

Original article

Precursor B-lineage acute lymphoblastic leukemia patients with aberrant natural killer cell and T cell – lineage antigen expression: experience from a tertiary cancer care center



Karthik Bommannan *, Jhansi Rani Arumugam, Venkatraman Radhakrishnan, Jayachandran Perumal Kalaiyarasi, Nikita Mehra, Tenali Gnana Sagar, Shirley Sundersingh

Cancer Institute (W.I.A.), Adyar, Chennai, India

ARTICLE INFO

Article history:

Received 20 April 2020

Accepted 6 August 2020

Available online 7 October 2020

Keywords:

CD56 positive precursor B lineage acute lymphoblastic leukemia
CD7 positive precursor B lineage acute lymphoblastic leukemia
Precursor B lineage acute lymphoblastic leukemia

ABSTRACT

Introduction: Flow cytometric immunophenotyping (FCI) plays a major role in diagnosing hematologic malignancies. In patients diagnosed with precursor B-lineage acute lymphoblastic leukemia (B-ALL), expression of certain non-lineage/cross lineage antigens is of prognostic and cytogenetic relevance. There is a paucity of studies that have comprehensively analyzed the clinical and laboratory profiles of B-ALL patients showing aberrant T/natural killer (NK) cell antigen expression.

Materials and methods: This is a prospective study where 152 consecutive B-ALL patients were analyzed for aberrant expression of T/NK cell antigens (CD1a, CD5, CD4, CD7, CD8 and CD56) by FCI. The clinical and laboratory profile of these T/NK-cell antigen-expressing B-ALL patients was statistically analyzed against conventional B-ALL patients.

Results: In our B-ALL cohort, CD5, CD7 and CD56 expression were observed in one, six and nine patients, respectively. CD56-expressing B-ALL patients were predominantly children (89%) and presented as standard clinical risk ($p = 0.010$) disease with frequent ETV6-RUNX1 fusion ($p = 0.021$) positivity. On the contrary, CD7-expressing B-ALL patients were adolescent-young adult/adult-age skewed (83%) and had an adverse cytogenetic profile ($p = 0.001$), especially for the frequent presence of BCR-ABL1 fusion ($p = 0.004$) and KMT2A rearrangement ($p = 0.045$). CD7-expressing B-ALL patients had inferior event-free survival ($p = 0.040$) than their CD56-expressing counterparts, but there was no significant difference in the overall survival ($p = 0.317$).

* Corresponding author at: Department of Oncopathology, Cancer Institute (W.I.A.), Adyar, Chennai, 600020 India.

E-mail address: bkkb87@gmail.com (K. Bommannan).

<https://doi.org/10.1016/j.htct.2020.08.012>

2531-1379/© 2020 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Conclusion: In comparison to conventional B-ALL patients, there are significant differences in the age, cytogenetic profile and event-free survival of T/NK-cell antigen-expressing B-ALL patients.

© 2020 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Flow cytometric immunophenotyping (FCI) plays a major role in the diagnosis and follow-up of patients with precursor B-lineage acute lymphoblastic leukemia (B-ALL). During FCI diagnosis of B-ALL, expression of certain non B-lineage antigens are of prognostic significance and provide clues towards underlying molecular-genetic aberrancies.¹ In this context, the cytogenetic and prognostic relevance associated with aberrant expression of myeloid lineage markers in B-ALL has been well documented.^{1,2}

English literature regarding aberrant expression of T-cell or natural killer (T/NK)-cell lineage antigens (i.e., CD1a, CD2, CD4, CD5, CD7, CD8 and CD56) in B-ALL patients dates back to 1989.^{2–13} Among this literature, only Seegmiller et al. and Hussein et al. have documented large cohorts (18 cases each) of T/NK cell antigen-expressing B-ALL patients, with only Hussein et al. having compared the clinical-laboratory profile of these patients against conventional B-ALL patients.^{2,3} Regarding these T/NK cell antigen-expressing B-ALL patients, individual antigen-wise clinical-hematologic profiles and their prognostic relevance is yet to be compared. In the current manuscript, we have documented our experience with CD1a, CD4, CD5, CD7, CD8 and CD56 antigen expression in patients with B-ALL and have compared the clinicopathologic relevance associated with aberrant expression of these individual antigens against each other.

Materials and methods

This study, in which all treatment-naïve B-ALL patients diagnosed between November 2017 and September 2019 were included, was conducted at a tertiary cancer care hospital. Patients in the age group of 1–14 years, 15–29 years and >30 years were considered as pediatric, adolescents-young adults (AYAs) and adults, respectively. Diagnosis of B-ALL was by morphologic assessment of Romanowsky-stained bone marrow aspiration and peripheral blood smears, followed by FCI. The diagnostic FCI panel (supplementary Table S1) and sample processing steps are as described in our previous publication.¹⁴ For the current study, surface expression of CD1a, CD4, CD7, CD5, CD8 and CD56 were evaluated on the leukemic B-lymphoblasts identified by sequential gating strategy. To identify the expression of our antigens of interest, normal B, T and NK lymphocytes within each corresponding sample served as internal controls. Patients with $\geq 20\%$ leukemic blasts expressing an antigen of interest were considered positive for that specific antigen.^{2,3} Patients without surface CD10 expression on blasts were classified as ‘pro

B-ALL’ immunophenotype and the remaining patients were categorized as ‘other precursor B-ALL’ immunophenotype.

Cytogenetic risk profiling was by conventional banding cytogenetics and interphase fluorescence *in situ* hybridization (i-FISH) to identify BCR-ABL1 fusion, ETV6-RUNX1 fusion, E2A-PBX1 fusion and KMT2A rearrangement. High-risk cytogenetics was defined by the presence of either BCR-ABL1 fusion, KMT2A rearrangement, hypo-diploidy or complex karyotype defined by the presence of ≥ 3 abnormalities including at least one structural abnormality.³ Clinical risk at diagnosis was assessed by the National Cancer Institute (NCI) criteria.³ Patients in the age group 1–18 years were treated with the Indian Childhood Collaborative Leukemia Group (ICiCle) protocol and patients over 18 years of age were treated with the Berlin-Frankfurt-Muenster 95 (BFM95) protocol. End induction (day 30) minimal residual disease (MRD) assessment was by FCI and samples with $\geq 0.01\%$ residual leukemic events were considered MRD positive.

The study was approved by the ethics committee of our Institute and was conducted adhering to the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Before treatment, informed consent was obtained from the patients (adults) or from parents or legal guardians (patients under 18 years of age).

Statistics

Statistical analysis was performed using Microsoft Excel 2016 and the statistical package for social sciences (version 23; IBM, Armonk, NY). For intergroup comparisons, the Fisher’s exact and Mann–Whitney U tests were used. With the date of disease diagnosis as a starting timepoint, Kaplan–Meier statistics was used to determine overall survival (OS) and event-free survival (EFS). Treatment induction failure ($\geq 5\%$ bone marrow blasts), disease relapse, or disease-related death were considered adverse events. The risk for an adverse event was determined by the Cox proportional hazard model (Wald test) using the following co-variables: NCI risk at diagnosis, cytogenetic risk, end-induction MRD status, CD7 expression and CD56 expression. All statistical tests were two-tailed and considered significant at a p -value ≤ 0.05 .

Results

During the study time frame, 152 treatment-naïve B-ALL patients were diagnosed at our Institute (Table 1). Among these patients, 14 opted against treatment, 3 died during the induction phase of treatment and the remaining 135 patients were tested for end-induction MRD. Among the 139

Table 1 – Clinical and laboratory profile of our B-ALL patients, in respect to their CD56 and CD7 expression status.

B-ALL patient category	All patients (n = 152)	CD56 expression		CD7 expression		P-value		
		CD56- (n = 143)	CD56+ (n = 9)	CD7- (n = 146)	CD7+ (n = 6)	CD56+ vs CD56-	CD7+ vs CD7-	CD56+ vs CD7+
Median age (range) in years	10 (0.2–64)	10 (0.2–64)	7 (2–62)	10 (0.2–64)	20 (3–50)	0.336	0.086	0.088
Age Group								
Pediatric	94	86	8	93	1			
AYA	32	32	0	29	3	0.188	0.021	0.014
Adult	26	25	1	24	2			
Sex (Male: Female)	1.8:1	1.6:1	2:1	1.7:1	2:1	0.822	0.856	1.00
Pro B-ALL (%)	8 (5)	7 (5)	1 (11)	7 (5)	1 (17)	0.394	0.202	0.756
Median (range) Hb in g/L	73 (22–149)	73(25–149)	67 (22–90)	73 (22–149)	71 (5–10)	0.336	0.921	0.388
Median(range) WBC count X10 ⁹ /L	10.3 (0.3–587)	10.3 (0.3–586)	6.8 (3.3–26.4)	10.2 (.3–587)	21.2 (3.5–175)	0.571	0.241	0.181
Median (range) Platelet X10 ⁹ /L	50 (10–563)	50 (10–563)	50 (20–457)	50 (10–563)	38 (15–71)	0.726	0.244	0.224
Median (range) BM blast %	89 (55–99)	89(78–94)	85(66–97)	89 (55–99)	82 (66–98)	0.926	0.869	0.864
Median (range) PB blast %	49 (4–98)	50(11–84)	41(4–88)	48 (6–98)	86 (5–98)	0.732	0.144	0.142
Splenomegaly (%)	67/152 (44)	63/143 (44)	4/9 (44)	66/146 (45)	2/6 (33)	0.501	0.567	0.398
Hepatomegaly (%)	56/152 (37)	52/143 (36)	4/9 (44)	55/146 (38)	1/6 (17)	0.626	0.296	0.246
Lymphadenopathy (%)	45/152 (30)	41/143 (28)	4/9 (44)	43/146 (30)	2/6 (33)	0.315	0.838	0.667
CNS involvement at diagnosis (%)	5/138 (4)	5/129 (4)	0/9 (0)	5/131 (4)	0/0 (0)	0.569	0.626	NA
Ploidy (%)								
Diploid	84/124 (68)	75/115 (65)	9/9 (100)	78/118 (66)	6/6 (100)			
High Hyperdiploid	18/124 (14)	18/115 (16)	0/9 (0)	18/118 (15)	0/6 (0)			
Low Hyperdiploid	14/124 (11)	14/115 (12)	0/9 (0)	14/118 (12)	0/6 (0)			
High Hypodiploid	04/124 (3)	4/115 (3.5)	0/9 (0)	4/118 (3)	0/6 (0)	0.728	0.864	NA
Low Hypodiploid	01/124 (1)	1/115 (1)	0/9 (0)	1/118 (1)	0/6 (0)			
Near Triploid	01/124 (1)	1/115 (1)	0/9 (0)	1/118 (1)	0/6 (0)			
Near Tetraploid	02/124 (2)	2/115 (2)	0/9 (0)	2/118 (2)	0/6 (0)			
NCI High-risk disease (%)	95/152 (62.5)	93/143 (65)	2/9 (22)	90/146 (62)	5/6 (83)	0.010	0.282	0.020
BCR-ABL1 fusion positive (%)	16/133 (12)	16/124 (13)	0/9(0)	13/127 (10)	3/6 (40)	0.278	0.004	0.024
ETV6-RUNX1 fusion positive (%)	14/133 (10.5)	11/124 (9)	3/9 (33)	13/127 (10)	1/6 (17)	0.021	0.616	0.475
KMT2A rearranged (%)	04/133 (3)	04/124 (3)	0/9 (0)	03/127 (2)	1/6 (17)	0.584	0.045	0.205
TCF3-PBX1 fusion positive (%)	05/133 (4)	05/124 (4)	0/9 (0)	05/127 (4)	0/6 (0)	0.539	0.620	NA
High Risk cytogenetics (%)	21/133 (16)	21/124 (17)	0/9 (0)	17/127 (13)	4/6 (67)	.202	0.001	0.006
End induction MRD positive (%)	56/135 (41)	53/127 (42)	3/8 (37.5)	52/129 (40)	4/6 (67)	0.814	0.200	0.280
Induction failure (%)	05/135 (4)	5/127 (4)	0/8 (0)	4/129 (3)	1/6 (17)	0.568	0.061	0.205
Relapse (%)	13/135 (10)	13/127 (10)	0/8 (0)	12/129 (9)	1/6 (17)	0.344	0.468	0.205
Adverse events (%)	25/138 (18)	25/130 (19)	0/8 (0)	22/129 (17)	3/6 (50)	0.170	0.024	0.018

AYA: Adolescent and young adult; B-ALL: B lineage acute lymphoblastic leukemia; BM: Bone marrow; CNS: the central nervous system; Hb: Hemoglobin; MRD: Minimal residual disease; NA: Not applicable; NCI: National cancer institute; PB: Peripheral blood; WBC: White blood cell.

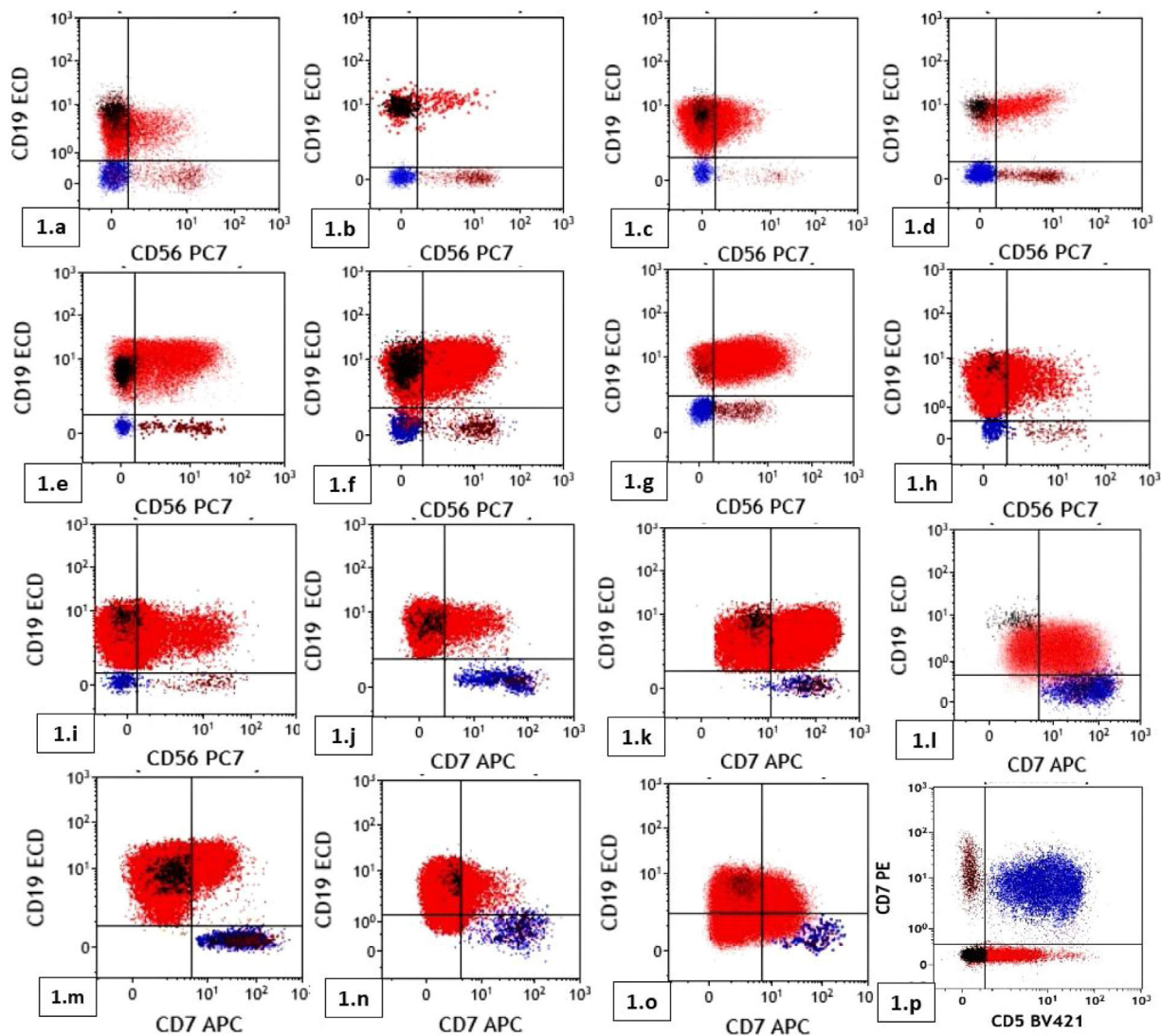


Figure 1 – Flow cytometric dot plots demonstrating precursor B-Lineage acute lymphoblastic leukemia patients with aberrant CD56 expression (1.a to 1.i), CD7 expression (1.j to 1.o) and CD5 expression (1.p). Red dots: leukemic blasts, Blue dots: T-lymphocytes, Black dots: B-lymphocytes and Brown dots: T/NK cells.

patients for whom conventional karyotyping was performed, metaphases were available for 124 patients. Interphase FISH for BCR-ABL1, ETV6-RUNX1 & TCF3-PBX1 fusions and KMT2A rearrangement were tested in 133 patients. The mean available follow-up had the duration of 10 months, ranging from 1 month to 22 months.

Among these 152 patients, aberrant expression of CD5, CD7 and CD56 was seen in 1 (0.7%), 6 (4%) and 9 (6%) patients, respectively (Figure 1 and Table 1). None of our patients had the expression of CD4, CD8, CD1a or the co-expression of any of the markers under study. The clinical-hematologic profile of these individual patients (n=16) with aberrant T/NK cell antigen expression is elucidated in Supplementary Table S2.

CD56+ B-ALL patients

Among CD56-expressing B-ALL patients (CD56+ B-ALL), the median age at diagnosis was 7 years, ranging from 2 to 62,

with only one adult patient. Clinicopathologic overview of CD56+ and CD56- B-ALL patients is elucidated in Table 1. The majority of our CD56+ B-ALL patients were diagnosed with NCI standard-risk disease ($p=0.010$) and had a higher frequency of ETV6-RUNX1 fusion ($p=0.021$) (Table 1). There was no significant difference in the OS ($p=0.349$) and EFS ($p=0.114$) between these two patient groups.

CD7+ B-ALL patients

The median age of our CD7+ B-ALL patients was 20 years, ranging from 3 to 50, with only one pediatric patient. These CD7+ B-ALL patients had a significantly higher frequency of BCR-ABL1 fusion ($p=0.004$), KMT2A rearrangement ($p=0.045$), high-risk cytogenetics ($p=0.001$) and adverse events ($p=0.024$). The Clinical-hematologic profile of CD7+ and CD7- B-ALL patients is summarized in Table 1. There was no

difference in the OS ($p = 0.648$) and EFS ($p = 0.187$) of B-ALL patients, in respect to CD7 expression.

CD56+ B-ALL vs CD7+ B-ALL patients

Compared to CD56+ B-ALL patients, CD7+ B-ALL patients were predominantly of AYA and adult age (11% vs 83%, $p = 0.014$), had more NCI high-risk disease (22% vs 83%, $p = 0.020$), high-risk cytogenetics (0% vs 67%, $p = 0.006$), frequent BCR-ABL1 fusion (0% vs 40%, $p = 0.024$) and were adverse events-prone (0% vs 50%, $p = 0.018$) (Table 1). Though CD7+ B-ALL patients fared poor EFS ($p = 0.040$) in comparison to the CD56+ B-ALL patients, there was no difference in OS ($p = 0.317$) between the two patient groups.

The risk for adverse event

On univariate analysis by the Cox proportional hazard model, patients with high-risk disease ($p = 0.042$, HR:1.663, 95% CI:1.017–2.718) and those with end-induction MRD positivity ($p = 0.003$, HR:2.137, 95% CI:1.293–3.533) had higher risk for an adverse event, whereas high-risk cytogenetics ($p = 0.857$, HR:1.051, 95% CI:0.611–1.808), CD7-expression ($p = 0.120$, HR:0.618, 95% CI: 0.337–1.134) or CD56-expression ($p = 0.322$, HR:0.043, 95% CI: 0.021–0.885) did not translate into increased risk for adverse events. On multivariate analysis, only end-induction MRD positivity was associated with a high risk for adverse events ($p = 0.005$, HR:2.076, 95% CI:1.254–3.438).

Discussion

Among 152 consecutive B-ALL patients, 16 (10.5%) had an aberrant expression of T/NK cell antigens under study, which is comparable to the experiences documented by Seegmiller et al. (9%) and Hussein et al. (13.4%).^{2,3} Similar to Seegmiller et al., CD56 was the most commonly encountered aberrant T/NK cell antigen in our B-ALL patients (6%), followed by CD7 expression seen in 4% of the patients.² In concordance with both Seegmiller et al. and Hussein et al., none of our patients had CD8 expression.^{2,3} However, in contrast to the above-mentioned studies, CD4 was not expressed in any of our B-ALL patients.

Our experience with B-ALL patients showing aberrant T/NK cell antigen expression was compared with English literature, in which more than 50 B-ALL patients have been evaluated using a common definition to determine antigen expression.

The frequency of CD56+ B-ALL patients in our cohort (6%) is concordant with the frequency of such cases reported in the literature and the pediatric skewness of our CD56+ B-ALL patients (89%) is supported by Seegmiller et al. (Table 2).² Regarding cytogenetic abnormalities, all of our CD56+ B-ALL patients were diploid and none had an adverse cytogenetic risk profile. The presence of higher ETV6-RUNX1 fusion positivity in 33% ($p = 0.021$) of our CD56+ B-ALL patients is well supported by the presence of 27% ($p = 0.002$) ETV6-RUNX1 fusion positivity observed by Seegmiller et al.² Though Aref et al. have documented BCR-ABL1 fusion positivity in 57% of their CD56+ B-ALL patients, none of our patients were BCR-ABL1 fusion-positive.⁴ This might be due to differences in

the age group of B-ALL patients being evaluated, with our cohort being pediatric-enriched, while Aref et al. evaluated only AYA/adult B-ALL patients.⁴

Among acute lymphoblastic leukemia patients, CD56 expression has been associated with higher induction failure, CNS infiltration at diagnosis and frequent CNS relapse.^{15,16} However, these studies have not made a distinction between B-ALL and T-ALL patients and have analyzed both diseases under the blanket category of acute lymphoblastic leukemia. Among studies that have categorically analyzed CD56 expression in B-ALL patients, only Aref et al. have evaluated for the presence of leukemic CNS infiltration.⁴ In their cohort of 70 AYA/Adult B-ALL patients, 6 of 7 (87%) CD56+ B-ALL patients had CNS infiltration at diagnosis. However, in our pediatric-dominant CD56+ B-ALL patients, none had CNS leukemic infiltration. This age-related propensity for leukemic CNS infiltration in CD56+ B-ALL patients has to be further investigated in larger cohorts.

To our knowledge, only 28 CD56+ B-ALL patients have been documented in the literature, with treatment response and disease outcome details being available for only 16 patients.¹⁷ Of these 16 patients, 50% had adverse events in the form of induction death in three, induction failure in two, relapse in one and two never having achieved remission.^{3,4} In contrast to these patients, none of our CD56+ B-ALL patient experienced an adverse event. This might be due to the differences in the treatment protocols used and the relatively short follow-up available in our study (mean follow-up of 10 months, ranging from 1 month to 22 months).

In the current study, the expression of CD56 did not have any detrimental effect on the OS ($p = 0.349$) and EFS ($p = 0.133$) of B-ALL patients. This is in contrast to both Aref et al. and Hussein et al., who have documented poor overall, event-free and relapse-free survival in CD56+ B-ALL patients.^{3,4} This incongruity could be due to a lesser number of CD56+ B-ALL patients documented by Hussein et al. (only three patients) and only adult patients being analyzed by Aref et al.

In our experience, CD56-expressing B-ALL patients were significantly associated with good risk features, such as favorable age at diagnosis (89% were pediatric), frequent ETV6-RUNX1 fusion positivity (33%) and absence of a high-risk cytogenetic profile. As compared to conventional B-ALL patients, CD56-expressing B-ALL patients did not experience any inferior EFS ($p = 0.114$) or OS ($p = 0.349$).

In contrast to conventional B-ALL patients, our CD7+B-ALL patients were predominantly AYA/Adults ($p = 0.021$), having a high-risk cytogenetic profile ($p = 0.001$) in the form of frequent BCR-ABL1 fusion positivity (10% vs 40%, $p = 0.004$) & KMT2A rearrangement (2% vs 17%, $p = 0.045$) and had encountered frequent adverse events (15% vs 50%, $p = 0.024$). Though statistically insignificant ($p = 0.061$), our CD7+ B-ALL patients also had higher frequency of treatment induction failure than CD7-B-ALL patients (3% vs 17%).

Our current understanding of CD7 expression in B-lymphoblasts is limited to 10 such patients, as documented in the literature.^{2,3} Through our manuscript, we have elucidated the clinical-laboratory profile of one of the largest CD7+ B-ALL cohorts ($n = 6$) available to date. The frequency of CD7+ B-ALL patients documented in our cohort (4%) is closely similar to the observation by Hussein et al. (4.4%), but is double the fre-

Table 2 – Comparison between our CD56+ B-ALL patients and cases reported in major works of literature.

Literature	Current	Hussein et al. ³	Seegmiller et al. ²	Aref et al. ⁴
Age group evaluated	All	All	All	AYA and Adult
Year	2019	2011	2009	2017
Country	India	USA	USA	Egypt
B-ALL patients evaluated	152	134	197	70
CD56+ patients (%)	9 (6)	3 (2.2)	10 (5)	7 (10)
Median age (Range) in years	7(2–62)	16 (13–16)	NA	NA (28–50)
Ped	8	01	09	00
Age Group (n)				
AYA	0	02	00	07 ^c
Adult	1	00	01	
Sex (Male: Female)	2:1	2:1	NA	1.3:1
Median (range) Hb in g/L	67(22–90)	NA	NA	77 (68–106)
Median(range) WBC count X10 ⁹ /L	6.8 (3.3–26.4)	NA	NA	18.3 (1.7–113)
Median (range) Platelet X10 ⁹ /L	50 (20–457)	NA	NA	140 (26–397)
Median (range) BM blast %	85(66–97)	NA	NA	80 (55–93)
Median (range) PB blast %	41(4–88)	NA	NA	90 (80–90)
Splenomegaly (%)	4/9 (44)	NA	NA	2/7 (29)
Hepatomegaly (%)	4/9 (44)	NA	NA	2/7 (29)
Lymphadenopathy (%)	4/9 (44)	NA	NA	4/7 (57)
CNS disease at baseline (%)	0/9 (0)	NA	NA	6/7 (87)
Diploidy (%)	9/9 (100)	NA	NA	NA
High-Risk cytogenetics (%)	0/9 (0)	1 ^a /3 (33)	NA	4/7 (57)
BCR-ABL1 fusion-positive (%)	0/9(0)	0/3	NA	4/7 (57)
ETV6-RUNX1 fusion-positive (%)	3/9 (33)	0/3	(27)	NA
KMT2A rearranged (%)	0/9 (0)	0/3	NA	NA
TCF3-PBX1 fusion-positive (%)	0/9 (0)	0/3	NA	NA
End induction MRD-positive (%)	3/8 (37.5)	NA	NA	NA
Induction failure (%)	0/8 (0)	1/3 (33)	NA	2/7 (29)
Induction death (%)	0/8 (0)	0/3 (0)	NA	3/7 (43)
Relapse (%)	0/8 (0)	0/1 ^b (0)	NA	NA

AYA: Adolescent and young adult; B-ALL: B-lineage acute lymphoblastic leukemia; BM: Bone marrow; CNS: the central nervous system; Hb: Hemoglobin; PB: Peripheral blood; MRD: Minimal residual disease; NA: Not available; WBC: White blood cell.

^a TP53 deleted.

^b Data of one patient as One patient left against medical advice and another had induction failure and was also treatment-refractory.

^c Distinction between AYA and Adults not available.

quency documented by Seegmiller et al. (2%).^{2,3} The AYA/adult age predominance (83%) of our CD7+ B-ALL patients is concordant with the observations by Seegmiller et al. (75%), but is strikingly discordant with Hussein et al. (17%), who have documented the maximum number of CD7+ B-ALL patients to date (Table 3).

Similar to Hussein et al., 67% of our CD7+ B-ALL patients had high-risk cytogenetics, however, we document a 40% incidence of BCR-ABL1 fusion positivity, which was not observed in any of the CD7+ B-ALL patients studied by Hussein et al.³ This might be due to the gross difference in the age at which CD7+ B-ALL patients were diagnosed in our study (83% were AYA/adult), as compared to the study by Hussein et al., in which 83% of the patients were pediatric. Moreover, KMT2A rearrangement was observed in 50% of the patients studied by Hussein et al. and was present in only 17% of our CD7+ B-ALL patients. We also observed a higher frequency (67%) of end-induction MRD positivity, which is a harbinger of relapse in B-ALL patients.

Though we report multiple adverse prognostic factors in our CD7+ B-ALL patients, there was no significant decline in the OS ($p = 0.648$) and EFS ($p = 0.104$) of these patients, as compared to CD7- B-ALL patients. This might be due to the short follow-up available in our study.

The univariate Cox regression analysis in the study by Hussein et al. documented the presence of NK/T cell antigen expression ($p = 0.0102$, HR:3.249, 95% CI:1.445–7.305), high-risk disease ($p = 0.0002$, HR:3.274, 95% CI:1.700–6.305) and high-risk cytogenetics ($p = 0.0002$, HR:3.146, 95% CI:1.717–5.767) as significant factors associated with increased risk for disease relapse. However, their multivariate Cox regression identified T/NK cell antigen expression as an independent predictor for disease relapse in B-ALL patients.

On univariate analysis of our cohort, irrespective of CD7 ($p = 0.120$, HR:0.618, 95% CI:0.337–1.134) or CD56 ($p = 0.322$, HR:0.043, 95% CI:0.0214–21.885) expression, the presence of high-risk disease at diagnosis ($p = 0.042$, HR:1.663, 95% CI:1.017–2.718) and end-induction MRD-positive status ($p = 0.003$, HR:2.137, 95% CI:1.293–3.533) were associated with a higher risk for an adverse event. The discordance of our analysis with Hussein et al. might be due to the lack of a longer follow-up in our study.

In our cohort, aberrant CD5 expression was present in only one pediatric B-ALL patient (supplement Table S1) and to date, only seventeen CD5-expressing B-ALL patients have been documented in the literature. Of these patients, 38% were pediatric, 46% were AYA and only 15% were adults.^{3,18–22} Among the 12 patients for whom cytogenetic data were avail-

Table 3 – Comparison between our CD7+ B-ALL patients and cases reported in major literature.

Literature		Current	Hussein et al. ³	Seegmiller et al. ²
Age group evaluated		All	All	All
Year		2019	2011	2009
Country		India	USA	USA
B-ALL patients evaluated		152	134	197
CD7+ patients (%)		6 (4)	6 (4.4)	4 (2)
Median age (Range) in years		20 (3–50)	5.4 (0.4–25)	NA
Age Group (n)	Ped	1	05	01
	AYA	3	01	
	Adult	2	00	03
Sex (Male: Female)		2:1	1:2	NA
Median (range) Hb in g/L		71 (5–10)	NA	NA
Median(range) WBC count X10 ⁹ /L		21.2 (3.5–175)	NA	NA
Median (range) Platelet X10 ⁹ /L		38 (15–71)	NA	NA
Median (range) BM blast %		82 (66–98)	NA	NA
Median (range) PB blast %		86 (5–98)	NA	NA
Splenomegaly (%)		2/6 (33)	NA	NA
Hepatomegaly (%)		1/6 (17)	NA	NA
Lymphadenopathy (%)		2/6 (33)	NA	NA
CNS disease at diagnosis		0/0 (0)	NA	NA
Diploidy (%)		6/6 (100)	NA	
High-Risk cytogenetics (%)		4/6 (67)	4/6 ^a (67)	NA
BCR-ABL1 fusion-positive (%)		3/6 (40)	0/6 (0%)	NA
ETV6-RUNX1 fusion-positive (%)		1/6 (17)	0/6 (0%)	NA
KMT2A rearranged (%)		1/6 (17)	3/6 (50%)	NA
TCF3-PBX1 fusion-positive (%)		0/6 (0)	0/6 (0%)	NA
End induction MRD-positive (%)		4/6 (67)	NA	NA
Induction failure (%)		1/6 (17)	0/6	NA
Induction death (%)		0/6 (0)	0/6 (0)	NA
Relapse (%)		1/6 (17)	4/6 (67)	NA

AYA: Adolescent and young adult; B-ALL: B-lineage acute lymphoblastic leukemia; BM: Bone marrow; CNS: Central nervous system; Hb: Hemoglobin; PB: Peripheral blood; MRD: Minimal residual disease; WBC: White blood cell; NA: Not available.

^a One patient had CDKN2A deletion.

able, 50% had high-risk cytogenetics in the form of complex karyotype (n=4), BCR-ABL1 fusion (n=1) and hypo-diploidy (n=1). Though 83% (10 of 12) of these patients had disease remission at the end of induction, the follow-up data available for 6 patients showed disease relapse in all. Even if the available literature portends a poor prognosis in CD5+ B-ALL patients, the paucity of comprehensive data prevents concrete conclusions.

Conclusion

In the current study, we have analyzed the clinical-pathologic profile of CD56- and CD7-expressing B-ALL patients and compared our experiences with the major literature. In our experience, CD56 expression is predominantly seen in pediatric B-ALL patients and is associated with significant ETV6-RUNX1 fusion positivity. On the contrary, CD7 expression is predominantly seen in AYA/adult B-ALL patients and is associated with adverse cytogenetics (especially BCR-ABL1 fusion) and is marred by frequent adverse events.

Limitations

Apart from the short follow-up, CD2 expression was also not analyzed in our patients. The distinction between 'common B-ALL' and 'pre-B-ALL' could not be made, as our acute

leukemia diagnosis panel does not include antibodies against kappa, lambda and cytoplasmic μ heavy chain. Hence, all CD10-positive B-ALL patients were classified under the 'other precursor B-ALL' category. As our single-tube 10-color MRD panel does not include CD56 and CD7, the stability of these antigens in MRD-positive cases could not be evaluated.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We would like to acknowledge Mrs. Arcot Radhakrishnan Abitha (senior scientific assistant) and Miss. Parkavi J (research assistant) for their efforts in flow cytometry sample processing and acquisition.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.htct.2020.08.012>.

REFERENCES

- Hrušák O, Porwit-MacDonald A. Antigen expression patterns reflecting genotype of acute leukemias. *Leukemia*. 2002;16(7):1233.
- Seegmiller AC, Kroft SH, Karandikar NJ, McKenna RW. Characterization of immunophenotypic aberrancies in 200 cases of B acute lymphoblastic leukemia. *Am J Clin Pathol*. 2009;132(6):940–9.
- Hussein S, Gill KZ, Sireci AN, Colovai AI, Small T, Emmons FN, et al. Aberrant T-cell antigen expression in B lymphoblastic leukaemia. *Br J Haematol*. 2011;155(4):449–56.
- Aref S, Azmy E, El-Bakry K, Ibrahim L, Mabed M. Prognostic impact of CD200 and CD56 expression in adult acute lymphoblastic leukemia patients. *Hematology*. 2018;23(5):263–70.
- Paietta E, Neuberg D, Richards S, Bennett JM, Han L, Racevskis J, et al. Rare adult acute lymphocytic leukemia with CD56 expression in the ECOG experience shows unexpected phenotypic and genotypic heterogeneity. *Am J Hematol*. 2001;66(3):189–96.
- Abdulateef N, Ismail MM, Aljedani H. Clinical significance of co-expression of aberrant antigens in acute leukemia: a retrospective cohort study in Makah Al Mukaramah, Saudi Arabia. *Asian Pac J Cancer Prev*. 2014;15(1):221–7.
- Alkayed K, Khattab E, Madanat F. Aberrant T-cell antigen expression in Jordanian children with B lymphoblastic leukemia. *Hematol Oncol Stem Cell Ther*. 2015;8(4):187–8.
- Chen W, Karandikar NJ, McKenna RW, Kroft SH. Stability of leukemia-associated immunophenotypes in precursor B-lymphoblastic leukemia/lymphoma: a single institution experience. *Am J Clin Pathol*. 2007;127(1):39–46.
- Bradstock K, Kirk J, Grimsley P, Kabral A, Hughes W. Unusual immunophenotypes in acute leukaemias: incidence and clinical correlations. *Br J Haematol*. 1989;72(4):512–8.
- Nakase K, Kita K, Miwa H, Nishii K, Shiku H, Nasu K, et al. Expression pattern of hybrid phenotype in adult acute lymphoblastic leukemia. *Cancer Detect Prev*. 2001;25(4):394–405.
- Iwamoto S, Deguchi T, Ohta H, Kiyokawa N, Tsurusawa M, Yamada T, et al. Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/Lymphoma Study Group. *Int J Hematol*. 2011;94(2):185–92.
- Gao C, Liu S-G, Yue Z-X, Liu Y, Liang J, Li J, et al. Clinical-biological characteristics and treatment outcomes of pediatric pro-B ALL patients enrolled in BCH-2003 and CCLG-2008 protocol: a study of 121 Chinese children. *Cancer Cell Int*. 2019;19:293.
- Griesinger F, Piro-Noack M, Kaib N, Falk M, Renziehausen A, Troff C, et al. Leukaemia-associated immunophenotypes (LAIP) are observed in 90% of adult and childhood acute lymphoblastic leukaemia: detection in remission marrow predicts outcome. *Br J Haematol*. 1999;105(1):241–55.
- Bommannan BK, Arumugam JR, Sundersingh S, Rajan PT, Radhakrishnan V, Sagar TG. CD19 negative and dim precursor B-lineage acute lymphoblastic leukemias: real-world challenges in a targeted-immunotherapy era. *Leuk Lympho*. 2019:1–7.
- Hu W, Wang X, Yang R, Bi L, Xie Y, Zhang Z, et al. Expression of CD56 is a risk factor for acute lymphocytic leukemia with central nervous system involvement in adults. *Hematology*. 2017;22(2):81–7.
- Ravandi F, Cortes J, Estrov Z, Thomas D, Giles FJ, Huh YO, et al. CD56 expression predicts occurrence of CNS disease in acute lymphoblastic leukemia. *Leukemia Res*. 2002;26(7):643–9.
- Neff J, Chen D. Pediatric Philadelphia-positive B lymphoblastic leukemia with CD56 expression and L2 morphology: Case report and review of the literature. *Hum Pathol: Case Rep*. 2017;8:9–12.
- Staley EM, Feldman AZ, Koenig RG, Hill B. CD5 positive B-ALL, a uniquely aggressive subcategory of B-ALL? A case report and brief review of the literature. *Pediatr Blood Cancer*. 2019;66(1):e27484.
- Ahmed D, Ahmed TA, Ahmed S, Tipu HN, Wiqar MA. CD5-positive acute lymphoblastic leukemia. *J Coll Physicians Surg Pak*. 2008;18(5):310–1.
- Sreedharanunni S, Kumar N, Khadwal A. CD5 positive B lymphoblastic leukemia: report of a case with review of literature. *Indian J Hematol Blood Transfus*. 2016;32(1):1–4.
- Peterson MR, Noskoviak KJ, Newbury R. CD5-positive B-cell acute lymphoblastic leukemia. *Pediatr Dev Pathol*. 2007;10(1):41–5.
- Mutreja D, Pati HP, Bansal D, Sharma RK, Jain S. Aberrant immunophenotypic expression of CD5 in a case of B acute lymphoblastic leukemia: a case report. *Indian J Hematol Blood Trans*. 2014;30(1):212–4.