Prognostic Value of Persistent Peripheral Blood and Bone Marrow Lymphoblasts on Day 15 of Therapy in Childhood Acute Lymphoblastic Leukemia as Detected by Flow Cytometry

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Abstract. Aim: The predictive value of residual disease measured by flow cytometry at day 15 of induction therapy was analyzed in 182 children treated for acute lymphoblastic leukemia (ALL). Materials and Methods: Peripheral blood (PB) and bone marrow were assessed for leukemia cells by morphology and flow cytometry at days 0, 8, and 15. Results: Absolute blast count (ABC) >200/μl in PB by day 15 assessed by flow cytometry predicted a lower probability of disease free survival (pDFS) (p=0.056). Patients with bone marrow lymphoblast (BML)>0.5% had a lower pDFS (p=0.002). Cumulative relapse incidence for patients with BML<0.5% was 8.9% vs. 47.1% (OR=4.6, p=0.036). In common/pre-B-ALL patients aged >10 years with BML>0.5%, pDFS value was significantly lower. In the multivariate analysis, the only significant factor with adverse prognostic value for pDFS was BML>0.5% (HR=5.3 p=0.030). Conclusion: BML>0.5% analyzed by flow cytometry at day 15 is possibly the strongest prognostic factor in pediatric ALL.

The speed of blast clearance during therapy is a major prognostic factor of outcome in childhood acute lymphoblastic leukemia (ALL) (1-3). Blast count in the peripheral blood (PB) on day 8, or in the bone marrow on day 15 and day 33, have been widely used to deliver risk-directed therapy (4-6). Another approach to measure the speed of leukemia clearance is the detection of minimal residual disease (MRD) during induction therapy (7, 8), or at days 33 and 78 (9, 10). The key factor of clinical relevance for most centers is the availability of measurement of residual disease at an early time point with a low-cost method. Presence of leukemia blasts during the early phase of therapy can be assessed by microscopy and, preferably, by flow cytometry (FC) or polymerase chain reaction (PCR) of the rearrangements of immunoglobulin (Ig) or T-cell receptor (TCR) gene segments (2, 3). Since PCR methods are laborious and costly, and thus unavailable to most centers, FC which is the method with acceptable sensitivity and wide availability in many centers/countries for hematological and immunological investigations (2, 11). FC in detection of residual disease, although formerly less standardized, may represent an important technology, due to its speed and cheaper availability (1). This technology enables most treatment centers to identify a high proportion of children with ALL who have a good early treatment response or are at high risk of leukemia relapse (11, 12).

In order to estimate the predictive impact of early blast reduction parameters, we investigated the potential of PB and bone marrow (BM) findings at diagnosis (day 0) and early follow-up time points (day 8, day 15, and day 33). In this analysis, we focused on the prognostic impact of residual disease measured by flow cytometry at day 15 of induction therapy.

Patients and Methods

A total of 182 children, including 98 males and 84 females, aged 1-19 years (median 5 years), consecutively diagnosed and treated for ALL between 1997 and 2010, were included in this study. Infants were excluded from this study. Median follow-up was 5.1 years (range, 0.5-13 years). Patients were recruited from the ALL-BFM-90 (n=78) (13) or ALL-IC-2002 (n=104) (2) protocols. Standard, intermediate-, and high-risk group (SRG, IRG, HRG) criteria were specifically characterized in each therapy protocol.

PBS was assessed for leukemia cells by morphology at days 0, 8, 15 and 33, while BM was analyzed by morphology and flow cytometry at days 0 and 15. PB absolute blast count (ABC) at day 15 was analyzed as a categorical value for the following cut-off values: 500 blasts/μl, 200 blasts/μl, and 100 blasts/μl. Early BM response...
by day 15 was assessed as: M1 – blast percentage <5%, M2 – blast percentage 5–<25%, and M3 – blast percentage 25% or higher. Bone marrow lymphoblasts (BML) data assessed by flow cytometry at day 15 were analyzed for following cut-off values: >10% blasts, >1% blasts and >0.5% blasts.

Leukemia-associated immunophenotypes were studied at diagnosis by using the following monoclonal fluorochrome-conjugated antibody combinations: CD10, CD19, CD20, CD34, CD45, CD11a for B-cell precursor ALL; and TdT, CD2, sCD3, CyCD3, CD4, CD5, CD7, and CD8 for T-ALL. Antibodies were purchased from BD Biosciences (San Jose, CA, USA), Dako/ DakoCytomation (Glostrup, Denmark), and Beckman/Coulter/ Immunotech (Marseille, France). At least two selected combinations were applied during follow-up for MRD detection for each patient. Analyses were performed on EPICS XL (Coulter, Miami, FL, USA) and Cytomics FC500 (Beckman-Coulter, Miami, FL, USA) flow cytometers.

According to blast immunophenotype, all patients were diagnosed as: pro-B (n=8), common/pre-B (n=150) and T-ALL (n=24). At day 15, blast morphology and PB smear (PB15) was assessed in 179 children, BM morphology (BM15) in 140 children and BML by flow cytometry in 91 children. Complete remission (CR) was defined as no physical signs of leukemia, BM with active hematopoiesis, and normal cerebrospinal fluid. Results of antileukemia therapy were presented as probability of disease-free survival (pDFS).

**Statistical analysis.** Baseline characteristics of all patients were summarized using descriptive statistics. Differences between groups were measured by chi-square test, Fisher exact test and Mann-Whitney U-test. The median survival of this patient group was estimated with a 95% confidence interval (CI). Kaplan-Meier curves were used to summarize DFS. Cox proportional hazards regression model was used to correlate each potential prognostic factor with a survival in univariate analysis. The factors that appeared to be important were then fitted together, and removed one at a time in a backward stepwise manner using the likelihood ratio test at a 0.05 level until all factors in the model were significant. A final check was made to ensure that no excluded factors would improve the fit.

**Results**

Patients characteristics and overall treatment results are presented in Table I. No differences in pDFS between patients treated with specific therapy protocol were found. Blast immunophenotype had no significant impact on pDFS.
The only two factors with p<0.1 for pDFS in univariate analyses (PB15 ABC<200; BML15<0.5%) were fitted together in the multivariate analysis. For all patients, the only significant factor with adverse prognostic value for pDFS was BML15>0.5% (hazard ratio=5.3; 95% CI=1.2-23; p=0.030). The only significant factor with adverse prognostic value for pDFS for subgroup of patients with common/pre-B phenotype was BML15>0.5% (hazard ratio=5.5; 95% CI=1.2-24; p=0.027). No prognostic factor was found when patients were analyzed in subgroups determined by the two therapy protocols.

Discussion

In this study, we have shown that FC analysis provided good information on MRD during induction therapy of ALL. We determined the favorable prognostic risk factor, based on the presence of <0.5% lymphoblasts in FC analysis in BM at day 15. With this factor, 30% of patients included in the analysis had an excellent outcome, pDFS with 91% with long-term follow-up.

With recently completed, risk-adapted treatment protocols for childhood ALL, most patients reached almost 80% of long-term remission (14-16). Further improvement is expected in better stratification of patients based on MRD studies. The optimal timing and method of MRD assessment are a matter of debate. Recently, several reports have been published dedicated to the role of FC studies of MRD during induction therapy of ALL.

Basso et al. in a study involving 815 patients with ALL, showed that MRD levels <0.1% measured by FC on day 15 of remission induction were a powerful prognostic factor that retained independent significance in a model including MRD levels determined by PCR amplification of antigen-receptor genes on days 33 and 78 from the start of remission induction (1). They also showed that classical risk factors such as blast immunophenotype, prednisolone response at day 8, or early BM response at day 15, had lower prognostic value with therapy protocol AIEOP-BFM-ALL 2000 (1).

Coustan-Smith et al., studied MRD by FC at day 19 in 248 patients and found that 46% were MRD negative (<0.01%). At this time point, patients could be segregated by these MRD results into two risk groups of almost equal size but with significantly different outcomes (11). Thus, the findings both of Basso et al. (1), and Coustan-Smith et al. (11) that MRD testing early in the treatment course can identify patients who are likely to have a superior treatment outcome and can possibly be spared from intensive chemotherapy might have an advantage, especially in resource-poor countries. Our study, performed in a country of central Europe, with relatively limited resources, corroborate results and conclusions of Italian and American research.

Studies of MRD at early time points such as day 15 can be performed proficiently for patients with simple and inexpensive combinations of antibodies (11). Moreover, the FC data can easily be standardized after being transferred via Internet to a central site for uniform analysis (17-19).
Interestingly, there are more data available that other simple tests based on morphology or FC analysis have strong prognostic value, better than previously accepted universal risk factors. Zweidler-McKay et al. showed that absolute lymphocyte count in PB higher than 1500/μl at day 29 of ALL therapy in children in protocol COG P9904/5/6 is a powerful prognostic factor with superior \( \text{HR}/p \)-value than MRD status at that day (20).

In conclusion, evidence is gathering that FC analysis of BM at day 15 of induction therapy in childhood ALL has strong prognostic value, comparable to that obtained by PCR MRD studies at later time points. In general, the presence of blasts by day 15 of therapy in childhood ALL is a poor prognosis factor. In this study, we determined an adverse prognostic factor based on the presence of >0.5% lymphoblasts in BM detected by FC. The unfavorable impact of BM blast count >0.5% by day 15 was especially prominent in common/pre-B patients aged above 6 years.

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References


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