Issue Highlights

Issue Highlight 2015

This issue of Cytometry includes four papers dealing with the utility of flow cytometry (FC) immunophenotyping, together with fine-needle aspiration cytology (FNAC) or standard core biopsy in the diagnosis of extra-hematological tissues obtained from patients with different types of malignancies. These include thyroid lymphomas, breast implant- associated anaplastic large-cell lymphomas, intracranial meningiomas, and brain tumors (1–4). FC studies are considered as a very useful tool for the diagnosis and classification of T, B and NK-cell lymphoid neoplasms, as well as of myeloid malignancies (5–10). Although FC is able to provide objective and quantitative results, even on very small samples and within few hours, it is not routinely applied in the evaluation of solid tissues suspected of lymphoma. The aim of the retrospective studies by Stacchini et al. and Montgomery-Goecker et al was to analyze the contribution of FC immunophenotyping to the detection of neoplastic cells in solid tissues suspicious for NHL (1,2). As far as the analysis of mengioma is concerned, the DNA ploidy and cell cycle phase determined by flow cytometry proved to be useful indicators for assessing biological behaviors in meningiomas. In fact, there was a significant difference in G0/G1 phase, S phase and mitoses fraction between benign and atypical/anaplastic meningiomas. Based on these findings, it can be speculated that this parameter represents tumoral grading and risk of recurrence in patients with meningiomas (3). With regards to brain tumors, it has been speculated that during surgery the differentiation between glioma versus gliosis or low-grade astrocytomas versus glioblastoma multiforme is important. Based on cell cycle analysis, low grade-gliomas can be distinguished from high-grade gliomas. Moreover, cell cycle analysis may have an additional prognostic role. Intraoperative cell cycle analysis permits not only the characterization of tumor aggressiveness but also the identification of tumor margins during glioma surgery.

The papers published in this issue provided evidence that FC is a reliable and precise tool for the evaluation of immunological markers as well as of cell cycle kinetic and proliferative activity on each tissue specimen. Furthermore, it can be speculated that FC, at least in these circumstances may avoid open biopsy, thus allowing tailored treatments with clinical and survival potential implications.

However, we do believe that only through the collaboration among expert practitioners expert in different fields will it be possible to achieve a correct diagnosis of these tissues. Overall, these manuscripts confirmed the efficacy of the use of FC for the diagnosis of these diseases, and provide objective, quantitative and rapid results for the evaluation of solid tissues suspected for lymphoma or other tumors (1–4).

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[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Two further papers discuss the inclusion of novel immunological markers in antibody panels for various leukemias (11,12). This is a critical issue especially in regions with limited resources. In that instance the minimal essential monoclonal antibody (MoAb) panel has to be delineated by experts with the main aim to provide basic principles and quality management guidelines (13,14). These guidelines should be comprehensive and must describe the minimal essential features of designing such a flow cytometry laboratory, and they should provide a list of equipment and supply needs and staffing features of a laboratory's design, setup, recommendations and product testing services, other than discussing the basic principles behind the most frequent procedures in FC immunophenotyping (16–19). This is a prerequisite to achieve the correct final diagnosis in given hematological disorders, based on a number of basic principles including the cost-effectiveness acceptable for services with different economic possibilities (14–19).

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The paper by De Vita et al. highlights the importance of interference between monoclonal antibodies as a source of error when analyzing multicolor flow cytometry data (20). The purpose of this report is to underline a technical variable due to epitope proximity that should be considered in the formulation of schemes of immune cell analysis. Knowing which MoAbs may interfere with the staining of other MoAbs in multicolor panels should be assessed beforehand, especially when the distance between epitopes is unknown and reagent panels are aimed to explore immune cell populations for which there are no defined reference intervals. This is a further report highlighting the necessity of standardizing the FC analysis in the various laboratories across the world.

The paper by Hedley and Keeney focuses on combined platelet enumeration and reticulated platelet (RP) determination by FC, with the scope of refining previous work and gating strategies distinguishing RP from mature platelets while incorporating accurate platelet enumeration into the analysis (21). These authors have shown that by combining CD41/CD61 platelet enumeration with thiazole orange, the RP percentage can be calculated by combining CD41/CD61 platelet enumeration with thiazole orange. However, the accurate RP percentage requires an effective gating strategy, as background fluorescence cursor placement is arbitrary. This method for enumeration of RP percentage combined with accurate platelet enumeration, particularly in the low range, could be useful in differentiating production from consumption issues in thrombocytopenia and monitoring response to therapy (21).

Dorwal et al. reported a subject with a typical CLL phenotype and Matutes score of five, showing the expression of two NK-associated immunological markers, namely CD56 and CD57 (22). These authors speculate that this entity may represent a rare subtype of CLL which needs to be studied more extensively for its prognostic implications. Although there have been multiple reports of expression of T cell associated markers on the B-CLL cells, the expression of CD56 and CD57 on B-CLL cells was never observed.

The paper by Whitby et al. reviewed current laboratory practices in FC for the enumeration of CD4+ T-lymphocyte subsets (23). These authors report the findings of a survey questionnaire issued to 1587 clinical FC laboratories to determine whether the UK NEQAS for Leucocyte Immunophenotyping Lymphocyte Subset External Quality Assessment (EQA) programme was suitable for current laboratory needs and practices, and to assess the impact of these responses on the clinical practice where CD4+ T-lymphocyte subsets analysis is undertaken.

The responses revealed major methodological variations between centers undertaking CD4+ T lymphocyte subset analysis. Based on these findings, it can be postulated that despite the availability of international guidelines, a lack of concordance amongst laboratory technique still exists. Such variation could adversely impact on patient care and clinical trial data, and therefore it is recommended that centers undertaking flow cytometric CD4+ T-lymphocyte subsets analysis urgently review their methodologies and normal ranges (23,24).

Levi et al. focuses on the development and implementation of a relatively novel methodology in order to optimize the bio-ferrography (BF) immunomagnetic isolation (IMI) procedure for the EGFR high positive circulating tumor cells (CTC) application (25). I would like to remind readers that BF allows the magnetic isolation of target cells and tissues on a microscope slide (ferrogram) and their microscopic, chemical, and biological characterization while preserving their original shape. The results observed are quite interesting, and due to the limited presence of CTCs in blood circulation, viable CTCs isolation technology needs to supply a very high recovery rate (26,27).

In the paper by DiGiuseppe et al., viSNE was used to detect minimal residual disease (MRD) in B lymphoblastic leukemia (28). viSNE is a recently developed computational tool based on the t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm that has been shown to be capable of detecting synthetic "MRD-like" populations of leukemic cells created *in vitro*. *In vivo* results are encouraging but need to be confirmed in larger series of patients through a comparison with a FC-based method (29–31).

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