

Age and Gender-Related Variability of Double-Negative T-cells in Paediatric Patients

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Introduction. Double negative cells (DNC) is a small population of T-lymphocytes that are negative for both CD4 and CD8; DNC comprise 1–5% of peripheral lymphocytes in adults, little is known about DNC in children. DNC may exhibit helper (particularly in AIDS) or regulatory and suppressive properties. DNC relation to other T-cell populations and their biological role remain unclear. Multicolor flow cytometry (at least 4 fluorochromes) is necessary for direct assessment of DNC. BD Multitest 6-Colour TBNK kit for lymphocyte subsets calculates DNC counts as an additional parameter during routine testing.

Aim, Material and Methods. The aim of the study was to evaluate age and gender-related variations of DNC count in paediatric patients tested in 2012–2015 in Children's Clinical University Hospital. Anonymised patients' data (age and gender) were obtained from the Clinical Laboratory LIS. The 6-colour TBNK test was performed by BD FACSCanto II flow cytometer, DNC were routinely measured by "% T-Sum" parameter and their absolute counts were calculated manually. MS Excel database was created, IBM SPSS v.21 software was used for Mann-Whitney and Wilcoxon test.

Results. Reports of 3411 representative consecutive samples entered the study. Median DNC relative content in the whole cohort was 3.8% of T-cells, median absolute count was 0.074E9/L. DNC counts significantly correlated with age ($p < 0.001$ for relative and $p = 0.009$ for absolute counts). Further analysis revealed that relative counts gradually increased until the age 7, then formed plateau until age 13, followed by decrease. Absolute counts increased until the age 2, remained high until the age 7 and then gradually decreased. Furthermore, significant difference between genders was observed, male patients tended to have higher numbers of DNC: their median relative count was 3.8% of T-cells vs 3.7% in girls ($p = 0.03$) and absolute – 0.078E9/L vs 0.069E9/L ($p < 0.001$). Comparison of yearly medians showed significant differences for both groups, too ($p = 0.002$ for relative counts and $p = 0.012$ for absolute counts).

Conclusions. DNC population varies significantly in paediatric patients. The study demonstrated the age-related dynamics of both relative and absolute DNC counts from infancy into adolescence. Unexpectedly, the study revealed gender-related variability, DNC counts in boys being higher. Biological and clinical significance of these findings is unclear and requires further research.

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