity.

DISCUSSION

The results of our study reveal that day 15 PB-MRD has a sensitivity of 64% in identifying concurrent day 15 BM-MRD, with 100% specificity. The positive and negative predictive values are 100% and 62.5%, respectively. On follow-up, none of the PB-MRD negative patients had disease relapse, while 67% of relapsed patients had PB-MRD positivity.

This study is limited by the fact that bone marrow examination was not repeated at end of induction phase as the institute's treatment-protocol used at the time of the study did not have this option, and hence, a comparison of mid-induction and end-induction MRD levels could not be done. In addition, a day 8 blast count by morphology and day 8 MRD analyses were also not attempted. A composite study with day 8, day 15, and day 35 (end-induction) MRD analysis with long term follow up, may yield information on optimal time or combination of time-points for attempting MRD analysis.

The strong relationship between end-induction BM-MRD levels and risk of relapse in childhood B-ALL is well documented [1,3-4,10]. MRD values are used to assess early treatment response, modify treatment intensity and estimate the optimal timing for hematopoietic stem cell transplantation [2,11-13]. In recent past, the concept of PB-MRD has come to lime light. There are few studies comparing the MRD levels in paired PB and BM samples collected at varying time points of treatment [5-6]. In 2002, Coustan-Smith, *et al*. [5] inferred that in T-ALL, PB-MRD was always positive if BM-MRD was positive (100% concordance) and hence PB-MRD can replace BM-MRD in T- ALL. The concept was not applicable for B-ALL, where the BM and PB MRD levels did not correlate and not all BM-MRD positive patients had concurrent PB-MRD positivity [5]. Similar conclusions were also derived by Van der Velden, *et al*. [14] in 2002, showing complete concordance between BM and PB MRD in T-ALL, but not in B-ALL [14]. Volejnikova, *et al*. [6] confirmed that PB-MRD analysis, specifically done on day 15 of induction had better prognostic correlation rather than the PB-MRD analysis done at later time points (day 33, week 12, pre- maintenance and post-maintenance). Authors were of the opinion that defining a low-risk group by PB-MRD negativity as early as day 15 will create an opportunity for de-escalating the therapy [6].

In conclusion, although end-induction BM-MRD presently remains irreplaceable, we suggest that day 15 PB-MRD could be considered as an early, minimally invasive and easily accessible MRD-screening option.

Acknowledgements: Prof Brent L Wood, Director, Hemato-pathology Laboratory, University of Washington, Seattle, USA, for his valuable inputs in standardizing our MRD experiment; and Mrs Kushum Chopra for helping in statistical analysis.

Contributors:MUSS: conceived and designed the study; KBBK: standardized and performed the flow cytometry experiment, collected clinical information, analyzed the data and wrote the manuscript; PB: helped in flow cytometry sample processing; MUS, NV, DB: made critical revisions of the manuscript.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Irving J, Jesson J, Virgo P, Case M, Minto L, Eyre L, *et al*. Establishment and validation of a standard protocol for the detection of minimal residual disease in B lineage childhood acute lymphoblastic leukemia by flow cytometry in a multi-center setting. Haematologica. 2009;94:870-4.
2. Schrappe M. Minimal residual disease: optimal methods, timing, and clinical relevance for an individual patient. Hematology Am Soc Hematol Educ Program. 2012; 2012:137-42.
3. Campana D. Minimal residual disease in acute lymphoblastic leukemia. Haemtology. 2010;1:7-12.
4. Conter V, Bartram C, Valsecchi M. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL2000 study. Blood. 2010;115:3206-14.
5. Coustan-Smith E, Sancho J, Hancock ML, Razzouk BI, Ribeiro RC, *et al*. Use of peripheral blood instead of bone marrow to monitor residual disease in children with acute lymphoblastic leukemia. Blood. 2002;100:2399-402.
6. Volejnikova J, Mejstrikova E, Valova T, Reznickova L, Hodonska L, Mihal V, *et al*. Minimal residual disease in peripheral blood at day 15 identifies a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with superior prognosis. Haematologica. 2011;96:1815-21.
7. O'Connor D, Jesson J, Bahey M, Eyre L, Lawson S. Analysis of early disease response in childhood acute lymphoblastic leukaemia: can peripheral blood replace bone marrow analysis? Br J Haematol. 2013;161:743-5.
8. Basso G, Veltroni M, Valsecchi MG, Dworzak MN, Ratei R, Silvestri D, *et al*. Risk of relapse of childhood acute lymphoblastic leukemia is predicted by flow cytometric measurement of residual disease on day 15 bone marrow. J Clin Oncol. 2009;27:5168-74.
9. Fronkova E, Mejstrikova E, Avigad S, Chik KW, Castillo L, Manor S, *et al*. Minimal residual disease (MRD) analysis in the non-MRD-based ALL IC-BFM 2002 protocol for childhood ALL: is it possible to avoid MRD testing? Leukemia. 2008;22:989-97.
10. Yamada M, Wasserman R, Lange B, Reichard BA, Womer RB, Rovera G. Minimal residual disease in childhood B-lineage lymphoblastic leukemia: persistence of leukemic cells during the first 18 months of treatment. N Engl J Med. 1990;323:448-55.
11. Brüggemann M, Raff T, Kneba M. Has MRD monitoring superseded other prognostic factors in adult ALL? Blood. 2012;120:4470-81.
12. Bader P, Kreyenberg H, Henze G. Prognostic value of minimal residual disease quantification before allogeneic stemcell transplantation in relapsed childhood acute lymphoblastic leukemia: The ALL-REZ BFM Study Group. J Clin Oncol. 2009;27:377-84.
13. Campana D. Role of minimal residual disease monitoring in adult and pediatric acute lymphoblastic leukemia. Haemtol Oncol Clin North Am. 2009;23:1083-98.
14. Van der Velden VH, Jacobs DC, Wijkhuijs AJ, Comans-Bitter WM, Willemse MJ, Hahlen K, *et al*. Minimal residual disease levels in bone marrow and peripheral blood are comparable in children with T cell acute lymphoblastic leukemia (ALL), but not in precursor-B-ALL. Leukemia. 2002;16:1432.