Indian J Hematol Blood Transfus (Jan-Mar 2023) 39(1):141–145
https://doi.org/10.1007/s12288-022-01541-1

SHORT COMMUNICATION

Cytogenetic Risk Stratification of B-Acute Lymphoblastic Leukemia and Its Correlation with Other Prognostic Factors

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Received: 24 November 2021 / Accepted: 11 April 2022 / Published online: 5 May 2022

Abstract Purpose of current study was to categorize WHO defined B-Acute Lymphoblastic Leukemia (B-ALL) cases into 3 cytogenetic risk groups (good, intermediate and poor) and to see their correlation with age, NCI risk criteria and treatment response. Clinical and diagnostic details were collected for 78 newly diagnosed B-ALL patients which included bone marrow morphology, flow cytometry immunophenotyping, karyotyping, FISH and RT-PCR. Study cohort comprised 44/78 (56.4%) children including 3 infants and 34/78 (43.6%) adults. Median age for paediatric group was 6 years (3 months–17 years) and for adults was 40.5 years (18 to 75 years). According to NCI risk criteria, excluding infants, 54 (72%) were high risk and 21 (28%) were standard risk. Clonal cytogenetic abnormality was detected in 59/78 cases (75.6%), while 19/78 (24.4%) cases showed normal karyotype. There was significant association of cytogenetic risk groups to age distribution (p value < 0.001) and NCI risk groups (p value < 0.001). There was no significant correlation of CNS involvement with cytogenetic risk groups ($p = 0.064$). Association of Day 8 steroid response and Day 15 bone marrow status with cytogenetic risk groups was significant ($p = 0.006$ and $p = 0.003$ respectively). Post treatment bone marrow status on Day 33 and Day 79 was available for 52 and 42 cases respectively. 9 adults died during induction phase. Day 33 post induction morphological remission was achieved in 51/52 cases (98%) and 1/52 (2.0%) were not in remission. Day 79 post induction morphological remission was achieved in 41/42 cases (98%) and 1/42 (2.0%) were not in remission. Day 33 or End of induction flow MRD (measurable residual disease) was negative in 39/52 (75.0%) patients and positive in 13/52 (25.0%) patients. Day 79 flow MRD was negative in 37/42 (88.1%) and positive in 5/42 (11.9%). Cytogenetic risk groups showed statistically significant Day 33 and Day 79 treatment response (morphologic remission: $p = 0.009$ and 0.003, flow MRD: $p = 0.004$ and $p = 0.012$ respectively). We concluded that cytogenetic risk groups showed statistically significant association with age, NCI risk criteria and treatment response.

Keywords B-acute lymphoblastic leukemia · Cytogenetic risk stratification · Flow MRD

Introduction

Cytogenetic abnormalities in chromosomal number and structure are seen in more than 80% of B-Acute lymphoblastic leukemia and have prognostic significance [1]. For decades, clinical trial groups studying ALL have utilized risk stratification schemes to assign patients to therapeutic regimens based on their estimated risk of treatment failure. The remarkable progress in the treatment of acute lymphoblastic leukaemia has been based on the adjustment of therapy to subgroups of leukaemia stratified by their prognostic implications [2].

Risk stratification systems for ALL utilize clinical factors such as age and WBC count along with incorporation of cytogenetic and genomic lesions of leukemic cells at diagnosis (e.g., favourable and unfavourable translocations) and response to therapy based on morphologic peripheral smear or bone marrow response and detection of MRD at end of induction and at later time points [3].

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The objective of the present study is to analyse correlation of WHO defined B- Acute Lymphoblastic Leukemia cytogenetic risk groups with age, NCI risk criteria and treatment response.

Materials and Methods

Data was collected from hospital information system for 78 newly diagnosed B-ALL patients registered at Medanta-The Medicity, between May 2018 to January 2022. Age, Total leucocyte counts at diagnosis along with post treatment Day 8 peripheral blood, Day 15, Day 33 and Day 79 bone marrow morphology were included for analysis. Diagnostic, Day 33 end of induction (EOI) and Day 79 Measurable residual disease (MRD) by Flowcytometric immunophenotyping was performed on bone marrow samples by 3 laser BD FACS Lyric instrument. Acute leukemia orientation tube (ALOT) based on Euroflow was run at the time of diagnosis along with 3 tube 8 colour flow cytometry panels which were run at the time of diagnosis to identify LAIPs and at later time points for MRD analysis (CD19, CD10, CD22, CD24, CD38, CD58, CD81, CD66c, CD15, CD200, CD73). Karyotyping was performed by G-banding and reported in accordance with ISCN 2016 nomenclature. 20 metaphases were analysed in all the cases. Real time qualitative PCR was performed by multigene PCR panel kit (3B Black Bio) for BCR-ABL, ETV6-RUNX1, TCF3-PBX1 and KMT2A (MLL) rearrangements in all the cases. FISH for cryptic chromosomal abnormalities using commercial fusion or break apart probes (Zytovision) for ETV6-RUNX1, BCR-ABL1 and MLL was done in cases detected to be positive by RT-PCR.

Results

Study cohort comprised 78 B-ALL patients of which 44/78 (56.4%) were children including 3 infants and 34/78 (43.6%) adults. Median age for paediatric group was 6 years (3 months—17 years) and for adults was 40.5 years (18 to 75 years). There were 45 Males (57.7%) and 33 Females (42.3%), Male to Female ratio was 1.4:1. Median TLC was 7734/μL (830–2,42,230/μL). Clonal cytogenetic abnormality was detected in 59/78 cases (75.6%), while 19/78 (24.4%) cases showed normal karyotype. Of total 78 cases included in the study, good risk cytogenetics comprised 21 (26.9%) cases, intermediate risk 32 (41.0%) and poor risk 25 (32.1%) cases. Good risk cytogenetics included Hyperdiploidy (51–65 chromosomes) and t(12;21)/ETV6-RUNX1. Intermediate group included t(1;19)/TCF3/PBX1, normal karyotype and other chromosomal abnormalities as mentioned in Table 1. Poor risk subtypes included t(9;22)/BCR-ABL, t(4;11)/KMT2A(MLL), complex karyotype (≥ 3 chromosomal abnormalities) and hypodiploidy (< 46 chromosomes) (Table 1). Good risk cytogenetics were more common in paediatrics, while poor risk in adults. Intermediate risk cytogenetics were almost equally distributed among children and adults. Association between good and poor cytogenetic risk groups and age distribution was significant (p value < 0.001). According to NCI risk criteria excluding infants, 54 (72%) were high risk (Age > 10 years and/or TLC $> 50{,}000/\mu L$) and 21 (28%) were standard risk (Age 1 to < 10 years and TLC $< 50{,}000/\mu L$). Majority of NCI high risk patients had intermediate and poor risk cytogenetic findings. The association between cytogenetic risk groups and NCI risk groups was statistically significant (p value < 0.001). CNS involvement was assessed at diagnosis in 56 patients of which 51 presented with CNS1 (< 5 WBC/μL with no blasts), 4 with CNS2 status (< 5 WBC/μL with blasts) and 1 case with CNS3 disease (> 5 WBC/μL with blasts). There was no significant association of CNS involvement with cytogenetic risk groups ($p = 0.064$). Post treatment Day 8 peripheral blood blast assessment was done in children, 37/38 (97.4%) had good prednisolone response (< 1000 blasts/μL) and 1/38 (2.6%) had poor steroid response. On Day 15 bone marrow, 32/38 (84.2%) children had M1 status ($< 5\%$ blasts) majority belonged to good risk and intermediate risk cytogenetics, 5/38 (13.2%) children had M2 status (5–25% blasts) of which 3 belonged to good risk and 2 to poor risk cytogenetic category and 1/38 (2.6%) had M3 status ($> 25\%$ blasts) who belonged to good risk cytogenetics. There was statistically significant association of Day 8 peripheral blood blast and Day 15 bone marrow status with cytogenetic risk groups ($p = 0.035$ & $p = 0.015$ respectively). Day 33 post induction morphological remission was achieved in 51/52 cases (98%) and 1/52 (2.0%) were not in remission. Day 79 post induction morphological remission was achieved in 41/42 cases (98%) and 1/42 (2.0%) were not in remission. Day 33 or End of induction (EOI) flow MRD (measurable residual disease) was negative in 39/52 (75.0%) patients and positive in 13/52 (25.0%) patients (3 belonged to good risk, 5 to intermediate and 5 to poor risk cytogenetic group). Thus, poor and intermediate risk cytogenetic groups each showed $\sim$ 38.5% Day 33 MRD positive cases. Day 79 flow MRD was negative in 37/42 (88.1%) and positive in 5/42 (11.9%) (1 belonged to good risk, 2 to intermediate and 2 to poor risk cytogenetic group). Day 79 positive MRD was higher in intermediate and poor risk cytogenetics (40% each) as compared to good risk cytogenetics (20%). Cytogenetic risk groups showed statistically significant Day 33 and Day 79 treatment response (morphologic remission: $p = 0.009$ and 0.003, flow MRD groups: $p = 0.004$ and $p = 0.012$ respectively) (Table 2).

Table 1 Frequency of recurrent and other cytogenetic abnormalities in B-Acute lymphoblastic leukemia

Cytogenetic risk group	Frequency N = 78	Recurrent cytogenetics and others	Frequency N (% of total)	Additional chromosomal abnormalities
Good risk	21 (26.9%)	Hyperdiploidy	15 (19.2%)	del(11q), del(13)(q12q22)
				add(19)(p13.3), del(6)(q21),del(7)(p13q11.2)del(17)(p13),i(9)(q10)
		t(12;21)/ETV6/RUNX1	6 (7.7%)	+ 21,+16, del(6)(q21q25)
				del(13)(q14),del(13)(q14)
Intermediate risk	32 (41.0%)	t(1;19)/TCF3/PBX1	4 (5.1%)	i(1)(q10)
		Normal karyotype	19 (24.4%)	–
		Other clonal abnormalities*	9 (11.5%)	–
Poor risk	25 (32.1%)	t(9;22)/BCR/ABL	16 (20.5%)	− 7, i(9)(q10), dic(7;9)(p11;p11)
				der(5)t(5;8)(q35;q13)
				i(8)(q10),del(9)(p22)
		t(4;11)/KMT2A	3 (3.8%)	der(19)t(1;19)(q12;p13), idem,i(8)(q10)
		Hypodiploidy	1 (1.3%)	–
		Complex karyotype (≥ 3 chromosomal abnormalities)	5 (6.4%)	–

*Abnormal (9p), Del(6), Dup(6), t(1;9)(p13;p12), der(22),del22(q13), t(8:22)(q24;q11) t(12;17)(p13;q11.2) dup(10)(q25q42), del(9)(p22) Inv(3)(q21q26), inv(7)(p13p21) der(10)t(1;10)(q23;q26)

Discussion

Most paediatric study groups classify patients into categories of standard, intermediate or average and high risk. Adults are generally divided into only standard-risk and high-risk groups. Age, leukocyte count at diagnosis, primary genetic abnormalities, response to therapy (which reflects the genetics of leukemic cells, the pharmacodynamics and pharmacogenomics) are strong prognostic indicators of outcome in paediatric B-ALL [4]. In the present study, paediatric patients were treated on modified Berlin, Frankfurt, and Munster 95 (BFM-95) protocol and adults on UKALL 14 protocol. In a study by AV Moorman et al. in 2010, UK MRC ALL97/99 randomized trial, analysis of fewer than 20 metaphases was classified as failure and was seen in 275 of 1694 samples (16%) [5]. Normal karyotype was seen in 219 of 1419 (15%) samples and clonal abnormality was detected in 1200 of 1419 (85%) samples. In our study, 20 metaphases could be analysed in all cases, normal karyotype was seen in 19/78 (24.4%) cases, while clonal chromosomal abnormality was detected in 59/78 cases (75.6%). Cytogenetic data from 1694 children with B-ALL included in UK MRC ALL97/99 were analysed and followed up for 8.2 years. Association between presentation cytogenetics, treatment response and relapsed risk group was examined. Two chromosomal abnormalities were associated with significantly better outcome (ETV6-RUNX1 and high hyperdiploidy, whereas five abnormalities were associated with increased risk of relapse (iAMP21, t(9;22), MLL, abnormal 17p and loss of 13q). In a study by Patkar et al., most patients harboured good-risk chromosomal abnormalities (n = 120, 48.9%), (n = 107, 43.7%) were classified as intermediate cytogenetic risk and a minority were classified into the high-risk group (n = 18, 7.3%). In comparison, our study showed more cases in high risk 25 (32.1%) and lesser in good risk 21 (26.9%), while intermediate risk cases showed similar frequency 32 (41.0%). Immunophenotypic MRD was detectable in 81 patients (33.1%), whereas the rest (n = 164, 66.9%) were negative for MRD at the end of induction [6]. Although cases were fewer in the present study, Day 33 flow MRD response were similar to above study, positive in 13/52 (25.0%) and negative in 39/52 (75.0%) patients. We also included Day 79 flow cytometric MRD analysis, which was positive in 5/42 (11.9%) and negative in 37/42 (88.1%).

Presence of minimal residual disease has been shown to be an independent prognostic marker in various studies depending on therapy and timing in the treatment protocol [7–10]. In a study by children's oncology group by Borowitz et al., a series of more than 1000 children with precursor-B- cell ALL, bone marrow MRD by four-color flow cytometry (at a level of more than 0.01%) was seen in approximately 22% of patients at the end of induction.

Table 2 Cytogenetic risk stratification of B-ALL cases and correlation with age, NCI risk criteria and treatment response

Parameter	Frequency n (%)	Cytogenetic risk groups frequency n (%)			*p* value
		Good risk	Intermediate risk	Poor risk	
Age n = 78					
Paediatric	44 (56.4)	18 (40.9)	19 (43.2)	7 (15.9)	< 0.001
Adult	34 (43.6)	3 (8.8)	13 (38.2)	18 (52.9)	
Gender n = 78					
Male	45 (57.7)	14 (31.1)	18 (40.0)	13 (28.9)	0.497
Female	33 (42.3)	7 (21.2)	14 (42.4)	12 (36.4)	
NCI risk group (excluding 3 infants) n = 75					
Standard	21 (28.0)	15 (71.4)	5 (23.8)	1 (4.8)	< 0.001
High	54 (72.0)	6 (11.1)	26 (48.1)	22 (40.7)	
CNS disease n = 56					
CNS 1	51 (91.0)	18 (35.3)	24 (40.0)	9 (17.6)	0.064
CNS 2	4 (7.1)	0 (0)	2 (50)	2 (50)	
CNS 3	1 (1.8)	1 (100)	0 (0)	0 (0)	
Day 8 PB steroid response n = 38					
Good	37 (97.4)	18 (48.6)	15 (40.5%)	4 (10.8)	0.006
Poor	1 (2.6)	0 (0)	0 (0%)	1 (100)	
Day 15 BM n = 38					
M1	32 (84.2)	15 (46.9)	14 (40.0)	3 (9.4)	0.003
M2	5 (13.2)	3 (60)	0 (0)	2 (40)	
M3	1 (2.6)	1 (100)	0 (0)	0 (0)	
Day 33 BM n = 52					
Remission	51 (98.0)	18 (35.0)	23 (45.0)	10 (20.0)	0.009
Not in remission	1 (2.0)	0 (0)	0 (0)	1 (100)	
Day 33 Flow MRD n = 52					
Negative	39 (75.0)	15 (38.5)	18 (46.0)	6 (15.0)	0.004
Positive	13 (25.0)	3 (23.1)	5 (38.5)	5 (38.5)	
Day 79 BM n = 42					
Remission	41 (97.6)	17 (41.5)	16 (39.0)	9 (22.0)	0.003
Not in remission	1 (2.4)	0 (0%)	0 (0%)	1 (100%)	
Day 79 Flow MRD n = 42					
Negative	37 (88.1)	16 (43.2)	14 (37.8)	7 (18.9)	0.012
Positive	5 (11.9)	1 (20.0)	2 (40.0)	2 (40.0)	

They also showed significant differences among MRD rates in genetically defined groups of patients [11]. Though, most patients with Ph + ALL were MRD + at high levels; high frequency of MRD positivity was seen in favourable trisomy patients. In a study by Schultz KR et al., COG risk classification scheme was used for division of B-precursor ALL into lower- (27%), standard- (32%), high- (37%), and very-high- (4%) risk groups based on age, white blood cell (WBC) count, cytogenetics, day-14 marrow response, and end induction minimal residual disease (MRD) by flow cytometry in COG trials for treatment algorithm [12]. In the present study, of total 78 cases included in the study, good risk cytogenetics comprised 21 (26.9%) cases, intermediate risk 32 (41.0%) and poor risk 25 (32.1%) cases. Post treatment, poor and intermediate risk cytogenetic groups each showed ~ 38.5% Day 33 MRD positive cases, followed by good risk cytogenetics ~ 23.1%. Day 79 positive MRD frequency was also similar, being higher in intermediate and poor risk cytogenetics (40% each) as compared to good risk cytogenetics (20%) (Table 2). After redefining risk groups based on positive MRD results, ~ 20% standard risk cases were included in high-risk category for treatment purpose.

Limitations of the present study are small number of de novo B-ALL cases and although risk stratification for B-ALL was done by karyotyping, FISH and RT-PCR for recurrent genetic abnormalities, outcome data in terms of event free survival, relapse and overall survival in various cytogenetic groups is not examined.

However, our study represents a single institute experience of B-ALL cases in paediatric and adults wherein, detailed diagnostics were done to risk stratify patients and follow-up flow cytometry MRD was assessed to monitor therapy and further refining risk stratification. Cytogenetic risk groups showed significant correlation with age, NCI risk criteria and treatment response. Present study highlights the importance of conventional cytogenetic analysis which remains as a gold standard for whole genome analysis and prognosis.

Funding No funding was received for conducting this study.

Declarations

Conflict of interest All authors declare no conflict of interest in writing this manuscript.

Ethics Approval Ethical committee approval was taken.

References

1. Zhou Y, You MJ, Young KH, Lin P et al (2012) Advances in the molecular pathobiology of B-lymphoblastic leukemia. Hum Pathol 43(9):1347–1362
2. Möricke A, Reiter A, Zimmermann M et al (2008) Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood 111(9):4477–4489
3. NCCN Practice guidelines in oncology (2014) Acute lymphoblastic leukemia. Version 1. NCCN.org
4. Pui C-H, Evans WE (2006) Treatment of acute lymphoblastic leukemia. N Engl J Med 354:166–178
5. Moorman AV, Ensor HM, Richards SM et al (2010) Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol 11(5):429–438
6. Patkar N, Subramanian PG, Tembhare P et al (2017) An integrated genomic profile that includes copy number alterations is highly predictive of minimal residual disease status in childhood precursor B-lineage acute lymphoblastic leukemia. Indian J Pathol Microbiol 60(2):209–213
7. Borowitz MJ, Pullen DJ, Shuster JJ, Viswanatha D, Montgomery K, Willman CL, Camitta B, Children's Oncology Group Study (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(8):1566–1572
8. Coustan-Smith E, Sancho J, Hancock ML, Boyett JM, Behm FG, Raimondi SC et al (2000) Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 96:2691–2696
9. Dworzak MN, Froschl G, Printz D, Mann G, Potschger U, Muhlegger N et al (2002) Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. Blood 99:1952–1958
10. van Dongen JJ, Seriu T, Panzer-Grumayer ER, Biondi A, Pongers-Willemse MJ, Corral L et al (1998) Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 352:1731–1738
11. Borowitz MJ, Devidas M et al (2008) Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood 111(12):5477–5485
12. Schultz KR, Pullen DJ, Sather HN et al (2007) Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). Blood 109(3):926–935