even after a significant expression difference from normal HSCs; none of these markers was of a predictive value alone. However, distinct expression patterns of these antigens can be correlated with response to treatment and disease outcome. Two response groups (good and poor) were defined based on treatment response and overall survival (one year and more for good response group). It was observed that a bright expression of CD116, CD135 and CD24 was associated with good response group while over expression of CD25, CD90 and CD123 was associated with a poor outcome, high LSC counts (median LSC% in poor outcome group: 35% when compared to 5% in good response group) and response to treatment. It was seen that LSC in good response group showed specific cell signaling when stimulated with different cytokines while multiple pathways were observed to be constitutively expressed in poor response group.

Conclusion: An initial LSC screening panel consisting of these markers at the time of diagnosis and knowledge of constitutively stimulated cell signaling pathways can act as a helpful predictive tool in acute leukemia.

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Activity of Aldehyde-Dehydrogenase in B-Cell and Plasma Cell Subsets of Monoclonal Gammopathy Patients

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Multiple myeloma (MM) is characterized by the presence of clonal plasma cells (PC) arising from malignant transformed B-cells. Aldehyde-dehydrogenase (ALDH) is an intracellular enzyme catalysing degradation of aldehydes to protect the cell against a toxic. It is active in hematopoietic stem and progenitor cells and its increased activity was detected also in some types of cancer stem cells.

The aim of this study was monitoring of ALDH activity in B-cell and plasma cell subsets in monoclonal gammopathy patients to identify potential source population of myeloma progenitors.

Bone marrow of 11 newly diagnosed MM and 9 MGUS patients were analysed by 8-color flow cytometry. Identification of $CD45^-$ immature, $CD45^+$ immature, naïve, memory (with/without isotype switch) and switched $CD27^-$ B, as well as plasmablasts and PCs ($CD19^+$PCs, $CD19^-$PCs and $CD138^{-/dim+}$ PCs) was performed according to expression of surface markers CD38, CD45, CD20, CD138, CD19, CD27 and IgM. Aldefluor assay was used to identify activity of ALDH in individual subsets. Rate of ALDH activity was assessed based on percentage of ALDH positive cells (%pos) and ratio (r) of median fluorescence intensity (MFI) of ALDH and MFI of negative control. Statistical significance of differences in continuous variables among groups of patients was analysed using nonparametric Kruskal-Wallis or Mann-Whitney U test test.

Higher ALDH activity in $CD45^-$ immature B in comparison with naïve B was found in both, MM and MGUS according to MFIr [1.32 (1.08-1.79) vs. 1.03 (0.72-1.59) and 1.34 (0.7-1.51) vs. 1.12 (0.65-1.18), resp., $p<0.05$], and in MM also according to %pos [8.0 (4.0-22.6) vs. 1.5 (0.1-27.6), $p<0.05$] . In MM there was found higher ALDH activity in $CD19^+$PCs, $CD19^-$PCs and $CD138^{-/dim+}$ PCs compared to naïve B based on MFIr [1.27 (0.77-1.64) ; 1.55 (0.80-2.44); 1.29 (0.66-1.93) and 1.03 (0.72-1.59), resp., $p<0.05$], as well as %pos [5.9 (1.3-19.7); 19.2 (0.4-52.9); 7.5 (0.4-35.1) and 1.5 (0.1-27.6), resp., $p<0.05$]. No difference was found among PC subsets and also between individual PC subsets and $CD45^-$ immature B. There was found higher ALDH activity in $CD19^-$PCs compared with naïve B based on both MFIr as well as %pos [1.52 (1.13-1.87) vs. 1.12 (0.65-1.18); 17.1 (12.6-43.8) vs. 3.1 (1.2-8.2), $p<0.05$], while no difference was found between $CD19^+$PCs and naïve B in MGUS. Moreover there was found higher ALDH activity in $CD19^-$PCs compared with $CD19^+$PCs based on MFIr [1.52 (1.13-1.87) vs. 1.18 (0.77-1.34) $p<0.05$], as well as %pos [17.1 (12.6-43.8) vs. 3.5 (1.3-11.5) $p<0.05$].

Increased ALDH activity in immature B-cells is probably caused by presence of very immature cells which retain some properties of stem cells. The rate of ALDH activity in $CD19^-$PC subsets is same as in immature B-cells, so it could indicate the presence of myeloma initiating cells exactly among these PCs subsets.

Supported by:

IGA NT12425 and GACR P304/10/1395.

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A Shape-Based Study of Platelet Activation with the Scanning Flow Cytometry

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Background: Blood platelets play a central role in hemostasis and are involved in many diseases, including thrombosis, hemorrhage, inflammation, and cancer. Platelet activation occurs normally in response to vessel wall injury or other physiological stimuli. It is very dangerous when become inappropriate, i.e., when platelets activate spontaneously or due to low dose of agonist. There are several approaches to assess the strength of platelet activation measuring the fraction of activated platelets with surface receptors labeling and the flow cytometry. We proposed an alternative technique based on the measurement of platelet shape, also being a marker of platelet activation (Moskalensky *et al, J. Biomed. Opt.* **18**, 017001, 2013). In current work, we present experimental data obtained for different patients using this technique.

Methods: We use the scanning flow cytometry, which is based on the measurement of angle-resolved light-scattering profiles (LSPs) of individual cells and on the solution of the inverse light-scattering (ILS) problem.

We measure 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. The measurement of platelets size and shape and hence the fraction of activated cells is possible without labeling and/or fixation. This reduces the sample preparation to the sole dilution of the whole blood or platelet plasma. Also an agonist (ADP or collagen) is added prior to measurement to induce platelet activation.

Results: The study of platelets with the scanning flow cytometry was performed for several patients. The platelets volume distributions determined by the solution of the ILS problem agree well with the results of standard analysis. The determined platelet shape show bi-modal distribution, corresponding to presence of resting and activated platelets. The ratio between resting and activated cells correlates well with the dose of agonist and varies from patient to patient.

Since no fixation step is required, a gradual transition of platelets from one state to another is visible over time, which can also be used for characterization of the platelet function. This transition occurs in first minutes after addition of agonist.

Conclusions: The shape-based approach to detect activated platelets can assist in diagnostics of platelet-associated disorders. It does not require labeling step, which facilitates rapid tests in clinical setting. Novel parameters, such as shape distribution and the time-

dependent activation course, are obtained using this approach. Further clinical studies are needed to correlate different disorders and these parameters.

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Flow Cytometry Analysis of Plasma Cell Phenotype in Extramedullary Relapse of Multiple Myeloma

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Background: Multiple myeloma (MM) is the second most common haematological malignancy in the world which is characteristic by a presence of clonal plasma cells (PCs). The introduction of new drugs (thalidomide, bortezomib, revlimid) has dramatically improved survival of MM patients, but MM still remains an incurable disease. Unfortunately, an increase in the incidence of extramedullary relapse of MM (EM), an aggressive mostly resistant entity with abysmal prognosis for patients has been reported. EM can affect any area of tissue - soft tissue involvement can be with or without relationship to bone marrow (BM). Finding a marker and/or phenotype allowing predict a risk of EM formation could help to improve treatment strategies and prolong patient life.

Aim: Analysis and comparison of PC phenotype in bone marrow and tumor tissue (TU) of EM cases.

Subjects and methods: There were analysed 28 MM patients (median age 62 years) with confirmed EM. Analysis of 25 bone marrows and 19 tumor tissues was done by polychromatic flow cytometry. Phenotype of $CD38^+CD138^+$ PC was analysed using CD19, CD20, CD27, CD28, CD44, CD56, CD81, CD117 and nestin. PCs from BM and/or TU were considered positive for given marker when its expression on PCs was higher than 20%.

Results: There were detected $CD38^+CD138^+$ PCs in all BM samples and in almost all TU samples (except 1 sample probably acquired not from tumor site). PC infiltration was significantly higher in TU then in BM [median of infiltration 51.7% (range 0.0-94.3) vs. 1.5% (0.01-93.9); $p<0.001$]. Although TU PCs have relatively similar phenotype like BM PCs, there was found significant decrease in median of expression of CD27 [18.1% in BM (range 0.5-92.0) vs. 1.8% in TU (0.0-63.0); $p=0.01$] and also in number of $CD27^+$ PCs [12/24 (50%) in BM vs. 2/16 (12.5%) in TU]. Moreover, no presence of $CD19^+$ normal PCs on tumor PCs was found even if mixture of $CD19^+$ and $CD19^-$ PCs was detected in BM ($p<0.001$). EM PCs (BM and/or TU) were almost negative for CD20 (5.3% $CD20^+$ PCs in BM and 6.3% in TU) and CD81 (12.5% $CD81^+$ PCs in BM and 14.3% in TU). On the other hand, EM PCs usually express nestin, a marker of stem/progenitor cells, which was found in 81.8% BM PCs and 100% of TU PCs; and EM PCs were always positive for CD44, a glycoprotein involved in cell-cell interactions, cell adhesion and migration. Adhesion molecule CD56, which loss is considered as a marker of extramedullary spread, was found on majority of EM PCs (70.8% $CD56^+$ PCs in BM and 75.0% in TU).

Conclusion: Flow cytometry analysis of BM PCs allows to identify a phenotype profile ($CD20^-CD27^-CD81^-CD44^+nestin^+$) related to possible extramedullary involvement in multiple myeloma.

Acknowledgements: This work was supported by MSM0021622434, IGA NT12425-4, IGA NT12130-4 and GACR P304/10/1395 grants.

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Normal Thymocyte Differentiation: Antigen Mapping and Multi-Dimensional Display in the Diagnosis of Thymoma

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Flow cytometric evaluation in cases of thymoma can be extremely beneficial in ruling out T-cell acute lymphoblastic leukemia. This requires the flow cytometrist to be thoroughly familiar with the expected phenotypic profiles expressed by differentiating human thymocytes. In normal thymus, early T-cell precursor stages expressing CD34 and CD10 (earliest precursor) and CD10 alone (late precursor) can be identified that are analogous to the two bone marrow stages of precursor B-cells. During thymocyte maturation, the T-cell precursors first up-regulate CD1 expression followed by CD4. Once CD4 expression density peaks, CD8 expression is initiated. As CD8 reaches peak surface density, CD3 surface expression is initiated. Initiation of CD8 acquisition is associated with CD34 down-regulation. As CD3 surface expression is up-regulated, CD10 expresssion is lost and the cells will ultimately down-regulate CD4 or CD8 to become a mature naive T-cell. We report here a case of thymoma presenting in the lung that on standard flow analysis was suspicious for T-cell ALL. The T-cells present were predominantly CD1 and CD4 positive with variable low-density CD3 and CD8 expression suggesting a more uniform precursor T-cell population. We utilized an antigen mapping technique (1,2) and multi-dimensional display to look for the characteristic patterns of thymocyte differentiation. By comparison to a normal thymus study, the thymoma-associated T-cell precursors demonstrated a decrease in peak CD8 density that gave the perception of a more phenotypically uniform population by standard analysis. On multi-dimensional display the classic patterns of thymocyte differentiation could be convincingly demonstrated. We present here detailed normal patterns of thymocyte antigen loss and acquisition for reference in evaluation of suspected cases of thymoma. We compare those patterns to the thymoma-derived precursors in the current case using an antigen mapping approach and multi-dimensional display to demonstrate the utility of this approach.

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236/B98

Flow Cytometric Quantification of the Reactive Oxygen Species Levels in Human Cord Blood, Mobilized Peripheral Blood and Adult Bone Marrow Progenitors

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Oxidative metabolism results from the balance between reactive oxygen species (ROS) and the antioxidant system. The level of ROS is an important regulator of hematopoietic stem cell (HSC) self-renewal and lifespan in murine hematopoiesis. It is established that murine HSCs have a low level of ROS which is correlated with stem cells properties (Jang YY and Sharkis SJ, Blood 2007) and we recently reported that *Gpx3*, a gene encoding for the ROS scavenger glutathione peroxydase 3, is a determinant of the selfrenewal of HSCs (Herault O *et al*, J Exp Med 2012). All these