

Routine Flow Cytometry of Paediatric Infectious Mononucleosis Demonstrates Significant Phenotypic Differences in Comparison to Normal Controls

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Introduction. Routine screening for immune deficiencies (ID) includes assessment of relative and absolute counts of lymphocyte (LYM) populations: T (subdivided to T4 and T8), B and NK cells. Multicolour flow cytometry (FC) is the method of choice; standardised 6-colour protocols allow detection of additional subpopulations like T4-/T8- double negative (DN), T4+/T8+ double positive (DP) T-cells or CD8+ NK (NK8).

Reactive proliferation of atypical T8 cells (infectious mononucleosis, IM) is a presentation of viral infections (usually EBV); children and adolescents are most commonly affected. LYM subpopulation test is non-diagnostic and thus rarely done in IM, though some cases present with signs of acquired ID.

Aim, Materials and Methods. The aim of the study was to investigate if, besides T8 cell hyperplasia, there are other changes of LYM subsets and expression of LYM antigens in paediatric MN.

23 EBV-related MN samples tested by BD FACSanto II flow cytometer, FACSCanto software and IVD 6-TBNK kit were retrospectively found in 2014–2016 FC files of Children's Clinical University Hospital Laboratory. Normal 243 age and gender-matched tests were used as controls.

DP cells were the main population (52% LYM) in one case, it was excluded. The rest 22 MN test files and the control files were exported into INFINICYT software (Cytognos), merged and analysed; the results were uploaded to MS Excel. Statistical analysis was performed by IBM SPSS v23 (Mann-Whitney U).

All LYM, main subpopulations (T, T4, T8, B and NK) and additional populations (DN and DP T-cells and NK8) were evaluated. Relative counts from LYM and WBC were calculated. Median fluorescence intensity (MFI) that defines antigen density on membrane and its CV that reflects the expression heterogeneity were estimated for the kit antigens CD3, CD16+56, CD45, CD4, CD19, CD8 and for optical parameters FSC (cell size) and SSC (complexity of cell structure).

Results. As expected, percentage of LYM, T and T8 cells was significantly increased and T4 and B cells decreased in comparison to controls; NK count was similar. In addition, the study revealed significant increase of DP and decrease of NK8 cells. CD45 MFI was significantly decreased with increased CV in MN cases in all studied populations, CD3 was decreased in T, T4, T8 and DP cells, CD4 in T4 and DP cells, CD8 in T8 and NK8 cells, CD16+56 in NK and NK8 cells. SSC was significantly increased in all studied populations, except NK.

Conclusions. As previously reported, the atypical cells were CD8+ with high SSC. The study revealed other significant alterations, including less studied populations. Of interest is the new data on the appearance of DP cells that are usually very few in normal samples. One patient demonstrated that in rare cases DP cells could constitute the main proliferating population.

Unexpectedly, the expression of most lymphoid antigens was decreased, which could be the result of cell immaturity and / or activation. Simultaneously, phenotypic heterogeneity (by fluorescence CV) was increased. The finding is novel.

Due to selection criteria and small number of MN tests, the cohort may be not completely representative. A larger prospective study would be necessary to confirm the findings.