Issue Highlight

Issue highlight – July 2018

This July issue of Clinical Cytometry B includes 11 original papers, 6 brief communications, 2 letters to the editor and a review article dealing with cancer-related mRNA expression analysis using a novel flow cytometry-based assay. The flow data from Depreter et al. showed a good correlation with the gold standard, RT-qPCR technique (1). Interestingly, *Wilms' tumor 1* (WT1) gene levels were shown to be significantly higher in AML patient samples with WT1 gene overexpression, previously defined by RT-qPCR. Moreover, *WT1* overexpression was distinguishable between heterogeneous cell populations and was also documented in rare leukemic stem cells (LSC). This study also showed *comparable results using fresh, short-term and long-term cryopreserved samples, thus allowing the analysis of RNA samples on a retrospective basis*. The paper by Depreter et al. provides crucial information concerning the ability to detect single-cell or rare cell population-specific gene expression changes in acute myeloid leukemia (AML) samples. In both adult and pediatric AML, persistence of rare LSCs are thought to be causative for the high relapse rates, therefore, an increasing need for advanced instrument analytical tools, able to elucidate coding and non-coding gene expressions at single-cell level, has emerged, and this study may help in clarifying this aspect (2–5).

Taking into account that the liquid biopsy is becoming a powerful diagnostic tool in solid tumor diagnosis, it is conceivable that this test may play a role for the detection of cancer cells from a tumor that are circulating in the blood and may help find cancer at an early stage. It may also be used to help plan treatment or to find out how well treatment is working or if cancer has come back (6,7). Furthermore, since accumulating biological evidence support the notion that current chemotherapy drugs will not be effective for leukemic stem cells killing, or at least has the capacity to target both the leukemic and normal stem cell populations, new strategies are required that specifically and preferentially kill the malignant stem cell population, while sparing normal stem cells. The possibility to detect LSC using this novel flow cytometry-based assay may allow the identification of suitable drugs targeting specifically LSC (6–8).

Original articles published in this issue were related to four main fields (1) immunophenotyping of lymphoproliferative disorders or normal cells, (2) the detection of a rare acquired genetic disorder Paroxysmal nocturnal hemoglobinuria (PNH), (3) immunology science with particular emphasis on monocytes, and (4) cell function.

In the paper by Starostka et al., a quantitative assessment of informative immunophenotypic markers in mature CD5-positive B-cell neoplasms was performed,

Francesco Lanza

and data showed that this approach may increase the diagnostic value of immunophenotyping in several chronic lymphoproliferative disorders (9). Based on their study, the most informative markers for the distinction of CLL/SLL, MCL, CD5+ MZL were the MFI values of the following markers: CD79b, CD20, CD23, CD43, CD38, CD11c, FMC7, CD200, kappa light chain, and their combinations. CD23 and CD200 were the most discriminant between CLL/SLL and MCL and CD23 plus CD79b between CLL/SLL and CD5+ MZL. However, this quantitative marker investigation failed to accurately distinguish MCL and CD5+ MZL. I, therefore, believe that this paper highlights the data mining methods for the analysis and selection of the most informative immunophenotypic markers and may help designing a predictive model, possibly minimizing the subjectivity of expert based assessment (10–12).

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As far as Dr. Auat's paper is concerned, a flow cytometry measurement of CD307a antigen expression in B-cell malignancies and during normal B-cell maturation has been investigated with the main aim to explore the diagnostic role played by this molecule during normal and leukemic differentiation (13). The results indicate that the flow cytometry assessment of CD307a expression could be helpful to distinguish CLL from MCL, and the latter from MZL. Although these results are not entirely conclusive, they provide a basis for further studies in a larger cohort of patients. Interestingly, B-lymphoblasts from acute lymphoblastic leukemia patients exhibited minimal expression of CD307a, displaying a similar expression pattern to that of normal B-cell precursors. This study may be considered in the construction and use of flow cytometric assays for minimal residual disease detection (14–16). The identification of novel antigenic markers to target the neoplastic cells through engineered monoclonal antibodies remains one of the main goals of the most recent treatment modalities, and this paper may provide valuable information in this research field (16).

The second theme covered in this issue is represented by PNH. PNH is an acquired clonal stem cell disorder characterized by intravascular hemolysis, deep venous thrombosis, bone marrow failure and increased susceptibility to infections. PNH is a stem cell disorder caused by a PIG-A gene mutation (17–19). As a consequence, blood cells completely or partially lack surface proteins that are tethered to the membrane through the glycosylphosphatidylinositol (GPI) anchor. The resulting absence of CD55 and CD59 molecules on red cells renders them sensitive to complement-mediated intravascular hemolysis and associated hemoglobinuria. Over the last 10 years, PNH testing by flow cytometry has become a common laboratory test in the clinical practice (20–22).

In the paper published by Blaha et al., authors tested the monoclonal anti-CD157 antibody clone SY11B5, for high sensitivity detection of PNH clones on the leukocyte population (23). Results showed that this reagent fails to detect a common polymorphic variant encoded by BST-1. The failure of anti-CD157 antibody clone SY11B5 to detect a common SNP can explain some CD157-negative cytometric data. This provides crucial knowledge for laboratories performing PNH analyses as such results can potentially lead to false-positive PNH interpretation. Due to the rare incidence of PNH, as well as rare genetic variations such as CD157, we suggest that flow cytometric analysis of PNH cells must not be based on lack of expression of just a single GPI-anchored marker. Rather a consistent deficiency of at least two markers on at least two different cell types is required.

In the second manuscript related to PNH, one of the leading scientist in this field of investigation, Rob Sutherland, compared high sensitivity 5-, 6, 7-color flow cytometry analysis using Becton-Dickinson and Beckman Coulters cytometers (24). Assessment of >40 PNH samples showed that the FLAER-based data derive virtually identical data to the non-FLAER results for neutrophils ($R2 = 1$) and monocytes ($R2 = 0.9999$). Furthermore, both Canto and Navios platforms gave rise similar data with 7-, 6-, and 5-color versions of the assay. Interestingly, analysis of non-PNH samples confirmed extremely low background rate of PNH phenotypes (neutrophils and monocytes) with all three approaches. In conclusion, this paper describes, for the first time a series of high-sensitivity, single tube flow assays for the simultaneous detection of GPI-deficient leukocyte subsets across both 3-laser Navios and Canto technologies. The 7-color variants of these assays confirm the earlier findings of Marinov et al. that FLAER is not an absolute requirement for high-sensitivity flow assays to detect PNH clones in PNH and related diseases (25). These results may be regarded as a practice changing approach (20–22,26).

Due to its high sensitivity, Lindemann et al. focused on the role played by the flow cytometry crossmatch (FCXM) for identifying an optimal living donor in kidney transplantation (27). In particular, the effect of ABO incompatibility on T cell flow cytometry crossmatch has been investigated, allowing authors to conclude that ABO incompatibility was associated with higher T-FCXM responses, especially in recipients with blood group O. This finding has major impact on the interpretation of flow crossmatch results. Based on these data, authors have postulated that current cut-off values need to be reassessed in the ABO incompatible setting (28).

Regarding Cecilia Langenskiold's investigation, authors established a flow cytometry antibody panel that can be used to determine granulocytes, monocytes and lymphocyte subset concentrations in fresh and frozen whole blood using TruCount technology (29). Using this method whole-blood samples can be frozen using a simple preparation method, and stored long-term before accurate determination of cell concentration. This allows for standardized analysis of the samples at a reference laboratory in multi-center studies. This methodological improvement may be helpful in routine flow cytometry analyses (30).

Wonner et al. investigated the effects of acute exercise on monocyte subpopulations in patients with metabolic syndrome (31). Results seem to demonstrate that strenuous exercise, even if it is not sufficient for weight loss, might exert a positive effect on vascular plaque formation by removing pro-inflammatory monocytes from the endothelium. However, as outlined by authors, this hypothesis needs to be further investigated, since strenuous exercise was able to mobilize the same amount of proinflammatory monocytes in MetS patients as in healthy persons; it is conceivable that the elevated basal level of these cells in MetS patients is likely to be caused by enhanced maturation rather than chronic mobilization. The removal of these monocytes from the endothelium might be part of the beneficial effect of exercise on vascular disease in long term studies (32,33).

Conventional data analysis of flow cytometry-based basophil activation testing requires repetitive, labor-intensive analysis that hampers efforts to standardize testing for clinical applications. Using an open-source platform, Patil et al. developed and implemented a

programmatic approach to the analysis of the basophil activation test (BAT) by flow cytometry. This novel method provided a high throughput objective approach to basophil activation analysis (34).

Del Zotto et al. published an interesting paper dealing with a rare genetic disorder, called Fibrodysplasia Ossificans Progressiva (FOP), caused by sporadic heterozygous mutations in *ACVR, that is* a gene which progressively leads to severe heterotopic ossification (35). The peripheral blood mononuclear cell immunophenotyping by flow cytometry in samples from this genetic disorder provided evidence for monocyte DNAM1 up-regulation, as well as significant differences in the expression profile of CXCR1 (CD181), CD62L, CXCR4 (CD184), and HL-DR molecules. Based on the notion that DNAM1 had been previously shown to play a pivotal role in monocyte migration through the endothelial barrier, the increased expression detected in patients' monocytes might suggest a role of this surface receptor during the early phases of FOP flare-ups in which the activation of the immune response is believed to represent a crucial event.

The original paper by Chutvanichkul dealt with labile iron pool (LIP) as a parameter to monitor iron overload and oxidative stress status in the erythrocyte population from β-thalassemia patients (36). LIP is intracellular non-protein bound iron that can generate oxygen radicals via the Fenton reaction resulting in oxidative cell damage. As a consequence, quantitative assessment of LIP may be helpful for detecting and monitoring the toxic iron status in iron overloaded patients. Based on authors results and speculation, it may be hypothesized that LIP assay may represent an alternative test to monitor the magnitude of iron overload and its consequent oxidative stress in β-thalassemia patients. LIP level may also be used as a marker for a careful evaluation of the therapeutic response to iron chelation treatment.

The investigation from the group of North Texas University had the main scope to study the effects of a single high-fat meal on monocyte adhesion molecule expression, CD36 expression and acLDL endocytosis using image-based flow cytometry. Results showed that consumption of a high-fat meal was associated with an increased adhesion molecules expression in both classical and non-classical monocytes, scavenger receptors, and propensity to form foam cells (37).

The current issue of Cytometry also includes six interesting brief communications and two letters to the editor, which all deserve a careful evaluation from the readers.

I do believe that the flow cytometry community is today very active, productive, and vital, and in the next years a lot of developments in this area will enrich our knowledge in many research and clinical disciplines, thus enhancing the use of flow cytometry as an ever-increasing powerful diagnostic tool.

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