BOOK OF ABSTRACTS
SCK•CEN-BA-18

12th BVAC/ABCA annual meeting

Annual meeting of the Belgian Society for Analytical Cytology: Book of Abstracts

Mons/Bergen, October 26, 2007

SCK•CEN
Boeretang 200
BE-2400 Mol
Belgium
http://www.sckcen.be

BOOK OF ABSTRACTS
SCK•CEN-BA-18

12th BVAC/ABCA annual meeting

Annual meeting of the Belgian Society for Analytical Cytology: Book of Abstracts

Mons/Bergen, October 26, 2007

SCK•CEN, Boeretang 200, BE-2400 MOL, Belgium

Contact: Sarah Baatout
Tel: 014/33.27.29
Fax: 014/33.35.12
E-mail: sarah.baatout@sckcen.be

Organising committee

Sarah Baatout

Studiecentrum voor Kernenergie
Centre d'étude de l'énergie Nucléaire
Boeretang 200
BE-2400 Mol
Belgium

http://www.sckcen.be

Status: Unclassified

Contents

Introduction

Programme

Title of Abstract

Name
Company
Address

☎ Telephone number
🖷 Fax number
💻 Email address

Abstract

Title of Abstract

Name
Company
Address

☎ Telephone number
🖷 Fax number
💻 Email address

Abstract

Title of Abstract

Name
Company
Address

☎ Telephone number
🖷 Fax number
💻 Email address

Abstract

Biographies

12th BVAC/ABCA annual meeting

“Rare event analysis"

Book of Abstracts

Belgian Society for Analytical Cytology

Mons/Bergen, Belgium
October 26, 2007

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

Belgische Vereniging voor Analytische Cytologie
Association Belge de Cytologie Analytique

PROGRAM

09:00	Registration desk open - Poster installation
09:50	Welcome & introduction by *D. Van Bockstaele*
10:00	Pitfalls in rare event analysis *L. Terstappen, PA, USA*
10:30	Indolent lymphomas: what the clinician expects from the laboratory *A. Bosly, Mont-Godinne, Belgium*
11:00	Coffee break + poster walking + exhibition visit
11:30	Clinical relevance of MRD studies by flow cytometry and molecular biology in acute leukemia *N. Straetmans, Jolimont, Belgium*
12:00	Basophil activation test by whole blood expression of CD203c in pediatric food allergy to milk, egg white and peanut *O. Lees, Rouen, France*
12:30	Detection of circulating tumor cells *I. Benoy, Antwerp, Belgium*
13:00	LUNCH + poster walking + exhibition visit
14:15	General assembly
14:30	Oral presentations (6 x 10 min)
15:30	Minimal residual disease in AML by flow cytometry *G.J. Schuurhuis, Amsterdam, The Netherlands*
16:00	Isolation of multipotent mesenchymal stromal cells and their use in translational research *A. Gothot, Liège, Belgium*
16:30	Endothelial progenitor cells: myth or reality? *F. Timmermans, Gent, Belgium*
17:00	Announcement of the super award winner (500 euro)
17:05	Closing

Sponsors

GOLD SPONSORS

analis
SCIENTIFIC INSTRUMENTS

PARTEC

BD

Dako

THE POWER INSIDE Luminex
xMAP TECHNOLOGY
Because you want to know more

SILVER SPONSORS

SERAGLU sarl

Roche

MACS
Miltenyi Biotec

Abbott

BRONZE SPONSORS

GOFFIN MEYVIS

OLYMPUS
Your Vision, Our Future

Belgian Association for Cytometry

www.cytometry.be

MEMBERS OF THE BOARD
(mandate from November 2005 to November 2007)

Christian DEMANET	President
Sarah BAATOUT	Vice-president
Dirk VAN BOCKSTAELE	Treasurer
Jan PHILIPPE	Board Secretary
Bernard CHATELAIN	Educational committee
Pieter DE SCHOUWER	Molecular Hematology
Bernard HUSSON	Newsletter
Peter MEEUS	Website
Nicole SCHAAF-LAFONTAINE	

OBJECTIVES OF THE ASSOCIATION

The Association was founded on 5 October 1995. Its objectives are:
- To promote the scientific collaboration in all fields of cytometry by: organising scientific meetings in the field of cytometry, flow cytometry, image cytometry, quantitative microscopy and molecular hematology.
- To exchange information between national and international society's with similar objectives.
- To promote the quality in cytometry by:
    - Encouraging and recommending quality controls.
    - Exchanging reagents for standardisation.
    - Establishing technical recommendations and recommendations for accreditation of laboratories.
- To promote education in all fields and at all levels of cytometry
- To promote scientific collaboration with the industry.

The association organises an annual meeting as well as 2 specialised workshops per year. It has around 100 members.

The membership fees are :
Full member : € 50;
PhDs and clinicians in training : € 25;
Students : free

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

SPEAKER PRESENTATION

Multiparameter strategies in rare event analysis.

Dirk VAN BOSCKTAELE, PhD, (BVAC/ABCA board).
Esoterix Clinical Trials Services, a LabCorp company,
Mechelen, Belgium.

Many "flow" departments now receive numerous requests to quantify rare events such as circulating haematopoietic progenitor cells (HPC), endothelial cells (EC) and endothelial progenitor cells (EPC). It is important to realise that reliably counting a rare event is an extremely difficult exercise that cannot be easily implemented in between the "classical" (*i.e.* frequent event) flow cytometric test samples and that there exists no definite consensus protocols for this purpose to date.

The ability to reliably count a rear event depends on the frequency of the rare event and on the signal (real rear events) to noise (spurious events) ratio. It is thus of utmost importance to not only recognise the rear events but also to be able to recognise the spurious events in order to exclude them, to realise what causes them and to minimise their occurrence.

Unfortunately it appears from literature that not always all efforts to guarantee the generation of a robust result are being exhaustively exploited. Every protocol should at least take into account all the precautions and available remedies.

These can be recapitulated as follows:

- Thoroughly **clean** the flow cytometer before rare event analysis.
- Never be tempted to increase the sample flow rate, rather increase the cell concentration but be sure to exclude coincidences using some form of **pulse shape analysis**.

Noise is made up of non-specific binding of fluorochromes and antibodies, cellular autofluorescence, disruptions in sample flow caused by cell clumps all giving rise to spurious events within every detection channel and some random (*i.e.* uncorrelated) noise.

- Increase the number of parameters to positively define your rare event: uncorrelated noise is diluted out by a power function of the # of parameters.
- Define the rare event with the brightest (PE chromophore) and tightest (low CV) positive marking.
- Include a negative marking for the rare event or include an unstained fluorescence parameter: gating on negative fluorescence will exclude autofluorescent cells.
- Use blocking serum (same species) before incubating with relevant antibodies.
- Label all potentially interfering cells using a fluorescence dump channel.
- Always include **time** as a parameter to facilitate identification and removal of event bursts, minor clogs, and other transient mechanical and electronic problems.

- Clearly define your denominator (total events) using a **nuclear stain**.
- Exclude **dead cells** that are notorious for spurious antibody binding
- Scatter backgating may help in excluding spurious events if the rare events appear tightly clustered.

After all these precautions are taken:

- Always **characterize total noise** using an appropriate negative control: i.e. using an isotype-matched irrelevant antibody at the same incubation concentration and with the same F/P ratio and with acquisition of the same amount of total events.
- Be prepared to measure **extremely large data files** (1 million of cells) on a dedicated flow cytometer, to reanalyse these in a scrutinous manner, to get results that appear underwhelming and to start from larger than "normal" blood volumes (a rare event with frequency in the order of $1/10^5$ cannot be retraced in a 100 µl blood sample).
- Always report your **precision**: rare event analysis is governed by Poisson statistics and you always end up with a minimal counting error equaling the square root of the actual counted events of interest. The more events one counts the better the precision will be.
- Beyond a certain limit, conditions become too cumbersome and **pre-enrichment techniques**, either in flow (fluorescence threshold triggering) or in bulk, are warranted.

All this should be carefully evaluated and validated for every given rare event type of analysis before one should proceed with data gathering on patient samples.

Ref.: Principles of rare event analysis by flow cytometry: Detection of injected dendritic cells in draining lymphatic tissue. AD Donnenberg and EM Meyer, Clinical Immunology

SPEAKER PRESENTATION

Tumor cells in blood of carcinoma patients

Leon WMM TERSTAPPEN, MD, PhD,
Chair Medical Cell Biophysics, University of Twente, The Netherlands
Chief Scientific Officer, Immunicon Corporation, USA

Arjan TIBBE, PhD
Branch Manager,
Immunicon Europe, The Netherlands

The threat in solid tumors is not the loss of function from the site the tumor arises, but dissemination of the disease and destruction of other organs. Cancer cells become dangerous when the cells break away from the primary site, enter the blood and find homes elsewhere in the body, i.e. form metastasis. The frequency of the dislodged tumor cells in the blood is extremely low. Technology was developed to immunomagnetically enrich cells of epithelial origin from 7.5mL blood in combination with fluorescent labeling of the cells and microscopic identification. Preclinical testing demonstrated a linear recovery of 1-1000 tumor cells spiked in 7.5 mL blood. In multi-center prospective clinical studies we demonstrated that Circulating Tumor Cells (CTC) were present in blood of a portion of patients with metastatic carcinomas, but not in blood of normal donors. The presence of CTC was associated with poor outcome. Repeated assessment of CTC in patients undergoing therapy for metastatic breast colorectal and hormone refractory prostate cancer was shown to be an effective means to predict outcome and monitor treatment. The therapy of patients with CTC detected after the first cycles of therapy is futile. Knowledge of the presence of treatment targets on CTC would provide a means to administer a therapy that has a larger chance of success. Expression level of specific drug related molecular targets can be detected on CTC, can be assessed at the protein or gene level and opens the road toward personalized medicine.

For more information, please read the two papers at the end of this book of abstracts and entitled "*Statistical Considerations for Enumeration of Circulating Tumor cells*" and "*Circulating tumor cells, disease progression, and survival in metastatic breast cancer*".

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

SPEAKER PRESENTATION

Indolent lymphomas : what the clinician expects from the laboratory

André BOSLY
Hematology Unit, UCL Mont-Godinne Hospital, Mont-Godinne, Belgium

Les lymphomes à faible degré de malignité, atteignant la lignée des lymphocytes B, peuvent être classés selon la WHO en 4 sous-types particuliers.

Les lymphomes lymphocytiques qui sont l'équivalent ganglionnaire de la leucémie lymphoïde chronique. Les lymphocytes ont le même phénotype et les traitements sont ceux qui s'appliquent à la leucémie lymphoïde chronique : alkylants, fludarabine, anthracycline, rituximab et alemtuzumab. Le pronostic se situe autour de 8 ans de médiane de survie.

Les lymphomes du tissu lymphoïde associés aux muqueuses (MALT) sont des lymphomes extra-ganglionnaires dont les plus fréquents sont ceux de l'estomac. Ils peuvent atteindre également le tube digestif, la thyroïde, le poumon, la dure-mère, la peau et les annexes oculaires. Dans un certain nombre de cas, l'influence d'une infection a pu être confirmée.
Les lymphomes du MALT de l'estomac sont liés à l'helicobacter pylori. Cette infection entraîne d'abord le développement de tissu lymphoïde au niveau de l'estomac, qui peut se transformer en un lymphome qui reste dépendant de l'helicobacter pylori ou qui devient indépendant, c'est notamment le cas lorsqu'il y a une translocation (11 ;18).
Les lymphomes du MALT de l'estomac vont d'abord être traités par antibiotiques puis, s'ils sont localisés, peuvent être traités par radiothérapie. Les traitements par alkylants et par rituximab sont également efficaces. Les autres localisations du MALT peuvent être traitées par chirurgie, radiothérapie, alkylants et rituximab.

Les lymphomes de la zone marginale sont spléniques ou ganglionnaires. Les lymphomes spléniques seront traités par splénectomie et ne seront traités que dans un petit nombre de cas par de la chimiothérapie.
Les lymphomes de la zone marginale ganglionnaires sont par contre de pronostic plus sévère et justifient l'association de rituximab et de chimiothérapie.

Les lymphomes folliculaires représentent l'entité de loin la plus fréquente parmi les lymphomes à faible degré de malignité de la lignée B. Ils représentent près d'un quart de l'ensemble des lymphomes. Ils sont caractérisés par la translocation (14 ;18) qui juxtapose l'oncogène BCL2 et les gènes de la chaîne lourde des immunoglobulines, entraînant au niveau du centre folliculaire une hyperexpression de BCL2.
Les facteurs de pronostic pour ces lymphomes sont le stade avancé, un taux élevé de LDH, la présence d'anémie, un nombre élevé de localisations extra-ganglionnaires et un âge de plus de 60 ans formant l'index pronostique FLIPI. Les rares stades localisés folliculaires peuvent être traités par radiothérapie seule. La majorité de ces lymphomes folliculaires sont à un stade avancé (III ou IV) et si, un tiers d'entre eux sont indolents et ne justifient pas d'emblée un traitement, ceux qui justifient un traitement ont vu leur pronostic complètement modifié par

l'association de rituximab et de chimiothérapie. Cette association est le standard que 4 études prospectives randomisées ont prouvé un avantage en survie par rapport à la chimiothérapie seule. On ne sait pas actuellement s'il faut compléter ce traitement par de la radioimmunothérapie ou la poursuite du traitement par anticorps monoclonal. Les intensifications thérapeutiques avec auto et allogreffe seront réservées chez les malades en rechute. L'allogreffe à conditionnement atténué est une option très attirante pour les malades jeunes car l'effet du greffon contre le lymphome a été bien démontré dans le cadre des lymphomes folliculaires.

Le dogme d'une maladie incurable doit être actuellement remis en question pour ces lymphomes folliculaires.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

SPEAKER PRESENTATION

Clinical relevance of MRD studies by flow cytometry and molecular biology in acute leukemia

Nicole STRAETMANS, Jolimont, Belgium

Abstract not available.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

SPEAKER PRESENTATION

Basophil activation test by whole blood expression of CD203c in pediatric food allergy to milk, egg white and peanut.

Olivier LEES, Celine TASSET, Laure COUDERC, Christophe MARGUET, Eric MALLET.

Immunopathology Lab, Clinical Pediatry, University Hospital Charles Nicolle, 1 rue de Germont, 76031 Rouen, France. Tel: 02 32 88 80 71; Fax: 02 32 88 81 86; e-mail: olivier.lees@chu-rouen.fr

1°) La réalisation du TAB, Test d'Activation des Basophiles, par cytométrie en flux, comme toute analyse biologique commence dès la phase pré-analytique.

Les prélèvements de sang veineux s'effectuent "au lit du malade" et vont permettre de techniquer en sang total avec lyse des hématies. Ceci est classique en cytométrie.

Le faible pourcentage de basophile dans le sang (de l'ordre de 1% des GB), et la fragilité de ces cellules incitent à effectuer un prélèvement le jour même de l'analyse. Un délai de 24h semble le maximum fiable.

Il faut utiliser un anti-coagulant et les tubes classiques sont soit le Citrate, soit l'EDTA, soit l'héparine.

2°) Ensuite, certains choix seront à faire pour la phase analytique.

L'utilisation d'enrichissement en GB par sédimentation du plasma peut être envisagée, par simple "buffy coat" ou utilisation de Dextran. Voir D.Ebo review Allergy 2006 p 130. La méthode d'isolement par ficoll est peu préconisée.

C'est la méthode en sang total qui est la plus souvent pratiquée. Elle comporte plusieurs aspects:

A - préstimulation –ou pas- par l'IL3. Il semble que ce soit nécessaire avec le CD63, et inutile voir néfaste avec le CD203c.

B - préparation de 3 tubes au minimum; ils correspondent à un:

= Test Négatif, qui sera la référence d'expression du marqueur choisi, basophiles au repos,

= Test Positif, avec un anti-IgE, référence de la capacité maximale d'expression des cellules,

= Test Allergène…Le choix, la source, et la dilution de l'allergène est un aspect primordial dans la réalisation du TAB.

C- choix des anticorps, marqueurs de sites cellulaires choisis. CD63, CD203c, fenêtres de sélection positives et négatives (CRTH2, CD3, CD123, HLA-DR….) sont au cœur du débat.

D – les méthodes de lyse des hématies sont nombreuses; certaines peuvent être préjudiciables à l'expression des basophiles. Des essais comparatifs doivent guider les choix.

E – Le bon repérage des rares Basophiles, en sang total est alors possible, mais sollicite l'attention du cytométriste.

3°) Méthodes et Patients.

Le TAB étudie les marqueurs surexprimés sur les basophiles activés. Le TAB utilisé est le « Kit Allergenicity » (Beckmann Coulter®). Il utilise le marqueur CD203c, le CRTH2 ou CD294 présent sur les basophiles, ainsi que le CD3. Nous avons choisi comme allergènes solubles les extraits cacahuète et blanc d'œuf de Stallergènes®, et du lait natif demi écrémé (Nactalia®). L'interprétation en cytométrie se fait de deux façons sur la fluorescence du CD203c : la variation de la moyenne d'intensité de fluorescence (MFI) combinée au calcul de l'index de MFI test allergène/témoin négatif (PBS), et le pourcentage de cellules CD203c positives. L'étude comporte 137 enfants répartis en 15 témoins sains, 45 nourrissons à risque atopique, et 78 enfants allergiques à un ou plusieurs allergènes: 30 allergiques au lait, 25 à l'œuf et 23 à l'arachide.

4°) L'expression des résultats en cytométrie en flux.

Elle se fait classiquement en pourcentage de cellules positives. Du fait de la présence au repos, et de l'observation de la sur-expression des marqueurs significatifs, l'interprétation en MFI (moyenne d'intensité de fluorescence), apparaît essentielle. L'index de stimulation Tpos/Tneg et Tallergène/Tneg est tres discriminant entre les sujets allergiques et non allergiques. Il va permettre d'aider à conclure le TAB.

5°) Résultats

- Valeurs des résultats du TAB :
  - population Témoins : index MFI Tpos/Tneg <2, % CD 203c activés < 15%,
  - populations allergiques : index MFI> 2, % CD 203c activés >20%,
  - validation du test selon 3 échelles : 1,5 – 2 + *faible*, 2-2,5 +, >2,5 + *fort*
  - validation des allergènes utilisés par étude CD 203c. Choix de dilution satisfaisante au 1/1000.
- Pour différentes populations étudiées, VPN atteint 100% (par comparaison avec test de réintroduction orale)
- % de non répondeurs = 8 à 11%

Le résultat du TAB est signifiant quand le rapport de MFI est supérieur à 2, ou si le pourcentage de cellules CD203c positives atteint plus de 20%. La spécificité du TAB est de 100%. La guérison de l'allergie confirmée par le succès du TPO est parfaitement corrélée à la négativité du TAB. Il est corrélé à l'intensité des manifestations allergiques présentées pour la population allergique au lait.

En conclusion.

L'application du TAB nous paraît apporter des résultats très prometteurs pour le suivi des allergies alimentaires chez l'enfant. Ce test pourrait devenir un modèle de réintroduction in vitro de l'allergène, et un marqueur prédictif de guérison de l'allergie. Un consensus coopératif multicentrique aiderait à répondre à propos du TAB à de nombreuses questions : quelles méthodes, quels marqueurs, quelles interprétations, quels contrôles de qualité CQI-CQE)… et dans quelles allergies ?

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

SPEAKER PRESENTATION

Detection of circulating tumour cells

Ina BENOY, PhD
Translational Cancer Research Group, Antwerp
www.tcrg.be
ina.benoy@telenet.be

In the Western world, nearly one out of ten people will be confronted with the diagnosis of a solid cancer. Because of increasing awareness and the implementation of screening methods, more tumours are being diagnosed at an early stage. The majority of these patients do not have evidence of metastatic disease using conventional diagnostic techniques. However, at this stage, haematogenous dissemination is responsible for the morbidity and mortality of the disease.

The most important and harmful step in the progression of solid tumours is the dissemination of cancer cells to other parts of the body. This process is called metastasis. The key players are disseminated tumour cells (DTC) which are distributed by blood circulation and can be found in the bone marrow in very low numbers.

The standard method to detect DTC is immunocytochemistry (ICC). Immunocytochemical detection assays involve monoclonal antibodies that bind to tumour-associated or histogenic markers; proteins which are expressed by disseminated tumour cells, but are absent on the surrounding normal cells. For epithelial tumours, cytokeratins (CK) have become the best characterised markers for the identification of disseminated tumour cells in blood and bone marrow.

The value of the cytological method is limited by a low sensitivity and is highly labour intensive since for each patient, 1 to 2 million cells need to be screened microscopically. Also this method has a high observer-dependency.
The application of molecular based methods like the polymerase chain reaction (PCR) technology, has much less restrictions. Both DNA and mRNA amplification methods are used to detect residual cancer cells in body fluids of patients with solid tumours. The sensitivity of these methods is high, but the specificity shows the limiting factor.

SPEAKER PRESENTATION

Analogous to the staging procedures in haematological malignancies, detection of minimal disease in bone marrow, defined as single or clumped disseminated cancer cells, has been suggested to be a more direct approach to select the metastasis-prone patients among the "good prognosis group" based on the TNM-staging (Braun and Pantel, 1998). Numerous studies have elaborated on this concept by detecting disseminated epithelial cells, or transcripts of supposedly specific markers for epithelial cells, in bone marrow aspirates (Diel, 1996; Braun, 2000; Janni, 2000; Gerber, 2001; Wiedswang, 2003; Benoy, 2004; Benoy, 2006a). In these studies, the bone marrow status is considered as a mirror for the efficacy of the metastatic process throughout the body, similar to detection of cancer cells in lymph nodes. Also the prognostic relevance of disseminated epithelial cells in bone marrow is clearly demonstrated by a number of large studies (Braun, 2000; Weinschenker, 2004; Braun, 2005). The vast majority of these studies used immunocytochemistry for cells detection. Currently, detection by PCR techniques is much less validated (Benoy, 2006a).

The detection of tumour cells in bone marrow is an obvious choice in cancer, since a majority of patients will develop bone metastases once dissemination has been detected clinically. Although bone marrow aspiration is an acknowledged clinical method, it remains cumbersome, especially if repetitive examinations are considered. Peripheral blood (PB) is an other organ to evaluate a patient for the presence of disseminated epithelial cancer cells, although tumour cells in blood and tumour cells in bone marrow do not necessarily have the same metastatic potential. Repetitive sampling of peripheral blood is accepted (Benoy, 2006b; Benoy, 2006c).

Detection of tumour cells outside the primary tumour, in blood and in organs relevant for subsequent metastasis formation, such as bone marrow, would serve three purposes that could be clinically useful: (1) as unambiguous evidence for an early occult spread of tumour cells; (2) as a relevant risk factor for subsequent metastasis and, thus, a poor prognosis; and (3) as a marker for monitoring treatment susceptibility. Finally, and perhaps as importantly in the long run, genotyping and phenotyping of circulating tumour cells and disseminated tumour cells should provide detailed insight into the metastatic process and permit direct exploration of targeted treatment strategies.

Reference List

Benoy IH, Elst H, Philips M, Wuyts H, Van Dam P, Scharpe S, Van Marck E, Vermeulen PB and Dirix LY. Prognostic significance of disseminated tumor cells as detected by quantitative real-time reverse-transcriptase polymerase chain reaction in patients with breast cancer. *Clin Breast Cancer* 2006a; 7: 146-152

Benoy IH, Elst H, Philips M, Wuyts H, Van Dam P, Scharpe S, Van Marck E, Vermeulen PB and Dirix LY. Real-time RT-PCR detection of disseminated tumour cells in bone marrow has superior prognostic significance in comparison with circulating tumour cells in patients with breast cancer. *Br J Cancer* 2006b; 94: 672-680

Benoy IH, Elst H, Van Dam P, Scharpe S, Van Marck E, Vermeulen PB and Dirix LY. Detection of circulating tumour cells in blood by quantitative real-time RT-PCR: effect of pre-analytical time. *Clin Chem Lab Med* 2006c; 44: 1082-1087

Benoy IH, Elst H, Van dA, I, Van Laere S, Van Dam P, Van Marck E, Scharpe S, Vermeulen PB and Dirix LY. Real-time RT-PCR correlates with immunocytochemistry for the detection of disseminated epithelial cells in bone marrow aspirates of patients with breast cancer. *Br J Cancer* 2004; 91: 1813-1820

Braun S and Pantel K. Prognostic significance of micrometastatic bone marrow involvement. *Breast Cancer Res Treat* 1998; 52: 201-216

Braun S, Pantel K, Muller P, Janni W, Hepp F, Kentenich CR, Gastroph S, Wischnik A, Dimpfl T, Kindermann G, Riethmuller G and Schlimok G. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. *N Engl J Med* 2000; 342: 525-533

Braun S, Vogl FD and Pantel K. Disseminated tumor cells (DTC) in bone marrow (BM) and clinical outcome: Final results of pooled analysis on 10-year survival of 4,703 breast cancer patients. *J Clin Oncol* 2005; 23: abstract nr 502

Diel IJ, Kaufmann M, Costa SD, Holle R, von Minckwitz G, Solomayer EF, Kaul S and Bastert G. Micrometastatic breast cancer cells in bone marrow at primary surgery: prognostic value in comparison with nodal status. *J Natl Cancer Inst* 1996; 88: 1652-1658

Gerber B, Krause A, Muller H, Richter D, Reimer T, Makovitzky J, Herrnring C, Jeschke U, Kundt G and Friese K. Simultaneous immunohistochemical detection of tumor cells in lymph nodes and bone marrow aspirates in breast cancer and its correlation with other prognostic factors. *J Clin Oncol* 2001; 19: 960-971

Janni W, Gastroph S, Hepp F, Kentenich C, Rjosk D, Schindlbeck C, Dimpfl T, Sommer H and Braun S. Prognostic significance of an increased number of micrometastatic tumor cells in the bone marrow of patients with first recurrence of breast carcinoma. *Cancer* 2000; 88: 2252-2259

Weinschenker P, Soares HP, Clark O and Giglio AD. Immunocytochemical detection of epithelial cells in the bone marrow of primary breast cancer patients: a meta-analysis. *Breast Cancer Res Treat* 2004; 87: 215-224

Wiedswang G, Borgen E, Karesen R, Kvalheim G, Nesland JM, Qvist H, Schlichting E, Sauer T, Janbu J, Harbitz T and Naume B. Detection of isolated tumor cells in bone marrow is an independent prognostic factor in breast cancer. *J Clin Oncol* 2003; 21: 3469-3478

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

SPEAKER PRESENTATION

Identification and characterization of small subpopulations of candidate leukemia initiating cells within the CD34+CD38- and side population (SP) of patients with acute myeloid leukemia

Gerrit Jan SCHUURHUIS, Monique TERWIJN, Bijan MOSHAVER, Angele KELDER, Guus H WESTRA, Anna VAN RHENEN, Gert J OSSENKOPPELE, Sonja ZWEEGMAN.

Department of Hematology, VU University Medical Center, Amsterdam, Netherlands.

Acute myeloid leukemia (AML) likely originates from the CD34+CD38- hematopoietic stem cell (HSC). The so-called side population (SP), defined by Hoechst 33342 dye, might offer an alternative/supplementary stem cell compartment, especially for cases without a CD34+CD38- compartment present. The relationship between both compartments is largely unknown. We found that the CD34+CD38- compartment can be subdivided in an AML and normal compartment, based on expression of AML stem cell specific antigen CLL-1: CLL-1 positive CD34+CD38- cells carry AML specific cytogenetic aberrations and initiate leukemia in NOD/SCID mice (van Rhenen, Blood 110:2659, 2007). Lineage aberrancies including CD7 (T-cell marker), CD19 (B-cell marker), CD56 (NK cell marker) and aberrant myeloid aberrancies (e.g. CD33+CD13-), enabled to further define AML and normal CD34+CD38- sub-compartments (van Rhenen, Leukemia 21:1700, 2007). Further studies now show that normal and AML stem cells can be discriminated by other parameters: in the majority of cases the normal stem cells is lower in both forward scatter (FSC) and side scatter (SSC). Also, expression of CD34 and of the pan myeloid marker CD45 differs between normal and AML stem cells (both usually higher in the normal stem cell compartment). These characteristics now allow to distinghuish between normal and AML stem cells both at diagnosis and in bone marrow in remission. These findings led us to investigate whether SP cells too have aberrancies and whether these define primitive AML stem cells. SP cells were detected in 40 of 47 AML patients with median frequency of 0.02% (range 0.002-7.6%). In the majority of cases there was also a CD34+CD38- compartment with a median frequency of 0.44% (range 0-27%), which is 22 fold higher (in all individual cases >1-fold) than SP frequency. SP cells were partly or completely positive for CLL-1 (median 53%), CD7 (35%), CD19 (20%) and CD56 (53%). SP cells in NBM (n=12; median frequency 0.12%) were completely negative for CLL-1 and lineage markers (median 0%, ranges 0-4% at maximum). These results strongly suggest that a considerable part of SP cells is malignant, which was confirmed by FISH analysis. This low frequency SP fraction was remarkably heterogeneous with at least 4 different subpopulations present: 1) 3 with lymphoid characteristics, ie CD7+ (median 7% of total SP), CD19+ (2%) and CD56+ (4%), all 3 $CD45^{high}$ and CD48+; 2) a presumably differentiated myeloid population (median 54% of SP population; range 4-91%), lower CD45 expression, CD48-negative, with relatively high forward and sideward scatter (FSC^{high}/SSC^{high}), high CD38 expression (median 82%) and usually with aberrant marker expression; 3) a low-frequency myeloid fraction with low FSC and SSC and low CD45 expression, CD48-negative, lower CD38 expression (median 48%), and negative for aberrant markers and 4) a similar population but with aberrant markers present. The latter population,

however, had an higher FSC/SSC compared to population 3, while CD34/CD45 expression was usually lower compared to population 3. This thus resembles the earlier described differences between normal and AML stem cells as seen in the CD34+CD38- compartment. The fourth presumably primitive, malignant population had median frequency (of WBC) of only 0.0018% (range 0.00016-0.0056%).. The putative primitive character of the AML FSC^{low}/SSC^{low} marker positive SP subpopulation was substantiated by suspension culture for 5 weeks with subsequent CFU assay (14 days): FSC^{low}/SSC^{low} cells had >200 fold clonogenic ability compared to FSC^{high}/SSC^{high} cells (population 2).
The CD34+CD38- content in the SP fraction was 20% at maximum. The putative interrelationship between both defined stem cell compartments is presently under investigation.

Conclusions: Both the CD34+CD38- and the SP stem cell compartment contain primitive subpopulations of marker negative, $FSC^{low}SSC^{low}$, $CD34/CD45^{high}$ normal stem cells and marker positive, FSC^{high}/SSC^{high}, $CD34/CD45^{low}$ and marker positive stem cells. The frequency may be as low as median 1:100,000 to $1:10^6$ (for SP cells), thereby approaching the presumed stem cell frequency in diagnosis AML. The definition of sub-compartments that are highly enriched for stem cells, in principle allows to define highly accurately new AML stem specific targets. Also it allows to discriminate between normal and AML stem cells under all conditionsof disease./treatment. The possibility to monitor effects of any therapy simultaneously on the normal and AML stem cell present in the same patient, opens the way to assess individualized therapeutic windows.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

SPEAKER PRESENTATION

Isolation of multipotent mesenchymal stromal cells and their use in translational research.

André GOTHOT
GIGA-R, Haematology Unit
University of Liège

Mesenchymal stem cells (multipotent stromal cells, MSC) reside in the stromal compartment of the hematopoietic bone marrow. Although present in small numbers in vivo, MSC may be easily isolated by plastic adhesion and expanded in cell culture. MSC are able to generate bone, cartilage, fat, and under specific conditions, liver, muscle and nerve. Numerous studies have suggested a potential use of MSC to repair degenerative or traumatic lesions, in organs where tissue repair is limited. Furthermore, MSC are endowed with immunosuppressive properties, and the potential to control graft versus host disease and rejection of allogenic hematopoietic stem cell transplants.

Isolation by plastic adhesion and ex vivo passaging is easy and cost-effective. Yet, passaged MSC are heterogeneous and may differ markedly from their primary in vivo counterparts. Differences may involve expression of homing receptors, secretion of cytokines or growth factors as well as differentiation potential, all of which could have a tremendous impact on the ability of MSC transplants to produce therapeutic effects.

How passaged MSC differ from primary MSC is not known, mainly because of the lack of consensus regarding the exact phenotype of the in vivo MSC. Prospective isolation by flow cytometric sorting would allow the characterization of MSC in the native state, a possible resolution of MSC subsets and fast quality control testing of BM preparations. Several membrane antigens have been proposed for the isolation of primary MSC : STRO-1, CD49a, CD271, D7-Fib, GD2, SSEA-4,… among others. The discriminative power and specificity of these markers will be discussed.

SPEAKER PRESENTATION

Endothelial progenitor cells: myth or reality?

Frank TIMMERMANS
Gent, Belgium

The article by Frank Timmermans on "*Endothelial Outgrowth Cells Are Not Derived From CD133 Cells or CD45 Hematopoietic Precursors*" can be found at the end of this book of abstracts.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°1 & SELECTED ABSTRACT FOR ORAL PRESENTATION

Immunosuppression induced by immature dendritic cells is mediated by TGF- β and IL-10 double-positive CD4+ regulatory T cells.

Nathalie Cools, Viggo F.I. Van Tendeloo, Evelien L.J.M. Smits, Marc Lenjou, Griet Nijs, Katrien Vermeulen, Dirk R. Van Bockstaele, Zwi N. Berneman, Peter Ponsaerts.

Laboratory of Experimental Hematology, Vaccine & Infectious Disease Institute, Faculty of Medicine, University of Antwerp, Belgium.

Objectives: To investigate the cellular mechanisms of immune suppression induced by human immature dendritic cells.
Materials and methods: In this study, we compared the in vitro allogeneic and autologous stimulatory capacity of both immature and mature human dendritic cells (DC) using following tests: (1) cytokine secretion assays for detection of inflammatory cytokine profile, (2) tetramer staining for detection of antigen-specific T cell expansion, (3) multiparameter cytokine flow cytometry for detection and characterization of induced regulatory T cell populations, and (4) functional evaluation of induced regulatory T cell populations through suppression assays.
Results: In this study, we found that both immature (i) and mature (m) DC types induced a significant increase in the number of transforming growth factor (TGF)-β and interleukin (IL)-10 double-positive CD4+ T cells within one week of autologous DC/T cell co-cultures. In iDC/T cell cultures, where antigen-specific T cell priming was significantly reduced as compared to mDC/T cell cultures, we demonstrated that the tolerogenic effect of iDC was mediated by soluble TGF-β and IL-10 secreted by CD4+CD25-FOXP3- T cells. In addition, the suppressive capacity of CD4+ T cells conditioned by iDC was transferable to already primed antigen-specific CD8+ T cell cultures. In contrast, addition of CD4+ T cells conditioned by mDC to primed antigen-specific CD8+ T cells resulted in enhanced CD8+ T cell responses, notwithstanding the presence of TGF-β+/IL-10+ T cells in the transferred fraction. In summary, we hypothesize that DC have an active role in inducing immunosuppressive cytokine-secreting regulatory T cells. We show that iDC-conditioned CD4+ T cells are globally immunosuppressive, while mDC induce globally immunostimulatory CD4+ T cells. Furthermore, TGF-β+/IL-10+ T cells are expanded by DC independent of their maturation status, but their suppressive function is dependent on immaturity of DC.
Conclusions: DC have important functions in T cell immunity and T cell tolerance. Previously, it was believed that T cell unresponsiveness induced by iDC is caused by the absence of inflammatory signals in steady-state in vivo conditions and by the low expression levels of costimulatory molecules on iDC. However, in this study we now indicate that iDC can also actively maintain peripheral T cell tolerance by the induction and/or stimulation of regulatory T cell populations. The latter is of major importance for the development of future immunotherapeutic strategies aiming immunological tolerance against auto-immune diseases.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°2 & SELECTED ABSTRACT FOR ORAL PRESENTATION

Which technique(s) to use when studying minimal residual disease at day 35 in childhood acute lymphoblastic leukemia?

Katrien Swerts[1], Barbara De Moerloose[1], Barbara Denys[2], Marleen Bakkus[3], Bruno Verhasselt[2], Yves Benoit[1], Jan Philippé[2]

[1]Department of Pediatric Hematology and Oncology, Ghent University Hospital, Ghent, Belgium
[2]Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium
[3]Laboratory of Hematology, UZ Brussel, Brussels, Belgium

Detection and quantification of minimal residual disease (MRD) in pediatric acute lymphoblastic leukemia (ALL) patients is important for monitoring therapeutic response and detection of early relapse. Currently, different methodologies for MRD detection are available (multiparameter flow cytometry (FC), detection of fusion genes or WT1 overexpression using real-time quantitative (RT-)PCR, and competitive PCR-based quantification of clonal immunoglobulin and T-cell receptor gene rearrangements). In this study, the MRD detection rates of these three techniques were compared. In addition, the relationship between the MRD results and the clinical outcome of the pediatric ALL patients was studied.

At the end of induction therapy (day 35), bone marrow samples from 107 pediatric ALL patients (90 precursor B-ALL and 17 T-ALL), treated according to EORTC protocol 58951, were analyzed using at least one of these three methods.

More MRD positive patients were identified by FC (8/107; 7%) or (RT-)PCR (8/50; 16%) compared to cytomorphology (2/107; 2%) or IgH/TCR rearrangement analysis (3/97; 3%). Five out of eight patients positive by FC were positive by at least one other technique; likewise 4/8 and 2/3 children positive by (RT-)PCR or IgH/TCR rearrangement analysis scored positive for at least one other detection method.

Several studies demonstrated that the presence of MRD in bone marrow at the completion of induction therapy is associated with an increased risk of relapse in pediatric ALL. At the conclusion of this study, 5 patients suffered from an early relapse (< 30 months after diagnosis), whereas a continuous complete remission of more than 30 months was reached in 60 children (median follow-up: 63 months). The MRD results of these patients (n=65) were used to calculate the positive predictive value (PPV) and the negative predictive value (NPV) of the different detection techniques at day 35. The PPV of FC, (RT-)PCR and IgH/TCR rearrangement screening was 25%, 50% and 50%, respectively. In order to increase the PPV, the results of at least two tests were combined. Only when FC and (RT-)PCR were combined, a PPV of 100% was reached. The NPV's of the individual or combined tests were at least 92%, illustrating the acceptable specificity of the applied detection techniques.

In conclusion, these preliminary data indicate that in our hands (RT-)PCR and IgH/TCR rearrangement analysis are superior to FC in identifying patients at risk of early relapse. MRD detection rates obtained by FC and (RT-)PCR are higher than those obtained by competitive PCR-based IgH/TCR rearrangement analysis. However, FC has a relatively high false positive rate (PPV=25%) and MRD screening based on real-time quantitative (RT-)PCR detection of fusion genes or WT1 overexpression is only possible in about 50% of pediatric ALL patients. Therefore, it is advisable to combine different detection methods in order to optimize the predictive value of MRD testing.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°3 & SELECTED ABSTRACT FOR ORAL PRESENTATION

A highly sensitive and robust method for the detection of minimal residual disease in B-cell chronic lymphocytic leukaemia

Hensen K, Franke S, Berten M, Jongen H, Peeters V, Rummens JL, Maes B

Laboratory of Experimental Haematology and Molecular Biology, Virga Jesse Hospital, Hasselt, Belgium

Background: The introduction of new therapeutic agents such as fludarabine and alemtuzumab, with or without autologous or allogeneic stem-cell transplantation, has resulted in increased complete remission rates in B-cell chronic lymphocytic leukaemia (CLL). Preliminary data have suggested that the absence of minimal residual disease (MRD) is an end point of therapy that, if achieved, translates into an improved survival. Future prospective clinical trials that aim toward achieving long-lasting complete remissions should include a test to assess MRD. However, techniques for assessing MRD in CLL show various sensitivity levels and lack standardization.
Aim: We have developed and validated a combined method to assess MRD in CLL using fluorescence-activating cell sorting (FACS) and interphase fluorescence in situ hybridization (FISH) for the detection of numerical chromosomal aberrations that occur in up to 80 % of CLL cases.
Methods: CLL cells were purified from the peripheral blood of CLL patients by FACS-Aria (BD, US) based on the CD19+CD5+ co-expression, with a purity of > 95 %, as assessed by microscopy and by reanalysing with flow cytometry. These CLL cells were shown to harbour either deletion 11q22.3 (ATM) or deletion 13q14 in > 95 %, by using dual colour FISH. Peripheral blood samples from normal individuals were spiked with the purified CLL cells with dilutions of 10-3 to 10-6 white blood cells (WBC). WBC from these spiked samples were subsequently labelled with CD19 and CD5 moAbs and analysed by FACS. CD19+CD5+ cell fractions were purified by FACS-Aria and analysed by FISH for either deletion 13q or deletion 11q.
Results: FISH detection of the specific chromosomal aberration in CD19+CD5+ purified cells allowed discrimination of CLL cells from normal precursor B-cells. Reproducible positive results, above cut-off levels of the probe, were demonstrated in all dilutions up to 10-5 or 10-6. Quantification was feasible using the percentage of CD19+CD5+ cells and the percentage of aberrant purified cells.
Conclusions: This approach for the detection and quantification of MRD in CLL reaches a sensitivity at least as high as and even higher than other methods, such as four-color flow cytometry or quantitative allele-specific PCR. It can be used for at least 80 % of CLL patients, including all CLL patients with poor prognosis as assessed by the presence of the deletion 11q (ATM) or the deletion 17p (p53). Furthermore, it allows easy standardization among laboratories, applying FACS cell sorting, as it is based on a two-colour labelling only and on FISH assays using commercially available probes. We are now clinically validating the method by assessing MRD levels in intensively treated CLL patients and we propose this method as a candidate approach for assessing the clinical impact of MRD detection in prospective clinical trials on CLL.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°4 & SELECTED ABSTRACT FOR ORAL PRESENTATION

Rare Event Analysis and Quantification of Circulating Endothelial Progenitor Cells: A Methodological Comparison of Six Flow Cytometric Approaches

Emeline MF Van Craenenbroeck[1], Katrien Vermeulen[2], Nathalie Cools[2], Dirk R Van Bockstaele[3], Viggo FI Van Tendeloo[2], Christiaan J Vrints[1], Viviane MA Conraads[1], Vicky Y Hoymans[1]

[1] ***Department of Cardiology, Center for Cell Therapy and Regenerative Medicine, University Hospital Antwerp, Antwerpen, Belgium***
[2] ***Laboratory of Experimental Hematology, Vaccine & Infectious Disease Institute (VIDI), Faculty of Medicine, University of Antwerp, Antwerpen, Belgium***
[3] ***Esoterix, LabCorp, Mechelen, Belgium.***

Objectives: The validity of endothelial progenitor cells (EPC) as biomarkers and their therapeutic potential for cardiovascular disorders depend on the accuracy of techniques used for enumeration. Despite extensive research, it is still undetermined how EPC should be defined exactly. Investigators have focused on the expression of vascular endothelial growth factor receptor 2 (VEGFR2) and CD34/CD133, delineating both endothelial commitment and progenitor characteristics. Secondly, the fact that circulating CD34+/VEGFR2+ cells represent only 0.0001% to 0.01 % of peripheral blood mononuclear cells poses the technical challenge of counting extremely rare events. Multiparameter flowcytometry and a colony forming unit (CFU)-assay are currently the most widely used techniques. The heterogeneity in definition and methods complicates cross-study comparisons and may lead to apparent paradoxical results. This study assessed the agreement between 6 flow cytometric methods and a CFU assay used for EPC quantification.

Methods: Venous blood samples were obtained from 30 healthy subjects on 2 different occasions. CD34+/VEGFR2+ cells were detected by multiparameter flow cytometry, starting from whole blood (A-C) or PBMC (D-F) and using different gating strategies:

A: CD34+/VEGFR2+ cells within lymphocyte region;
B and D: exclusion of autofluorescent cells based on CD3 negative selection;
C and E: exclusion of autofluorescence and cell aggregates (pulse shape analysis by FSCarea/FSCpeak);
F: exclusion of autofluorescence, cell aggregates and non-nucleated cells.

Additionally, PBMC were cultured in Endocult® medium to assess CFU numbers.

POSTER n°4 & SELECTED ABSTRACT FOR ORAL PRESENTATION

Method	Starting material	Membrane markers	Gating strategy
A	200 µl WB	CD34, VEGFR2	Lymphocyte gating
B	200 µl WB	CD34, VEGFR2, CD3	CD3 negative selection
C	200 µl WB	CD34, VEGFR2, CD3	CD3 negative selection FSCarea/FSCpeak for single cells
D	PBMC from 2 ml PB	CD34, VEGFR2, CD3	CD3 negative selection
E	PBMC from 2 ml PB	CD34, VEGFR2, CD3	CD3 negative selection FSCarea/FSCpeak for single cells
F	PBMC from 2 ml PB	CD34, VEGFR2	Draq 5 nuclear stain FSCarea/FSCpeak for single cells Backgating

WB= whole blood; PB= peripheral blood

Results: The intra-observer agreement for the different gating strategies was excellent with an intra-class correlation coefficient (ICC) ranging from 0.867 to 0.974. Moderate agreement was found between methods B-C and D-E, which differed only in the exclusion of cell aggregates (ICC 0.647 and 0.530 respectively). Comparison of methods B and D (same gating strategy, but whole blood for method B and PBMC for method D) showed poor agreement (ICC 0.178). The same holds true when method C and E were compared, resulting in an ICC of 0.249. This was also the case for techniques that considerably differed with regard to gating strategies (A-B, A-F, B-F). CFU numbers did not correlate with flow cytometric quantification (all $p > 0.05$).

Conclusions: Agreement between methods for EPC quantification is moderate to poor, which may generate apparent controversial results. Although each protocol is highly reproducible, this study cautions against the comparison of results obtained with different enumeration techniques. With the prospect of a consensus protocol, the principles of rare event analysis require attention.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°5 & SELECTED ABSTRACT FOR ORAL PRESENTATION

Preparing for JAK2 V617F targeted therapy: development of a highly sensitive and simple real-time RT-PCR method for JAK2 V617F transcript quantification

Brigitte Maes, Rita Smets, Greet Bries, Vincent Madoe, Veerle Peeters, Jean-Luc Rummens

Laboratory of Experimental Haematology and Molecular Biology, Virga Jesse Hospital, Hasselt, Belgium

Background: The identification of the JAK2 V617F mutation, occurring in almost all cases of polycythemia vera (PV) as well as in approximately 60 % of cases of essential thrombocythemia (ET), has strongly simplified the diagnosis and classification of these diseases. Recently, it has been suggested that the JAK2 V617F burden may have prognostic significance. In addition, new promising JAK2 V617F targeted drugs are under development. Aim: We developed a novel real-time RT-PCR method for quantification of JAK2 V617F transcripts that would allow determination of JAK2 V617F burden at diagnosis as well as the evaluation of the response to newly developed therapeutic agents.
Methods: RT-PCR reactions were performed on a Rotor-Gene 3000 (Westburg) in single tubes with 1 set of primers and two differently labelled, allele-specific TaqMan probes, directed to respectively wild-type (WT) and mutant (Mut) JAK2 sequences (1 mismatch). Probes were adapted by Locked Nucleic Acid (LNA) modification for increased hybridization specificity and enhanced allelic discrimination. Standard curves were constructed with JAK2 V617F WT and Mut plasmids. Results are expressed as percentage of JAK2 V617F of total JAK2. Whole peripheral blood or bone marrow samples of a total of 54 JAK2 V617F positive cases, including 23 untreated and 7 conventionally treated PV cases and 19 untreated and 5 conventionally treated ET cases, were analysed. In addition, also 30 peripheral blood samples of normal individuals were analysed.
Results: Reaction efficiencies of this single tube assay for JAK2 Mut and JAK2 WT were equal (97 %). Quantities down to 10 copies of JAK2 Mut plasmid amongst WT cDNA and patient JAK2 V617F cDNA diluted down to 0,09 % into WT cDNA could be reliably detected. Low intra- and inter-assay variabilities ensure good reproducibility of the assay. None of the negative control samples showed any increase of the fluorescent signal derived from the Mut probe, demonstrating the high specificity of the assay and no requirement for defining a cut-off value. For PV patient samples, the assay showed mean JAK2 V617F quantities of 82 % for untreated cases versus 56 % for treated cases. Untreated ET cases showed a significantly lower mean JAK2 V617F % compared to untreated PV cases (55 % versus 82 %).
Conclusion: We have developed a robust and simple method for quantification of JAK2 V617F transcripts that is more sensitive than all previously described methods. It provides the potential to evaluate the prognostic significance of the JAK2 V617F burden at diagnosis as well as the response to JAK2 V617F targeted therapy that will become available in the near future.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°6 & SELECTED ABSTRACT FOR ORAL PRESENTATION

Modifications of gravity-sensitive signaling pathways by FGF in endothelial cells.

Myriam Ghardi[1,2], Hanane Derradji[1], Claudia Ulbrich[3], Kriss Westphal[3], Markus Wehland[3], Johann Bauer[4], Manfred Infanger[5], Reinhold Kreutz[3], Sonia Vadrucci[6], Marcel Egli[6], Augusto Cogoli[6], Daniela Grimm[3], Bernard Chatelain[2] and Sarah Baatout[1]

[1]Laboratory of Molecular and Cellular Biology, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium; [2]Laboratory of Experimental Hematology and Oncology, UCL Mont-Godinne, Yvoir, Belgium; [3]Institute of Clinical Pharmacology and Toxicology, Charité-Universitätsmedizin Berlin, Germany; [4]Max-Planck Institute of Biochemistry, Martinsried, Germany; [5]Department of Trauma and Reconstructive Surgery, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany; [6]Zero-g Lifetec GmbH and Space Biology Group, ETH Zurich, Zurich, Switzerland.

Prolonged exposure of astronauts to space radiation and extended microgravity has revealed profound physiological and clinical changes in the health. Many of the health problems thought to be explainable by the effects of microgravity include a decrease in the heart and the respiratory rates, a loss of body weight, changes in bone calcium, a redistribution of body fluids with greater amount in the upper body, a decrease in muscle tissue, a weakening of veins and arteries in the legs as well as an underproduction of red blood cells leading to anemia. Furthermore, microgravity induces a shift of body fluid from the lower to the upper part of the body that mainly occurs by exchange of water and solutes across the endothelial cell layer (which provides the principal barrier to transport of material between the blood and the underlying tissue). Furthermore, alterations in the vascular pressure associated with microgravity changes the transvascular pressure differential which drives the fluid transport from blood to the tissues.

In this report, we were interested in further investigate the in vitro effects of microgravity on endothelial cells. In particular, the EA.hy926 endothelial cell line (EC) was cultured under normal gravity (1g) and microgravity (µg) conditions in the presence or absence of basic fibroblast growth factor (bFGF). Gene expression, apoptosis, extracellular markers and cytokine release were therafter studied. First, we showed that µg early increased endothelin-1 (ET-1) as well as TGF-ß1 gene expression whilst bFGF reduced ET-1 and the enzyme endothelial nitric oxide synthase (eNOS) gene expression. Secondly, investigating apoptosis, we observed that µg induced an elevation of Bax, Fas, activated caspase 3 and p53 protein levels whilst bFGF exerted synergistic effects on Bax and p53 only; bFGF reducing Bcl-2 and caspase-3 in µg cultures. We thereafter investigated the effects of µg and bFGF on extracellular matrix proteins and showed that µg as well as bFGF increased the basic cellular amount of collagen type I and that laminin and fibronectin productions were upregulated to a larger extent by µg than by bFGF. Finally, since growth factors or interleukins released into

the supernatant may affect cells by binding to their corresponding receptors, we measured the release of cytokines in the culture supernatants and showed that bFGF triggered the release of IL-6, IL-8 and TGF-ß1. No effect of µg or bFGF was observed on IL-1, adiponectin and TNF-α.

In conclusion, these results show that microgravity alters the impact of bFGF on the intracellular signaling network, by regulating functions that differ depending on the gravity conditions.

This research is partly financed by the European Space Agency and the Belgian Science Policy (PRODEX contract n°C90303). M Ghardi is supported by a doctoral grant from SCK●CEN/UCL.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°7

Effects of acute oxidative stress on the physiology of *Arthrospira* sp. PCC8005

Nicolas Morin[1, 2], *Sarah Baatout*[1], *Annick Wilmotte*[2], *Larissa Hendrickx*[1], *Max Mergeay*[1]

[1] Laboratory of Molecular and Cellular Biology, Belgian Nuclear Research Center (SCK•CEN), Mol, Belgium
[2] Center for Protein Engineering, Institute of Chemistry B6, Sart-Tilman, University of Liège, Liège, Belgium

At the time being, space research is entering a new era. Scientists all around the world are combining their thoughts towards pushing the human frontier one step further, as numerous projects are expected for the next twenty years, including the implementation of a lunar base or the organization of a human trip to Mars. Nevertheless, in order to attend such ambitious research programs, the development of life support systems has become a necessity in order to ensure the safety of man in space by providing him with food, water, oxygen, and recycling his wastes. Among the different projects actually under development, the MELiSSA (Micro-Ecological Life Support System Alternative) project of the European Space Agency presents the particularity of combining bacteria, higher plants and photosynthetic microorganisms to process the wastes, recreate the atmosphere and produce biomass.

Due to its performant oxygen production, along with its valuable nutritional properties, the cyanobacterium *Arthrospira* sp. PCC8005 has been selected as the primary oxygen producer and complementary food source in the MELiSSA recycling loop. As a life support system organism, *Arthrospira* will hence undergo different stresses induced by the environmental conditions encountered in space. Among these conditions, oxidative stresses (including cosmic radiations, UV or high light) might have an important effect on the metabolism of this photosynthetic organism.

In order to study the effects of oxidative stress on *Arthrospira*, we tested the evolution of different physiological parameters (membrane permeability, membrane potential, redox activity, viability/vitality and chlorophyll autofluorescence) after a pulse exposition of one hour to different concentrations of hydrogen peroxide (H_2O_2), using flow cytometry. The results obtained allow us to determine a response curve of *Arthrospira* in function of H_2O_2 concentration, and to highlight a certain range of resistance of this organism to oxidative stress. This will be of major importance in the pursuit of the study of space-related environmental effects and in the future design of in-flight monitoring methods.

The presented project is financially supported by the European Space Agency and the Belgian Science Policy (Belspo) (MELISSA/BIORAT Genetic stability study - phase 2 (MELGEN 2) TEC-MCT/2006/3501/In/CL). We are grateful to C. Lasseur and F. Marty, both from ESTEC/ESA, for their support and advice.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°8

Potential of BAL fluid examination for the diagnosis of pulmonary sarcoidosis.

E. Van Gastel, Marijke Reynders, L. Verfaillie, L. Smet, W. Renmans. M. De Waele.

Departments of Haematology and Pneumology, UZ Brussel, 1090 Brussel.

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology most frequently presenting with hilar lymphadenopathy and pulmonary infiltration. The diagnosis is made by the finding of noncaseating epitheloid-cell granulomas in a bronchial biopsy after exclusion of all other causative agents. During the last few years cytological examination and immunophenotyping of broncho-alveolar lavage (BAL) fluid are often performed to support the diagnosis. In this study the potential of this approach is examined.

BAL fluid of 249 patients with pulmonary disease was examined including 40 patients with sarcoidosis and 209 patients with other lung disorders such as COPD, asthma, pneumonia, other interstitial lung disorders, neoplasms and tuberculosis. White and red blood cells were counted and a differential count was made on a cytocentrifuge preparation stained with May Grünwald Giemsa. Lymphocyte subsets were enumerated with monoclonal antibodies and flow cytometry.

The number of red or white blood cells in the BAL fluid were not significantly different between sarcoidosis and non-sarcoidosis patients (Mann-Whitney test). Sarcoidosis patients had a lower percentage of neutrophils and a higher percentage of lymphocytes in their BAL fluid. In addition they showed a higher percentage of T-lymphocytes (CD3+) and of CD4+ T cells, a lower percentage of CD8+ T lymphocytes and a higher CD4/CD8 ratio than non-sarcoidosis patients. With ROC analysis the highest area under the curve was found for the % CD4+ T cells (0.765), the CD4/CD8 ratio (0.758) and the % CD8+ T cells (0.738). The cut-off points in the ROC analysis were 57% for CD4+ T-cells, 27% for CD8+ T-cells, 3.0 for CD4/CD8 ratio, 10% for % neutrophils and 7.5% for % lymphocytes. The highest sensitivity at the cut-off point was found for % lymphocytes (87.5%) and % neutrophils (86%), but the specificity was low, 44.5% and 67.5% respectively. The sensitivity for % CD4+T cells, % CD8+ T-cells and the CD4/CD8 ratio at their cut-off points was 77.5%, 65% and 60% respectively, while the specificity was 70.8%, 69.4% and 82.3%. The negative predictive value at the cut-off point for all these parameters was above 90%.

In conclusion, a high percentage of lymphocytes or of CD4+ T-cells, a high CD4/CD8 ratio and low percentages of lymphocytes or CD8+ T cells in BAL fluid are in favour of a diagnosis of pulmonary sarcoidosis. However a considerable overlap with the findings in other lung diseases exists. Only extreme values, like a CD4/CD8 ratio above 7.3, occurred only in sarcoidosis. Therefore BAL fluid examination cannot replace the bronchial biopsy for the diagnosis of pulmonary sarcoidosis.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°9

Neutrophils CD64 expression level is a valuable inflammatory marker in haematology patients.

G Detry, A. Dubois, B Husson, L boon-Falleur, J Cadrobbi and A Delannoy
Centre Hospitalier de Jolimont, Haine Saint Paul, Belgium

Plasma membrane receptor for the cristallisable fragment (Fc) of Immunoglobulins G (IgG) have been shown to consist of three main types: FcgRI (CD64), FcgRII (CD32) and FcgRIII (CD16). All three belong to the immunoglobulin gene superfamily and play an important role in the immune response, ea in the antibody dependent cell cytotoxicity phagocytosis and immune complexes clearence. CD64 is a high affinity receptor for IgG and constitutively expressed on monocytes, macrophages and eosinophils. The level of expression of this receptor on neutrophils is low but is upregulated by various interleukines such as INFg. Several papers have described neutrophils CD64 expression levels as an help in the identification of septic patients, particularly in the newborns. We have evaluated in this study whether the assesment of CD64 expression on neutrophils could add any sensitivity or specificty in the diagnosis of septic shock in patients hospitalised in haematology unit.

We studied 16 control subjects with no evidence of infectious problems, 30 septic patients from the emergency or intesive care units with blood cultures of gram positive and gram negative bacteria (two differents positive blood cultures were required for gram positive cocci) and 33 patients hospitalised in the haematology unit. For this last group of patients, CD64 index was realised as a prospective test for septicemia with every blood cultures. All the CD64 assays for the septic patients and patients hospitalised in the haematology unit were compared with CRP and fibrinogen level.

CD64 indexes were measured on neutrophils with the Leuko64^{TM} detection kit and used as described by the manufacturer (Trillium diagnostic, LLC, Brewer, USA) kindly provided by IQ products. Data were acquired on a Flacsort (Decton Dickinson, San Jose, USA). CRP were measured by the CRP Latex assays (Olympus) on an Olympus AU600 analyser. Fibrinogen were measured with the thrombin reagent (Dade-Behring) on a CA-7000 analyser (Sysmex).

The statistical power of the CRP level and CD64 index assays for the positive predictive value of septicemia was evaluated with a Receiving-Operating Curve analysis. The proposed cut-off levels for CRP level and CD64 index assays are 8.5 mg/dL and 1.8 respectively. With these cut-off values, the CRP assays shows the best overall diagnostic power with a specificity of 61% and a sensitivity of 97%. The CD64 index assays has a specificity of 34% and a sensitivity of 90%.In conclusion, CD64 is a valuable assays for the diagnosis of septic shock in haematological patients but has several disadvantages compared to CRP level assays. CD64 index determinations are expensive, time consumming, required costly instruments and some technical skills in the interpretation of data. Nevertheless, this approach can be appropriate in some categories of patients such as newborns in witch CRP level may not be a reliable assay for the diagnosis or the exclusion of septicemia.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°10

AML with monocytic component associated to bundles of Auer rods : a case report

[a]J. Cadrobbi, [a]G. Detry, [a]B. Husson, [a]L. Demol, [b]L. Michaux, [b]N. Straetmans and [b]A. Delannoy
[a]Laboratory, Hôpital de Jolimont, Haine-Saint-Paul, Belgium
[b]Haematology Unit, Hôpital de Jolimont, Haine-Saint-Paul, Belgium

Acute myelocytic leukemia (AML), French-American-British (FAB) subtype M3 is defined by either the translocation t(15;17)(q22;q21) or the fusion of *PML* and *RARα* genes. The classic form shows abnormal promyelocytes with heavy granulation and bundles of Auer rods.
AML-M5 is a subtype in wich monocytic population predominate with two distinct morphologic subcategories, M5a and M5b and is usually related with rearrangements of the MLL (11q23) gene.
We report a case of AML with monocytic component associated to bundles of Auer rods.
A 65-year-old woman presented in our hospital with leucopenia, trombocytopenia and with 10 % of anarchic promyelocytes in peripherical blood.
Marrow aspiration showed 12 % of abnormal promyelocytes and 69 % of immature monocytic cells (monoblasts and promonocytes). Cytochemistry and flow cytometry confirmed the presence of this two populations.
Flow cytometry immunophenotyping revealed 7 % of promyelocytes and 42 % of monocytic cells.
Promyelocytes showed self-fluorescence and expressed CD117, CD13, CD33 and CD9. HLA-DR, CD34 and CD66b were absent.
The monocytic population expressed HLA-DR, CD11c, CD36, CD38, CD33 and CD4 (weak). CD34 and CD117 were negative. In addition, absence of CD 14 and weak expression of CD13 were two abnormal markers.
Immunocytochemistry with antibodies against the PML gene product didn't shown a characteristic nuclear multigranular pattern.
Cytogenetic analysis revealed the chromosome translocation t(9;11)(p22;q33) and FISH (fluorescence in situ hybridation) confirmed involvement of the MLL gene and the absence of a translocation of the RARalpha gene. Then, the final diagnosis of AML M5b was made.
This case illustrates the necessity of a mutli-modality diagnostic approach including morphology, cytochemistry, flow cytometry, immunocytochemistry and conventional cytogenetic study.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°11

Dendritic cells in atherosclerosis: their involvement in the pathogenesis of plaque rupture

Ilse Van Brussel[a], Emily A. Van Vré[a], Hidde Bult[b], Christiaan J. Vrints[a], Johan M. Bosmans[a]
Divisions of [a]Cardiology and [b]Pharmacology, University of Antwerp, Wilrijk

***Objective**:* Recently a decrease of circulating dendritic cells (DCs) in patients with unstable coronary syndromes was reported. To test whether this decrease was one of the causes or just a result of plaque rupture, percutaneous transluminal coronary angioplasty (PTCA) was used as a model of plaque rupture. In a second, histological part, the presence of DC-precursors and more differentiated DC-types in the atherosclerotic plaque was investigated.

***Methods**:* Blood samples of 21 patients were collected before, immediately after and 17-24 h. after angioplasty. Flow cytometry was used to determine the number of circulating myeloid (MDC, BDCA-1^+) and plasmacytoid (PDC, BDCA-2^+) subtypes, regulatory T-cells (Tregs) were characterized as $CD3^+$, $CD4^+$ and $CD25^+$, but $CD127^-$. CRP and troponin I were measured in serum. In the histological study, 6 endarterectomy specimens were immuno-histochemically stained with antibodies against S100 and fascin (more mature DC), CD11c and BDCA-1 (MDC), CD123 and BDCA-2 (PDC), CD3 (T cells), CD68 (macrophages), CD14 (monocytes) and vWF (endothelial cells).

Results: The in vivo study showed a rise in the number of circulating MDC (8083 $\pm$ 661 vs 10617 $\pm$ 1008; p = 0.004) and regulatory T cells (20878 $\pm$ 2049 vs 26609 $\pm$ 3642; p = 0.06) 17-24 h. after the angioplasty, whereas the circulating PDC number tended to be reduced (5672 $\pm$ 778 vs 4594 $\pm$ 531; p = 0.06). Furthermore, an increase of troponin I (0.055 $\pm$ 0.06 vs 0.283 $\pm$ 0.447; p = 0.006) with a similar tendency for hsCRP (p = 0.06) pointed to myocardial necrosis and an inflammatory response. Immunohistochemistry showed the presence of both MDC and PDC in atherosclerotic plaques. There were more MDC than PDC, but DC-precursors were less numerous than more mature DCs. Mature DC tended to be correlated with T cells (p = 0.06), but not with CRP (p = 0.7).

***Conclusions**:* PTCA provoked an acute inflammatory response, with significant increases of MDC 17-24 h. after the procedure. The elevated troponin I pointed to discrete myocardial necrosis that appears during the angioplasty. Although the analysis of plaque rupture was possibly confounded by the other factors, our data suggest a rather causal role of DCs in the process of plaque rupture. The histological observations support the hypothesis of an infiltration of DC precursors from the circulation to the atherosclerotic lesion.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°12

Evaluation of the Beckman Coulter® FP 1000 Cell Preparation System in the pre-analytical phase of flow cytometric whole blood lymphocyte immunophenotyping.

Barbara Denys, Simon Lambert and Jan Philippé
Department of Clinical Chemistry, Microbiology and Immunology,
Ghent University Hospital, Ghent, Belgium

Introduction: Multiparametric immunophenotyping in whole blood with the fluorescently labelled monoclonal antibodies CD45, CD19, CD3, CD4 and CD8 is a frequently used combination of markers in clinical laboratories. Currently, this technique is labour-intensive and time-consuming because of the need for manual sample preparation.

Aim: The objective of this study was to evaluate an automated preparation system to be used in combination with our flow cytometer (Beckman Coulter® FC500 Cytomics). We wished to create a flexible automated cytometric solution that can reduce work load but offers equal analytical quality.

Materials and Methods: We compared different fix&prep system on the Beckman Coulter® FP 1000 prototype Cell Preparation System with the accredited manual procedure. Initially, comparisons were made on 112 randomly selected patient samples. An optimized protocol was validated on another 41 samples.

Results: Initially, we observed a decreased forward scatter (FS) signal of all lymphocytes with the FP 1000 protocol, resulting in an underestimation of in particular the fraction CD19+ cells. Adjustment of flow cytometer gain parameters did not solve the problem. However, since Versalyse™ Lysing Solution (Beckman Coulter®) used in the FP 1000 protocol does not contain any fixative, in contrast to OptiLyse C (Beckman Coulter®), used in the manual sample preparations that contains formaldehyde, we inferred this could explain the different scatter properties. Indeed, 41 whole blood samples analysed on FP 1000 after adding PBS-formaldehyde 1% to each mixture showed an increased FS-signal comparable to that found with the manual method.

Passing and Bablok regression comparing the manual preparation method (OptiLyse C protocol, abscissa) and the adapted automated FP 1000 method (Versalyse™ with addition of formaldehyde, ordinate) yielded a good correlation for the different markers (Table, n=41):

Whole Blood	Correlation Coëfficient	Slope	Intercept
CD19	0,995 95% CI: 0,991 to 0,997	1,01 95% CI: 0,98 to 1,07	-0,31 95% CI: -1,19 to 0,21
CD3	0,989 95% CI: 0,980 to 0,994	0,98 95% CI: 0,92 to 1,03	0,95 95% CI: -3,12 to 4,99
CD4	0,996 95% CI: 0,992 to 0,998	1,02 95% CI: 0,99 to 1,05	-1,03 95% CI: -1,75 to -0,21
CD8	0,995 95% CI: 0,990 to 0,997	1,00 95% CI: 0,97 to 1,03	-0,10 95% CI: -1,26 to 0,71

During the evaluation period, the instrument was considered as user friendly with options to work on-line that could improve work load. A technical problem regarding evaporation of the monoclonal antibody reagents was detected. This was in part solved by putting piercable caps on the reagent bottles and it will be completely solved in the near future by installing a refrigerating element.

Conclusion: After optimization of the lysing solution, results obtained with the automated FP 1000 Cell Preparation System were comparable to the manual procedure. We conclude that the instrument, with either little technical adaptations, can improve workflow, especially for lymphocyte subset immunophenotyping. FP 1000 allows to work in standardised conditions and reduces the risk for errors. Moreover, the FP 1000 is easy to use with limited skills and experience needed.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°13

A correct gating strategy plays an important role in rare event detection of endothelial progenitor cells.

Remco Koninckx[1,2]; Hanne Jongen[1]; Paul Steels[2]; Marc Hendrikx[2,3]; Paul Dendale[4]; Dominique Hansen[4]; Jean-Luc Rummens[1,2]; Karen Hensen[1]

[1] Laboratory of Experimental Hematology, Virga Jesse Hospital, Hasselt, Belgium; [2] Laboratory of Physiology, Faculty of Medicine and School of Life Sciences, Hasselt University,Biomedical Research Institute and Transnational University Limburg, Belgium; [3] Department of Cardiothoracic Surgery, Virga Jesse Hospital, Hasselt, Belgium; [4] Rehabilitation and Health Centre (ReGo) Virga Jesse Hospital, Hasselt, Belgium

Endothelial progenitor cells (EPCs), mainly present in bone marrow, are stem cells, which have the capacity to differentiate into mature endothelial cells. Several studies suggested that EPCs could contribute in neovascularisation and re-endothelialisation. Although EPCs are lowly abundant (0,0001 to 0,01%) in circulation, they can be mobilized from the bone marrow to peripheral blood by several factors such as vascular disorders, systemic administration of cytokines or as recently described by physical exercise. Moreover, EPC dysfunction can give important information on the course of various diseases ranging from cancer to cardiovascular disorders. Indeed, the number of circulating EPCs and their migratory activity are reduced in patients with diabetes, coronary artery disease (CAD), and in subjects with multiple coronary risk factors. Consequently, detection and enumeration of EPCs in peripheral circulation can serve as an indicator of poor prognosis. EPCs can be characterised by the detection of the haematopoietic stem cell antigens CD133 and CD34 in addition to the endothelial marker VEGFR-2.

In the present study, a six-colour multiparameter flowcytometric panel was optimised using following combinations of monoclonal antibodies: CD3-FITC; CD15-FITC; CD133-PE; CD14-PerCP; CD34-PE-Cy7; VEGFR2-APC and CD45-APC-Cy7. We validated our protocol by comparing EPC numbers before and after physical exercise. For the accurate detection of cells with such low frequencies, it was necessary to build in several controls. At first, fluorochrome matched isotype controls were used in combination with fluorescence minus one (FMO), in order to have a good estimate of non-specific binding of antibodies to cells. In addition, the FITC channel was reserved as dump channel using anti-CD3- and anti-CD15-FITC conjugated monoclonal antibodies. Nevertheless, background noise between 0.001% and 0.006% was measured, which is significantly compared to the concentrations EPCs represent in blood. By optimising the gating strategy, background noise could be substantially diminished to levels of 0.00008%. Moreover, when the optimised gating strategy was used, a statistically significant difference of EPC numbers before and after exercise could be obtained. The absolute number of CD34+/CD133+/VEGFR2+ cells per ml blood changed nearly 2-fold (from 5,92 ± 6.09 before exercise to 11.01 ± 9.60 after exercise ($p=0.04$) $n=10$).

In conclusion, we developed a six-colour flowcytometric assay to detect EPCs in circulation. Our results indicated that the use of a good gating strategy is very important for correct analysis and should be taken into consideration when performing rare event detection.

12th annual meeting of the Belgian Association for Cytological Analysis,
"Rare event analysis", Mons, 26/10/2007.

POSTER n°14

Radiation-induced damage in human blood cells.

Kharman Faraj Akram, Hanane Derradji, Mazin Manuel Elias, Sarah Baatout.*

Laboratory of Molecular and Cellular Biology, Belgian Nuclear Research Center, SCK•CEN, Mol, Belgium;
****Institute of laser for postgraduate studies, University of Baghdad, Baghdad, Iraq.***

Molecular biological markers of radiation response are thought to be of potential use for monitoring the progress of radiation therapy and even for predicting outcome early in a treatment regimen. They are also possible tools for monitoring potentially exposed populations after a radiological accident or a "dirty bomb" incident. Although still in its infancy as a scientific discipline, the study of radiation biomarkers includes DNA mutation, chromosome aberrations, apoptosis as well as protein and gene expression array technologies. However, additional studies are needed to validate candidate biomarkers (molecular and proteic) for applied biological dosimetry applications and that could provide early and rapid information after exposure to radiation. In this respect, flow cytometry as well as multiplex array assay technology are promising tools. Flow cytometry is a general method to rapidly analyse individually large numbers of cells using light-scattering and fluorescence measurements. The power of this method lies both in the wide range of cellular parameters that can be concomitantly determined as well as in the ability to obtain information on large cell populations. We are currently studying the effects of low and high doses (0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2 and 4 Gy) of X- and γ-rays on DNA damage, chromosome aberrations and apoptosis in human white blood cell populations. The low doses reflect the doses received by nuclear workers in case of a nuclear incident whilst the high doses correspond to a conventional radiotherapeutical treatment. For the detection of radiation-induced apoptosis and DNA damage, flow cytometry is used. Multiplex array assay is chosen for the measurement of the cytokines released in the plasma or culture medium. The analysis of chromosomal aberrations is the classical method and is assumed to be a sensitive method capable of detecting changes caused by ionising radiation in the cell genome of the living organism. Dicentric and tricentric chromosomes result from asymmetrical interchanges between two and three chromosomes, respectively. Dicentrics, can be analyzed with greater efficiency than any other aberration type (> 95%) and it is their frequency that is generally used for estimations of radiation dose. For this purpose, blood samples were collected, diluted into culture medium supplemented with phytohaemagglutinine for T-lymphocyte activation, irradiated and then incubated for 48 hrs. The cells were then blocked in metaphasis by adding colcemid and then spread for chromosomal preparations. Giemsa staining was then performed and chromosome aberrations were finally detected under the microscope. Our results of the metaphase observations after irradiation of the human blood are in accordance with previous studies that showed an increase in the frequency of chromosomal aberrations in function of the dose received.

Concerning the detection of radiation-induced apoptosis and cell cycle change, blood samples were collected from donors. White blood cells were separated, cultured, irradiated, incubated for 72 hrs and finally fixed in ethanol. Cells were then stained with propidium iodide and analysed by flow cytometry. We showed that radiation caused a cell cycle delay dominated by a period of G2-arrest which was proportional to the position of the cells in the cell cycle and to the dose delivered. Two other methods are now being implemented to first precisely estimate radiation-induced DNA damage by flow cytometry using a specific marker of double strand breaks induced by ionising radiation, namely, γH2AX. Concomitantly, we are also developing a strategy to optimize and validate radiation-responsive protein biomarkers using the multiplex array assay technology for high-sample throughput analysis after having selected radiation-responsive protein targets that have shown a robust radiation dose response demonstrated using gene-expression microarray.

K. F. Akram is supported by a doctoral grant from the University of Baghdad (Iraq) and SCK●CEN (Belgium).