

Flow Cytometry Immunophenotyping News from ASH 2013-

CHAPTER 2

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LYMPHOCYTE SUBPOPULATIONS

Description and/or quantification of several lymphocyte subpopulations have been evaluated in a varied number of hematological malignancies.

Response to therapy in **CML** patients has been evaluated by 2 groups. **Colom Fernández et al.** made a thorough description of the immunophenotypic changes that dasatinib produces on the immunophenotype of NK-cells from patients diagnosed with chronic myeloid leukemia (n=19). After comparison with healthy donors (n=12) and patients treated with imatinib (n=9), NK-cells in DA-treated patients expressed a highly differentiated phenotype: CD57^{high}, Nkp30^{low}, Nkp46^{low} and CD161^{low} ([Abstract 1475](#)).

Sopper et al. focused on 50 newly diagnosed CML patients receiving nilotinib as frontline therapy, and described its immunomodulatory effects on a wide range of lymphocyte subsets. These authors conclude that changes after therapy are most likely due to normalization of the peripheral blood compartment, and that a specific immunological signature associated to response to nilotinib was not identified ([Abstract 2731](#)).

Two groups concentrated on description of lymphocyte subsets in **CLL** patients. **J.Rueter et al.** used a specific FC analysis program to identify distinct cell type distributions after comparison of controls (n=13) and CLL patients (n=55) ([Abstract 2864](#)).

Grigore et al. compared the distribution of several cell types in untreated CLL patients (n=85) and CLL patients with advanced stage who failed to respond to 1 or more lines of treatment (n=63). Treated patients had significantly reduced counts of total and memory normal B-cell subsets. Also, the absolute count of CD4⁺ T cells and CD4/CD8 double negative TCRαβ cells were significantly lower in patients with >1 line of treatment ([Abstract 4158](#)).

Three groups analysed different immune cell populations in the context of transplantation. **T. Hahn et al.** evaluated a cohort of 70 MM patients who received high dose melphalan and autologous hematopoietic stem cell transplantation. They confirmed the association of absolute lymphocyte count recovery with prolonged progression-free (PFS) and overall survival (OS). They also showed that an increase in the relative proportions of CD4⁺ and CD8⁺ effector cells, and a decrease in CD4⁺ and CD8⁺ central memory and Treg populations demonstrated that the pre-ASCT effector cell phenotype contributed to faster absolute lymphocyte count recovery, and improved PFS/OS. ([Abstract 3348](#))

M.V. Gonçalves et al. analysed the impact of NK subpopulations before and at different points after recovery on allogeneic hematopoietic stem cell transplantation. The study included 111 patients (most of them diagnosed with acute leukemia), who received BM (46%), umbilical cord (32%), or peripheral blood (22%) from unrelated (n=90) or related donors (n=21). This Brazilian group concluded that low counts of the subpopulation CD56⁺⁺/CD16⁻ in the first week after HSCT are associated with increased non-relapse related mortality ([Abstract 4625](#)).

D.M. Warad et al. used a combination of whole blood quantitative FC and bioinformatics to generate immune profiles among patients with graft-versus-host disease (GVHD) who could benefit from extracorporeal photopheresis (ECP), a therapy that has demonstrated efficacy in a proportion of GVHD patients that are steroid refractory. They found that patients who

responded to ECP had lower numbers of lymphocytes, CD4 T cells, CD4⁺CD69⁺ T cells, and CD8⁺CTLA4⁺ than healthy controls and non-responders ([Abstract 3260](#)).

The last abstracts concerning lymphocyte subpopulations concentrate on patients with Hodgkin disease and sickle cell disease. Considering promising immunotherapy regimen drugs for patients with classical Hodgkin lymphoma, **J.M.K. Silva *et al.*** evaluated the behaviour of peripheral blood regulatory and effector CD4 T cells through surface expression of CTLA-4 and CD127, respectively. They found a negative correlation between these 2 molecules: the percentage of CD4⁺CTLA-4⁺ T cells was significantly increased at diagnosis as compared with healthy controls, and decreased after therapy, and, conversely, CD4⁺CD127⁺ T lymphocytes were decreased at diagnosis, with values increasing after therapy. ([Abstract 4240](#))

Finally, **R.S. Nickel *et al.*** evaluated how chronic transfusion (CT) and hydroxyurea (HU) affected several immune populations in 111 children diagnosed with sickle cell disease (SCD). They found that these children had quantitative differences in many different immune cell populations compared to age and race matched healthy controls. HU treatment appeared to bring the absolute number of these cell subsets closer to those of healthy controls, while CT did not. ([Abstract 2208](#))

MATURE B-CELL NEOPLASMS

Three groups focused their studies on CLL. **T. Köhnke *et al.*** aimed to improve the CLL diagnostic score from Dr. Matures through the addition of CD200. They concluded that CD200⁺/CD23⁺/CD5⁺/FMC7⁻ and low or absent CD79b on CD19⁺ B-cells exhibited a significantly higher specificity than the classical score system, and maintained very high sensitivity ([Abstract 4150](#)).

E. Cornet *et al.* determined the incidence of atypical CLL defined as those cases with typical immunophenotypic features of CLL, absence of histological evidence of mantle and marginal cell lymphoma, and lack of all the diagnostic criteria of CLL proposed by Hallek *et al.* (Blood 2008). Among 1,819 B-cell chronic lymphoproliferative cell disorders diagnosed by flow cytometry in a single centre between 2000 and 2013, 127 cases (7%) met the diagnostic criteria of atypical CLL. The authors discuss the importance of a multidisciplinary approach for the identification of this entity considering its different outcome as compared with CLL ([Abstract 1770](#)).

Finally, **P. Bulian *et al.*** presented a worldwide multicenter analysis in order to validate the prognostic significance of CD49d in a series of 2,972 patients with CLL. Using a cut-off point of ≥30% of neoplastic cells expressing CD49d, this molecule emerged as the strongest flow cytometry-based predictor of overall survival and treatment free survival for patients with CLL ([Abstract 672](#)).

Two different groups concentrated on the study of diffuse large B-cell lymphoma (**DLBCL**). Patients with this disease can be divided into prognostic subgroups according to the cell of origin of the lymphoma: germinal centre B-cell-like (GCB) and the activated B-cell-like (ABC). The Gene Expression Profile is the “gold standard” method for defining these prognostic groups. An interesting work from **M. Kusakabe *et al.***, designed a 10-color flow panel for DLBCL cell lines, in an attempt to propose FC as a useful tool to predict the cell origin from freshly disaggregated patient lymph node biopsy specimens. Five out of 6 GCB and 4 out of 6 ABC DLBCL cell lines were

correctly assigned. CD27 and BCL6, and CD44 and IRF4, were the most informative markers for the identification of GCB and ABC subgroups, respectively ([Abstract 4301](#)).

Another study from **R.M. de Tute *et al.*** investigated the frequency of peripheral blood clonal B-cell populations and B-cell subset abnormalities in 358 newly diagnosed DLBCL patients. Abnormalities were detected in 52% of cases, and B-cell lymphopenia was the most frequent finding (94/278). They also highlight that presence of a circulating clonal population with a germinal-centre phenotype was highly predictive of the GCB DLBCL subtype. (84% GC-type populations detected were in GCB cases) ([Abstract 3013](#)).

Finally, **S. Fujiwara *et al.*** evaluated the expression of CD25 in a large number of patients (n=437) diagnosed of high and low-grade mature B-cell lymphomas and discussed the diagnostic and prognostic implications on its expression on different B-cell lymphomas ([Abstract 4308](#)).

MULTIPLE MYELOMA (MM) and related disorders

A. Behdad *et al.* presented a sensitive 9-color (cytoplasmic κ /cytoplasmic λ /CD45/CD38/CD56/CD138/CD19/CD117/CD20), 11-parameter, two-tube FC assay for diagnosing patients with paraproteinemias, and searching MRD in MM. Evaluation of 363 samples and comparison of the FC results with morphology, immunohistochemical studies and serum/urine M-protein measurements were used to validate this assay. ([Abstract 3129](#))

M. Schmidt-Hieber *et al.* investigated the biological significance of CD56 expression on clonal plasma cells. According to their work, CD56⁺ MM PCs had a counterpart of CD56⁺ normal PC, and were associated with unique disease characteristics: t(4;14), DNA hyperdiploidy, and a higher frequency of major bone lesions. ([Abstract 751](#))

W.I. Gonsalves *et al.* presented 2 different works exploring the prognostic value of flow cytometry quantification of circulating clonal plasmatic cells (PCs) in patients with MM. In their first work, they evaluated 158 consecutive patients with newly diagnosed MM and, according to their findings, a cut-off of ≥ 400 clonal events/150,000 gated mononuclear events predicted for a median time-to-next-treatment of 14 months and OS of 32 months. The authors concluded that increased circulating PCs on multiparametric FC is an independent prognostic biomarker in newly diagnosed MM. ([Abstract 1842](#))

In their second work, a large series of 647 patients with relapsed MM were studied. They found that circulating clonal PCs were more likely to be detected in patients with actively relapsing disease compared with plateau phase (43% vs. 4%, $P < 0.001$); none of the CR patients and $< 5\%$ of the remaining plateau phase patients had any detectable circulating clonal PCs. Also, patients with actively relapsing disease and ≥ 100 circulating PCs had a worse OS with a median of 12 months. ([Abstract 754](#))

S. Kosuri *et al.* confirmed that, also in patients with relapsed MM who had undergone T-cell depleted allogeneic hematopoietic stem cell transplantation, multiparameter FC detection of MRD provides additional information to assess the overall disease status. In their series (35 patients), FC had a negative predictive value of 100%, allowed an early detection of frank relapse in 18% of cases and allowed the identification of false positive marrow relapses distinguishing malignant PCs from proliferating recovering marrow PC post-transplant. ([Abstract 4647](#))

Based on the assessment that plasma cell immunophenotype has prognostic value in MM and MGUS, **S. Sachchithanantham *et al.*** evaluated its prognostic value in a series of 48 patients diagnosed with systemic AL amyloidosis. In their work, CD56 expression correlated with cardiac involvement and CD27 with a poorer haematological response. Both markers seemed independent markers of poor prognosis in systemic amyloidosis, with a median OS <2 months in patients expressing the two of them. ([Abstract 3120](#))

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

N. Obara *et al.* presented the results of a nationwide multicenter prospective observational study focused on the detection of minimal PNH-type cells (below 0.01%) in patients with BM failure syndromes. A uniform high-resolution flow cytometry-based method, using a combination of FLAER, and a cocktail of monoclonal antibodies including CD55 and CD59, enabled the detection of PNH-type granulocytes and erythrocytes, and offered equal accuracy in the 6 participant laboratories. ([Abstract 1241](#)).

S.J. Richards *et al.* presented a 22 year experience of detecting and monitoring PNH clones in 705 patients using FC. At diagnosis, PNH clone sizes were larger in patients with haemolytic PNH as compared to aplastic patients (median values of 32.35% vs 0.34% within red cells and 90.61% vs 2.58% within granulocytes). Interesting findings were that in aplastic patients, the risk of evolution into haemolytic PNH was related to the size of the granulocyte PNH clone at diagnosis: clones <1% did not evolve to hemolytic PNH, and progression to haemolytic disease was observed in patients with a clone size >5%. For patients presenting with hemolytic disease, PNH granulocyte clones continued to increase in size in 44% of cases. In most patients with with >95% granulocyte PNH clones, the clone remained stable over time (many on Eculizumab therapy), and only 8% of patients showed a gradual fall in clone size ([Abstract 3718](#)).

PLATELETS AND ERYTHROCYTES

Erythrocytes are the target of the study of **S. H. Park *et al.***, as they compared 3 methods for the screening of hereditary spherocytosis (HS). After evaluating 151 abnormal samples (30 with HS), and 140 normal controls these authors concluded that, in contrast to what is recommended in the 2011 update UK guidelines for the diagnosis and management of hereditary spherocytosis, both the eosin 5-maleimide (EMA) binding test and the flow cytometric osmotic fragility test, should be used as screening tests for the diagnosis of HS ([Abstract 3425](#)).

N. Hezard *et al.* adapted flow cytometry to assess abnormalities in different platelet molecules in 52 patients diagnosed with essential thrombocytopenia. However, these authors raised the question of which should be the method of choice for quantification: molecules per platelet vs molecules per platelet pool, since opposite results were obtained with each of them ([Abstract 4089](#)).

Finally, **V. Deutsch *et al.*** presented a modified version of a functional flow-cytometry assay for the diagnosis of heparin-induced thrombocytopenia (HIT). As compared to the standard antigenic assays, flow turned out to be a sensitive and highly specific test for the reliable diagnosis of HIT ([Abstract 1130](#)).

LIST OF ABBREVIATIONS

ALL: acute lymphoblastic leukemia

AML: acute myeloid leukemia

BM: bone marrow

CLL: chronic lymphocytic leukemia

DLBCL: diffuse large B-cell lymphoma.

FC: flow cytometry

MDS: myelodysplastic syndrome

MGUS: monoclonal gammopathy of uncertain significance

MM: multiple myeloma

MPN: myeloproliferative neoplasms

MRD: minimal residual disease

OS: overall survival

PC: plasma cells

PNH: paroxysmal nocturnal hemoglobinuria

WBC: white blood count