Hematological Disorders (B136 – B154)

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Detection of Residual Tumor Load and Pre-leukemic Cell Clone Using Combined Pheno- and Genotyping at Single Cell Level

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Background: After accidents, cancer is the second leading cause of death among children between age 1 to 14 years in the Western World and acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood. Since the amount and dynamics of residual tumor load presented during induction therapy is an independent prognostic factor in this entity, it is used increasingly in modern treatment protocols to define risk groups. We examined the tumor dilution in patients with ALL harboring t(12;21) translocation (*ETV6/RUNX1* gene fusion) following therapy using a combined cell-, RNA- and DNA based method.

Methods: 55 bone marrow samples withdrawn from 14 children according to the ALL IC-BFM 2002 protocol were investigated. Combined CD10 immunophenotyping and *ETV6/RUNX1* fluorescent in situ hybridization analysis was performed on cytospin preparations using automated microscopy. Results were compared to data acquired using reverse transcription polimerase chain reaction (PCR) targeting the *ETV6/RUNX1* fusion gene. If significant discrepancy between the results of cell- and RNA-based techniques was observed, the *ETV6/RUNX1* gene fusion was examined at DNA level using multiplex-PCR as well. The study was conducted in accordance with the Basic Principles of the Declaration of Helsinki.

Results: An average of 27,000 cells per sample were analyzed using the cell based method having sensitivity and specificity of 98.67% and 99.97%, respectively. A cell population positive for both CD10 and *ETV6/RUNX1* fusion was detected in 89%, 30%, 13% and 36% of cases at day 15, day 33, week 12 and month 5, respectively. Significant *ETV6/RUNX1* expression was not detected after day 15. In cases, where discordant results had been obtained using the cell- and the RNA based methods, additional chromosomal aberrations also detected at the time of diagnosis were found in cells positive for *ETV6/RUNX1* fusion at days 15 and 33. However, at week 12 and month 5, these secondary changes were not observed suggesting the presence of pre-leukemic cells instead of residual tumor load. The DNA-based multiplex-PCR analysis successfully validated the results of the cell based method.

Conclusions: Various PCR based techniques and flow cytometry are the most preferred methods to detect minimal residual disease in treated cancer patients. However, the molecular genetic techniques are not cell-based methods providing only indirect estimation regarding the real tumor load; furthermore, pathological phenotype other than that of normal cells is not always present making the interpretation of flow cytometry results difficult. These issues prove the relevance of our cell-based method collecting combined phenotypic and genotypic features at single cell level. What is more, we were also able to detect pre-leukemic clones that has great importance in the monitoring of therapy of children with ALL. These cell populations can serve as early indicators of an impending late relapse typical to this entity.

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Simultaneous Determination of Size and Shape of Human Blood Platelets Using Light-Scattering Flow Cytometry

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Background: blood platelets play a pivotal role in hemostasis. They are involved in many diseases, including myocardial infarction, stroke, peripheral vascular disease, cancer, and many infections. It is, therefore, important to evaluate platelet function. Different approaches have been developed, including those based on light transmission aggregometry, impedance counting, flow cytometry and others. But there is lack of techniques that directly assess platelets morphology, while several studies confirmed that it is related to some diseases. For instance, the platelet shape change during activation can be enhanced due to hypertension. Also a fraction of platelets can undergo activation without shape change in acute and recent cerebrovascular ischemia.

Methods: We used the light-scattering flow cytometry. This method is based on measurement of angle-resolved light-scattering patterns (LSPs) of individual cells and on solution of the inverse light-scattering (ILS) problem. We measured LSPs with the Scanning Flow Cytometer fabricated by CytoNova Ltd. (Novosibirsk, Russia, http://cyto.kinetics.nsc.ru/). The solution of ILS problem is based on fitting an experimental LSP with theoretical ones, modeling platelets as oblate spheroids. To accelerate the fit a precalculated database of 500000 LSPs is used. Thus volume, aspect ratio, and refractive index of each platelet in a sample are obtained. Additionally to these estimates (best-fit values) of platelet characteristics the method assesses the quality of fit by confidence regions of these estimates.

Results: We present a new approach for accurate and statistically reliable measurement of platelets morphology, including both size and shape, using light-scattering flow cytometry. We tested the new method on blood samples of healthy donors. Platelet volume distributions acquired by our method was comparable to conventional curves measured with Coulter counter. Aspect ratio values are also in good agreement with literature data. Moreover, aspect ratio distributions of intact and activated platelets were significantly different.

Conclusions: The presented method allows complete characterization of population of blood platelets. In particular, it can be used for label-free detection of activated platelets in blood. Another advantage of our approach is that microaggregates of platelets, if present, can be directly separated from single cells. It opens a way for a detailed study of early stage of platelets aggregation.

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Flow Cytometry-Based Enumeration and Functional Characterization of a Novel Regulatory Cell Expressing CD8 Phenotype from Multiple Myeloma Patients

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