Application and prognostic relevance of CD34 detection in immunophenotyping of pediatric acute B lymphoblastic leukemia

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Abstract. – OBJECTIVE: We aimed to investigate the application of CD34 detection in immunophenotypic discrimination and its prognostic relevance in children with acute B-lymphoblastic leukemia (B-ALL).

PATIENTS AND METHODS: A retrospective analysis was conducted on clinical follow-up data of 105 children with newly diagnosed B-ALL treated at our hospital from January 2022 to December 2023. Based on the expression of CD34 in the bone marrow, patients were divided into a CD34 positive group (positive cells ≥10%) and a CD34 negative group (positive cells <10%). The study compared the positive rates of common leukemia cell antigens, clinical characteristics, initial treatment responses, and long-term follow-up outcomes between the two groups.

RESULTS: Among all 105 B-ALL cases, 87 children (82.9%) had bone marrow CD34 positive cells ≥10%, classified into the CD34 positive group, while the remaining 18 children (17.1%) had bone marrow CD34 positive cells <10%, classified into the CD34 negative group. The CD34 positive group exhibited significantly higher positive rates of CD13 expression, standard-risk B-ALL, and risk stratification than the CD34 negative group. In contrast, the proportions of early pre-B-ALL, *E2A-PBX1* fusion gene, and *MLL-AF4* fusion gene were significantly lower in the CD34 negative group, with statistically significant differences ($p<0.05$). No significant differences were found in the positive rates of leukemia cell antigens such as CD10, CD19, CD20, CD22, CD79a, CD13, CD33, and CD38 between the two groups ($p>0.05$). The occurrence rates of minimal residual disease (MRD) and relapse after induction chemotherapy in the CD34 positive group were significantly lower than those in the CD34 negative group ($p<0.05$). However, the sensitivity to the first prednisone treatment and bone marrow treatment efficacy on the 19th and 33rd days after chemotherapy showed no significant differences between the groups ($p>0.05$).

CONCLUSIONS: A higher positive rate of bone marrow CD34 expression in children with B-ALL is associated with a favorable prognosis. Children with negative CD34 expression are relatively more prone to MRD and tumor relapse after chemotherapy.

Key Words:
    CD34, Acute B-lymphoblastic leukemia, Children, Immunophenotyping, Prognosis.

Introduction

Acute B-lymphoblastic leukemia (B-ALL) is a malignant neoplastic disease originating from the abnormal proliferation of B-cell lineage lymphocytes in the bone marrow, predominantly occurring in children under the age of 9 years. B-ALL is characterized by the excessive proliferation of abnormal primitive and immature cells in the bone marrow that suppress normal hematopoiesis, leading to bleeding, anemia, infections, and infiltration of organs such as the spleen, liver, and lymph nodes. Currently, acute leukemia is recognized for its high heterogeneity, with a complex immunophenotype. The detection of various monoclonal antibodies can more accurately identify the immunological characteristics of leukemia cells, aiding in the clinical assessment of the condition and prognosis. This has been a research focus in recent years[1-7]. CD34, a highly glycosylated type I transmembrane glycoprotein primarily expressed on the surface of hematopoietic stem/progenitor cells, diminishes and disappears as the cells mature. Recent studies[8-12] have found that CD34 plays a crucial role in mediating cell adhesion, participating in the transport and

colonization of hematopoietic stem cells, and in the homing of lymphocytes. However, there are few reports on its correlation with the prognosis of B-ALL patients. Therefore, this study aims to investigate the expression level of CD34 in the bone marrow of children with B-ALL, focusing on its value in immunophenotypic identification and its correlation with prognosis.

Patients and Methods

General Information

A total of 105 children with B-ALL who were diagnosed and initially treated at our hospital from January 2022 to December 2023 were involved in the study.

Inclusion criteria were: (1) patients diagnosed with B-ALL based on routine blood tests, genetic testing, bone marrow morphology, chromosomes, and cellular immunophenotyping; (2) age at diagnosis ranged from 3 months to 15 years; (3) all had complete bone marrow biopsy, with clinical and follow-up data that were comprehensive and reliable. Exclusion criteria were: (1) patients who did not complete a full course of induction chemotherapy and were unable to undergo an efficacy evaluation for induction remission; (2) those who had received chemotherapy or other anti-leukemia treatments before their first visit to our hospital; (3) those with severe primary diseases or malignant tumors in other major organs.

The study has been approved by the Ethics Committee of Anhui Children's Hospital, with the approval number EYLL-2022-018 (approval date 23.02.2022). All research activities were conducted in accordance with the ethical guidelines of China and international ethical standards. All participants involved in this study have provided their written informed consent. Before collecting any personal data, we fully explained the purpose of the study, procedures, potential risks, and benefits to the participants, ensuring their participation was entirely voluntary and that they had the right to withdraw at any time.

Methods

Bone marrow cellular immunophenotype detection

All children underwent routine bone marrow aspiration, and the immunophenotype of lymphoblastic leukemia cells in the bone marrow was detected using flow cytometry. This included B-lymphocyte antigens such as CD10, CD19, CD20, CD22, cCD79a, myeloid antigens such as CD13, CD33, and nonspecific antigens like CD34, CD38. All antibodies were purchased from BD Biosciences (Franklin Lakes, NJ, USA). Children with bone marrow CD34 positive cells $\geq 10\%$ were classified as positive and grouped into the CD34 positive group, while the rest were categorized into the CD34 negative group.

Data collection, treatment, and follow-up

Data on all children's gender, age at diagnosis, subtypes of B-ALL, bone marrow cell fusion genes (including *BCR-ABL, MLL-AF4, E2A-PBX1, TEL-AML1*, etc.), presence or absence of central nervous system infiltration, white blood cell count at diagnosis, and risk stratification were collected. All children were diagnosed with B-ALL for the first time after completing relevant examinations and tests. They initially received prednisone monotherapy for one week at a dose of 60 mg/(m²·d). On the 8th day, prednisone sensitivity was assessed based on the absolute count of immature cells in the peripheral blood. Subsequently, the VDLD regimen (vincristine + daunorubicin + L-asparaginase + dexamethasone) was used for induction remission chemotherapy. Bone marrow status was re-examined on the 19th and 33rd days after chemotherapy to assess early treatment efficacy, including complete remission (CR), partial remission (PR), and non-remission (NR). Additionally, on the 19th day after chemotherapy, flow cytometry was used to determine the presence or absence of minimal residual disease (MRD) in the bone marrow, and subsequent treatment plans were formulated based on the condition and treatment response. All children were followed up regularly until December 31, 2023, comparing the relapse and mortality rates after chemotherapy between the two groups.

Statistical Analysis

SPSS 26.0 statistical software (IBM Corp., Armonk, NY, USA) was used to analyze all study data. Qualitative data were presented as the number of children (n) and percentage (%). For ordinal data (e.g., risk stratification), the Wilcoxon rank-sum test was used to compare the ordinal data between the CD34 positive and negative groups. For binary or unordered qualitative data (e.g., gender), the Chi-square test was used for intergroup comparisons. If the smallest expected frequency was <1, Fisher's exact test was chosen for intergroup comparisons. Survival analysis was conducted using the Kaplan-Meier method, and intergroup comparisons were

made using the Log-rank test. A *p*-value<0.05 was considered statistically significant.

Results

Comparison of Positive Rates of Leukemia Cell Antigen Expression between CD34 Positive and CD34 Negative Groups

Among all 105 cases of B-ALL children, 87 had bone marrow CD34 positive cells ≥10%, with a positive rate of 82.9%, classified into the CD34 positive group. The remaining 18 children (17.1%) had bone marrow CD34 positive cells <10%, classified into the CD34 negative group. As shown in Table I, the positive rate of CD13 expression in the CD34 positive group was significantly higher than in the CD34 negative group, with a statistically significant difference (*p*<0.05). The comparison of positive rates of leukemia cell antigens such as CD10, CD19, CD20, CD22, CD79a, CD13, CD33, and CD38 between the two groups showed no significant difference (*p*>0.05).

Comparison of Clinical Characteristics Between CD34 Positive and CD34 Negative Groups

As shown in Table II, the proportion of children with common B-ALL and standard-risk stratification in the CD34 positive group was significantly higher than in the CD34 negative group. In contrast, the proportions of early pre-B-ALL, *E2A-PBX1* fusion gene, and *MLL-AF4* fusion gene were significantly lower in the CD34 negative group, with statistically significant differences (*p*<0.05).

Comparison of Initial Treatment Response Between CD34 Positive and CD34 Negative Groups

As shown in Table III, the occurrence rate of MRD after induction chemotherapy in the CD34 positive group was significantly lower than in the CD34 negative group, with a statistically significant difference (*p*<0.05). However, the sensitivity to the first prednisone treatment and the bone marrow treatment efficacy on the 19th and 33rd days after chemotherapy showed no significant differences between the two groups (*p*>0.05).

Comparison of Follow-up Outcomes Between CD34 Positive and CD34 Negative Groups

The follow-up results indicated that by December 31, 2023, among all 105 cases of B-ALL children, 5 experienced relapses after chemotherapy, with a relapse rate of 4.8%, and there were no deaths. The relapse rate in the CD34 positive group was 2.3%, significantly lower than the 16.7% in the CD34 negative group, showing a statistically significant difference (*p*=0.035 <0.05).

Discussion

Acute leukemia is a highly heterogeneous group of malignant hematologic diseases with complex pathogenesis. The proliferation of leukemia cells in the bone marrow suppresses normal hematopoiesis, severely endangering human health. Historically, the diagnosis and prognostic assessment of this disease have primarily relied on morphological criteria. In recent years, immunological testing of leukemia cells has increasingly become an important reference for disease diagnosis, classification, and prognostic evaluation, with more and more valuable biological markers emerging[13-17].

CD34, a highly glycosylated leukocyte differentiation antigen discovered in the 1980s with a molecular weight of 105-120 kDa, serves as an adhesion molecule within the sialomucin family.

Table I. Comparison of positive rates of leukemia cell antigen expression between CD34 positive and CD34 negative groups.

Leukemia Cell Antigens	CD34 Positive Group (n=87)	CD34 Negative Group (n=18)	χ^2 value	*p*-value
CD10	79 (90.8)	14 (88.9)	0.036	0.850
CD19	87 (100)	18 (100)	-	-
CD20	21 (24.1)	4 (22.2)	0.017	0.896
CD22	84 (96.6)	15 (83.3)	2.694	0.101
CD79a	81 (93.1)	16 (88.9)	0.016	0.900
CD13	25 (28.7)	0 (0)	5.297	0.021
CD33	7 (8.0)	0 (0)	0.528	0.467
CD38	83 (95.4)	18 (100)	-*	1.000

*The Fisher's exact test was used.

Table II. Comparison of clinical characteristics between CD34 positive and CD34 negative groups.

Characteristics	CD34 Positive Group (n=87)	CD34 Negative Group (n=18)	Statistical values	*p*-value
Gender			χ^2=1.821	0.177
Male	49 (56.3)	7 (38.9)		
Female	28 (40.0)	12 (34.3)		
Age at Diagnosis (years)			χ^2=0.270	0.604
0-9	79 (90.8)	15 (83.3)		
≥10	8 (9.2)	3 (16.7)		
Subtype of B-ALL			χ^2=8.484	0.014
Common B-ALL	76 (87.4)	11 (61.1)		
Early pre-B-ALL	4 (4.6)	4 (22.2)		
Pre-B-ALL	7 (8.0)	3 (16.7)		
TEL-AML1 Fusion Gene			χ^2=0.447	0.504
Positive	24 (27.6)	3 (16.7)		
Negative	63 (72.4)	15 (83.3)		
E2A-PBX1 Fusion Gene			χ^2=21.025	<0.001
Positive	2 (2.3)	7 (38.9)		
Negative	85 (97.7)	11 (61.1)		
BCR-ABL Fusion Gene			χ^2=0.528	0.467
Positive	7 (8.0)	0 (0)		
Negative	80 (92.0)	18 (100)		
MLL-AF4 Fusion Gene			4.316	0.038
Positive	4 (4.6)	4 (22.2)		
Negative	83 (95.4)	14 (77.8)		
Central nervous system infiltration at diagnosis			-	-
Present	0 (0)	0 (0)		
Absent	87 (100)	18 (100)		
Leukocyte count at first diagnosis (×10⁹/L)			χ^2=0.298	0.585
<50	75 (86.2)	14 (77.8)		
≥50	12 (13.8)	4 (22.2)		
Risk Stratification			Z=2.539	0.011
low risk	49 (56.3)	5 (27.8)		
Intermediate risk	33 (37.9)	9 (50.0)		
High risk	5 (5.7)	4 (22.2)		

B-ALL: B-cell Acute Lymphoblastic Leukemia; TEL-AML1: Translocation Ets Leukemia-Acute Myeloid Leukemia; E2A-PBX1: E2A-Pre-B-cell leukemia transcription factor 1; BCR-ABL: Breakpoint Cluster Region-Abelson proto-oncogene; MLL-AF4: Mixed Lineage Leukemia-Family Member 4.

The CD34 antigen, primarily expressed on the surface of human and other mammalian hematopoietic stem cells or progenitor cells, diminishes and eventually disappears as cells mature. Hence, the CD34 antigen possesses high-stage specificity and is commonly used as a criterion for selecting hematopoietic stem cells or progenitor cells. While CD34 lacks lineage specificity and can be expressed in most types of leukemia cells, reports on its correlation with the prognosis of children with B-ALL are scarce. In response, this study retrospectively analyzed the clinical and follow-up data of 105 children with newly diagnosed B-ALL treated at our hospital from January 2022 to December 2023. Children with bone marrow CD34-positive cells ≥10% were considered positive. The results showed that the positivity rate of CD34 was as high as 82.9%, similar to the 73.8% reported by Borowitz et al[18], indicating that about 4/5 of newly diagnosed children with B-ALL exhibit CD34 positive expression in the bone marrow, while the positivity rate of CD34 in normal bone marrow is typically less than 5%, warranting clinical attention. Our findings revealed no significant difference in the positive rates of common leukemia cell antigens such as CD10,

Table III. Comparison of initial treatment response between CD34 positive and CD34 negative groups.

Treatment response	CD34 Positive Group (n=87)	CD34 Negative Group (n=18)	X² value	p-value
First Prednisone Treatment Outcome			1.213	0.271
Sensitive	82 (84.3)	15 (83.3)		
Insensitive	5 (5.7)	3 (16.7)		
Bone marrow Treatment efficacy on day 19 after chemotherapy			1.513	0.219
CR	72 (82.8)	12 (66.7)		
PR or NR	15 (17.2)	6 (33.3)		
Bone marrow treatment efficacy on day 33 after chemotherapy			-*	0.075
CR	86 (98.9)	16 (88.9)		
PR or NR	1 (1.1)	2 (11.1)		
MRD			3.946	0.047
Present	10 (11.5)	6 (33.3)		
Absent	77 (88.5)	12 (66.7)		

*The Fisher's exact test was used. CR: complete remission; PR: partial remission; NR: non-remission; MRD: minimal residual disease.

CD19, CD20, CD22, CD79a, CD13, CD33, and CD38 between CD34 positive and negative groups ($p>0.05$), suggesting that CD34 expression is not significantly correlated with the expression of most common leukemia cell antigens and should be separately tested in immunophenotyping. Clinically, compared to CD34 negative children, CD34 positive children had higher proportions of common B-ALL and standard-risk stratification at diagnosis and lower proportions of early pre-B-ALL, *E2A-PBX1* fusion gene, and *MLL-AF4* fusion gene ($p<0.05$), indicating that children with CD34 positive B-ALL might have a lower disease risk and potentially better prognosis. Previous reports[19-22] on the correlation between CD34 expression and prognosis in acute leukemia patients have been inconsistent, possibly related to leukemia type, ethnicity, age, and other factors. Dakka et al[21] indicated that in B-ALL, CD34-positive patients are more common in males aged 1-10 years, with white blood cell (WBC) count $<50\times10^9$/L, CD10 positivity, and hyperdiploid leukemia being more prevalent. However, the opposite was observed in patients with T-lymphoblastic leukemia, where CD34 expression was associated with age >10 years, WBC count $>50\times10^9$/L, diploid leukemia cells, and CD10 negativity. This study showed that the occurrence rate of MRD and relapse after induction chemotherapy in the CD34 positive group was significantly lower than in the CD34 negative group ($p<0.05$), suggesting that CD34 expression is related to a favorable prognosis, possibly because CD34-positive leukemia cells are more susceptible to apoptosis induced by anti-leukemia treatment[22]. Furthermore, we found that children with CD34-positive B-ALL are less likely to exhibit *E2A-PBX1* and *MLL-AF4* fusion genes, both associated with poor prognosis or chemotherapy resistance. This study has several limitations, including being a single-center study with a relatively small number of cases and significant individual differences. The impact factors on prognosis are multifaceted, and the results need to be confirmed by further multicenter prospective studies.

Conclusions

A high positive rate of bone marrow CD34 expression in children with B-ALL is associated with a favorable prognosis. Children with negative CD34 expression are relatively more prone to the occurrence of minimal residual disease and tumor relapse after chemotherapy.

Authors' Contributions

The contributions to this paper are as follows: Wei Zha and Yue Yuan were responsible for the study design, data analysis, and manuscript writing; Ting Yang and Lijuan Zhu were responsible for data collection and verification; Weiyu Kong and Jiajia Zhuo provided research materials and tools; Wei Zha was responsible for the final review and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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