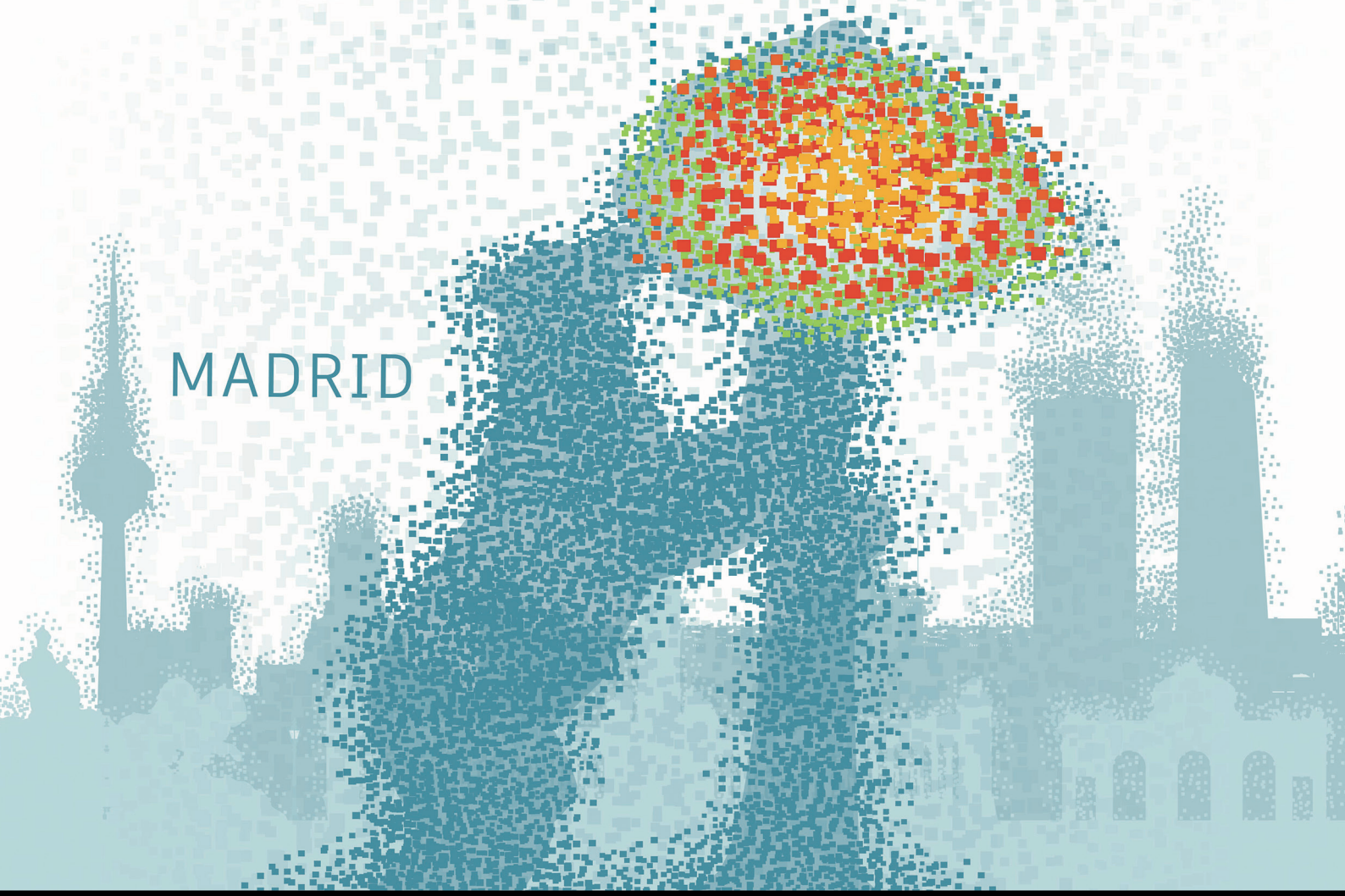
SIC2023

XXIII

CONGRESS OF THE IBERIAN SOCIETY OF CYTOMETRY

19 - 21 April, 2023



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SIC SOCIEDAD IBÉRICA DE CITOMETRÍA

Hotel Meliá Avenida de América. Madrid

XVIII CONGRESS
OF THE IBERIAN SOCIETY
OF CYTOMETRY

00 Contents

01

Welcome Letters

Julia Almeida
President
Iberian Society of Cytometry

Dear colleagues, dear friends,

It is my pleasure to welcome you all to the first SIC Congress after the COVID-19 pandemic in an entirely face-to-face format, so during the meeting we will be able to interact and network again, in a more personalized way that behind the screens.

I also take this opportunity to thank for all the hard work done by the Organizing and Scientific Committees of SIC2023 Congress, chaired by Lola Martínez and José Carlos Segovia, welcoming us in the wonderful and cosmopolitan city of Madrid, and providing an interesting program, which includes innovative topics that cover all the different scientific areas of interest for our Society, from basic to clinical cytometry. Of course, it is also my pleasure to welcome all the great scientists invited to participate as speakers, and to thank all of them for their commitment to share their recent studies and research.

I am sure that SIC2023 Congress will bring people together now again in a face-to-face way; I encourage you to have an active and enthusiastic participation and to enjoy Madrid.

With warm regards.

Lola Martínez
José Carlos Segovia
Local organizers
XVIII Congress of the Iberian Society of Cytometry
Madrid, 19th-21st April, 2023

Dear colleagues,

It is a pleasure to inform you that the next biannual congress of our society, the Iberian Society of Cytometry (SIC), will be held in Madrid at the Hotel Avenida América, from April 19th to 21st, 2023.

After the hard years of the pandemic, which forced us to communicate mainly through our computer screens, we are very pleased to inform you that, this new edition of our biannual congress (if there are no unexpected circumstances preventing it), will be again face-to-face. During these two and a half days we will have the opportunity to meet again and tell each other about our work and experiences in person, something we dare say we were all wanting and needing.

We have prepared a congress that will cover the applications of cytometry in diverse fields, including clinical, environmental basic research. We have given special importance to the presentation of new technologies that can complement the results obtained by conventional cytometry. This includes particularly the new developments in full spectral cytometry and massive multiparametric analysis, which are maybe called to be the next standard in flow cytometric analysis.

In addition, we have prepared some pre-conference courses that we hope will be useful not only for newcomers to this discipline, but also to update the most experienced.

Do not hesitate and book this event in your agendas. We are waiting for you in Madrid!

Best Wishes.

02
Committees

ORGANIZING COMMITTEE

José Carlos Segovia
CIEMAT-UCM. Madrid, Spain

Rebeca Sánchez
CIEMAT-UCM. Madrid, Spain

Beatriz Álvarez
Centro Nacional de Investigaciones Cardiovasculares (CNIC) Madrid, Spain

Julia García-Lestón
Centro Nacional de Investigaciones Oncológicas (CNIO). Madrid, Spain

Lola Martínez
Centro Nacional de Investigaciones Oncológicas (CNIO). Madrid, Spain

SCIENTIFIC COMMITTEE

Beatriz Álvarez
Centro Nacional de Investigaciones Cardiovasculares (CNIC) Madrid, Spain

Alberto Álvarez
Universidad de Extremadura. Badajoz, Spain

María Arroz
Centro Hospitalar de Lisboa Ocidental. Lisbon, Portugal

Marco A. Fernández
Institut d'Invenstigació Germans Trias I Pujol. Barcelona, Spain

Julia García-Lestón
Centro Nacional de Investigaciones Oncológicas (CNIO). Madrid, Spain

Carlos Lázaro
CatLab, Barcelona, Spain

Concepción Marañón
Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica (GENYO). Granada, Spain

Lola Martínez
Centro Nacional de Investigaciones Oncológicas (CNIO). Madrid, Spain

Marta Monteiro
Instituto Gulbenkian de Ciência. Lisbon, Portugal

Andre Mozes
Champalimaud Foundation. Lisbon, Portugal

José Enrique O'Connor
Universidad de Valencia. Valencia, Spain

Rebeca Sánchez
CIEMAT-UCM. Madrid, Spain

José Carlos Segovia
CIEMAT-UCM. Madrid, Spain

Dolores Subirá
Hospital Universitario de Guadalajara. Guadalajara. Spain

Neus Villamor
Hospital Clínic. Barcelona, Spain

03

General Information

GENERAL INFORMATION

VENUE

Hotel Meliá Avenida América
C/ Juan Ignacio Luca de Tena, 36
28027 Madrid (Spain)
Tel: + 34 914 23 2400
Web: www.melia.com

ORGANIZED BY

Sociedad Ibérica de Citometría

TECHNICAL SECRETARIAT

Tel: +34 91 002 90 17
Website www.sic-congress.com
e-mail: sic2023@imagineevents.es
Tel: +34 91 002 9017

TECHNICAL SECRETARIAT SCHEDULE

Wednesday 19th April: 8.00h -19.00h
Thrusday 20th April: 8.00h -19.00h
Friday 21th April: 8.30h -14.00h

CONGRESS BADGE

On arrival, at the registration desk you will receive your name badge. The organization kindly as you to carry always your badge with you at the Congress, during all sessions and social events.

CLOSING DINNER

Closing dinner will be held on Thrusday 20th at Ramses Restaurant.
Pl. de la Independencia, 4. Madrid.
Time: 21:00h. Transfer by bus will be available from the venue.
Please check the schedule at technical secretariat.

04
Programme

PROGRAMME AT GLANCE

TIME	WEDNESDAY 19th APRIL		
8:00-18:00	REGISTRATION		
9:00-11:00	**WORKSHOP 1 (Zaragoza III)** How to succeed in High-content Cytometry – Basics Sponsored by IZASA Scientific & Miltenyi Biotec	**WORKSHOP 2 (Tenerife I)** Detection of Nanoparticles (EVs, virus & nanoplastics) by FC: Nanocytometry Sponsored by Beckman Coulter and Miltenyi Biotec	**WORKSHOP 3 (Tenerife II)** Chronic Lymphoproliferative Disorders – Case Presentations
11:00-11:30	COFFEE BREAK (Zaragoza I-II)		
11:30-13:30	**WORKSHOP 4 (Zaragoza III)** High-content Cytometry Data Analysis - data analysis challenge by OMIQ, FCSExpress, FlowJo, Infinicyt, Tercen, AltraBio Sponsored by IZASA Scientific and DeNovo Software (Dotmatics)	**WORKSHOP 5 (Tenerife I)** Systematic multicolour panel design in the era of spectral cytometry	**WORKSHOP 6 (Tenerife II)** Measurable residual disease in B-cell precursor acute lymphoblastic leukemia (BCP-ALL)
13:30-14:30	LUNCH/REGISTRATION		
14:30-14:40	WELCOME SESSION (Zaragoza III-IV)		
14:30-15:40	**OPENING LECTURE (Zaragoza III-IV)** Florian Mair (ETH, Zurich): Disentangling human tumor-unique immune phenotypes from non-malignant tissue inflammation using multi-omic single cell analysis		
15:45-16:15	BREAK		
15:45-16:15	COMMERCIAL TALK I BD - Enzifarma (Zaragoza III)	COMMERCIAL TALK II Standard Biotools (Tenerife I)	COMMERCIAL TALK III Palex - Cytek (Tenerife II)
16:20-18:15	**PARALLEL SESSION I (Zaragoza III)** Flow Cytometrry in Advanced Therapies **PARALLEL SESSION I (Zaragoza III)** Flow Cytometrry in Advanced Therapies		
18:20-19:00	SIC WORKING GROUPS/GECLID (Zaragoza III & Tenerife I)		
19:00-21:00	WELCOME COCKTAIL/EXHIBITION OPENING/POSTERS (Zaragoza I-II)		

TIME	THURSDAY 20th APRIL	
8:00-18:00	**REGISTRATION**	
9:00-10:45	**PLENARY SESSION I (Zaragoza III-IV)** Beyond Technology	
10:50-11:20	**COFFEE BREAK (ZARAGOZA I-II)**	
15:45-16:15	COMMERCIAL TALK IV BD - Enzifarma (Tenerife I)	COMMERCIAL TALK V Bonsai Lab (Tenerife II)
11:30-13:30	**PARALLEL SESSION III (Zaragoza III)** Leukemia & Lymphoma	**PARALLEL SESSION IV (Tenerife I)** Small particle cytometry Sponsored by Beckman Coulter
13:30-14:30	**LUNCH (Zaragoza I-II)**	
14:30-15:00	COMMERCIAL TALK VI Beckman Coulter (Tenerife I)	COMMERCIAL TALK VII Palex - Cytek (Tenerife II)
15:00-17:00	**PARALLEL SESSION V (Zaragoza III)** Erythroid & Platelet disorders	**PARALLEL SESSION VI (Tenerife I)** SARS-CoV-2
17:00-18:45	**PLENARY SESSION II (Zaragoza III-IV)** Environmental Cytometry	
18:45-20:00	**DRINKS/COMMERCIAL EXHIBITION/POSTERS (Zaragoza I-II)**	
20:00-23:00	**GALA DINNER**	

TIME	FRIDAY 21st APRIL	
9:00-11:00	**PARALLEL SESSION VII (Tenerife I)** Quality Assurance in Flow Labs	**PARALLEL SESSION VIII (Zaragoza III)** Immunodeficiencies & Non-Hematological Diseases
11:00-11:30	**COFFEE BREAK (ZARAGOZA I-II)**	
11:00-11:30	COMMERCIAL TALK VIII Miltenyi Biotec (Tenerife I)	COMMERCIAL TALK IX Tercen (Tenerife II)
11:30-12:30	**CLOSING LECTURE (Zaragoza III-IV)** Jacques Van Dongen (LUMD, Leiden): New Challenges in Immunomonitoring by Flow Cytometry	
12:30-13:00	**SIC ANNUAL GENERAL MEETING (AGM) (ZARAGOZA III-IV)**	
13:00-13:30	**SIC AWARDS CEREMONY & CLOSING REMARKS (ZARAGOZA III-IV)**	
13:30	**DEPARTURE**	

PROGRAMME
19th April 2023

08:00-18:00 **REGISTRATION**

9:00-11:00 **Pre-Congress WORKSHOPS I, II & III**

WORKSHOP I (Sala Zaragoza III) - **How to succeed in High-content Cytometry – Basics.**
Concepción Marañón (GENyO. Pfizer-University of Granada), María Beatriz Álvarez (CNIC, Madrid), Marta Monteiro (Instituto Gulbenkian da Ciência), André Mozes (Champalimaud Foundation).
Sponsored by IZASA Scientific and Miltenyi Biotec.

WORKSHOP II (Sala Tenerife I) - **Detection of Nanoparticles (extracellular vesicles, virus and nanoplastics) by Flow Cytometry: NanoCytometry.**
Alberto Álvarez-Barrientos (Universidad de Extremadura), Jordi Petriz (Germans Trias I Pujol Research Institute. Badalona), Enrique O'Connor (Universidad de Valencia).
Sponsored by Beckman Coulter and Miltenyi Biotec.

WORKSHOP III (Sala Tenerife II) - **Chronic Lymphoproliferative Disorders – Case Presentations.**
María Jorge Arroz (Centro Hospitalario Lisboa Occidental), Dolores Subirá (Hospital Universitario de Guadalajara).

11:00-11:30 Sala Zaragoza I-II **COFFEE BREAK**

11:30-13:30 **Pre-Congress WORKSHOPS IV, V & VI**

WORKSHOP IV (Sala Zaragoza III) - **High-content Cytometry Data Analysis - data analysis challenge by OMIQ, FCSExpress, FlowJo, Infinicyt, Tercen, AltraBio.**
Paulina Rybakowska (GENyO. Pfizer-Universidad de Granada), Katrien L. Quintelier (VIB-UGent, Ghent), Geoff Kraker (OMIQ), Andrea Valle (DeNovo Software), Ioannis Panetas and Javier Silvan (BD), Alexandre Gouy (Tercen), Julien Nourikyan (AltraBio).
Sponsored by IZASA Scientific and DeNovo Software (Dotmatics).

WORKSHOP V (Sala Tenerife I) - **Systematic multicolor panel design in the era of spectral cytometry (advance session – some cytometry experience required).**
Florian Mair (ETH, Zurich), Rui Gardner (MSKCC, New York) and Lola Martínez (CNIO, Madrid).

WORKSHOP VI (Sala Tenerife II) - **Measurable residual disease in B-cell precursor acute lymphoblastic leukemia (BCP-ALL).**
Montserrat Torrebadell (Hospital Sant Joan de Déu, Barcelona) y Neus Villamor (Hospital Clinic Barcelona).

13:30-14:30 Sala Zaragoza I-II **LUNCH**

14:30-14:40 Sala Zaragoza III-IV **WELCOME**

Local committee: Jose-Carlos Segovia / Lola Martínez

14:40-15:40 Sala Zaragoza III-IV **OPENING LECTURE**
Chairs: Lola Martínez / José-Carlos Segovia

Disentangling human tumor-unique immune phenotypes from non-malignant tissue inflammation using multi-omic single cell analysis.
Florian Mair (ETH, Zurich).

15:45-16:15 **BREAK**

15:45-16:15 **COMMERCIAL SESSIONS I, II & III**

BD - Enzifarma (Sala Zaragoza III) – **Experiencing sorting with the spectrally enabled FACSymphony S6.**
Rui Gardner (MSKCC, New York).

Standard BioTools (Sala Tenerife I) – **Ready-to-use 45+ marker panels to measure activation, cytokine production and cytotoxicity from <300ul of whole blood.**
Ernesto Marcos López (Standard BioTools).

Palex - Cytek (Sala Tenerife II) – **Multiparametric characterisation of telomere length in T cells from PLWH by Flow-FISH.**
Macedonia Trigueros Peña (Universidad de Barcelona).

16:20-18:15 Sala Zaragoza III-IV **PARALLEL SESSION I – Flow Cytometry in Advanced Therapies**
Chairs: José-Carlos Segovia / Neus Villamor

Advanced flow cytometry diagnosis of immune dysregulation in pediatric diseases. An effective tool for the use of precision medicine.
Rafael Correa (Instituto de Investigación Sanitaria Gregorio Marañón, Madrid).

Development of transposon-based CAR-T cells for the treatment of patients with lymphoma.
Begoña Díez-Cabezas (CIEMAT-CIBERER/IIS-FJD, Madrid).

Gene editing to generate new Pompe Disease models and medicines.
Javier Molina-Estévez (GENyO, Granada).

Short oral presentations:

Analysis of innate responses induced by Mesenchymal stromal/Stem Cell-based therapy in an experimental model of colitis by Flow Cytometry Technology.
Mercedes López-Santalla (CIEMAT/CIBER-ER/IIS-FJD/UAM. Madrid).

Conformational changes of PD-L1 in circulating MDSCs and prediction of escape from immunotherapy in NSCLC patients receiving anti-PD-1/PD-L1 checkpoint inhibitors.
Roser Salvia Cerdà (Germans Trias I Pujol Research Institute, Badalona).

MIDKINE drives immune evasion by rewiring dendritic dell differentiation and immunogenicity.
Xavier Catena (CNIO, Madrid).

16:20-18:15 Sala Tenerife I **PARALLEL SESSION II – Non-Human Flow Cytometry**
Chairs: Concepción Marañón / Marta Monteiro

Non-human primate models to explore Natural Killer cell development and function in simian immunodeficiency virus (SIV) infection.
Caroline Petitdemange (Institut Pasteur, Paris).

The choreography of T cell selection in the thymus and its implication in the establishment of protective immune responses.
Nuno L. Alves (i3S, Porto).

Short oral presentations:

Design and validation of novel flow cytometry panels to analyze a comprehensive range of peripheral immune cells in C57BL/6J mice.
Ainara Barco-Tejada (Universidad Carlos III, Madrid).

Assessment of immunologic changes in peripheral blood during the progression of Clostridioides difficile infection (CDI) in mouse models.
Elena Blázquez-López (Instituto de Investigación Sanitaria Gregorio Marañón, Madrid).
Sexual dimorphism in the anti-tumor immune responses elicited by the combination of fasting and chemotherap+A8:G59y.
Andrés Pastor-Fernández (IMDEA Food Institute, Madrid).

18:20-19:00 Sala Zaragoza III-IV **SIC WG: Controles de Calidad SIC.**
Alba Torres Valle (CIC-USAL, Salamanca).

18:20-19:00 Sala Tenerife I **Asamblea de Usuarios del Programa de Garantía externa de calidad Calidad SIC- GECLID.**
Carmen Martín (Centro de Hemoterapia y Hemodonación, Valladolid).

19:00-21:00 Sala Zaragoza I-II **WELCOME COCKTAIL/COMMERCIAL EXHIBITION/POSTERS**

PROGRAMME
20th April 2023

08:00-18:00 **REGISTRATION**

09:00-10:45 Sala Zaragoza III-IV **PLENARY SESSION I – BEYOND TECHNOLOGY**
Chairs: Jose-Carlos Segovia / Lola Martínez

Cytometric analysis of hematopoietic tissues using 3D quantitative microscopy.
César Nombela-Arrieta (University of Zurich).

STAbbing Tumors: Engineering T Cells for in vivo Secretion of Tumor-Specific T Cell Engagers.
Luis Alvarez Vallina (H12O-CNIO, Madrid).

Computational tools for high-dimensional cytometry data analysis.
Katrien Quintelier (VIB-Ugent, Ghent).

10:50-11:20 Sala Zaragoza I-II **COFFEE BREAK**

10:50-11:20 **COMMERCIAL SESSIONS IV & V**

BD - Enzifarma (Sala Tenerife I) – **Re- Discover Flow Cytometry and Spectral Cell Sorting: see what you sort, sort what you see.**
Mark Dessing (European Center for Single Cell Solutions Manager, BD).

Bonsai Lab (Sala Tenerife II) – **Exploring immunology with single cell multiomics.**
Agnieszka Ciesielska (Science & Technology Advisor 10X Genomics. Europe, Middle East & Africa).

11:30-13:30 Sala Zaragoza III-IV **PARALLEL SESSION III – LEUKEMIA & LYMPHOMA**
Chairs: Dolores Subirá / María Jorge Arroz

Flow cytometry for monitoring of patients with leukemia and lymphoma.
Alberto Orfao (CIC-USAL, Salamanca).

Next-Generation Flow Cytometry baseline prediction of NPM1 gene mutation in acute myeloid leukemia.
Sergio Matarraz (CIC-USAL, Salamanca).

Short oral presentations:

Soluble BCMA is a promising biomarker for monitoring patients with multiple myeloma.
Laura Moreno (Hospital Universitario de Guadalajara).
Immune profiling of blood T-lymphocytes in systemic mastocytosis.
Alba Pérez Pons (Centro de Investigación del Cáncer. USAL).

Flow Cytometry as a tool for diagnosis and elucidation of pathogenesis in CD2+ B-Lymphoblastic leukemia with transdifferentiation to monocytic lineage.
Rubén Ballester González (Hospital Universitario Ramón y Cajal, Madrid).

11:30-13:30 Sala Tenerife I **PARALLEL SESSION IV – SMALL PARTICLE CYTOMETRY**
Chairs: Alberto Álvarez-Barrientos / André Mozes
Sponsored by Beckman Coulter

Flow cytometry as a powerful tool for phenotypic characterization of seminal extracellular vesicle subsets in livestock species.
Isabel Barranco Cascales (Universidad de Murcia).

Nanoparticle-based flow cytometry for the analysis of nanoplastics and SARS-CoV-2 control: General considerations and troubleshooting.
Jordi Petriz (Germans Trias I Pujol Research Institute, Badalona).

Intestinal bacteria as biosensors in chronic inflammatory diseases.
Hyung Dong Chang (DRZF, Berlin).

Short oral presentations:

Nanoplastics represent a significant fraction of inhaled air, can overcome biological barriers and accumulate in human tissues.
Roser Salvia Cerdà (Germans Trias I Pujol Research Institute, Badalona).

Real-time environmental monitoring of Mycobacterium bovis in a multi-host transmission scenario.
André C. Pereira (Center for Ecology, Evolution and Environmental Changes –cE3c, Lisbon).

From full spectrum to conventional cell sorting – a method to isolate phytoplankton using autofluorescence.
Marta Monteiro (Instituto Gulbenkian da Ciência, Portugal).

13:30-14:30 Sala Zaragoza I-II **LUNCH**

14:30-15:00 **COMMERCIAL SESSIONS VI & VII**

Beckman Coulter (Sala Tenerife I) – **Use of CytoFLEX SRT cell sorter for phenotypic and functional characterization of patients with immune dysregulation.**
Marta Lopez Nevado (Servicio de Inmunología, Hospital 12 de Octubre, Madrid).

Palex - Cytek (Sala Tenerife II) – **Next-generation cell mimics double as apoptosis and TBNK controls and efficient flow cytometry training tools.**
Jordi Petriz (Germans Trias I Pujol Research Institute, Badalona).

15:00-17:00 Sala Zaragoza III-IV **PARALLEL SESSION V – ERYTHROID & PLATELET DISORDERS**
Chairs: María Beatriz Álvarez Flores / Rebeca Sánchez-Domínguez

Erythropathology and Flow Cytometry.
Enrique Colado (Hospital Universitario Central de Asturias, Oviedo).

PNH: From Diagnosis to Therapy.
Angelina Lemes (Hospital Universitario de Gran Canaria Doctor Negrín, Gran Canaria).

Analysis of Platelet functions by Flow Cytometry.
Enrique O'Connor (Universidad de Valencia).

Short oral presentations:

Fetomaternal Hemorrhage quantification, 10 years' experience in a University Hospital.
Enrique Colado (Hospital Universitario Central de Asturias, Oviedo).
Flow Cytometry as a powerful tool for Malaria drug discovery.
Sara Viera Morilla (GSK Global Health Medicines R&D, Tres Cantos, Madrid).

Different cellular kinetics after mobilization treatment in a Pyruvate Kinase deficiency model.
Isabel Ojeda Pérez (CIEMAT, Madrid).

15:00-17:00 Sala Tenerife I **PARALLEL SESSION VI – SARS-CoV-2.**
Chair: Jorge Carrillo

Cellular and humoral functional responses after BNT162b2 mRNA vaccination, an original approach for Spectral flow cytometry during the COVID pandemic.
Roberto Lozano and Jaime Valentín-Quiroga (IdiPAZ, Madrid).

Immunophenotypic characterization of long COVID.
Sara Morón-López (IrsiCAIXA, Badalona).

Short oral presentations:

Long-Term Monitoring by Flow Cytometry of T-Cell Memory and In Vitro Activation Response in Immunosupressed Patients after COVID-19 Vaccination.
Guadalupe Herrera (UCIM-Incliva Foundation. University of Valencia).

Altered migratory profile of circulating mononuclear cells after COVID and influenza infection.
Francisco Pérez Cózar (GENyO. Pfizer-Universidad de Granada).

Immune dysregulation throughout COVID-19 vaccination in hematological patients with mature B-cell neoplasms.
Esperanza Martín-Sánchez (SARS-CoV-2 Center for Applied Medical Research. CIMA, Universidad de Navarra).

17:00-18:45 Sala Zaragoza III-IV **PLENARY SESSION II – ENVIRONMENTAL CYTOMETRY**
Chairs: Alberto Álvarez-Barrientos / Marta Monteiro

Microalgal activity affected by the chemical contamination of water could increase the climate change problem.
Ángeles Cid (Universidade da Coruña).

Applications and challenges of flow cytometry DNA analyses on fungal biology and plant pathology.
Pedro Talhinhas (University of Lisbon).

Characterization of microbial populations in different marine environments using Flow Cytometry.
Jesús M. Arrieta (Instituto Oceanográfico Canarias, Santa Cruz de Tenerife).

18:45-20:00 Sala Zaragoza I-II **DRINKS/COMMERCIAL EXHIBITION/POSTER SESSION**

21:00-23:00 **GALA DINNER**

PROGRAMME
21st April 2023

09:00-10:00 Sala Tenerife I **PARALLEL SESSION VII – QUALITY ASSURANCE IN FLOW LABS**
Chairs: Catarina Martins / Lola Martínez

How to face Accreditation.
Catarina Martins (Universidade NOVA de Lisboa).

Reglamento Europeo 746 y Acreditación.
Isabel de la Villa (ENAC, Madrid).

Attaining ISAC SRL Recognition – Insights.
Kathleen Daniels (ISAC SRL Emerging Leader) ONLINE.

Short oral presentation:

Normaflow: survey of Latin-American's flow cytometry laboratories for ISO accreditation and certification.
Jordi Petriz (Germans Trias I Pujol Research Institute, Badalona).

09:00-10:00 Sala Zaragoza III-IV **PARALLEL SESSION VIII – IMMUNODEFICIENCIES AND NON-HEMATOLOGICAL DISEASES**
Chairs: Carlos Lázaro / Garbiñe Roy

Activated gut-homing CD8 + T cells for coeliac disease diagnosis of patients on a gluten-free diet.
Concepción Núñez (Instituto de Investigación Sanitaria Hospital Clínico San Carlos, Madrid).

Monitoring the immune system in pediatric brain tumours.
Tiago Carvalheiro (Princess Massima Center of Pediatric Oncology, Utretch).

Dissection of the pregerminal center B cell maturation in CVID.
Lucía del Pino-Molina (IdiPAZ, Madrid).

Short oral presentations:

Evaluation of a new age-matched criteria which will improve the identification of late-onset combined immunodeficiency patients.
Alba Torres Valle (IBMCC, USAL-CSIC, Salamanca).

The Relevance of Flow Cytometry in the Etiologic Diagnosis of Pleural Effusions.
María Jorge Arroz (Centro Hospitalario Lisboa Occidental).

Subset analysis reveals key features and biomarkers of non-muscle invasive bladder cancer tumour microenvironment.
Víctor Manuel García Martínez (CIEMAT, Madrid).

11:00-11:30 Sala Zaragoza I-II **COFFEE BREAK**

11:00-11:30 **COMMERCIAL SESSIONS VIII & IX**

Miltenyi Biotec (Sala Tenerife I) – **MACSQuant® Tyto® Cell Sorter: the flow revolution in cell sorting.**
Alex Adán (Flow Cytometry Application Specialist, Miltenyi Biotec).

Tercen (Sala Tenerife II) - **Tercen: high-dimensional flow cytometry data analysis made easy.**
Alexandre Gouy (Application Specialist, Tercen).

11:30-12:30 **CLOSING LECTURE**
Chairs: Julia Almeida / José-Carlos Segovia

New Challenges in Immunomonitoring by Flow Cytometry.
Jacques Van Dongen (University of Leiden)

12:30-13:00 Sala Zaragoza I-II **SIC Annual General Meeting (AGM)**

13:00-13:20 Sala Zaragoza I-II **SIC Awards Ceremony**

Best Oral Communication Award (Enzifarma – SIC)
Best Poster Award (BD – SIC)
Best Clinical Cytometry Work Award (Sysmex – SIC)
SIC Outstanding Career Awards - Enrique O'Connor (2021) and **2023 Awardee**

13:20-13:30 Sala Zaragoza I-II **Closing Remarks**
(Lola Martinez / José-Carlos Segovia)

13:30 Sala Zaragoza I-II **Departure**

05

Oral Presentations

ORAL PRESENTATIONS

19th April 2023

OPENING LECTURE

Florian Mair
ETH, Zurich

DISENTANGLING HUMAN TUMOR-UNIQUE IMMUNE PHENOTYPES FROM NON-MALIGNANT TISSUE INFLAMMATION USING MULTI-OMIC SINGLE CELL ANALYSIS

Anti-tumor immunotherapies have achieved remarkable successes in the treatment of cancer, but major challenges remain. An inherent weakness of current treatment approaches is that the therapeutically targeted pathways are not restricted to tumours, but are also found in other tissue microenvironments.

Despite great efforts to define inflammatory processes in the tumour microenvironment, our understanding of tumour-unique immune alterations is limited by a knowledge gap regarding the immune cell function in inflamed human tissues. Here, in an effort to identify such tumour-enriched immune alterations, we used complementary single-cell analysis approaches to interrogate the immune infiltrate in human head and neck squamous cell carcinomas and site-matched non-malignant, inflamed tissues.

Our analysis revealed a large overlap in the composition and phenotype of immune cells in tumour and inflamed tissues. Computational analysis identified tumour-enriched immune cell interactions, one of which yields a large population of regulatory T cells (Tregs) that is highly enriched in the tumour and uniquely identified among all haematopoietically-derived cells in blood and tissue by co-expression of ICOS and IL-1 receptor type 1 (IL-1R1). We provide evidence that these intratumoural IL-1R1+ Treg cells had responded to antigen recently and demonstrate that they are clonally expanded with superior suppressive function compared with IL-1R1- Treg cells.

Overall, our work highlights how combining several single-cell techniques with subsequent validation experiments can provide critical new insight into the function of immune cells in human tissues.

PARALLEL SESSION I – FLOW CYTOMETRY IN ADVANCED THERAPIES

Rafael Correa
Instituto de Investigación Sanitaria Gregorio Marañón, Madrid

ADVANCED FLOW CYTOMETRY DIAGNOSIS OF IMMUNE DYSREGULATION IN PEDIATRIC DISEASES. AN EFFECTIVE TOOL FOR THE USE OF PRECISION MEDICINE

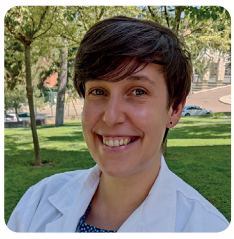
There are a wide range of diseases that are caused by dysregulation of the immune system. This imbalance can lead to an exacerbated response of certain immune populations and a severe inflammatory process. The existing arsenal of biological therapies that can block or inhibit these immune processes at different levels is extensive, but in most cases, there is no precise diagnosis to identify the appropriate target for each patient. In the case of pediatric patients this limitation is even greater, given the small volume of blood that can be safely obtained from these patients. For this reason, advanced analysis protocols are needed to enable a correct diagnosis and to correctly prescribe these therapies. Advances in immunological knowledge and in the technologies that allow its study, open the possibility of making a more precise diagnosis and developing personalized medicine treatments.

Taking advantage of the multi-parameter flow cytometry technology, our researchers in the Laboratory of Immune-regulation at the IISGM have developed a pro-

tocol for exhaustive immune analysis in pediatric samples. This technique allowed us to analyse frequencies and absolute counts of more than 100 subpopulations of lymphocytes, granulocytes, monocytes and NK cells in less than 0.5 ml of blood. This tool has been employed with success for the diagnosis and treatment of an infant in serious danger of death caused by a rare dermatologic disease.

The thorough study of the immune system employing our protocol allowed us to identify in the patient an immune profile characterized by very high levels of both Th17 cells and IL-17A producing CD4+ T cells. In consequence, the patient was treated with a new biological treatment in order to diminish the inflammation. After the treatment, the patient showed remarkable clinical improvements, notably a weight gain, which has rescued her from grave danger of death.

The use of flow cytometry allows the identification of the immune profile of pediatric patients with immune related diseases. This precision diagnosis could provide insight of the severity of the inflammation; the potential existing treatments that could help improve the symptoms and, therefore, the quality of life of these patients.



Begoña Díez-Cabezas

CIEMAT-CIBERER/ IIS-FJD. Madrid

DEVELOPMENT OF TRANSPOSON-BASED CAR-T CELLS FOR THE TREATMENT OF PATIENTS WITH LYMPHOMA

TranspoCART is a project conceived to develop an innovative CAR-T cell therapy for lymphoma patients based on a viral vector-free gene-transfer strategy. A Sleeping Beauty transposon minicircle (SBmC) carrying a CAR consisting of the 4-1BB CAR endodomain, an anti-CD19 ScFv and a truncated EGFR -as a safety switch- was designed for this clinical trial. Our optimized manufacturing protocol showed transposition efficacies ranging between 30 and 60% in both healthy donors and patients' T cells, with efficient cell expansion rates (between 29.6x and 31.8x), compatible with clinical applications. Extensive in vitro characterization of TranspoCART19 cells showed an enriched stem-cell memory/central memory phenotype, no signs of cell exhaustion and high level of specific cytotoxicity against tumour cell lines and primary tumour cells expressing CD19. No detectable levels of both the SBmC and the transposase protein were found at the end of the expansion period. NALM6 xenograft models using immunodeficient NSG mice were used to evaluate the in vivo antitumoral efficacy of TranspoCART19 cells. Treated animals showed an improved survival compared to untreated mice, similar to the one observed in mice treated with lentivirally-produced CAR-T cells. Biodistribution and toxicity studies revealed that TranspoCART19 cells administration is safe. Additionally, cetuximab administration in CAR-T cell treated NALM6 xenograft mice depleted TranspoCART19 cells, proving its efficacy. Finally, two independent GMP facilities carried out TranspoCART19 cell manufacturing GMP validations, and showed that the manufacturing procedure is highly efficient and reproducible. These encouraging results support the initiation of a clinical trial with the TransproCART19 cell product.

Javier Molina-Estévez

GENyO, Granada

GENE EDITING TO GENERATE NEW POMPE DISEASE MODELS AND MEDICINES

There are increasing and solid records supporting the clinical use of Hematopoietic Stem and Progenitor Cells (HSPC) ex vivo gene therapy to treat several, otherwise uncurable, monogenic diseases. Pioneer trials relied on tamed viral components acting as vectors which provided robust and proficient gene transference at the expense of genotoxicity. Recent strategies have upgraded these tools to safer recombinant vectors or shifted towards implementation of gene editing. The later provides accurate control on the chromosome and locusthat is going to be altered, and the number of therapeutic gene copies that can be inserted. However, it's novelty requires a thorough characterization of every new tool and clinical application in terms of safety and efficiency.

We aim to use tissue resident macrophages and circulating myeloid cells originated from gene-edited HSPCs for paracrine cross-correction of monogenic diseases, such as Pompe Disease. Therefore, we focused in finding a CRISPR/Cas9 guide that efficiently targeted an intronic region from a gene expressed

upon myeloid progenitor commitment: CX3CR1. We demonstrated that our guide efficiently targets the 4th intron while it does not disturb physiological expression of the host gene. Furthermore, we tested this locus as a safe harbor by introducing reporter cassettes within. Using a promoter-less or a strong viral promoter configuration, we documented efficient integration of our cassette and unaltered CX3CR1expression in edited lymphoid and myeloid cell lines. Interestingly, we found increased levels of CX3CR1 after editing cord blood CD34 cells in vitro, along with a skewed reporter expression inclined towards differentiated myeloid lineages.

Preliminary evaluation of gene-edited-HSPC transplantation into a NSG mice suggests that their safety and biodistribution justify further exploratory and translational evaluation of this approach that could provide a clinical platform to treat Pompe Disease.

PARALLEL SESSION II – NON-HUMAN FLOW CYTOMETRY

Caroline Petitdemange
Institut Pasteur, Paris

NON-HUMAN PRIMATE MODELS TO EXPLORE NATURAL KILLER CELL DEVELOPMENT AND FUNCTION IN SIMIAN IMMUNODEFICIENCY VIRUS (SIV) INFECTION

Non-human primates (NHP) are essential models for the development of therapies and vaccines. They also represent the only animal model for studying the physiopathology of HIV infection. One major anatomical reservoir of HIV in humans and of SIV in NHP are lymph node (LN) B cell follicles (BCF) where HIV/SIV persists through replication in follicular helper T cells. Natural hosts of SIV, such as African green monkeys (AGM) do not progress to disease. We demonstrated that natural killer (NK) cells mediate a strong control of SIVagm infection in secondary lymphoid organs. In order to study the underlying mechanisms of this potent NK-cell mediated control in SIVagm-infected AGMs, we developed a broad range of tools to analyze NK cells in distinct NHP species, and in particular in AGMs and cynomolgus macaques. We determined combinations of cell-surface markers that allow to identify NK cells in distinct NHP tissues, as well as protocols for sorting NK cells from blood and tissues of distinct NHP species and for NK cell in situ imaging analyses. This allowed us to identify a unique CXCR5 and IL-15 dependent NK cell localization within BCF and the expansion of terminally differentiated NK cells in secondary lymphoid organs of SIVagm-infected AGMs. These terminally differentiated NK cells displayed an adaptive transcriptional profile with increased MHC-E restricted cytotoxicity in response to nonamer peptides derived from the Env leader sequence of SIV. In contrast, such NK cell differentiation was found to be impaired in SIVmac infection of macaques (MAC). Given the potential importance of this HLA-E/peptide/NK axis in the control of SIVagm infection, we currently study the role of the SIVagm nonamer Env leader sequence peptide in vivo. Furthermore, we compare the NK cell profiles identified in the NHP to that in a human cohort of early anti-retroviral treated people living with HIV.

Nuno L. Alves
i3S, Porto

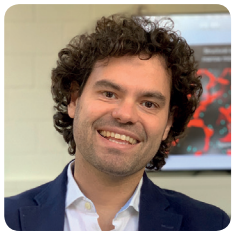
THE CHOREOGRAPHY OF T CELL SELECTION IN THE THYMUS AND ITS IMPLICATION IN THE ESTABLISHMENT OF PROTECTIVE IMMUNE RESPONSES

T cells are chief white blood cells that protect our organism from infectious diseases and cancer. As we age, T-cells become less effective in fighting infection and they respond less well to vaccines. T cells are generated in the thymus, which makes this organ an essential element in establishing T cell immunity. Given the present interest in T cell-mediated immunotherapy, there is a growing need to understand how T-cell development is controlled in the thymus. The development of immunologically competent T cells is promoted by inductive microenvironments formed by thymic epithelial cells (TECs). Still, how TECs select functionally diverse T cells capable of responding to foreign threats is not fully comprehended. Particularly, the selection of CD4 T cells depends on interactions between TCRs expressed on T cell precursors and self-peptides:MHC II complexes presented by cortical TECs (cTECs). Although the macroautophagy/

autophagy-lysosomal protein degradation pathway is implicated in CD4 T cell selection, the molecular mechanism that controls the generation of selecting MHC II ligands remains elusive. LAMP2 is a well-recognized mediator of autolysosome maturation. I will discuss experimental evidence that points to a new role for LAMP-2 in T cell differentiation. Genetic inactivation of Lamp2 in thymic stromal cells specifically impaired the development of CD4 T cells that completed positive selection, without misdirecting MHC II-restricted cells into the CD8 lineage. Mechanistically, defects in autophagy in lamp2-deficient cTECs were linked to alterations in MHC II processing. These alterations in TECs led to a profound reduction in CD4 T cells and a restriction in the diversity of their TCR repertoire, limiting the immune response of the host to pathogenic Listeria. This study reveals a new molecular axis that regulates the production of T cells and that, as such, is deterministic of protective anti-microbial T cell responses.

20th April 2023

PLENARY SESSION I – BEYOND TECHNOLOGY



César Nombela-Arrieta
University of Zurich

CYTOMETRIC ANALYSIS OF HEMATOPOIETIC TISSUES USING 3D QUANTITATIVE MICROSCOPY

Bone marrow (BM) cavities are the primary sites of continuous and highly regulated blood cell production and reservoirs for long-term immune memory. Within BM tissues, a rare population of self-renewing and mulitpotent hematopoietic stem cells (HSCs) continuously differentiates into progenitors, which in turn generate mature blood derivatives to compensate for the rapid turnover of these cells in the organism. Beyond the different hematopoietic subsets and terminally differentiated immune cells, the BM contains a complex and heterogeneous stromal compartment made of cells of vascular, mesenchymal or neural origin, which crucially participate in the regulation of hematopoiesis and HSC maintenance. In the last decades, this unparalleled complexity of the BM at compositional and functional levels has been dissected through the use of flow cytometry. Nonetheless, flow cytometric techniques do not allow to capture fundamental spatial and morphological information, and miss the vast majority of stromal cells, which are lost during the mechanical dissociation of BM tissues.

To generate spatial maps of BM components, gain information on functional interactions between key cell types and obtain accurate quantifications of stromal cell types, we have developed 3D quantitative microscopy (3D-QM) of hematopoietic tissues. In this workflow we combine advanced tissue processing and clearing protocols for the generation of 3D reconstructions of entire BM cavities with cellular and subcellular resolution, with deep learning-assisted computational tools for the automatic detection of various anatomical landmarks in 3D image data sets. Through the use of 3D-QM, we currently investigate the distribution of HSCs in specific locations to unravel their preferential interactions in specific niches, we describe the homeostatic topology of stromal networks and we study the profound functional and structural remodeling of the complex BM tissue landscape during pathological conditions such as chronic infections, myeloablative insults and hematological neoplasias.

Luis Álvarez Vallina
H12O-CNIO, Madrid

STABBING TUMORS: ENGINEERING T CELLS FOR IN VIVO SECRETION OF TUMOR-SPECIFIC T CELL ENGAGERS

Cancer immunotherapeutic approaches based on the redirection of T cell activity toward Tumor-Associated Antigens are actively being investigated. The impressive clinical success of the continuously intravenously infused bispecific T cell-engaging (TCE) antibodies, and of engineered T cells expressing chimeric antigen receptors (CAR-T cells) in hematological malignancies, has led to renewed interest in a novel cancer immunotherapy strategy that combines features of antibody- and cell-based therapies. This emerging approach is based on the endogenous secretion of small-sized Fc-free TCEs by gene-modified T

cells (STAb-T cells). Adoptive transfer of genetically modified STAb-T cells secreting anti-CD19 TCEs (STAb-T19) or anti-CD1a TCEs (STAb-T1a) have demonstrated potent anti-tumor activity in hematologic preclinical xenograft models. Our findings suggest that the continued antibody secretion, allows an efficient recruitment of the endogenous T cell pool, resulting in fast and effective elimination of cancer cells that may prevent leukemia frequently associated with CAR-T therapies.

Katrien Quintelier
VIB-Ugent, Ghent

COMPUTATIONAL TOOLS FOR HIGH-DIMENSIONAL CYTOMETRY DATA ANALYSIS

The number of parameters that can be measured with cytometry techniques has increased a lot. Manually evaluating all markers and marker combinations is time-consuming and often infeasible and that is where the field of computational cytometry comes in. The default analysis pipeline consists of three main steps: pre-processing and quality control, clustering, and downstream (statistical) analysis. The quality control (i.e. removing low quality events and detecting and solving batch effects) is especially important in a computational setting, because of the garbage in, garbage out principle. The overall pipeline will be discussed and some computational tools like PeacoQC for quality control, CytoNorm for batch effect removal and FlowSOM for clustering will be highlighted.

PARALLEL SESSION III – LEUKEMIA & LYMPHOMA

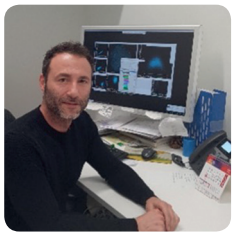
Alberto Orfao
CIC-USAL, Salamanca

FLOW CYTOMETRY FOR MONITORING OF PATIENTS WITH LEUKEMIA AND LYMPHOMA

In recent years, the treatment of leukemia and lymphoma has undergone significant changes that have led to greater response rates and prolonged patient survival both in the settings of high-dose chemotherapy (HDT) followed by stem cell transplantation (ASCT) and through the use of new targeted drugs and immune therapy. Because of such improvements, conventional criteria used to assess complete response (CR) have progressively become insufficient to evaluate the quality of response in a significant fraction of the patients, highlighting the need for more sensitive techniques.

In the past, both conventional (4-10 colour) flow cytometry and ASOqPCR have shown to be relatively sensitive approaches to monitor the effect of therapy in leukaemia and lymphoma through the detection of low to minimal levels of measurable (residual) disease (MRD) at the 10-4 to 10-5 level. Subsequent technological advances in the speed of digital flow cytometers have enable the advent of high-sensitive next-generation flow cytometry (NGF) MRD assays, capable of identifying down to 10-6 residual neoplastic cells among millions of bone marrow cells from patients with acute and chronic leukaemia and multiple myeloma based on objective and automated analytical approaches. Such increased sensitivity proved to allow the novel NGF-MRD assays to be broader applicable in BM and blood samples with very important practical implications. More recently, the introduction of antibody- and CAR-T targeted immune therapies, together with the exponential growth of the multicolour capabilities of spectral flow cytometers has fostered the development of more comprehensive antibody monitoring panels and assays which go beyond MRD detection, to the simultaneous identification of MRD and potential targets for e.g., antibody and/or CART-cell mediated immune therapies on residual leukaemia/lymphoma cells, the identification of in vivo expanded blood circulating CART cells and their detail sub-setting, and monitoring of the effect of therapy on the residual innate and adaptative immune cells.

Altogether, this has progressively brought flow cytometry as a key tool for treatment monitoring and treatment-decision making processes in leukaemia and lymphoma patients both in the settings of clinical trials and real-world clinical practice.



Sergio Matarraz
CIC-USAL, Salamanca

NEXT-GENERATION FLOW CYTOMETRY BASELINE PREDICTION OF NPM1 GENE MUTATION IN ACUTE MYELOID LEUKEMIA

Occurrence of insertion mutations of the nucleophosmin gene (NPM1mut) is among the most frequent genetic events described in acute myeloid leukemia (AML) and is overall associated with a relatively favourable prognosis. However, clonal evolution of NPM1mut leukemic cells and disease outcome is importantly modulated by comutation patterns of concurrent competing leukemic cell clones. Over years, an increasing amount of phenotype-genotype associations are being unravelled in AML which may contribute to early diagnosis and risk-adapted therapy for these patients. However, leukemia cells from NPM1mut AML patients present at diagnosis with heterogeneous patterns of lineage commitment, including early myeloid maturation arrests and/or more mature traits of myelomonocytic differentiation. More in detail, this translates into a broad spectrum of NPM1mut associated phenotypes, from immature CD117+HLA-DR+ leukemia cells showing minimal differentiation features to more differentiated (e.g. CD34-) monocytic (e.g. CD11b+CD14+ and/or CD64+) and/or neutrophil lineage (e.g. CyMPO+ HLA-DR-) phenotypes, these latter cases (partially) resembling the phenotypic profile of acute monoblastic/monocytic subtypes (e.g. AML with KMT2A-rearranged or CBFB-MYH11) and acute promyelocytic leukemia with PML-RARA (APL), respectively. Such heterogeneity has hampered the identification of baseline immunophenotypic patterns that might accurately predict for NPM1mut AML.

Herewith, we characterized the distribution and immunophenotype of different subsets of bone marrow (BM) leukemia cells studied at diagnosis of a large series of 377 AML patients, including 201 NPM1mut AML cases. Our major goal was to identify reliable phenotypic profiles that could be used for fast screening for the NPM1mut and/or FLT3-ITD in newly diagnosed AML patients. In addition, novel next-generation flow cytometry approaches for automated immunophenotypic discrimination of NPM1mut AML vs. potentially overlapping AML categories (e.g. APL) will be introduced.

PARALLEL SESSION IV – SMALL PARTICLE CYTOMETRY

Isabel Barranco Cascales
Universidad de Murcia

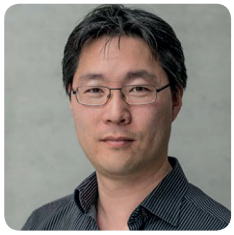
FLOW CYTOMETRY AS A POWERFUL TOOL FOR PHENOTYPIC CHARACTERIZATION OF SEMINAL EXTRACELLULAR VESICLE SUBSETS IN LIVESTOCK SPECIES

In the last years, extracellular vesicles (EVs), membrane-delimited nanoparticles (30-to-1000 nm) released by all functional cells into the extracellular milieu, have become an attractive target for scientific community. EVs play an essential role as messengers in cell-to-cell communication, delivering their bioactive cargo (proteins, lipids and RNAs) from donor to recipient cell for triggering a specific response in them. EVs has been isolated from all body fluids, where different EV-subsets can be found, making them a challenge to study. The lack of accurate methodologies for isolating/characterizing EV-subsets lead to a poor definition of their composition/function. In the last years, advances in engineering have allowed to develop high-resolution flow cytometers allowing to multiparametric analysis of EVs. Flow cytometry-based method enables distinguishing between EVs vs non-EV particles, giving valuable information about number, size, and phenotype of EVs at single level. Recently, our research group has performed a phenotypical characterization of EV-subsets isolated from pig seminal plasma by flow cytometry, following the International Society of Extracellular Vesicles guidelines. Seminal plasma is a complex fluid secreted mainly by male accessory sex that accompanies sperm during and after ejaculation. This fluid contains a large and heterogeneous population of EVs that can clustered in different subsets, which one will be involved on modulating different sperm physiological processes. In this context, flow cytometric analysis is offered as essential tool for identifying seminal EV-subsets allowing to reach useful information about phenotypic parameters and putative specific function of each EV-subset.

Jordi Petriz
Germans Trias I Pujol Research Institute, Badalona

NANOPARTICLE-BASED FLOW CYTOMETRY FOR THE ANALYSIS OF NANOPLASTICS AND SARS-COV-2 CONTROL: GENERAL CONSIDERATIONS AND TROUBLESHOOTING

Flow cytometry is a highly sensitive tool to study plastic nanoparticles, with advantages over other techniques, such as Raman spectroscopy, which can resolve generally 1-2 µm particle sizes. One direct method to analyze the nanoplastics (NPs) that the human body can accumulate consists of measuring the NPs levels in peripheral blood. We have used Nile Red (NR), a red phenoxazone dye that binds to the surface of plastics and neutral lipids, was used to determine the presence in blood of the most common plastics: low density polyethylene, polystyrene, polyethylene terephthalate, and polyamide.Previous work assessing the levels of MPs or NPs in human peripheral blood has been conducted by Fourier Transform Infrared (FT-IR) spectrometry, by Scanning Electron Microscopy coupled with an Energy Dispersion Detector (SEM-EDX), and by double shot pyrolysis - gas chromatography/mass spectrometry. The flow virometric assay can be used as a potential method to detect RNA viruses. It allows the rapid detection of viral particles and shows high sensitivity and specificity, and can be used complementary with any other technique for virus detection, such as RT-qPCR and RAD methods. The combination with fluorescent monoclonal antibodies against SARS-CoV-2 spike glycoprotein help to confirm the specific detection of the virus. Furthermore, the implementation of this flow virometric method will allow early detection of infection by running rapid high throughput analysis of a large scale of population groups, necessary to detect the presence of asymptomatic individuals. We will discuss the conceptual basis for quantitative fluorescence measurements using flow cytometry, cross-calibration results of different sub-micron beads and instrument models as well as guidelines for flow cytometry data presentation.



Hyung Dong Chang
DRZF, Berlin

INTESTINAL BACTERIA AS BIOSENSORS IN CHRONIC INFLAMMATORY DISEASES

Chronic inflammatory diseases are often associated with changes in the microbiota, generally termed dysbiosis. We have developed an analysis method, which allows phenotyping of microbiota on the single-cell level by multi-parameter flow cytometry. We determine phenotypic properties of bacteria, such as coating with host immunoglobulins and the expression of surface sugars, to capture the immunological context of their recognition by the host and to reflect metabolic conditions, adhesion ability and cell-crosstalk, respectively. By machine-learning, phenotypic signatures can be delineated which are specific for different chronic inflammatory diseases independently of taxonomic alterations. Thus, microbial phenotyping on the single cell level reveals that intestinal bacteria can serve as biosensors allowing disease classification with the potential for disease monitoring and patient stratification but also for identification of bacterial populations or microbiota conditions potentially relevant in disease pathogenesis.

PARALLEL SESSION V – ERYTHROID & PLATELET DISORDERS

Enrique Colado
Hospital Universitario Central de Asturias, Oviedo

ERYTHROPATHOLOGY AND FLOW CYTOMETRY

Erythropoiesis involves proliferation and differentiation of small populations of hematopoietic stem cells resident in the bone marrow into mature red blood cells. The determination of the cellular composition of the blood is a valuable tool in the diagnosis of different diseases and monitoring of therapy. Different flow cytometry immunophenotyping techniques can be routinely used to characterize the heterogeneous cell populations present in the blood and the hematopoietic cell differentiation and maturation pathways of the bone marrow. In this session, we will discuss the role of flow cytometry in the study of erythropoiesis and classical red blood cell disorders including reticulocyte analysis; quantitative methods for detection of Fetal–Maternal Haemorrhage (FMH); Sickle Cell Disease (SCD); Hereditary Spherocytosis (HS) and related disorders; Paroxysmal Nocturnal Hemoglobinuria (PNH); and red cell survival.

Angelina Lemes
Hospital Universitario de Gran Canaria Doctor Negrín, Gran Canaria

HEMOGLOBINURIA PAROXÍSTICA NOCTURNA: ¿DÓNDE ESTAMOS?

En los últimos años, la "antigua" hemoglobinuria nocturna paroxística (HPN) ha logrado nuevos avances en términos de comprensión de su fisiopatología, del enfoque diagnóstico, la optimización de la terapia y el desarrollo dinámico de nuevos agentes terapéuticos.

En esta revisión se describe en donde estamos en el momento actual en cuanto a la fisiopatología y el proceso de diagnóstico de la HPN. El despistaje de HPN se recomienda en pacientes con hemólisis intravascular, síndromes de insuficiencia medular adquirida y en casos de trombosis con localizaciones inusuales. A pesar de la disponibilidad de directrices de consenso para el diagnóstico y monitorización de la HPN, aún se encuentran discrepancias sobre cómo se llevan a cabo sus pruebas diagnósticas, dando lugar a variaciones técnicas que pueden conducir a un diagnóstico incorrecto.

También se describen los determinantes del tratamiento moderno, como las estrategias de inhibidores del C5 del complemento frente al alotrasplante de células madres hematopoyéticas.

Durante los últimos 20 años, la terapia de la HPN se ha basado en la inhibición del complemento terminal mediante anticuerpos bloqueantes, pero recientemente una gran cantidad de nuevos medicamentos que interfieren con la cascada del complemento proximal y terminal, están en desarrollo. Así se presentan los primeros inhibidores aprobados de la vía del complemento proximal que previenen principalmente la hemólisis extravascular y se destacan sus potenciales beneficios.

Enrique O'Connor
Universidad de Valencia

ANALYSIS OF PLATELET FUNCTIONS BY FLOW CYTOMETRY

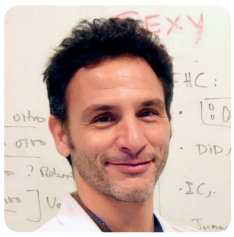
Platelets are essential for the onset and regulation of hemostasis, coagulation, endothelial-cell support and wound repair. On the other hand, platelets are involved in pathological conditions, including thrombosis, inflammation, infection and cancer. Platelets sense their environment through activating or inhibiting cell-surface receptors connected to fast-response transduction pathways. Platelet activation leads to sequential processes of shape change, aggregation, secretion of intracellular factors and shedding of membrane microparticles. These changes contribute locally to form and stabilize the hemostatic plug, while ensuring coagulation, attracting circulating platelets and leukocytes, and promoting repair of vessel lessions. Interestingly, the same platelet functions that elicit their protective action may lead to life-threatening conditions when improperly activated or disregulated. Moreover, platelet function may be affected by congenital or acquired conditions, including hemato-oncological disorders and therapy side-effects.

These facts have promoted the study of platelet function in many clinical areas. Flow cytometry (FCM) has paid attention to platelets as early as the 1980s, and novel FCM platelet assays refflect the technical improvements in Cytomics. Currently, FCM is the choice tool to undertake platelet studies in near-physiological conditions. FCM assays of platelets in whole blood use small amount of sample, minimize artifactual activation of platelets and maintain the cellular heterogeneity of circulating blood elements.

In this way, FCM allows to follow platelet maturation and destruction, to detect congenital defects of platelets and to provide risk factors related to platelet hypo- or hyper-reactivity in vivo. Such risk factors are based on the activation responses of individual platelets, their interactions with other blood cells and the secretion/shedding of extracellular molecules and microparticles.

Moreover, functional ex-vivo real-time assays reveal fast responses of platelets when challenged with agonists. The repertoir of FCM platelet assays allows also to monitor therapy at the patient level, as well as to control the quality of platelet hemoderivates.

PARALLEL SESSION VI – SARS-CoV-2.



Roberto Lozano and Jaime Valentín-Quiroga
IdiPAZ, Madrid

CELLULAR AND HUMORAL FUNCTIONAL RESPONSES AFTER BNT162B2 MRNA VACCINATION, AN ORIGINAL APPROACH FOR SPECTRAL FLOW CYTOMETRY DURING THE COVID PANDEMIC

We have analyzed BNT162b2 vaccine-induced immune responses in naive subjects and individuals recovered from coronavirus disease 2019 (COVID-19), both soon after (14 days) and later after (almost 8 months) vaccination. We design a 40-color panel to obtain a fully complete screening of the immune system in order to check the profile pre and after vaccination using a Cytek Aurora spectral system. Plasma spike (S)-specific immunoglobulins peak after one vaccine shot in individuals recovered from COVID-19, while a second dose is needed in naive subjects, although the latter group shows reduced levels all along the analyzed period. Despite how the neutralization capacity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mirrors this behavior early after vaccination, both groups show comparable neutralizing antibodies and S-specific B cell levels late post-vaccination.

When studying cellular responses, naive individuals exhibit higher SARS-CoV-2-specific cytokine production, CD4+ T cell activation, and proliferation than do individuals recovered from COVID-19, with patent inverse correlations between humoral and cellular variables early post-vaccination. However, almost 8 months post-vaccination, SARS-CoV-2-specific responses are comparable between both groups. Our data indicate that a previous history of COVID-19 differentially determines the functional T and B cell-mediated responses to BNT162b2 vaccination over time.

Sara Morón-López
IrsiCAIXA, Badalona

IMMUNOPHENOTYPIC CHARACTERIZATION OF LONG COVID

Long COVID can be developed by individuals after an infection with SARS-CoV-2 as described by the WHO. Although this condition is more commonly described in adults, it can occur in children and adolescents with a wide range of estimated prevalence of 1-25%. Little is known about the role of the immune system in longCOVID. The objective of this study is to compare immune cells populations in paediatric populations with and without longCOVID.

PLENARY SESSION II – ENVIRONMENTAL CYTOMETRY

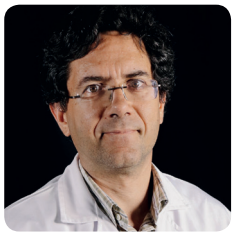
MICROALGAL ACTIVITY AFFECTED BY THE CHEMICAL CONTAMINATION OF WATER COULD INCREASE THE CLIMATE CHANGE PROBLEM

In the aquatic environments, phytoplankton account for approximately half the production of organic matter on Earth, due to their metabolic capacity to fix CO2, producing O2. Some studies have concluded that global phytoplankton concentration has declined over the past century.

Angeles Cid
Universidade da Coruña

Eukaryotic microalgae are part of this phytoplankton and their cellular characteristics make them suitable to be analyzed by flow cytometry. Our studies based on functional flow cytometry showed an additional concern, since growth of this kind of cells is affected by chemical contamination of water ecosystems, but not only. Energy producing metabolism (mainly photosynthesis related parameters), cellular homeostasis (intracellular pH, membrane potential) cell signalling (intracellular calcium, ROS level) or apoptosis-related processes were altered when microalgae were exposed to persistent (metals, pesticides) and emerging pollutants, such as pharmaceuticals or personal care products.

These alterations will need to be considered at a global scale, since geochemical cycling or fisheries depends on these microorganisms.



Pedro Talhinhas
University of Lisbon

APPLICATIONS AND CHALLENGES OF FLOW CYTOMETRY DNA ANALYSES ON FUNGAL BIOLOGY AND PLANT PATHOLOGY

Flow cytometry is considered the state-of-the-art technique for nuclear DNA content estimation. In an era where genomes are sequenced each time faster and cheaper, genome size estimation should precede genome sequencing which often is not the case, leading sometimes to the use of inadequate sequencing strategies. Fungal genome sizes vary over 1600x, ranging from 2.2 (Encephalitozoon romaleae) to 3706 Mbp (Jafnea semitosta), but the patterns of such variations differ across fungal phylogeny, with some phylogenetic groups exhibiting little genome size variations and others encompassing major shifts. In several fungal clades, genome size variation seems to accompany evolution and to relate to fungal life-styles: the genome sizes of saprophytic fungi and those of fungi that interact with animals tend to be smaller than those of fungi that interact with plants, either in mutualism or in parasitism. Fungal genomes are in average smaller than those of plants or animals and the use of flow cytometry for fungal genome size measurement and nuclear cycle analysis is challenging, requiring optimization of isolation procedures, DNA standards and stains. This communication will thus focus on applications and challenges of flow cytometry DNA analyses on fungal biology and plant pathology, aiming to promote the use of this technique in mycology and phytopathology as a fast, low-cost and reliable approach to help solving research questions.

Jesús M. Arrieta
Instituto Oceanográfico Canarias, Santa Cruz de Tenerife

CHARACTERIZATION OF MICROBIAL POPULATIONS IN DIFFERENT MARINE ENVIRONMENTS USING FLOW CYTOMETRY

Flow cytometry has become one of the basic tools in aquatic microbial ecology, allowing the rapid and efficient analysis of large numbers of individual cells in large numbers of samples. Determining the size, DNA content and natural fluorescence properties of microbial cells provides information on the identity, abundance and even the physiological state of some of the key components of marine food webs. Using flow cytometry allows the routine detection of the most abundant microbial cells in natural waters like viruses, heterotrophic bacteria, cyanobacteria and even small eukaryotes. This makes flow cytometry a valuable tool for understanding the complex interactions and processes within microbial communities in aquatic environments.

In this presentation you will find some examples of how we are using flow cytometry to characterize microbial populations in different marine environments from surface to deep waters and their response to different natural phenomena like a) hydrothermal emissions from a submarine volcano, b) lava and ash inputs from a subaerial volcano into the coastal ocean and c) counting the number of microbes in airborne desert dust and in air over the open ocean.

21st April 2023

PARALLEL SESSION VII – QUALITY ASSURANCE IN FLOW LABS

Catarina Martins
Universidade NOVA de Lisboa

HOW TO FACE ACCREDITATION

Since its first publication in 2003, the recognition of ISO 15189 “Medical laboratories – Requirements for quality and competence” as the leading quality standard for medical laboratories has significantly grown. In Europe, countries like France, Ireland or Belgium made it mandatory, at least in some fields of medical laboratories. The recent EU Regulation for In Vitro Diagnostic Medical Devices (IVD-R 2017/746) also reinforces the standard’s importance by setting accreditation as a requirement for all clinical laboratories performing laboratory developed tests within the EU.

In the Iberian Peninsula, despite the increasing number of accredited laboratories, the coverage of flow cytometry laboratories and assays is still very low compared to areas like clinical chemistry or microbiology. Shortage of staff and time

are amongst the usual difficulties and limitations identified, but flow cytometry laboratories also face specific technical challenges like method validation, lack of normalized approaches, or the availability of quality control platforms and programs.

Following the update on the parent standard ISO/IEC 17025 in 2017, the revision of ISO 15189 led to the publication of its fourth edition in 2022. There are no fundamental changes, but mostly a structural reorganization of the standard to align with ISO/IEC 17025, at the same time translating the evolution of medical laboratories in the past decade, now with an important emphasis on risk management and patient welfare and safety. Whatever strategy is followed in each country, by December 2025, accredited medical laboratories shall comply with the new standard. Thus, this transitional period represents an opportunity for flow cytometry laboratories to address accreditation by the only global standard for medical laboratories. Empowered by the growing discussions on issues like measurement uncertainty and validation, or the tools and recommendations made available in recent years, flow cytometry labs can start a new chapter, redefining efforts, and strategies to face accreditation.

Isabel de la Villa
ENAC, Madrid

REGLAMENTO EUROPEO 746 Y ACREDITACIÓN

La acreditación es la herramienta establecida a escala internacional para generar confianza sobre la correcta ejecución de las actividades de evaluación de la conformidad (ej: laboratorios clínicos), permitiendo diferenciar a los organismos que han demostrado disponer de la competencia técnica necesaria para realizarlas y que ofrecen fiabilidad en sus resultados de los que no lo han demostrado.

ENAC es el Organismo Nacional de Acreditación designado por el Gobierno en aplicación del Reglamento (CE) nº 765/2008 que reggula el funcionamiento de la acreditación en Europa.

El reglamento europeo 2017/746 sobre los productos sanitarios para diagnóstico un vitro establece una excepción en su aplicación a los productos fabricados y utilizados exclusivamente en centros sanitarios establecidos en la Unión, cuando se cumplan unas condiciones entre las que se encuentra “que el laboratorio del centro sanitario sea conforme a la norma ISO 15189 o, en su caso, a las disposiciones nacionales aplicables, incluidas las relativas a la acreditación”.

La acreditación otorgada por un organismo de acreditación reconoce la competencia técnica de un laboratorio para realizar unas actividades determinadas ya que el conjunto de sus procesos, procedimientos y recursos (personal, equipos, instalaciones) han permitido demostrar que realizan sus actividades técnicas de manera solvente.

Sin embargo, la acreditación no debe entenderse como un reconocimiento de aspectos específicos como personal, equipos o métodos/procedimientos de la entidad fuera de su contexto. Es decir, no debe entenderse que un método/procedimiento incluido en el alcance de acreditación de un laboratorio ha obtenido un reconocimiento específico fuera de su utilización en el laboratorio que obtuvo la acreditación. La acreditación no debe entenderse como un reconocimiento o autorización de un método de laboratorio determinado.

Kathy Daniels
ISAC SRL
Emerging Leader

ATTAINING ISAC SRL RECOGNITION - INSIGHTS

The ISAC SRL Recognition Program, launched in the Fall of 2021, aims to identify and acknowledge cytometry laboratories who have put in significant efforts to adhere to best practices, with the long-term goal to generate a level of professionalism across SRLs that increases the overall quality and reproducibility of flow cytometry. In this session we will provide an overview of the Recognition Program, detailing it’s purpose, how to apply, feedback from recognized labs and data from the first two application cycles. Additionally, we will discuss the perspective of a reviewer who has partaken in judging applicants to the Recognition Program.

PARALLEL SESSION VIII – IMMUNODEFICIENCIES AND NON-HEMATOLOGICAL DISEASES

María Concepción Núñez Pardo de Vera

Instituto de Investigación Sanitaria Hospital Clínico San Carlos, Madrid

ACTIVATED GUT-HOMING CD8 + T CELLS FOR COELIAC DISEASE DIAGNOSIS OF PATIENTS ON A GLUTEN-FREE DIET

Coeliac disease (CD) is an immune-mediated systemic disease, which is triggered by gluten ingestion in genetically susceptible individuals. Current diagnosis requires that patients are following a gluten containing diet. However, a long life gluten-free diet (GFD) constitutes the treatment of CD and many patients adopt a GFD on their own, precluding a proper diagnosis.

In 2018, our research group proposed a diagnostic test for individuals following a GFD based on detecting activated gut-homing CD8+ T cells in blood after a 3-day gluten challenge. These T cells can be detected by flow cytometry using only four markers (CD8, CD103, ß7 and CD38), providing a simple method of easy implementation in clinical practice. In 2021, we showed that the suggested test provides accurate CD diagnosis with 95% specificity and 97% sensitivity. This test is very useful in diverse situations observed in daily clinical practice such as subjects following a GFD and needing review of the initial diagnosis due to absence of or incomplete original testing (serological screening and confirmatory biopsy), previous discrepant results or slow or non-responsiveness to the GFD. It appears as an alternative to the current long gluten challenge (usually >6 weeks) followed by serological and biopsy testing, which is refused by many patients worried about the clinical consequences.

Additionally, it provides a standardized gluten challenge protocol consisting of 3 days supplying 10 g of gluten/day, which must be combined with two days of sample (blood) collection and analysis (before gluten challenge and 6 days later). It offers highly reproducible results for fresh analysis, but also for sample processing and analysis after 24 hours, opening the possibility of sending samples to a reference center.

Tiago Carvalheiro

Princess Massima Center of Pediatric Oncology, Utretch

MONITORING THE IMMUNE SYSTEM IN PEDIATRIC BRAIN TUMOURS

Brain tumors are the most common solid cancers in childhood, compromising 25% of the pediatric cancer diagnoses, and are the leading cause of cancer-related death in children. In particular, the prognosis of pediatric high-grade brain tumors is dismal. The combination of aggressive tumors in a delicate environment limits the therapeutic options. Studies on brain tumor biology and immune responses in adults outweigh pediatric tumor immunology knowledge. Even though many processes are similar in children and adult immunity, there are substantial age-specific developmental differences. A thorough comprehension of the pediatric brain tumor microenvironment and its influence on local and systemic immune system is essential for the translation of preclinical immune neuro-oncology research into clinical trials that improve current treatment options for these patients.

Spectral flow cytometry is an ultimate technique that offers the ability to perform complex multicolor analysis of cellular parameters. Because of its capacity for extensive panels, this method provides a comprehensive solution to investigate a substantial number of cellular characteristics in a single experiment, making it particularly useful for analyzing unique samples.

We have established a comprehensive and standardized immune monitoring platform that utilizes a combination of advanced technological solutions, such as spectral flow cytometry, to examine a wide range of immune parameters in various pediatric brain tumor entities. This platform enables us to track changes in the immune system during treatment, including the impact of surgery, radiotherapy, and chemotherapy.

Gaining a comprehensive understanding of the tumor microenvironment and immune landscape in brain malignancies is crucial to pave the path towards effective immunotherapeutic strategies in pediatric neuro-oncology.

Lucía del Pino-Molina
IdiPAZ, Madrid

DISSECTION OF THE PREGERMINAL CENTER B CELL MATURATION IN CVID

Common Variable Immunodeficiency (CVID) is characterized by defective antibody production and hypogammaglobulinemia with impaired differentiation of mature post-germinal-center (GC) class-switched memory B-cells (MBC). Flow cytometry immunophenotyping has become of great relevance for its diagnosis and classification. In this collaborative multicentric study the EuroFlow PID 8-color Pre-GC B-cell tube, standardized sample preparation procedures (SOPs) and innovative data analysis tools, were used to characterize the maturation profile of pre-GC B-cells in 100 CVID patients, vs 62 age-matched healthy donors (HD).

The Pre-GC B-cell tube allowed identification within pre-GC B-cells of three subsets of maturation associated immature B-cells and three subpopulations of mature naïve B-lymphocytes. CVID patients showed overall reduced median absolute counts (vs HD) of the two more advanced stages of maturation in immature B cells. This was associated with an expansion of CD21- CD24- and CD21- CD24++ naïve B-cell counts above normal values. Aditionally, reduced IgMD+ and IgMD- MBC counts were found to be below normal values CVID patients, always together with severely reduced/undectatable circulating blood plasmablasts.

Comparison of the maturation pathway profile of pre-GC B cells in blood of CVID patients vs HD using EuroFlow software tools showed systematically altered patterns in CVID. These consisted of: i) a normally-appearing maturation pathway with altered levels of expression of >1 (CD38, CD5, CD19, CD21, CD24, and/or smIgM) phenotypic marker (57/88 patients; 65%) for a total of 3 distinct CVID patient profiles (group 1: 42/88 patients, 48%; group 2: 8/88, 9%; and group 3: 7/88, 8%) and ii) CVID patients with a clearly altered pre-GC B cell maturation pathway in blood (group 4: 31/88 cases, 35%). Our results show that maturation of pre-GC B-cells in blood of CVID is systematically altered with up to four distinctly altered maturation profiles.

CLOSING LECTURE

Jacques Van Dongen
University of Leiden

NEW CHALLENGES IN IMMUNOMONITORING BY FLOW CYTOMETRY

Any form of tissue damage will instantly recruit immune cells to control this damage and induce repair. This is valid for infectious diseases, inflammatory diseases, auto-immune diseases, and trauma as well as for many different types of medical interventions (surgery, irradiation, cytotoxic drugs, immunosuppressive treatment and immunotherapies). Obviously, dependent on the type of tissue damage, the degree and type of immune response will differ.

Thanks to many new developments in flow cytometry, cellular immune responses are stepwise better understood, particularly due to the EuroFlow-based analysis of differentiation, maturation and activation pathways with multi-color (>35-color) flowcytometry. Depending on the clinical setting, the focus of the cellular studies should be adapted to consecutive steps, such as in infection, vaccination and surgical interventions, were several mature immune subsets play consecutive roles. In contrast, specific immature subsets should be studied to understand reconstitution and regeneration during & after specific cytotoxic therapies or after stem cell transplantation. In targeted immunotherapies, such as in hemato-oncology, immune monitoring during & after therapy should be combined with minimal residual disease, regrowth of the normal targeted cells and the immune therapy itself, particularly in case of CAR T-cells.

Finally, in addition to the EuroFlow-based pathway concepts, also high levels of standardization appear to be critical to get comparable results at different sites within the same clinical protocols. This concerns, the sample processing, the choice of instruments (allowing acquisition of 25,000 to 30,000 cells per second), acquisition of 5 to 10 million cells, usage of at least 25 colors, no complex requirements for compensation settings, and comparability & stability of instruments at different sites (and overtime). This is why European collaboration should be fostered in order to obtain comparable results between many different clinical sites in international treatment protocols.

06

Short Oral Presentations

SHORT ORAL PRESENTATIONS

19th April 2023

PARALLEL SESSION I – Flow Cytometry in Advanced Therapies

Mercedes Lopez-Santalla
CIEMAT/CIBER-ER/IIS-FJD/UAM. Madrid

ANALYSIS OF INNATE RESPONSES INDUCED BY MESENCHYMAL STROMAL/STEM CELL-BASED THERAPY IN AN EXPERIMENTAL MODEL OF COLITIS BY FLOW CYTOMETRY TECHNOLOGY

Inflammatory bowel diseases (IBD) consist of chronic inflammatory disorders that mainly involved the intestinal mucosa. Current treatments for IBD have significant side effects as well as a lack of response in a significant number of patients. Mesenchymal stem/stromal cells (MSCs) may be a therapeutic alternative due to their immunomodulatory and tissue regeneration properties. Among the mechanisms participating in the inhibition of intestinal inflammation, the induction of regulatory immune responses has been involved in the beneficial effects of MSC-based therapy.

The aim was to characterize myeloid populations associated with the beneficial long-term effects of MSC therapy in a clinically relevant model of colitis.

MSCs were infused into dextran sulphate sodium (DSS)-induced colitic mice during the induction phase of the disease. Following a latency period, mice were re-challenged with DSS. Myeloid populations were analysed by flow cytometry at different time points in different organs and tissues.

After the latency period, increased levels of Ly6G+CD11b+ myeloid cells were noticed in the peritoneal cavity (PC), spleen (SP), and bone marrow (BM) in MSC-treated compared to untreated colitic mice. Ly6G+CD11b+ cells from PC co-expressed F4/80, CD206, and CX3CR1, markers of alternative-activated macrophages from BM origin. Based on the expression of Ly6C and Ly6G markers, the Ly6G+CD11b+ cells from SP were defined as myeloid-derived suppressor cells and, from BM, immature myeloid cells. These phenotypes were also confirmed by the intracellular expression of iNOS, Arg-1, IL-10, and by inhibition of T-cell proliferation. During the 2nd challenge and in parallel to a reduction in the disease activity index, an increase of IL-10+Arg1+F4/80+Ly6G+CD11bdim regulatory cells and a decrease of GM-CSF+TNFα+IL-6+iNOS+IFN+Ly6G+/dimCD11b+ inflammatory cells were observed in the colon in MSC-treated compared to untreated colitic mice. These findings obtained by flow cytometry technology suggest that MSC-based therapy can imprint innate immune memory-like responses that confer sustained protection against intestinal inflammation in the long term.

Roser Salvia Cerdà
Germans Trias i Pujol Research Institute (IGTP), Universitat Autònoma de Barcelona, Badalona

CONFORMATIONAL CHANGES OF PD-L1 IN CIRCULATING MDSCS AND PREDICTION OF ESCAPE FROM IMMUNOTHERAPY IN NSCLC PATIENTS RECEIVING ANTI-PD-1/PD-L1 CHECKPOINT INHIBITORS

Anti-PD-1/PD-L1 immunotherapy (IT) is indicated in non-small cell lung cancer (NSCLC) stages III-IV without driver gene mutations, and has improved the survival of patients. However, the 5-year survival rate is still only 26%. Myeloid-derived suppressor cells (MDSCs) are circulating cells that express PD-L1 and can infiltrate and proliferate in the tumor environment, inducing immunosuppression.

In this work, we have studied the conformational changes of PD-L1 in MDSCs and their potential value in assessing prediction of escape from IT in NSCLC patients receiving checkpoint inhibitors.

Peripheral blood from stage III-IV NSCLC patients (n=37) was processed immediately prior to IT, using minimal sample perturbation protocols. Briefly, blood was stimulated with phorbol esters (PMA), which resulted in conformational changes of PD-L1 and increased epitopic target accessibility. MDSCs were identified as HLA-DRlo/-CD33+CD11b+ and analyzed on the Attune™ NxT flow cytometer (Thermo Fisher). Comparative analysis of the conformational dynamics was assessed using the PD-L1 index (PD-L1i), obtained by calculating the difference of PD-L1 mean fluorescence intensity between stimulated PD-L1+MDSC and non-stimulated MDSCs, divided by two times the standard deviation of the non-stimulated populations.

Responders and non-responders were classified according to PD-L1i and progression-free survival (PFS). With PD-L1i >5.88, disease progression occurred in 58.33% patients, showing significant differences when comparing with a PD-L1i of ≤5.88, where only 7.69% underwent disease progression (p-value = 0.0042 and 95% CI = 3.17-NA). Overall survival (OS) was significantly worse in the group with higher PD-L1i (58.33% vs 23.07% deaths, respectively; p-value = 0.035; 95% CI = 7.53-NA). The conformational dynamics of PD-L1 in MDSCs has been shown to be a good biomarker for predicting NSCLC progression in patients receiving anti-PD-1/PD-L1 IT. Importantly, this approach uses a non-invasive and rapid flow cytometry assay with a promising biomarker for the evaluation of immunotherapy escape.

Xavier Catena Parrado

National Cancer Research Center, CNIO, Madrid

MIDKINE DRIVES IMMUNE EVASION BY REWIRING DENDRITIC CELL DIFFERENTIATION AND IMMUNOGENICITY

Malignant melanomas are characterized by a high potential to metastasize and evade immune surveillance. Immunotherapy has revolutionized treatment in melanoma, still, a significant fraction of melanoma patients displays intrinsic or acquired immune resistance. Thus, a pending question in the field is to understand how immune resistant environments are generated. We have recently identified MIDKINE (MDK) as promoter of melanoma metastasis, and a driver of resistance to immunotherapy thought the rewiring of macrophages.

We aimed to further characterize the roles of MDK in the regulation of the immune system, specifically dendritic cells (DCs). DCs are professional antigen-presenting cells whose main function is to regulate the establishment of efficient anticancer responses. However, aggressive tumors may impair DC function. The precise aims of this project were: i) assess whether MDK interferes with DC differentiation and ii) function. iii) Further study the effects of MDK on antigen presentation and in consequence, its impact on the immune response against melanoma. iv) And finally, to validate our results in melanoma patients.

In this project, we combined transcriptomic and computational analyses, flow cytometry immunophenotyping and in vivo and in vitro functional analyses to comprehensive profile dendritic cell populations rewired by MDK.

Here, we have unveiled the impact of MDK on DCs at four levels: MDK reduces DC infiltration in melanoma and lymphoid organs by blocking their differentiation at the bone marrow; MDK inhibits DC-mediated antigen presentation by blocking DC key functions. Consequently, MDK-educated DCs trigger a dysfunctional T response that results in an increased resistance to ICB.

Finally, we have identified an MDK-educated DC gene signature that stratifies melanoma patients with differing overall survival and deficient response to ICBs. In conclusion, MDK hinders anticancer immune responses by interfering with DC biology facilitating resistance to immunotherapy. Together our results may hold translational relevance for therapeutic intervention in melanoma.

PARALLEL SESSION II – NON-HUMAN FLOW CYTOMETRY

Ainara Barco-Tejada
Hospital General Universitario Gregorio Marañón, Madrid

DESIGN AND VALIDATION OF NOVEL FLOW CYTOMETRY PANELS TO ANALYZE A COMPREHENSIVE RANGE OF PERIPHERAL IMMUNE CELLS IN C57BL/6J MICE

Flow cytometry is a widely used technique to diagnose several immune-related diseases in humans in peripheral blood (PB). However, in murine models, it presents several limitations, such as the meager amount of PB available , narrow variety of commercial antibodies and fluorochromes and high inter-subject variability in immune populations

The aim was to define novel cytometry panels to analyze extensively immune cell populations in mice from 100uL of PB and test the panels in lipopolysaccharide (LPS) lung inflammation model.

Twelve C57BL/6J mice were randomized into two groups: LPS, (n=6) undergoing intratraqueal inoculation of LPS (5mg/kg) in 100 µL of saline; and control (n=6). Fresh blood was collected from the maxillary venous sinus on day 1 pre-inoculation, and days 1 and 3 post-inoculation (p.i). Whole blood was labeled with 27 antibodies distributed in two panels, and acquired in a 16 channels Flow Cytometer. Peripheral populations were assessed overtime, overlapping innate and adaptive immune subsets.

LPS-induced inflammation was rapidly observed at day 1 p.i, by an increase of granulocytes frequency and numbers, correlated with the mice's weight loss. Interestingly, a decline in the pro-inflammatory Th1 and NK subsets was observed in the same group. Rare cellular populations have shown high variability, likely due to very low cells/µL. For all these reasons, our panels allowed us to analyze most physiological peripheral immune cellular subsets from a minimal amount of whole blood and can permit us to detect immune changes due to rapid inflammation induction. This longitudinal study could help in determining the best timing for future experimental designs, using minimal blood and with a minimal impact on the animal status, avoiding unnecessary animal sacrifices and thus, reducing sample size

Elena Blázquez-López
Instituto de Investigación Sanitaria Gregorio Marañón, Madrid

ASSESSMENT OF IMMUNOLOGIC CHANGES IN PERIPHERAL BLOOD DURING THE PROGRESSION OF CLOSTRIDIOIDES DIFFICILE INFECTION (CDI) IN MOUSE MODELS

Current evidence establishes a causality relation between inflammation, and the development of many pathologies, such as CDI, one the most common and severe nosocomial infections. Its prognosis and recurrence prediction are still an unsatisfied need, just based on clinical criteria which are not sufficiently accurate. Therefore, the determination of immune profile could throw relevant information about the progression and gravity of CDI.

The aim was to evaluate the evolution of the immunological profile in peripheral blood (PB) on a mouse model of CDI over time.

CDI was induced in 20 female C57BL/6 mice following Cussó et al. (DOI: 10.1007/s11307-019-01408-4) protocol. Mice were divided into mild (ribotype 001, low toxin production) and severe (ribotype 027, high toxin production) groups. Clinical signs score (CSS) was obtained for each animal over time. PB samples were collected at basal, pre-infection (0d), post-infection (+2d) and recovery (+15d); and incubated with 2 multiparametric antibodies panels to analyse lymphoid and myeloid populations. Then erythrocytes were lysate and samples were acquired by flow cytometry using a MACSQuant® Analyzer 16 Flow Cytometer, and the results were analysed using Kaluza Analysis Software.

Both groups showed an increase of activated and effector CD4+ T cells post-infection which recovered after 15 days. Also, B lymphocytes values raised progressively according to CDI progression, in particular plasmablasts percentages were higher at recovery, while monocytes (Mo) and dendritic cells (DC) were decreased post-infection, compared to basal levels.

However, severe group at recovery presents increased values of activated CD8+ T cells, Th17 cells and pro-inflammatory Mo and DC compared to mild group, indicative of more intense inflammatory responseThis result was consistent with the CSS since severe group failed to recover. In conclusion, our flow cytometry strategy allows us to detect PB immunological changes on CDI mouse models that can discriminate between CDI severity degrees.

Andrés Pastor-Fernández
IMDEA Food Institute, Madrid

SEXUAL DIMORPHISM IN THE ANTI-TUMOR IMMUNE RESPONSES ELICITED BY THE COMBINATION OF FASTING AND CHEMOTHERAPY.

Fasting during chemotherapy treatment enhances anti-tumor efficacy in mice and humans. In turn, fasting shows a marked sexual dimorphism in its metabolic effects, but little is known about the influence of sex in the benefits of fasting on chemotherapy.

B16F10 melanoma or MC38 colon carcinoma cells were engrafted in C57BL/6JOlaHsd mice and subjected to two cycles of chemotherapy (doxorubicin or oxaliplatin) plus 48 hour fasting. This protocol was also performed in castrated males and in females implanted with testosterone. For immunophenotyping, inguinal lymph nodes, peripheral blood and tumors were analysed. For classical cytometry we generated four different panels: lymphoid (10 markers), subtypes of CD4 (8 markers), exhausted CD8 (6 markers) and myeloid (9 markers:). For high-dimensional analysis, two panels were generated: B16F10 panel (17 markers) and MC38 panel (19 markers).

Here, we showed that fasting can enhance the anti-tumor effects of certain chemotherapeutic agents in a sex-specific manner in B16F10 or MC38 allografts. Fasting strongly improved the anti-tumor B16F10 effects of doxorubicin only in males, and testosterone levels were critical for this fasting-mediated benefits. In turn, B16F10 tumors treated with oxaliplatin exhibited a beneficial effect in both sexes, more prominent in males. In contrast, fasting was beneficial in both sexes in the treatment of MC38 allografts plus oxaliplatin. Fasting led to different immune responses depending on the tumor and chemotherapeutic agent used. In B16F10 tumor, fasting increased anti-tumoral NK and NKT infiltration in males and increased pro-tumoral exhaustion of CD8 in females. In MC38, fasting increased anti-tumoral effector CD8 T cells and type I CD4 cells, and reduced tumor-associated macrophages in both sexes. These findings highlight the potential for sex-specific differences in response to fasting and the importance of considering tumor type and chemotherapeutic agent when studying the effects of fasting on the immune system and cancer treatment.

20th April 2023

PARALLEL SESSION III – LEUKEMIA & LYMPHOMA

Laura Moreno
Hospital Universitario de Guadalajara

SOLUBLE BCMA IS A PROMISING BIOMARKER FOR MONITORING PATIENTS WITH MULTIPLE MYELOMA

B cell maturation antigen (BCMA) is expressed in all normal and malignant plasma cells (PCs). BCMA and its proliferation-inducing ligand (APRIL) play an important role in the long-term the survival of these cells. The enzyme γ-secretase is responsible for the removal of BCMA from PCs. For this reason, it is possible to find soluble BCMA (sBCMA) in peripheral blood (PB).

This study measured the amount of sBCMA in the PB of patients with multiple myeloma (MM) in order to explore its value for monitoring this disease.

PB of 5 healthy donors and PB and BM aspirates from 19 patients (12 newly-diagnosed and 7 with relapse/refractory disease) with MM were studied. The concentration of sBCMA and APRIL was evaluated by the ELISA technique in PB and BM. The percentage of infiltration of PCs in BM was evaluated by flow cytometry.

MM patients showed a higher concentration of sBCMA in PB than healthy donors (mean 610.5 ng/mL vs 16.01 ng/mL, p<0.0003). Reganding APRIL, differences were also found between patients with MM and healthy donors, being higher in the control group (mean 138.3 pg/mL vs. 373 pg/mL, p<0.03). In MM patients, mean value of sBCMA detected in BM was 474.1 ng/mL and showed a strong correlation with sBCMA PB levels (R2=0.86 p<0.0001). Finally, a strong correlation between PCs infiltration in BM and the sBCMA detected in PB from MM patients was demonstrated too (R2=0.69, p=0.002). In summary, PB sBCMA can predict the percentage of PCs infiltration in BM. This new biomarker it would be useful for monitoring to disease including non-secretory MM patients. In addition, sBCMA could influence the selection of the appropriate dose of anti-BCMA therapies such as monoclonal antibodies or CART cells currently on the rise. Further translational studies and evaluation are warranted.

Alba Pérez Pons

Centro de Investigación del cáncer, USAL

IMMUNE PROFILING OF BLOOD T-LYMPHOCYTES IN SYSTEMIC MASTOCYTOSIS

Systemic mastocytosis (SM) includes a heterogeneous group of disorders, resulting from an expansion of clonal mast cells (MC) associated with gain-of-function KIT-mutations that lead to MC activation and the release of MC mediators. These might alter the tumour microenvironment with potential consequences on adaptative immune cells, such as T- and NK-cells.

The aim was to investigate the distribution of NK-cells, T-cells and their subsets in blood of SM patients with distinct disease subtypes, and its relationship with other disease features.

We studied 111 SM patients - 37 bone marrow mastocytosis (BMM), 65 indolent SM (ISM), 9 aggressive SM (ASM)- and 83 age--matched healthy donors (HD). The distribution of blood NK-cells, T-cells and their subsets was investigated by spectral flow cytometry and the EuroFlow Immunemonitoring panel, and correlated with multilineage KITD816V, alpha-tryptasemia genotypes (HAT) and other disease features.

SM patients showed decreased (vs HD) median blood counts (cells/µl) of NK-cells (212 vs 393; p<0.001), cytotoxic T-cells (517 vs 621; p=0.002) and several functional subsets of TCD4+ cells such as Th1 (112 vs 134; p=0.006), Th2-effector-memory (4.1 vs 4.4; p=0.012) and TH22-terminal-effector (0.3 vs 0.4; p=0.001) cells, whereas T-follicular-helper (163 vs 133; p=0.009) and Th1/Th17-like T regulatory cells (0.9 vs 0.7; p=0.003) showed increased values (vs. HD, respectively). Interestingly, different immune profiles were found among distinct diagnostic subtypes of SM, where e.g., ASM patients displayed decreased numbers of total and several maturation-associated and functional subsets of TCD4+ cells. In turn, ISM patients with multilineal KIT had decreased Th2-cell counts (51 vs 65 cells/µl vs MC-restricted cases; p=0.018), whereas HAT+ cases displayed increased Th1/Th17-cell counts (120 vs 77 vs HD; p=0.023) in blood. These results suggest an altered distribution of blood NK and T-lymphocytes in SM, which relates to disease subtype, the pattern of involvement of hematopoiesis by KITD816V and a HAT genotype.

Rubén Ballester González

Hospital Universitario Ramón y Cajal, Madrid

FLOW CYTOMETRY AS A TOOL FOR DIAGNOSIS AND ELUCIDATION OF PATHOGENESIS IN CD2+ B-LYMPHOBLASTIC LEUKEMIA WITH TRANSDIFFERENTIATION TO MONOCYTIC LINEAGE

In B-lymphoblastic leukemia (B-ALL), the lineage change of aberrant B precursors to a myeloid lineage during treatment is an extremely rare event, which may cause doubts about the initial diagnosis and the continuation of the established treatment. In recent years, a subgroup of B-ALL with CD2 expression at diagnosis has been described. After initiation of treatment, these patients present a higher probability of transdifferentiation towards monocytoid lineage cells.

We present a clinical case with the most relevant immunophenotypic findings and explore the possible reasons that justify this phenotypic change.

Flow cytometry analysis of bone marrow (BM) aspirates were performed using a FACSCantoII cytometer at diagnosis, 15 and 30 days post-treatment. At day

+15, a cell culture was performed in presence or absence of methylpredinisolone, cells were extracted at days +2 and +5 and the cell subset percentages, cell death and proliferation were evaluated.

Diagnosis of common B-ALL according to EGIL classification was established, with aberrant CD2 expression. Measurable residual disease (MRD) study on day +15 revealed 28% of aberrant precursors, with a clear differentiation of the original lymphoblasts towards monocytoid cells, being able to establish up to 3 populations. The results obtained in cell culture showed that the presence of methylprednisolone did not modify the percentages of the different subpopulations. The results showed greater proliferation and less cell death in cells with monocytoid differentiation, without appreciating differences due to the presence of methylpredinosolone. On day, +30 aberrant blasts of any type of lineage were not observed. This case demonstrates that despite the clear transdifferentiation of the original blasts, neither the initial diagnosis nor its treatment should be changed. In vitro study revealed that during treatment, cells with monocytoid differentiation showed greater proliferative capacity and resistance to cell death, which explains the behavior of this leukemia.

PARALLEL SESSION IV – SMALL PARTICLE CYTOMETRY

Roser Salvia Cerdà

Germans Trias i Pujol Research Institute (IGTP), Universitat Autònoma de Barcelona, Badalona

NANOPLASTICS REPRESENT A SIGNIFICANT FRACTION OF INHALED AIR, CAN OVERCOME BIOLOGICAL BARRIERS AND ACCUMULATE IN HUMAN TISSUES

Nanoplastics (NPs) are plastic particles between 1-1000nm. The accumulation of NPs has been demonstrated in human tissues, such as blood or placenta, while in others it remains unstudied.

The aim was to analyze the accumulation of NPs in human bronchoalveolar lavage (BAL), cerebrospinal fluid (CSF), lymph nodes (LN), biological fluids (BL) and peripheral blood (PB), as well as PB from mice living under SPF confinement.

Organic matter was degraded in KOH (1%) at 60°C in a dry block for 10 days. 20µl of the digested sample was diluted in deionized water and stained with 2µl Nile Red for 15min at room temperature. Submicron microspheres were used for calibration. All samples were analyzed on the Attune™ NxT flow cytometer, with accumulation of NPs measured in triplicate.

NPs were detected in all tissues analyzed. Specimens with highest accumulation of NPs were LN and PB, with a median of 7 million NPs per mg of tissue (range: LN 1.02- 17.57 million NPs/mg; PB, 5.11-13.19 million NPs/mg). BAL showed lower accumulation (5 million NPs/mg), and high dispersion between samples (0.55-36.92 million NPs/mg). Pleural liquid, seroma, and CSF, showed lower accumulation of NP, with values of 2 million NPs/mg (range 0.14-0.44 million NPs/mg) in CSF. NP levels in the blood of mice were significantly lower than in humans (0.6 vs. 7 million NPs/mg; mice range: 0.27- 1.08 million; p-value<0.0001). NPs can cross biological barriers, as in the case of CSF accumulation. NPs can enter living organisms through inhalation, as confirmed by detection in BAL. In mice, the lower accumulation of NPs could be related to the air quality in facilities equipped with HEPA filters that clear larger particles. Further studies will be needed to understand the impact of NPs in living organisms as well as their entry, accumulation and elimination pathways.

André C. Pereira

Centre for Ecology, Evolution and Environmental Changes (cE3c), Lisbon

REAL-TIME ENVIRONMENTAL MONITORING OF MYCOBACTERIUM BOVIS IN A MULTI-HOST TRANSMISSION SCENARIO

In Iberia, Mycobacterium bovis, causing animal tuberculosis (TB), circulates in a multi-host system including cattle, wild boar, and red deer. Transmission occurs via direct or indirect contact with infected animals shedding bacteria through several routes. Current knowledge postulates interspecific transmission is favoured by animal contact with natural substrates (soil, water, etc.) contaminated with infected droplets and fluids. However, methodological constraints

have hampered the assessment of M. bovis environmental contamination and the validation of this hypothesis.

With a new real-time monitoring tool, we assessed the burden and viability of M. bovis in natural substrates from a TB hotspot area and assessed the phylogenomic relatedness of environmental and animal isolates.

Sixty-five natural substrates were collected nearby the International Tagus Natural Park region. These included sediments, sludge, water, and food deployed at unfenced feeding stations. The tripartite workflow included quantification and sorting of different M. bovis cell populations: total, viable, and quiescent. This integrated methodology is based on flow cytometry applying 5-carboxyfluorescein diacetate acetoxymethyl ester to differentiate metabolically active from inactive cells, fluorescence in situ hybridization with an IS6110-specific probe, and fluorescent-activated cell sorting. Taxonomically labelled sorted cells were sequenced after whole-genome enrichment for M. bovis. The phylogenomic analysis explored the signatures of environmental and animal (> 200) M. bovis isolates recovered from the study area.

Thirty-eight percent of samples were positive for M. bovis (mean 3.4x10^4 cells/g or /L), with only 12% showing metabolically active cells (8.9x10^3 cells/g or /L). After a resuscitation step, that number raised to 54%. Environmental isolates were assigned to the two major M. bovis lineages circulating in Portugal, being phylogenetically related to animal isolates. This pioneer multi-tool framework highlights the importance of environmental contamination in indirect transmission and offers additional resources for surveillance, potentially guiding the implementation of biosecurity and control measures.

Marta Monteiro
Instituto Gulbenkian de Ciência, Portugal

FROM FULL SPECTRUM TO CONVENTIONAL CELL SORTING – A METHOD TO ISOLATE PHYTOPLANKTON USING AUTOFLUORESCENCE

Phytoplankton are a diverse group of mostly unicellular photosynthetic organisms critical for the global carbon cycle. In fundamental research, it is possible to use and manipulate phytoplankton strains isolated from oceanic waters and maintained in culture collections to investigate a number of biological questions.

In our flow cytometry facility, we aimed to establish a method to isolate by cell sorting different species of marine phytoplankton that have been co-cultured under different conditions to support research projects.

We have been focusing, in particular, on Phaeodactylum tricornutum, Nannochloropsis granulata, Dunaliella tertiolecta, Amphidinium carterae and Tisochrysis lutea. Although the different species had distinct morphologies and the light scatter properties were not identical, in most cases there was a significant overlap that prevented their resolution using those parameters alone. Using full spectrum flow cytometry, we characterized the spectral signatures of each phytoplankton species cultured individually.

These signatures were based on the endogenous autofluorescence, which is conferred by the differential chlorophyl content, as well as other pigments and metabolites. As expected, similarity indices were very high, but, with the exception of Phaeodactylum, spectral signatures allowed the resolution of all species. Based on the differences in maximal and minimal fluorescence emission in the spectral signatures, we prepared a customized optical configuration for the conventional cell sorter to discriminate the different species.

After testing different nozzle sizes and numbers of sorted cells, and without replacing the sheath fluid by sea water, we established a gating strategy that enabled a highly pure isolation from a heterogeneous suspension of four species that could successfully expand in culture after sorting.

In conclusion, our methodology will enable to specifically isolate with a high speed and degree of purity distinct phytoplankton species from co-cultures for further downstream characterization. This opens new opportunities to address important questions in ecology and evolution.

PARALLEL SESSION V – ERYTHROID & PLATELET DISORDERS

Enrique Colado Varela
Hospital Universitario Central de Asturias, Oviedo

FETOMATERNAL HAEMORRHAGUE CUANTIFICATION. 10 YEAR EXPERIENCE IN AN UNIVERSITY HOSPITAL

Fetomaternal hemorrhage (FMH) refers to the entry of fetal blood into the maternal circulation before or during delivery. This pathological condition presents wide clinical heterogeneity, including devastating consequences for the fetus such as neurologic injury, stillbirth, or neonatal death.

In 2012, a clinical protocol for flow cytometry-based detection of fetal erythrocytes in maternal samples was established in cases of fetal/neonatal anemia (FA) and unexplained fetal loss (uFL) in order to quantify FMH and guide clinical decisions.

The Fetal Cell Count Kit (IQP Products, Rozenburglaan, The Netherlands) including anti-carbonic anhydrase (CA) and anti-fetal hemoglobin (HbF) was used to discriminate the different RBC populations in maternal blood. Calculation of FHM volume was performed according to Mollison BMJ 1972. Significant bleed was defined as any bleed exceeding 2mL of fetal erythrocytes according to BCSH guidelines.

69 cases corresponded to uFL, while 14 corresponded to fetal/neonatal anemia. In 8/83 cases, a significant bleeding was detected. The median FMH volume was 75mL (range 2.4 to 132mL). 28% of FA showed a significant bleeding (range 2.4 to 122mL). In two cases with significant bleeding (one corresponding to uFL and one to FA) the mother was D negative and the fetus/baby was D positive. FMH volume was 103 and 122mL respectively, therefore supplementary doses of anti-D immunoglobulin for the prevention of haemolytic disease of the newborn (HDN) had to be administered and clearance of fetal erythrocytes was subsequently confirmed. Correct quantification of FMH using flow cytometry is feasible and provides valuable clinical information on the origin of uFL and FA. In cases with D antigen discrepancy, communication with the obstetric team allows the calculation of anti-D immunoglobulin dose required to cover the reported bleeding and advice regarding follow-up samples to check for clearance of fetal cells.

Sara Viera Morilla
GSK Global Health Medicines R&D, Tres Cantos, Madrid

FLOW CYTOMETRY AS A POWERFUL TOOL FOR MALARIA DRUG DISCOVERY

Malaria is a vector-borne disease caused by parasites from the genus Plasmodium and transmitted to human by the bite of an Anopheles mosquito. This disease that affects over 200 million people every year. P. falciparum, responsible of the majority of the deaths, infects almost exclusively human erythrocytes.

The use of humanized mouse models to study this disease have been crucial in the past years to find new drugs to fight malaria. GSK Pf-Hu (Plasmodium falciparum-Humanized) mice model and Flow Cytometry analysis described in this work has been used for preclinical studies of different assets that are currently on clinical phases.

Parasite growth in Pf-Hu mice blood is reproducible and shows all erythrocytic stages of parasite (rings, trophozoites and schizonts). In mice peripheral blood, parasite is detected by flow cytometry, using a combination of DNA dye (YOYO-1) and a monoclonal antibody specific for mouse erythrocytes (TER119-PE). Parasite is shown as the population YOYO-1+/TER119-PE- in a bidimensional FL1/FL2 dot plot, tacking advantage autofluorescence we are able to discriminate the different erythrocytes stages.

Flow cytometry analysis allows the discrimination of parasite population sensible to the drug treatment when Pf-Hu mice model is used for the screening of new antimalarial compounds. Different standard antimalarial drugs have been used for the setting up of this analysis strategy and it has been demonstrated that a simple bidimensional flow cytometry analysis allows to obtain valuable information to decipher the mechanism of action when little is known about

the drugs tested. “The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents” “All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals.”

Isabel Ojeda Pérez
CIEMAT, Madrid

DIFFERENT CELLULAR KINETICS AFTER MOBILIZATION TREATMENT IN A PYRUVATE KINASE DEFICIENCY MODEL

Human pyruvate kinase deficiency (PKD), an autosomal recessive disorder produced by mutations in the PKLR gene, is the most common cause of chronic non-spherocytic hemolytic anemia. This deficiency alters the erythrodifferentiation and gives rise to anaemic phenotypes. We have previously shown that the anaemic phenotype has an impact on bone marrow niche by altering cellular subsets. An imbalance in the most immature compartment has been demonstrated but it has not been studied how this alteration could affect physiological processes, such as mobilization.

Our aim has been to study cellular kinetics after pharmacological induced Mobilization of peripheral blood progenitor cells in a anemic PKD mice model.

We have designed a 13 colour panel that includes both stem and more mature committed hematopoietic markers such as CD3, CD45R/B220, Ly-6A/E (Sca 1), Ly-6G, Ly-6C, CD11b (Mac-1), CD16/32, CD34, CD71, CD117(c-Kit), TER-119, CD135, F4/80 antigen and viability marker. The samples were acquired in an 4L BD LSRFortessa X-20™ with a total of 20 detectors. The panel was applied at different time points (0, 30´, 90´, 120´, 180´and 24h). Blood samples were also analyzed in a haematological counter(Sysmex XN-1000V).

Results shown an altered kinetic of recovery of white blood cell populations after mobilization in mutant animals. Surprisingly, the results have also shown that the severity of the erythroid phenotype improves after mobilization with Plerixafor at least at the mature level. The number of cells expressing cKit in mutant´s blood at 90´ was also increased after pharmacological treatment compared to healthy control. Whether these kinetic differences are due to intrinsic defects to the progenitor/mature fractions themselves and/or to disturbed niche signals by the constant erytrhoid bias towards the erythroid path remains unclear.

PARALLEL SESSION VI – SARS-CoV-2.

Guadalupe Herrera
Cytometry Service, Central Research Unit (UCIM), Incliva Foundation-University of Valencia

LONG-TERM MONITORING BY FLOW CYTOMETRY OF T-CELL MEMORY AND IN VITRO ACTIVATION RESPONSE IN IMMUNOSUPRESSED PATIENTS AFTER COVID-19 VACCINATION

T cells are essential for protection against infection by natural- and vaccine-induced immunity. Monitoring T-cell functions is crucial to follow-up COVID-19 vaccination in immunosuppressed patients. Flow cytometry (FCM) has revealed multiple immunological features and consequences of COVID-19 disease.

The aim was to apply FCM for assessing the efficiency of COVID-19 vaccines in immunosuppressed patients, based on circulating antigen-specific T cells, memory subpopulations and cytokine production by T cells upon in vitro activation.

Twenty-two immunosuppressed patients and 21 controls were studied at months one, three, six and twelve along mRNA vaccination. Circulating CD8 T cells, specific for a Sars-Cov-2 Spike epitope were detected with APC-conjugated dextramer (Immudex). Appropriate DuraClone panels (Beckman Coulter) were used for immunophenotyping, enumeration of T cell-memory subpopulations and quantitation of intracellular cytokines. Assays were run on Gallios flow cytometer (Beckman Coulter) and analyzed with Kaluza software (Beckman Coulter). For statistical analysis, Mann-Whitney test was applied.

Patients presented lower and heterogeneous levels of antigen-specific CD8 cells. Patients showed persistent decrease of CD4 lymphocytes and total lymphocytes and T cells were decreased at 3 months and 6 months. CD28-CD27+ CD4 cells increased, while CD28+CD27+ CD8 cells decreased at 3 and 6 months. There was a marked increase of TEMRA CD4 cells. These findings suggest impaired T cell co-stimulatory function. CD4 cells from patients showed lower production of IL-2 and lower production of cytokines by IL-2+ cells, but greater production of IFNγ and TNFα without IL-2 expression. CD8 lymphocytes from patients showed lower IL-2 expression but were more efficient in producing TNF-α and/or IFN-γ in the absence of IL-2. Our data on immunosuppressed individuals show heterogeneous antigen-specific CD8 memory and lower T-cell activation responses. Our FCM approach may be applied for personalized follow-up of high-risk patients. Sponsored by Fundación Mutua Madrileña (IRAS-VAC Project).

Francisco Pérez Cozar

Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), PTS, Granada, Spain

ALTERED MIGRATORY PROFILE OF CIRCULATING MONONUCLEAR CELLS AFTER COVID AND INFLUENZA INFECTION

Clinical symptoms following resolution of Sars-CoV-2 infection are known as long-COVID. The incidence and prevalence of chronic symptoms after acute viral infections have gained strong interest over the course of the COVID-19 pandemic. Acute post-infection sequelae have also been observed in other respiratory viral infections, including influenza virus.

Here, we performed a deep-immune characterization of a cohort of convalescent COVID-19 (post-COVID) and post-influenza (post-FLU) patients in order to describe the immune landscape after acute infection.

We collected peripheral blood samples from patients who recovered from COVID-19, 3 months after they were discharged from hospital (n = 83), and from post-FLU patients. Immune phenotyping on PBMC was performed using 40-plex mass cytometry, and the levels of 45 circulating cytokines were quantified by Luminex. We also included 17 pre-pandemic healthy donors. Principal component analysis (PCA) and K-means clustering were performed.

PCA revealed notable differences in the immunological profile, especially related to migration, among healthy donors, post-FLU and post-COVID patients, with the expression of chemokine receptors CCR4, CCR6, CXCR3 and CXCR5 on lymphoid and myeloid cells being the most contributing features to the PCA. A logistic regression model was able to classify all individuals according to their diagnosis with good accuracy. Using the 50 features with the highest contribution to PCA, K-means revealed four clusters of individuals. Two of these clusters corresponded to post-COVID patients, which differed in age of individuals but did not correlate with post-COVID symptoms or acute severity.

Our results showed immune abnormalities in post-COVID patients three months after hospital discharge, and to a lesser extent in post-FLUindividuals, compared to healthy donors. We were able to divide post-COVID individuals into two immunological groups. The immune alterations in post-COVID were independent of acute or post-syndrome symptoms.

Esperanza Martin-Sánchez

Center for Applied Medical Research - CIMA Universidad de Navarra

IMMUNE DYSREGULATION THROUGHOUT COVID-19 VACCINATION IN HEMATOLOGICAL PATIENTS WITH MATURE B-CELL NEOPLASMS

Patients with mature B-cell neoplasms are at risk of severe infections and poor vaccine immunogenicity. The cell-specific extent of immune dysfunction of these patients prior and during vaccination is poorly characterized.

The aim was characterize the immune response to vaccination in patients with mature B-cell neoplasms using COVID-19 vaccines as a case study.

This study included 82 patients with mature B-cell neoplasms (7 CLL, 46 lymphoma, 29 MGUS/myeloma) and 96 age-matched health care practitioners (HCP). A total of 1104 blood and serum samples were collected before and at days 7 and 14 after the first dose, as well as at days 7 and 62 after the second.

Also before and 17 days after a booster dose. Immune profiling was performed using multidimensional and computational flow cytometry that systematically analyzed 56 cell-types per sample. Serum levels of IgM, IgG and IgA against four viral-antigens were quantified using a multiplex-microsphere-based flow assay. SARS-CoV-2-specific CD8 T cells were quantified using a dextramer panel of spike, membrane, ORF3 and nucleocapsid proteins.

Using HCP as a reference of immune response to COVID-19 vaccination, we observed significant longitudinal deviations in 2/3 granulocytic, 6/6 antigen-presenting cell, 14/18 CD4 and 10/12 CD8 T-cell, and 17/17 B-cell subsets. Accordingly, anti-RBD IgM, IgG and IgA indexes were significantly lower in patients vs HCP. An immune dysregulation longitudinal cumulative score was associated with impaired anti-RBD IgG levels after the second dose.

While SARS-CoV-2-specific CD8 T cell percentage after the second dose was similar between patients and HCP, there was an enrichment of TEMRA cells in the former. Furthermore, the booster failed to increase virus-specific CD8 T-cell and antibody levels in patients. This study uncovers the cell-specific extent of immune dysfunction in patients with mature B-cell neoplasms and how it affects the efficacy of vaccination strategies such as for COVID-19.

21st April 2023

PARALLEL SESSION VII – QUALITY ASSURANCE IN FLOW LABS

Jordi Petriz
IGTP, Badalona, Barcelona

NORMAFLOW: SURVEY OF LATINAMERICAN'S FLOW CYTOMETRY LABORATORIES FOR ISO ACCREDITATION AND CERTIFICATION

Normalflow is a non-governmental organization (NGO) recently formed by scientists from Argentina, Colombia, Spain, Ireland and UK.

The aim is to encourage and support flow cytometry laboratories in Latinamerica to obtain International Organization of Accreditation and Certification (ISO) 15189 and 9001 respectively

Normaflow sent a survey to twelve Latinamerican countries: Argentina, Bolivia, Chile, Colombia, Costa Rica, Ecuador, El Salvador, Mexico, Perú, Republica Dominicana, Uruguay and Venezuela. The survey was done through SurveyMonkey.co.uk, ten questions were sent including the type of institution and others relevant to QAS: does the laboratory have a Quality Manual and identify the pre, post and analytical, processes based on the ISO 15189 and ISO 9001, are these well documented and other QAS related questions.

We obtained 83 responses: 27 Argentina, 2 Bolivia, 4 Chile, 7 Colombia, 3 Costa Rica, 2 Ecuador, 1 El Salvador, 24 Mexico, 9 Perú, 1 República Dominicana, 1 Uruguay and 2 Venezuela.

The type of Institution was: 67,9% clinical laboratories, 26,2% State Universities, 1,2% Industry and 4,8% other. 69,5% stated that their institution had documented and implemented a QAS. 48,2% agreed that their QAS was based on standards ISO 15189 and 9001. 56,6% of laboratories had Quality Manual but 24,1% didn't. Only 56,8% agreed that the Quality policy was following the ISO 15189 and 9001 standards.

Leaders of the institutions were making progress to achieve these standards. This survey gave us a glimpse in Latinamerican's flow cytometry laboratories compliant with ISO 15189 and 9001.

The conclusion from this small sample is that there is a need in Latinamerican's flow cytometry laboratories to be compliant with ISO. Normaflow is an organization with the aim to encourage and facilitate laboratories in their own language to obtain ISO accreditation offering expertise in QAS and orientation on how to obtain these with advice and practical help.

PARALLEL SESSION VIII – IMMUNODEFICIENCIES AND NON-HEMATOLOGICAL DISEASES

Alba Torres Valle

Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS)

EVALUATION OF A NEW AGE-MATCHED CRITERIA WHICH WILL IMPROVE THE IDENTIFICATION OF LATE-ONSET COMBINED IMMUNODEFICIENCY PATIENTS

Several studies indicate that a group of Common Variable Immunodeficiency (CVID) patients actually suffer from late-onset combined immunodeficiency (LOCID), a different disease with a T-cell defect associated with a more aggressive clinical behavior. Some criteria based on naïve CD4+ T-cell counts have been proposed for LOCID (the DEFI and Freiburg criteria). However, these criteria have been arbitrarily established without considering age-related variations in the reference values for naïve CD4+ T cells.

The aim was to search a new age-matched criteria to better discriminate LOCID among patients with a CVID-like clinical phenotype.

In this multicentric study, 157 CVID patients were studied in parallel to 270 healthy donors using 8-colors flow cytometry based on the Euroflow standard operation procedures.

Using the new age-matched criteria, a higher number of LOCID patients was observed (n=56) vs. the Freiburg (n=35) and DEFI (n=21) classifications. The age-based criteria was particularly efficient to identify LOCID patients, among the 18-39y (36% vs. 23% and 9%; $p<0.01$) and 40-59y (46% vs. 28% and 19%; $p<0.001$) CVID age-groups. Frequency of infections were associated with IgG and IgA serum levels at diagnosis, but presence of non-infectious complications were exclusively found among individuals with low naïve CD4+ T-cell counts, independently of the LOCID criteria. However, the age-based criteria showed a higher sensitivity for identifying of patients suffering autoimmune cytopenia (66% vs. 41% and 24%), enteropathy (60% vs. 31% and 31%) splenomegaly (60% vs. 40% and 24%), hepatomegaly (69% vs. 42% and 35%), lymphadenopathy (71% vs. 49% and 27%), granulomas (70% vs. 44% and 25%) and interstitial lung disease (59% vs. 47% and 24%) than the Freiburg and DEFI criteria, respectively. In conclusion, the new age-matched criteria to identify CVID patients with decreased naïve CD4+ T-cell counts results in a more accurate diagnosis of LOCID, associated with more severe clinical phenotypes.

Carlos Duarte

Centro Hospitalar de Lisboa Ocidental, Hospital de São Francisco Xavier

THE RELEVANCE OF FLOW CYTOMETRY IN THE ETIOLOGIC DIAGNOSIS OF PLEURAL EFFUSIONS

Pleural fluid samples are the most frequent body fluids immunophenotyped in the clinical laboratory. Pleural effusions are associated with various pathological processes. It is extremely difficult to distinguish reactive lymphocytes from hematopoietic malignancy or mesothelial cells from metastases based on cytomorphology alone, even for the most experienced Pathologist. Flow Cytometry is crucial in contributing to diagnose and monitor hematopoietic and metastatic disease in body fluids.

This study is a single-institution retrospective review of the flow cytometric analysis of pleural fluid samples from 2018 until 2022. Our goal was to identify the percentage of neoplastic samples and also the percentage of cases in which it was the first result to establish the suspicion of a diagnosis.

A screening 8-color tube (BD OneFlow™ LST) was used to discriminate reactive from abnormal lymphocytes. In case abnormal lymphocytes were identified a more extensive characterization was obtained using 8- color specific panels. Epithelial cells were identified by the positivity of CD326 (DAKO™ clone Ber-EP 4) and negativity of both CD45 and CD33.

513 pleural fluid samples from 432 patients were studied. The results of 302 samples (58.9%) presented no evidence of malignancy. The remaining 211 samples (41.1%) were positive for hematopoietic neoplasms (13.1%), and carcinoma (27.9%) respectively. The hematopoietic neoplasms included 2 LMA, 1 NK-NHL, 7 T-NHL and 57 B-CLPD (CLL and NHL). In 50 cases (23.7% of the positive cases)

patients without prior clinical established diagnosis were newly diagnosed based on flow cytometry results. Flow cytometry for pleural fluids is of great utility for hematopoietic malignancy or carcinoma metastasis, being sometimes the only study that raises the suspicion of a diagnosis given the large number of cells analyzed, the short time of response and the accuracy to identify and classify the abnormal cells, not only at diagnosis but also for patient follow-up.

Víctor Manuel García Martínez

Molecular Oncology Unit CIEMAT, Madrid, Spain

SUBSET ANALYSIS REVEALS KEY FEATURES AND BIOMARKERS OF NON-MUSCLE INVASIVE BLADDER CANCER TUMOUR MICROENVIRONMENT

Non-muscle invasive bladder cancer (NMIBC) has been treated for decades with immunotherapy with relatively good success rates. Nontheless, to date we lack a full characterisation of the microenvironment of this type of tumours. Besides, immune check-point inhibitors has entered the treatment scheme in this disease. A better understanding of the composition of these tumours could help to better patient stratification and management.

The aim was to use flow cytometry to characterise non-muscle invasive tumours in terms of subset composition and immune check-point inhibitor expression in order to find celular markers that may help stratify patients.

98 bladder cancer patients were enrolled along three years in this study, from which we collected paired tumour and non-pathological samples. We digested freshly removed tissue samples into single cell suspensions and stained cells with two flow cytometry panels to characterise the following subsets: Urothelial/tumour cells, macrophages and M2-like macrophages, neutrophils, conventional dendritic cells type 1 and 2, T cells, NK cells, endotelial cells, mesemchymal cells and fibroblasts. Results were analysed using manual gating and unbiased computactional methods.

We found that NMIBC tumours present all the cell subsets analysed. Compared to non-patholocal tissue, tumours display high macrophage infiltration and several signs of inflammation. Stroma infiltration and landscape varied significantly according to tumour stage. PD-L1 expression was similar between tumour and non-pathological samples in urothelial cells, but higher in the tumour microenvironment. Finally, we used an agnostic approach to find biomarkers associated to tumour grade and found that M2-like macrophages and activated fibroblasts are more abundant in high grade tumours. Our findings confirm that NMIBC tumours are infiltrated with immune and non-immune cells, and that increased PD-L1 expression comes from microenvironment cells. We also provide novel markers to help in tumour grading, of key importance for patient classification.

07
Posters

1. POSTER

HIGH-SENSITIVITY FLOW CYTOMETRY ASSAY IMPROVES THE DIAGNOSTIC YIELD OF MALIGNANT PLEURAL EFFUSIONS

AUTHORS

Subirá, D.[1], Barriopedro, F.[1], Moreno, L.[1], Martínez, R.[1], Chara, L.[2], Castelao, J.[3], Candia, A.[4], Fernández, J.[3]

AUTHORS AFFILIATION

(1) Flow Cytometry Unit. Hematology Department. Hospital Universitario de Guadalajara.
(2) Oncology Department. Hospital Universitario de Guadalajara. Guadalajara.
(3) Neumology Department. Hospital Universitario de Guadalajara. Guadalajara.
(4) Pathology Department. Hospital Universitario de Guadalajara. Guadalajara.

ABSTRACT

Background: Diagnosing malignant pleural effusion (MPE) is challenging when cytology does not detect malignant cells in pleural fluid and patients lack a history of cancer.

Aims: To investigate whether implementation of a systematic analysis of pleural effusions by flow cytometry immunophenotyping (FCI) has any impact on the diagnosis of MPE.

Methods: Over 7 years, 570 pleural fluid samples from patients with clinical suspicion of MPE secondary to lymphoma or epithelial tumors, were submitted for FCI analysis. For screening of epithelial malignancies, a 3-color monoclonal antibody panel including anti-EpCAM (clone Ber-EP4) was added to a cell-rich sample, and 5 x 106 events were finally acquired in the flow cytometer. The limit of detection and quantification defined the level of sensitivity of the FCI assay. FCI results, informed as “malignant” or “no-malignant”, were blinded to cytology. Final diagnosis of MPE was established by clinicians based on all available information.

Results & Conclusion: FCI correctly diagnosed 453/522 samples (87.3%) suitable for FCI and cytology comparison. A final diagnosis of MPE was established in 182 samples: FCI identified 141/182 (77.4%) as compared to 94/182 (51.6%) by cytology ($p<0.0001$); most of them were epithelial-cell malignancies (117 vs. 81; $p<0.0001$). MPE not detected by cytology had significantly lower percentages of EpCAM+ cells as compared to cytology positive cases (0.02% vs. 1%; $p<0.0001$). Of note, 29/52 MPE (55.8%) only detected by FCI were new diagnosis of cancer, and 20 were lung tumours. Except for mesothelioma, FCI had better sensitivity than cytology for other lung subtypes, being particularly relevant squamous cell carcinoma (50% vs. 14.3%) and neuroendocrine tumours (86.7% vs. 33.3%).

In summary, this high-sensitivity FCI protocol significantly increases the diagnostic yield of pleural effusions, accelerating the diagnostic process in clinical practice. Even clinical laboratories with limited resources can easily implement the small panel designed to discard epithelial-cell malignancies.

3. POSTER

HIGH-AFFINITY FC IGE RECEPTOR AND OMALIZUMAB: ANY RATIONALE FOR FLOW CYTOMETRY STUDIES?

AUTHORS

Subirá, D.[1], Ekatherini Tsopanas, E.[2], Moreno, L.[1], Barriopedro, F.[1], Martínez, R.[1], Notario, R.[1], Pastor, M.[4], Beitia, J.[2], Vega, A.[2]

AUTHORS AFFILIATION

(1) Flow Cytometry Unit. Hematology Department. Hospital Universitario de Guadalajara.
(2) Allergy Department. Hospital Universitario de Guadalajara. Guadalajara.
(4) Dermatology Department. Hospital Universitario de Guadalajara. Guadalajara.

ABSTRACT

Background: Omalizumab is a monoclonal antibody that binds IgE and high-affinity IgE receptor (FcεRI) indicated in severe antihistamine-refractory chronic urticaria (CU).

Aims: To investigate by flow cytometry (FC) the expression of FcεRI in UC patient's candidates to receive omalizumab.

Methods: We evaluated 35 healthy controls and 52 antihistamine-refractory CU patients treated with monthly 150 mg omalizumab. Some required up-dosing. Twenty-three patients were monitored before and after therapy. FC design included CD123, HLA-DR, CD3 and FcεR1α (clone AER-37) reagents and acquisition of at least 1 x 106 events. Levels of FcεRIα on basophils and plasmacytoid dendritic cells (pDc) were expressed as median fluorescence intensity (MFI).

Results & Conclusion: Controls (n=35) and patient´s samples (n=96) showed higher levels of FcεRIα on basophils than on pDc (r2= 0.679). To normalise the wide range of expression, a ratio between basophils and T cells (negative control) MFI was calculated. Three levels of FcεRIα expression were observed: high (ratio ≥200), intermediate (ratio ≥100 and <200), and low (ratio <100). The distribution of FcεRIα levels was different in controls and pre-therapy samples (n=35) (p=0.015), with high levels in 20/35 patients (p<0.001). There was a weak positive correlation between serum IgE and FcεRIα expression (r2= 0.25). Anti-thyroid antibodies, basophils or pDc numbers could not predict FcεRIα levels. Both cells downregulated FcεRIα levels after first-therapy evaluation, but decline did not correlate with clinical response. Most patients with lower baseline levels (14/15; 93.3%) presented some kind of clinical improvement as compared to 13/18 patients (72%) with high levels (p=ns). In turn, 8/18 patients (44%) with high levels and 3/15 (20%) with lower levels needed updosing (p=ns).

In summary, while serial FC studies had no clinical interest, baseline data identified patients with low/intermediate FcεRIα levels who were more prone to respond to this omalizumab dosage. Larger studies are warranted to confirm these preliminary findings.

4. POSTER

ANGIO-IMMUNOBLASTIC T-CELL LYMPHOMA WITH CD8 FOLLICULAR PHENOTYPE IN A PEDIATRIC PATIENT WITH TET2 MUTATION

AUTHORS

Martinez, M.[1], Caldirola, M.[2], Garcia, A.[2], Bernacchia, A.[2], Tejada, M.[2], Gomez Raccio, A.[2], Gaillard, M.[2]

AUTHORS AFFILIATION

(1) Bioquemistry. Hospital de niños Ricardo Martinez. Buenos Aires Argentina.
(2) Hospital De niños Ricardo Gutierrez. Buenos Aires. Argentina.

ABSTRACT

Background: Nodal T-follicular helper cell lymphoma, angioimmunoblastic type (nTFHL-AI) is an aggressive subtype of peripheral Tcell-lymphoma with T follicular helper phenotype, characterized by characterized by recurrent mutations in several epigenetic modifier such as TET2 and DNMT3A. nTFHL-AI usually presents at a median age of 60-64 being extremely rare in children. TET2 is highly expressed in hematopoietic stem cells and plays important roles in hematopoiesis. Germline TET2 loss of function is a novel inborn error of immunity associated with immunodeficiency, autoimmunity and lymphoproliferation with EBV susceptibility.

Aims: Describe a pediatric patient with angioimmunoblastic Tcell-lymphoma (AITL) and TET2 mutation.

Results & Conclusion: Case report: A 10 years old (yo) girl born from non-consanguineous parents. At 8yo she was admitted to hospital due to prolonged fever, myalgia, periorbital edema, non-infectious and non- malignant lymphoproliferation with elevated inflammatory markers. Normal bone marrow (BM) biopsy. Autoinflammatory syndrome was suspected due to clinical and laboratory features. At 9yo she developed a new non-infectious lymphoproliferation event with fasciitis. BM biopsy showed myeloid hyperplasia with myelodysplastic focal changes. She received high dose corticosteroids with mild clinical and no laboratory parameters response. At 10yo she presented central nervous system (CNS) compromise with abnormal CNS CT and MRI. Biopsies: normal lymph node and liver with prominent CD8+Tcells infiltrate in bowel. Immunological laboratory findings showed: leukocytosis with CD8 lymphoproliferation, expanded CD3+TcRαß+CD4-CD8- double-negative Tcells, low cTfh skewed towards th2 and low memory B cells. Molecular testing revealed a novel probably pathogenic de novo TET2 heterozygous non-sense variant (p.Arg1237*). Thereafter, she developed tonsillar hypertrophy and mediastinal widening. Tonsillar biopsy: AITL compatible. Flow cytometry: clonal Tcells: CD3+CD8+CD2+CD5+CD7+CXCR5+ICOS+/PD1+CD27+CD28+CD45RA-CCR7-HLADR+CD4-CD56-CD16-

CD94-Perforin-GranzymeB-TRBC1- (tonsils and peripheral blood) with BM and cerebrospinal fluid compromise. She started chemotherapy with non-response and died during treatment. Our findings confirm a strong link between TET2 deficiency, immune dysregulation, and lymphomagenesis, with an extremely rare early onset angioimmunoblastic Tcell-lymphoma.

7. POSTER

CYTOKINES PROFILE DURING PLASMODIUM FALCIPARUM INFECTION IN A HUMANIZED MICE MODEL

AUTHORS

Cortés Gil, L.[1]

AUTHORS AFFILIATION

(1) Scientist, GSK Global Health Medicines R&D. Tres Cantos, Madrid.

ABSTRACT

Background: Malaria is a parasitic vector-borne disease that affects over 200 million people every year, with around 100 countries officially considered malaria endemic regions. Plasmodium parasites are transmitted by the bite of infected female mosquitoes of the genus Anopheles. Blocking or reducing malaria transmission rates constitutes one of the main strategies to achieve global malaria eradication. The humanized mouse model for in vivo studies with P. falciparum is a preclinical tool used for Drug Discovery in Malaria, this model has been used for the dose prediction of assets that are currently in Clinical development.

Aims: In this work we study inflammatory cytokines profile during engraftment and infection protocol with the human malaria parasite Plasmodium falciparum in NSG mice. NSG mice are immunodeficient, they lack T, B and NK cells, but have active innate immune cells.

Methods: We have measured different cytokines included in the inflammatory CBA kit for mice, and the expression of these cytokines through the course of the infection of asexual stages of the human parasite.

Results & Conclusion: We have set up a starting point of cytokines profile in the mouse model and we are able to determine the differences in this pattern in Host Directed Therapies assay, for preclinical studies in Malaria Drug Discovery. We have detected different inflammatory cytokines during the different stages of the protocol. The most expressed are MCP-1 expressed during engraftment process and IL6 during the infection and parasite exponential growth.

"The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents".

"All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals".

8. POSTER

FLOW CYTOMETRY FOR SCREENING TOXIC DOSE EFFECT OF MYCOTOXINS INDIVIDUALLY AND COMBINED IN BREAST CANCER CELLS, LEUKEMIA CELLS, AND FRESH PERIPHERAL MONONUCLEAR CELLS

AUTHORS

Juan-García, A.[1,2], Juan, C.[1], Martínez, L.[2]

AUTHORS AFFILIATION

(1) Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia.
(2) Spanish National Cancer Research Center (CNIO), Institute of Health Carlos III – Ministry of Science and Innovation, Madrid.

ABSTRACT

Background: Beauvericin (BEA), Enniatin B (ENN B), and Ochratoxin A (OTA) are mycotoxins produced by fungi spp. Their main effect on several organs and systems is associated with chronic exposure going from estrogenic disorders, and renal failure to cancer (in animals and humans). OTA belongs to Group 1 according to the International Agency for Research in Cancer (IARC) and it has legislated limited values; not happening for BEA nor ENN B. Exposure to mixtures of mycotoxins occurs through food intake in daily consumption.

Aims: To evaluate the implication of BEA, ENN B, and OTA individually and combined in producing cytotoxicity in: breast cancer (MDA-MB-231) cells, leukemia cells (HL-60) and in fresh peripheral blood mononuclear cells (PBMCs).

Methods: Cells were treated for 4 and 24h at the following concentrations: from 0 to 16μM, from 0 to 8μM, and from 0 to 30μM for BEA, ENN B, and OTA, respectively. Binary and tertiary mixtures were also tested. Assays were carried out in 96 well/plate and cell death was measured by DAPI dye. Measurement was performed by using a FACS Canto II (BD) in multi-plate reading mode, collecting 10000 cells/well (n=8).

Results & Conclusion: Individual treatment OTA exerted the greatest cytotoxicity for PBMC cells (IC50 0.5 μM) while ENN B for HL-60 (IC50 0.25 μM) and MDA-MB-231 (IC50 0.15 μM). In binary combination ENN B + OTA resulted to exert the greatest cytotoxicity for HL-60 and MDA-MB-231 cells; while BEA + OTA in PBMC cells. Triple combination resulted to be highly cytotoxic for PBMC cells compared to HL-60 and MDA-MB-231 cells. PBMC were the most sensible cells for all three mycotoxins. The presence of OTA in any of the combinations had the greatest toxicity. The potential effects of synergism, addition and antagonism will be necessary to study in order to further set the limit for those mycotoxins not legislated. Acknowledgments: This work has been supported by the Spanish Ministry of Science and Innovation PID2020-115871RB-100. AJ-G would like to acknowledge GVA- Conselleria d´Innovació, Universitats, Ciència i Societat Digital for the BEST-2022 Grant (CIBEST/2021/145)

9. POSTER

POMEGRANATE EXTRACT IMPROVES INTRACELLULAR BASAL LEVELS OF REACTIVE OXYGEN SPECIES IN BLOOD CELLS FROM A MURINE AGING MODEL

AUTHORS

Verdu Coloma, D.[1], Valls Arrufat, A.[1], Herrera, G.[1], Serna García, E.[1], Viña Ribes, J.[1]

AUTHORS AFFILIATION

(1) Universitat de València.

ABSTRACT

Background: The free radical theory of aging provided a background for many laboratories working in this area. Reactive oxygen species (ROS) are oxygen-derived by-products that include hydroxyl radical (HO•), superoxide radical (O2–) and hydrogen peroxide (H2O2). ROS have been observed in normal aging. Furthermore, mitochondria play a main role in cellular aging because of they are a source of ROS.

Aims: The objective of the present study was to evaluate whether the supplementation with pomegranate extract (PE) to aged mice could prevent the increased generation of ROS in blood cells.

Methods: We analyzed intracellular ROS as well as mitochondrial ROS using fluorescent dyes from blood cells of young (10 months) and aged mice (22 months) compared with aged mice supplemented with PE (22 months) for four months. ROS levels were measured by flow cytometry using the intracellular fluorescence dye dihydrorhodamine123 (DHR123) and mitochondria peroxy yellow 1 (Mitophy1). The mean ± S.E.M. from four replicates was determined for each experimental group. All data were analyzed using ANOVA with Graphpad Prism 8 software.

Results & Conclusion: Our study shows that aging lymphocytes, monocytes, and neutrophils have increased basal cytoplasmatic ROS as well as mitochondrial H2O2. We have observed that aged animals supplemented with PE were protected against oxidative damage. Mitochondrial H2O2 was lower in neutrophils from aged mice with PE. In the same group of supplemented mice, we observed that the increase in cytoplasmatic ROS was reversed in neutrophils, monocytes, and lymphocytes. For this reason, PE could modulate as adaptive as innate immune response. The data suggest that the enhanced ROS production in aging may be prevented with prebiotics such as PE.

10. POSTER

POMEGRANATE EXTRACT PROTECTS BLOOD CELL POPULATIONS FROM INDUCED OXIDATIVE DAMAGE IN A NORMAL AGING MURINE MODEL. NEW APPROACH TO AN IN VIVO RESILIENCE STUDY

AUTHORS

Verdu Coloma, D.[1], Valls Arrufat, A.[1], Herrera, G.[1], Serna García, E.[1], Viña Ribes, J.[1]

AUTHORS AFFILIATION

(1) Universitat de València.

ABSTRACT

Background: Resilience in aging is the ability to recover from or adapt to stress and maintain or restore one's physical, psychological, or emotional equilibrium. Interventions to increase physiological resilience in aging include regular physical exercise, a low-calorie diet and prebiotics and probiotics supplementation. For this reason, one of studied nutraceuticals is pomegranate (Punica granatum), which could enhance a protected response before the damage.

Aims: The objective of the present study was to evaluate whether the supplementation with pomegranate extract (PE) to aged mice could improve the response to an inducible oxidative stress by increasing resilience capacity

Methods: We analysed intracellular as well as mitochondrial basal levels of ROS in leukocytes, neutrophils and lymphocytes and monocytes from aged mice (22 months) compared with aged mice supplemented with PE for four months. Blood from both aged groups was treated with an oxidant agent, tert-butyl hydroperoxide (tBHP) and was compared with non-oxidised blood. ROS levels were measured by flow cytometry using the intracellular fluorescence dye dihydrorhodamine123 (DHR123), 2,7- Dihydrodichlorofluorescein diacetate (DCF) and mitochondria peroxy yellow 1 (Mitophy1). The mean ±

S.E.M. from four replicates was determined for each experimental group. All data were analysed using ANOVA with Graphpad Prism 8 software.

Results & Conclusion: Leukocyte populations were induced to oxidative damage with tBHP and PE supplementation caused a decrease in total intracellular ROS levels using the DCF dye. In addition, mitochondrial H2O2 was lower in neutrophils from aged mice with PE supplementation detected with Mitophy1 dye. Nevertheless, peroxide and peroxynitrite levels did not change, probably due to neither NO, superoxide, nor hydrogen peroxide alone acting to oxidize DHR123. The data suggest that resilience capacity in aging is enhanced by PE supplementation by reducing ROS after induced oxidative stress.

11. POSTER

HYPERTENSION INCREASES BASAL INTRACELLULAR PEROXYNITRITE LEVELS IN LEUKOCYTES OF MALE BUT NOT FEMALE RATS

AUTHORS

Valls Arrufat, A.[1], Suarez, A.[1], Verdu Coloma, D.[1], Guerra - Ojeda, S.[1], Herrera, G.[2], Serna García, E.[1], Mauricio, M.[1]

AUTHORS AFFILIATION

(1) Department of Physiology, University of Valencia.
(2) Flow Cytometry Unit, IIS INCLIVA, Fundación Investigación Hospital Clínico Valencia, 46010 Valencia, Spain.

ABSTRACT

Background: Superoxide anion (O2-) reduces nitric oxide (NO) bioavailability by transferring its extra electron to NO to form peroxynitrite (ONOO-), a potent oxidant. This reaction has important biological effects by reducing the vasodilation. Moreover, superoxide anion increases vasoconstriction by releasing inositol triphosphate. These effects are increased in spontaneously hypertensive rats (SHR), therefore the vascular tissue of SHR could have more sensitivity to oxidative stress contributing to the progression of the disease.

Aims: Since leukocytes circulate in the bloodstream and can reflect cardiovascular oxidative stress providing useful information on pathological condition, we aimed to measure basal intracellular ROS levels in leukocytes from male and female SHR compared to their respective controls (WKY rats).

Methods: Basal intracellular levels of ROS in leukocytes: neutrophils, lymphocytes, and monocytes from 16-week-old male and female SHR and WKY rats were measured by flow cytometry using the intracellular fluorescence dyes dihydrorhodamine123 (DHR123), dihydroethidium (DHE) and diaminofluorescein (DAF). Blood from all four groups was treated with a potent oxidant agent, tert-butyl hydroperoxide (tBHP) and compared with non-oxidised blood. The mean ± S.E.M. from four replicates was determined for each experimental group. All data were analysed by ANOVA using Graphpad Prism 8.

Results & Conclusion: Our results for male showed that fluorescence for DHR123, an indicator of H2O2 and peroxynitrite levels, was increased in leukocytes from SHR compared to WKY rats, without any differences in DHE, an indicator of O2- levels, or DAF, an indicator of NO levels. It is probable that changes in the levels of O2- and NO were not observed due to their consumption in the reaction to form ONOO-. Regarding female, we did not observe any change in SHR versus WKY suggesting that the resilience capacity against the increased oxidative stress induced by hypertension is higher in females versus males.

12. POSTER

IS FLOW CYTOMETRY REALLY NOT USEFUL IN THE DIAGNOSIS OF HODGKIN LYMPHOMA?

AUTHORS

Álvarez Flores, M.[1], Sopeña Corvinos, M.[2], Guillén Santos, R.[2], Cava Valenciano, F.[2]

AUTHORS AFFILIATION

(1) Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid.
(2) Laboratorio Clínico de la Comunidad de Madrid UR Salud, Madrid.

ABSTRACT

Background: Hodgkin and Reed-Sternberg cells are large and fragile, making them difficult to study by flow cytometry. For that reason, the HL diagnosis is based on immunohistochemical and cytomorphological pathological studies.

Aims: Characterize the CD71 expression pattern on CD4+ T cells in patients with HL and to design a simple flow cytometry algorithm to complement the histopathological diagnosis of HL. The study proposes a conventional staining protocol with a simple panel and a well-defined analysis strategy. The cut-off of CD71 ratio was based in the obtained ROC curve, that classifies diagnostic groups as suggestive (≥0.5) or non-suggestive (< 0.5) from HL.

Methods: A total of 143 samples of suspected lymphoma were studied: 111 lymph nodes, 25 fine needle aspirations and 7 core needle biopsies. The antibody panel consisted of five tubes (Table 1). One for the detection of B clonality and a second for the T lymphocyte subpopulations and HL (tubes 1 and 2). When no clonal B population was detected in tube 1, a fluorescence minus one control panel (FMO control) was implemented to assess the expression of CD71, CD30, and CD15 (tubes 3- 5). The cells were acquired in a FACSCanto II cytometer.

Tabla 1: The antibody panel for B cell clonality and Hodgkin lymphoma analysis

TUBE	FITC	PE	PerCP-Cy7	PE-Cy7	APC	APC-H7	V450	V500
1	Kappa	Lambda	CD19	CD5	CD10	CD20	CD45	HLA-DR*
2	CD15	CD30	CD4	CD8	CD71	CD3	CD45	KI67*
3	FMO	CD30	CD4	CD8	CD71	CD3	CD45	
4	CD15	FMO	CD4	CD8	CD71	CD3	CD45	
5	CD15	CD30	CD4	CD8	FMO	CD3	CD45	

Tube 1: analysis of B populations for clone detection.
Tubes 2-5: Hodgkin Lymphoma analysis.

Results & Conclusion: Application of the CD71 ratio algorithm yielded a sensitivity of 82% and specificity of 87%, with 84.61% of patients correctly diagnosed, not bad results considering the limitations of flow cytometry in HL diagnosis. Moreover, the panel is simple, and the antibodies are commonly used in most clinical laboratories. Although histopathology remains the definitive diagnostic tool for HL, the flow cytometry can be useful as a support tool in the diagnosis of HL.

14. POSTER

DIFFERENCES IN PAEDIATRIC DISEASES (KAWASAKI AND MULTISYSTEM INFLAMMATORY SYNDROME) USING FLOW CYTOMETRY: ON THE WAY TO A DIAGNOSTIC AND THERAPEUTICAL TARGET

AUTHORS

Kennedy Batalla, R.[1], Fernández Cooke, E.[2], Aracil Santos, J.[3], Saavedra, J.[4], García Salido, A.[5], Albendea Gómez, T.[6], Cano Nieto, M.[1], González, F.[4], Martín Puig, S.[7], Correa, R.[1]

AUTHORS AFFILIATION

(1) Laboratorio de Inmuno-regulación, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid.
(2) Unidad de Infectología Pediátrica. Hospital Universitario 12 de Octubre. Madrid.
(3) Enfermedades Infecciosas Pediátricas. Hospital Universitario La Paz. Madrid.
(4) Pediatría. Hospital General Universitario Gregorio Marañón. Madrid.
(5) Fundación de Investigación Biomédica Hospital Infantil Universitario Niño Jesús. Madrid.
(6) Centro Nacional de Investigaciones Cardiovasculares (CNIC). Madrid.
(7) Laboratorio de Homeostasis del Oxígeno en el Sistema Cardiovascular. Instituto de Investigaciones Biomédicas Alberto Sols, (IIBM), Consejo Superior de Investigaciones Científicas & Universidad Autónoma de Madrid (CSIC-UAM).

ABSTRACT

Background: MIS-C (Multisystem Inflammatory Syndrome in Children) is a new pathological entity caused by a late response to the infection by SARS-CoV-2 in some children. At first, it was confused with another pediatric affection: Kawasaki Disease (KD) with which they share numerous clinical features, including cardiac alterations that can end in an acquired heart disease that will impact these children's lives in the future. To prevent these future complications, early diagnosis and treatment are crucial.

Aims: Therefore, our aim was to exhaustively characterize the immune response of these patients in the peak of the inflammatory response and once recovered to understand the underlying immune mechanisms and be able to aid differential diagnosis.

Methods: Whole blood samples were obtained from acute and recovery phases of MIS-C and KD individuals and non-fever control children. A multiparametric flow cytometry analysis was carried out that permitted the characterization of 106 different immune populations with a very small volume of blood (400ul).

Results & Conclusion: Our analysis shows strong cytopenias in most immune populations (especially lymphocytes) excepting neutrophils and dendritic cells (DCs) of MIS-C individuals when compared to the non-fever control group. Although MIS-C and KD are both inflammatory disorders, there are differences between major subsets that could help discriminate between them. Major differences are found in monocytes, granulocyte, and T lymphocytes populations although distribution of T CD4+ subsets are similar between groups (except for Th17 percentage). However, when recovery phases were studied in deep, most of the immune populations showed a steady non-inflammatory state more like non-fever control children excluding some parameters that show differences between MIS-C and KD.

Despite the similarities between KD and MIS-C, our flow cytometry analysis has allowed us to characterize differences in immune subsets that could help reduce the time to diagnose these pathologies.

15. POSTER

PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA AND FLOW CYTOMETRY: CASE SERIES OF A PORTUGUESE HOSPITAL

AUTHORS

Barbosa Mendes, C.[1], Soares, R.[2], Santos, M.[3], Fonseca, S.[3], Oliveira, L.[3], Santos, F.[3], Martins Pereira, F.[3], Tente, D.[4], Lau, C.[3], Teixeira, M.[3]

AUTHORS AFFILIATION

(1) Departamento de Patologia, Centro Hospitalar Universitário de Santo António, Porto.
(2) Serviço de Patologia Clínica, Centro Hospitalar Médio Tejo, Tomar, Portugal.
(3) Laboratório de Citometria; Unidade de Diagnóstico Hematológico Margarida Lima, Serviço de Hematologia Clínica, Centro Hospitalar Universitário de Santo António, Porto, Portugal.
(4) Serviço de Anatomia Patológica; Centro Hospitalar Universitário de Santo António, Porto, Portugal.

ABSTRACT

Background: Primary mediastinal large B-cell lymphoma (PMBCL) is a mature aggressive large B-cell lymphoma of thymus B cells which appears in the mediastinum, representing 2-3% of non-Hodgkin lymphomas and 7% of diffuse large B-cell lymphomas. Usually it occurs in young adults, being more frequent in females. The variable and nonspecific clinical presentation makes the diagnosis a challenge. Mediastinal mass flow cytometry immunophenotyping may be an asset for rapid diagnostic and therapeutic guidance.

Aims: To perceive and evaluate the contribution of immunophenotyping by flow cytometry in the diagnosis of PMBCL.

Methods: Retrospective study of patients diagnosed with PMBCL between 2015 and 2022 in a single tertiary Portuguese hospital.

Results & Conclusion: During the analyzed period, PMBCL was diagnosed in five patients, aged between 22 and 48 years, of which two were male and three females. The clinic at presentation was nonspecific. Among the initial symptoms there was a predominance of chest pain, cough, and absence of symptoms B. One patient presented with superior vena cava syndrome, in which thoracic CT showed an anterior mediastinal voluminous mass (>90mm), with invasion and stenosis of the local vascular structures. Flow cytometry immunophenotyping was performed in fine needle biopsy specimens from three of the five patients and in needle core biopsy of the remaining two patients. Mature phenotype B cells was identified. The expression of CD23 and CD30 was evaluated in four of the five samples (all of them were CD23 positive and two positive for CD30) and three samples were tested for CD200 (all positive), raising the suspected diagnosis of PMBCL, later confirmed by pathological study.

Flow cytometry immunophenotyping should be considered a viable and complementary approach to the pathological study, despite not being the gold-standard, since it increases initial diagnostic suspicion due to its rapid execution, interpretation and result that is important to guide the initial treatment.

16. POSTER

ESTIMATING ADMISSIBLE TOTAL ERROR IN LYMPHOCYTE POPULATIONS' ANALYSIS BY FLOW CYTOMETRY: 10 YEARS' RESULTS OF SPANISH SOCIETY FOR IMMUNOLOGY EQAS GECLID-SEI

AUTHORS

San Segundo, D.[1], Comins Boo, A.[1], Pérez Pla, F.[1], Irure Ventura, J.[1], Martín Alonso, M.[2], López Hoyos, M.[1], Blanco Peris, L.[2]

AUTHORS AFFILIATION

(1) University Hospital Marqués de Valdecilla. Santander.
(2) Hemotherapy and Hemodonation Center of Castilla y León. Valladolid.

ABSTRACT

Background: Flow cytometry analyses of lymphocyte subpopulations (T, B, NK) are crucial for an increasing number of clinical algorithms and research workflows such as those for leukaemia, lymphoproliferative disorders, immunodeficiencies, or CAR-T therapies' monitoring. Growing quality and safety standards for in vitro diagnostic medical devices (IVD) are to be implemented by every clinical laboratory to fulfil the European directive IVD-Regulation EU 2017/746, fully applicable in the EU since May 26th, 2022.

Aims: To estimate admissible Total Error (aET) values of percentage and absolute number of lymphocyte subpopulations based on the current state of the art (SOTA) using real data from a proficiency testing scheme of the Spanish Society for Immunology (GECLID program) and to compare them with previously published specifications based on biological variability (BV)

Methods: 44,998 results from 75 laboratories were analysed from 2012 to 2021. The quantitative scheme includes both percentage and absolute numbers of CD3+, CD4+, CD8+, CD19+, and NK cells. % TE was calculated as: [(reported value – robust mean)/robust mean] *100/laboratory/parameter. The cut off for aTE was set at 80% best results of the laboratories.

Results & Conclusion: The previously aTE specifications calculated in 2019 based in BV were widely achieved by the laboratories included in the study. The SOTA aTE was calculated for 2 different sets of data, the first with all data from 2012-2021, and a second data from 2017-2021. The aTE for each parameter from 2017-2021 was lower than that calculated from the whole dataset: for % of CD3+, CD4+, CD8+, CD19+, NK cells the aTE was (3.6vs4.1vs6.0); (5.8vs6.4vs11.3); (7.2vs8.0vs10.7); (10.5vs12.7vs16.8) and (17.1vs24.4vs34.4) calculated based on SOTA(17/21), SOTA(12/21) and desirableBV, respectively. The decrease of % aTE observed with 2017-2021 data could be related to technical advances in flow cytometry. The aTE specifications for lymphocyte subsets' determination based on proficiency testing results are an useful tool for analytical improvement.

17. POSTER

THE IMPORTANCE OF GOOD CONTROLS IN POLYCHROMATIC AND SPECTRAL FLOW CYTOMETRY

AUTHORS

Fernández Delgado, I.[1], ILIE, A.[1], García-Lestón, J.[1], Martínez, L.[1]

AUTHORS AFFILIATION

(1) Flow Cytometry Core Unit, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain.

ABSTRACT

Controls are important in all aspects of science, whether they are biological or technical controls. Particularly in multicolor flow cytometry, it is required several types of controls to ensure instrument performance and proper experimental set up. Among those controls there are some particularly important ones, not only the biological, but also single color as they will be needed to calculate properly the compensation or unmixing matrixes. Without compensation or unmixing it will be difficult to discern our populations properly in a high dimensional multicolor experiment and to be sure that the panel we are using and the instrument settings are at its best possible. Resolution will not only depend on the panel design but also on the quality of the sample and sensibility of the instrument. However, many times is a challenge to identify good single color controls that will allow the proper calculation of the compensation or unmixing matrix. Are single color controls in cells a better approach than using commercial comp beads? what other options we might have if beads do not rend good results and we are limited by sample size?

Good controls are a must to succeed in our high-content flow analysis. In this work, we explore and highlight the different single controls options available and compare the advantages or disadvantages they may have both in polychromatic and spectral cytometry.

21. POSTER

BULK LYSIS PROCEDURES ALTER TARGET CELL POPULATION COUNTS

AUTHORS

Garcia Rico, L.[1], Salvia Cerdà, R.[1], Ward, M.[2], Petriz, J.[1]

AUTHORS AFFILIATION

(1) Institut Germans Trias i Pujol. Badalona.
(2) Bussiness System Specialist. Thermo Fisher Scientific. Eugene.

ABSTRACT

Background: Rare cell detection by flow cytometry (FCM) is of extreme importance in the evaluation of measurable residual disease (MRD). To achieve high sensitivity (<1 in 10^5 cells), the minimum number of acquired cells must be considered, and conventional immunophenotyping protocols fall short of these numbers. To overcome this, a pre-erythrocyte lysis prior to immunophenotyping called “bulk” lysis (BL) is a standardized approach that allows the analysis of millions of cells required for high-sensitivity MRD detection. However, this additional step has been associated with significant cell loss, along with other adverse effects.

Aims: Evaluate BL protocols and compare them with minimal sample preparation (MSP) protocols to detect potential over or underestimates of rare cells when using these methods.

Methods: An MRD model was generated using fresh peripheral blood obtained from healthy donors and a K562 cell line with stable EGFP expression at known frequencies (from 10 to 0.01%). Samples were then prepared with BL and MSP protocols and evaluated with FCM, excluding necrotic cells with propidium iodide. Samples were acquired in triplicate on the Attune™ NxT Flow Cytometer (Thermo Fisher).

Results & Conclusions: For all frequencies of K562 cells, a significant decrease of this population was detected in the BL samples compared with MSP samples (e.g., for 10%: 8.4±0.15% EGFP cells for MSP samples and 1.7±0.26% EGFP cells for BL samples; p-value: <0.0001, 95% CI: -7.2 to -6.1). Regarding non- necrotic cells, we found significant differences in frequency, being lower in BL samples (e.g., for 10%: 98.9±0.10% non-necrotic cells for MSP samples and 38.9±2.98% non-necrotic cells for BL samples; p-value: 0.0038, 95% CI: -76.9 to -44.5). These results are of great interest in evaluating the potential effects of “bulk” lysis protocols and in obtaining the final count, especially in over or under estimation, such as in measurable residual disease.

22. POSTER

MPDCP AN ENTITY ALREADY RECOGNIZED IN 5TH EDITION OF THE WHO

AUTHORS

Pallarés Borràs, M.[1], Lázaro, C.[2], Vidal Martínez, J.[2]

AUTHORS AFFILIATION

(1) CATLAB/Hospital Universitari Mutua Terrassa.
(2) Departamento de Citometría. CATLAB.

ABSTRACT

Background: An 80-years-old male arrived to the hospital with dyspnea and pruritic skin lesions. The physical exploration showed fever and eroded papules over his entire body surface. Our laboratory findings: Hemoglobin: 94g/L; White blood cell: 145*10^9/L; Platelets: 42*10^9/L; Absolute neutrophil count: 1.46*10^9/L; Blasts: 60%; Glomerular Filtrate: 20.84ml/min/1.73m^2.

The diagnostic orientation was an acute leukemia with high risk of tumor lysis due to hyperleukocytosis and renal failure. The plan was a complete study of bone marrow aspirate, dermic biopsy and herpesvirus PCR.

Aims: The aim of the case is to publicize the mature plasmacytoid dendritic cells proliferation (MPDCP) entity and differentiate it from the blastic plasmacytoid dendritic cell neoplasm (BPDCN), as well as highlighting the importance of detecting by immunophenotype the dendritic cells that can be associated with Acute Myeloid Leukemia (AML).

Methods: Flow cytometry immunophenotyping was performed on fresh bone marrow. Sample was examined with Euroflow antibody panels designed to study AML and was acquired on FASCLyric cytometer, acquisition was performed with FACSSuite software (BD Biosciences) and analyzed by Infinicyt (Cytognos). Standardization was applied using CS&T Beads following the protocols of Euroflow Consortium.

Results & Conclusions: The results of the immunophenotype revealed 54,92% of Myeloid Blasts and additionally 11,72% of mature plasmacytoid dendritic cells (pDCs)(HLA-DR+, CD123++, CD4+, CD36+, CD38+, CD56-).

It is important to remember that there are two distinct types of neoplastic counterparts for pDCs; one is BPDCN, described in the WHO, and the other is MPDCP, only briefly mentioned and now recognized at the 5th edition of the WHO. The mature pDCs are characterized by HLA DR+ CD123++ CD56− CD4+ CD38+ immunophenotype.

A cutaneous infiltrate composed of pDCs is frequently found in patients with MPDCP, like in this case. The final diagnostic was an AML with pDCs with FLT3ITD and IDH2 mutations. Skin biopsy also described dendritic cells and the herpesvirus PCR was negative.

25. POSTER

VALIDATION OF CYTOCHECK SPACHIP® PH DETECTION KIT BY FLOW CYTOMETRY

AUTHORS

Garcia Leston, J.[1], Quilez Alvarez, A.[2], Hernandez Pinto, A.[2], Martínez, L.[1]

AUTHORS AFFILIATION

(1) Flow Cytometry Core Unit, National Centre for Cancer Research (CNIO), Madrid.
(2) Arrays for Cell Nanodevices, SL (A4Cell), Madrid.

ABSTRACT

Regulation of Intracellular pH is crucial for the maintenance of the normal function of the cells as it plays an important role in many processes such as vesicle trafficking, cellular metabolism or cell signalling. Traditionally, several fluorescently labelled probes have been used for intracellular pH measurement. However, these probes may not be suitable for measuring pH changes during long periods as they can suffer a gradual loss of fluorescence due to leakage out of the cell or degradation by the intracellular enzymatic activity.

CytoCHECK SPAChip® pH detection kit is a novel technology that uses lab-in-a-cell nanodevices, which consist of fluorescently labelled silicon particles (SPA-chip®). The SPAchips can be internalised in the cytosol of cells and measure intracellular and extracellular pH levels by changes in fluorescence intensity. These particles are non-toxic for the cells and they can remain in the cytosol of the cells for up to a month allowing monitorization of the cells for long periods of time.

In this study, we aim at evaluating the performance of the CytoCHECK SPA-Chip® pH detection kit in several adherent and suspension cancer cell lines after treatment with antimycin A (an inhibitor of cellular respiration).

The method includes the generation of a calibration curve exposing cells to buffers with known pH and the interpolation of the test sample. The changes in fluorescence intensity were measured by flow cytometry and internalization of the SPAchips checked by confocal microscopy.

Moreover, we wanted to evaluate if the analysis with a spectral flow cytometer would improve the resolution of the signal given by the SPAchips by removal of the cell auto-fluorescence.

26. POSTER

RELATIONSHIP BETWEEN NORMAL T AND B CELL DISTRIBUTION IN PERIPHERAL BLOOD AND KINETICS OF TUMORAL CLONE IN B-CLL PATIENTS

AUTHORS

Solano Ramos, F.[1], Criado García, I.[2], Moreno Paredes, N.[1], Gomez Fernandez, C.[1], Moreno Nombela, S.[1], Lerma Verdejo, A.[3], Luts, I.[3], García Vela, M.[3], Almeida, J.[2], Orfao, A.[3]

AUTHORS AFFILIATION

(1) Hospital Nuestra Señora del Prado, Talavera de la Reina.
(2) Centro de investigación del cáncer, Salamanca.
(3) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain.

ABSTRACT

Background: A landmark in the biology of CLL is the interaction of tumoral B cells and their microenvironment, particularly the normal circulating lymphoid population. These interactions could explain the altered distribution of absolute counts of normal residual B- and T- cells, and the high incidence of infections in these patients. However, the role of the microenvironment in the kinetics of tumor B cells is incompletely understood.

Aims: Study the interactions between tumoral cells and their microenviroment.

Methods: A total of 58 patients with CLL (Stage 0/A Rai Binet) were followed-up for a median period of 34 months. Every patient was categorized into “stable” or “dynamic” group according to the evolution of the B-cell clone size in peripheral blood. Clinical and biological parameters related to disease progression were recorded, as well as immunophenotypic studies of normal B- and T- cells subpopulations.

Results & Conclusions:

- There were no significant differences between these two groups in terms of age, gender, and relevant clinical or biological data used in daily practice.
- Despite no overall differences in the absolute counts of IgM+ and IgM- memory B cells (MBC) being observed between two groups, the IgM+/IgM- MBC ratio at baseline was significantly lower for Stable cases compared to the Dynamic group (median [range]: 1.6 [0.30-40] vs. 6.1 [0.46-208]); p=0.038.
- The number of TCRαß CD4+CD8+dim T cells was significantly higher among individuals with Stable clones compared to Dynamic ones (median [range]: 70 [5.6-562] cells/µL vs. 21 [5.4-197] cells/µL at baseline and 45 [1.1-696] cells/µL vs. 24 [6.8-326] cells/µL at follow-up.
- The risk of progression (need to be treated) was significantly higher in dynamic group.

This study found alterations in the distribution of absolute counts of circulating normal residual B- and T- cell subpopulations among CLL patients vs. healthy donors, but also significant differences among CLL patients depending on the kinetics of the clonal population.

27. POSTER

TOWARDS STANDARDIZATION OF FLOW CYTOMETRIC ANALYSIS OF REACTIVE OXYGEN- AND NITROGEN SPECIES FOR IN VITRO STUDIES USING CELL LINES

AUTHORS

Javega, B.[1], Herrera, G.[2], O'Connor, E.[1]

AUTHORS AFFILIATION

(1) Laboratory of Cytomics, Dpt. Biochemistry and Molecular Biology, University of Valencia.
(2) Cytometry Service, Central Research Unit (UCIM), Incliva Foundation-Valencia University, Valencia.

ABSTRACT

Background: Reactive Oxygen- (ROS) and Nitrogen (RNS) species are at the basis of oxidative- and nitrosative stress, which are inextricably linked to key physiopathological processes. Fluorescence-based analysis of ROS and RNS is an important application of flow cytometry (FCM), but detection and quantitation of individual ROS or RNS is challenging, because of the biological complexity of the processes involved and inherent limitations of fluorescent probes.

Aims: To provide recommendations in order to optimize the detection and quantification of relevant ROS and RNS of interest for functional in vitro studies cell lines using FCM.

Methods: All FCM experiments were performed with Jurkat cells using Gallios or Cytomics FC500 cytometers (Beckman Coulter). Initially, the range of toxicity of several model donors of ROS or RNS was studied and their IC50 was established by standard FCM viability assays. Then, a series of fluorogenic substrates or fluorescent probes employed in ROS or RNS analysis by FCM were titrated against fixed non- toxic concentrations of ROS- or RNS donors. After having thus defined the optimal conditions for fluorescent staining and sub-lethal treatment (doses below IC50), we studied systematic combinations of the fluorescent reagents at optimal staining with the selected ROS- or RNS donors at different concentrations. The changes in mean fluorescence intensity in live cells were normalized as the ratio of fluorescence intensity of donor-treated cells over the untreated and stained cells. Statistical significance was determined by the one-way ANOVA test.

Results & Conclusions: Based upon our normalized results we issue recommendations for selecting the fluorescent reagents more appropriate for the different ROS or RNS, as well as for including viability markers and suitable positive- and negative biological controls in the experiments. Such recommendations are expected to improve the specificity and sensitivity of ROS and RNS analysis by FC M.

28. POSTER

PD-1 EXPRESSION IN PERIPHERAL T CELLS OF PATIENTS WITH IDIC-15, A RARE DISEASE OF NEURODEVELOPMENT

AUTHORS

O'Connor, E.[1], Montagud, C.[1], Javega, B.[1], Herrera, G.[2], García-Pérez, M.[3], Orquín, M.[4], Codoñer-Franch, P.[5], Alonso-Iglesias, E.[6]

AUTHORS AFFILIATION

(1) Laboratory of Cytomics, Dpt. Biochemistry and Molecular Biology, University of Valencia.
(2) Cytometry Service, Central Research Unit (UCIM), Incliva Foundation-University of Valencia, Valencia, Spain.
(3) Genetics, Incliva Foundation and Valencia University, Valencia, Spain.
(4) Family and Community Medicine Service, Health Center Ontinyent III, Ontinyent, Spain.
(5) Dpt. of Pediatrics, Obstetrics and Gynecology, Valencia University-Doctor Peset University Hospital, Valenca, Spain.
(6) Dpt. Biochemistry and Molecular Biology, Valencia University, Valencia, Spain.

ABSTRACT

Background: Idic15 syndrome is a rare disease of neurodevelopment caused by variable duplications in the q11-q13 region of chromosome 15. As in other neurodevelopmental pathologies, many Idic15 patients show enhanced susceptibility to infections, and we have previously shown alterations in T, B and NK cells in these patients. Programmed Cell Death-1 (PD-1) is a co-inhibitory receptor involved in the regulation of T-cell activation, differentiation, effector function and memory. In chronic infections, sustained PD-1 expression may impair protection against pathogens.

Aims: To study by FCM the expression of PD-1 protein in CD4 and CD8 peripheral T-cells in Spanish Idic- 15 patients and age- and sex-matched healthy individuals.

Methods: DuraClone panels (Beckman Coulter) were used for immunophenotyping T cell-memory subpopulations and for quantitation of PD-1 expression. Assays were run on Gallios flow cytometer with Kaluza software (Beckman Coulter). For statistical analysis, Mann-Whitney and Spearman tests were applied.

Results & Conclusions: We found no significant differences between controls and patients in the abundance of circulating CD4 or CD8 cells expressing PD-1 nor intensity of PD-1 expression per cell. Interestingly, PD-1 expressing CD4 ($p=0.02$) or CD8 ($p=0.08$) cells were more abundant in women than in men in both groups. Moreover, we found an age-dependent decrease of the intensity of expression in PD- 1+ CD4 cells in both controls and patients, independent of their gender. We found lower numbers of PD- 1 expressing CD4 ($p=0.09$) and CD8 ($p= 0.01$) cells in Idic-15 patients with repeated infections, together with a non-significant decrease of PD-1 expression per cell. No changes in PD-1 expression were observed in patients stratified by their genetic lession or neurological symptoms. Our findings suggest that PD-1 expression may change according to age and sex. PD-1 may be involved in the susceptibility of Idic-15 patients to infection. Sponsored by the "One House One Life" (Great Chance SLU).

30. POSTER

MONOCYTE-DERIVED SUBPOPULATIONS IN CLL PATIENTS COMPARED WITH HEALTHY DONORS

AUTHORS

Moreno Paredes, N.[1], Lerma Verdejo, A.[1], Moreno Nombela, S.[1], Criado Garcia, I.[4], Teodosio, C.[2], Perez Peris, M.[3], Martinez Mayoral, M.[3], Blazquez Sanchez, R.[3], Jimenez, R.[3], Solano Ramos, F.[1]

AUTHORS AFFILIATION

(1) Hematology Service. Hospital Ntra. Sra. Del Prado. Talavera de la Reina.
(2) Centro de Investigación del Cáncer. Universidad de Salamanca.
(3) Cytometry Service. Hematology. Hospital Ntra. Sra del Prado. Talavera de la Reina.

ABSTRACT

Background: Chronic lymphocytic leukaemia (CLL), a biologically and clinically heterogeneous condition, have an immunotolerant microenvironment that modulate CLL cells viability and survival.

In node biopsy samples and tissue models it has been observed that interactions between tumoral and monocyte-derived immune cells favours tumour cells growth. An elevated monocyte count in peripheral blood is related to accelerated disease progression and to time free of treatment.

Aims: Our study pretends to explore monocyte-derived subpopulations in CLL patients and its relationship with healthy donors standardized by age and gender.

Methods: We have analysed several monocyte-derived subpopulations in peripheral blood by multiparametric flow cytometry, as well as neutrophils, eosinophils, and lymphocytes in 17 CLL patients and 48 healthy controls.

Samples were stained and acquired according to Euroflow requirements in a FACSCANTO II cytometer. This samples had been analysed according published Euroflow strategy.

Results & Conclusions: Absolute number of neutrophils, total monocytes, and its subpopulations: classical monocytes (cMo) (CD62L+ and CD62L-), intermediate (iMo), and non-classical monocytes (ncMo) Slan+ are significantly superior in CLL patients group than in controls (p= 0.0001). ncMo Slan- are inferior in CLL patients than in controls but this difference was not significant (p=0.05).

Despite of insufficient number of CLL samples the differences observed in our study in absolute number of monocytes and neutrophils agrees with published bibliography. These differences could be due to myeloid niches occupation by the tumour. It will be necessary more studies with more samples to establish the relationship of this findings with clinical behaviour of this disease.

32. POSTER

FLOW CYTOMETRY AS A CONTRIBUTION TO THE DIAGNOSTIC ORIENTATION AND STAGING OF CHILDHOOD SOLID TUMORS – AN ONCOLOGY CENTER 1 YEAR EXPERIENCE

AUTHORS

Fernandes, B.[1], Faria, C.[1], Palmeira, C.[1], Sousa, E.[1], Godinho, I.[1], Barreto, J.[1], Afonso, M.[1], Martins, G.[2]

AUTHORS AFFILIATION

(1) IPO-Porto, Porto.
(2) Department of Clinical Pathology of Instituto Português de Oncologia Francisco Gentil do Porto. Porto.

ABSTRACT

Background: Pediatric cancer is a relatively rare and heterogeneous group of hematological and non- hematological malignancies. Multiple procedures are required for diagnosis and classification. Early diagnosis and classification are particularly important for therapeutics and adequate patient management, improving health outcome. Flow cytometry (FCM) has been recently suggested to be an accurate and valuable aid in the management of these pediatric patients due to its multiparametric and rapid quantitative features.

Aims: To evaluate the performance of FCM in paediatric solid tumour cells screening, in comparison to histology.

Methods: Samples of all the children with a clinical suspicion of a solid tumor in 2022 were selected (n=19). Patient ages ranged between 1 and 15 years old. A total of 19 samples; 8 mass biopsies, 2 lymph nodes and 9 bone marrow samples were processed by FCM, using the EuroFlow Solid Tumor Orientation Tube. FCM results were compared with histological results, available from each patient's file.

Results & Conclusions: Eleven samples were identified as positive by both FCM and histology, comprising: 5 Neuroblastomas, 5 Ewing/Ewing-like Sarcomas and 1 Medullary Thyroid Carcinoma. One bone marrow sample was found to be positive by FCM and negative by histology (Neuroblastoma). The remaining 7 samples were considered negative by both techniques.

Regarding the sample type, all the mass biopsies were positive. Comparing to positive bone marrow samples, mass biopsies had a consistently higher tumor cell count by FCM.

A proportion of overall agreement of 94.7% (18 out of 19 samples) was observed. The only disagreement between the techniques (positive by FCM, negative by histology) may be related to a higher sensitivity of FCM.

These results seem to support the valuable contribution of FCM mainly for solid tumors pediatric staging where bone marrow samples frequently contain few and scattered pathological cells.

34. POSTER

FLOW CYTOMETRY ANALYSIS OF TROGOCYTOSIS IN CAR-T CELLS AGAINST HNSCC CELL LINES

AUTHORS

Lopez, A.[1], Vela, P.[1], Sánchez Domínguez, R.[1], Alberquilla, O.[1], Casado, J.[1]

AUTHORS AFFILIATION

(1) CIEMAT/IIS-FJD/CIBERER.

ABSTRACT

Background: Trogocytosis is the intercellular transfer of membrane-associated molecules documented in different biological processes. This process has been extensively studied in immune system cells and can alter the immune response. Lymphocytes expressing a synthetic chimeric receptor (CAR-T cells) against tumor antigens have shown clinical results against several types B leukemias. Recent work has described that trogocytosis processes in CAR T cells, which acquire the tumor antigen from the target cell, may have a negative impact on this antitumor therapy.

Aims: An in-vitro model of trogocytosis using CAR-T cells against the pan-ErbB family is studied by flow cytometry in the presence of EGFP+ head and neck carcinoma cell lines (HNSCC) overexpressing ErbB molecules. The percentage of lymphocytes acquiring ErbB molecules that are trog+ (CD3+ErbB+EGFP-), trog- (CD3+ErbB-EGFP-) and tumor cells (CD3-ErbB+EGFP+) is analysed at different time points in CAR+ and CAR- subpopulations.

Methods: Expression of CAR with 41BB as co-stimulatory domain and directed against ErbB molecules, is obtained by lentiviral transduction. The target cells are cell lines derived from HNSCC patients (VU-1131 and VU-1365) that overexpress ErbB molecules and were transfected to express EGFP/luc. CAR T cells are added to the adherent lines at 5:1 (E:T) ratio and cytotoxicity is analysed at different time points by bioluminescence. Subsequently, CAR-T cells are resuspended and the presence of ErbB tumor antigen is analysed by labelling with CD3-PE, CAR (EGFb+ bStreptavidin-BV711), ErbB1-APC.

Results & Conclusions: Trogocytosis is evident in CAR-expressing lymphocytes after 24 hours of interaction with target cells. Notably, trog+ lymphocytes show intermediate DAPI staining, indicating a possible fratricide process. Using markers to identify effector and target cells, in-vitro trogocytosis processes can be quantified in CAR-T cells after interaction with their target cell.

37. POSTER

EXPANDED TH1 CELLS IN LOCID AND CVID PATIENTS ARE ASSOCIATED WITH AUTOIMMUNE CYTOPENIAS AND INTERSTITIAL LUNG DISEASE

AUTHORS

Torres Valle, A.[1], Neirinck, J.[2], De Arriba, S.[3], Aragon, L.[4], Silva, S.[5], Serrano, C.[6], Subirá, D.[7], Ruiz Mercado, M.[8], Gonzalez-Granado, L.[9], Marcos, M.[10], Inés, S.[3], Martins, C.[11], Hofmans, M.[12], De Vriendt, C.[12], Albarrán, B.[13], Hurtado, G.[14], Barez, A.[15], Arenas-Caro, P.[4], Madruga, I.[16], Martín, A.[17], Bastida, J.[17], Dávila, I.[18], Contreras-Sanfeliciano, T.[20], Jara, M.[21], Prieto, C.[22], Kerre, T.[23], Haerynck, F.[24], Sousa, A.[25], Prada, Á.[4], Bonroy, C.[12], Van Dongen, J.[26], Pérez-Andrés, M.[27], Orfao, A.[27]

AUTHORS AFFILIATION

(1) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), Uni,
(2) Department of Laboratory Medicine, Ghent University Hospital; Department of Diagnostic Sciences, Ghent University, Ghent, Belgium.
(3) Servicio de Pediatría, Hospital Universitario de Salamanca, Salamanca, Spain.
(4) Immunology Department, Donostia University Hospital, Osakidetza Basque Health Service, San Sebastián, Spain.
(5) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.
(6) Servicio de Inmunología, Fundación Jiménez Díaz, Madrid, Spain.
(7) Flow Cytometry Unit, Department of Hematology, Hospital Universitario de Guadalajara, Spain.
(8) Hospital Costa del Sol, Hematologia y Hemoterapia, Marbella, Spain.
(9) Immunodeficiencies Unit. Hospital 12 de octubre. Research Institute Hospital 12 octubre (i+12), Madrid, Spain.
(10) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain.
(11) NOVA Medical School/Faculdade de Ciências Médicas Universidade Nova de Lisboa, Lisbon, Portugal.
(12) Department of Laboratory Medicine, Ghent University Hospital; Department of Diagnostic Sciences, Ghent University, Ghent, Belgium.
(13 Hematology, Complejo Asistencial Universitario, Palencia, Spain.
(14) Servicio de Hematología, Complejo Hospitalario de Navarra, Pamplona, España.
(15) Department of Hematology, Complejo Asistencial de Ávila, Spain.
(16) Servicio de Medicina Interna, Hospital Universitario de Salamanca, Spain.
(17) Servicio de Hematología, Hospital Universitario de Salamanca, Institute for Biomedical Research of Salamanca, Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de salud Carlos III, Madrid, Spain.
(18) Servicio de Alergia, Hospital Universit

ABSTRACT

Background: Several alterations of CD4+ T-cell subsets have been reported in common variable immunodeficiency (CVID) patients, although most studies did not discriminate patients with an underlying defect in CD4+ T-cell production fulfilling late-onset combined immunodeficiency (LOCID) criteria.

Aims: To evaluate the relationship between CD4+ T-cell compartment alterations and the clinical behavior of CVID and LOCID patients.

Methods: CD4+ T-cell subsets were analyzed in 53 CVID and 19 LOCID patients,

in parallel with 146 healthy donors (4-88 years) using EuroFlow-based flow cytometry methods.

Results & Conclusions: Higher percentage of patients with decreased T-cell subset counts were observed in LOCID vs. CVID patients, including Treg (84% vs. 61% of patients respectively), Th2 (100% vs. 45%), Th17 (95% vs. 49%), and Th1/Th2 (63% vs. 28%), as compared to age-reference values. In contrast, few LOCID and CVID patients showed decreased TFH (5% and 2%), Th1 (21% and 20%), and Th1/Th17 (16% and 14%) counts. Multivariant analysis showed two clearly distinct subgroups of LOCID, those with higher Th1 counts presenting with a higher frequency of autoimmune cytopenia (90% vs. 22%, p=0.005) and interstitial lung disease (60% vs. 11%, p=0.04), together with lower frequency of non-respiratory infections (50% vs. 100%, p=0.02). In addition, four CVID subgroups were identified, one of them without alterations in the Th compartment and the others were identified based on Th1 and TFH cell alterations, with a significantly higher frequency of autoimmune cytopenia in CVID cases with higher Th1 cells (88% vs. 18% vs. 8% vs. 44%, p<0.001). To conclude, LOCID patients show more severe T-cell defects (Th2, Th17, and Th1/Th2) than CVID patients. Increased counts of Th1 CD4+ T cells in blood, was strongly associated with the presence of autoimmune cytopenia in both LOCID and CVID cases, together with interstitial lung disease in LOCID patients, but not in CVID patients.

38. POSTER

EVIDENCE OF BICLONALITY OF BLOOD TAB-CELL EXPANSIONS SHOWING AN APPARENTLY NORMAL TRBC1+/TRBC1- RATIO USING THE TRBC1-BASED FLOW-CYTOMETRY APPROACH

AUTHORS

Morán Plata, F.[1], Carretero Domínguez, S.[2], Oliva Ariza, G.[2], Fuentes-Herrero, B.[2], Balanzategui, A.[3], Bárcena, P.[2], González González, M.[2], Alcoceba Sánchez, M.[3], Orfao, A.[4], Almeida, J.[2]

AUTHORS AFFILIATION

(1) Centro de Investigación del Cáncer. Salamanca.
(2) Translational and Clinical Research Program, Cancer Research Center (IBMCC, CSIC – University of Salamanca); Cytometry Service, NUCLEUS; Department of Medicine, University of Salamanca, Salamanca, Spain. IBSAL, Salamanca, Spain. CIBERONC ISCIII, Madrid, Spain.
(3) Department of Haematology, University Hospital of Salamanca (HUS-IBSAL), CIBERONC, and Cancer Research Institute of Salamanca-IBMCC (CSIC-USAL University), Salamanca, Spain.
(4) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain.

ABSTRACT

Background: TRBC1-based flow-cytometry approach has emerged as a new method to assess TαB-cell clonality. Normal-(polyclonal) TαB-cells show a polytypic/bimodal TRBC1 pattern (TRBC1+/TRBC1- ratio of 0.66±0.07 in blood), whereas monoclonal TαB-cells show a monotypic profile (100% TRBC1+ or TRCB1-). Here we describe two cases carrying suspicious/aberrant TαB-cells in blood with TRBC1+/TRBC1- ratios of 0.58 and 0.25, in which monoclonality was confirmed in each subset (TRBC1+ and TRCB1-).

Aims: To describe for the first time the presence of biclonality in blood TαB-cell expansions with apparently normal TRBC1+/TRBC1- ratios.

Methods: Case#1. A 39-year-old male with lymphocytosis maintained for four years without clinical evidence of CLPD.

Case#2. A 60-year-old male blood donor, included in a population-based screening for lymphoid clones, with normal blood-cell count and no clinical manifestations.

Peripheral blood samples were analyzed by flow-cytometry, using the EuroFlow-based Lymphoid_Screening_Tube plus anti-TRCB1, followed by extended panels for further characterization. PCR-based TRB/G and TRBJ1/J2 gene rearrangement assays were performed to confirm clonality on FACSorted cells.

Results & Conclusions: Case#1 was referred for an expansion (1361 events/µL) of TαBCD3+CD4+CD7- cells with a TRBC1+/TRBC1- ratio of 0.58. In-depth flow-cytometry analysis revealed a suspicious (CD2++CD7-/+dimCD8-/+dimCD62L+) TαBCD4+ population with a CD27-CD45RA+cyGranzyme B+ terminal-effector phenotype (36.6% TRBC1+ and 63.4% TRBC1-). TCR gene rearrangement analysis of purified cell-fractions confirmed that they corresponded to non-related clonal populations, the TRBC1- population was found to have functionally rearranged TRBJ2 sequences.

In Case#2, a TαBCD3+CD8+CD5- population (986 events/µL) with a TRBC1+/TRBC1- ratio of 0.25 was detected in the screening tube. Both TRBC1+ (CD2+dimCD7-CD8+CD16+) and TRBC1- (CD2-/+dimCD7+CD8+dimCD16+) populations of terminal-effector cells were confirmed to correspond to different (unrelated) clones.

In summary, our results show that for a correct evaluation of suspicious/expanded TαB-cells, both the TRBC1 expression pattern and the presence of phenotypic aberrancies must be considered.

39. POSTER

ANALYSIS OF THE IMMUNOLOGICAL PROFILE OF SIX GOOD SYNDROME PATIENTS

AUTHORS

Torres Valle, A.[1], De Arriba, S.[2], Aragon, L.[3], Serrano, C.[4], Marcos, M.[5], Melero, J.[6], Arenas-Caro, P.[3], Jara, M.[7], Prieto, C.[8], Prada, Á.[3], Van Dongen, J.[9], Pérez de Andrés, M.[9], Orfao, A.[9]

AUTHORS AFFILIATION

(1) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.
(2) Servicio de Pediatría, Hospital Universitario de Salamanca, Salamanca, Spain.
(3) Immunology Department, Donostia University Hospital, Osakidetza Basque Health Service, San Sebastián, Spain.
(4) Servicio de Inmunología, Fundación Jiménez Díaz, Madrid, Spain.
(5) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain.
(6) Servicio de Inmunología y Genética, Hospital Infanta Cristina, Complejo Hospitalario Universitario de Badajoz, Badajoz, Spain.
(7) DNA Sequencing Service (NUCLEUS), University of Salamanca, Salamanca, Spain. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), number CB16/12/00400, Instituto de Salud Carlos III, Madrid, Spain,
(8) Bioinformatics service (NUCLEUS), University of Salamanca, Salamanca, Spain.
(9) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain; Department of Immunology, Leiden University Medical Center.

ABSTRACT

Background: Classically, Good's syndrome (GS) was defined as a rare association of thymoma and hypogammaglobulinemia, suggesting that it might be a subset of Common Variable Immunodeficiency. However, the knowledge of the disease is hampered by the incomplete and inconsistent reports about the disease due to low prevalence of the disease (1.5 cases per million).

Aims: A more detailed dissection of the immune cells in a significant set of patients would contribute to understand the pathophysiology of the disease.

Methods: Up to 350 immune subpopulations were analyzed in six GS patients (53-79 years) from 4 different hospitals and 49 age-matched controls using next-generation flow cytometry.

Results & Conclusions: All patients consistently presented with lack of B-cells (<0.02 cells/μL). In addition, alterations were also found in other immune subsets including significantly reduced counts of total CD4+T-cells, NK-cells, neutrophils, basophils, eosinophils, CD141+ and plasmacytoid dendritic cells (DCs) ($p \leq 0.05$), as compared to age-matched controls. Decreased of total CD4+ T-cell counts was due to reduced numbers of naïve, central memory and transitional memory cells and, in addition, lower Treg, TFH, Th2, Th17, Th22, Th1/Th17 and Th1/Th2 cells ($p \leq 0.01$). However, number of Th1 cells was not different from age-matched controls ($p > 0.05$). Interestingly, counts of naïve CD8+ and TCRγδ+ T-cells were normal, meanwhile central and transitional memory CD8+ and TCRγδ+ T-cells were reduced ($p \leq 0.01$). However, total TCRγδ+ T-cells tends to be higher ($p = 0.07$) due to statistically significant expanded terminally differentiated cells ($p \leq 0.01$). All the other immune subsets analyzed were within the normal range. In conclusion, together with the lack of B-cells, a complex profile of alterations was observed in GS patients including a large spectrum of defects in the adaptative (CD4+ T-cells) and innate cells (NK-cells, eosinophils, neutrophils, basophils, DCs). In contrast, expanded TCRγδ+ T-cells and normal counts of Th1 CD4+ and CD8+ T-cells were observed.

40. POSTER

OVEREXPRESSION OF THE EXOSOME MARKER CD63 IN BONE MARROW MYELOMA PLASMA CELLS IMPACTS IN THE OSTEOLYTIC PRESENTATION OF THE DISEASE

AUTHORS

Sanoja-Flores, L.[1], Reinoso-Segura, M.[2], Reguera-Ortega, J.[2], Carrillo-Cruz, E.[2], Martín-Chacón, E.[2], Ruiz, V.[2], Garzón-López, S.[3], Cauto-Caro, M.[4], Pérez-Simón, J.[2], Caballero-Velázquez, T.[2]

AUTHORS AFFILIATION

(1) Instituto de Biomedicina de Sevilla.
(2) Department of Hematology, Institute of Biomedicine of Seville (IBiS), University Hospital Virgen del Rocío of the "Consejo Superior de Investigaciones Científicas" (CSIC), University of Seville, Seville, Spain. Centro de Investigación Biomédica en Red de Cáncer (CIBER-ONC), Instituto Carlos III, Madrid, Spain. Number CB16/12/00480.
(3) Department of Hematology, University Hospital of Jerez, Jerez de la Frontera, Cádiz, Spain.
(4) Department of Hematology, Institute of Biomedicine of Seville (IBiS), University Hospital Virgen de Valme of the "Consejo Superior de Investigaciones Científicas" (CSIC), University of Seville, Seville, Spain.

ABSTRACT

Background: Typically, disseminated multiple myeloma (MM) curses with high counts of blood circulating tumour plasma cells (CTPC), which is in contrast to macrofocal MM patients. However, its biological meaning is unknown. We hypothesize that the overexpression of CD63 in the surface of bone marrow (BM)-myelomatous plasma cells (mPC), could be a mechanism to spread the tumour into extramedullary sites.

Aims: To explore the expression of CD63 among BM-myelomatous and normal PC (nPC) compartments and its correlation with disease features.

Methods: Overall, 34 paired BM and blood samples from 17 newly-diagnosed MM patients, were analysed. Samples were processed using the EuroFlow bulk-lysis protocol, stained by antibody panel containing (at least) CD138-BV421/CD45-BV650/CD19-BV786/CD38-FITC/CD63-PerCPCy5.5/CD56-PECy7, for final measurement in the spectral 4L-CytekAurora cytometer. Evaluation of CD63 expression (Infinicyt software) was performed by calculating a ratio from BM-mPC vs nPC median fluorescence intensity values (rCD36).

Results & Conclusions: MM cases were grouped according to rCD63 levels from ≤1 (median: 0.5, range: 0.1-0.9; n=11), to >1 (3.8, 1.3-54.4; n=6). Thus, MM patients with rCD63>1 showed lower counts of blood- CTPC contrary to rCD63≤1 cases [0.0027% (0.0003% to 0.07%); vs 0.09% (<0.0002% to 5.4%); p=0.04], respectively. Also, overexpression of CD63 in BM-mPC was related to minimum medullary tumour burden [1.1% BM-mPC (0.2% to 39.7%) vs 10.4% (0.4% to 45.5%); p=0.08], and a highly proportion of osteolytic lesions by imaging techniques (100%; 5/5 vs 55%; 6/10) than rCD63≤1 MM cohort, respectively. Moreover, the above results translated into higher levels of serum calcium [10.4 (9.3 to 13.1) vs 8.9 (8.6 to 10.5) mg/dL; p=0.014], and creatinine [1.5 (0.8 to 7.4) vs 0.7 (0.5 to 1.5) mg/dL; p=0.027] among >1 vs ≤1 rCD63 patients, respectively.

Conclusion: Overexpression of the exosome marker CD63 in BM-mPC could be implied in the presentation of bone myeloma profile.

41. POSTER

CLOSE LONG-TERM LONGITUDINAL IMMUNE-MONITORING ALLOWS THE IDENTIFICATION OF IMMUNE PROFILES ASSOCIATED WITH THE SEVERITY OF INFECTION BY SARS-COV-2

AUTHORS

Fuentes-Herrero, B.[1], Oliva-Ariza, G.[1], Carbonell, C.[2], Lecrevisse Q.[1], Perez-Pons, A.[1], Torres-Valle, A.[1], Pozo, J.[1], Martín-Oterino, J.[3], González-López, Ó.[1], López-Bernús, A.[4], Bernal-Ribes, M.[1], Belhassen-García, M.[4], Pérez-Escurza, O.[1], Pérez de Andrés, M.[5], Vazquez, L.[6], Hernández-Pérez, G.[7], García Palomo, F.[8], Leoz, P.[6], Costa-Alba, P.[11], Pérez-Losada, E.[12], Yeguas, A.[6], Santos Sánchez, M.[1], García-Blázquez, M.[13], Morán- Plata, F.[1], Damasceno, D.[14], Botafogo, V.[1], Muñoz-García, N.[1], Fluxa, R.[15], Contreras-Sanfeliciano, T.[16], Marcos, M.[3], Almeida, J.[14], Orfao, A.[5]

AUTHORS AFFILIATION

(1) Translational and Clinical Research Program, Cancer Research Center (IBMCC, CSIC – University of Salamanca); Cytometry Service, NUCLEUS; Department of Medicine, University of Salamanca, Salamanca, Spain. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.
(2) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.
(3) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain.
(4) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain. Department of Infectious Diseases, University Hospital of Salamanca, Centro de Investigación de Enfermedades Tropicales de la Universidad de Salamanca (CIETUS), Salamanca, Spain.
(5) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain.
(6) Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain. Department of Hematology, University Hospital of Salamanca, Salamanca, Spain.
(7) Department of Internal Medicine, University Hospital of Salamanca, Salamanca, Spain.
(8) Spanish National DNA Bank Carlos III, University of Salamanca, Salamanca, Spain.

ABSTRACT

Background: COVID-19 pandemic has resulted in high morbidity and mortality worldwide; although most cases are mild/asymptomatic, 15%-20% of patients have severe disease. The relationship between the immune response and the clinical heterogeneity and outcome of the disease remains to be fully elucidated.

Aims: To analyze in blood the kinetics of leukocyte populations and anti-SARS-CoV-2 antibodies, and the viral load, through close monitoring along the infection, also according to the disease severity.

Methods: 802 samples from 343 patients (median 57 years -y-, from 18-99y) were analyzed, including 23 cases (n=132 samples) studied every 24h-48h during the

acute phase. The distribution of cell-populations was analyzed by flow-cytometry; plasma levels of anti-virus N-protein antibodies were determined by ELISA. Results were relativized from the day of symptoms onset (d0) and normalized by age with healthy donors (n=928). SARS-CoV-2 viral load was quantified in plasma by RT-PCR (n=303 samples).

Results & Conclusions: Eosinopenia, lymphopenia (mainly T-lymphocytes -all subpopulations-) and neutrophilia were evident early (d+2), followed by an increase in plasma-cells (d+10-d+15), returning to normality from d+50 onwards; most patients had detectable plasma antibodies (IgM, IgG and IgA) early from d0. Eosinopenia, lymphopenia and neutrophilia were more pronounced (but with a later recovery to normality) in more severe patients, while the plasma-cell peak was higher (albeit delayed) vs. milder patients. Also, plasma levels of specific IgG and IgA, and viral load were increased in more severe cases. In conclusion, there are unique immunological profiles of each leukocyte population, particularly during the acute phase, with more severe patients showing a greater increase/decrease in affected populations, antibody levels and viral load, but with a delay in reaching peak/nadir or returning to normality. Precise knowledge of the kinetics of immune cells and antibodies in blood and their profiles associated with severity contribute to a more accurate prognosis in COVID-19.

42. POSTER

KI-67 EXPRESSION ASSESSMENT BY FLOW CYTOMETRY IN THE DIAGNOSIS OF HUMAN B-CELL LYMPHOMA

AUTHORS

Castillo, F.[1], Iruretagoyena, M.[2], Spralja, B.[3], Morales, C.[2], Díaz, J.[4], Ernst, D.[5]

AUTHORS AFFILIATION

(1) Clínica Alemana de Santiago.
(2) Laboratorio Clínico, Clínica Alemana de Santiago.
(3) Laboratorio Anatomía Patológica, Clínica Alemana de Santiago.
(4) Departamento de Oncología, Clínica Alemana de Santiago.
(5) Laboratorio Clínico, Clínica Alemana de Santiago. Departamento de Oncología, Clínica Alemana de Santiago. Instituto de Ciencia e Innovación en Medicina (ICIM), Facultad de Medicina, Universidad del Desarrollo, Santiago, Chile.

ABSTRACT

Background: Ki-67 is a nuclear protein associated with cellular proliferation and is used as a prognostic marker in the classification of B-cell lymphomas. Immunohistochemistry (IHC) of histological samples is the gold standard.

Aims: The aim of this study was to evaluate Ki-67 expression on samples of mature B-cell neoplasms by flow cytometry (FCM) and to compare the results with Ki-67 by IHC.

Methods: Bone marrow aspirate and tissue biopsy specimens were obtained for routine lymphoma screening. Flow cytometric analysis was performed according to Euroflow standard LST screening. Positive samples were further characterized. Ki-67 expression was incorporated into the characterization of chronic B-cell neoplasms using a PerCP-Cy5.5 fluorochrome-conjugated monoclonal antibody (Biolegend) after cell fixation and permeabilization. Ki-67 expression was calculated as all positive cells among the neoplastic cells (%). Ki-67 expression by FCM was compared with Ki-67 expression by IHC (semi- quantitative). Patient information was collected from the electronic medical record.

Results & Conclusions: Between January 2021 and November 2022, a total of 51 samples were positive in LST and characterized for B-chronic malignancies. There were 4 bone marrow and 47 tissue samples. Overall, there were 28 low-grade (54.9%) and 23 high-grade (45.1%) B-cell lymphomas. Ki-67 expression by flow cytometry showed a significant Spearman correlation coefficient of r=0.925, (95% CI 0.870 - 0.957, $p < 0.0001$). A Bland-Altman plot showed a negative constant bias of -13.35 with limits of agreement ranging from -46.87 to 20.16. This bias is greater in low-grade lymphoma (-19.43) as opposed to high- grade lymphoma (-5.09). To our knowledge, this is the first study to correlate Ki-67 by FCM and IHC in human B-cell lymphomas. Ki-67 expression evaluated by flow cytometry can be easily integrated into screening strategies and may become a useful marker to discriminate the diagnosis of human B-cell lymphomas. Further studies are required to validate Ki-67 assessment by flow cytometry.

44. POSTER

T CELL CLONALITY EVALUATION IN SCREENING OF SAMPLES SUSPECT LYMPHOMAS BY FLOW CYTOMETRY WITH TRBC1

AUTHORS

Castillo, F.[1], Iruretagoyena, M.[2], Spralja, B.[3], Morales, C.[2], Díaz, J.[4], Ernst, D.[5]

AUTHORS AFFILIATION

(1) Clínica Alemana de Santiago.
(2) Laboratorio Clínico, Clínica Alemana de Santiago.
(3) Laboratorio Anatomía Patológica, Clínica Alemana de Santiago.
(4) Departamento de Oncología, Clínica Alemana de Santiago.
(5) Laboratorio Clínico, Clínica Alemana de Santiago. Departamento de Oncología, Clínica Alemana de Santiago. Instituto de Ciencia e Innovación en Medicina (ICIM), Facultad de Medicina, Universidad del Desarrollo, Santiago, Chile.

ABSTRACT

Background: The identification of T-cell lymphomas is challenging due to the limitations of currently available T-cell clonality assays. The recent description of a monoclonal antibody (mAb) specific for the T cell receptor ß constant region 1 (TRBC1) provides a new specific alternative for the discrimination of clonal T cells.

Aims: The main objective of this study was to evaluate anti-TRBC1 mAb for the identification of T cell clonality by flow cytometry in normal and pathological samples received in our laboratory using a custom designed T cell clonality panel.

Methods: We used 10 normal peripheral blood samples to determine the normal expression of TRBC1 and the TRBC1+/- ratio on CD3+CD4+ and CD3+CD8+ cells. We also evaluated 59 patient samples submitted for routine lymphoma screening by flow cytometry using the Euroflow Lymphoma Screening Tube (LST) and a T-cell clonality tube CD45/TRBC1/CD2/CD7/CD4/TRCγδ/CD3. The results were compared with morphological studies, PCR clonality tests and/or clinical history.

Results & Conclusions: We observed that the TRBC1+/- ratio in normal samples was between 0.33 and 1.1 for TαßCD4+ cells and between 0.22 and 1.0 for TαßCD8+ cells. Monophasic TRBC1 expression was identified in 18 samples from patients with T-cell malignancies. Inclusion of TRBC1 in routine suspected lymphoma samples improves the detection of T cell clones compared to our established CD4/CD8 ratio. The results of these analyses correlate with histopathological findings.

47. POSTER

LARGE-SCALE IMMUNE MONITORING OF SYSTEMIC AUTOIMMUNE DISEASE PATIENTS BY MASS CYTOMETRY

AUTHORS

Rybakowska, P.[1], Van Gassen, S.[2], Barturen, G.[1], Pérez Sánchez, C.[3], Ibáñez-Costa, A.[3], Varela, N.[1], Ortega Castro, R.[3], Fernández-Roldán, C.[4], Jiménez-Moleón, I.[5], Ortego, N.[4], Raya, E.[5], Aguilar Quesada, R.[6], López- Pedrera, C.[3], Collantes, E.[3], Saeys, Y.[7], Alarcón-Riquelme, M.[8], Marañón, C.[1]

AUTHORS AFFILIATION

(1) GENYO, Center for Genomics and Oncological Research Pfizer/University of Granada/ Andalusian Regional Government, Granada.
(2) Department of Applied Mathematics, Computer Science and Statistics, Ghent University. Data Mining and Modeling for Biomedicine, VIB Center for Inflammation Research, Ghent.
(3) Maimonides Institute for Research in Biomedicine of Cordoba (IMIBIC)/Reina Sofia University Hospital/University of Córdoba, Córdoba.
(4) Servicio de Medicina Interna. Unidad de Enfermedades Autoinmunes Sistémicas. Departamento de Medicina, Universidad de Granada. Hospital Universitario San Cecilio, P.T.S. Granada.
(5) Servicio de Reumatología. Hospital Universitario San Cecilio, P.T.S. Granada.
(6) Biobanco del Sistema Sanitario Público de Andalucía, Andalusian Public Health System Biobank.
(7) Department of Applied Mathematics, Computer Science and Statistics, Ghent University, Ghent, Belgium.
(8) Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

ABSTRACT

Background: Systemic autoimmune diseases (SADs) like systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjögren's syndrome, and mixed connective tissue disease are diagnosed using different clinical and laboratory criteria. Differential diagnosis is difficult due to internal heterogeneity and overlapping symptoms across diseases. Therefore, molecular studies are needed to disentangle heterogeneity.

Aims: Explore differences and similarities between SADs and build a reclassification framework using mass cytometry.

Methods: Blood samples collected from 125 individuals, including above mentioned SADs, undifferentiated patients, and controls, were stained with a 39-plex antibody panel and acquired on a HELIOS. Cell frequencies, median signal intensities (MSI), and 45 plasma proteins measured by a multiplexed Luminex assay were analyzed. Kruskal-Wallis's test was used to compare diagnostic categories, and a linear model comparing CTRs and SADs together with a Monte Carlo reference-based consensus clustering (M3C) was done to reclassify patients.

Results & Conclusions: Differentially expressed features were observed between different diseases, regarding the frequency and activation level across various cell populations. None of them were disease specific. The regression model between SADs and CTRs detected 14 differentially expressed features, and the M3C clustering identified 3 stable patient clusters (C). C1 had the highest expression of CD95 and the lowest of PD-L1 in granulocytes. The highest levels of CD38 and CD25 in basophils, CD11c+ NK, and T regs were also observed. C1 and C2 were opposite to each other and C3 presented a transitional phenotype, being the most like CTRs. Higher levels of disease activity-associated cytokines, IP10, TRAIL, and low levels of the immunomodulatory cytokine TGFß, were seen in C1.

The distribution of diagnosis across different clusters confirms disease heterogeneity. In summary, SADs patients can be classified into phenotypically similar groups that could benefit from the same line of treatment, regardless of their primary diagnosis.

IMI, PRECISESADS, (GA#115565), 3TR, (GA#831434), EFPIA

48. POSTER

A CUT-OFF VALUE ADJUSTMENT FOR THE DUODENAL LYMPHOGRAM INCREASE THE DIAGNOSTIC ACCURACY OF CELIAC DISEASE IN ADULTS

AUTHORS

García Bravo, L.[1], Villegas Mendiola, Á.[1], Rodriguez Vicente, N.[1], Alonso Arenillas, B.[1], Mohamed Mohamed, K.[1], Mansilla Ruíz, M.[1], Rodríguez Pereiro, A.[1], Fernández Arquero, M.[1], Sánchez Ramón, S.[1], Guzmán Fulgencio, M.[1]

AUTHORS AFFILIATION

(1) Department of Immunology. Hospital Clínico San Carlos. Madrid

ABSTRACT

Background: Celiac disease (CD) is an immune-mediated disorder elicited by gluten intake. Duodenal lymphogram analyzes intraepithelial lymphocytes (IELS) and has been proven to be a complementary tool in the diagnosis of CD. The typical finding is an increase of total intraepithelial lymphocytes (IELsT) and TCR-γδ+ IELs, plus a concomitant decrease in CD3- IELs.

Aims: To determinate cut-offs for IELsT, TCR-γδ+ and CD3- with the highest sensitivity and specificity for the diagnosis of CD and to compare these subpopulations of IELs between celiac and non-celiac patients (other digestive pathologies).

Methods: A total of 120 patients were distributed into two categories: I) CD (n=43) and II) non-CD (n=77) based on serology results, Marsh 1 to Marsh 3 histological damage, celiac genetics study (haplotype HLA- DQ2/DQ8) and a clinical and serological remission after a gluten-free diet. The study of duodenal lymphogram was done by applying flow cytometry in duodenal biopsy samples. ROC curve and Mann- Whitney U test were used to perform the statistical analysis. Values were expressed as % or mean±standard deviation ($p \leq 0.05$).

Results & Conclusions: The ROC curve showed the threshold with the highest pooled sensitivity (72.09%) and specificity (97.41%) for CD diagnostic: IELsT≥9%, TCR-γδ+≥15 and CD3-≤7%.

When we compared duodenal lymphogram among CD and non-CD patients, we observed significantly higher mean values for IELsT in CD than those obtained in non-CD (15.23±6.65 versus 11.81±9.28, $p=0.001$). As expected, the results showed significantly higher mean values of TcR-γδ+ in CD group compared with non-CD (27.32±11.03 versus 9.55±7.51, $p<0.0001$). In contrast, mean values of CD3- were significantly lower in CD compared with non-CD (5.93±8.42 versus 17.11±13.49, $p<0.0001$).

Our results highlight the requirement of a cut-off value adjustment for duodenal lymphogram to achieve an accurate differential diagnosis of CD with other digestive pathologies. Further studies are necessary to study clinical and laboratory parameters that modify the values of duodenal lymphogram.

49. POSTER

FOOTPRINT ANALYSIS OF VDJ SEGMENTS IN CLONAL B CELLS PURIFIED FROM MALE AND FEMALE SUBJECTS WITH LOW COUNT MONOCLONAL B-CELL LYMPHOCYTOSIS (MBLLOW)

AUTHORS

Serrano Lozano, J.[1], Rodríguez-Caballero, A.[1], Fuentes-Herrero, B.[1], Oliva Ariza, G.[1]

AUTHORS AFFILIATION

(1) Cancer Research Center, Institute of Cancer Molecular and Cellular Biology (IBMCC), University of Salamanca-The Spanish National Research Council (USAL-CSIC), and Department of Medicine and Cytometry Service, Nucleus Research Support Platform from University of Salamanca (NUCLEUS), University of Salamanca, Salamanca, Spain.

ABSTRACT

Background: Despite the significant advances in the identification and phenotypic characterization of small CLL-like B-cell clones (<50 clonal B-cells/μL) of "healthy" adults with MBLlow by next-generation flow cytometry, their molecular characterization remains challenging.

Flow cytometric studies report higher frequency of MBLlow, high-count monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia in males than females. The analysis of VDJ segments conforming the IGHV sequence of clonal B-cells in males versus females contributes to give some clues about the weight of different factors (i.e., clonal hematopoiesis, hormones, immunosenescence) affecting the emergence and progression of these B-cell expansions.

Aims: To compare the B-cell receptor molecular (IGHV) signature in male and female subjects with MBLlow, using a new adapted workflow to extremely low clonal B-cell numbers.

Methods: 37 CLL-like MBLlow clones were identified and sorted by high-sensitivity flow cytometry (FACSariaIII flow cytometer) (EuroFlowTM Lymphoid Screening Tube) from blood of healthy individuals: 19 females -median age: 68 (47-88)- and 18 males -median age: 65 (43-81)-. A new multiplex PCR protocol for IGHV sequencing was applied in 34 samples with low numbers of clonal B-cells (2,33±3,04 clonal B- cells/μL) and an old standardized BIOMED PCR protocol, entailing previous DNA purification, was applied in 3 samples (9,38±10,36 clonal B-cells/μL).

Results & Conclusions: Some tendency to differential use of VDJ segments was observed in females compared to males. IGHV1 (1/18 vs 5/19; P=0,184) and IGHD1 (0/18 vs 4/19; P=0,105) were more frequently found in females, whereas IGHJ5 and IGHJ6 were more common in males (8/18 vs 3/19; P=0,076). Despite these differences of VDJ segments in males versus females, IGHV-mutated clones were predominantly found in both groups (males: 12/18 -66%- vs females: 14/19 -73,6%- with IGHV-mutated clones).

From the molecular point of view, clonal B-cells in males show VDJ footprints compatible with more immature B-lymphocytes with theoretically increased sensitivity to transformation compare with their mature counterparts.

51. POSTER

AMMONIUM CHLORIDE RESISTANT ERYTHROCYTES ARE ENRICHED IN ECHINOCYTIC CELLS AS DEMONSTRATED BY LIVE CELL IMAGING ANALYSIS AND ACOUSTIC FLOW CYTOMETRY

AUTHORS

Garcia Rico, L.[1], Salvia Cerdà, R.[1], Ward, M.[2], Petriz, J.[1]

AUTHORS AFFILIATION

(1) Institut Germans Trias i Pujol. Badalona.
(2) Thermo Fisher Scientific. Eugene.

ABSTRACT

Background: Conventional immunophenotyping protocols use ammonium chloride to lyse the vast majority of erythrocytes. Sample preparation can result in incomplete lysis of erythrocytes, particularly in abnormal blood samples, marrow, or cord blood specimens, making the discrimination of white cell populations difficult.

Aims: Evaluate the combination of high speed bright-field imaging and precision flow cytometry to identify highly resistant erythrocytes incubated with ammonium chloride-based solutions.

Methods: Peripheral blood was lysed as described using ammonium chloride lysis buffer. Samples were acquired on the Invitrogen™ Attune™ CytPix™ Flow Cytometer (Thermo Fisher), using the Attune™ NxT™ No-Wash, No-Lyse Filter Kit for violet laser side scatter (SSC) detection, which offers a robust assay with minimal sample manipulation.

Results & Conclusions: Highly resistant erythrocytes ranged from 4.56 to 11.97% of total acquired events. Importantly, more than 90% of lysing resistant red blood cells consisted in echinocyte-like cells, as confirmed by live cell imaging analysis on the CytPix™. Here we show the potential of imaging acoustic cytometers for developing new approaches for the evaluation of red blood cell disorders and the effects of storage and ageing on changes or damage to RBCs membranes. The combination of acoustic orientation, light-scattering and live cell imaging could be a helpful tool that could be applied to the immediate evaluation of the quality of erythrocytes.

52. POSTER

M-PROTEIN CONCENTRATION AND BLOOD AND BONE MARROW CLONAL PLASMA CELLS IN THE EARLY DIAGNOSIS AND CLASSIFICATION OF MONOCLONAL GAMMOPATHIES

AUTHORS

Pérez-Escurza, O.[1], Þórðardóttir, H.[2], Óskarsson, J.[2], Sanoja-Flores, L.[3], Pozo, J.[4], Durie, B.[5], Van Dongen, J.[6], Kristinsson, S.[2], Orfao, A.[4]

AUTHORS AFFILIATION

(1) Translational and Clinical Research Program, Cancer Research Center (IBMCC, CSIC – University of Salamanca); Cytometry Service, NUCLEUS; Department of Medicine, University of Salamanca, Salamanca, Spain. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain.
(2) Faculty of Medicine, University of Iceland, Iceland.
(3) CIBERONC, Instituto Carlos III, Madrid, Spain. Institute of Biomedicine of Seville, Department of Hematology, University Hospital Virgen del Rocío of the Consejo Superior de Investigaciones Científicas (CSIC), University of Seville, Seville, Spain.
(4) Department of Medicine, Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), University of Salamanca (USAL), Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; Biomedical Research Networking Centre Consortium of Oncology (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain.
(5) Department of Hematology and Oncology, Cedars-Sinai Medical Center, Los Angeles, CA, USA.
(6) Immunology Department, Leiden University Medical Center, The Netherlands.

ABSTRACT

Background: Monoclonal gammopathy (MG) of undetermined significance (MGUS) is a premalignant condition characterized by the accumulation of clonal plasma cells (cPC) in bone marrow (BM) and a serum/urine M-component, which increase following progression to smoldering (SMM) and multiple myeloma (MM). Additionally, circulating (C) cPC is a new adverse prognostic biomarker.

Aims: We evaluated the relationship between serum M-protein concentration and the number of BM and blood cPC in different diagnostic subtypes of MG based on a group of 76 Icelandic individuals recruited in the iSTOPMM study.

Methods: We analyzed 152 paired BM and blood samples from donors (≥40y) with a serum M-component by mass spectrometry. Detection and enumeration of cPC and cB-cells was performed by high-sensitive next-generation-flow.

Results & Conclusions: Based on IMWG diagnostic criteria, 55/76 donors had MGUS, 12/76 SMM, 1/76 MM and 7/76 smoldering Waldenström macroglobulinemia (SWM). Median (range) M-protein concentration (1g/L; 0.1-17.2g/L) significantly increased from MGUS (0.8g/L; 0-8.8g/L) to SMM+MM (5g/L; 0-15.8g/L) (p=0.001) and SWM (2.5g/L; 0.4-17.2g/L) (p=0.051). Likewise, the median percentage (range) of BM cPC and/or cB-cells also increased from MGUS (0.31%; 0-22.2%) to both SMM+MM (1.4%; 0.08-17.8%) (p=0.02), and SWM (1.2%; 0-61%) (p=0.03), while no significant differences were found in blood CcPC (median number of cells/μL; range) in MGUS (0; 0-1.053); SMM+MM (0.02; 0-4.8); SWM (0; 0-32.2). Serum M-protein concentration moderately correlated with the percentage of BM cPC (rho=0.52; p<0.001), and cPC plus cB-cells (rho=0.53; p<0.001), and to a lesser extent also with CcPC (rho=0.24; p=0.03), but not with CcPC plus cB-cell counts (rho=0.19; p=0.11). Subjects with <1 vs ≥1g/L serum M-protein showed significantly different median of 0.07% vs 0.66% BM cPC (p<0.001), and of blood CcPC (p=0.01). Overall, our results show a close relationship between the serum M-component concentration and the percentage of cPC in BM and blood, which can help optimize the diagnostic approach of early vs advanced stage MG.

53. POSTER

STUDY OF CD4+ LYMPHOCYTE SUBSETS IN COMMON VARIABLE IMMUNODEFICIENCY (CVID): NEW CONTRIBUTIONS TO DIAGNOSIS

AUTHORS

Pereiro Rodríguez, A.[1], Mohamed Mohamed, K.[1], Guerra Galán, T.[1], Palacios Ortega, M.[1], Mansilla Ruíz, M.[1], Villegas Mendiola, Á.[1], García Bravo, L.[1], Guzmán Fulgencio, M.[1], Fernández Arquero, M.[1], Sánchez Ramón, S.[1]

AUTHORS AFFILIATION

(1) Servicio de Inmunología, Hospital Clínico San Carlos, Madrid

ABSTRACT

Background: Common variable immunodeficiency (CVID) is the most common clinical primary immunodeficiency, characterized by a defect in B cell differentiation which leads to hypogammaglobulinemia. However, alterations in the function of T cells have also been described, affecting the T-B cell interaction. T cell defects could explain the defective antibody production, but also the development of other complications, such virus infection, gastrointestinal disease, autoimmunity or inflammation.

Aims: In this study, we assessed a new flow cytometry panel for the diagnosis of CVID patients.

Methods: We studied 5 CVID patients and 5 healthy controls (HC) for CD4+ T lymphocytes subsets. Twelve-color flow cytometry panel and analysis of circulating lymphocytes was made. Results were analyzed by U de Mann-Whitney statistical test.

Results & Conclusions: We observed a decrease in CD4+ T lymphocytes and naïve CD4+ T cells in CVID patients compared with HC ($p<0.05$ and $p<0.01$, respectively). The percentage of effector memory (EM) CD4+ T cells was significantly higher in CVID patients than HC ($p<0.01$). An increase in T helper 1 (Th1) lymphocytes in CVID was also observed ($p<0.01$). One CVID patient had diminished regulatory T (Treg) cells and a marked expansion of Th1 lymphocytes which might explain the development of autoimmunity and GLILD in this patient.

Our data show that the analysis of CD4+ T lymphocytes by flow cytometry provides valuable diagnostic information in CVID patients, which, added to the classical diagnostic criteria, could increase diagnostic efficiency with clinical relevance.

54. POSTER

IMPACT OF JAK-INHIBITORS IN INNATE AND ADAPTIVE CELL SUBSETS IN RHEUMATOID ARTRITIS

AUTHORS

Comins Boo, A.[1], Fernández Cabero, J.[1], Lasa Teja, C.[1], Loricera, J.[1], Corrales, A.[1], Bejerano, C.[1], Martínez Taboada, V.[1], San Segundo, D.[1], Blanco, R.[1], López-Hoyos, M.[1]

AUTHORS AFFILIATION

(1) University Hospital Marqués de Valdecilla. Santander.

ABSTRACT

Background: Rheumatoid arthritis (RA) is a multifactorial autoimmune diseasecharacterized by chronic jointinflammation with different treatment options. The new JAK-STAT inhibitors (JAKinibs) are a promising therapeutic approach. Different JAKinibs are currently approved for RA treatment. However, the JAK-STAT signaling target differs from JAKinibs in non-selective and JAK-selective.

Aims: To assess the potential differential impact of JAKinibs in innate and adaptive immune cells in RA patients by flow cytometry.

Methods: 73 RA patients treated during a mean of 21.5 months withJAKinibs (non-selective: Baricitinib (Bari) or Tofacitinib (Tofa) and JAK-1 selective:Upadacitinib (Upa)or Filgotinib (Filgo) were recruited. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient Ficoll. Multiparametric flow cytometry was performed for immunophenotype characterization of different subsets of Monocytes, NK, T, and B cells acquired inNavios EX cytometer (Beckman Coulter).

Results & Conclusions: 35 (48%) with Bari, 13 (18%) with Tofa, 8 (11%) with Upa, and 17 (23%) with Filgo- treated RA patients were compared. Asignificant decrease in the frequency of classical monocytes in Tofa compared with Filgo and Upa (80.2 and 87.3, and 88.7, respectively; p values: 0.044 and 0.011) was observed.Moreover, CD45RO+Th17 cells with BARI vs. TOFA (8.7 vs. 18.3) were significantly reduced ($p<0.001$). Contrary, treatment with Tofa reduced CD45RO+Th1 cells vs.others (38.1 vs.Bari46.3, $p=0.043$, vs. Filgo47.9, $p=0.005$ vs.Upa51.5, p value=0.011).

The JAK-STAT inhibition by JAKinibs impacts innate and adaptive immune system cells differently. The non-selective JAKinibs:Tofacitinib in RA patients reduces the frequency of classical monocytes and Th1 cells, whereas Baricitinib reduces Th17 cells. Contrary, JAK-1 selective JAKinibs (Upadacitinib and Filgotinib) showed a lower impact on these immune cells.Extensive studies should be addressed to define better the modulation of JAK-STAT pathways by JAKinibs and the different impacts on immune-mediated diseases.

55. POSTER

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM: A RARE DIAGNOSIS

AUTHORS

Matos Silva, M.[1], Ramos, I.[2], Fernandes, B.[3], Faria, C.[3], Palmeira, C.[3], Sousa, E.[3], Godinho, I.[3], Henrique, R.[4], Martins, Â.[2], Mariz, J.[2], Martins, G.[3]

AUTHORS AFFILIATION

(1) Department of Clinical Pathology of Centro Hospitalar do Tâmega e Sousa. Penafiel.
(2) Department of Onco-Hematology of Instituto Português de Oncologia Francisco Gentil do Porto, Porto.
(3) Department of Clinical Pathology of Instituto Português de Oncologia Francisco Gentil do Porto, Porto.
(4) Department of Anatomical Pathology of Instituto Português de Oncologia Francisco Gentil do Porto, Porto.

ABSTRACT

Background: Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) is a rare and aggressive neoplasm. The precise incidence of this malignancy is difficult to estimate since there have been recent changes in nomenclature and diagnostic criteria.

Most patients present with cutaneous lesions, with or without bone marrow (BM) or central nervous system (CNS) involvement. Extreme cases can develop leukemic dissemination and are associated with a worse prognosis.

Aims: To raise awareness about BPDCN manifestations and immunophenotypical characteristics by sharing a case report.

Methods: Clinical data was collected from the electronic medical record. Immunophenotyping was performed using the BD FACSCANTO II flow cytometer and cell analysis was done with Infinicyt software.

Results & Conclusions: A 49-year-old male with a cutaneous violaceous lesion on the right shoulder that developed 2 years prior, had now developed several similar new lesions on the scalp and trunk. A biopsy of the shoulder lesion was performed and investigation with flow cytometry immunophenotyping of the BM showed 2,63% of blasts CD4+, CD 25-/+, CD36+, CD38-/+, CD56++, CD71+, CD123+, HLADR++, CD2-, cCD3-, CD7-, CD9-, CD11b-, CD11c-, CD13-, CD14-, CD15-, CD16-, CD19-, CD20-, CD22-, CD33-, CD34-, CD35-, CD41-, CD42B-, CD61-, CD64-, CD105-, CD117-, CD203c-, MPO-, NG2-, TdT-, which suggested the diagnosis of BPDCN. Flow cytometry immunophenotyping of the liquor showed CNS involvement. The histology of the cutaneous lesion corroborated the diagnosis.

The diagnosis of BPDCN stage IV was made and patient was proposed to first line treatment chemotherapy with Hyper-CVAD followed by allogenic hematopoietic cell transplant. After the first cycle, there was no evidence of CNS involvement, and after 4 cycles a BM assessment for minimal residual disease was negative. He is presently on the 7th cycle and with no clinical evidence of disease and has a related BM donor.

57. POSTER

STUDY OF THE CLONALITY OF T CELLS WITH CD7- PHENOTYPE IN THE VITREOUS HUMOR OF A PATIENT WITH SUSPECTED OCULAR LYMPHOMA

AUTHORS

Ballester González, R.[1], Rodríguez Martín, E.[1], Roy Ariño, G.[1], Manterola Navarro, E.[1], Ciancas, E.[1], Villar Guimerans, L.[1], Roldán Santiago, E.[1]

AUTHORS AFFILIATION

(1) Ramón y Cajal University Hospital. Madrid,

ABSTRACT

Background: Differential diagnosis between pathologies that involve cellular infiltrate in the vitreous body is complex due to the overlapping of their clinical manifestations, although the pathogenesis of these diseases may be disparate. There are studies that postulate the possibility of using flow cytometry (FC) as a complementary diagnostic technique. However, the antigenic profiles of the cell populations in the vitreous humor (VH), where the volume obtained and the cellularity are scarce, are not accurately known.

Aims: Clinical case of a patient with bilateral panuveitis, vitritis and retinal infiltrates who underwent diagnostic/therapeutic vitrectomy. Samples were analyzed to rule out infectious disease or lymphoma as the main suspected diagnosis.

Methods: The antigenic profile of VH cell populations was determined by FC using a FACSCanto II cytometer. Clonality analysis in T lymphocytes was established with the Vbeta Repertoire TCR kit. Cytokine levels in VH were performed by using the Human Th1/Th2/Th17 CBA kit.

Results & Conclusion: Immunophenotyping by FC showed absence of B lymphocytes, increased CD4/CD8 ratio (75,4%/2,8%) and loss of CD7 antigen in most of the T population. The TCR Vbeta Repertoire study ruled out clonality of the CD7- T cells. Cytokine determination in the VH revealed a highly inverted IL- 10/ IL-6 ratio (17 pg/ml / 4.099 pg/ml). Microbiological analyses were negative and histological study of a skin punch sample revealed non-necrotizing sarcoid granulomas.

The vitrectomy was initially suggestive of ocular lymphoma. However, CF clonality study ruled out this diagnosis despite of the presence of an anomalous T cell phenotype. The presence of an elevated CD4/CD8 ratio and sarcoid skin granulomas, reoriented the diagnostic suspicion towards a possible ocular sarcoidosis.

62. POSTER

BAFF-R EXPRESION DINAMIC AFTER ADMINSTRATION OF AN ORAL PROTEASOME INHIBITOR AS PROPHYLAXIS FOR CHRONIC GRAFT VERSUS HOST DISEASE

AUTHORS

Jimenez-Leon, M.[1], Delgado-Serrano, J.[2], Sanoja-Flores, L.[2], Ruiz, V.[2], Lopez-Corral, L.[3], Valcarcel, D.[4], Ferra- Coll, C.[5], Caballero-Velázquez, T.[2], Pérez-Simón, J.[2]

AUTHORS AFFILIATION

(1) Hospital Universitario Virgen del Rocio.
(2) Department of Hematology, Institute of Biomedicine of Seville (IBiS), University Hospital Virgen del Rocío of the "Consejo Superior de Investigaciones Científicas" (CSIC), University of Seville, Seville, Spain.
(3) Haematology Department, Complejo Asistencial Universitario de Salamanca-IBSAL, Centro de Investigación del Cáncer-IBMCC, Salamanca, Spain.
(4) University Hospital Vall d'Hebron, Barcelona, Spain.
(5) Servicio de Hematología Clínica, Institut Català d'Oncologia-Hospital Germans Trias i Pujol, Badalona; Institut de Recerca Contra la Leucèmia Josep Carreras, Universidad Autónoma de Barcelona, Barcelona, España; Universitat de Vic, Universitat de Central de Catalunya, Vic, Barcelona, España.

ABSTRACT

Background: Chronic graft-versus-host disease (cGVHD) continues being a major cause of late post- transplant morbidity and mortality. One of the most consistent B-cell associated abnormalities identified in cGVHD is the high levels of soluble B-cell activating factor (BAFF). BAFF and its principal receptor on B- cells, BAFF receptor (BAFF-R/ CD268), are critical factors for normal B-cell maturation and survival.

Aims: The aim of the present study is to evaluate the effect of a second-generation oral proteasome inhibitor administration, that has been shown in preclinical models to prevent GVHD, on the different B- cells subsets.

Methods: 73 participants (33 control and 39 treatment group) were enrolled in a prospective trial. Patients were randomized to receive or not ixazomib from d+100 up to 18 months postrasplant. B-cell subsets and the expression of BAFF-R were quantified by multiparametric flow cytometry on samples obtained on days +100 and +180 post-transplantations. Peripheral blood samples were collected in EDTA tubes and stained immediately using the following antibodies anti-CD38 (FITC); anti-CD27 (PE); anti-CD5 (PerCP-Cy5.5); anti-CD19 (PE-Cy7); anti-SIgM (APC); anti-CD24 (APCH7); anti-CD268 (V450) and anti-CD45 (V500). Samples were acquired using CANTO-II Cytometer (BD Immunocytometry Systems) and analyzed using Infinicyt software (Cytognos S.L.).

Results & Conclusion: No differences in total B-cells and naïve, transitional, un-switched memory B-cells, switched memory B-cells and plasmablasts were detected between control and treatment groups. Nevertheless, a significant difference of the mean fluorescence intensity (MFI) of BAFF-R on day +180 (median: 8093; range: 1343-16496) respect +100 (6625, 39-13883) was detected in the control group (p=0.036). Furthermore, the increase of BAFF-R was significant higher in the control as compared to the treatment group (p=0.02) and its was significantly increased in patients with severe cGVHD (p=0.011) (-310; -8954-11437 vs 4435, -1264-8283).

Expression of BAFF-R is significantly increased in patients with cGvHD and decreases in patients receiving ixazomib as cGvHD prophylaxis.

66. POSTER

APPLICATION OF APTAMERS IN THE IDENTIFICATION OF LEUKEMIC STEM CELLS AND NORMAL HEMATOPOIETIC STEM CELL AND PROGENITORS BY FLOW CYTOMETRY

AUTHORS

Fernández, G.[1], Pérez De Camino Gaisse, B.[2], Soto, C.[2], Salgado, R.[2], Izquierdo Barba, I.[3], Serrano Del Castillo, C.[4], Serrano, J.[5], Llamas Sillero, P.[2], González, V.[6], Serrano López, J.[7]

AUTHORS AFFILIATION

(1) Aptus Biotech S.L.
(2) Hematology Department, Hospital Universitario Fundación Jiménez Díaz, UAM, Madrid, Spain.
(3) Departamento de Química en ciencias Farmacéuticas, Facultad de Farmacia, Universidad Complutense de Madrid, Instituto de Investigación Sanitaria Hospital 12 de Octubre i+12, Plaza Ramón y Cajal s/n Madrid, 28040, Spain.; CIBER de Bioingeniería Biomateriales y Nanomedicina CIBER-BBN, Madrid, 28040, Spain.
(4) Immunology department, Hospital Universitario Fundación Jiménez Díaz, UAM, Madrid, Spain.
(5) Hematology Department, Reina Sofía University Hospital/Maimonides Biomedical Research Institute of Córdoba (IMIBIC)/University of Córdoba, Spain.
(6) Grupo de Aptámeros, departamento de Bioquímica-Investigación, IRYCIS-Hospital Ramón y Cajal, 28034 Madrid, Spain.
(7) Experimental Hematology Lab, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain.

ABSTRACT

Background: Leukaemic stem cells (LSCs) are the malignant transformation of haematopoietic stem and progenitor cells (HSCPs). Both LSC and HSCP are quite similar and share molecular markers, cell cycle stages and circadian rhythms, making it difficult to distinguish between them. Aptamers are artificial short single-stranded oligonucleotides, selected and generated through and in vitro molecular method. Size of aptamers differ from 20 to 80 nucleotides. They have high binding affinity and specificity towards a wide range of targets.

Aims: Here we searched for new surface molecules based on aptamer technology that discriminate between normal and leukaemic progenitor cells.

Methods: We isolated CD34+ cells from normal bone marrow or mobilized product and primary acute myeloid leukaemia samples. Then, we performed Cell-SELEX approach to describe a specific library of aptamers against LSC. Several bioinformatics tools have been used to predict their three-dimensional structure. LSC-aptamers were labeled with Cy7. To validate our LSC library we used flow cytometry, and the analysis was carried out in FACSDiva software and FlowJo v10.

Results & Conclusion: After six rounds of screening assay, a LSC-library of nine aptamers showed the highest affinity to distinguish LSC-CD34+ and not HSCP-CD34+. LSC library was validated in primary AML samples (N=20) and normal bone marrow samples (N=10). 3 out of 9 aptamers retain their specific affynity for LSC-CD34+. Conclusion: we have successfully developed an innovative strategy to characterize new possible surface markers on LSC. The precise identification of those surface molecules should be the subject of further studies.

67. POSTER

EXTRACELLULAR VESICLES FROM SORTED MYELOID-DERIVED SUPPRESSOR CELLS MAINTAIN SPECIFIC STAINING FROM SORTING

AUTHORS

Marquina Rodríguez, Á.[1], Ortega, M.[2], García-Lorena, P.[3], Barroso-García, G.[4], Arandilla, A.[4], González López, M.[1], Clemente, D.[2], Vila-del Sol, V.[1]

AUTHORS AFFILIATION

(1) Extracellular Vesicles Unit-UVEx, Hospital Nacional de Parapléjicos, SESCAM; Flow Cytometry Unit, Hospital Nacional de Parapléjicos, SESCAM, Toledo.
(2) Neuroimmuno Repair Group, Hospital Nacional de Parapléjicos, SESCAM, Toledo; Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Carlos III Health Institute, Madrid (Spain).
(3) Neuroimmuno Repair Group, Hospital Nacional de Parapléjicos, SESCAM, Toledo.
(4) Extracellular Vesicles Unit-UVEx, Hospital Nacional de Parapléjicos, SESCAM; Proteomics Unit, Hospital Nacional de Parapléjicos, SESCAM, Toledo.

ABSTRACT

Background: Myeloid-Derived Suppressor Cells (MDSCs) are a heterogeneous population of immature myeloid cells with immunosuppressive activity, related to the recovery of the clinical symptoms observed in experimental autoimmune encephalomyelitis (EAE), the animal model of Multiple Sclerosis (MS). Extracellullar Vesicles (EVs) are important mediators of cellular communication, and their function is mediated by their protein and RNA cargo. To evaluate the functional role of MDSCs-derived EVs on immunosuppression, their characterization needs to be addressed.

Aims: Characterization of MDSCs-derived EVs by Flow Cytometry (FC) and western blot (WB)

Methods: CD11b+ Ly-6Chi Ly-6G-/lo MDSCs were isolated from spleens of EAE mice by FACS and cultured in free-EVs medium for 24 hours. EVs were isolated by centrifugation, ultrafiltration, and Size Exclusion Chromatography (SEC). EVs were detected by FC using anti-CD45, anti-Ly-6C for MDSCs and anti-CD81 antibodies, following MIFlowCyt-EV guidelines. Results were corroborated by WB.

Results & Conclusion: EVs isolated from MDSCs culture medium expressed CD45 and Ly-6C, specific marker in sorted MDSCs. Ly-6C and CD81 proteins were detected by WB in EVs. To improve EVs isolation method, SEC was introduced in the protocol. FC analysis of SEC fractions without staining showed events with fluorescence in B525/40 channel, and VSSC signals compatible with EVs. Ly-6C protein was detected by WB in the same fractions, suggesting that fluorescence might be due to residual antibody used for MDSCs-sorting in EVs surface. By contrast, CD11b was not detected on non-stained EVs. Next, MDSCs were sorted adding anti-CD81, and isolated EVs analysed by FC. Only few EVs were CD81+ Ly-6C+. Staining with anti-CD81 of pooled EVs fraction revealed that CD81 was not co-expressed with Ly-6C in large EVs, whereas there was CD81 dim expression on small EVs. In conclusion, EVs from previously sorted cells maintain antibodies bounded to high-expressed proteins on their surface, and it must be considered its effect in later functional studies.

68. POSTER

TUMOR-BONE MARROW INTERPLAY IN MELANOMA INVOLVING THE GROWTH FACTORS MIDKINE AND PLEIOTROPHIN

AUTHORS

Poluha, L.[1], Leal Lasarte, M.[1], Catena Parrado, X.[1], Juan Larrea, N.[1], Martínez, L.[1], Soengas, M.[1]

AUTHORS AFFILIATION

(1) Centro Nacional de Investigaciones Oncologicas, Madrid.

ABSTRACT

Bone marrow mesenchymal stromal cells (MSC) constitute a heterogeneous population of cells with key implications in the hematopoietic microenvironmental niche both under steady-state and stress conditions. Furthermore, it has been demonstrated that bone marrow MSCs are recruited to primary tumors and metastasis where they are functionally important for tumor growth and invasion. In addition, it has been reported that bone marrow MSC are able to differentiate into distinct subpopulations of cancer-associated fibroblasts, which have distinct functions regulating immune surveillance, tumor growth and angiogenesis. However, the exact functions of MSC in the tumor microenvironment are not well characterized, as it is reported that MSC can either promote or inhibit tumor progression.

Our group is interested in whether and how melanoma cells modulate bone-marrow precursors, MSC in particular.

Using a series of animal models combined with comprehensive analyses of patient tissue biopsies and datasets, we have previously identified the growth factor MIDKINE (MDK) as a new driver of pre-metastatic niches in melanoma. Moreover, our laboratory has identified reported MDK as an immune suppressor.

Analyzing single-cell RNA sequencing data from bone marrow cells, MDK is particularly expressed in a subtype of reticular cells (Cxcl12-positive). These cells do not express the same levels of pleiotrophin (PTN), a protein that is highly homolog to MDK. This distinct expression of MDK and PTN may offer the possibility of differing roles in melanoma progression and modulation of immunomodulatory signals.

70. POSTER

ANTITUMORAL ROLES OF MIDKINE VIA B CELLS

AUTHORS

Juan Larrea, N.[1]

AUTHORS AFFILIATION

(1) Centro Nacional de Investigaciones Oncológicas. Madrid.

ABSTRACT

Background: Metastasis to lymph nodes (LN) is a frequent and early event in melanoma dissemination and is associated with poor prognosis. Tumor-draining LN are the first lymphoid sites that encounter tumour-associated antigens and secreted factors. While the intrinsic role of the LNs should be to ultimately favour the recognition and clearance of malignant cells, pro-tumoral responses can be activated depending on a variety of intrinsic and extrinsic cues. The role of macrophages as well as dendritic cells has long been studied as major Antigen Presenting Cells (APCs). B cells can also act as APCs (in addition to their inherent function in antibody production), but the extent to which these activities of B cells are engaged at pre-metastatic niches is not well understood. Moreover, B cells can acquire immune suppressive traits (i.e., acting as Bregs), but the underlying contribution of this antitumoral role in melanoma has yet to be defined. Our group has identified the growth factor MIDKINE (MDK) as a tumor- secreted protein that favors metastasis to lymph nodes and visceral sites by generating an immune- suppressive microenvironment.

Aims: Define whether and how MDK affects B cell function, both, during melanoma genesis and in the response to immunotherapy.

Methods: Using different models of melanoma with different levels of MDK expression, we have found an inverse correlation between MDK expression and the number of B Cells in the tumor draining LN. Flow cytometry, single-cell RNASeq.

Results & Conclusion: Preliminary data suggest that that MDK arrests B Cells in the draining LNs, which may reduce antigen presentation and antibody production, ultimately favouring tumour progression.

71. POSTER

TREATMENT SCHEME AFFECTS LYMPHOCYTE SUBSETS IN SLE PATIENTS

AUTHORS

Jimenez de las Pozas, Y.[1], López Brull, Á.[1], Castañón Morales, S.[1], Gonzalo Hernández, R.[1], Sánchez Pernaute, O.[1], Sánchez Blázquez, M.[1], Serrano, C.[1]

AUTHORS AFFILIATION

(1) H.U. Fundación Jiménez Díaz. Madrid.

ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease where lymphocytes play a relevant pathogenic role such as generation of memory B cells, plasma cells and long- lived effector T cells. Despite its importance, there are few studies that describe its role in classifying patients or defining clinical outcomes.

Aims: To compare leukocyte and lymphocyte subsets from SLE patients with different treatment schemes.

Methods: Retrospective study of T subpopulations, NK cells, B cells and plasma cells (PC) in a cohort of treated SLE patients. Complete blood count, IgG, IgA, IgM immunoglobulin levels and clinical data were collected after reviewing medical records.

Results & Conclusion: We studied 39 patients (mean age 49±13 years, 95% women) with SLE in treatment with: corticoids (CS, in any scheme) 26%; immunomodulators (IMD, in any scheme) 54%; rituximab (RTX) 8%; and others,13%. Median immunoglobulin levels, B, T and NK lymphocytes were within age reference values. We observed differences between patients treated with IMD and patients treated with CS in absolute leukocyte count (4775 cel/ul vs 7507 cel/ul, p=0.0038), monocytes (434 cel/ul vs 692 cel/ul, p=0.0014) and lymphocytes (1429 cel/ul vs 2538 cel/ul, p=0.0029).

In the analysis of the different lymphocyte subsets, we found that 50% of the patients present NK lymphopenia without any differences in median NK lymphocytes between groups. Patients treated with IMD had a lower absolute T cell count compared to CS treated patients (1180 cel/ul vs 2181 cel/ul, p=0.0037) due to CD4 lymphocytes (640 cel/ul vs 1282 cel/ul, p=0.0003).

Regarding B cells, we could not find any differences between groups, nevertheless, 50% of our patients present more than 2% of PC. There were not any differences in serum immunoglobulins between any group.

There are no significant changes in SLE patients treated with different therapeutic regimens. However, NK lymphopenia and presence of PC are common in some patients.

72. POSTER

LYMPHOCYTE SUBSETS IN A IGA DEFICIENCY COHORT THAT INCLUDES PATIENTS WITH CELIAC DISEASE

AUTHORS

López Brull, A.[1], Jimenez de las Pozas, Y.[1], Gonzalo Hernández, R.[1], Castañón Morales, S.[1], Farrais Villalba, S.[1], Sánchez Blázquez, M.[1], Serrano, C.[1]

AUTHORS AFFILIATION

(1) H.U. Fundación Jiménez Díaz. Madrid.

ABSTRACT

Background: Celiac disease (CD) is a chronic digestive and autoimmune disorder. It has been associated with functional asplenia and a certain degree of immunodeficiency. The incidence of CD is higher in patients with IgA deficiency (IgAD) and it is unknown whether this associated risk factor may affect the immunological characteristics of these patients.

Aims: To study differences between immunological status in patients with IgAD with and without CD.

Methods: TCD3, TCD4, TCD8, BCD19, NK lymphocyte populations and B cell subpopulations (naive, memory, plasma cells) were performed in a group of IgAD. Total IgG, IgA, IgM and IgG1, IgG2, IgG3 and IgG4. Clinical data were recorded after reviewing medical records.

Results & Conclusion: We studied 77 patients with IgAD of which 15 (19.5%) have CD. We found a similar incidence of other autoimmune diseases in both CD and non-CD patients (20%vs 21%) being autoimmune thyroiditis the most prevalent. TCD3, TCD4, TCD8, BCD19, NK and B lymphocyte subpopulations were within reference values. Comparing CD vs non-CD IgAD patients, we found significative differences between absolute CD3 lymphocyte counts (1804±990cel/ul vs 1525±502cel/ul p<0.0001), CD4 (1067±615cel/ul vs 866±308cel/ul p=0.0006) and CD19 (418± 311cel/ul vs 221±117cel/ul p=0.0009). Regarding immunoglobulin levels, we found 30/77 with IgG levels above reference values (>1600 mg/dL) however, when we compare IgG subclasses median levels, we do not find any differences with the reference population.

We could not find any differences between IgAD patients and healthy controls according to literature in the different lymphocyte subsets. Although CD tended to have more total B cells, these patients did not present a decrease in memory populations, a marker of functional asplenia. The increase in IgG levels described in some IgA is not associated with changes in memory B populations. Patients with IgAD and CD do not present greater immunosuppression data than IgAD patients without CD.

73. POSTER

ASSESSMENT OF HEMATOPOIETIC STEM CELLS AND COMMITTED PROGENITOR SUBSETS IN PRIMARY SAMPLES OF CORD BLOOD, BONE MARROW AND MOBILIZED PERIPHERAL BLOOD

AUTHORS

Sánchez Domínguez, R.[1], Alberquilla, O.[1], Ojeda Pérez, I.[1], Navarro Ordoñez, S.[1], Río Galdo, P.[1], Quintana Bustamante, Ó.[1], Gimeno Martínez, R.[2], Segovia, J.C.[1]

AUTHORS AFFILIATION

(1) CIEMAT-CIBERER / IIS-FJD
(2) IMIM

ABSTRACT

Background: Hematopoietic stem cell transplantation (HSCT) is the transplant of multipotent hematopoietic stem and progenitor cells (HSPCs) to regenerate the hematopoietic system. HSCT is the most extended cell therapy since patients suffering from malignant or non-malignant hematopoietic disorders benefit from it. HSCT success depends on the quality and number of the infused cells. Different sources, such as bone marrow (BM), mobilized peripheral blood (mPB) and umbilical cord Blood (CB), of HSPCs are used in the clinics. However, there are experimental and clinical pieces of evidence describing differences in the repopulating capabilities, such as engraftment and reconstitution kinetics, among the different HSPC sources.

Aims: To find factors able to explain the differences in hematopoietic engraftment of BM, mPB and CB, as sources of HSC, we aim to characterize the composition in the HSPC subset of these three sources.

Methods: Here we describe two flow cytometry panels to characterize HSPC subset in CB, mPB and BM, based on the differential expression of the stem cells markers by flow cytometry. Panel 1 consists in determining the expression of CD34, CD38, CD45RA, CD90 and CD7 (panel 1) and some additional functional tests, such as in vitro differentiation potential and colony forming studies. Additionally, panel 2 was used to investigate HSPC hierarchy in CD34+ cells deeply by analyzing of CD38, CD10, CD7, CD135, CD90, CD45RA and CD49f.

Results & Conclusion: We have demonstrated that the highest proportion of CD45RA-CD90-, highly enriched in MPP progenitors (a step forward of HSC) is in mPB. Additionally, there is an increment in common myeloid progenitor (CMP) and in megakaryocyte/erythrocyte progenitor (MEP) subsets in mPB in comparison to CB or BM. These differences might explain the faster recovery of immune system or neutrophil/platelets recovery reported after mPB transplantation. Understanding differences at the HSPC compartment in different CD34+ sources might improve the efficacy of HSCT.

74. POSTER

ESTABLISHMENT OF BASIC QUALITY GUIDELINES TO STUDY OF PLATELET ACTIVATION IN A BONE MARROW FAILURE SYNDROME BY LACISEP

AUTHORS

Alberquilla, O.[1], Sánchez Domínguez, R.[1], Lozano Vinagre, M.[1], Palma Barqueros, V.[2], Navarro Ordoñez, S.[1], Sevilla, J.[3], Díaz De Heredia, C.[4], Bueren, J.[1], Río Galdo, P.[1], Segovia, J.C.[1]

AUTHORS AFFILIATION

(1) CIEMAT-CIBERER / IIS-FJD. Madrid.
(2) Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, Murcia, Spain.
(3) Hospital Niño Jesus, Madrid.
(4) Hospital Vall d´Hebron, Barcelona.

ABSTRACT

Background: Fanconi anemia (FA) is a rare disease mainly characterized by bone marrow failure, congenital abnormalities, and cancer predisposition. The follow-up of the hematopoietic stem and progenitor cells (HSPCs) in the bone marrow and peripheral blood of these patients has been routinely evaluated in the Spanish FA patients. Among other features, the patients are characterized by a low platelet count due to the bone marrow failure progression and a decreased platelet reactivity.

Aims: Our aim has been to establish the basic quality guidelines to study the platelet content and activation in human FA peripheral blood to apply it the follow up of both untreated and treated patients by different approaches.

Methods: To this purpose, platelet were labeled with CD41/CD62/PAC1 antibodies, specific binding of labeled Fibrinogen-Alexa488 and analized after stimulation with physiologic agonists such as adenosine diphosphate (ADP 25µM), Phorbol myristate acetate (PMA 200nM) and Thrombin Receptor Activator Peptide 6 (PAR-1 50µM). We assessed by flow cytometry platelet function in 16 FA patients and healthy donors peripheral blood samples harvested using citrate.

Results & Conclusion: Our results have shown a diminished activation of platelets in our FA cohort in comparison with healthy donors. Both signal of PAC-1 and Fibrinogen-Alexa488 decreased after stimulation with PMA while no differences were observed after PAR-1 and ADP stimulus.

In summary, flow cytometry is a feasible strategy to analyze platelet function in FA patients and will contribute to understand the pathophysiology of the thrombocytopenia in FA and other bone marrow failure syndromes.

75. POSTER

CONGENITAL LEUKEMIA OR TRANSIENT ABNORMAL MYELOPOIESIS? A CASE REPORT

AUTHORS

Santos, F.[1], Martins Pereira, F.[2], Ventura, V.[2], Moreira, R.[3], Henriques PInto, D.[4], Menezes, F.[5], Ferreira, J.[6], Loureiro, I.[6], Lau, C.[2], Teixeira, M.[2]

AUTHORS AFFILIATION

(1) Cytometry laboratory, Centro Hospitalar Universitário de Santo António. Porto.
(2) Cytometry Laboratory, Unidade de Diagnóstico Hematológico Margarida Lima, Centro Hospitalar Universitário de Santo António. Porto.
(4) Pediatric and Neonatology Department, Centro Hospitalar Entre Douro e Vouga. Santa Maria da Feira.
(5) Neonatal Intensive Care Unit, Pediatric and Neonatology Department, Centro Hospitalar Entre Douro e Vouga. Santa Maria da Feira.
(6) Clinical Pathology Department, Centro Hospitalar Entre Douro e Vouga. Santa Maria da Feira.

ABSTRACT

Background: Children with Down Syndrome (DS) have a 500-fold increased incidence of Acute Megakaryoblastic Leukemia (AMKL). Neonates with DS are also predisposed to exhibit Transient Abnormal Myelopoiesis (TAM), with circulating blasts indistinguishable from AMKL by routine methods. Most TAM patients are clinically asymptomatic at presentation, but they may develop mild to severe symptoms if blasts infiltrate some organs. The main difference is that TAM typically undergoes spontaneous remission in the first months, contrary to AMKL. Diagnosis is essential to decide initial therapy, but it can be challenged.

Aims: Demonstrate the importance of flow cytometry (FCM) in differential diagnosis between TAM and acute leukemia in patients with DS.

Methods: We describe a case of TAM in a neonate with prenatal diagnosis of Trisomy 21 (T21).

Results & Conclusion: A late pre-term neonate (36w3d) with T21 was admitted in Neonatal Intensive Care Unit with transient tachypnea of the newborn and type 1 respiratory failure. On physical examination, he presented tachypnea, hypotension and global hypotonia, without peripheral lymphadenopathies or organomegalies. Cell Blood Counts (CBC) revealed 52000 leucocytes/µL, hemoglobin of 19.8 g/dL and platelet count of 60000/µL. At peripheral blood smear there were 43.5% blasts with high nucleus- cytoplasm ratio, lax chromatin, and visible nucleoli. FCM showed blasts with positivity for immaturity (CD34, HLA-DR), myeloid (low CD13, CD33, CD117) and T-cell (CD7) markers, without expression of CyCD3, CD41, CD42b, CD56, cyCD79a or MPO. Possible TAM was assumed. Patient underwent oxygen therapy and hyperhydration. CBC normalized after 1 month, without abnormalities in FCM study. The patient remains well after 10m of follow-up.

TAM is rare, but it should be a diagnostic hypothesis in presence of a neonate with peripheral blasts. This case illustrates that TAM blasts are virtually indistinguishable from AMKL blasts, but FCM can help to differentiate them. This patient only required supportive treatment and progressively recovered without chemotherapy.

76. POSTER

THE ROLE OF FLOW CYTOMETRY IN THE DETECTION AND CHARACTERIZATION OF GAMMA- DELTA T-CELL ENTITIES: MONOMORPHIC EPITHELIOTROPIC INTESTINAL T-CELL LYMPHOMA WITH LUNG INVOLVEMENT AND ITS IMMUNOPHENOTYPIC COMPARISON WITH OTHER GAMMA-DELTA T-CELL PROLIFERATIONS

AUTHORS

Martín Moro, F.[1], Ballester González, R.[1], Piris Villaespesa, M.[1], Martín Rubio, I.[2], García-Cosío Piqueras, M.[1], Rodríguez Martín, E.[1], Chen, S.[2], Roldán Santiago, E.[1], Delgado Trillo, I.[2], García Vela, J.[3]

AUTHORS AFFILIATION

(1) Ramón y Cajal University Hospital. Madrid.
(2) University Hospital of Getafe. Madrid.
(3) University Hospital Puerta de Hierro. Madrid.

ABSTRACT

Background: Gamma-delta (γδ) T-cell lymphomas are rare and aggressive neoplasms with diagnostic complexity, being the multiparametric flow cytometry (FCM) a technique scarcely use in some cases.

Aims: To compare the phenotype of γδ T-cell entities based on the presentation of a clinical case of monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL).

Methods: FCM was assessed by an eight-colour instrument (BD FACSCanto II) using the LST and T-CLPD panels (EuroFlow). Five cases of γδ T-cell entities were studied, one in bronchoalveolar lavage (MEITL) and four in peripheral blood: hepatosplenic T-cell lymphoma (HSTL), γδ T-cell large granular lymphocytic leukemia (γδ T-LGL), γδ T-cell acute lymphoblastic leukemia (γδ T-ALL), and reactive γδ T-cell expansion. Differences in antigen expression were analysed by comparison of mean fluorescence intensities.

Results & Conclusion: The MEITL case consisted in a 46-year-old male with lung involvement by the disease. FCM assessed in the bronchoalveolar lavage demonstrated an infiltration of abnormal T-cells with the following immunophenotype: CD3-/+ γδ+ CD45+ CD4- CD8+ CD5- CD56++ CD2+low CD197- CD45RA+ CD57+het CD94- CD16-/+low. When compared with the other cases, the combination of CD3/CD4/CD8/CD5/CD56/CD16/CD57/CD94 was useful to classify each case. MEITL was the only one CD4-/CD8+ case, being the other tumoral ones CD4- and variably CD8-/+low. CD5 was -/dim in all tumoral cases, as well as CD3 was dim in HSTCL, γδ T-ALL and MEITL; γδ T-cells in the reactive case maintained bright expression of CD3 and CD5. CD56 was +/++ in HSTCL, γδ T-LGL and MEITL, while the higher CD16 expression was detected in HSTCL and γδ T-LGL. The higher expression of CD57 was seen in γδ T-LGL and MEITL, being CD94 bright in HSTCL, γδ T-LGL and reactive cases. To conclude, FCM can characterize γδ T- cell entities by a simple marker combination, and the assessment of samples beyond blood and bone marrow may be informative in those rare cases.

79. POSTER

MEASURABLE RESIDUAL DISEASE (MRD) ASSESSMENT IN THE MANAGEMENT OF MULTIPLE MYELOMA (MM) IN A REAL-WORLD SETTING

AUTHORS

Caetano, J.[1], Coelho, I.[2], Lúcio, P.[1], João, C.[1]

AUTHORS AFFILIATION

(1) Hemato-Oncology Unit - Champalimaud Clinical Center. Champalimaud Foundation.
(2) Hematology Department - Portuguese Institute of Oncology Lisbon.

ABSTRACT

Background: Growing evidence suggests that the goal of Multiple Myeloma (MM) treatment should be to achieve the deepest remission possible. Patients with Measurable Residual Disease (MRD) negativity showed longer progression-free and overall survival. Next Generation Flow (NGF) represents a standardized, sensitive, and available approach for MRD assessment. IInvestigation on its effectiveness to support routine clinical decisions is ongoing.

Aims: Evaluate the feasibility of implementing MRD assessment by NGF in real-world MM patient management.

Methods: Real-world data from newly diagnosed MM patients was gathered between 2014 and 2018 at 2 institutions. Patients' treatment followed standard-of-care guidelines, according to their eligibility or ineligibility for autologous stem cell transplant (ASCT). Bone marrow (BM) aspirates were obtained for MRD evaluation using NGF according to EuroFlow guidelines.

Results & Conclusion: A total of 140 BM samples from 91 patients (59 male, 32 female; median age 68) were included. 10 patients had high-risk disease. In our cohort, 65% (59) of patients were in VGPR or better. All patients had at least 1 MRD evaluation, 40% (36) had 2, and 14% (13) had 3 or more. The sensitivity threshold was 10-5 in 50% of analyzed samples. In the remaining, this sensitivity level was not attained, mostly due to insufficient sample volume, low cellularity and/or hemodilution. MRD negativity rate overall was 30%. Noteworthy, 40% of high-risk patients achieved MRD negativity. Of the patients that had at least 2 evaluations, 54% maintained MRD negativity throughout the assessment period. Interestingly, after a median follow-up of 48 months of the 3 patients with MRD evaluations at least 12 months apart, all had sustained MRD negativity.

Our experience highlights that effective monitoring of MRD by NGF is feasible and can be incorporated as a biomarker of response in routine patient care. Real-world studies with long-term follow-up MRD data can provide critical insights to maximize treatment benefits.

81. POSTER

USEFULNESS OF IMMUNO-PHENOTYPING IN THE EARLY DIAGNOSIS OF POST-TRANSPLANT LYMPHO-PROLIFERATIVE DISEASE (PTLD): A CASE REPORT

AUTHORS

Portela Piñeiro, V.[1], Díaz Arias, J.[1], Mosquera Orgueira, A.[1], García Losada, J.[1], Rodríguez Mansilla, M.[1], Jorge Ríos, N.[1], Vallejo Llamas, J.[1]

AUTHORS AFFILIATION

(1) Complexo Hospitalario Universitario of Santiago de Compostela.

ABSTRACT

Background: Post-transplant lymphoproliferative disease (PTLD) is a rare, high-severity complication that occurs because of immunosuppression in the setting of allogeneic haematopoietic stem cell transplantation (alloHSCT) (approximately 2%). Most PTLD arise from B-cells and are usually related to Epstein-Barr virus infection. In the 2017 World Health Organization histological classification, six types are included, being the monomorphic, most of them. Immunophenotyping also provides information for the characterisation of PTLD, with 63% being centroblastic, 11% Burkitt or Burkitt-like, 22% plasmacytic features and 4% anaplastic.

Aims: The aim is to demonstrate the important role of immunophenotyping in PTLD diagnosis.

Methods: A case diagnosed with the EuroFlow panel in our centre was selected because of its medical interest and infrequency.

Results & Conclusion: We present a 58-year-old patient diagnosed in April 2022 with IgA lambda plasma cell leukaemia (15% of plasma cells in periheral blood (PB)), presenting 23.63% of atypical plasma cells in bone marrow (BM) at diagnosis with the following immunophenotype: CD38+, CD138+, CD19-, CD56+lo/+, CD45-, CD117-, CD27+lo, CD81+. She received treatment with chemotherapy first, and an alloHSCT was infused on 25/10. She was admitted due to fever on 30/12. Bone marrow immunophenotyping was performed, finding 0.13% in BM of atypical plasma cells with an immunophenotype different from that of the diagnosis, and 2.5% in PB (cytoplasmic kappa monoclonality, CD38+, CD138+, CD19+, CD56-, CD45+, CD117-, CD27+, CD81+), with undetectable minimal residual disease. FISH concluded that the origin of the clone came from the donor cells. This led to a diagnosis of PTLD and targeted treatment could be initiated. Use of immunophenotyping is helpful in the diagnosis of PTLD, as it offers the following advantages:

- Speed in obtaining the diagnosis, compared to histopathology.
- Characterisation of the abnormal phenotype, which may not be differentiated in the histological study. This could reduce the mortality associated with PTLD.

82. POSTER

USING FLOW CYTOMETRY TO UNCOVER CIRCULATING AND PERITONEAL NK CELL PROFILES IN ENDOMETRIOTIC WOMEN

AUTHORS

Martins, C.[1], Lourenço Reis, J.[2], Dias, M.[3], Borrego, L.[3], Ramos Lima, J.[3]

AUTHORS AFFILIATION

(1) NOVA Medical School/Faculdade de Ciências Médicas Universidade Nova de Lisboa, Lisbon, Portugal.
(2) Serviço de Ginecologia-Obstetrícia, Hospital da Luz Lisboa.
(3) Immunology, CHRC, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisboa, Portugal.

ABSTRACT

Background: Endometriosis is a chronic inflammatory disease characterized by the presence of endometriotic tissue outside the uterine cavity. It affects 10% of women of reproductive age, often associated with infertility. An increasing number of studies correlate the etiopathogenesis of endometriosis with immunological abnormalities, particularly in NK cells, their receptors, and functions.

Aims: Characterize the expression of NK receptors in women with endometriosis, to assess their potential clinical application in diagnostic and prognostic approaches.

Methods: Patients operated for endometriosis and control patients with benign gynaecological conditions were enrolled. Peritoneal fluid (PF) and peripheral blood (PB) samples were collected during surgery and analysed by flow cytometry (BD FACS Canto II), with CD45, CD3, CD8, CD56, CD57, activating receptors (CD16, CD96), inhibitory receptors (PD-1, KIR2DL1/CD158a, NKG2A, TIGIT, TIM-3, LAG-3, CD161) and cytokine receptor IL18Ra. Data analysis was performed with Infinicyt and FlowJo software.

Results & Conclusion: In the 38 endometriosis patients and 15 controls assessed, we were able to identify differences in the local and circulating subsets. As expected, two major circulating NK subsets were identified: the most abundant CD56dimCD16Hi (57-99% in patients; 86-97% in controls), and a less represented CD56HiCD16dim. This more immature subset was significantly increased in patients (p=0.039), also showing higher proportions of cells expressing CD57 (p=0.057). In PF samples 4 subsets were identified: the most abundant were CD56+/dimCD16Hi (23-87% in patients; 23-73% in controls) and CD56HiCD16- (5-81% in patients; 9-67% in controls), with smaller amounts of CD56HiCD16+ and CD56dimCD16- NK cells. Despite patients showed enlarged amounts of cytotoxic CD56+/dimCD16Hi cells, no significant differences were encountered. However, patients showed increased PF CD8+CD16Hi NK cells (p=0.010), CD56+CD16+PD1+ NK cells (P=0.010) and CD158a+NKG2a+ NK cells (p=0.021). Briefly, NK profiles are different in PB and PF, showing distinctive features in endometriosis patients that may hold a potential for prognostic and therapeutic applications.

83. POSTER

CELLULAR IMMUNE RESPONSES TO SARS-COV-2 INFECTION IN PAEDIATRIC PATIENTS

AUTHORS

Martins, C.[1], Dias, M.[2], Chasquerira, M.[3], Brito, M.[4], Milheiro Silva, T.[4], Matos, M.[4], Lopes, M.[2], Crespo, H.[2], Borrego, L.[5], Paixão, P.[6]

AUTHORS AFFILIATION

(1) NOVA Medical School/Faculdade de Ciências Médicas Universidade Nova de Lisboa, Lisbon, Portugal.
(2) Immunology, CHRC, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisboa, Portugal.
(3) CHRC, Infection, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisboa, Portugal.
(4) Hospital D. Estefânia, Centro Hospitalar Universitário de Lisboa Central (CHULC).
(5) Immunology, CHRC, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisboa, Portugal. Serviço de Imunoalergologia, Hospital da Luz Lisboa.
(6) CHRC, Infection, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisboa, Portugal. Serviço de Patologia Clínica, Hospital da Luz Lisboa.

ABSTRACT

Background: Despite SARS-CoV-2 infection in children is usually asymptomatic/mild, it can evolve to severe and life-threating illness, though children are less hospitalized than adults. However, in the current evolving scenario of SARS-CoV-2 infection there is still much to know about immunity in children, in both natural infection and vaccination settings.

Aims: To assess specific immune responses in children after SARS-CoV-2 infection.

Methods: Blood samples were collected from children recruited at Hospital D.Estefânia with a positive SARS-CoV-2 PCR/antigenic test, at infection and >6 months after. Humoral immunity was evaluated by chemiluminescent immunoassays, while cellular responses to S and N proteins were assessed by flow cytometry (Act-T4 Cell™ kit, Cytognos), after a 48h-stimulation period. Data analysis was performed with FlowJoTM and statistical analysis with GraphPadPrism.

Results & Conclusion: Fifty-eight children were assessed during active SARS-CoV-2 infection (41% females; mean age=6.7y), including an additional group of 12 vaccinated prior to infection. Also, 20 children were assessed at least 6-mo post-infection (7 previously assessed at infection). Cellular responses to S and N were detectable in 84% and 86% of children during infection, respectively, with a strong positive correlation between them ($p<0,0001$; $r=0,824$). Children<10y showed lower cellular responses (S and N) compared to those ≥10y, despite presenting similar responses to non-specific stimulus (PHA). Not surprisingly, vaccinated children had stronger responses to S, but not N, compared to non-vaccinated ($p=0,006$). Six months after infection, both anti-S and anti-N responses were detectable, with the small cohort followed from acute infection showing slightly increased responses to both in the later timepoint, though without statistical significance. In conclusion, children display immune responses against SARS- CoV-2 sustained even 6 months after infection, with vaccination boosting initial responses, which can also behave in an age-dependent manner.

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EXHIBITION
FLOORPLAN

8	BD-ENZIPHARMA
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9	BONSAILAB
14	IMMUNOSTEP
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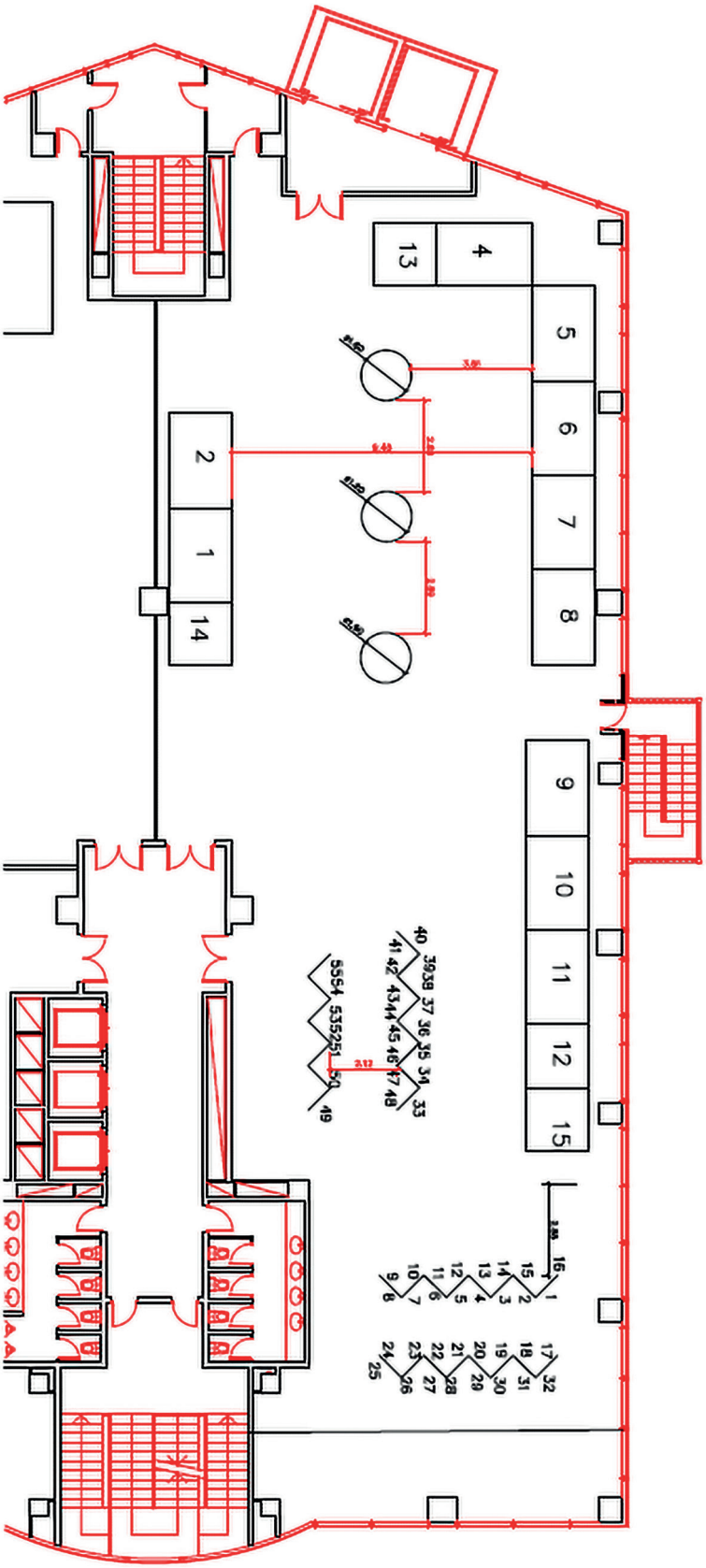
10-11	PALEX-CYTEK
13	PARALAB
12	STANDARD BIO
6-7	SYSMEX
15	300 K SOLUTIONS



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Calle Alcalá 414, oficinas 4ª planta, 28027 Madrid
Tel: +34 913 612 600
secretaria.tecnica@citometria.org