PROCEEDINGS OF THE XXVII NATIONAL CONFERENCE OF CYTOMETRY

Centro Congressi Fiera

Ferrara
14—17 Ottobre 2009

EDITED BY
R. DE VITA and G. MAZZINI

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SOCIETÀ ITALIANA DI CITOMETRIA
c/o Unità Tossicologia e Scienze Biomediche
ENEA Centro Ricerche Casaccia s.p. 016
Via Anguillarese, 301 - 00123 Roma
tel.: 06 30484671 fax: 06 30484891
e-mail: devita@enea.it
http://biotec.casaccia.enea.it/GIC/

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XXVII National Conference of the Italian Society of Cytometry GIC

October 14—17, 2009
Ferrara - Italy

Following the first experience in 2005, also this year an issue of Cytometry is partly dedicated to the programme and abstracts of the National Conference of the Italian Society of Cytometry, GIC. The XXVII edition of the Conference has been organized in October 2009 in Ferrara City, Italy. From 1995 on, UNESCO has included the historical centre of Ferrara in the list of World Cultural Heritage as a wonderful example of a town planned in the Renaissance and still keeping its historical centre intact. Its beauty has been linked to one of the most important courts in the political scenario of the 15th-16th century: the Estense court, which was one of the major actors in that precious season we call the Renaissance period.

As far as the GIC meeting is concerned, we want to stress the fact that all abstracts were carefully reviewed by the Scientific program Committee and published here in full and categorized by scientific track (1. cell cycle and apoptosis; 2. environmental sciences and toxicology, 3. hematology, 4. immunology, 5. methodology and technology, 6. oncology).

Following a continuous growth in these years, to date there are over 850 members actively involved in educational programs, promotion of quality controls programs, drafting/validation of guidelines and accreditation, providing information for people involved that actively work in the field of basic and applied cytometry.

This year, a great number of abstracts (>100) have been selected by the Scientific Committee among those submitted by basic and clinical researchers operating in the various Italian Institutions.

Each session involved invited lectures and was focused on the emerging role of cytometry techniques in Hematology, Stem Cell Biology, Immunology, Oncology and Environmental Sciences and Toxicology.

In addition, different topics of general interest in biological and medical sciences, new data on the study of a rare disease such as PNH, accreditation, standardization of ZAP70 measurement across Italy, and on the Methodological and Technological advances were reviewed by experts from Italy. Two of these lectures were dedicated to the loss of two "top" scientists, Prof Bruno Rotoli (Naples) and Prof Antonio Tabilio (Perugia). Both of them tirelessly helped young researchers and research students, and they were active in disseminating research findings to and communicating with the public. We do all miss them!

The Conference had been also characterized by a round table dealing with the possible interactions between parental scientific Societies having different levels of interest in cytometric techniques and applications Since many years ago the GIC Society did promote such kind of scientific interactions.

A substantial contribution was obtained from the principal industries in the field that have been located in a large exhibition area inside the conference center.

This national event is growing each year and, once again, represents Italian cytometry's scientific contribution to the international community.

Guest Editors:
R. De Vita - G. Mazzini

Francesco Lanza
GIC President

observed also in thawed samples. The extension of this approach to a larger series of JMML samples will help to investigate further biological insights of JMML and to evaluate to what extent this new assay may be considered as a tool for the diagnostic work-up of JMML patients.

CD22, CD79b, CD81 AND CD200 HIGHLY SPECIFIC MARKERS CAN ENHANCE THE FLOW CYTOMERIC (FC) DIAGNOSIS AND MONITORING OF B-CLL AND B-NHL

Calzavara E., Testi M.A., Cabras A.D., Carbone A. and Aiello A. S.C.
Anatomia Patologica 3, Fondazione IRCCS Istituto Nazionale Tumori, Milano
antonella.aiello@istitutotumori.mi.it

FC diagnosis of B-NHL is challenging. Even CLL, the most common low grade disorder, can be misdiagnosed, especially when CD5 or CD23 are present at low density or weak FMC7 staining is observed. For this reason, new markers (CD200) or disease-specific antibody combinations (CD81/CD22/CD79b on CD19/CD5 B cells) have been recently identified for CLL immunophenotypic characterisation and MRD monitoring.

We have introduced the newly described antibodies in our FC practice to improve immunophenotypic profile definition, differential diagnosis and MRD detection in CLL and other B-NHL.

PB, BMA and pleural effusion samples from 63 consecutive B-NHL patients (46 CLL, 4 FL, 4 MCL, 4 MZL, 1 HCL, 1 LPC, 3 BL) were tested with CD79b, CD200 and the CD81/CD22 antibody combination.

CD22 and CD81 were downmodulated in all but 4 CLL and in 2/4 MCL. Likewise, CD81 was weak in 1/1 HCL, 2/4 MZL and 1/4 FL. Interestingly, in 2/3 BL and in 1 transformed FL, CD81 was up-regulated to levels comparable to immature B-cells. In 20 patients (CLL, MZL, LPC), the CD81/CD22 combination was used for highly sensitive and specific monitoring of MRD. As expected, CD79b was undetectable or dim in CLL, while CD200 was highly expressed in all but 2 CLL and in 1/1 HCL; surprisingly, bright CD200 was also found in 2 MCL. Conversely, 2/4 FL, 4/4 MZL, 1/3 MCL and 3/3 BL turned out CD200-. According to the FC data, histological re-evaluation and molecular investigation were performed in 3 cases (1 CLL and 2 MCL), allowing correct re-classification.

These data confirm that the CD81, CD22, CD79b represent a powerful tool for the immunophenotypic characterisation and follow-up monitoring of CLL. Likewise, if confirmed on a larger series of cases, CD200 may represent an interesting marker for the differential diagnosis of both low and high grade B-NHL, with possible clinical implication in the field of target therapies.

MULTIPARAMETRIC FLOW CYTOMETRIC CHARACTERIZATION OF CIRCULATING STEM CELL SUBSETS IN PATIENTS WITH MIOCARDIAL INFARCTION: CORRELATION WITH CLINICAL AND BIOLOGICAL DATA

Campioni D.,[1] Gambetti S.,[2] Monti M.,[2] Rizzotto L.,[1] Cavazza C.,[2] Cangiano E.,[2] Ferrari L.,[1] Moretti S.,[1] Ceconi C.,[2] Lanza F.,[1] Ferrari R.,[2] and Cuneo A.[1]
[1]Department of Biomedical Sciences and Advanced Therapies, Hematology Section, University of Ferrara, Ferrara-Italy
[2]Department of Clinical Sperimental Medicine Section of Cardiology, University of Ferrara, Italy
cmpdni@unife.it

In an attempt to better understand the functional and immunophenotypic characteristics of circulating stem cell subsets we investigated and analysed 120 patients from 1st to the 6th days after acute MI. The presence of the different stem cell subsets were correlated to the in vitro functional characteristics of hematopoietic/endothelial progenitor cells and to the different patient's clinical parameters to clarify which could be regarded as the most predictive of long-term clinical follow-up. The multiparametric flow cytometric protocol was necessary to overcome immunophenotypic misunderstanding since the different hemopoietic and endothelial stem cells populations share numerous surfaces markers.

The analysis of the CD34+ cells (ranging from 0,024 to 0,037%) subsets showed that CD34+cells defined by the expression of in particular the CD117 (c-kit) or CD146 markers are higher after five days of MI. No significative number of circulating EPC were observed. Short term clonogenic assay showed an high number of in vitro generated BFU-E (burst forming unit erithroblasts) at five days after MI that correlated with the percentage of the circulating CD117+ and CD146+ CD34+CD45+ stem cells. The presence of in vitro EPC colonies was found only ten days after MI in 3% of patients only. On the other hand, 52% of patients showed the presence of in vitro endothelial-like monocytic colonies 5 days after MI. Patients displaying high number of BFU-E resulted characterized by no cardiovascular events at nearly 6 months follow up, and this may represent an important role in myocardial repair. All these patients had no smoking habits, and this data confirm previous studies results in which stem cells mobilization was lower in smoking subjects. In the same group of patients there was an higher number of diseased vessels. These data would be useful for clinical setting.

This study was supported by Programma di Ricerca medicina Rigenerativa Regione Emilia Romagna-Università 2007-2009.

IMMUNOPHENOTYPIC HETEROGENEITY OF MESENCHYMAL STROMAL CELLS: MULTIPARAMETRIC FLOW CYTOMETRIC ANALYSIS

Campioni D.,[1] Rizzo R.,[2] Stignani M.,[2] Lanzoni G.,[3] Bonsi L.,[3] Alviano F.,[3] Cuneo A.,[1] Bagnara GP.,[3] Baricordi OR.,[2] and Lanza F.[1]
[1]Department of Biomedical Sciences and Advanced Therapies, Hematology Section, Azienda Ospedaliera-Universitaria Arcispedale S.Anna, Ferrara-Italy
[2]Department of Experimental and Diagnostic Medicine, Laboratory of Immunogenetics, Section of Medical Genetics, University of Ferrara, Italy
[3]Department of Histology, Embryology and Applied Biology, University of Bologna, Stem Cell Research Centre, University of Bologna, Italy.
cmpdni@unife.it

So far, the immunophenotypic profile of freshly isolated and ex-vivo expanded human mesenchymal stromal

cells (hMSCs) has been confined to single or dual staining analysis especially in normal subjects. The lack of specific markers complicate the in vivo hMSC detection. The immunophenotype of cultured hMSC is not still elucidated. Although hMSCs are reported to be uniformely positive for CD90, CD105, CD73, we specifically investigated the hMSC immunophenotype after ex vivo expansion in relation to different parameters such as different culture conditions (serum free, additional use of platelet-lysate, cytokines), culture age, hMSC source (different tissues and normal versus pathologic donors, such as hematological malignancies, HM) and contaminant CD45pos hematopoietic cells and/or endothelial cells. The human primary MSC immunophenotype was also compared to that of different transformed tumor cell lines phenotype. Based on these observations, we observed hMSC immunophenotypic modulation in particular of CD44, CD10, CD146 in relation to the hMSC source and culture passages.

The downregulation of CD90 expression is documented on bone marrow-derived hMSC treated with angiogenic cytokines and in some HM patients and is also related to a diminished immunomodulant MSC capacity. Of interest is the expression of the CD34 stem cell marker that is universally known to be negative on hMSC but that we found to be positive uniquely on hMSC from lipoaspirates.

The lack of "MSC" markers such as CD90, CD73, CD106 and CD146 expression was observed on some cancer cell lines from colon and epatocarcinomas. The results confirm an immunophenotypic heterogeneity of cultured hMSC that could have different clinical implications. The study of hMSC cell immunophenotypic subsets could be useful before their use in transplantation setting.

ELEVATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND STROMAL DERIVED FACTOR AFTER MYOCARDIAL INJURY MOBILIZES CD34+ CELLS IN CARDIOVASCULAR PATIENTS

Cangiano E.,[1] Cavazza C.,[1] Campo G.,[1] Valgimigli M.,[2] Malagutti P.,[1] Fileti L.,[1] and Ferrari R.[2]

[1]Chair of Cardiology, S.Anna Hospital-University of Ferrara, C.so Giovecca 203, Ferrara, Italy

[2]Cardiovascular Research Centre, Salvatore Maugeri Foundation, IRCCS Ferrara, Italy

Objective: it is not known whether bone marrow cells (BMC) mobilization in human is triggered by necrosis, ischemia or both. Recognition of the trigger is important to improve therapeutic use of BMC. The aim of this work is to test the role of necrosis, ischemia or both in BMC mobilization in patients with cardiovascular disease. Methods: we studied three groups of patients (P) : group 1 with pure ischemia (24 P with unstable angina); group 2 with pure necrosis (28 P undergoing transcatheter radiofrequency ablation, without CAD); group 3 with ischemia + necrosis (30 P with transmural myocardial infarction). As control groups we studied 27 P with angiographically documented stable angina (C1), and 20 P without CAD undergoing coronary artery angiography for valvular diseases or cardiomiopathy (C2). CD34+ cells and cytokines were monitorated at: T_0 (baseline), 48 hours and 5, 7, 10, 14 days thereafter. **Results**. In the groups with necrosis (1 and 2), there was a significant increase of CD34+ cells at T_3 and T_4 (after 7 and 10 days, respectively). The peak of mobilization was observed ten days after the necrotic event (2.8 ± 1.4 vs. 5.9 ± 1.9 in the group 1, $p=0.03$; and 3 ± 1.5 vs. 5.6 ± 2 in the group 2, $p=0.04$; respectively). There was a correlation between CD34+ and SDF and VEGF peak values ($r=0.77$ and $r=0.63$, respectively), but no correlation between peaks of CK-MB or troponin and CD34+. **Conclusions**: necrosis, but not ischemia, causes an increase of VEGF and SDF1and a CD34 mobilization. CD34 mobilitation is not correlated to the extension of necrosis.

This study was supported by Programma di Ricerca medicina Rigenerativa Regione Emilia Romagna-Università 2007-2009.

MULTIPARAMETER IMMUNOPHENOTYPING BY FLOW CYTOMETRY IN MULTIPLE MYELOMA: DEFINING RANGES OF NORMAL EXPRESSION AND THEIR DIAGNOSTIC UTILITY

Elisa Cannizzo,[1,2] Emanuele Bellio,[2] Judith A. Ferry,[1] Robert P. Hasserjian,[1] Aliyah R. Sohani,[1] Michelle E. Dorn,[1] Craig Sadowski,[1] Janessa J. Bucci,[1] Mario Petrini,[2] Giovanni Carulli,[2] and Frederic Preffer[2]

[1]Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, USA

[2]Department of Oncology, Transplants and Advances in Medicine, Section of Hematology, University of Pisa, Pisa, Italy

elisacannizzo@libero.it

Background: In order to appropriately study multiple myeloma (MM) utilizing flow cytometry (FC) it is necessary to be able to distinguish between the normal and abnormal plasma cells (PCs). Numerous studies have reported on the immunophenotype of PC cell neoplasms, but very few have examined the immunophenotype of normal PCs. In this study, an objective definition of normal range of expression for each antigen was found on normal control PC obtained from orthopaedic resections. Using these new ranges of normal expression (new method) is different from using a static 20% cut-off described in the literature (traditional method0. These newly calculated normal ranges for each antigen were applied to patients' data, and compared to histologic and immunohistochemical findings.

Methods: Bone marrow samples from 55 patients with plasma cell neoplasms and 15 normal controls were studied. A minimum of 100 PC were analyzed for each patient and control sample. An 8-color staining method was applied to study the immunophenotype of PCs, using a BD FACSCanto II.

Results: CD19 correlated with histology by both the traditional and new methods, but had superior correlation by the new method.

Conclusions: This report is the first 8-color immunophenotypic study of MM in which a "range of normal expression" for each antigen is defined. This is a critical step to discern which PCs antigens are of diagnostic importance.