Paediatric Leukemia Phenotype Assessed by Flow Cytometry and Immunohistochemistry: Comparative Analysis

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Introduction. Acute leukemia (AL) is the most common malignancy in children, with therapy response varying from good to excellent. Modern laboratory techniques are to a great degree responsible for the favorable prognosis, ensuring reliable diagnosis of AL subvariants and treatment monitoring; immunophenotyping and genetics are the main diagnostic approaches. There are two practical ways to assess phenotype in leukemia setting: immunohistochemistry (IHC) on histological material (usually bone marrow) and flow cytometry (FC) of marrow or blood suspension. Each method has advantages: FC is fast and allows simultaneous detection of multiple antigens, while cell morphology and infiltration pattern are visible on IHC. Histological processing may damage or unmask antigenic structures, so IHC and FC are not analogues by definition. Very few comparative data on IHC and FC results have been published.

Aims. The aim of the study is to retrospectively check the IHC and FC results of paediatric AL patients for similarity and to define the areas of discrepancy.

Material and Methods. Patients tested on single platforms were selected: FC performed by FacsCanto II cytometer (Becton-Dickinson) according to Euroflow protocols, and IHC stained by automatic AutostainerLink 48 (DAKO). 41 patient treated at the Children's Clinical University Hospital in 2011–2014 had both FC and IHC performed simultaneously at diagnosis, and thus were suitable for the study. 29 patients had been diagnosed as B-acute lymphoid leukemia (ALL), 3 T-ALL, 1 Burkitt lymphoma, 1 chronic myeloid leukemia in blast crisis and 1 juvenile myelomonocytic leukemia. FC files from the Hospital Clinical Laboratory database were reviewed for the study by INFINICYT v.1.5 software; IHK slides from the Pathology archive were microscopically reevaluated. Antigens CD1a, CD3, CD4, CD5, CD7, CD8, CD10, CD15, CD19, CD20, CD34, CD45, CD79a, CD99, CD117, HLA-DR, MPO and TdT were evaluated (304 pairs altogether). From 2 to 16 antigens were available for comparison in individual cases. MS Excel database was designed; Wilcoxon signed ranks test for differences was performed by using IBM SPSS v.21.

Results. Differences were found in 95 pairs (31.3%); interpretation of positivity differed only in 22 pairs (7.3%), in remaining cases a degree of antigen expression was different. FC was more sensitive in 61/95 case of discrepancy (64.2%), and in 15/22 cases of the critical difference. Statistically, the results of IHC and FC were not different ($Z = -0.287$, $p = 0.774$). There were more than 20 pairs available for antigens CD3, CD10, CD20, CD34, CD45, CD79a, MPO and TdT; these markers were further analyzed. There was no statistical difference between IHC and FC detection of CD3 ($p = 0.157$), CD20 ($p = 0.102$), CD45 ($p = 0.08$) and MPO ($p = 0.655$). FC was significantly more sensitive for CD10 ($p = 0.034$) and CD34 ($p = 0.003$), and IHC was more sensitive for TdT ($p = 0.023$).

Conclusions. The study demonstrated an unexpectedly high degree of concordance between IHC and FC: 68.7% of the results were analogous and 92.7% compatible for diagnostic purposes. Overall, FC was slightly more sensitive; the difference was significant for such diagnostically important antigens as CD10 and CD34. Contrariwise, IHC seems to be more suitable for TdT detection than FC in Euroflow setting. Very good agreement of IHC and FC results makes it possible to use the methods as mutual external quality controls for antigens that are used by both protocols.