Residual Hemopoiesis in Paediatric Acute Lymphoid Leukemia: Assessment by Multicolour Flow Cytometry

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Introduction. Acute lymphoblastic leukemia (ALL) is the most common paediatric malignancy with excellent response to therapy (Pui, 2008). Flow cytometry (FC) is a rapid and sensitive method that is routinely used for diagnosing and monitoring ALL (Peters, 2011). Though evaluation of blast phenotype is the main application of FC, it provides additional information on non-neoplastic cells in the sample (Craig et al., 2008). The condition of residual hemopoiesis is obviously important; still, there are a few published studies of cytometrically defined benign cells in bone marrow (BM) and blood of ALL patients.

Aim, Material and Methods. The aim of the study was to retrospectively evaluate residual hemopoiesis in BM and blood of paediatric ALL at diagnosis. FC was performed by BD FacsCantoII with 3-laser (2 + 4 + 2) configuration, using 8-colour Euroflow protocol (van Dongen, 2012) – orientation ALOT tube and 4-tube T or B-protocol. Files were re-evaluated for the study by Infinicyt software; blasts, total myeloid cells, neutrophils, normal lymphocytes, normal B and normal T and NK cells were defined. Statistics were performed by MS Excel and IBM SPSS v.21, Mann-Whitney test was used for differences and Spearman for correlations.

Results. 68 consecutive cases of ALL diagnosed in 2011–2015 entered the study. Anonymised patient data were obtained from the Children's Clinical University Hospital IS (B- or T-cell variant, primary case or relapse, leucocyte count in BM and blood). There were 57 primary cases and 11 relapses; B-cell ALL was diagnosed to 60 patients, T-cell ALL in 8 patients. Only BM was tested in 10 cases, only blood in 12 cases, both BM and blood tests were available in 46 cases BM: median myeloid cell count was 3.3E9/L, neutrophils 2.6E9/L, normal lymphocytes – 3.9E9/L, B-cells 0.7E9/L, T/NK-cells 3.0E9/L. Absolute counts of all reactive populations significantly positively correlated with blast count and total leukocyte count in BM. Blood: median total myeloid cell count was 1.7E9/L, neutrophils 1.4E9/L, normal lymphocytes 4.4E9/L, B-cells 0.7E9/L; T/NK-cells 3.4E9/L. Likewise in BM, normal blood cells significantly positively correlated with blast count (except neutrophils) and with leukocyte count in blood. There was significant correlation between all populations' BM and blood counts. Total myeloid cell and neutrophil counts both in BM and blood were significantly higher in T-cell ALL in comparison to B-cell ALL ($p = 0.01$ in all tests). Primary cases and relapses significantly differed by the counts of BM total myeloid cells ($p = 0.016$), neutrophils ($p = 0.018$), B-cells ($p = 0.007$) and blood B-cells ($p = 0.007$), T-cells ($p = 0.007$), normal lymphocytes ($p = 0.004$).

Conclusions. Unexpectedly, in spite of a marked relative reduction of residual hemopoiesis, median absolute counts of normal leukocyte populations in BM and blood were normal or even increased, and strongly positively correlated with blast and leukocyte counts. The dispersion of values was very high, thus, a larger cohort and deeper analysis is necessary to understand the phenomenon. In clinical terms, the finding may explain the relatively fast rebound of hemopoiesis after highly intensive chemotherapy employed for the treatment of paediatric ALL; an attempt to correlate initial findings with tests at later time points may be of interest. Finally, comparison with adult data may be indicated to find out if the reasonably well-preserved normal hemopoesis in ALL at presentation is an exclusively paediatric phenomenon.