Comparison of minimal residual disease detection by flow cytometry and quantitative PCR in children with acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood. Although almost all patients achieve complete remission, 20% of patients suffer from relapse. Patient with risk of relapse can be discerned by slower reduction of minimal residual disease (MRD). Flow cytometry (FC) is often used for MRD detection but still has limited impact on clinical decision. B-cell regeneration presents major challenge for specifity of cytometric MRD evaluation. Multi-colour cytometry-FACS sorting and detection of rearranged immunoglobulin and/or TCR genes using RQ-PCR (real-time quantitative polymerase chain reaction) were combined for MRD detection in this study. FC is cheaper and easier in comparison with molecular methods; the most complicated phase is the data analysis and intepretation. Data interpretation is based on deep knowledge of immunophenotype of normal bone marrow. High speed cell sorters nowadays enable effective population separation using up to 11 parameters. The populations were sorted and subsequently level of MRD by RQ-PCR was assessed. Combination of these two methods allowed PCR quantification of leukaemic cells with greater sensitivity, and testing the specifity of chosen immunophenotypic characteristic of residual leukaemic cells. Using these combined techniques, four to eight colour panels were tested for MRD monitoring. For proper MRD detection, Ig/TCR RQ PCR needs adequately concentrated DNA. Using sorted cell fractions, samples with lower DNA concentrations are usually obtained, thus it was indispensable to test efficacy of Ig/TCR RQ PCR in samples containing variable cell number. Experiments were performed using defined number of sorted cells (100000, 10000, 1000 and 100) of leukemic cell line REH, which corresponded to usual number of cells in target populations.

Key words: Minimal residual disease (MRD), Acute lymphoblastic leukemia, Flow cytometry, Quantitative PCR, Immunophenotypic characterization, MRD quantification