Flow Cytometry is Significantly more Sensitive than Routine Microscopy for Detecting Peripheral Blasts in Pediatric Acute Lymphoblastic Leukemia

Irina Shakalova, Sergey Nikulshin [1], *Zhanna Kovalova, Iveta Tolstikova* [1], *Dagne Gravele* [1]

Rīga Stradiņš University, Latvia
[1] *Children's Clinical University Hospital, Latvia*

Introduction. Acute leukemia is the most frequent malignancy in children; acute lymphoblastic leukemia (ALL) accounts for 75% cases. Early and correct diagnosis is essential for effective treatment, but it could be complicated in pediatric setting, for, in contrast to adults, only 50% childhood ALL present with leukocytosis and 20% have leukopenia and no blasts at presentation. Blast morphology is different as well; in some cases tumor population is hardly distinguishable from normal cells. Thus, reliable evaluation of blast count becomes imperative for successful treatment; traditionally used routine morphology may not be sufficiently sensitive.

The aim, materials and methods. The aim of the study was to compare sensitivity of two reference methods for detecting blasts in peripheral blood – microscopy and flow cytometry. Data of 72 pediatric ALL patients (age 0–17 years) treated at Children's Clinical University Hospital Department of Oncohematology in 2007–2012 were enrolled. ALL diagnosis was unquestionably proven by phenotype of blood and/or bone marrow blasts in all cases. Data on 110 relevant blood samples simultaneously tested for blasts by microscopy and flow cytometry were available: 69 samples were of ALL at primary presentation, 14 samples at relapse and 27 at day 8 of treatment. 93 samples were of B-cell ALL and 17 were of T-cell ALL. Microscopy was performed by highly experienced cytologists; panel diagnosis was practiced in case of doubt. Flow cytometry was carried out by 4-color flow cytometer Coulter Epics XL (Beckman-Coulter, USA) in 2007–2010 and 8-color flow cytometer FacsCanto II (Becton-Dickinson, USA) since 2011. Data were analyzed by Microsoft Excel and IBM *SPSS* Statistics v.21. t-test and two-sided correlations (Spearman rho) were calculated.

Results. Though the percentage of blasts obtained by two methods significantly correlated ($p = 1.9E-18$), there was a crucial difference in results for the whole cohort ($t = 7.8$, $p = 3.5E-12$), flow cytometry providing higher counts in all but 2 cases. The difference was more evident in B-ALL ($p = 7.8E-10$) than in T-ALL ($p = 0.0004$), in children 0–10 year old ($p = 7.7E-9$) than in older patients ($p = 0.0001$), at presentation ($p = 1.6E-10$) than at day 8 ($p = 0.03$), when microscopy returned low blast count < 5% ($p = 7.6E-7$) than in cases of overt blastosis > 30% ($p = 0.006$). Still, the difference remained statistically significant in all these groups. Absolute blast counts of the whole cohort were significantly higher when determined by flow cytometry ($p = 0.005$). Blasts > 5% were found in 80.7% primary patients by flow and in 49.4% by microscopy. Diagnostic blastosis (> 30%) was defined in 65.1% primary cases by flow and only in 31.4% by microscopy.

Conclusions. Determination of blast counts by flow cytometry was found to be significantly more sensitive than routine cytology. Moreover, when paired to flow, microscopy turned out to be ineffective in screening for blast cells in two thirds of primary cases with blastosis and missed the ALL diagnosis in half of the patients with diagnostic blast count. Both low leukocyte counts at presentation and the specific "pediatric" morphology may be responsible for the phenomenon. Thus, considerable caution should be advised when screening for pediatric ALL by microscopy alone.