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Research paper

No prognostic significance of immunophenotypic changes at the end of remission induction therapy in children with B-lineage acute lymphoblastic leukemia

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ABSTRACT

Detection of aberrant antigen expression in acute lymphoblastic leukemia (ALL) by flow cytometric is proposed for the quantification of minimal residual disease (MRD). There are few studies that investigate the stability of the antigen expression in children with B lineage ALL at the end of remission induction therapy and determine its prognostic impact. Between 2010 and 2015, 691 bone marrow specimens of childhood ALL were sent at diagnosis for immunophenotypic characterization, and follow-up samples for MRD were analyzed on day 33. Of these, 155 patients with MRD more than or equal to 0.01% were eligible for the study. Immunophenotypic studies were performed by multiparametric flow cytometry using four-colour monoclonal antibody combinations. Overall, 86 of 155 (55.5%) cases showed phenotype shifts at least one marker. CD19 was the most stable markers. By contrast, CD20 was significantly different between diagnosis and day 33 in nearly one third of the cases. Shifts of antigen expression was not significantly associated with EFS, RFS or OS ($P > 0.05$). Multivariate analysis showed that WBC and *BCR-ABL* have independent prognostic value in childhood ALL. Changes in antigen expressions were commonly occurred at the end of induction and not associated with prognostic value in patients whose MRD were positive on day 33.

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common cancer diagnosed in the pediatric population. Approximately 3000 children in the United States are diagnosed with ALL every year [1]. The survival rate of pediatric ALL has increased from less than 10% to 90% during the past four decades, due to the improvements in the efficacy of multiagent chemotherapy and stratification of treatment intensity [1].

Minimal residual disease (MRD) in ALL can be measured by means of flow cytometric detection of aberrant patterns of antigens that distinguish them from normal hematopoietic cells or by means of polymerase-chain-reaction amplification of clonal antigen-receptor gene rearrangements [2–5]. Previous studies have shown that detection of MRD at the end of remission induction therapy is a powerful predictor of clinical outcome in childhood ALL [6–10].

Several studies have focused on the immunophenotypes of the leukemic cells at diagnosis compared to that observed at relapse. Immunophenotypic changes between diagnostic and relapse have been observed in up to 72% of patients with ALL [11,12]. These changes include either gain/loss of lineage-associated antigens or lineage switch [11,13,14]. Loss or acquisition of CD10 is a frequent change in ALL patients [15,16]. In childhood ALL, an overall change in the immunophenotype at relapse does not seem to influence the outcome of therapy [15].

However, little is known about the changes in antigen expressions on leukemic cells at the end of induction and their relation with prognosis. In this study, we analyzed the data of antigenic shift at the end of induction treatment in children with B-lineage ALL to assess the influence of these changes on the prediction of relapse by flow cytometry.

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2. Methods

2.1. Patients

From July 2010 to November 2015, a cohort of 691 children with B lineage ALL were consecutively diagnosed and treated at West China Second University Hospital, Sichuan University, Chengdu, China. Of these, 155 patients who were flow cytometric MRD-positivity (≥0.01% of leukemia blasts) at the end of induction were included in this study. Bone marrow samples were sent to the Department of Laboratory Medicine of the hospital at diagnosis for immunophenotypic characterization, and follow-up samples for MRD analysis were sent at induction end (day 33). All cases were diagnosed as ALL according to the French-American-British (FAB) morphological and cytochemical criteria [17]. Relapse was defined as the reappearance of leukemic cells in BM (> 20% blasts) after complete remission (CR). Immunophenotype and MRD were performed by indirect immunofluorescence on flow cytometry. According to their cytogenetic and molecular genetics outlined by the Chinese Childhood Leukemia Group (CCLG) protocols (ALL-2008) (Fourth Revision), all the patients were classified into three groups as high risk (HR), intermediate risk (IR) and low risk (LR). The following presenting features were evaluated: age (< 1, 1 to < 10, ≥10 years), gender, risk stratification (LR, IR, or HR), leukocyte count (< 20, 20 to < 100, ≥100× 10^9/L), FAB morphology (L1, or L2), immunophenotype at diagnosis (pro-B-ALL, common-ALL, or pre-B-ALL), and cytogenetic abnormalities (*BCR-ABL*, *MLL-AF4*, *TEL-AML1*, or *EA-PBX1*). The study was approved by the ethical committee of our hospital, and informed consent forms were signed from parents or guardians.

2.2. Flow cytometric analyses

All samples were processed as previously described within 24 h after collection [18,19]. Aliquots of bone marrow were adjusted to sample concentrations of 10–20 × 10[^]9/L in phosphate-buffered saline (PBS) solution for immunophenotypic and MRD analysis. The specimens were stained with a variable combination of fluoresceinated, phycoerythrinated, allophycocyaninated or peridinin chlorophyll protein conjugated monoclonal antibodies to CD10, CD19, CD20, CD22, CD34, and CD45 (Becton Dickinson, San Jose, CA, USA; or Beckman Coulter, Hialeah, FL). After incubation for 15 min in the dark at room temperature, samples were lysed using a commercially available red cell lysing solution (Becton Dickinso, San Jose, CA, USA), then washed in PBS and resuspended in 0.5 ml of PBS before running on a FACSCalibur flow cytometer (Becton Dickinso, San Jose, CA, USA). Sample was analyzed on BD Cell-Quest™ Pro software (Becton Dickinson, San Jose, CA, USA). Instrument setup was optimized daily with calibrite beads (Becton Dickinson) for quality control purposes. A minimum of 10,000 events was collected for immunophenotype at diagnosis, and 500,000 events collected for MRD analysis. Nonviable cells were excluded from analysis using forward scatter and side scatter (SSC). Gating of cell populations was performed using CD45 and SSC parameters. Leukemic cells were identified using an immunological gate based on CD19 expression associated with SSC and they were discriminated from normal B lymphocytes by immunophenotypes, as previously described in our study [19]. It has been indicated that hematogones are normal B-cell precursors, including CD34+ CD38+ CD10+ CD20- CD22+ CD19+, and $CD34^{-/lo}$CD38+ CD10+ CD20+ CD22+ CD19+. The expression was recorded as positive or negative using a combination of isotype negative control or comparing with built-in control populations. The specimen was considered positive if more than 20% of blast cells expressed the antigen. Antigen gain or loss was defined by conversion from antigen negativity to positive, or vice versa.

2.3. Fusion transcript analyses

According to manufacturer's instructions, the total RNA of each sample was extracted with Trizol (Invitrogen Life Technologies, Carlabad, CA, USA). A multiples reverse transcription polymerase chain reaction (RT-PCR) was performed to detect simultaneously the following fusion transcripts of ALL: *BCR-ABL*, *TEL-AML1*, *E2A-PBX1* and *MLL-AF4*.

2.4. Statistical methods

Statistical analysis was performed using SPSS 13.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA). Categorical variables were compared to Chi-square test or Fisher's exact test when necessary. The duration of event-free survival (EFS) was measured from the day of diagnosis to the first negative event (failure to induce remission, relapse, or death from any cause) or to the last follow-up date. Relapse-free survival (RFS) was measured from the time of CR to the time of relapse or last follow-up. Overall survival (OS) was measured from the diagnosis of ALL to the date of death or last follow-up. Comparison between curves was performed with the log-rank test and survival was plotted with Kaplan-Meier curve. The Cox proportional hazards regression model was used to draw multivariate regression. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Clinical features

From these 155 patients, 90 were male and 65 female cases with a median age of 4 years (range 1–15 years of age). The clinical and laboratory information of patients at diagnosis were summarized in Table 1. The EFS, RFS and OS ranged from 1 to 65 months (median, 22 months), 1–64 months (median, 21 months) and 1–65 months (median, 23 months), respectively.

Table 1
Characteristics of 155 children with B-Lineage ALL.

Variable	Value
Age, years (Median, range)	4 (1–15)
Gender	
Male	90 (58.1%)
Female	65 (41.9%)
Risk stratification	
Low risk	37 (23.9%)
Intermediate risk	95 (61.3%)
High risk	23 (14.8%)
Laboratory features (Median, range)	
WBC (×10^9/L)	10.0 (1.0–601.2)
Absolute neutrophil count (×10^9/L)	1.1 (0–54.1)
PB blasts (×10^9/L)	3.1 (0–550.0)
Hemoglobin (g/L)	75 (29–148)
Platelet (×10^9/L)	55 (3–644)
BM blasts (%)	87.5 (26.0–97.5)
FAB	
L1	64 (41.3%)
L2	91 (58.7%)
Immunophenotype at diagnosis	
Pro-B	15 (9.7%)
Common	108 (69.7%)
Pre-B	32 (20.6%)
Cytogenetic abnormalities	
BCR-ABL	12 (7.7%)
MLL-AF4	3 (1.9%)
TEL-AML1	23 (14.8%)
E2A-PBX1	8 (5.2%)