Aberrant myeloid antigen co-expression is correlated with high percentages of CD34-positive cells among blasts of acute lymphoblastic leukemia patients: an Indian tertiary care center perspective

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Background

Aberrant myeloid antigen (MA) co-expression and high expression of CD34 antigen on the blasts of acute lymphoblastic leukemia (ALL) patients are independently reported to have a role in pathogenesis and prognosis. This study was conducted to determine whether these two parameters are related.

Methods

A total of 204 cases of ALL were included in an analysis of blast immunophenotypic data. CD34 expression was categorized as low when less than 50% of blasts were CD34-positive (CD34^{low}) and as high when 50% or more were CD34-positive (CD34^{high}).

Results

Of 204 cases of ALL, 163 and 41 were of B-cell origin (B-ALL) and T-cell origin (T-ALL), respectively. Of all cases, 132 (64.7%) showed co-expression of MA and among these, 101 (76.51%) were CD34^{high}, while the remaining 31 (23.48%) were CD34^{low}. Of 72 cases without MA co-expression, 25 (34.72%) were CD34^{high} and 47 (67.25%) were CD34^{low}. Furthermore, of 163 cases of B-ALL, 111 showed co-expression of MA and 84 of these were CD34^{high}. Of 52 cases of B-ALL without MA expression, 22 were CD34^{high}. Among 41 cases of T-ALL, 21 co-expressed MA, 17 of which were CD34^{high}. Moreover, all 20 cases of T-ALL without co-expression of MA were CD34^{low}. These differences were statistically significant.

Conclusion

We observed a strong correlation between aberrant MA expression and CD34^{high} expression on the blasts of ALL. We hypothesize that these different patient subsets may represent unique prognostic characteristics.

Key Words   CD34, Acute lymphoblastic leukemia, Immunophenotyping, Aberrant myeloid antigen co-expression, Flow cytometry

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a hematological malignancy characterized by accumulation of immature progenitor cells in the bone marrow, peripheral blood, and, occasionally, the central nervous system [1]. Aberrant myeloid antigen (MA) expression in ALL has been reported to be highly variable, as detected by flow cytometry [2-4].

In adult ALL patients, 38% of B cell-lineage ALL (B-ALL) and 24% of T cell-lineage (T-ALL) cases have been shown to co-express CD13 and/or CD33 [4, 5].

Knowledge about the association of aberrant MA co-expression with adverse prognostic factors, clinical and hematologic features, and their prognostic significance is controversial in ALL [5, 6]. CD34 is expressed on the surface of immature hematopoietic progenitor cells that comprise 1−2% of all nucleated cells [7], and is involved in cellular

adhesion and mediates resistance to apoptosis. CD34 expression has been associated with poor prognosis in acute myeloid leukemia (AML) patients in many studies, and is possibly associated with increased drug resistance [7, 8].

Although aberrant MA and high CD34 expression have significant roles in the prognosis of ALL, their relationship is largely unknown. Thus, a broader understanding of the role of immunobiology in prognosis of ALL is needed. The aim of the present study was to determine whether high CD34 expression on blasts of ALL patients is associated with aberrant MA co-expression.

MATERIALS AND METHODS

Study design

This study was conducted in the Department of Hematology of a tertiary care center in India during the period of January 2011 to December 2012. Diagnosis of acute leukemia was based on morphological examination of bone marrow aspirate smears, including cytochemistry along with flow cytometric immunophenotyping. All the cases of ALL (B-ALL and T-ALL) were included in the study, while cases of AML and lymphomas with bone marrow spills were excluded from the study.

Flow cytometric immunophenotyping

Bone marrow aspirate samples were processed using a standard stain-lyse-wash method for flow cytometric immunophenotyping. The samples were processed within 24 h of collection. The monoclonal antibodies used were: CD45 (PerCP-Cy5.5), CD13 (PE), CD33 (APC), CD10 (PE-Cy7), CD19 (FITC), CD7 (FITC), CD117 (APC), CD34 (PE-Cy7), HLA-DR (PE), MPO (FITC), c CD79a (PE), c CD3 (PE-Cy7), Tdt (APC), CD64 (FITC) and CD11c (APC). All of these antibodies were purchased from BD Biosciences (San Jose, CA, USA). Initial incubation of monoclonal antibodies for 15–30 min at room temperature in the dark followed by lysis and washing of debris/unlysed RBCs were performed and samples were acquired using a BD-FACS Canto system. A control sample without monoclonal antibody was prepared and acquired in the same way. All analyses and interpretation were carried out using the FACS-Diva software (BD Biosciences). Aberrant MA expression was defined as ≥20% of blasts showing one or more of CD13, CD33, and CD117 antigens. CD34 expression was categorized as low when less than 50% of blasts were CD34-positive (CD34^{low}) and as high when 50% or more of blasts were CD34-positive (CD34^{high}). Differences between groups were analyzed by a two-tailed Fisher's exact test; P values of <0.05 were considered statistically significant.

Table 1. Demographic details of the different subsets of the patients analyzed.

Patient subgroup	Gender (N)		Age (Median, years) [Range]	TLC (Median) [Range]	Hb (Median) [Range]	Plt (Median) [Range]	Blast % (Median) [Range]
	M	F					
Total ALL	150	54	22 [2–58]	16,030 [4,050–52,160]	7.0 [3–13.5]	30,000 [10,000–160,000]	81 [22–98]
ALL with co-exp and CD34 ≥50%	76	25	22 [2–58]	10,500 [3,500–48,000]	7.1 [4–12.2]	38,000 [10,000–120,000]	76.5 [20–94]
ALL with co-exp and CD34 <50%	26	05	31 [4–59]	8,800 [5,500–70,000]	6.65 [3.2–11.3]	21,000 [14,000–160,000]	64.5 [28–87]
ALL without co-exp and CD34 ≥50%	11	14	22 [1–52]	20,300 [8,800–96,000]	7.6 [4.1–12.3]	39,000 [12,000–150,000]	83 [23–93]
ALL without co-exp and CD34 <50%	35	11	18.5 [2–60]	33,000 [10,000–67,000]	6.6 [4–11.6]	22,000 [18,000–170,000]	86 [24–88]
B-ALL with co-exp and CD34 ≥50%	61	23	22 [4–49]	5,500 [6,700–76,000]	5.7 [4.4–12.3]	25,500 [12,000–140,000]	67.5 [20–84]
B-ALL with co-exp and CD34 <50%	24	3	31 [6–53]	8,000 [4,400–46,000]	6.4 [4.5–11.3]	19,000 [8,000–90,000]	63.5 [25–97]
B-ALL without co-exp and CD34 ≥50%	10	15	22 [3–59]	20,300 [12,000–54,000]	7.6 [4.5–12.6]	39,000 [18,000–140,000]	83 [22–96]
B-ALL without co-exp and CD34 <50%	19	8	20 [2–58]	23,360 [4,400–58,000]	5.9 [5.6–11.3]	25,000 [10,000–110,000]	74 [25–93]
T-ALL with co-exp and CD34 ≥50%	15	02	22 [5–42]	132,850 [11,000–175,000]	7.4 [6.3–10.1]	30,000 [12,000–88,000]	85.5 [22–87]
T-ALL with co-exp and CD34 <50%	02	02	18.5 [7–56]	59,000 [8,000–96,000]	9.15 [3–10.4]	87,500 [12,000–160,000]	70 [24–96]
T-ALL without co-exp and CD34 ≥50%	-	-	-	-	-	-	-
T-ALL without co-exp and CD34 <50%	16	03	16 [4–49]	67,910 [4,500–110,000]	8.0 [4.1–11.2]	19,000 [10,000–78,000]	89 [26–88]

Abbreviations: Hb, hemoglobin; TLC, total leukocyte count; Plt, platelet.

Table 2. Aberrant myeloid antigen co-expression and its correlation with blast CD34 percentage.

Patients subgroup	CD34 at ≥50% level [CD34^{high}]	CD34 at <50% level [CD34^{low}]	P
A.			0.0001
· Total ALL patients with myeloid co-expression (N=132) (64.70%)	N=101 (76.51%)	N=31 (23.48%)	
· Total ALL patients without myeloid co-expression (N=72) (35.29%)	N=25 (34.72%)	N=47 (65.27%)	
B.			0.0001
· B-ALL with myeloid co-expression (N=111) (68.09%)	N=84 (75.67%)	N=27 (24.32%)	
· B-ALL without any myeloid co-expression (N=52) (31.90%)	N=22 (42.30%)	N=30 (57.69%)	
C.			0.0001
· T-ALL with myeloid co-expression (N=21)	N=17 (80.95%)	N=4 (19.04%)	
· T-ALL without myeloid co-expression (N=20)	N=0 (0.0%)	N=20 (100%)	

Abbreviations: ALL, acute lymphoblastic leukemia; T-ALL, T cell acute lymphoblastic leukemia; B-ALL, B cell acute lymphoblastic leukemia.
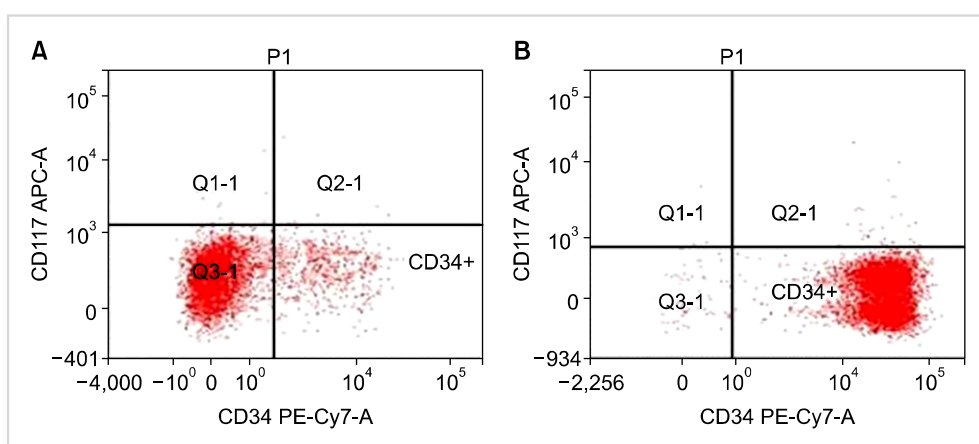

Fig. 1. Flow cytometric dot plots representing variable CD34 positivity in blasts of acute lymphoblastic leukemia. Dot plot (**A**) is representative of CD34^{low} and dot plot (**B**) is representative of CD34^{high}.

RESULTS

The present study included 204 cases, of which 156 were males and 48 were females, with a median age of 22 years (range, 2 to 61 years). Demographic details of all the patients are shown in Table 1. The most common symptoms were weakness, fatigue, and fever. Of the 204 ALL cases, 163 (79.90%) were diagnosed as B-ALL and 41 (20.09%) were diagnosed as T-ALL.

Total ALL patient group

Out of the total 204 ALL patients (Group A) recruited, 132 (64.70%) patients co-expressed one or more of the MA markers (CD13/CD33/CD117). The remaining 72 (35.29%) patients did not show any MA expression. Of the 132 cases of ALL with MA co-expression, 101 (76.51%) were CD34^{high} and 31 (23.48%) were CD34^{low}. Among the 72 patients lacking any MA co-expression, only 25 (34.72%) were CD34^{high} and 47 (65.27%) were CD34^{low}, and the difference between these groups was statistically significant ($P<0.001$; Table 2; Fig. 1, 2).

B-ALL patient subgroup

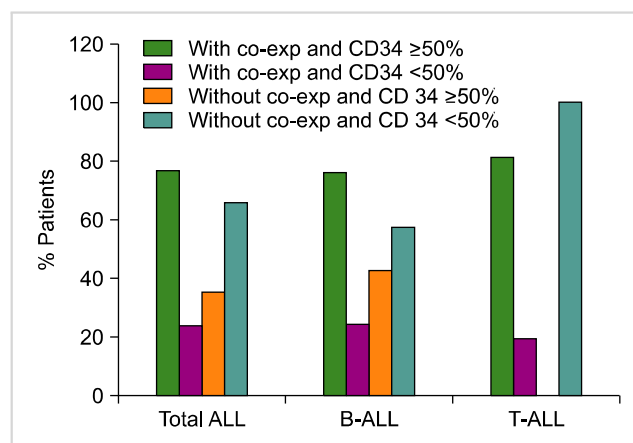On further analysis of the 163 cases of B-ALL (Group

Fig. 2. Frequencies of different patient subsets with respect to their association of myeloid antigen co-expression and CD34 percentage.

B), 111 (68.09%) showed aberrant MA co-expression, whereas 52 (31.90%) were MA-negative. Of 111 cases of B-ALL with MA co-expression, 84 (75.67%) were CD34^{high} and 27 (24.32%) were CD34^{low}.

Of 52 cases of B-ALL lacking any MA expression, 22 (42.30%) were CD34^{high} and 30 (57.69%) were CD34^{low}. High CD34 expression was more strongly associated with cases

of B-ALL that co-expressed MA than those without MA, and the difference was statistically significant (P=0.0001; Table 2, Fig. 2).

T-ALL patient subgroup

Of 41 cases of T-ALL (Group C), 21 (51.21%) showed co-expression of MA. Among these 21 cases, 17 (80.95%) were CD34^{high} and 4 (19.04%) were CD34^{low}. Intriguingly, none of the remaining 20 cases of T-ALL, which lacked MA co-expression, were CD34^{high}. CD34^{high} expression was associated with all cases of T-ALL with co-expression of MA, and the difference in this association between Group A and B was statistically significant (P=0.0001; Table 2, Fig. 2).

DISCUSSION

CD34 is expressed on the surface of immature hematopoietic progenitor cells and is involved in cellular adhesion and mediates resistance to apoptosis. Differential CD34 expression in the blasts of acute leukemia patients has been reported to be a significant prognostic indicator. High percentages of CD34-positive cells have been shown to correlate with poor prognosis in AML patients in several studies [7, 8]. AML blast resistance to apoptosis has been shown to positively correlate with the percentage of CD34 cells [8]. The cut-off percentage of CD34 for prognostic assessment is very controversial and ranges from 5 to 20% [9]. One study showed that AML patients expressing a high CD34 percentage (more than the cut-off value) had a 12-month relapse rate of 61%. However, the prognostic significance of CD34 expression in the blasts of ALL patients remains a matter of significant debate.

CD34 is usually expressed in the early phases of B-cell development, and low percentages of CD19, CD34 and CD10 positive cells in normal bone marrow are typical [10]. It is well known that most (around 70%) of the common B-ALL (CD10$^+$) cases are CD34-positive; however, a large percentage of phenotypically less mature pre B-ALL cases (CD10$^-$) are CD34-negative [11, 12]. In T-ALL, more than 40% of cases are CD34 positive, independent of the presence or absence of T-cell immaturity markers [13]. In our study, we found that among the blasts from ALL patients with MA expression, 76.51% were CD34^{high} (>50% level) and only 23.48% of patients were CD34^{low} (<50%), whereas among ALL patients with no detectable MA expression, 34.72% were CD34^{high} and 65.27% were CD34^{low}. In addition, by categorizing B- and T-ALL separately, these results were similar to that of total ALL patients, as the value of CD34 was consistently high for the MA-positive patient subgroup and was low for the MA-negative subgroup. The differences between all these subgroups of ALL patients were statistically significant (P<0.001) when analyzed by a two-tailed Fisher's exact test. Our data clearly indicate that there is a strong correlation between MA co-expression and CD34^{high} in the blasts of ALL patients.

The association of CD34 expression with specific genotypes in ALL has been extensively reported. B-ALL with t(1;19) (q23;p13) has been consistently demonstrated to be CD34-negative, while t(12;21) (p13;q22), adult B-ALL with t(9;22), and t(4;11) (q22;q23) are known to be CD34-positive [14-17].

The clinical relevance of CD34 expression in ALL is controversial as it has been reported to be associated with poor prognosis by some groups but not by others [18-20]. Interestingly, CD34 expression has also been reported to be associated with favorable prognostic markers such as low peripheral WBC counts, absence of central nervous system involvement, and hyperdiploidy. In multivariate analysis, CD34 positivity had an independent favorable prognostic effect on event-free survival [10]. In contrast to pediatric ALL, CD34 expression in adult ALL is associated with poor prognosis [10, 20]. Aberrant MA expression in ALL has been reported to exhibit variable frequency and differing outcomes among different study groups [2-4]. The same scenario exists for CD34 expression in blasts of ALL [15-17]. Although it is known that aberrant MA expression as well as the percentage of CD34-positive cells play significant prognostic roles in ALL, knowledge about their interrelatedness is almost nil. Therefore, in the present study, we explored this lacuna to attain a broader understanding of the pathobiology of ALL.

As reported by Pui *et al.* [18], Thomas *et al.* [20], and others indicating CD34 expression as an adverse prognostic marker in ALL, and similarly for aberrant MA co-expression by Drexler [2], Craddock *et al.* [21], and others, we hypothesize that ALL patients with MA co-expression and high CD34 expression may reflect a more aggressive form of ALL as compared to those with MA co-expression and low CD34 expression. Patients without MA co-expression who also have lower CD34 expression may represent a relatively good prognostic patient subset. Patients with either MA co-expression alone or with higher CD34 expression alone may represent an intermediate prognostic subset. However, as discussed above, several studies [4, 10, 17] have also found contradictory results with respect to correlations between these two parameters and clinical factors and prognosis; the prognostic spectrum of these patient subgroups may prove to be entirely different. Because the scope of our investigation did not include follow-up survival data for these patients, we could not provide any evidence in this regard and the question of long-term prognostic outcome remains to be addressed. Further studies that analyze clinical outcomes based on our prognostic algorithm could unveil a more advanced and efficient approach for prognostic assessment in ALL patients at earlier stages of the disease, which may also aid in clinical management. Since both of these factors are involved in the pathogenesis of ALL, further knowledge about their association may reveal information for immunobiology and prognosis of these patients more extensively and in a more efficient way.

In conclusion, we retrospectively analyzed the correlation between aberrant MA co-expression and the percentage of CD34-expressing cells among the blasts of ALL, and found

a positive correlation between them. We hypothesize that the ALL patients with/without AMA co-expression and with high/low CD34 co-expression may reflect different subsets of patients showing unique prognostic characteristics that remain to be further investigated for their long-term prognostic potential.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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