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The ratio of absolute lymphocyte count at interim of therapy to absolute lymphocyte count at diagnosis predicts survival in childhood B-lineage acute lymphoblastic leukemia

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ABSTRACT

Absolute lymphocyte count (ALC) after therapy has been reported to be an independent prognostic factor for clinical outcome in leukemia. This study mainly analyzed ALC at interim of therapy on day 22 (ALC-22) and the ratio of ALC-22 to ALC at diagnosis (ALC-0) on the impact of survival and the relation of ALC to lymphocyte subsets in 119 pediatric B-lineage acute lymphoblastic leukemia (B-ALL) patients. Univariate analysis revealed that ALC-22/ALC-0 ratio <10% was significantly associated with inferior overall survival (OS) (hazard ratio (HR) = 12.24, $P = 0.0014$) and event-free survival (EFS) (HR = 3.3, $P = 0.0046$). In multivariate analysis, ALC-22/ALC-0 ratio remained an independent prognostic factor for OS (HR = 6.92, $P = 0.0181$) and EFS (HR = 2.78, $P = 0.0329$) after adjusting for age, white blood cell (WBC) count and minimal residual disease (MRD) status. A Spearman correlation test showed that CD3+ T cells had a negative correlation with ALC-0 ($r = -0.7204$, $P < 0.0001$) and a positive correlation with ALC-22 ($r = 0.5061$, $P = 0.0071$). These data suggest that ALC-22/ALC-0 ratio may serve as a more effective biomarker to predict survival in pediatric B-ALL and ALC is mainly associated with CD3+ T cells.

1. Introduction

Initial studies have shown that early absolute lymphocyte count (ALC) recovery after hematopoietic stem cell transplantation (HSCT) is associated with survival in both children and adults with hematological malignancies, such as acute lymphoblastic leukemia [1,2], acute myeloid leukemia [3,4] and lymphoma [5,6]. These results suggest that ALC is associated with an increased graft-*versus*-tumor effect and might serve as an indicator of bone marrow recovery. In the non-transplant setting, ALC, absolute neutrophil count (ANC) and absolute monocyte count (AMC) at diagnosis have been reported to be the prognostic factors in non-leukemia hematological diseases [7–9] and other solid tumors [10–12]. These findings further confirm the role of immune cells in cancer therapy. While in leukemia frequently with abnormal white blood cell (WBC) count at diagnosis, ALC after induction treatment has been reported to be a prognostic factor for clinical outcome. In the pediatric setting, several studies have shown the relation of ALC to survival with different cut-off values (350 cells/μl [13,14], 500 cells/μl [15], 1000 cells/μl [14,16] and 1500 cells/μl [14,17,18]) and different time points during induction therapy on day 15 [13,16], day 28 [13,16], day 29 [14,17], day 36 [18] and day 43 [15] in either ALL or AML. Also, MRD measurement by using flow cytometry or molecular techniques and other traditional prognostic factors such as age, WBC and cytogenetics have been used to predict outcome in leukemia [19,20].

Despite the predictive role of ALC with survival after chemotherapy in pediatric ALL has been evaluated by several studies, some issues remain to be addressed: (1) previous studies always have demonstrated the impact of ALC on survival in ALL (both B-ALL and T-ALL) as a whole cohort, which has not given a clear idea for either B-ALL or T-ALL; (2) although studies have suggested that ALC after therapy may reflect the degree of bone marrow recovery in ALL [15,21], it may be partially affected by ALC at diagnosis due to abnormal initial WBC count in leukemia. So whether ALC after therapy reflects the actual hematopoietic recovery remains unclear; (3) various ALC cut-off values and time points have caused difficulties in clinical practice, thus it is necessary to find more appropriate and simpler prognostic indicators to overcome the diversity; (4) natural killer (NK) cells and T cells have been shown to target hematopoietic

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malignancies and play an important role in maintaining remission [22–24], however, it is unclear whether ALC is associated with T cells, NK cells or even B cells responsible for its effect. Notably, in our previous study, we showed that higher ALC at interim of the induction therapy is significantly correlated with lower MRD level at interim of therapy in childhood B-ALL [25]. Based on the above observations, we evaluated the prognostic value of ALC at the mid-course of induction therapy (ALC-22) and the ratio of ALC-22 to ALC at diagnosis on day 0 (ALC-0) with survival in 119 consecutive, newly diagnosed childhood B-ALL patients. Blood cell recovery according to ALC-22 and ALC-22/ALC-0 ratio and the correlation of ALC and lymphocyte subsets were also investigated.

2. Methods

2.1. Patients

From August 2006 to June 2009, a total of 119 children (age ≤ 18 years) with newly diagnosed B-ALL at the Children's Hospital of Zhejiang University School of Medicine were enrolled in this study. Patients with expression of other lineage molecules such as myeloid or T lineage markers were excluded to avoid the possible impact of abnormal lineage cells on the level of ALC. Treatment regimens were described elsewhere [26]. In brief, the treatment regimen included 7 days of prophase treatment with prednisone and followed by 4 weeks of induction therapy consisting of four drugs (vincristine, daunorubicin, L-asparaginase and dexamethason) according to the protocol NPCAC97. This study was approved by the Ethics Committee of Children's Hospital, Zhejiang University School of Medicine, and informed consents were obtained from all patients' parents or guardians in accordance with the declaration of Helsinki.

2.2. Analysis

General medical records were reviewed to determine gender, age, cytogenetics, WBC, ANC, red blood cell (RBC) count, hemoglobin (Hb) and platelets (Plts). ALC values calculated from the complete blood cell count (CBC) were obtained at the time of diagnosis on day 0, at the mid-course of induction therapy on day 22 and at the end of induction therapy on day 36. MRD values were determined at interim of the induction therapy on day 22 and were assayed with a combination of CD19/CD10/CD34/CD45 antibodies, which has been described elsewhere [26]. Lymphocyte subset analyses were obtained at diagnosis with CD3/CD4/CD8 and CD3/CD56/CD16/CD19 antibody combinations by flow cytometry. The lymphocytes were mainly gated as T cells (CD3+) which were further gated as CD4+ T cells (CD3+CD4+) and CD8+ T cells (CD3+CD8+), B cells (CD3−CD19+) and NK cells (CD3−CD16+CD56+).

2.3. Statistical methods

Receiver operating characteristic (ROC) curve analysis was used to determine the cut-off values of the ALC-0, ALC-22, and ALC-22/ALC-0 ratio. The value with maximum sensitivity and specificity was selected as the best cut-off value. OS was measured as the time between the first day of diagnosis and the date of death from any cause or the last follow-up and EFS was calculated from the first day of diagnosis to the date of first event (disease progression, second malignancy, relapse or death of any causes) occurrence or last follow-up. OS and EFS rates were estimated by Kaplan–Meier analysis and compared by using the log-rank test. Potential risk factors for OS and EFS outcome were evaluated in univariate and multivariate analyses with the Cox proportional hazards regression model. Categorical variables between groups were compared by using Chi-square tests. Continuous variables between groups were evaluated by using Mann–Whitney *U* test or Wilcoxon signed-rank test. Spearman's rank correlation coefficients were used to evaluate the associations for continuous variables. Statistical analysis of the data was performed using the SPSS 19.0 software package (SPSS, Chicago, IL, USA). A two-sided *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Cut-off values for the ALC-0, ALC-22 and ALC-22/ALC-0 ratio

The cut-off values of ALC-0, ALC-22 and ALC-22/ALC-0 ratio were selected from the ROC curve analysis based on its utility as a marker for the survival status of death/survival. The ALC-0 value of 15.7×10^9/L had an AUC of 0.79 (95%CI, 0.65–0.93, $P = 0.001$) with a sensitivity of 67% and a specificity of 86% (Fig. 1A). The ALC-22 value of 0.9×10^9/L had an AUC of 0.71 (95%CI, 0.57–0.86, $P = 0.0198$) with a sensitivity of 69% and a specificity of 73% (Fig. 1B). The most discriminative cut-off value of ALC-22/ALC-0 ratio was 10% with an AUC value of 0.83 (95%CI, 0.74–0.93, $P = 0.0003$) on the ROC curve (75% sensitivity and 83% specificity) (Fig. 1C).

3.2. Patient characteristics

The median age of the study group at diagnosis was 4.4 years (range: 1.1–14.3 years). The 5-year OS and EFS rates estimated for the entire cohort were 89.91% and 79.83%, respectively. Distributions of baseline characteristics for these patients were presented in Table 1 according to the ALC-22/ALC-0 ratio (≥10% *vs.* <10%). Patients with ALC-22/ALC-0 ratio <10% was more likely to have higher initial WBC count ($\geq 50 \times 10^9$/L) ($P < 0.0001$), higher initial LDH level (≥normal) ($P < 0.0001$), and higher MRD-positive status (≥0.01%) ($P = 0.0015$) on day 22. Although ALC-22/ALC-0 ratio <10% was more correlated with older age (≥10 years), male and unfavorable cytogenetics as compared with ALC-22/ALC-0 ratio ≥10%, no statistical significance was observed ($P > 0.05$).

3.3. Prognostic significance of ALC-22/ALC-0 ratio

Twelve (10.1%) of 119 patients had died in this cohort. Recurrence (8/12) and progression (4/12) of the disease were the main causes of death. To determine which time point was the best to predict survival, we also analyzed ALC at the end of therapy on day 36 (ALC-36) with survival. However, ALC-36 was not significantly associated with OS or EFS when analyzed as a continuous variable ($P > 0.05$). Otherwise, patients with ALC-22 $\geq 0.9 \times 10^9$/L had an inferior 5-year OS rate (80% *vs.* 94.94%, $P = 0.0276$, Fig. 2A) and 5-year EFS rate (70% *vs.* 84.81%, $P = 0.1295$, Fig. 2B) as compared with ALC-22 $< 0.9 \times 10^9$/L. ALC-22/ALC-0 ratio <10% was significantly associated with a lower 5-year OS rate (72.97% *vs.* 95.56%,

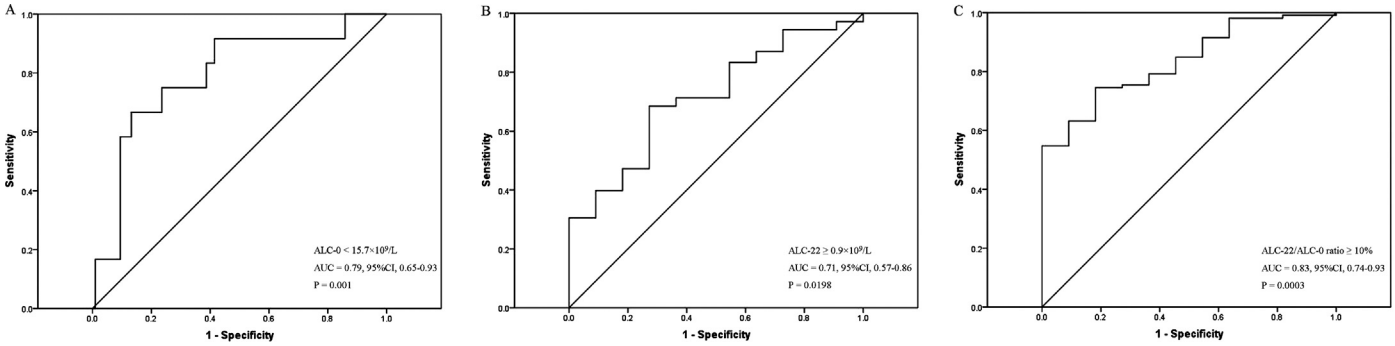

Fig. 1. Receiver operating characteristic (ROC) curve and area under the curve (AUC) for ALC-0 (A), ALC-22 (B), and ALC-22/ALC-0 ratio (C). ALC, absolute lymphocyte count; CI, confidence interval; ALC-0, measured at diagnosis (day 0); ALC-22, measured at interim of induction therapy on day 22.